

Table S1 The disease index analysis in Jinyou 38 and Xintaimici in cucumber

Cucumber cultivars	Numbers	Days post-inoculation (dpi)	Disease index
Jinyou 38	77	7	14.85
Xintaimici	75	7	72.33

Note: High resistance (HR), $0 < DI \leq 15$; Moderate resistance (R), $15 < DI \leq 35$; Resistance (MR), $35 < DI \leq 55$; Susceptible (S), $55 < DI \leq 75$; High susceptible (HS), $DI > 75$.

Table S2 The primers of qRT-PCR used in this study

Analysis	Primer name	Sequence(5'-3')
<i>CsPR1-1a</i> for qRT-PCR in transgenic cucumber	<i>CsPR1-1a</i> -F	GGCAGCCCAGACTTCTCAGC
	<i>CsPR1-1a</i> -R	GCATCTCACTTTGGCACATCCTA
<i>CsPR2</i> for qRT-PCR in transgenic cucumber	<i>CsPR2</i> -F	AAGACAACATCCTCAACTTTGC
	<i>CsPR2</i> -R	CTGCTGAAAGAATTCCAGTGTG
<i>CsPR3</i> for qRT-PCR in transgenic cucumber	<i>CsPR3</i> -F	CATTTCTCTCATCCTTTGGCAG
	<i>CsPR3</i> -R	GATATCGAAATCAACGCCATCC
<i>CsPYL2</i> for qRT-PCR in transgenic cucumber	<i>CsPYL2</i> -F	CAGAAATCATTGATGGACGACC
	<i>CsPYL2</i> -R	TGAAACATCAGCAAGTGACTTG
<i>CsPP2C2</i> for qRT-PCR in transgenic cucumber	<i>CsPP2C2</i> -F	TATTCCCGATCCAGAAGTCATG
	<i>CsPP2C2</i> -R	CAAGCATTGAGAGGTAATCTGC
<i>CsSnRK2.2</i> for qRT-PCR in transgenic cucumber	<i>CsSnRK2.2</i> -F	GCAGATCCAGAGACGAGAATAA
	<i>CsSnRK2.2</i> -R	TCAATACTCTGTGTTGCCTCAT
<i>CsABI5</i> for qRT-PCR in transgenic cucumber	<i>CsABI5</i> -F	GAATCGCCATTACAGTCTGAAC
	<i>CsABI5</i> -R	AACTCATCCAATGTGAGTGAGT
<i>CsRbohD</i> for qRT-PCR in transgenic cucumber	<i>CsRbohD</i> -F	GCGTAACATTACTGGTGATTCC
	<i>CsRbohD</i> -R	ATTCTTCCATCAGCATCCGTAT
<i>CsRbohF</i> for qRT-PCR in transgenic cucumber	<i>CsRbohF</i> -F	ATCGGACGATATTACACAGCTT
	<i>CsRbohF</i> -R	ACAATCACCAGAACGAATACCT
Cucumber <i>CsActin</i> gene for and qRT-PCR	Actin-F	TCGTGCTGGATTCTGGTG
	Actin-R	GGCAGTGGTGGTGAACAT

Table S3 List of primers used in the study

Analysis	Primer name	Sequence(5'-3')
Sequencing of the cDNA of <i>CsMLO1</i>	<i>CsMLO1</i> -F	GATATCATGGCGGGGGCAGCCGGTGGCAA
	<i>CsMLO1</i> -R	GCGGCCGCTCATTCAACTCTATCAAATGAAAAGTC
Sequencing of the cDNA of <i>CsMLO2</i>	<i>CsMLO2</i> -F	GGATCCATGGCTGAATGTGGAACAGAG
	<i>CsMLO2</i> -R	CTCGAGTCATTTGGCAAATGAGAAGTCTGAG
qRT-PCR for <i>CsMLO1</i>	<i>RTCsmLO1</i> -F	ATGCGTTGGCTAGAGCTAAGATG
	<i>RTCsmLO1</i> -R	AGGTGTCTCTTGCAAACCTAAACC
qRT-PCR for <i>CsMLO2</i>	<i>RTCsmLO2</i> -F	GTCATTCACCTCACTGGAAAGTGG
	<i>RTCsmLO2</i> -R	AGTGACAGCATCTTGGCCTATCG
<i>CsMLO1</i> -silencing vector	<i>TRVCsmLO1</i> -F	GAATTCGTGGCAGAGGCCCTTCGCAAC
	<i>TRVCsmLO1</i> -R	GAGCTC TCATTCAACTCTATCAAATGA
<i>CsMLO2</i> -silencing vector	<i>TRVCsmLO2</i> -F	gtgagtaaggttaccgaattcCACCACTCAGCCAAGAAG
	<i>TRVCsmLO2</i> -R	ggcctcgagacgcgtgagctcTCATTTGGCAAATGAGAA
<i>CsMLO1</i> -overexpression vector	<i>LucCsMLO1</i> -F	tctgagctctcagaATGGCGGGGGCAGCCGGTGG
	<i>LucCsMLO1</i> -R	cataagcttctgcagTTCAACTCTATCAAATGAAA
<i>CsMLO2</i> -overexpression vector	<i>LucCsMLO2</i> -F	ctgagctctcagaATGGCTGAATGTGGAACAGA
	<i>LucCsMLO2</i> -R	cataagcttctgcagTTTGGCAAATGAGAAGTCTG
Chimeric primer for <i>CsMLO1</i> -nLuc	<i>CsMLO1-nLuc</i> -F	ACAACGATTGCGCCCTCTCCATCTC
	<i>CsMLO1-nLuc</i> -R	CCTCGATATGTGCATCTGTAAAAGC
Chimeric primer for <i>CsMLO2</i> -nLuc	<i>CsMLO2-nLuc</i> -F	GTTGCCTCCTTCTTCACACCATAGC
	<i>CsMLO2-nLuc</i> -R	CCTCGATATGTGCATCTGTAAAAGC

ATGGCGGGGGCAGCCGGTGGCAAGTCGCTGGAGCAAACACCGACATGGGCCGTTGCCGTTGTTGCTTTGCTCGTCATCTCTATT	90
M A G A A G G K S L E Q T P T W A V A V V C F V L L V I S I	30
TTCATCGAATATAGTCTCCATCTTATCGGACATTGGCTAAAGAAGAGACACAAACGGCGTTGTTTGAAGCATTAGAGAAGATCAAATCA	180
F I E Y S L H L I G H W L K K R H K R A L F E A L E K I K S	60
GAGCTTATGTTATTGGGGTTTATATCATTGCTACTAACGGTGGGGCAAGGACCAATAACGGAGATATGTATTCCACAACATGTAGCTGCA	270
E L M L L G F I S L L L T V G Q G P I T E I C I P Q H V A A	90
ACGTGGCATCCATGTACAAAGGAAAGAGAAGATGAGATGAACAAGAGGTGGAGAAATCTGTGGAACATTGGGCTCTAATCGCCGGAGA	360
T W H P C T K E R E D E M N K E V E K S V E H L G L N R R R	120
CTCCTTATCTCCTCGGAAATGGTGAAGATTTCGGCGGAGTTTGGCCGCTGCGGGAGGAGAGGATAAATGTGCCGCCAAGGGTAAAGCT	450
L L H L L G N G E S F R R S L A A A G G E D K C A A K G K A	150
TCCTTTATTCAGCAGATGGAATTCATCACTTCATATCTTCATTTTTGTGTTGGCTGTTTTTCATGTTTTGTATTGTGTCTAACCTAT	540
S F I S A D G I H Q L H I F I F V L A V F H V L Y C V L T Y	180
GCGTTGGCTAGAGCTAAGATGAGGAGTTGGAAAACATGGGAAAAGAGACCAAAACTGCTGAATACCAATTCTCACATGATCCAGAGAGG	630
A L A R A K M R S W K T W E K E T K T A E Y Q F S H D P E R	210
TTTAGGTTTGAAGAGACACCTCATTGGGAGAAGACATTTGAGCTTTTGGACCAAAAATCCTGCCTTGATGTGGATCGTTTGTTCCTC	720
F R F A R D T S F G R R H L S F W T K N P A L M W I V C F F	240
AGACAATTTGAAGATCTGTCCAAAAGTTGATTACTTGACATTAAGACATGGGTTTATAATGGCACATTTAGCACCTCAAAGTCATACA	810
R Q F V R S V P K V D Y L T L R H G F I M A H L A P Q S H T	270
CAATTTGATTTCAAAAATACATTAATAGATCCCTTGAAGAAGACTTCAAAGTTGTTGTGGGAATCAGCCCACCAATTTGGTTCTTTGCT	900
Q F D F Q K Y I N R S L E E D F K V V V G I S P P I W F F A	300
GTTCTATTTCTCCTCTCAAACACTCACGGTTGGAGGGCGTATCTATGGCTGCCATTTCATCCACTAATCATTTTGTCTGTTGATGGAA	990
V L F L L S N T H G W R A Y L W L P F I P L I I L L L I G T	330
AAATTGCAAGTGATCATAACGAAAATGGCACTAAGAATACAAGAAAGAGGTGAAGTAGTGAAGGGCGTGCCGGTGGTGGACCTGGCGAT	1080
K L Q V I I T K M A L R I Q E R G E V V K G V P V V E P G D	360
GACCTCTTTGGTTAATCGACCTCGCCTTATCTTTATCTCACTCACTTGTCTCTTTCAAATGCCTTCCAAGTTGCCTTCTTTGCT	1170
D L F W F N R P R L I L Y L I N F V L F Q N A F Q V A F F A	390
TGGACTTGGTATGAGTTTGGGTGAATCTTGCTTCCATGAGCATATAGAAGATGTGGTATCAGAATTTCTATGGGGTGCCTGTGTACAA	1260
W T W Y E F G L N S C F H E H I E D V V I R I S M G V L V Q	420
ATCCTTTGCAGTTATGTTACTCTTCCTTTTATGCACTAGTCACTCAGATGGGTTCAACAATGAAGCCAATATATTCAATGAGAGAGTG	1350
I L C S Y V T L P L Y A L V T Q M G S T M K P T I F N E R V	450
GCAGAGGCCCTTCGCAATTTGGTACCCTCGGCTCGAAAGCACATCAAACACAACCGGGTTCGGTCACTCCAATGTGCGCCGACCCGCC	1440
A E A L R N W Y H S A R K H I K H N R G S V T P M S S R P A	480
ACCCGACTCACAGCATGTCACCTGTCCACCTTCTCCGACTACAAGAGTGAAGTCGATAGCTTCCACACCTCACCGAGAAGTCCACCG	1530
T P T H S M S P V H L L R H Y K S E V D S F H T S P R R S P	510
TTGACACCGATCGTTGGGACAACGATTCGCCCTCTCCATCTCGCCATGTTGATGGTTCGCTTCGTCACAACCCACGTTGAGATGGGA	1620
F D T D R W D N D S P S P S R H V D G S S S S Q P H V E M G	540
GGTTATGAAAAGATCCCGTGAATCAAGTTCGTTCTCAAGTTGATCCGGTTCACCATCTCGAAACCGCAATCAACATGAGATTCATATT	1710
G Y E K D P V E S S S S Q V D P V Q P S R N R N Q H E I H I	570
GGAGGCCCAAAGACTTTTCATTTGATAGAGTTGAATGA	1749
G G P K D F S F D R V E *	582

Figure S1 Nucleotide and deduced amino acid sequences of cucumber *CsMLO1* cDNA encoding an MLO homolog protein. The deduced amino acid sequences are given below the nucleotide sequences. The transcriptional start site is shown in bold type and the termination codon is marked by an asterisk (*). The amino acids shown in a solid box reveal a domain of the MLO-related protein, a member of the plant integral membrane proteins.

ATGGCTGAATGTGGAACAGAGCAGCGTACTTTGGAAGATACCTCAACTTGGGCTGTGCGGTTGTTGTTTTTCTTGGTTGTATTTC	90
M A E C G T E Q R T L E D T S T W A V A V V C F F L V V I S	30
ATCTTCATTGAACATGTCATTACCTCACTGGAAGTGGCTGGAGAAAAGGCACAAGCCAGCTCTTGTGAAGCTCTAGAAAAGGTTAAA	180
I F I E H V I H L T G K W L E K R H K P A L V E A L E K V K	60
GCAGAGCTTATGCTATTGGGATTCATATCCCTACTTCTAACGATAGGCCAAGATGCTGTCACTCAAATTTGTGTTTCGAAAGAGCTTGCA	270
A E L M L L G F I S L L L T I G Q D A V T Q I C V S K E L A	90
GCAACTTGGCTTCCCTGTGCAGCAAGAGCTAAAACAGGAGTAAAAGTTGCGAAGAACAGTCGCTTTAGACTTCTGAATTTTTAGATCCT	360
A T W L P C A A R A K T G V K V A K N S R L R L L E F L D P	120
GACTATGGTTCGAGGCGTATTTTAGCCTCGAAAGGAGATGATGCATGCGCTAAGAGGGGCCAACTCGCTTTCGTGTCGGCATATGGAATC	450
D Y G S R R I L A S K G D D A C A K R G Q L A F V S A Y G I	150
CATCAGCTCCATATTTTCATCTTCGTATTGGCTGTCTCCATGCTCATACTGCATCATAACTTTGGCTTTTGGCAGAACGAAGATGAGC	540
H Q L H I F I F V L A V F H V L Y C I I T L A F G R T K M S	180
AAATGGAAGGCTGGGAGGATGAAACCAAGACAATGAATACCAGTACTATAATGATCCAGCAAGATTTAGATTGCTAGAGATACTACG	630
K W K A W E D E T K T I E Y Q Y Y N D P A R F R F A R D T T	210
TTTGGACGCCGACACTTGAGCTTCTGGAGTCGTACACCAATTTCCCTCTGGATTGTTGTTTCTTCAAACAGTTCCTTGGATCAGTTACC	720
F G R R H L S F W S R T P I S L W I V C F F K Q F F G S V T	240
AAGGTTGATTACATGACACTGAGACATGGATTTCATCGTTGCACATCTTGACCCCGAAGTGAAGTAAAATTTGATTTCCACAAATACATT	810
K V D Y M T L R H G F I V A H L A P G S E V K F D F H K Y I	270
AGCAGATCTCTGGAAGACGACTTTAAAGTTGTTGTGGGATTAGTCCCGCAATGGCTATTTGCTGTTCTCTTCATCCTAACCAATACA	900
S R S L E D D F K V V V G I S P A M W L F A V L F I L T N T	300
AATGGTGGTATTATATCTATGGCTGCCTTTTCATCTCCTTAATTATAATCTTATGGTGGGAACAAGCTCCATGTTATTATAACTCAT	990
N G W Y S Y L W L P F I S L I I I L L V G T K L H V I I T H	330
ATGGGTTGACAAATCAAGAAAGGGTTCATGTTGTGAAGGTGTTCCGGTCGTTCCAGCTCGGGATGACCTGTTTGGTTTGGACGTCCA	1080
M G L T I Q E R G H V V K G V P V V Q P R D D L F W F G R P	360
CAACTTATTCTCTTCGATCCACTTTGTTCTCTTATGAATGCATTTGAGCTTGCCTTCTTTGCTTGGACCTTATGCATTTAAGTGG	1170
Q L I L F L I H F V L F M N A F Q L A F F A W T T Y A F K W	390
ATGGGTTGTTTCCATCAGCGAGTTGAAGATATTGTCATCAGACTCTCAATGGGGTTATCATACAAGTTCTCTGAGTTATGTCACACTC	1260
M G C F H Q R V E D I V I R L S M G V I I Q V L C S Y V T L	420
CCACTCTATGCTTTGGTTACTCAGATGGGCTCTAACATGAGACCAACCATTTTCAACGACCGAGTGGCAACGGCATGAAGAAGTGGCAC	1350
P L Y A L V T Q M G S N M R P T I F N D R V A T A L K N W H	450
CACTCCGCAAGAACATGAAGCAGCACCGCAACCCAGACAGTACCTCACCATTCTCAAGCAGGCCAGCTACTCCAACCTCACGGCATG	1440
H S A K K N M K Q H R N P D S T S P F S S R P A T P T H G M	480
TCTCCTATTACCTTCTGCACAAACATCAGCATGGCAGCACATCTCCAGGCTATCCGATGCCGAACCCGATCGTTGGGAAGAGTTGCCT	1530
S P I H L L H K H Q H G S T S P R L S D A E P D R W E E L P	510
CCTTCTCACACCATAGTAGAGCCCCCATCATGATAATCATCAAGATCAACAAGAACAATCTGAGACAATAATTAGAGAACAGGAGATG	1620
P S S H H S R A P H H D N H Q D Q Q E Q S E T I I R E Q E M	540
ACAGTTCAAGGACCAAGTTCAAGTGAACCGGTTCCATAACAGTCTGCTCGCCCTCATCAGGAAATCACTAGGACTCCATCAGACTTC	1710
T V Q G P S S S E T G S I T R P A R P H Q E I T R T P S D F	570
TCATTTGCCAAATGA	1725
S F A K *	574

Figure S2 Nucleotide and deduced amino acid sequences of cucumber *CsMLO2* cDNA encoding an MLO homolog protein. The deduced amino acid sequences are given below the nucleotide sequences. The transcriptional start site is shown in bold type and the termination codon is marked by an asterisk (*). The amino acids shown in a solid box reveal a domain of the MLO-related protein, a member of the plant integral membrane proteins.

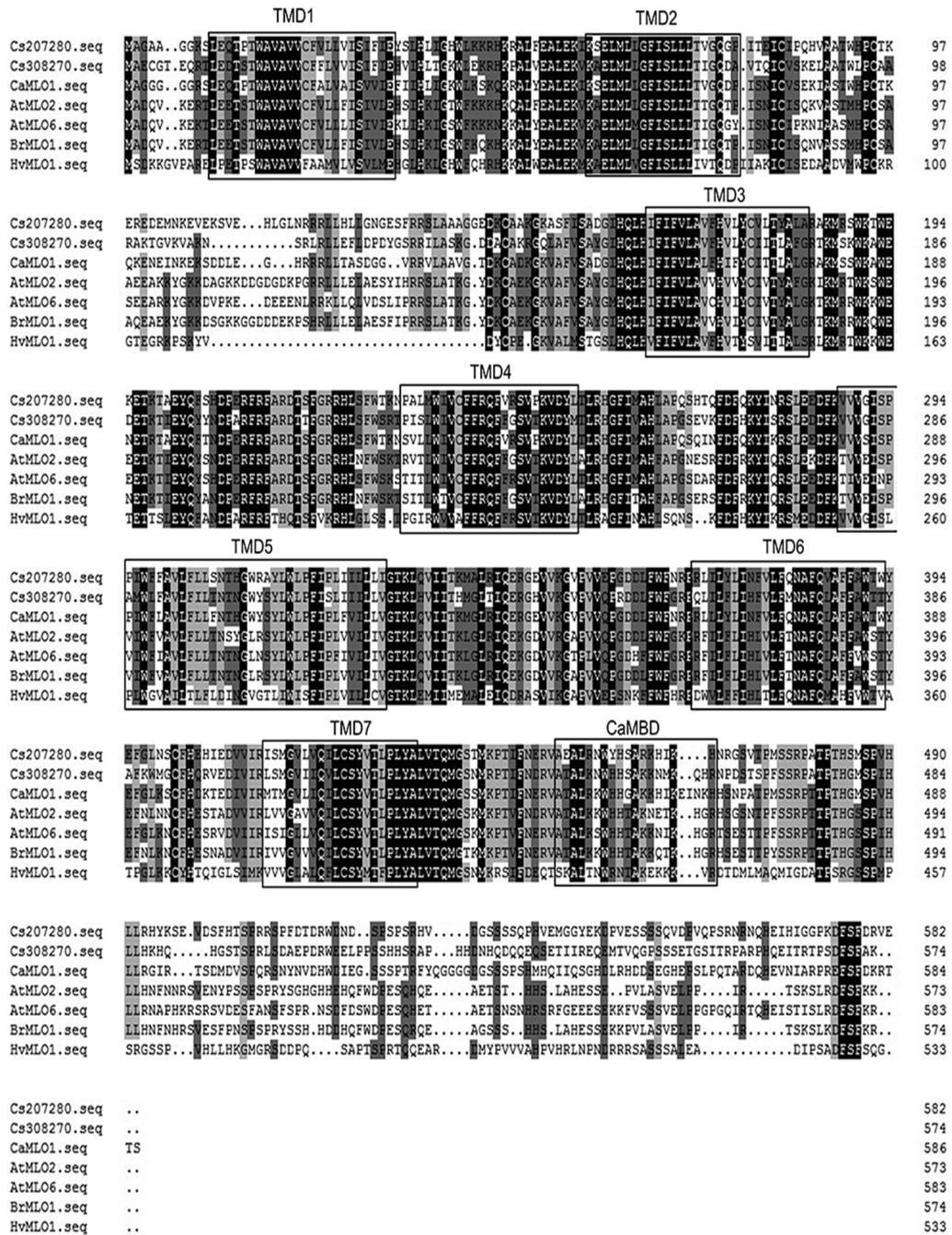


Figure.S3 Sequence analysis to CsMLO1 and CsMLO2 in cucumber. Comparison of the deduced amino acid sequence of Cs207280 (CsMLO1) and Cs308270 (CsMLO2) cDNA with other plant MLOs from pepper (*Capsicum annuum*, MLO1, accession no. AAX31277), Arabidopsis (*Arabidopsis thaliana*, MLO2 and MLO6, accession no. Q9SXB6 and Q94KB7), turnip (*Brassica rapa*, MLO1, accession no. AAX77014) and barley (*Hordeum vulgare* subsp. *vulgare*, MLO1, accession no. P93766). The conserved seven transmembrane domains (TMD1 to TMD7) and the calmodulin-binding domain (CaMBD) of the MLO protein were shown in solid boxes.

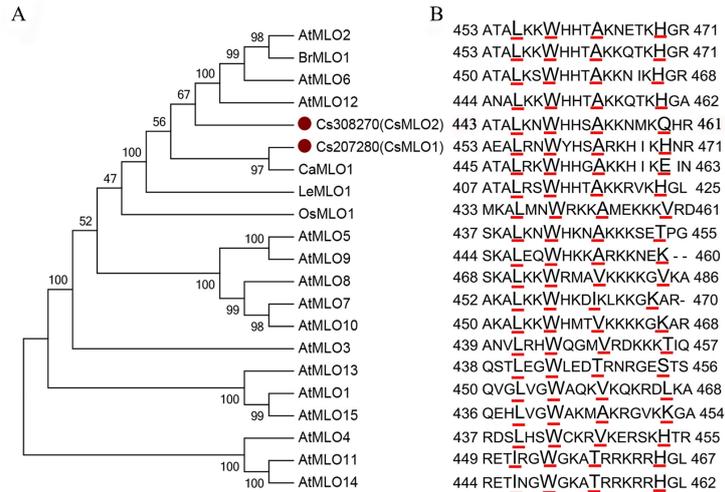


Figure S4. Phylogenetic analysis of CsMLO1/CsMLO2 and analysis of the CaM-binding domain (CaMBD) sequences of CsMLO1/CsMLO2. **(A)** Phylogenetic analysis of plant mildew resistance locus O (MLO) proteins. **(B)** Alignment of the putative CaMBD sequence of CsMLO1 (Alanine⁴⁵³ to Argine⁴⁷¹) and CsMLO2 (Alanine⁴⁴³ to Argine⁴⁶¹) with the corresponding regions of MLO families. The names of MLO families listed in **(A)** and their putative CaMBD sequences are aligned in the same order. The hydrophobic amino acids corresponding to a Ca²⁺-dependent CaM-binding motif and the highly conserved tryptophan (W) residues were the capital letters.

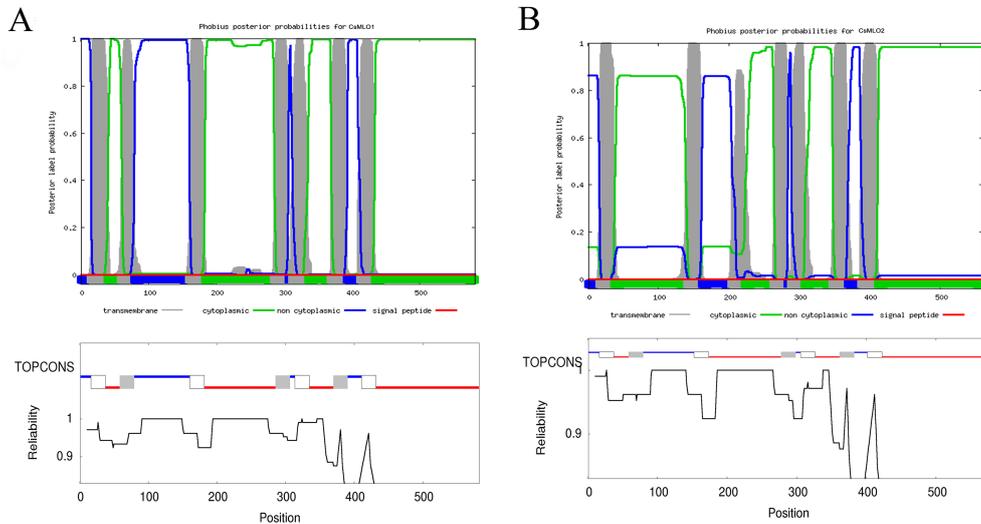


Figure S5. The predicted transmembrane topology of the CsMLO1 and CsMLO2 proteins analyzed. Cytoplasmic and non-cytoplasmic represented the intracellular and extracellular locations, respectively, using the online tools Phobius (<http://phobius.sbc.su.se/>) and TOPCONS (<http://topcons.cbr.su.se/>).

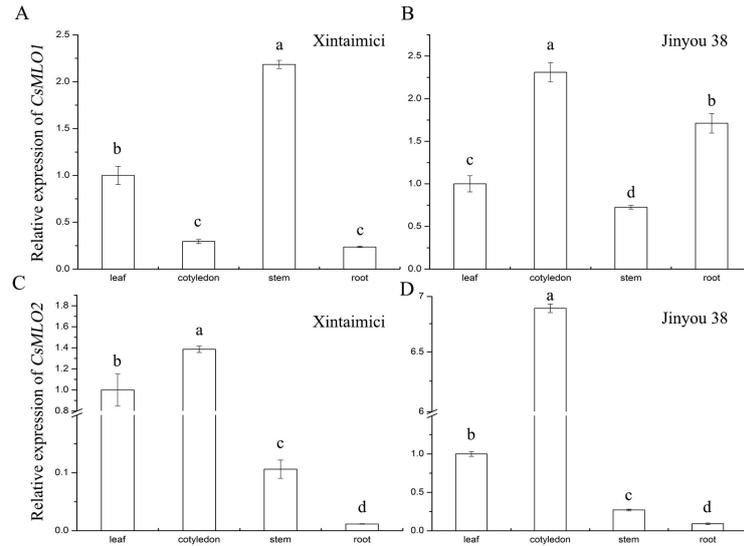


Figure S6. Expression pattern of *CsMLO1* and *CsMLO2* in cucumber. Spatial expression patterns of *CsMLO1* and *CsMLO2* in leaves, cotyledons, stems and roots according to reverse-transcription quantitative PCR (RT-qPCR). Expression analysis of candidate genes using the $2^{-\Delta\Delta Ct}$ method. Data are means \pm standard deviations from three biological replicates per cultivar. The lowercase letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$). (A) Expression pattern of *CsMLO1* in susceptible cultivar. (B) Expression pattern of *CsMLO1* in resistant cultivar. (C) Expression pattern of *CsMLO2* in susceptible cultivar. (D) Expression pattern of *CsMLO2* in resistant cultivar. The expression level in the leaf was normalized as 1. Data represent means \pm SE of three biological replicates. Letters indicate significant differences at $P < 0.05$ compared with the leaf by Student's *t*-test.

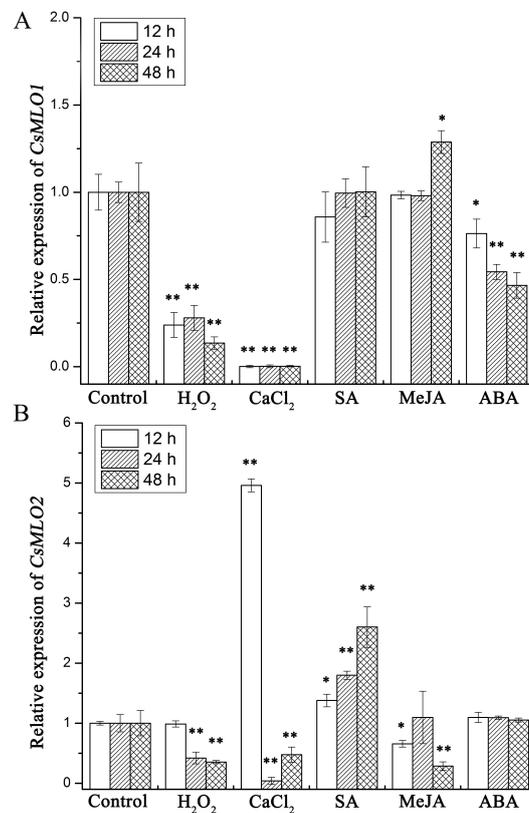


Figure S7. Expression patterns of *CsMLO1* and *CsMLO2* genes in response to plant hormones. (A) RT-qPCR analyses of *CsMLO1* transcripts in resistant variety under plant hormones treatments such as hydrogen peroxide (H_2O_2 , 10 μM), abscisic acid (ABA, 100 μM), salicylic acid (SA, 1 $\text{mmol}\cdot\text{L}^{-1}$), methyl jasmonate (MeJA, 100 $\mu\text{mol}\cdot\text{L}^{-1}$) and CaCl_2 (10 $\text{mmol}\cdot\text{L}^{-1}$). (B) RT-qPCR analyses of *CsMLO2* transcripts in resistant varieties under plant hormones treatments. The relative expression levels of *CsMLO1* and *CsMLO2* in cucumber plants at variety time points were compared with the mock control, which was set to 1. Expression analysis of candidate genes using the $2^{-\Delta\Delta\text{Ct}}$ method. Data are means \pm standard deviations from three biological replicates per cultivar. The asterisks indicated a significant difference (Student's *t*-test, * $P < 0.05$ or ** $P < 0.01$).