genes

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Article

Discovery and Characterization of a Novel Tomato *mlo* Mutant from an EMS Mutagenized Micro-Tom Population

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Supplementary Materials

Table S1. Primer pairs used in this study.

Primer name	Forward primer (5′- 3′)	Reverse primer (5′- 3′)	PCR product size
*SlMLO1*_full-length	TTGACATTTCCCCTTCTTCTTA	TACAAAATCATTGCCATTTGAA	1743 bp
*SlMLO1*_seqA	CCCTTTCTGAAATCCTTACTCC		
*SlMLO1*_seqB	TATTGCTAGTTGGCACAAAACT		
HRM_marker	TGGCTAAAGCACGGTCTA	CTGGATCTTGCAACACTGTCA	406 bp
35S_promoter	GCTCCTACAAATGCCATCA	GATAGTGGGATTGTGCGTCA	195 bp
NPTII_marker	TCGGCTATGACTGGGCACAAC	AAGAAGGCGATAGAAGGCGA	722 bp
SlEf1α	ACAGGCGTTCAGGTAAGGAA	GAGGGTATTCAGCAAAGGTCTC	120 bp
On_ITS	CGCCAAAGACCTAACCAAAA	AGCCAAGAGATCCGTTGTTG	90 bp

Table S2. Genotyping and phenotyping of eight progenies (BC_1S_1) derived from two (i.e. BC_{1_1} and BC_{1_3}) of the three BC_1 crosses M200 × MT.

M200 x MT	Number of susceptible plants	Number of resistant plants	Expected 3S:1R		HRM profiles		
			X^2	*p*	Wild-type allele	heterozygous	*m200* allele
BC_1S_1_1-1	10	6	2.07	0.15	2	8	6
BC_1S_1_1-2	13	4			4	9	4
BC_1S_1_1-4	13	6			3	10	6
BC_1S_1_1-10	12*	7			4	7	7
BC_1S_1_3-1	14	4	0.45	0.50	4	10	4
BC_1S_1_3-6	11	7			4	7	7
BC_1S_1_3-7	17	2			4	13	2
BC_1S_1_3-9	11	8			2	9	8

S = susceptible, R = resistant.
* One plant could not be genotyped with the HRM marker.

Table S3. Genotyping and phenotyping of the progenies (F_2) of three crosses between the resistant M200 plant and the tomato cv Moneymaker (M200 × MM).

M200 x MM	Number of susceptible plants	Number of resistant plants	Expected 3S:1R		HRM marker profiles		
			X2	p	Wild-type allele	heterozygous	*m200* allele
F_2_1	25	14	2.47	0.12	9	16	14
F_2_2	27	11	0.32	0.57	11	16	11
F_2_3	25*	14	2.47	0.12	5	19	14

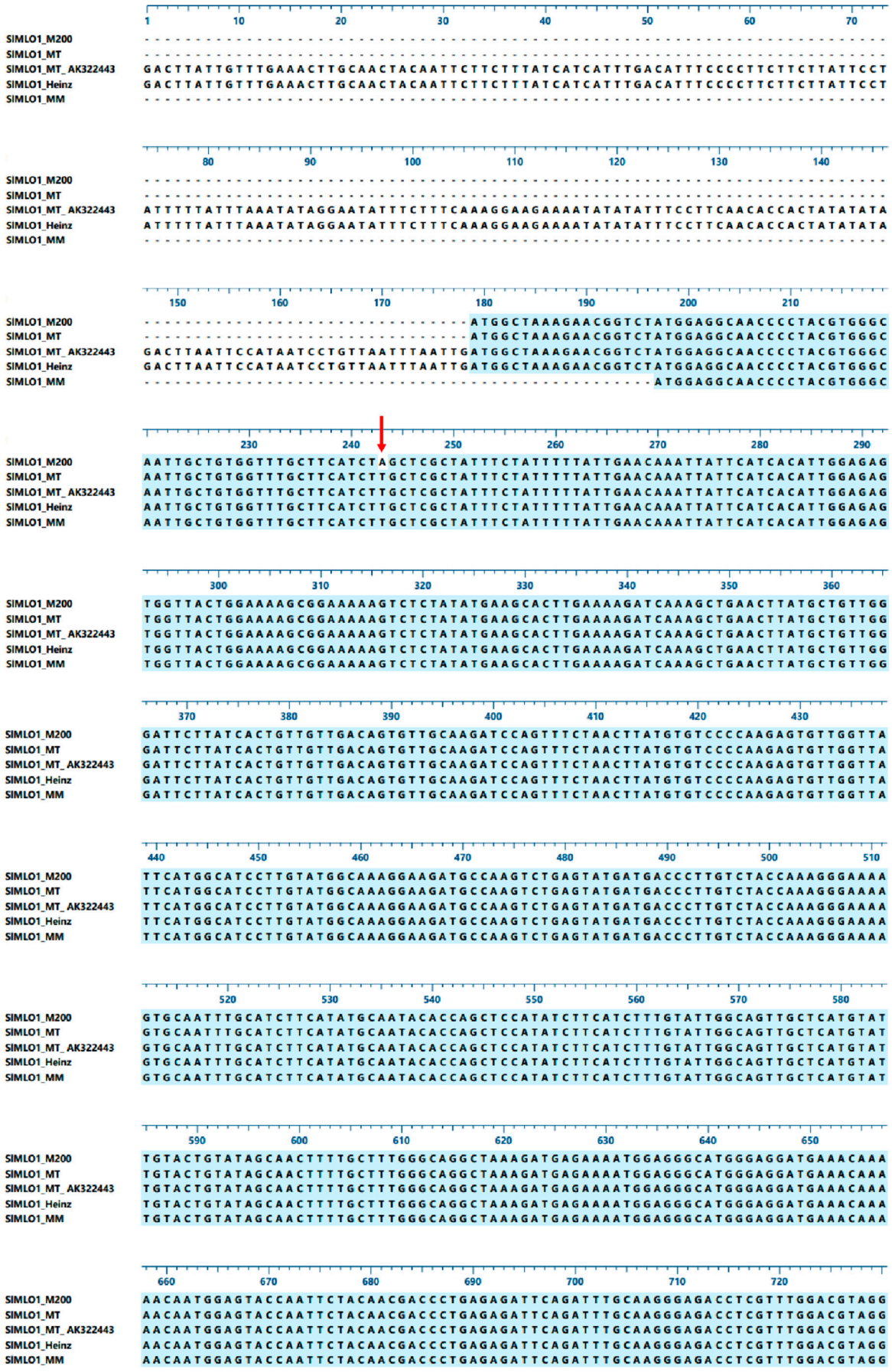
S = susceptible, R = resistant.
* One plant could not be genotyped with the HRM marker.

Table S4. Overview of the *mlo*-mutants described in the literature.

Plant species	*MLO* gene	Allele name	Amino-acid exchange	Location	Origin	Reference
Pea (*Pisum sativum*)	*PsMLO1*	*er1-1*	S227 → stop	2IC	natural origin	[1]
		er1-2	aberrant splicing variants	n.a.	natural origin	
		er1-3	G288 → stop	TM4	natural origin	
		er1-4	ΔA91 (in frame shift)	miss 1EXTRA &TM1	natural origin	
		er1-5	W190 → stop	2IC	DES	[2]
		er1-6	L353P	TM6	natural origin	[3]
		n.a.	Q226 → stop	2IC	ENU	[4]
		n.a.	312Y → stop	TM5	ENU	
Petunia (*Petunia* x *hybrida*)	*PhMLO1*	n.a.	S130L	TM3	EMS/TILLING	[5]
		n.a.	G176E	2IC	EMS/TILLING	
Rose (*Rosa multiflora*)	*RhMLO4*	n.a.	V519 → stop	C-terminal	natural origin	[6]
		n.a.	T532 → stop	C-terminal	natural origin	
Tomato (*Solanum lycopersicum*)	*SlMLO1*	*m200*	L22 → stop	TM1	EMS	Present study
		ol-2	Q198 → stop	2IC	natural origin	[7]
		Slmlo1.1	M403 → stop	3EXTRA	natural origin	[8]
		n.a.	Small deletion	TM7	CRISPR-Cas9	[9]
Apple (*Malus domestica*)	*MdMLO19*	n.a.	E405 → stop	3EXTRA	natural origin	[10]
Grapevine (*Vitis vinifera*)	*VvMLO3*	n.a.	INDEL	3EXTRA	CRISPR-Cas9	[11]
Cucumber (*Cucumis sativus*)	*CsaMLO1*	n.a.	V170G	TM3	natural origin	[12]
			V472I	C-terminal	natural origin	
			V557I	C-terminal	natural origin	
	CsaMLO8	n.a.	K178 → stop	2IC	natural origin	[13]
			K487 → stop	C-terminal	natural origin	
			Δ174	3IC, part of TM5 & TM6	natural origin	[14]
Tobacco (*Nicotiana tabacum*)	*NtMLO1*	n.a.	Q198R	2IC	PCR error during cloning	[15]
	NtMLO2	n.a.	6 aberrant splicing variants	2IC	natural origin	[16]
		n.a.	2 aberrant splicing variants		natural origin	
Arabidopsis thaliana	*AtMLO2*	*Atmlo2-8* (*pmr2-2*)	G66R	TM2	EMS	[17]
Barley (*Hordeum vulgare*)	*HvMLO*	n.a.	V76L	TM2	natural origin	[18]
		mlo-12	F240L	2IC	NMU	
		mlo-28	T222I	2IC	NaN3	
		mlo-1	W162R	2IC	X-rays	
		mlo-6	3 aberrant splicing variants	n.a.	EMS	
		mlo-44	1 aberrant splicing variant	n.a.	NaN3	
		mlo-16	1 aberrant splicing variant	n.a.	EMS	
		mlo-30	2 aberrant splicing variants	C terminal	EMS	
		mlo-11	aberrant splicing variants	n.a.	natural origin	
		mlo-2	A349T	TM6	X-rays	
		mlo-34	W423 → stop	C-terminal, CaMBD	EHOES	
		mlo-36	W357 → stop	TM6	iso-PMS	
		mlo-39	Q351 → stop	TM6	iso-PMS	
		mlo-43	Q210 → stop	2IC	NaN3	
		mlo-32	E35 → stop	TM1	NaN3	
		mlo-31	G276 → stop	TM5	NaN3	
		mlo-35	H231L	2IC	iso-PMS	
		mlo-37	S71F	1EXTRA	iso-PMS	
		mlo-38	G318R	3IC	iso-PMS/NaN3	
		mlo-40	G264D	TM4	NaN3	
		mlo-41	R209K	2IC	NaN3	
		mlo-42	S187L	2IC	X-rays	
		mlo-5	M1I	N-terminus	EMS	
		mlo-8	M1V	N-terminus	EMS	
		mlo-7	G226D	2IC	EMS	
		mlo-9	R10W	N-terminus	EMS	
		mlo-13	V30E	TM1	EMS	

Plant species	*MLO* gene	Allele name	Amino-acid exchange	Location	Origin	Reference
Barley (*Hordeum vulgare*)	*HvMLO*	*mlo-17*	S31F	TM1	EMS	[18]
		mlo-26	L270H	TM4	EMS	
		mlo-27	G318E	3IC	EMS	
		mlo-29	P334L	3IC	NaN3	
		mlo-33	A306T	3IC	NaN3	
		mlo-3	frame shift P396	TM7	gamma-rays	
		mlo-4	frame shift W159	2IC	X-rays	
		mlo-10	deletion F182 and T183	2IC	gamma-rays	
		n.a.	W159R	2IC	SDM	
		n.a.	W162A or W163E	2IC	SDM	
		n.a.	E163R	2IC	SDM	
		n.a.	S187F	2IC	SDM	
		n.a.	D219	2IC	SDM	
		n.a.	D251N	TM4	SDM	
		n.a.	L307A	3IC	SDM	
		n.a.	P320A	3IC	SDM	
		n.a.	P324A	3IC	SDM	
		n.a.	F329A	3IC	SDM	
		n.a.	W330A	3IC	SDM	
		n.a.	F331A	3IC	SDM	
		n.a.	R333A	3IC	SDM	
		n.a.	C367A	3EXTRA	SDM	
		n.a.	C98A	1EXTRA	SDM	
		n.a.	C86A	1EXTRA	SDM	
		n.a.	C114A	1EXTRA	SDM	
		n.a.	P395G	TM7	SDM	
		mlo-11(*cnv2*)	aberrant splicing variants	n.a.	natural origin	[19]
Wheat (*Triticum aestivum*)	*TaMLO-A1*		P325L	3IC	EMS/TILLING	[20]
	TaMLO-A1		A354V	TM6	EMS/TILLING	
	TaMLO-B1		G296E	TM5	EMS/TILLING	
	TaMLO-B1		T297I	3IC	EMS/TILLING	
	TaMLO-B1		R313W	3IC	EMS/TILLING	
	TaMLO-B1		S315N	3IC	EMS/TILLING	
	TaMLO-B1		G319R	3IC	EMS/TILLING	
	TaMLO-B1		A320T	3IC	EMS/TILLING	
	TaMLO-B1		T345M	TM6	EMS/TILLING	
	TaMLO-D1		V316T	3IC	EMS/TILLING	
	TaMLO-D1		G319R	3IC	EMS/TILLING	
	TaMLO-D1		A320T	3IC	EMS/TILLING	
	TaMLO-D1		P321S	3IC	EMS/TILLING	
	TaMLO-D1		V323I	3IC	EMS/TILLING	
	TaMLO-D1		P335L	3IC	EMS/TILLING	
	TaMLO-D1		T345M	TM6	EMS/TILLING	
	TaMLO-A1		aberrant splicing variants and T78K	2IC	CRISPR-Cas9	[21]

n.a., not available; TM: Transmembrane; IC: Intracellular; EXTRA: Extracellular; DES: diethyl sulfate; ENU: N-ethyl-N-nitrosourea; EMS: Ethyl methanesulfonate; NMU: N-Nitroso-N-methylurea; NaN3: sodium azide; SDM: site-directed mutagenesis; EHOES: Ethyl hydroxyethanesulfonate; iso-PMS: isopropyl-methane-sulfonate; INDEL: insertion and deletion.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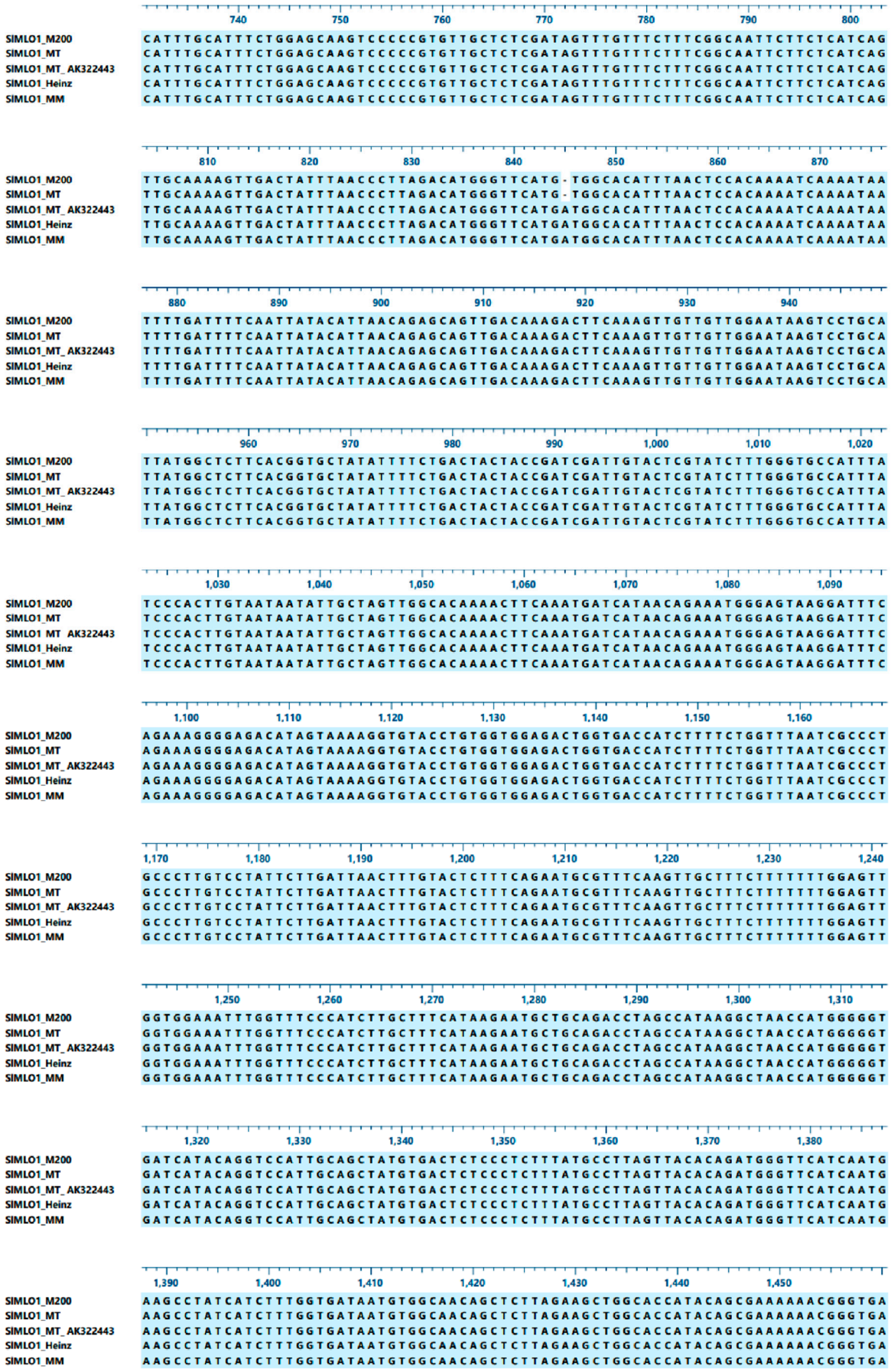
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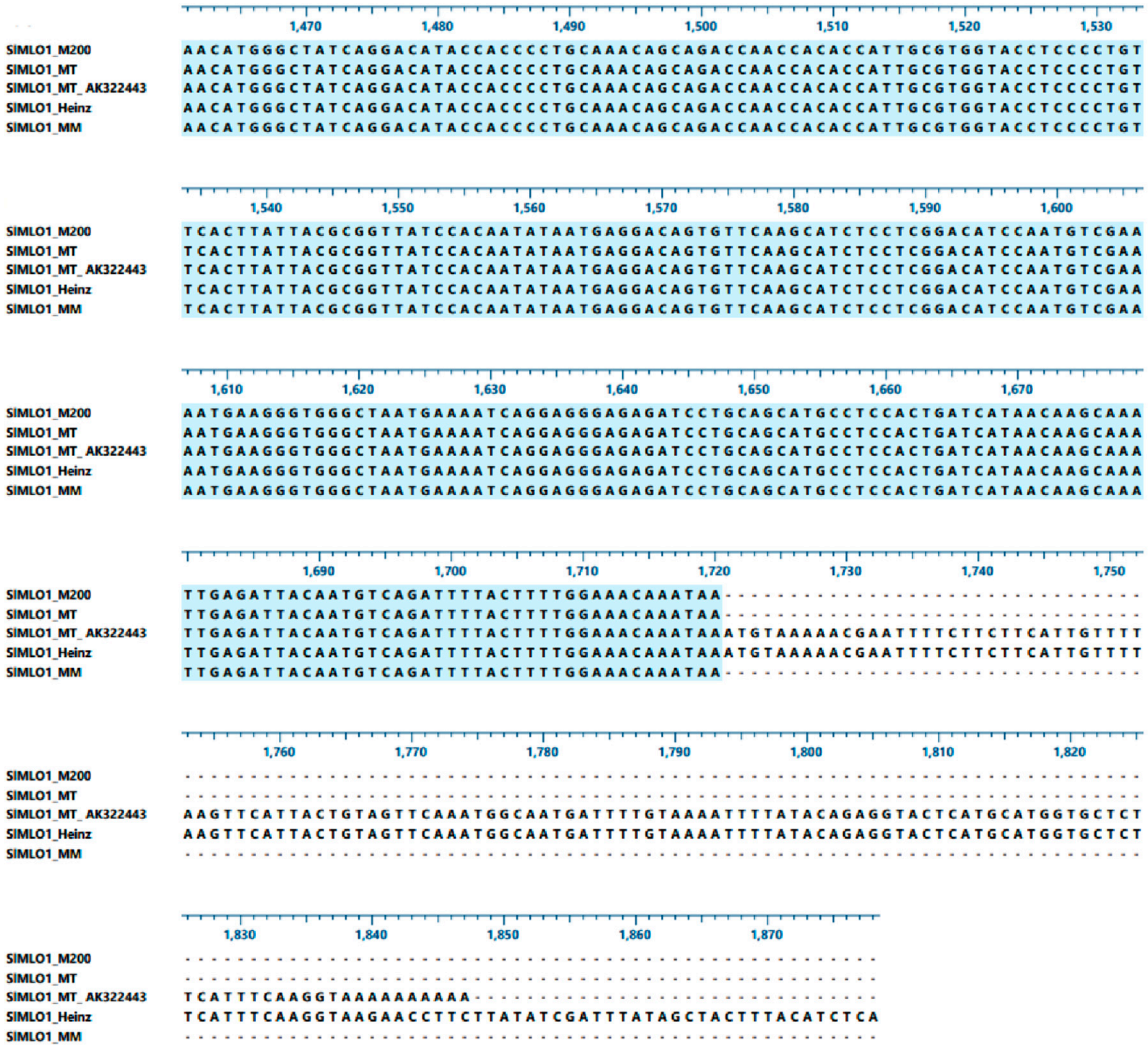
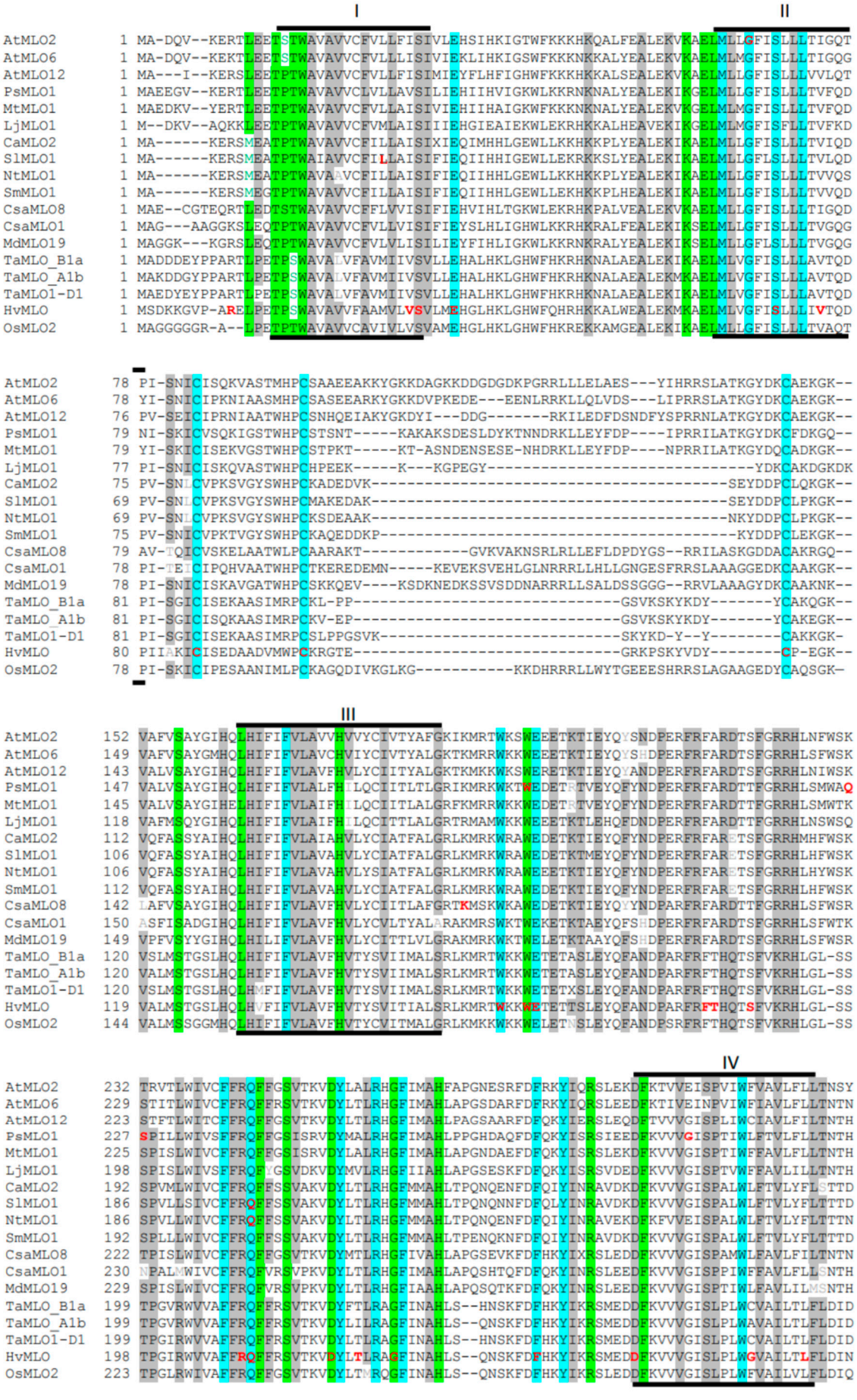


Figure S1. Nucleotide alignment of the *SlMLO1* sequence experimentally obtained from the tomato cv Micro-Tom (MT), the full-length transcript AK322443 of MT *SlMLO1* obtained from NCBI, the *SlMLO1* gene in M200, MM leaf cDNA sequence of *SlMLO1* obtained from Zheng et al. [22] and the one from the cv Heinz as in the SGN database Solyc04g49090.3). Arrow indicates the base change T → A responsible for the premature stop codon in M200 plants.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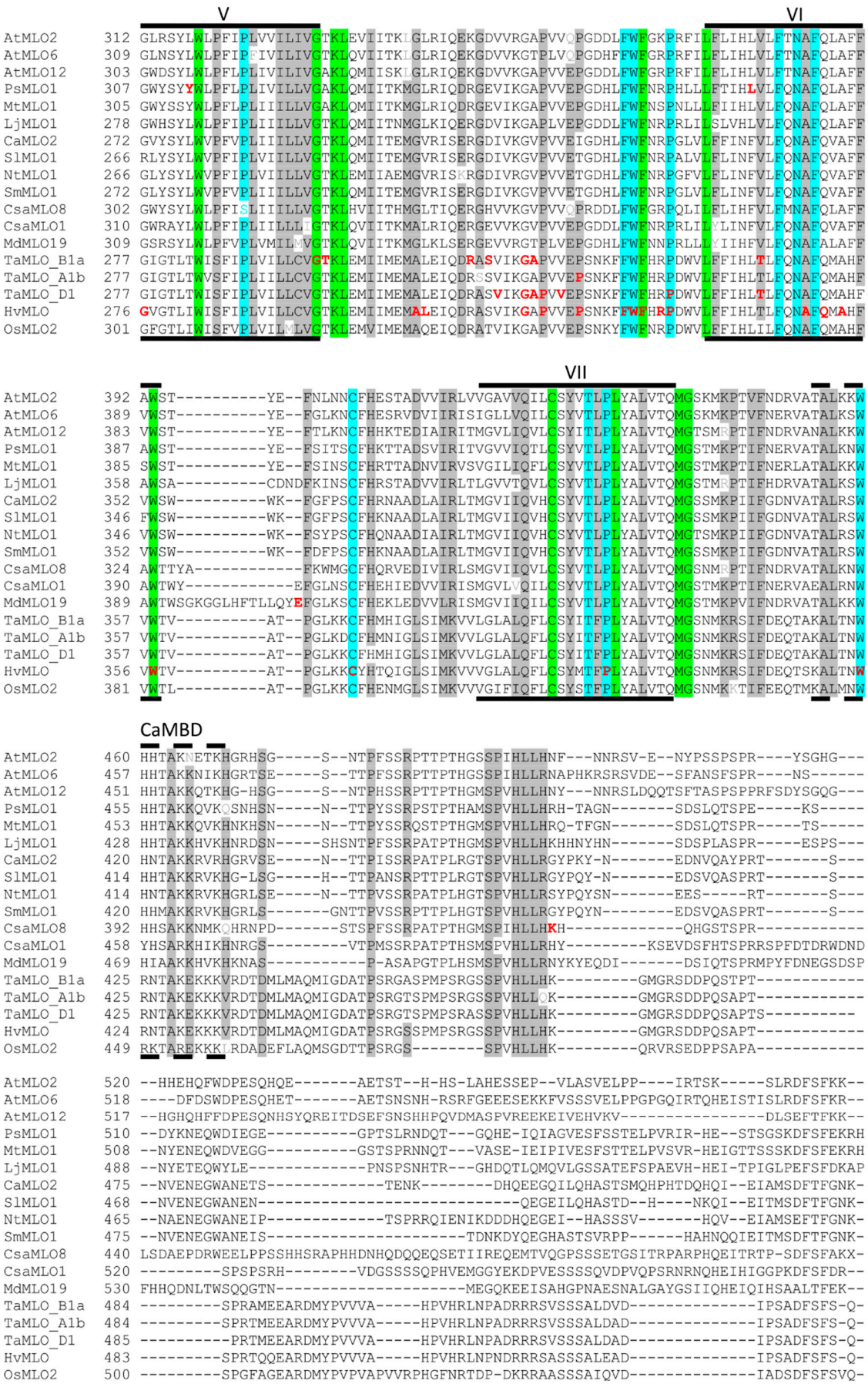
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TaMLO_D1 277 GIGTLTWISFIPLVILLCVGTKLEMIIMEMALEIQDRASVIKGAPVVEPSNKFFWFHRPDWVLFFIHLTLFQNAFQMAHF
HvMLO 276 GVGTLIWISFIPLVILLCVGTKLEMIIMEMALEIQDRASVIKGAPVVEPSNKFFWFHRPDWVLFFIHLTLFQNAFQMAHF
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AtMLO12 383 VWST----------YE--FTLKNCFHHKTEDIAIRITMGVLIQVLCSYITLPLYALVTQMGTSMRPTIFNDRVANALKKW
PsMLO1 387 AWST----------YE--FSITSCFHKTTADSVIRITVGVVIQTLCSYVTLPLYALVTQMGSTMKPTIFNERVATALKNW
MtMLO1 385 SWST----------YE--FSINSCFHRTTADNVIRVSVGILIQFLCSYVTLPLYALVTQMGSTMKPTIFNERLATALKKW
LjMLO1 358 AWSA----------CDNDFKINSCFHRSTADVVIRLTLGVVTQVLCSYVTLPLYALVTQMGSTMRPTIFHDRVATALKSW
CaMLO2 352 VWSW----------WK--FGFPSCFHRNAADLAIRLTMGVIIQVHCSYVTLPLYALVTQMGSSMKPIIFGDNVATALRSW
SlMLO1 346 FWSW----------WK--FGFPSCFHKNAADLAIRLTMGVIIQVHCSYVTLPLYALVTQMGSSMKPIIFGDNVATALRSW
NtMLO1 346 VWSW----------WK--FSYPSCFHQNAADIAIRLTMGVIIQVHCSYVTLPLYALVTQMGTSMKPIIFGDNVATALRSW
SmMLO1 352 VWSW----------WK--FDFPSCFHKNAADLAIRLTMGVIIQVHCSYVTLPLYALVTQMGSSMKPIIFGDNVATALRSW
CsaMLO8 324 AWTTYA----------FKWMGCFHQRVEDIVIRLSMGVIIQVLCSYVTLPLYALVTQMGSNMRPTIFNDRVATALKNW
CsaMLO1 390 AWTWY----------EFGLNSCFHEHIEDVVIRISMGVLVQILCSYVTLPLYALVTQMGSTMKPTIFNERVAEALRNW
MdMLO19 389 AWTWSGKGGLHFTLLQYEFGLKSCFHEKLEDVVLRISMGVIIQILCSYVTLPLYALVTQMGSTMKPVIFNDRVATALKKW
TaMLO_B1a 357 VWTV----------AT--PGLKKCFHMHIGLSIMKVVLGLALQFLCSYITFPLYALVTQMGSNMKRSIFDEQTAKALTNW
TaMLO_A1b 357 VWTV----------AT--PGLKDCFHMNIGLSIMKVVLGLALQFLCSYITFPLYALVTQMGSNMKRSIFDEQTAKALTNW
TaMLO_D1 357 VWTV----------AT--PGLKKCFHMHIGLSIMKVVLGLALQFLCSYITFPLYALVTQMGSNMKRSIFDEQTAKALTNW
HvMLO 356 VWTV----------AT--PGLKKCYHTQIGLSIMKVVVGLALQFLCSYMTFPLYALVTQMGSNMKRSIFDEQTSKALTNW
OsMLO2 381 VWTL----------AT--PGLKKCFHENMGLSIMKVVVGIFIQFLCSYSTFPLYALVTQMGSNMKKTIFEEQTMKALMNW
AtMLO2 460 HHTAKNETKHGRHSG-----S--NTPFSSRPTTPTHGSSPIHLLHNF---NNRSV-E--NYPSSPSPR----YSGHG---
AtMLO6 457 HHTAKKNIKHGRTSE-----S--TTPFSSRPTTPTHGSSPIHLLRNAPHKRSRSVDE--SFANSFSPR----NS------
AtMLO12 451 HHTAKKQTKHG-HSG-----S--NTPHSSRPTTPTHGMSPVHLLHNY---NNRSLDQQTSFTASPSPPRFSDYSGQG---
PsMLO1 455 HHTAKKQVKQSNHSN-----N--TTPYSSRPSTPTHAMSPVHLLHRH-TAGN-------SDSLQTSPE-----KS-----
MtMLO1 453 HHTAKKQVKHNKHSN-----N--TTPYSSRQSTPTHGMSPVHLLHRQ-TFGN-------SDSLQTSPR-----TS-----
LjMLO1 428 HHTAKKHVKHNRDSN-----SHSNTPFSSRPATPTHGMSPVHLLHKHHNYHN-------SDSPLASPR-----ESPS---
CaMLO2 420 HNTAKKRVRHGRVSE-----N--TTPISSRPATPLRGTSPVHLLRGYPKY-N-------EDNVQAYPRT-------S---
SlMLO1 414 HHTAKKRVKHG-LSG-----H--TTPANSRPTTPLRGTSPVHLLRGYPQY-N-------EDSVQASPRT-------S---
NtMLO1 414 HNTAKKRVKHGRLSE-----N--TTPVSSRPATPLHGTSPVHLLRSYPQYSN-------EES-----RT-------S---
SmMLO1 420 HHMAKKRVKHGRLS-------GNTTPVSSRPTTPLHGTSPVHLLRGYPQYN--------EDSVQASPRT-------S---
CsaMLO8 392 HHSAKKNMKQHRNPD-------STSPFSSRPATPTHGMSPIHLLHKH----------QHGSTSPR----------
CsaMLO1 458 YHSARKHIKHNRGS---------VTPMSSRPATPTHSMSPVHLLRHY---------KSEVDSFHTSPRRSPFDTDRWDND
MdMLO19 469 HIAAKKHVKHKNAS-----------P-ASAPGTPLHSMSPVHLLRNYKYEQDI-------DSIQTSPRMPYFDNEGSDSP
TaMLO_B1a 425 RNTAKEKKKVRDTDMLMAQMIGDATPSRGASPMPSRGSSPVHLLHK---------GMGRSDDPQSTPT----------
TaMLO_A1b 425 RNTAKEKKKVRDTDMLMAQMIGDATPSRGTSPMPSRGSSPVHLLQK---------GMGRSDDPQSAPT----------
TaMLO_D1 425 RNTAKEKKKVRDTDMLMAQMIGDATPSRGTSPMPSRASSPVHLLHK---------GMGRSDDPQSAPTS----------
HvMLO 424 RNTAKEKKKVRDTDMLMAQMIGDATPSRGSSPMPSRGSSPVHLLHK---------GMGRSDDPQSAPT----------
OsMLO2 449 RKTAREKKKLRDADEFLAQMSGDTTPSRGS--------SPVHLLHK---------QRVRSEDPPSAPA----------
AtMLO2 520 --HHEHQFWDPESQHQE-------AETST--H-HS-LAHESSEP--VLASVELPP----IRTSK-----SLRDFSFKK--
AtMLO6 518 ----DFDSWDPESQHET-------AETSNSNH-RSRFGEEESEKKFVSSSVELPPGPGQIRTQHEISTISLRDFSFKR--
AtMLO12 517 --HGHQHFFDPESQNHSYQREITDSEFSNSHHPQVDMASPVREEKEIVEHVKV----------------DLSEFTFKK--
PsMLO1 510 --DYKNEQWDIEGE----------GPTSLRNDQT---GQHE-IQIAGVESFSSTELPVRIR-HE--STSGSKDFSFEKRH
MtMLO1 508 --NYENEQWDVEGG----------GSTSPRNNQT---VASE-IEIPIVESFSTTELPVSVR-HEIGTTSSSKDFSFEKRH
LjMLO1 488 --NYETEQWYLE-------------PNSPSNHTR---GHDQTLQMQVLGSSATEFSPAEVH-HEI-TPIGLPEFSFDKAP
CaMLO2 475 --NVENEGWANETS-------------TENK--------DHQEEGQILQHASTSMQHPHTDQHQI--EIAMSDFTFGNK-
SlMLO1 468 --NVENEGWANEN----------------------------QEGEILQHASTD--H----NKQI--EITMSDFTFGNK-
NtMLO1 465 --NAENEGWANEIP-------------TSPRRQIENIKDDDHQEGEI--HASSSV-------HQV--EIAMSEFTFGNK-
SmMLO1 475 --NVENEGWANEIS----------------------TDNKDYQEGHASTSVRPP------HAHNQQIEITMSDFTFGNK-
CsaMLO8 440 LSDAEPDRWEELPPSSHHSRAPHHDNHQDQQEQSETIIREQEMTVQGPSSSETGSITRPARPHQEITRTP-SDFSFAKX-
CsaMLO1 520 ---------SPSPSRH--------VDGSSSSQPHVEMGGYEKDPVESSSSQVDPVQPSRNRNQHEIHIGGPKDFSFDR--
MdMLO19 530 FHHQDNLTWSQQGTN----------------------MEGQKEEISAHGPNAESNALGAYGSIIQHEIQIHSAALTFEK--
TaMLO_B1a 484 ---------SPRAMEEARDMYPVVVA-----HPVHRLNPADRRRSVSSSALDVD--------------IPSADFSFS-Q-
TaMLO_A1b 484 ---------SPRTMEEARDMYPVVVA-----HPVHRLNPADRRRSVSSSALDAD--------------IPSADFSFS-Q-
TaMLO_D1 485 ----------PRTMEEARDMYPVVVA-----HPVHRLNPADRRRSVSSSALDAD--------------IPSADFSFS-Q-
HvMLO 483 ---------SPRTQQEARDMYPVVVA-----HPVHRLNPNDRRRSASSSALEAD--------------IPSADFSFS-Q-
OsMLO2 500 ---------SPGFAGEARDMYPVPVAPVVRPHGFNRTDP-DKRRAASSSAIQVD--------------IADSDFSFSVQ-

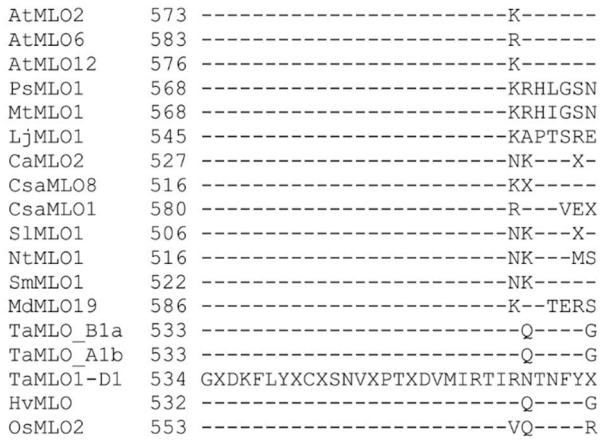

Figure S2. Protein alignment of functionally characterized MLO sequences of *Arabidopsis* thaliana AtMLO2, -6, and -12 (GenBank accession numbers NP172598, NP176350, and NP565902) [17], *Pisum sativum* (pea) PsMLO1 (GenBank accession number FJ463618) [1], *Medicago truncatula* (barrel clover) MtMLO1 (GenBank accession number HQ446457) [1], *Lotus japonicus* LjMLO1 (GenBank accession number AY967410) [1], *Capsicum annuum* (pepper) CaMLO2 (GenBank accession number AFH68055) [23], *Cucumis sativus* (cucumber) CsaMLO1 and -8 (GenBank accession numbers Csa1M085890.1 and Csa5M623470.1) [12,14], *Solanum lycopersicum* (tomato) SlMLO1 (GenBank accession number NP001234814) [7], *Nicotiana tabacum* (tobacco) NtMLO1 (GenBank accession number KM244716) [15], *S. melongena* (eggplant) SmMLO1 (GenBank accession number KM244717) [24], *Malus domestica* (apple) MdMLO19 (GenBank accession number MDP0000168714) [10], *Triticum aestivum* (wheat) TaMLO-A1b, TaMLO-B1a, and TaMLO-D1 (GenBank accession numbers AX063298, AF361932, and AX063296) [25,26], *Hordeum vulgare* (barley) HvMLO (GenBank accession number Z83834) [27] and *Oryza sativa* (rice) OsMLO2 (GenBank accession number AF384030) [25]. Highlighted in green and in light blue are the conserved amino acids among the whole MLO family indicated by Kusch et al. [28] and by Elliott et al. [29], respectively. Amino acids highlighted in gray refer to the ones reported to be under negative selection by Appiano et al. [30]. Letters displayed in green, light blue or gray indicate synonymous amino acid exchanges in each of three categories above described. Letters in red bold indicate amino acids identified in *mlo*-mutants for each of the plant species described above. Black lines indicate the position of the transmembrane domains which have been numbered with romans numbers.

References

1. Humphry, M.; Reinstädler, A.; Ivanov, S.; Bisseling, T.; Panstruga, R. Durable broad-spectrum powdery mildew resistance in pea er1 plants is conferred by natural loss-of-function mutations in PsMLO1. *Mol. Plant Pathol.* **2011**, *12*, 866–878, doi:10.1111/j.1364-3703.2011.00718.x.
2. Pavan, S.; Schiavulli, A.; Appiano, M.; Marcotrigiano, A.R.; Cillo, F.; Visser, R.G.F.; Bai, Y.; Lotti, C.; Ricciardi, L. Pea powdery mildew *er1* resistance is associated to loss-of-function mutations at a *MLO* homologous locus. *Theor. Appl. Genet.* **2011**, *123*, 1425–1431, doi:10.1007/s00122-011-1677-6.
3. Sun, S.; Fu, H.; Wang, Z.; Duan, C.; Zong, X.; Zhu, Z. Discovery of a Novel *er1* Allele Conferring Powdery Mildew Resistance in Chinese Pea (*Pisum sativum* L.) Landraces. *PLoS ONE* **2016**, *11*, e0147624, doi:10.1371/journal.pone.0147624.
4. Santo, T.; Rashkova, M.; Alabaça, C.; Leitão, J. The ENU-induced powdery mildew resistant mutant pea (*Pisum sativum* L.) lines S(*er1mut1*) and F(*er1mut2*) harbour early stop codons in the *PsMLO1* gene. *Mol. Breed.* **2013**, *32*, 723–727, doi:10.1007/s11032-013-9889-x.
5. Jiang, P.; Chen, Y.; Wilde, H.D. Identification and mutagenesis of disease susceptibility genes of *Petunia hybrida*. *Plant Cell, Tissue Organ. Cult.* **2016**, *126*, 117–125, doi:10.1007/s11240-016-0982-9.
6. Kaufmann, H.; Qiu, X.; Wehmeyer, J.; Debener, T. Isolation, Molecular Characterization, and Mapping of Four Rose *MLO* Orthologs. *Front. Plant Sci.* **2012**, *3*, 244, doi:10.3389/fpls.2012.00244.
7. Bai, Y.; Pavan, S.; Zheng, Z.; Zappel, N.F.; Reinstädler, A.; Lotti, C.; De Giovanni, C.; Ricciardi, L.; Lindhout, P.; Visser, R.; et al. Naturally Occurring Broad-Spectrum Powdery Mildew Resistance in a Central American Tomato Accession Is Caused by Loss of *Mlo* Function. *Mol. Plant Microbe Interac.* **2008**, *21*, 30–39, doi:10.1094/mpmi-21-1-0030.
8. Kim, D.; Jin, B.; Je, B.I.; Choi, Y.; Kim, B.S.; Jung, H.J.; Nou, I.S.; Park, Y. Development of DNA markers for *Slmlo1.1*, a new mutant allele of the powdery mildew resistance gene *SlMlo1* in tomato (*Solanum lycopersicum*). *Genome* **2018**, *61*, 703-12, doi: 10.1139/gen-2018-0114
9. Nekrasov, V.; Wang, C.; Win, J.; Lanz, C.; Weigel, D.; Kamoun, S. Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. *Sci. Rep.* **2017**, *7*, 1–6, doi:10.1038/s41598-017-00578-x.
10. Pessina, S.; Angeli, D.; Martens, S.; Visser, R.G.; Bai, Y.; Salamini, F.; Velasco, R.; Schouten, H.J.; Malnoy, M. The knock-down of the expression of *MdMLO19* reduces susceptibility to powdery mildew (*Podosphaera leucotricha*) in apple (*Malus domestica*). *Plant Biotechnol. J.* **2016**, *14*, 2033–2044, doi:10.1111/pbi.12562.

11. Wan, D.-Y.; Guo, Y.; Cheng, Y.; Hu, Y.; Xiao, S.; Wang, Y.; Wen, Y.-Q. CRISPR/Cas9-mediated mutagenesis of *VvMLO3* results in enhanced resistance to powdery mildew in grapevine (*Vitis vinifera*). *Hortic. Res.* **2020**, *7*, 1–14, doi:10.1038/s41438-020-0339-8.
12. Berg, J.A.; Appiano, M.; Bijsterbosch, G.; Visser, R.G.F.; Schouten, H.J.; Bai, Y. Functional characterization of cucumber (*Cucumis sativus* L.) Clade V *MLO* genes. *BMC Plant Biol.* **2017**, *17*, 1-15, doi: 10.1186/s12870-017-1029-z
13. Nie, J.; Wang, Y.; He, H.; Guo, C.; Zhu, W.; Pan, J.; Li, D.; Lian, H.; Pan, J.; Cai, R. Loss-of-function mutations in *CsMLO1* confer durable powdery mildew resistance in cucumber (*Cucumis sativus* L.). *Front. Plant Sci.* **2015**, *6*, 1155. doi.org/10.3389/fpls.2015.01155
14. Berg, J.A.; Appiano, M.; Martínez, M.S.; Hermans, F.W.K.; Vriezen, W.H.; Visser, R.G.F.; Bai, Y.; Schouten, H.J. A transposable element insertion in the susceptibility gene *CsaMLO8* results in hypocotyl resistance to powdery mildew in cucumber. *BMC Plant Biol.* **2015**, *15*, 243, doi:10.1186/s12870-015-0635-x.
15. Appiano, M.; Pavan, S.; Catalano, D.; Zheng, Z.; Bracuto, V.; Lotti, C.; Visser, R.G.F.; Ricciardi, L.; Bai, Y. Identification of candidate *MLO* powdery mildew susceptibility genes in cultivated Solanaceae and functional characterization of tobacco *NtMLO1*. *Transgenic Res.* **2015**, *24*, 847-858, doi: 10.1007/s11248-015-9878-4
16. Fujimura, T.; Sato, S.; Tajima, T.; Arai, M. Powdery mildew resistance in the Japanese domestic tobacco cultivar Kokubu is associated with aberrant splicing of *MLO* orthologues. *Plant Pathol.* **2016**, *65*, 1358–1365, doi:10.1111/ppa.12498.
17. Consonni, C.; Humphry, M.E.; Hartmann, H.A.; Livaja, M.; Durner, J.; Westphal, L.; Vogel, J.; Lipka, V.; Kemmerling, B.; Schulze-Lefert, P.; et al. Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nat. Genet.* **2006**, *38*, 716–720, doi:10.1038/ng1806.
18. Reinstädler, A.; Müller, J.; Czembor, J.H.; Piffanelli, P.; Panstruga, R. Novel induced *mlo* mutant alleles in combination with site-directed mutagenesis reveal functionally important domains in the heptahelical barley Mlo protein. *BMC Plant Biol.* **2010**, *10*, 31, doi:10.1186/1471-2229-10-31.
19. Ge, X.; Deng, W.; Lee, Z.Z.; Lopez-Ruiz, F.J.; Schweizer, P.; Ellwood, S.R. Tempered *mlo* broad-spectrum resistance to barley powdery mildew in an Ethiopian landrace. *Sci. Rep.* **2016**, *6*, 1-10, doi: 10.1038/srep29558
20. Acevedo-Garcia, J.; Spencer, D.; Thieron, H.; Reinstädler, A.; Hammond-Kosack, K.; Phillips, A.L.; Panstruga, R. *mlo*-based powdery mildew resistance in hexaploid bread wheat generated by a non-transgenic TILLING approach. *Plant Biotechnol. J.* **2016**, *15*, 367–378, doi:10.1111/pbi.12631.
21. Wang, Y.; Cheng, X.; Shan, Q.; Zhang, Y.; Liu, J.; Gao, C.; Qiu, J.-L. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nat. Biotechnol.* **2014**, *32*, 947–951, doi:10.1038/nbt.2969.
22. Zheng, Z.; Appiano, M.; Pavan, S.; Bracuto, V.; Ricciardi, L.; Visser, R.G.F.; Wolters, A.-M.A.; Bai, Y. Genome-Wide Study of the Tomato *SlMLO* Gene Family and Its Functional Characterization in Response to the Powdery Mildew Fungus *Oidium neolycopersici*. *Front. Plant Sci.* **2016**, *7*, 380, doi:10.3389/fpls.2016.00380.
23. Zheng, Z.; Nonomura, T.; Appiano, M.; Pavan, S.; Matsuda, Y.; Toyoda, H.; Wolters, A.M.A.; Visser, R.G.F.; Bai, Y. Loss of function in *Mlo* orthologs reduces susceptibility of pepper and tomato to powdery mildew disease caused by *Leveillula taurica*. *PLoS ONE* **2013**, *8*, e70723, doi: 10.1371/journal.pone.0070723
24. Bracuto, V.; Appiano, M.; Ricciardi, L.; Göl, D.; Visser, R.G.F.; Bai, Y.; Pavan, S. Functional characterization of the powdery mildew susceptibility gene *SmMLO1* in eggplant (*Solanum melongena* L.). *Transgenic Res*. **2017**, *26*, 323-330, doi: 10.1007/s11248-016-0007-9
25. Elliott, C.; Zhou, F.; Spielmeyer, W.; Panstruga, R.; Schulze-Lefert, P. Functional conservation of wheat and rice *Mlo* orthologs in defense modulation to the powdery mildew fungus. *Mol. Plant Microbe Interact*. **2002**, *15*, 1069-1077, doi: 10.1094/MPMI.2002.15.10.1069
26. Várallyay, É.; Giczey, G.; Burgyán, J. Virus-induced gene silencing of *Mlo* genes induces powdery mildew resistance in *Triticum aestivum*. *Arch. Virol*. **2012**, *157*, 1345-1350, doi: 10.1007/s00705-012-1286-y
27. Büschges, R.; Hollricher, K.; Panstruga, R.; Simons, G.; Wolter, M.; Frijters, A.; van Daelen, R.; van der Lee, T.; Diergaarde, P.; Groenendijk, J.; et al. The Barley *Mlo* Gene: A Novel Control Element of Plant Pathogen Resistance. *Cell* **1997**, *88*, 695–705, doi:10.1016/s0092-8674(00)81912-1.
28. Kusch, S.; Pesch, L.; Panstruga, R. Comprehensive Phylogenetic Analysis Sheds Light on the Diversity and Origin of the MLO Family of Integral Membrane Proteins. *Genome Biol. Evol.* **2016**, *8*, 878–895, doi:10.1093/gbe/evw036.
29. Elliott, C.; Müller, J.; Miklis, M.; Bhat, R.A.; Schulze-Lefert, P.; Panstruga, R. Conserved extracellular cysteine residues and cytoplasmic loop–loop interplay are required for functionality of the heptahelical MLO protein. *Biochem. J.* **2005**, *385*, 243–254, doi:10.1042/bj20040993.
30. Appiano, M.; Catalano, D.; Martínez, M.S.; Lotti, C.; Zheng, Z.; Visser, R.G.F.; Ricciardi, L.; Bai, Y.; Pavan, S. Monocot and dicot MLO powdery mildew susceptibility factors are functionally conserved in spite of the evolution of class-specific molecular features. *BMC Plant Biol.* **2015**, *15*, 1–10, doi:10.1186/s12870-015-0639-6.