

## Supplementary information

### **Msp40 effector of root-knot nematode manipulates plant immunity to facilitate parasitism**

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### Supplemental Table S1.

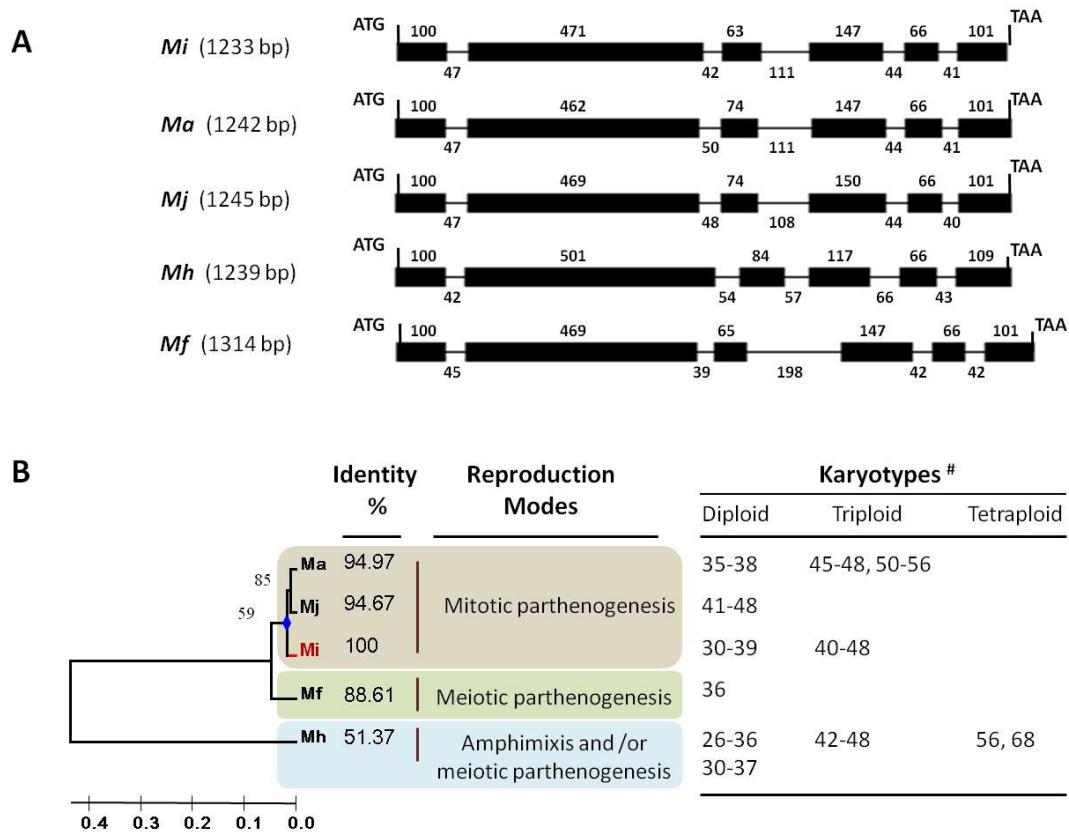
The platforms about nematodes genome and transcripts used for *Msp40* homologies screening.

<b>Species</b>	<b>Types</b>	<b>Blast server links</b>
<i>M. incognita</i>	Root-knot nematode	<a href="http://meloidogyne.toulouse.inra.fr/blast/blast.html">http://meloidogyne.toulouse.inra.fr/blast/blast.html</a>
<i>M. hapla</i>	Root-knot nematode	<a href="http://www.pngg.org/cbnp/index.php?option=com_wrapper&amp;Itemid=8">http://www.pngg.org/cbnp/index.php?option=com_wrapper&amp;Itemid=8</a>
<i>M. floridensis</i>	Root-knot nematode	<a href="http://nematodes.org/genomes/meloidogyne_floridensis/">http://nematodes.org/genomes/meloidogyne_floridensis/</a>
<i>G. rostochiensis</i>	Potato cyst nematodes	<a href="http://nematode.net/NN3_frontpage.cgi?navbar_selection=speciestable&amp;subnav_selection=Globodera_rostochiensis">http://nematode.net/NN3_frontpage.cgi?navbar_selection=speciestable &amp;subnav_selection=Globodera_rostochiensis</a>
<i>G. pallida</i>	Potato cyst nematode	<a href="http://www.sanger.ac.uk/cgi-bin/blast/submitblast/g_pallida">http://www.sanger.ac.uk/cgi-bin/blast/submitblast/g_pallida</a>
<i>H. glycines</i>	Soybean cyst nematode	<a href="http://nematode.net/NN3_frontpage.cgi?navbar_selection=speciestable&amp;subnav_selection=Heterodera_glycines">http://nematode.net/NN3_frontpage.cgi?navbar_selection=speciestable &amp;subnav_selection=Heterodera_glycines</a>
<i>B. xylophilus</i>	Pine wood nematode	<a href="http://www.genedb.org/blast/submitblast/GeneDB_Bxylophilus">http://www.genedb.org/blast/submitblast/GeneDB_Bxylophilus</a>
<i>C. elegans</i>	Free-living nematode	<a href="http://www.wormbase.org/tools/blast_blat">http://www.wormbase.org/tools/blast_blat</a>
Other nematode species		<a href="http://blast.ncbi.nlm.nih.gov/Blast.cgi">http://blast.ncbi.nlm.nih.gov/Blast.cgi</a>

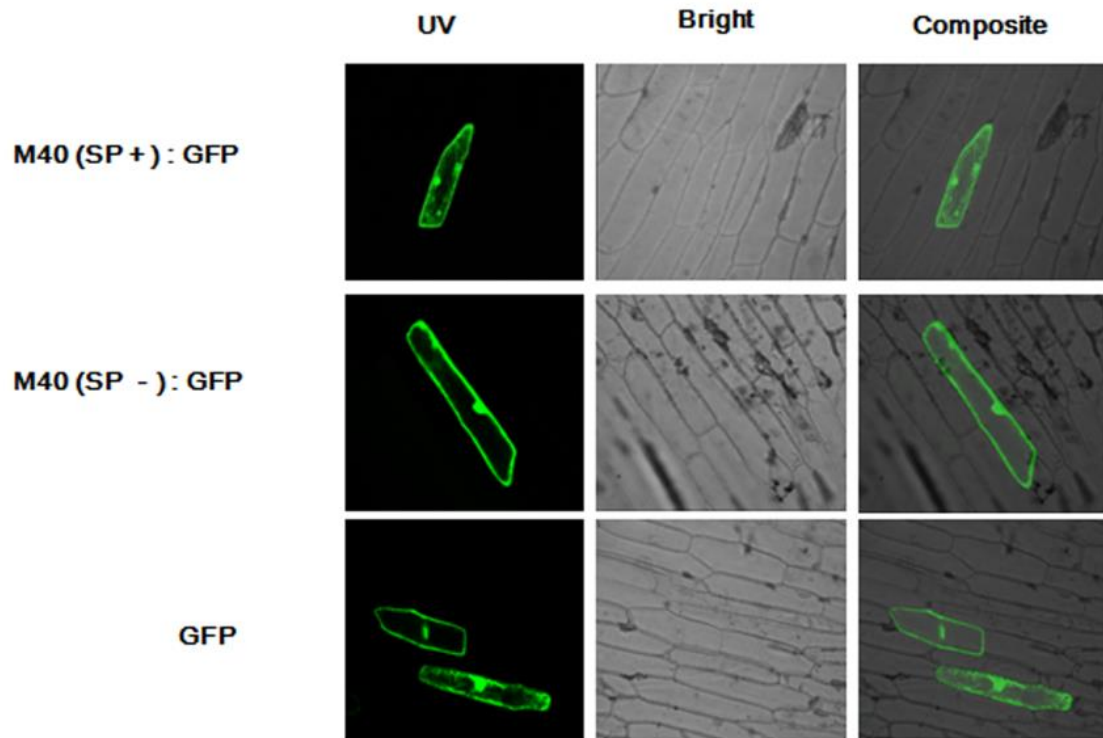
**Supplemental Table S2. Overview of the reference and target genes used in this study.** (The underlined regions represent cleavages sites of restriction enzymes.)

Genes	Primers	Primer sequences (5'–3')	Length	Usage
<i>Msp40</i> (AY422833)	Msp40 cds F	GGTCATTCTTATAACTAAAAACCTTCAAAC	1150bp	Full length cDNA and DNA cloning
	Msp40 cds R	AATTACGAGAAACACGGATGAATAGAT		
	M40 qRT-F1	GCAAAGAATCATGTAATGGGATACTG	244bp	qRT-PCR
	M40 qRT-R1	TGAGACAATCAGAAATCATAGTCGGT		
	M40 Sb F	CCATTATTGCTTTTGCTTACCCTT	857bp	Southern blotting
	M40 Sb R	GCACAATCAGAAATCATAGTCGGT		
	M40-- F1 KpnI	CGGG <u>G</u> TACCATGGAGGAGGATAAGGAAG	945bp	Subcellular localization
	M40--F2 KpnI	CGGG <u>G</u> TACCATGGAGGAGGATAAGGGAGA	882bp	
	M40-- R1 XbaI	TG <u>C</u> TAGAAGCAGCAGCTGCTGCAAGT		
	Msp40 P1S	AAGATGCTGAGAGTGCAGAGGAG	356bp	in situ hybridization
	MSP40 P1A	CTTGTCATCTTCTCCTTTTATCC		
	M40-NcoI	TTG <u>C</u> ATGGATGGAGGAGGATAAGGAAGACAT	948bp	Host expression
	M40-BstE II	TTT <u>G</u> TGACCCTTAAGCAGCAGCTGCTG		
	TS1-BglII	CC <u>A</u> GATCTAAGATGCTGAGAGTGCAGAGGAG	354bp	In vivo RNAi 1
	TS1-EcoRI	CC <u>G</u> AATTCCTTGTCATCTTCTCCTTTTATCC		
	TS1- Sall	CC <u>T</u> GCAAGGTCGACCTTGTCATCTTCC		
	TS1-BamHI-BstE II	AATT <u>G</u> GATCCGGTGACCAAGATGCTGAGAGTGC		
	TS2 -BglII	TTG <u>A</u> GATCATGTAATGGGATACTGC	292bp	In vivo RNAi 2
	TS2- EcoRI	TTG <u>G</u> AATTCAGCTGCTGCAAGTCTCTT		
	TS2 -Sall	A <u>A</u> GTCGACAGCTGCTGCAAGTCTCTT		
TS2-BamHI-BstEII	CGC <u>G</u> GATCCGGTTACCCATGTAATGGGATACTGC			
Msp40_SmaI	TAT <u>C</u> CCGGGATGGAGGAGGATAAGGGAG	882bp	Suppression of PCD	
Msp40_SaII	CTA <u>G</u> TGCACTTAAGCAGCAGCTGCTGCA			
<i>α-Tublin</i> (CK983765)	Mi Tub qRT-F	AAAAGAGGCTGAGGGTTGTGATTG	206bp	qRT-PCR (Control)
	Mi Tub qRT-R	GAACAGAAAGAGTTGCGTTGTAGGG		
CHAS intron (EU049859)	CHAS F	CGTTTATCTTAATGGCTCTTC	1312bp	RT-PCR
	CHAS R	CCTGCAAATTGACCAAAAAAG		
<i>FRK1</i> (AT2G19190)	FRK1 qRT_F	TGCAGCGCAAGGACTAGAG	108bp	qRT-PCR
	FRK1 qRT_R	ATCTTCGCTGGAGCTTCTC		
<i>PAD4</i> (AT3G5243)	PAD4 qRT_F	GCCGCTTTCACCCACTTTGG	102bp	qRT-PCR
	PAD4 qRT_R	GAGAGATTGGTTCCGAGCAGAGG		
<i>WRKY29</i> (AT4G23550)	WRKY29 qRT_F	ATCCAACGGATCAAGAGCTG	120bp	qRT-PCR
	WRKY29 qRT_R	GCGTCCGACAACAGATTCTC		
<i>WRKY33</i> (AT2G38470)	WRKY33 qRT_F	GCTGCTATTGCTGGTCACTCC	201bp	qRT-PCR
	WRKY33 qRT_R	GGTCTCCTGTTTGGTTCTTCC		
<i>CYP81F2</i> (At5g57220)	CYP81F2 qRT_F	GTGAAAGCACTAGCGAAGC	183bp	qRT-PCR
	CYP81F2 qRT_R	ATCCGTTCCAGCTAGCATCA		
<i>UBP22</i> (AT5G10790)	UBP22 qRT_F	GCCAAAGCTGTGGAGAAAAG	159bp	qRT-PCR (control)
	UBP22 qRT_R	TGTTTAGCGGAACGGATAC		

**Supplemental Fig S1. Alignment analysis for *Msp40* homologies.** (A) Genomic structure of the *Msp40* homologies in five *Meloidogyne* species, exons are represented by black boxes and introns by a line. (B) Phylogenetic tree constructed by the neighbor-joining method, based on the derived *Msp40*-like proteins from five *Meloidogyne* species. The three *Msp40*-based clades corresponding to the divergence for reproduction modes and cytogenetical karyotypes among the tested species. “#”, data referenced from the previous description mainly (Castagnone-Sereno, 2006), *M. floridensis* belongs to diploid with only one karyotype (2n=36) (David et al., 2013), and other *Meloidogyne* species contained diverse chromosome numbers and aneuploid. The triploid and tetraploid were putative karyotypes.



**Supplemental Fig S2. Subcellular localization of MiMsp40 in plant cells.** *MiMsp40* cDNA with and without signal peptide-coding sequence were fused to the joined GFP reporter gene respectively, and expressed in onion epidermal cells by particle bombardment. GFP fluorescence is mainly localized in the cytoplasm and the nucleus for both constructs.



**Supplemental Fig S3. Effect of plant host-derived RNAi of *MiMsp40* on *M. incognita* infection.** (A) Root galls development of control (WT) and transgenic lines (TS1, TS2) of two different *MiMsp40* dsRNA cassette (TS1 RNAi and TS2 RNAi). (B) RT-PCR of the single-stranded CHAS intron of *MiMsp40* dsRNA hairpins confirmed the expression of TS1 and TS2 RNAi constructs in independent transgenic *Arabidopsis* lines.

