10.1071/FP13103_AC

Supplementary Material: *Functional Plant Biology*, 2014, 41(2), 133–143.

Supplementary Material

Manipulation of methyl jasmonate esterase activity renders tomato more susceptible to *Sclerotinia sclerotiorum*

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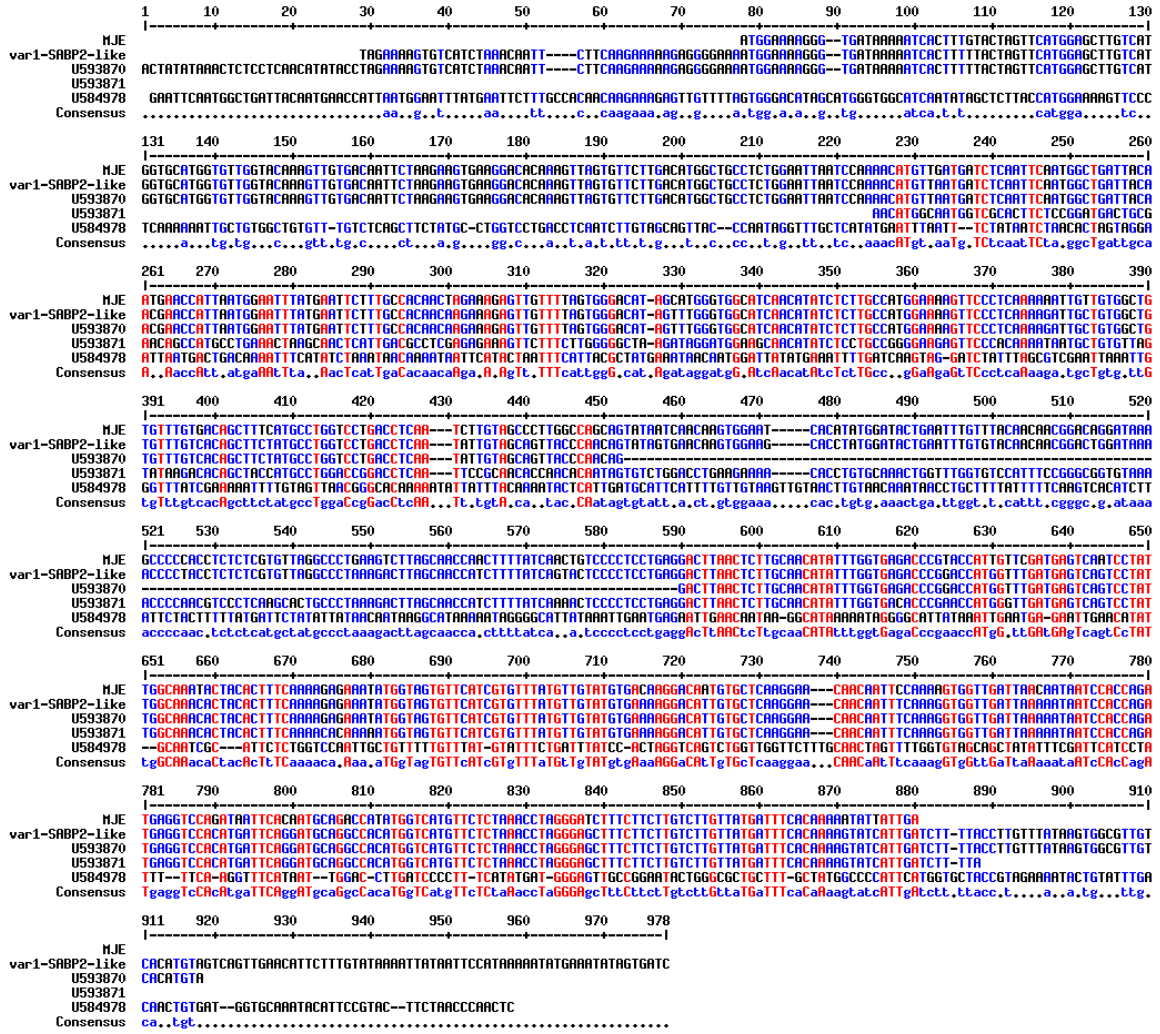

Fig. S1. Comparative alignment of sequences with similarity to the sequence of the MJE. Genes with sequence similarity to the RNAi fragment were identified by BLAST search using the SOL web site (http://solgenomics.net/tools/blast/index.pi and sequence alignment was performed using MultAlin (http://multalin.toulouse.inra.fr/multalin/). Red colour indicates nucleotides which are identical in all sequences. The RNAi sequence (position 509-671) is underlined in black.

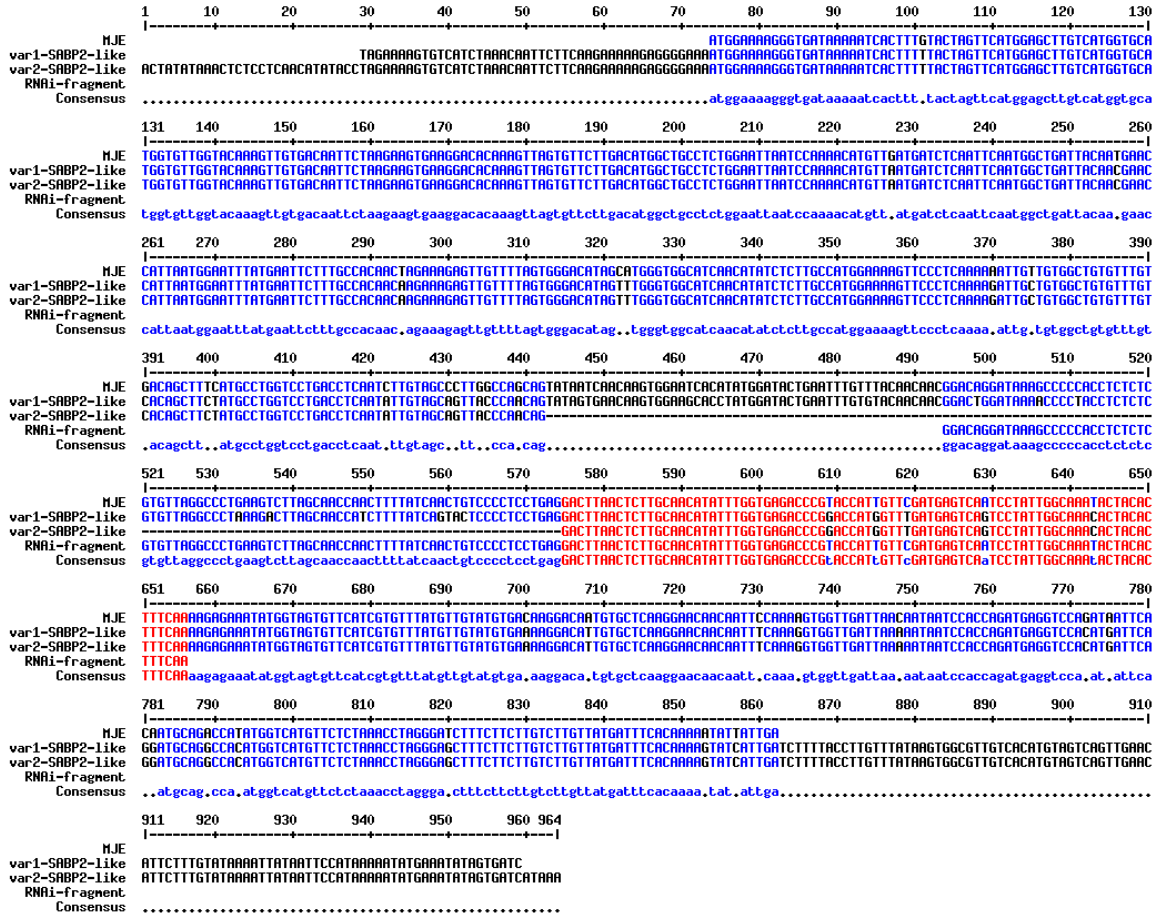

Fig. S2. Comparative alignment of sequences with similarity to the sequence of the RNAi fragment. Genes with sequence similarity to the RNAi fragment were identified by BLAST search using the website http://blast.ncbi.nlm.nih.gov/Blast.cgi and sequence alignment was performed using MultAlin (http://multalin.toulouse.inra.fr/multalin/). Red colour indicates nucleotides which are identical in all sequences. Var1 and var2 are splice variant 1 (var1) and splice variant 2 (var2) of the gene SABP2-like with the ID LOC101251956. U593870 corresponds to splice variant 2 of the gene SABP2-like shown in Suppl. Fig. S1 with the last 65 BP missing.

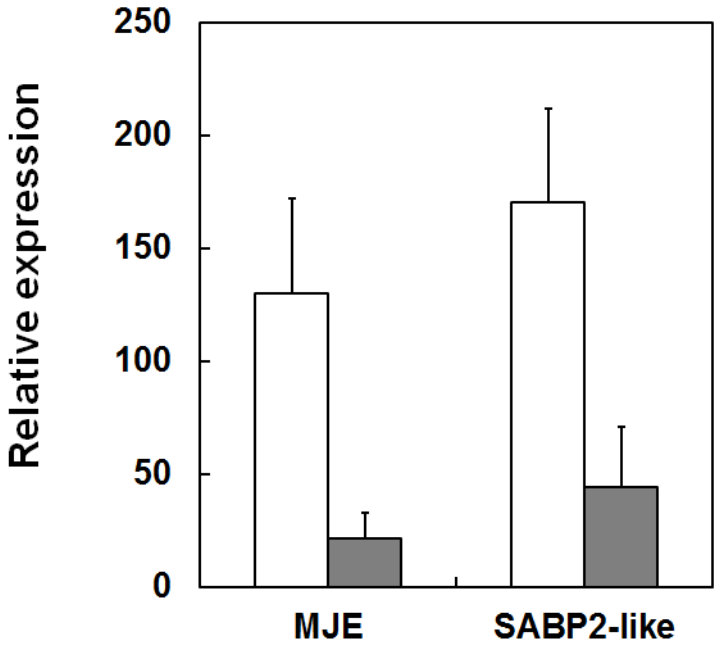

Fig. S3. Expression of *MJE* and *SABP2-like* in the RNAi line (grey bars, line 3-4-17) and the control line (white bars). Expression is relative to 10000 molecules *EF-1a*. The mean of at least three biological replicates + sd is shown. Stars indicate significant differences (*: $p< 0.05$; **: $p<0.01$). Similar results were obtained with another line and in independent experiments. The primer combination used gives products of different length with splice variant 1 and 2. Only the longer product of splice variant 1 was detectable. Using a second primer pair yielding the same product with both splice variants also showed similar reduction in transcript levels.

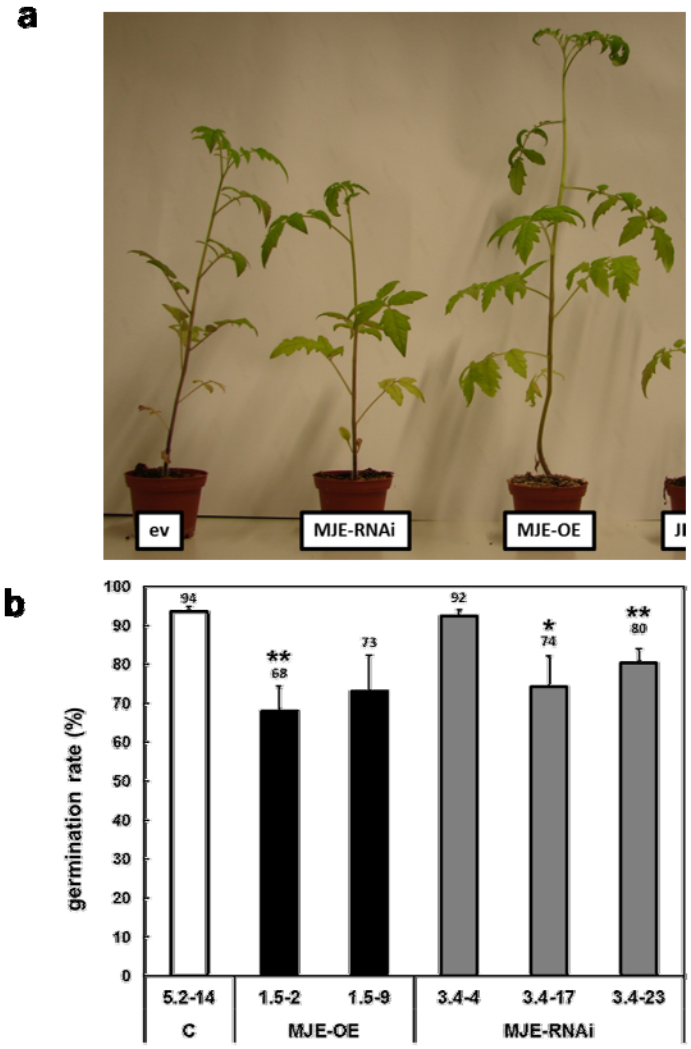

Fig. S4. Growth and germination rate of lines with altered activity of MJE. The picture shows the growth habitus of eight week old representative plants (a). The germination rate is the mean of at least six boxes with nine seeds each (b).

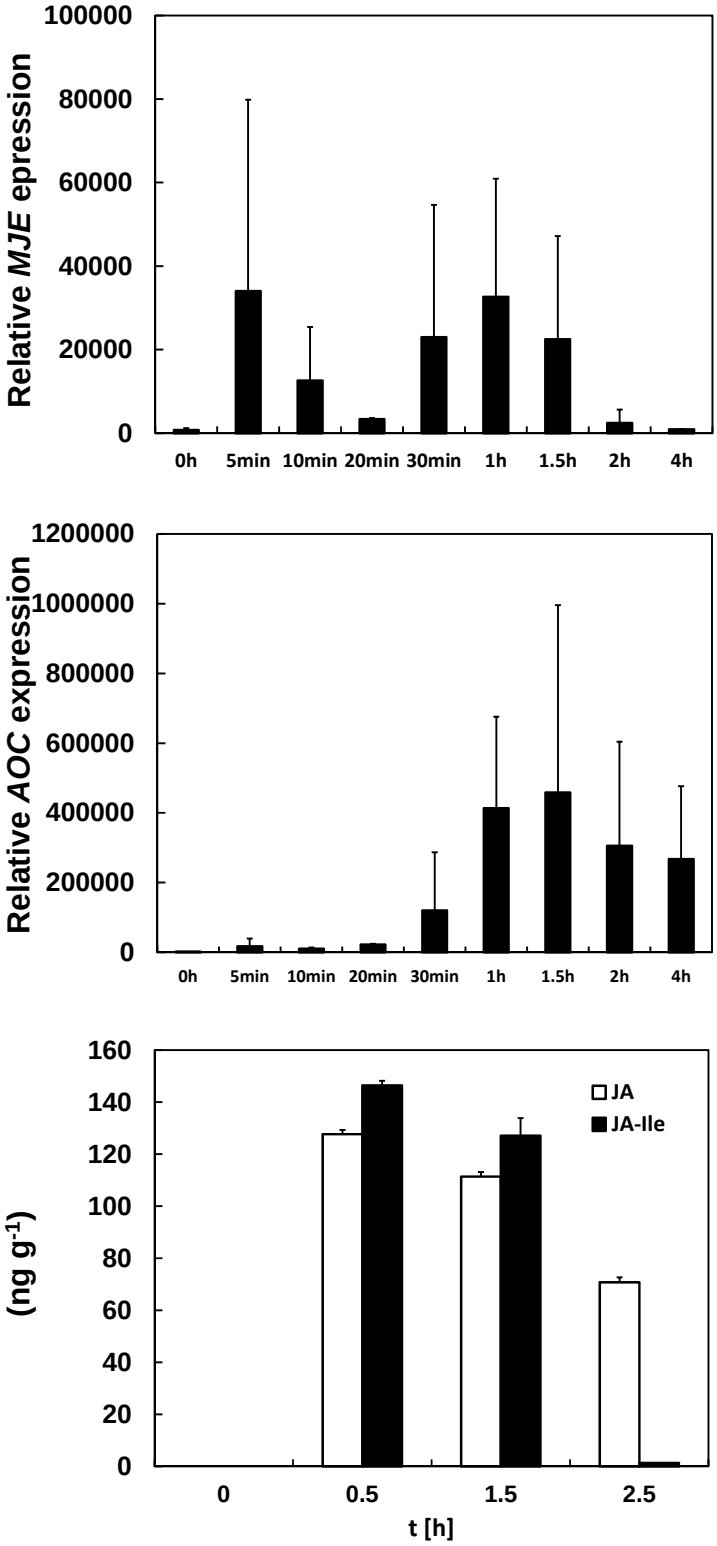

Fig. S5. Time course of wound-induced oxylipin accumulation and gene expression. JA and JA-Ile levels (a) and expression of *MJE* (b) and *AOC* (c) at different time points after wounding. Expression is relative to 10000 molecules *EF-1a*. The mean of at least three biological replicates + sd is shown.

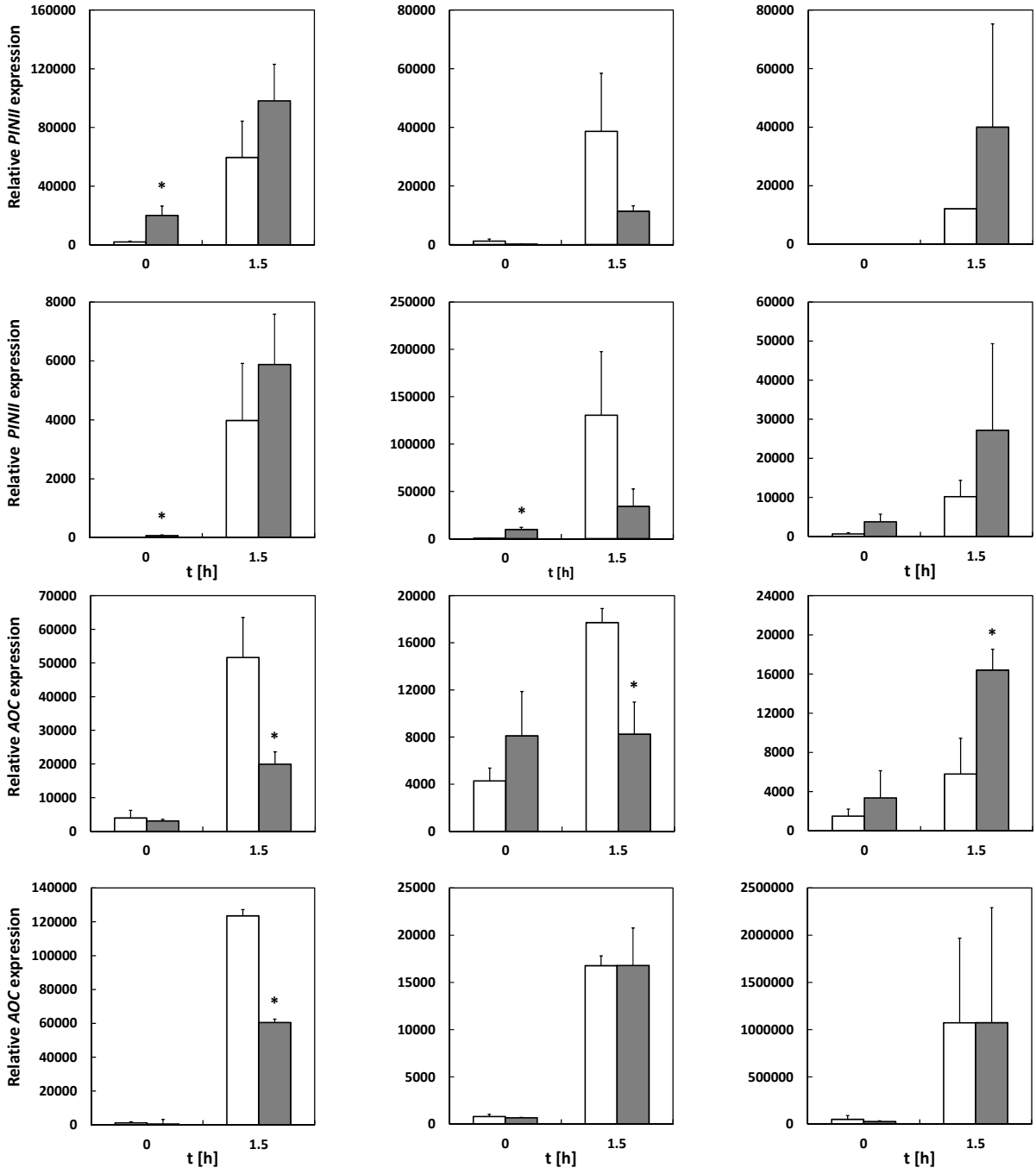

Fig. S6. Expression of *AOC* and *PINII* in plants with MJE-RNAi scion grafted on wild type stocks. Lower leaves of the wild type stock were wounded and expression was analysed after 1.5 h in distal, unwounded MJE-RNAi leaves (grey bars). As control wild type grafted on wild type was used (white bars). Expression is relative to 10000 molecules *EF-1a*. Shown are six independent experiments. V1, V2, V3 V4 were performed with line 3.4-17, V5 with line 3.4-23 and V6 with line 3.4-4. Each experiment shows the mean of at least three replicates + sd. Stars indicate significant differences (*: $p < 0.05$).

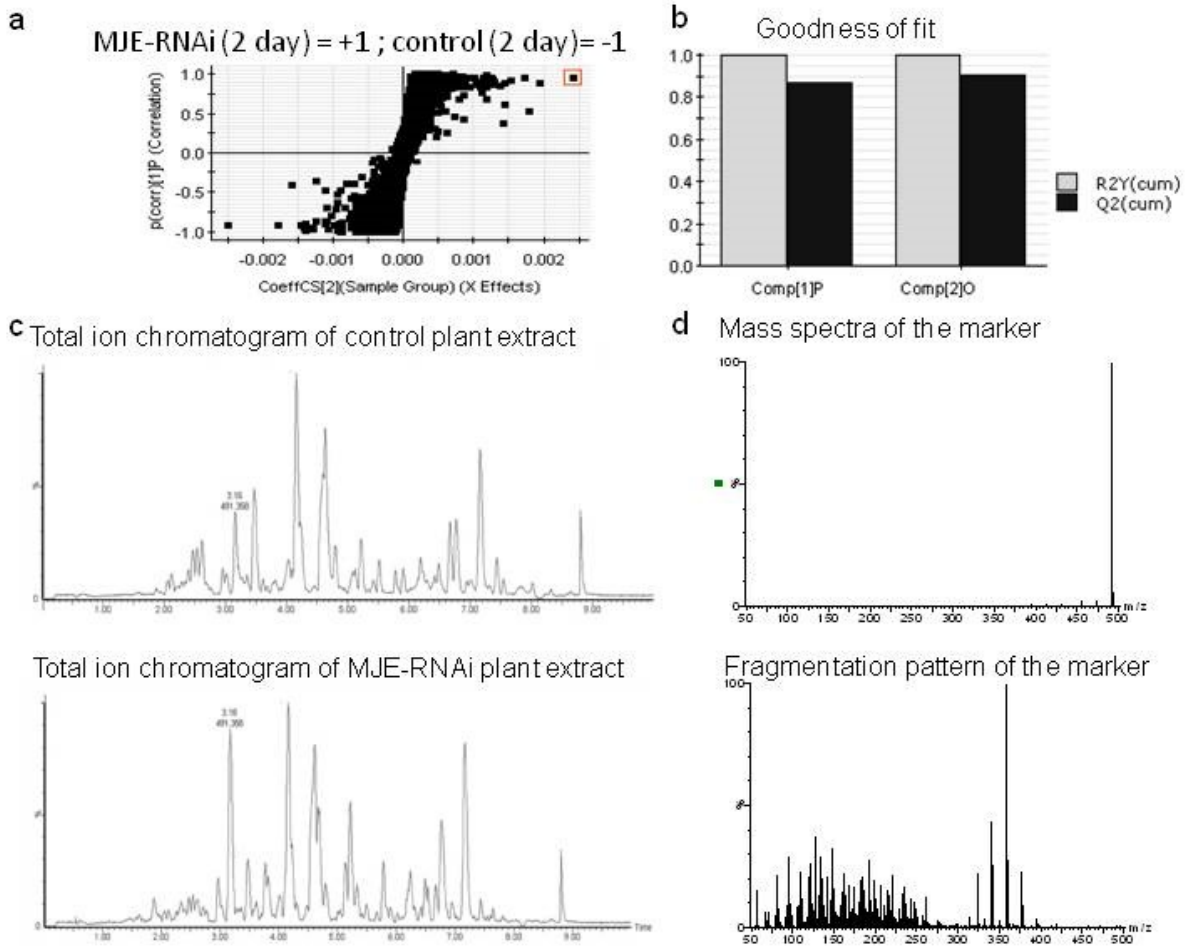

Fig. S7. Metabolomic fingerprinting of MJE-RNAi and control line. S-Plot visualizing the metabolite (red square) characteristic for MJE-RNAi compared to the control two day after *Sclerotinia* infection (a), goodness of fit indices of the OPLS-DA model (b), total ion chromatogram of the control plant (top) and MJE-RNAi (bottom) extract (c) mass spectra (top) and fragmentation pattern (bottom) of the marker (d). The first component of the PLS-DA statistical analysis was the infection time (1 dpi and 2 dpi) and the second component separated the control plants with empty vector from the MJE-RNAi line although the model showed poor goodness of fit (Q2 and R2Y of 0.2 and 04). Thus orthogal multivariate method (OPLS-DA) was applied since the paired data structure underlying the cross-over design considerably improves the interpretability of the multivariate solution (Westerhuis *et al.* 2010). One metabolite showed more than two-fold higher abundance in MJE-RNAi plants compared to the control plants after two days and also after one day inoculation with *S. sclerotiorum*. The identified marker had a retention time of 3.17 min and elemental composition of $C_{26}H_{50}O_8$ considering the accurate m/z of 491.358 at positive ESI and the

isotope distribution pattern. Searching in different databases no structure proposal for the formulae was found. The fragmentation pattern of the metabolite showed persistent water loss. At high collision energy (CID 25 eV) the structure broke down into small fragments that made the structure elucidation impossible. The marker was clearly detectable measuring hyphae extracts of *S. sclerotiorum* cultivates grown on agar plate indicating that this compounds originates from the fungus.

Table S1. Sequences of primers used for construct generation

Description		Sequence 5`-3`	ID
MJE-RNAi sense	for	CTCGAGCCTGAAGTCTTAGCAACC	LOC544264; GI41814856
	rev	GGTACCACAAGAAGAAAGATCCCT	
MJE-RNAi antisense	for	TCTAGACCTGAAGTCTTAGCAACC	LOC544264; GI41814856
	rev	AAGCTTACAAGAAGAAAGATCCCT	
MJE-OE	for	CGTTCTAGAATGGAAAAGGGTGATAAAAATCAC	LOC544264; GI41814856
	rev	TATGAGCTCTCAATAATATTTTTGCGAA	

Table S2. Sequences of primers used for qRT-PCR

Target		Sequence 5`-3`	ID
MJE	for	CCACCTCTCTCGTGTTAGGC	LOC544264; GI41814856
	rev	CTGGACCTCATCTGGTGGA	
AOC	for	ATCGCCGTTCAGGGAGC	AJ308481; GI40643242
	rev	TCAGTGCGGCCCCTTC	
PINII	for	CACAGGGTACAAGGGTTGCTA	JN091682; GI387604156
	rev	TATTTGCCTTGGGTTCATCAC	
EF-1α	for	TACTGGTGGTTTTGAAGCTG	X14449; gene ID 544055
	rev	AACTTCCTTCACGATTTCATCATA	
Actin (S. sclerotiorum)	for	TCTTGAGAGCGGTGGTATCC	XM_001589919; GI:156048003
	rev	GATGATGGTGCAAGAGCAGT	
SABP2-like	for	CCTCAATATTGTAGCAGTTAC	LOC101251956; GI:460377800
	rev	ACGCCACTTATAAACAAGGTA	

Table S3. Contents of the media used for generating transgenic plants

Induction medium:

MS-222	6.6 g L^{-1}
$NaPO_4$ [400 mM]	313 µL
BAP	1 µg mL^{-1}
Acetosyringone [50 mM]	10 µL
Antibiotics	50 µg mL^{-1}
adjust to	10 mL

SSR-agar medium (pH 5.7):

MS-231	4.4 g L^{-1}
Sucrose	20 g L^{-1}
Zeatin	1 µg mL^{-1}
Kanamycin	50 µg mL^{-1}
Ticarcillin	160 µg mL^{-1}
Cefotaxime	50 µg mL^{-1}
Micro-agar	8 g L^{-1}
adjust to	1 L

RI medium (pH 5.7):

MS-231	4.4 g L^{-1}
Sucrose	20 g L^{-1}
Kanamycin	50 µg mL^{-1}
Ticarcillin	160 µg mL^{-1}
Cefotaxime	50 µg mL^{-1}
Micro-agar	8 g L^{-1}
adjust to	1 L

MS2 medium (pH 5.4):

MS222	4.4 g L^{-1}
Sucrose	20 g L^{-1}
Gelrite	3 g L^{-1}
adjust to	1 L

2N1B medium (pH 5.8):

MS-222	4.4 g L^{-1}
Sucrose	20 g L^{-1}
NAA	2 µg mL^{-1}
BAP	1 µg mL^{-1}
Acetosyringone	40 µg mL^{-1}
Glucuronic acid	160 µg mL^{-1}
Micro-agar	8 g L^{-1}
adjust to	1 L

MSO Medium (pH 5.4):

MS-231	4.4 g L^{-1}
Glucose	30 g L^{-1}
adjust to	1 L

Table S4. Sensitivity of MJE-RNAi line and control line to MeJA

Root elongation of control and MJE-RNAi line on medium with or without 10 µM MeJA. Shown is the mean of 12–15 seedlings ± s.d. Similar results were obtained in three independent experiments

	Control line	MJE-RNAi
Root length ± s.d. control medium	13.6 ± 5.5	12.9 ± 4.4
Root length ± s.d. MeJA medium	3.8 ± 1.4	3.5 ± 0.8
% inhibition	72	73

Reference

Westerhuis JA, van Velzen EJ, Hoefsloot HC, Smilde AK (2010) Multivariate paired data analysis: multilevel PLSDA versus OPLSDA. *Metabolomics* **6**, 119–128. doi:10.1007/s11306-009-0185-z