

SUPPLEMENTAL DATA

Supplemental Method

Transient expression in Arabidopsis protoplasts and immunoprecipitation

The coding sequences of *SINAT5DN* and the *MtSINA* genes were cloned into a pRT104-derived vector (Töpfer et al., 1989) as N-terminal fusion with 3x HA and 3x MYC epitopes, respectively. Protoplasts from Arabidopsis ecotype Columbia were prepared as described (Dangl et al., 1987) and cotransfected with the polyethylene glycol-mediated method (Kiegerl et al., 2000). Proteins were obtained with extraction buffer (25 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 15 mM EGTA, 15 mM NaCl, 15 mM pNO₂PhenylPO₄, 15 mM β-glycerophosphate, 1 mM dithiothreitol, 1 mM NaF, 0.1 mM Na₃VO₃, 0.5 mM phenylmethylsulfonylfluoride, 10 μg/ml leupeptin and aprotinin, 0.1% Tween, and 10% glycerol); total protein concentration was determined with the Protein Assay kit (Bio-Rad, Hercules, CA). For immunoprecipitation, 300 μg protein extract was incubated with rat monoclonal anti-HA antibody 3F10 (Roche Diagnostics, Brussels, Belgium) and 25 μl of protein G Sepharose 4 Fast Flow beads (GE-Healthcare, Little Chalfont, UK) for 2 h at 4°C. Immune complexes were washed 3 times in 500 μl extraction buffer and protein complexes coupled to the anti-HA Protein G beads were dissolved in Tris-glycine-SDS sample buffer (Novex, San Diego, CA) supplemented with 100 mM β-mercaptoethanol, boiled for 5 min at 95°C, and loaded on SDS-PAGE gels according to Laemmli (1970). Proteins were transferred electrophoretically onto an Immobilon-p polyvinylidene difluoride membrane (Millipore, Bedford, MA). After blocking at 4°C in 5% skimmed milk in 100 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween-20, the efficiency of the immunoprecipitation was verified with the monoclonal 12CA5 anti-HA mouse antibody (Roche Diagnostics). MYC-tagged proteins were detected with the monoclonal 9E10 anti-cMYC mouse antibody (Roche Diagnostics). The secondary anti-mouse-IgG antibody coupled to horseradish peroxidase was detected with a chemiluminescence detection kit according to the manufacturer's instructions (Perkin-Elmer Life Sciences, Boston, MA).

Literature cited

Dangl JL, Hauffe KD, Lipphardt S, Hahlbrock K, Scheel D (1987) Parsley protoplasts retain differential responsiveness to u.v. light and fungal elicitor. *EMBO J* **6**: 2551-2556

- Kiegerl S, Cardinale F, Siligan C, Gross A, Baudouin E, Liwosz A, Eklöf S, Till S, Bögre L, Hirt H, Meskiene I** (2000) SIMKK, a mitogen-activated protein kinase (MAPK) kinase, is a specific activator of the salt stress-induced MAPK, SIMK. *Plant Cell* **12**: 2247-2258
- Laemmli UK** (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685
- Töpfer R, Gronenborn B, Schell J, Steinbiss H-H** (1989) Uptake and transient expression of chimeric genes in seed-derived embryos. *Plant Cell* **1**: 133-139

Table S1. Overview of primers used for amplifying ORFs, RT-PCR and qRT-PCR analyses

Gene, use, amplicon length (bp)	Forward primer	Reverse primer
<i>SINAT5</i> (product length 600 bp)	AAGCGTTTACGAGCTCCTTG	CCCAGTGCCTCCAACCTTCTA
<i>MtSINA1</i> , ORF, 999	ATGGATTTGGATAGTATTGAGTGT	TCAGCTGCAAAGATTTGGTATA
<i>MtSINA2</i> , ORF, 969	ATGATCAACCATTTCATATCTCAGATCT	CTAGCTACACATGTTGGGTGTG
<i>MtSINA3</i> , ORF, 912	ATGACATCTAGTAATCCTTTCTTTGATGA	TCACTGCTCTTTCCAAATCCTT
<i>MtSINA4</i> , ORF, 972	ATGGAGTTGGATAGCATTGAGTGT	TCAGCTACATAGATTAGGTATGCAC
<i>MtSINA5</i> , ORF, 927	ATGGATTCAGATAGTACTGTTTCTTCATT	CTAGCTACACAAGTTGGGTATGCA
<i>MtSINA6</i> , ORF, 912	ATGGCATTGAGTAATCCACTCTTT	TCATTGTTCTTTCCAAATCCTACCA
<i>MtSINA1</i> , qRT-PCR	AGGGTACACCACGGAGTATTCG	AGTTCCTTCCTATCACCACCAGAG
<i>MtSINA2</i> , qRT-PCR	AGCATATCCAACAACAATCGTGAC	AACGGTTTTTCATGATTCAGCAAC
<i>MtSINA3</i> , qRT-PCR	CAGTCTTGAAGGAGGTGGTAATGG	GCGGTGGCTTCCCTAATGC
<i>MtSINA4</i> , qRT-PCR	TTTGGTGAGAGTTGATGGAGTTGG	TGACACTGGTGAATAGGAGGGTAC
<i>MtSINA5</i> , qRT-PCR	GTCTTCTTTCATCATTTCAAACATACCC	ATCAATGAAGAAACAGTACTATCTGAATCC
<i>MtSINA6</i> , qRT-PCR	TGAAAACCTCAAACCCACCAAAACC	CCCAGCGGCCCTGAAGC
Translation elongation factor 1 α (<i>MtELF1α</i>), RT-PCR	TTGGACATGTCGACTCTGGA	GCCTGGGAGGTTCCAGTAA
Translation elongation factor 1 α (<i>MtELF1α</i>), qRT-PCR	ACTGTGCAGTAGTACTTGGTG	AAGCTAGGAGGTATTGACAAG

Supplemental Fig. S1. Alignment of SINA protein sequences of *M. truncatula*, Arabidopsis, rice, poplar and Siah1. The RING finger motif and the SINA domain are marked by asterisks and dollar signs, respectively.

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OsSINA5 1  -----MDMDSVECLSLPDS-SMDVDDVDGGGVSHHHHHHHALPPLPAGVAVGVGPGGRAFPKANVAGVGGGAAGAP 73
OsSINA1 1  -----MDVDSVECLSLPDS-SSSSGAGAGGGG-----GGGGGGVGAMDAADDVGLALHAHGALLAAAA 58
OsSINA3 1  -----MDMASIECVSYSDSMDDDDDDGVGVGVVSH-----LPRPILVKPSSAAAAVNVVVVSAGSGGGGAGG 61
PtSINA8 1  -----M-----
SINAT5 1  -----METDSIDSVIDD----DEIHQK-----HQFSSTKSQGG- 29
AtSINA4 1  -----METDSMECVSSTG---NEIHQNG-----NGHQSYQFSSTKTHGA 37
PtSINA7 1  -----MDFDNIECVSSSDGLDEDEIHHHNLQHHL-----HQFASPKPHVNNNSNG 47
PtSINA9 1  -----M-----VN-----NNHNNG 8
MtSINA1 1  -----MDLDSIECVSSSDGMDEDEIQHRILPHHQ-----QHHSSEFSSSLKPRS GG 47
MtSINA4 1  -----MELDSIECVSSSDGMDEDEIHS-----HHHHSEFSSSTKARNGG 39
AtSINA3 1  -----MDLDSMDCTSTMDVTDDEEIHQDR-----HSYASVSKHHHTN 37
PtSINA3 1  -----M-----
MtSINA2 1  MINHSYLRFHCDICLKMETGGVDSSILS-----LTMEEAEHHHH 40
MtSINA5 1  -----MDS---DSTVSS-----LIMDEDLHP-H 20
OsSINA6 1  -----MASVTYLDL---AHSEVIDPPKN-----EEMLDVTELVDD 32
OsSINA4 1  -----MASVTYIDD---SGSEVIDPPK-----TEVLDVTELAGD 31
PtSINA2 1  -----MT-----IRNKPEVIDPPQN-----KMDTDIVECVNS 27
PtSINA4 1  -----MASSNLFDDIRNKPEVIDPPQN-----KDLTDIGESVND 35
MtSINA3 1  -----MTSSNPFDDIRSRP-GVDPPQT-----EESTEIPELVND 34
MtSINA6 1  -----MALSNFLFDDIRSNI-DVDPPQK-----EESTDVGILVND 34
AtSINA2 1  -----MAPGGSALKEVMESNSTGMDYE-----VKTAKVEVNNNK 34
AtSINA1 1  -----MAPGGSALKEALESNSTGV DYE-----VKMAKVEANS-K 33
PtSINA6 1  -----MAPGGSALKEVLEFHSTVADCD-----VANSKSENNI-T 33
PtSINA10 1  -----MAPGGSALKEVLEL---VAECD-----IATSKSENNI-A 30
PtSINA1 1  -----M-----MATSAAELRGS 11
PtSINA5 1  -----MAPGGIINKEEIESRIAYLDHDTG-----HPATSNAELRGS 36
OsSINA2 1  -----MAPGSSIVTDIPESDCVSDGLS-----EALTGIRLDGDS 34
Siah1 1  -----M-----MSRQTATALPTGT 13

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OsSINA5 74  AAGGAVAGGGGPGGPPATS-VHELLECPVCTNSMYPPIHQCHNGHTLCSTCKARVHNRCPCTCRQELGDIRCLALEKVAE 152
OsSINA1 59  AARAAACSKGGGGGAAAGS-VHELLECPVCTNSMYPPIHQCHNGHTLCSTCKARVHNRCPCTCRQELGDIRCLALEKVAE 138
OsSINA3 62  GGGGVGVVAGAP-AVPPATS-VHELLECPVCTNSMYPPIHQCHNGHTLCSTCKTRVHNRCPCTCRQELGDIRCLALEKVAE 139
PtSINA8 1  -----VHELLECPVCTNSMYPPIHQCHNGHTLCSTCKTRVHNRCPCTCRQELGDIRCLALEKVAE 59
SINAT5 29  -ATVVIS-----PATS-VYELLECPVCTNSMYPPIHQCHNGHTLCSTCKSRVHNRCPCTCRQELGDIRCLALEKVAE 98
AtSINA4 38  AAAAVTNIIVGPTATAPATS-VYELLECPVCTNSMYPPIHQCHNGHTLCSTCKRVHNRCPCTCRQELGDIRCLALEKVAE 116
PtSINA7 47  -IANVVG---GPTVIAPATS-VHELLECPVCTNSMYPPIHQCHNGHTLCSTCKTRVHNRCPCTCRQELGDIRCLALEKVAE 122
PtSINA9 8  -NTIVVG---GPTAIAPATS-VHELLECPVCTNSMYPPIHQCHNGHTLCSTCKTRVHNRCPCTCRQELGDIRCLALEKVAE 83
MtSINA1 48  NNNHGV1---GSTAIAPATS-VHELLECPVCTNSMYPPIHQCHNGHTLCSTCKTRVHNRCPCTCRQELGDIRCLALEKVAE 123
MtSINA4 40  ANINNIL---GPTAIAPATS-VHELLECPVCTNSMYPPIHQCHNGHTLCSTCKTRVHNRCPCTCRQELGDIRCLALEKVAE 115
AtSINA3 38  NNTTNVN-AAAGLLPTTTS-VHELLECPVCTNSMYPPIHQCHNGHTLCSTCKARVHNRCPCTCRQELGDIRCLALEKVAE 115
PtSINA3 1  -----VHELLECPVCTNSMYPPIHQCHNGHTLCSTCKTRVHNRCPCTCRQELGDIRCLALEKVAE 59
MtSINA2 41  QFSS--ISKLLNN-APTPTS-VHLLDLCPCVCTNSMYPPIHQCHNGHTLCSTCKTRVHNRCPCTCRQELGDIRCLALEKVAE 116
MtSINA5 21  QFSSSTSKLHNSNGTPTSTS-VHELLECPVCTNSMYPPIHQCHNGHTLCSTCKTRVHNRCPCTCRQELGDIRCLALEKVAE 99
OsSINA6 33  HTQHS PK-----PNAMVSGNVRELECPVCLNAMYPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 106
OsSINA4 32  PVPHSPK-----PNVVVSSVRELECPVCLNAMYPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 105
PtSINA2 28  PAKTALK-----PNVTVSSVRELECPVCLNAMYPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 101
PtSINA4 36  PAQTALK-----PNVTVSSVRELECPVCLNAMYPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 109
MtSINA3 35  PIQTUVK-----PNGTVSSVRELECPVCLNAMYPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 108
MtSINA6 35  PAQTALK-----PTGTVLSSVRELECPVCLNAMYPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 108
AtSINA2 35  PTKGSA--GIGKYGIHSNNGVYELLECPVCLNAMYPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 112
AtSINA1 34  PTKSGSG--SIGKF--HSSNGVYELLECPVCLNAMYPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 109
PtSINA6 34  PTKGTVV--LGGKHGVYSNNGVYELLECPVCLNAMYPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 111
PtSINA10 31  PTKGTVV--LSGKHGVYSNNGVYELLECPVCLNAMYPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 108
PtSINA1 12  HFRKAATP-NTGNLQKQSTSNMQLDLCPCVCTNSMYPPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 90
PtSINA5 37  PFRKAATP-STGNLQKQSTSNMQLDLCPCVCTNSMYPPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 115
OsSINA2 35  TCKPWSTS--LVTVELSSLTGLNDLLECPVCTNSMYPPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 112
Siah1 14  SKCPPSQRPALTGTASNNDLASLLECPVCFDYVLPPIHQCHNGHTLCSSGCKPRVHNRCPCTCRHELGNIRCLALEKVAA 92

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OsSINA5 153  SLELPCKYCSIGCPEIFPYYSKIKHEAQCSFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 232
OsSINA1 139  SLELPCKYCSIGCPEIFPYYSKIKHEAQCMFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 218
OsSINA3 140  SLELPCKYYSIGCPEIFPYYSKIKHEAQCFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 219
PtSINA8 60  SLELPCKYMSIGCPEIFPYYSKIKHEAICNFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 139
SINAT5 99  SLELPCKYNYIGCGLGIFPYYSKIKHEAQCFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 178
AtSINA4 117  SLELPCKEYNYIGCPEIFPYYSKIKHEAICNFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 196
PtSINA7 123  SLELPCKYNYIGCPEIFPYYSKIKHEAICNFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 202
PtSINA9 84  SLEFPCKEYNYIGCPEIFPYYSKIKHEAICNFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 163
MtSINA1 124  SLELPCKYYSIGCPEIFPYYSKIKHEAICNFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 203
MtSINA4 116  SLELPCKYYSIGCPEIFPYYSKIKHEAICNFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 195
AtSINA3 116  SLELPCKHMSIGCPEIFPYYSKIKHEAICNFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 195
PtSINA3 60  SLELPCKYMSIGCPEIFPYYSKIKHEAICNFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 139
MtSINA2 117  SLEFPCKYYSIGCPEIFPYYSKIKHEAICNFRPNCPYAGSECAVAGDIPFLVAHLRDDHKVDMHSGCTFNHRVYKSNPR 196

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Supplemental Fig. S2. Nucleotide sequence alignment (690 bp assembled conserved regions) used to design the phylogenetic tree shown in Fig. 1B.

OsSINA5	AGCGTGGAGCTGCTCGAGTGCCCCGTCTGCAACATGTTCCCGCCCATCCA	[50]
OsSINA1	AGCGTGGAGCTGCTCGAGTGCCCCGTCTGCAACATGTACCCGCCGATCCA	[50]
OsSINA3	AGCGTCGAGCTGCTCGAGTGCCCCGTCTGCAACATGTACCCGCCCATCCA	[50]
PtSINA8	---GTTGAGCTCCTTGAATGCCCTGTTTGTAAATATGTACCCTCCTATTCA	[47]
PtSINA3	---GTTGAGCTCCTTGAATGCCCTGTTTGTAAATATGTACCCTCCTATTCA	[47]
PtSINA7	AGTGTGAGCTCCTAGAAATGTCCTGTTTGTAAATATGTACCCACCAATTCA	[50]
PtSINA9	AGTGTGAGCTGCTAGAAATGTCCTGTTTGTAAATATGTACCCACCAATCCA	[50]
MtSINA1	AGTGTGAGTTGTTGGAATGTCCTGTTTGTAAATATGTATCCTCCAATTCA	[50]
MtSINA4	AGTGTGAAATGCTTGAATGTCCTGTTTGTAAATATGTACCCTCCTATTCA	[50]
AtSINA3	AGTGTGAGCTTCTCGAATGTCCTGTCTGCAATATGTACCCTCCCATCCA	[50]
MtSINA2	AGTGTGATCTTCTTGAATGTCCGGTTTGTAAATATGTACCCTCCAATCCA	[50]
MtSINA5	AGTGTGAGCTTCTTGAATGTCCTGTTTGTAAATATGTACCCTCCAATCCA	[50]
SINAT5	AGCGTTGAGCTCCTTGAATGCCCTGTCTGCAATATGTACCCACCAATCCA	[50]
AtSINA4	AGCGTGGAGCTTCTGGAGTGCCCTGTCTGTTATATGTATCCTCCTATCCA	[50]
OsSINA6	GGCGTGGAGCTACTGGAATGCCCTGTGTGTAATATGTATCCTCCAATTCA	[50]
OsSINA4	AGCGTGGAACTGCTTGAATGTCCAGTCTGCAGCATGTATCCTCCCATCCA	[50]
PtSINA2	AGCGTTGAATTATTGGAGTGTCCCGTGTGCAATATGTATCCTCCAATTCA	[50]
PtSINA4	AGCGTTGAATTACTGGAGTGTCCCGTGTGCAATATGTATCCTCCAATTCA	[50]
MtSINA3	AGCGTTGAGCTTTTAGAGTGCCCTGTGTGCAATATGTACCCTCCAATCCA	[50]
MtSINA6	AGCGTTGAGCTTCTAGAGTGTCCGTGTGTAATATGTACCCTCCAATCCA	[50]
AtSINA2	AATGTTGAGCTTCTTGAATGTCCCGTCTGTAATATGTACCCTCCAATCCA	[50]
AtSINA1	AATGTTGAGCTCCTTGAATGTCCGGTTTGTAAATATGTATCCTCCAATTCA	[50]
PtSINA6	AATGTGGAGCTCCTTGAGTGTCCCTGTCTGTAATATGTACCCTCCAATCCA	[50]
PtSINA10	AATGTGCAACTCCTTGAGTGTCCCTGTCTGTAATATGTATCCTCCAATTCA	[50]
PtSINA1	AGTATGGACCTTCTTGACTGTCCCTGTTTGCCTATGTACCCTCCAATTTT	[50]
PtSINA5	AGTATGGACCTTCTTGATTGTCCCTGTTTGTACCATGTATCCTCCAATTTT	[50]
OsSINA2	ACGTTGGATTTGCTCGAGTGTCCAGTCTGCAACATGCGCCACCTATACT	[50]
Siah1	AATTTGAGTCTTTTTGAGTGTCCAGTCTGCGACGTGTTACCGCCCATCT	[50]
OsSINA5	CCAGTGAATGGACACACTTTATGTTTCATGCAAGAGAGTCAACCGGTGCC	[100]
OsSINA1	CCAGTGAATGGACACACTCTGTGTTCCCTGCAAAAGGGTGAACCGTTGCC	[100]
OsSINA3	CCAGTGAATGGTCATACTCTGTGTTTCATGCAAAAGAGTGAACCGGTGCC	[100]
PtSINA8	TCAGTGAATGGCCATACACTCTGTTCATGTAAACGAGTAAATCGGTGTC	[97]
PtSINA3	TCAGTGAATGGCCACACACTCTGTTCATGTAAACGAGTAAATCGGTGCC	[97]
PtSINA7	TCAGTGAATGGGCACACGCTATGTTCCCTGTAAGAGGGTAAATCGCTGCC	[100]
PtSINA9	TCAGTGAATGGGCACACGCTATGTTCCCTGTAAGAGGGTAAATCGCTGCC	[100]
MtSINA1	TCAGTGAACGGTCACACATTGTGTTCCCTGTAAGAGGGTAAATCGATGTC	[100]
MtSINA4	CCAGTGAATGGCCACACACTATGTTCCCTGCAAAAGAGTGAACCGATGTC	[100]
AtSINA3	TCAGTGAATGGACATACGTTGTGTTTCATGTAAAGAGGGTAAACCGCTGCC	[100]
MtSINA2	TCAGTGAATGGACATACCCCTTTGTTTCATGTAAAGAGGGTAAACAGATGTC	[100]
MtSINA5	TCAGTGAATGGGCATACCCCTATGTTTCATGTAAAGAGGGTAAACAGGTGCC	[100]
SINAT5	TCAGTGAATGGACATACTTTATGTTTCATGTAAAGAGTGAATCGGTGTC	[100]
AtSINA4	TCAGTGAATGGACATACATTGTGCTCGTGTAAAGAGGGTAAACAGGTGTC	[100]
OsSINA6	TCAGTGAATGGTCATACTCTATGTTCCCTGCAAGAGGGTAAACCGCTGCC	[100]
OsSINA4	TCAGTGAATGGTCATACTCTATGTTCCCTGCAAAAGGGTAAATCGCTGTC	[100]
PtSINA2	TCAGTGAATGGTCACACATTATGTTCCCTGCAAGAGAGTTGGCCGATGCC	[100]
PtSINA4	CCAGTGAATGGCCACACATTATGTTCTTGTCAAGAGGGTAAACCGATGCC	[100]
MtSINA3	CCAGTGAATGGTCACACTCTATGCTCCTTGTCAAAAGAGTGCACCGGTGCC	[100]
MtSINA6	TCAGTGAATGGTCACACTATATGCTCATGCAAAAGAGTAAACCGGTGCC	[100]
AtSINA2	TCAGTGAATGGCCACACATTATGTTTCATGCAAAAGAGTGAACACATGCC	[100]
AtSINA1	TCAGTGAACGGCCACACATTGTGTTTCATGCAAAAGAGTGAACACATGCC	[100]
PtSINA6	CCAGTGAATGGACACACTTTATGTTTCGTGCAAGAGAGTAAACTGTTGCC	[100]
PtSINA10	CCAGTGAATGGACACACTCTATGTTTCATGCAAGAGAGTAAACTGTTGCC	[100]
PtSINA1	CCAGTGAATGGCCACACTCTATGTTTCATGCAAGAGAGTAAACTGTTGCC	[100]
PtSINA5	CCAGTGAATGGCCACACTCTATGTTTCATGCAAGAGAGTGAACACTGTTGCC	[100]
OsSINA2	GCAGTGAATGGTCACACAATATGCTCCTTGTCAAGAGGGTGAATCATTGCC	[100]
Siah1	TCAATGTAGTGGCCATCTTGTGTTGTAGCTGTGCAAGCTC---TGTTGTC	[97]

OsSINA5 CTACCTGCAGACAAGAGCTCGGTGATATTAGGTGCTTGGCACTGGAGAAA [150]
OsSINA1 CTACATGCAGACAAGAGCTTGGTGTATATCAGGTGTTTGGCCCTGGAGAAA [150]
OsSINA3 CAACCTGTCGACAGGAGCTTGGTGTATATTAGATGTCTAGCATTGGAGAAA [150]
PtSINA8 CCACCTGTAGACAGGAGCTTGGTGTACATTAGATGTCTAGCTTTGGAAAAA [147]
PtSINA3 CCACTTGTAGACAGGAGCTTGGTGTACATTAGATGTCTAGCATTGGAAAAA [147]
PtSINA7 CCACATGTAGGCAGGAGCTTGGAGACATTAGATGTTTGGCCCTAGAAAAAG [150]
PtSINA9 CTACTTGCAGGCAGGAGCTCGGAGACATTAGGTGTTTAGCCCTAGAGAAG [150]
MtSINA1 CGACCTGTAGACAAGAGCTTGGAGATATTAGGTGTCTGGCACTGGAAAAAG [150]
MtSINA4 CGACTTGTAGACAAGAGCTTGGAGATATCAGGTGTCTAGCACTGGAGAAG [150]
AtSINA3 CAACTTGTAGACAAGAGCTCGGTGATATCCGTTGTTTGGCACTGGAAAAA [150]
MtSINA2 CAACTTGCAGGCAAGAGCTAGGCGATATAAGGTGTTTAGCATTGGAGAAA [150]
MtSINA5 CAACTTGCAGACAGGAAGCTGGGCGATATTCCGTTGTTTAGCATTGGAAAAA [150]
SINAT5 CAACGTGTAGACAAGAGCTTGGAGATATTAGATGTTTAGCTCTTGAGAAA [150]
AtSINA4 CCACATGTAGACAAGAAGCTTGGAGATATTAGATGTTTAGCTCTTGAGAAA [150]
OsSINA6 CAACTTGCAGACATGAATTGGGTAACATAAGATGCCTTGCTCTGGAAAAAG [150]
OsSINA4 CAACTTGTAGGCATGAAGTGGGTAATATTAGATGTCTTGCTCTCGAGAAG [150]
PtSINA2 CCATTTGCAGGCATGAAGTGGCAACATTAGATGTCTTGCGCTAGAGAAA [150]
PtSINA4 CCACTTGCAGGCACGAAGCTCGGCAACATTAGATGTCTTGCACTAGAGAAA [150]
MtSINA3 CTACTTGTGCGCATGAGCTCGGCAATATTAGATGTCTGGCATTGGAGAAG [150]
MtSINA6 CTACTTGTAGGCATGAGCTGGGAAATATTAGATGTCTAGCACTGGAGAAG [150]
AtSINA2 CTACATGTCGCTACGAGCTTGGTAACATAAGATGCTTAGCTCTTGAGAAA [150]
AtSINA1 CTACTGCCGCTATGAGCTTGGTAACATAAGATGCTTAGCTCTTGAGAAG [150]
PtSINA6 CTACTTGCCGCTATGATCTTGGAAATATACGCTGTTTGGCTTTGGAGAAA [150]
PtSINA10 CTACTTGCCGCTATGATCTTGGAAATATACGGTGCTTGGCTTTGGAGAAA [150]
PtSINA1 CAATTTGCCGAGGAGAGCTAGGAAATATAAGGTGCTTGGCTCTGGAAAAA [150]
PtSINA5 CAATTTGCCGAGGAGAAGCTAGGAAATATAAGGTGCTTGGCTCTGGAGAAA [150]
OsSINA2 CTACTTGTGCGCAAGAAGCTGGGTAATATCAGATGTTTGGCTCTTGAGAAG [150]
Siah1 CAACTTGCCGGGGCCCTTTGGGATCCATTGCAACTTGGCTATGGAGAAA [147]

OsSINA5 GTAGCAGAGTCACTCGAGCTTCCTTGCAAGTATTCTTTAGGTTGCATTTT [200]
OsSINA1 GTAGCTGAATCGCTTGGAGCTTCCCTGTAAGTACTCTTTGGGGTGCATCTT [200]
OsSINA3 GTGGCTGAATCGCTTGGAGCTACCCTGCAAAATATTCACTAGGTTGTATCTT [200]
PtSINA8 GTAGCTGAATCACTCGAGCTACCTTGCAAAATACTCACTTGGATGCATTTT [197]
PtSINA3 GTAGCTGAATCACTTGGAGCTGCCTTGCAAAATACTCGCTTGGATGCATTTT [197]
PtSINA7 GTGGCTGAGTCACTAGAAGCTGCCTTGCAAAATAACTTGGGGTGCATATT [200]
PtSINA9 GTGGCTGAGTCACTGGAATTTCCCTTGCAAAATTAAGTGGGGTGCATATT [200]
MtSINA1 GTTGCTGAATCACTCGAGTTACCATGCAAAATACTCTCTTGGATGTATCTT [200]
MtSINA4 GTGGCTGAATCACTTGAAGTTACCCTGCAAGTACTCCCTCGGATGTATATT [200]
AtSINA3 GTAGCCGAATCACTTGAATTTCCCTGTAAACACTCACTTGGATGTATCTT [200]
MtSINA2 ATAGCAGAATCACTTGAATTTCCCTGTAGATACTCTCTTGGTTGTATATT [200]
MtSINA5 ATAGCAGAATCACTTGAATTTCCCTGCGATACTCTGTTGGCTGTATATT [200]
SINAT5 GTAGCTGAGTCGCTTGGAGTTACCCTGCAAGTATAATCTTGGATGCATTTT [200]
AtSINA4 GTAGCCGAGTCGCTTGGAGCTGCCTTGCAAGTTAATCTGGGATGTATCTT [200]
OsSINA6 GTAGCTGCATCACTAGAGCTTCCATGCAAAATAACTTTGGGGTGCATATA [200]
OsSINA4 GTGGCTGCGTCGCTTGAAGTTCCATGCAAGTACAAGTTCGGGTGTATTTA [200]
PtSINA2 GTTGCTGCATCTCTTGAAGTTCCATGTATTTATAGTTTTGGGTGCATATA [200]
PtSINA4 GTTGCTGCATCTCTTGAAGTTCCATGTAAATATAGTTTTGGGTGCATATA [200]
MtSINA3 GTGGCTGCATCACTAGAAGTTCCGTGTAATATGGTTTTGGGTGCATATA [200]
MtSINA6 GTGGCTGCATCTTTTGCCTCCCTGTAAATTCGATTTTGGGTGCATATA [200]
AtSINA2 GTTGCGAGTCTTTGGAAGTTCCATGCCGGTACAATTTGGGGTGTATTTT [200]
AtSINA1 GTTGCGGAATCATTGGAAGTTCCATGCCGGTACAATTTAGGGTGTATTTT [200]
PtSINA6 GTTGCGAATCATTGGAGCTGCCCTGCAAAATTCAGCTTAGGATGCATTTT [200]
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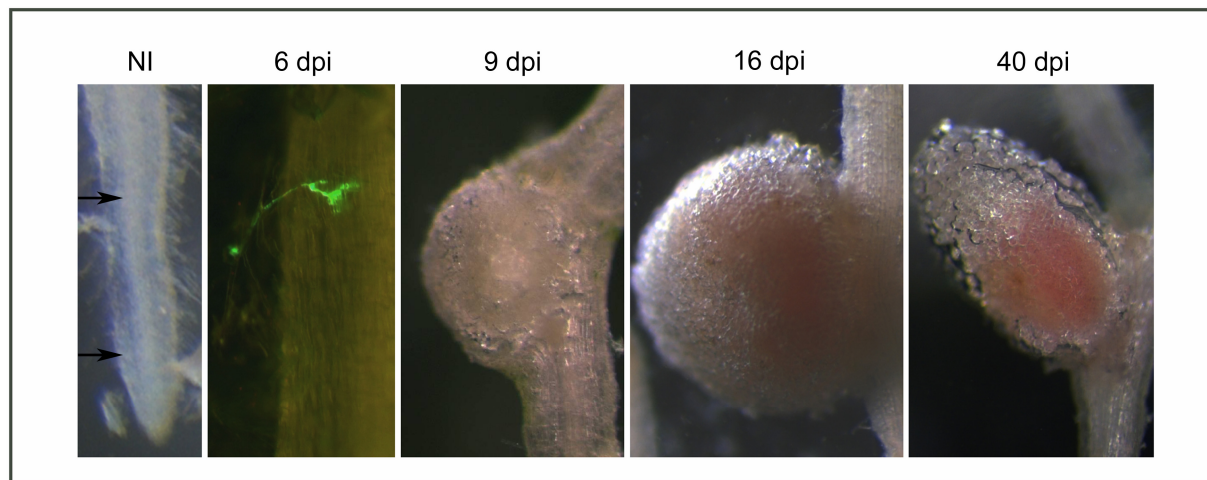
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 AtSINA2 ATCATCCCAAGGAACCTCGCACTCTACTTCTCTGGAGGTGATAGGCAAGA [650]
 AtSINA1 ATAATCCCTAGAAACTTGGCTTTGTACTTCTCTGGCAGTGACAAAGAAGA [650]
 PtSINA6 ATTATTCAAAGGAATTTGGCTCTCTACTTCTCTGGTGGGGATAGGCAAGA [650]
 PtSINA10 ATTATTCAAAGGAATTTGGCTCTCTACTTCTCTGGTGGGGATAGGAAAGA [650]
 PtSINA1 ATCATTCAAGAGAACTTGGCACTTTTCTCTCCGGAGGGGATCGGCAGGA [650]
 PtSINA5 ATCATTCAAGAGAACTTGGCACTTTTCTTTTCTGGAGGGGAACGGCAGGA [650]
 OsSINA2 ATCATCCACCGGAACATGGCCCTCTTCTCTCCGGCGGCAACAGGCAGGA [650]
 Siah1 GTCTTTGACACCAGCATTGCACAGCTTTTTGCAGAAAATGGCAATTTAGG [641]

OsSINA5 GCTGAAGCTGAGGGTGACCGCCGTATCTGGAAAGAACAG [690]
 OsSINA1 GCTGAAGCTGAGGATCACCGCCGGATCTGGAAGGAGCAG [690]
 OsSINA3 GTTGAATTCGCGGGTACCGGCCGAATCTGGAAGGAACAG [690]
 PtSINA8 GTTAAAGCTGCGAGTTACAGGACGGATATGGAAAGAACAG [687]
 PtSINA3 GTTAAAGCTGCGAGTTACAGGACGGATATGGAAAGAACAG [687]
 PtSINA7 GCTGAAGCTTAGAGTTACCGGGAGGATATGGAAGGAACAA [690]
 PtSINA9 GCTGAAGCTAAGAGTTACCGGGAGGATATGGAAGGAACAA [690]
 MtSINA1 ACTGAAATTAAGAGTACAGGAAGAATATGGAAAGAACAA [690]
 MtSINA4 GTTGAAACTTAGAGTACCGGAAGAATATGGAAGGAACAG [690]
 AtSINA3 GCTGAAACTTCGAGTCACTGGAAGGATATGGAAAGAGCAA [690]
 MtSINA2 GTTGAAACTAAGAGTAACAGGAAGGATATGGAAAGAGCAG [690]
 MtSINA5 GCTGAAGTTACGAGTAACAGGAAGGATATGGAAAGAACAG [690]
 SINAT5 ACTGAAACTTAGAGTCACTGGAAGAATCTGGAAAGAGCAA [690]
 AtSINA4 GCTTAAACTTAGAGTCACTGGTAAAATCTGGAAAGAGCAA [690]
 OsSINA6 GCTCAAATTCGCGTGTACCGGAAGGATTTGGAAGGAGCAG [690]
 OsSINA4 GCTCAAATTCGCGGTCACTGGGAGAATTTGGAAGGAACAG [690]
 PtSINA2 ATTGAAGCTTCGCGGTGACTGGTTCGATTTGGAAGGAACAG [690]
 PtSINA4 ATTGAAGCTTAGGGTGACTGGTTCGAATTTGGAAGGAACAG [690]
 MtSINA3 ATTGAAGCTACGAGTTACCGGAAGGATTTGGAAGAGCAG [690]
 MtSINA6 GTTGAAGCTAAGGGTTACTGGTAGGATTTGGAAGGAACAA [690]
 AtSINA2 ACTCAAGTTGAGAGTGACTGGACGAATCTGGAAAGAGGAG [690]
 AtSINA1 GCTCAAGTTGCGAGTCACTGGACGGATTTGGAAGGAAGAA [690]
 PtSINA6 GCTGAAATTCGAGGGTTACTGGTTCGTGTATGGAAAGAAGAA [690]
 PtSINA10 GCTGAAGTTGAGGGTTACTGGTTCGTGTATGGAAAGAAGAA [690]
 PtSINA1 GTTAAAACGTAAAGTTTCAGGACGGATTTGGAAGGAACAG [690]
 PtSINA5 GTTAAAACGTAAAGTTTCAGGACGGATTTGGAAGGAACAG [690]
 OsSINA2 GCTCAAGCTTCGCGGTACCGGCCGCATCTGGAAGGAGCAA [690]
 Siah1 CATCAATGTAACATTTCCATGTGT----- [666]

Supplemental Fig. S3. Stereomicroscopical images representing the different stages of nodule development that were harvested for qRT-PCR analysis.

From uninoculated roots, the zone just above the tip between the arrows, was harvested at the same time as the 6-dpi tissue. At 6 dpi, root sections were collected on which invading infection threads were seen. At 9 dpi, incipient nodule primordia were taken that were still white, a sign for premature symbiosis. At 16 dpi, round nodules were isolated that were pinkish, indicating the presence of leghemoglobin and initiation of nitrogen fixation, and at 40 dpi elongated fixing nodules were collected (see "Materials and Methods").



Supplemental Fig. S4. Co-immunoprecipitation of MtSINA1 and MtSINA3 with SINAT5DN. Immunoblots with the anti-cMYC antibody for detection of MtSINA1 and MtSINA3 proteins fused to the 3x MYC tag are shown. The upper and lower panel visualize the MYC-MtSINA proteins (33-kD fragment), present in the protein extracts before (input, 1/10 amount of the immunoprecipitated fraction) and after immunoprecipitation with the anti-HA antibody, respectively. Lanes 1 and 3 correspond to extracts from protoplasts transfected with only MtSINA3 and MtSINA1, respectively, whereas lanes 2 and 4 correspond to extracts of protoplasts cotransfected with HA-SINAT5DN (HA-SDN) and MYC-MtSINA3 and MYC-MtSINA1, respectively. Arrows mark the 33-kD band of the MtSINA proteins. The 25-kD band observed in the bottom panels corresponds to the size of the light chain of the anti-HA antibody used for immunoprecipitation. The 37-kD band in the input fraction is due to background signal in the extracts.

