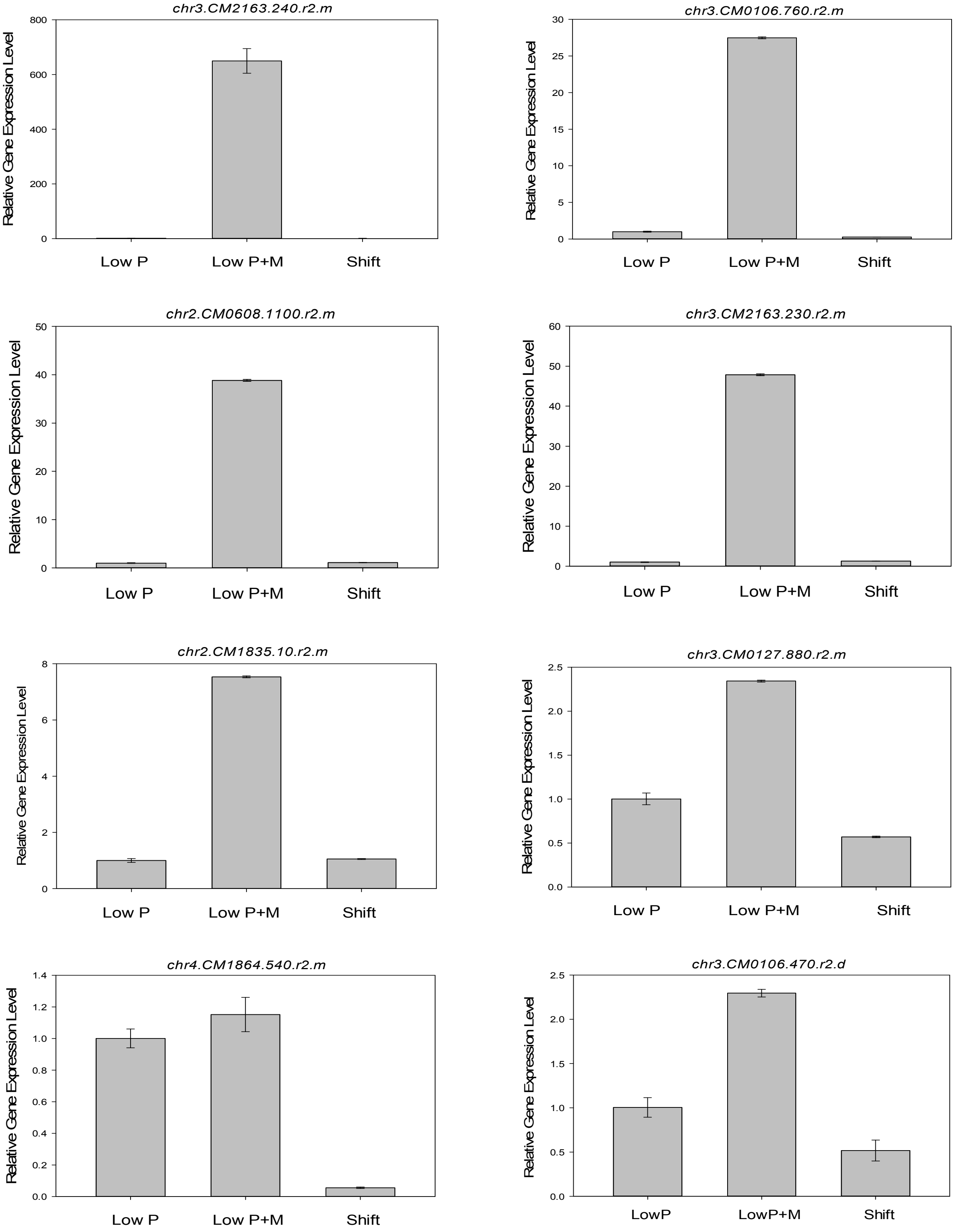
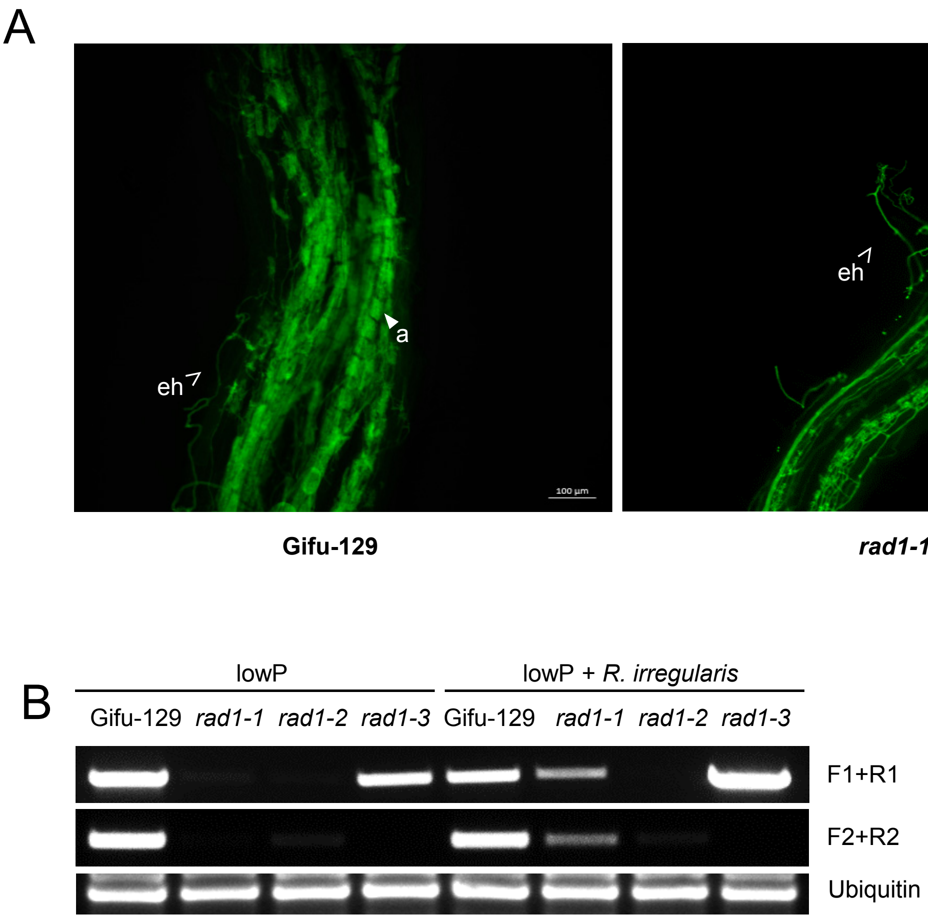
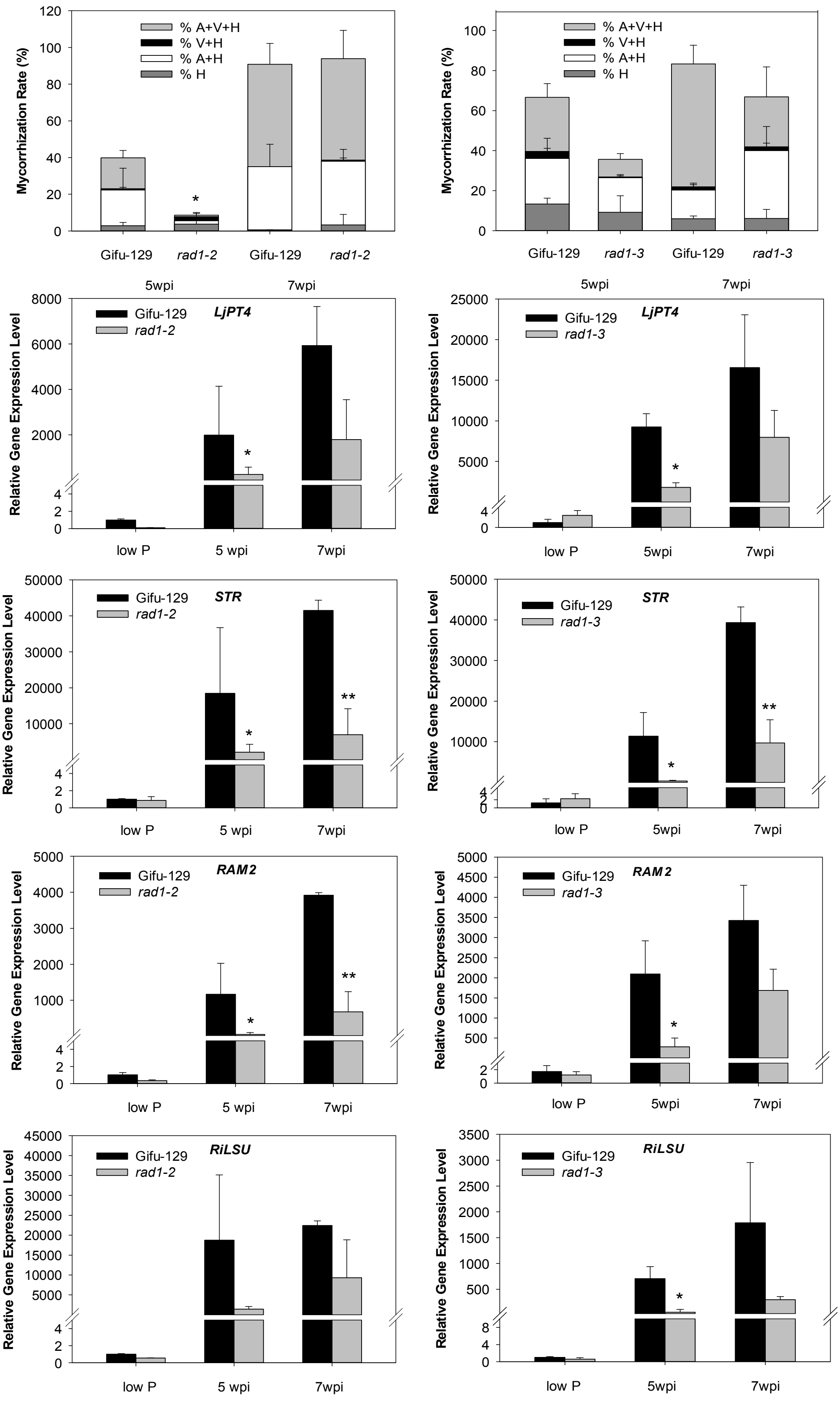


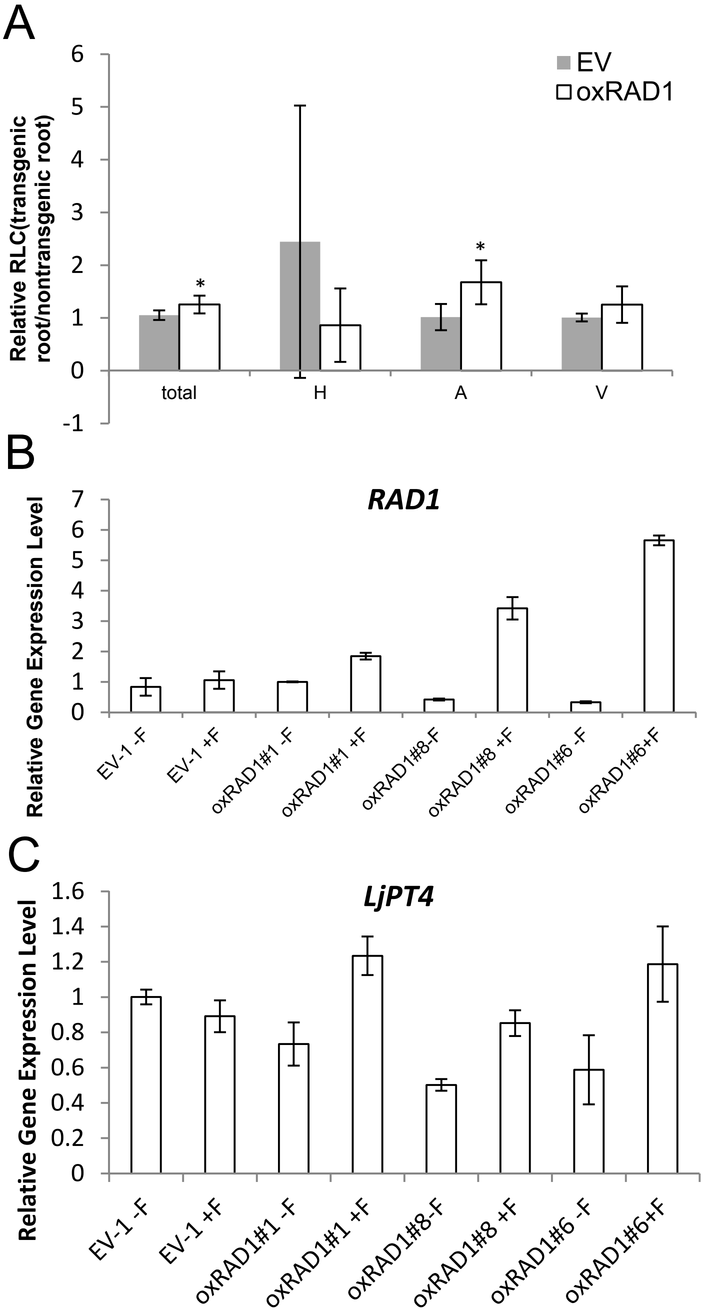
Supplemental Fig. S1. AM-inducible transcription factor genes repressed by high Pi condition. Gifu-129 plants grown at low Pi (5 µM) conditions were used as controls. After inoculation with *R. irregularis*, plants were grown at low Pi conditions for 8 weeks or 6 weeks at low Pi and re-supplied for two weeks with high Pi (7.5mM). Roots of four plants in one pot were harvested as one sample. Three technical replicates were used to calculate SD.



Supplemental Fig. S2. Mycorrhiza morphology in *rad1-1* and *RAD1* transcript analysis in *rad1 LORE1a* insertion lines.
(A) Arbuscules in Gifu-129 and *rad1-1* in the presence of *R. irregularis* at 8wpi. eh, external hypha. a, arbuscule. da, degenerated arbuscule. Scale bars are 100 µm.
(B) *RAD1* transcripts were detected in Gifu-129, *rad1-1, rad1-2* and *rad1-3* in the presence or absence of *R. irregularis* at 6wpi. 32 cycles were performed. Representative results from one out of three independent experiments are shown.



Supplemental Fig. S3. Mycorrhization rate and AM-inducible marker gene expression in *rad1-2* and *rad1-3* at 5 and 7 wpi .Three biological replicates were utilized. Error bars represent SD. Student's t-test was used to calculate confidence level. * indicates *P*<0.05. ** indicates *P*<0.01. Similar results was obtained from another independent experiment.

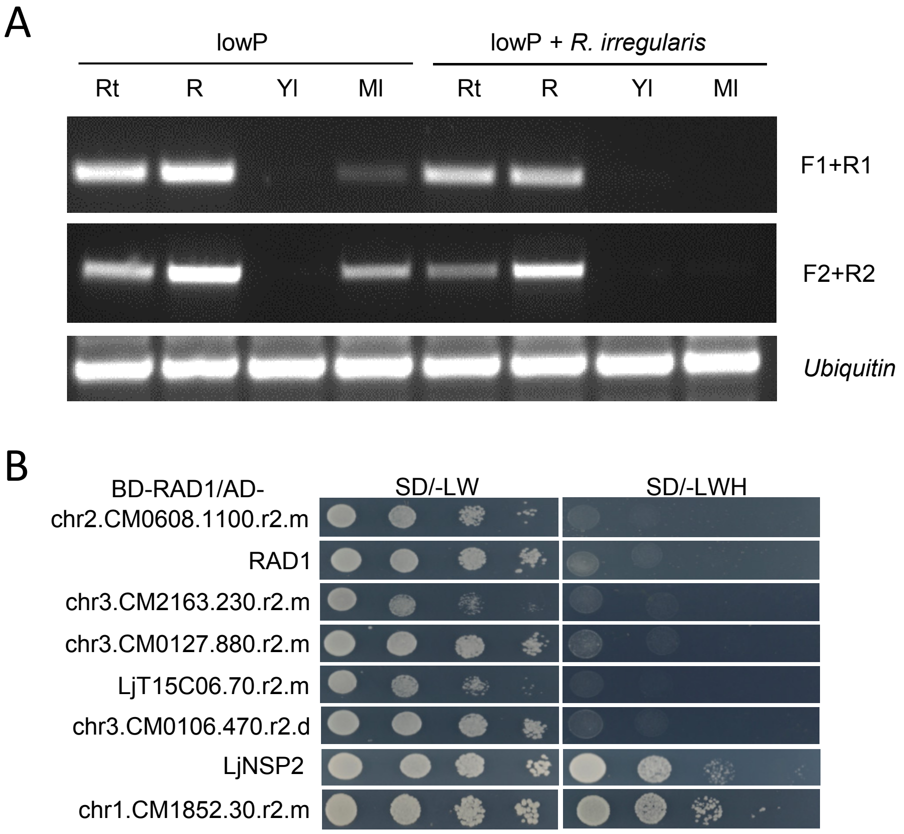


Supplemental Fig. S4. Mycorrhizal structures and *RAD1* gene expression in transgenic hairy roots overexpressing chimeric *35Spro:RAD1*.

(A) Relative root length colonized (RLC) in wild type hairy roots transformed with the empty vector (EV) or with a construct containing 35S*pro*:*RAD1* (oxRAD1). Percentage of RLC from transgenic hairy roots with EV (*n*=3) or oxRAD1 (*n*=8), respectively, was quantified. Relative RLC was calculated comparing the RLC from the transgenic red fluorescent hairy roots with the RLC from non-transgenic roots on the same composite plants. Significant differences of means between EV and oxRAD1 were determined by Student's t-test (* indicates $P<0.05$).

(B) *RAD1* gene expression is shown in transgenic red fluorescent hairy roots (+F) and non-transgenic non-fluorescent roots (-F) from three composite oxRAD1 plants and one control plant with EV in presence of *R. irregularis*. Error bars are from technical replicates.

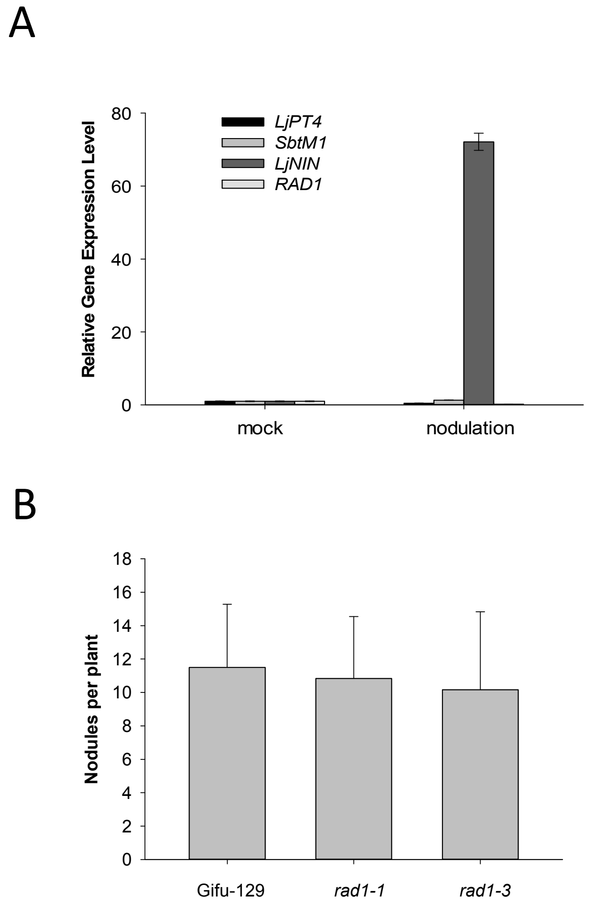
(C) *LjPT4* gene expression in transgenic fluorescent hairy roots (+F) and non-transgenic roots (-F) from three composite oxRAD1 plants and one control plant with EV in presence of *R. irregularis*. Error bars are from technical replicates.



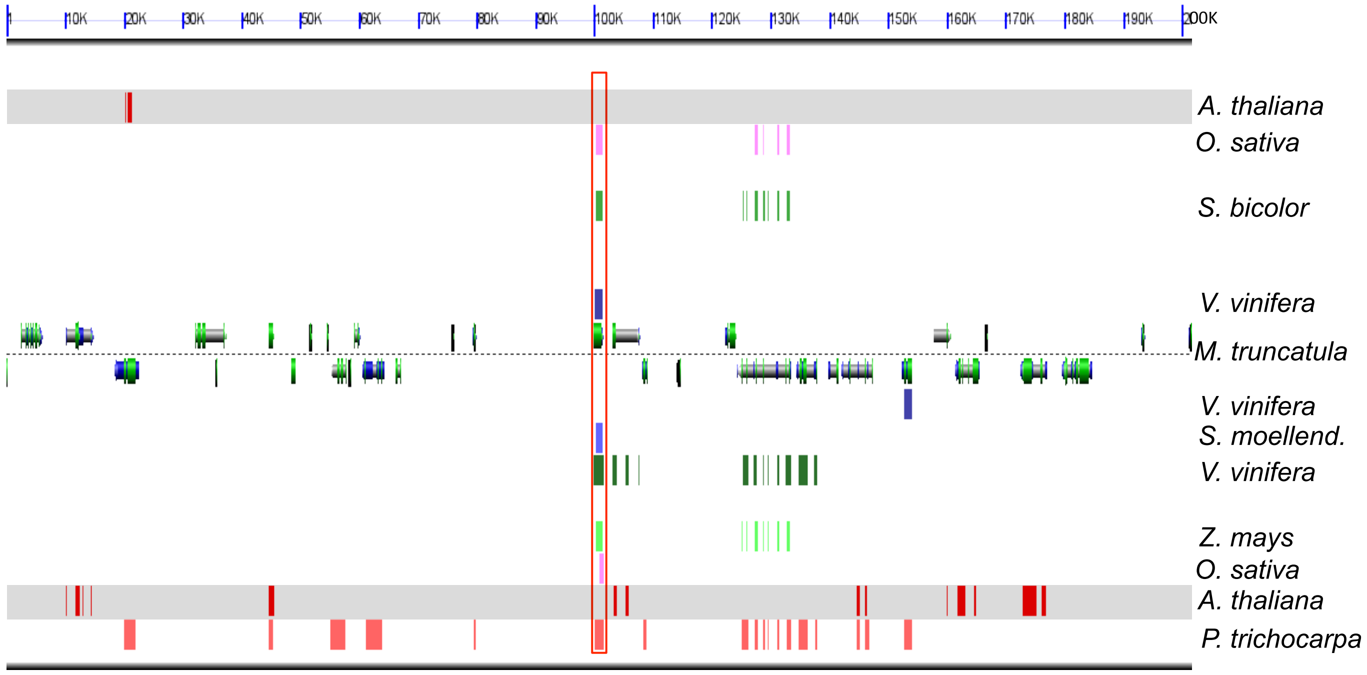
Supplemental Fig. S5. *RAD1* gene expression profile in mycorrhizal and non-mycorrhizal *Lotus* and GRAS proteins that interact with RAD1.

(A) RT-PCR analysis of *RAD1* transcript levels in different tissues of wild type in the presence or absence of *R. irregularis*. Rt, root tip; R, root ; Yl, young leaves; Ml, mature leaves. For *Ubiqutin* transcript amplification 28 PCR cycles, for *RAD1* 32 PCR cycles were used.

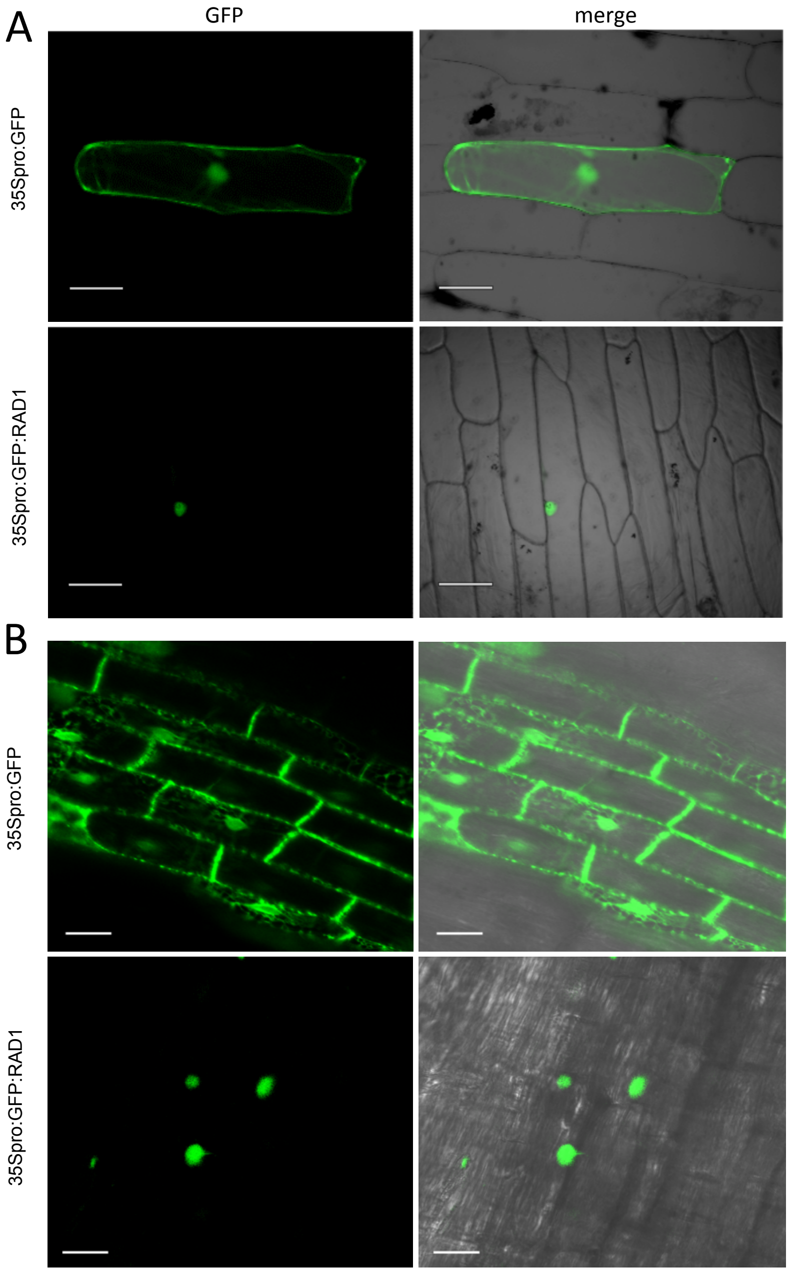
(B) Y2H analysis of interaction of RAD1 with mycorrhiza-regulated GRAS proteins. RAD1 was fused to the GAL4 DNA binding domain (BD). An AP2 transcription factor (chr2.CM0608.1100.r2.m) fused to the GAL4 activation domain (AD) was used as negative control. SD/-LW and SD/-LWH indicate a synthetic dropout medium lacking Leu and Trp and lacking Leu, Trp, and His, respectively.



Supplemental Fig. S6. *RAD1* gene expression in nodulated roots and nodulation in *rad1-1* and *rad1-3.*
(A) Relative expression levels of *LjPT4*, *SbtM1*, *LjNIN* and *RAD1* genes detected by qRT-PCR in nodulated plant roots.
(B) The number of nodules per plant in *rad1-1*, *rad1-3* and Gifu-129 is given (n=6). Two experiments were performed independently with similar results. Error bars represent SD.



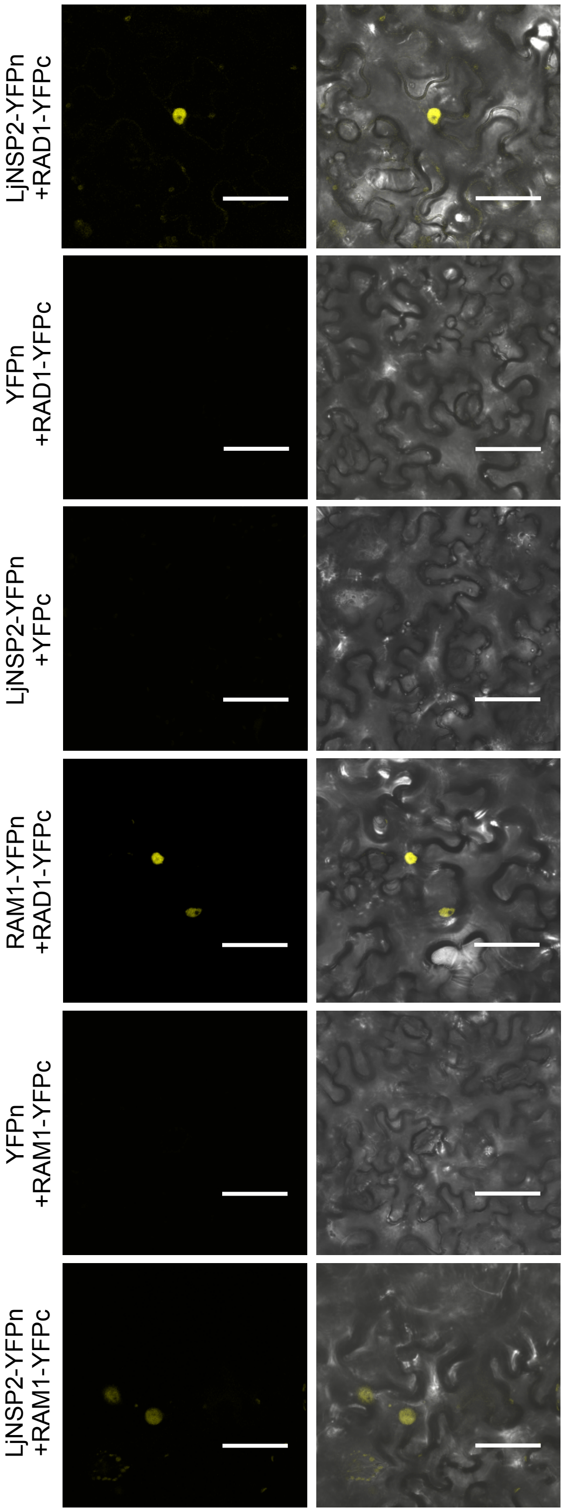
Supplemental Fig. S7. Synteny analysis of *RAD1* on AM host species and non-host *Arabidopsis thaliana*. Synteny analysis of a ~200 kb region encompassing the *RAD1* locus in *M. truncatula*. Green and blue boxes above and below the dashed lines represent *Medicago* genes. Orthologous genes in other species are indicated above and below: *Arabidopsis thaliana* (red), *Populus trichocarpa* (orange), *Vitis vinifera* (dark blue – RAD1-like; dark-green RAD1), *Sorghum bicolor* (green), *Oryza sativa* (pink), *Zea mays* (light green) and *Selaginella moellendorffii* (violet). The RAD1 locus is red-boxed.



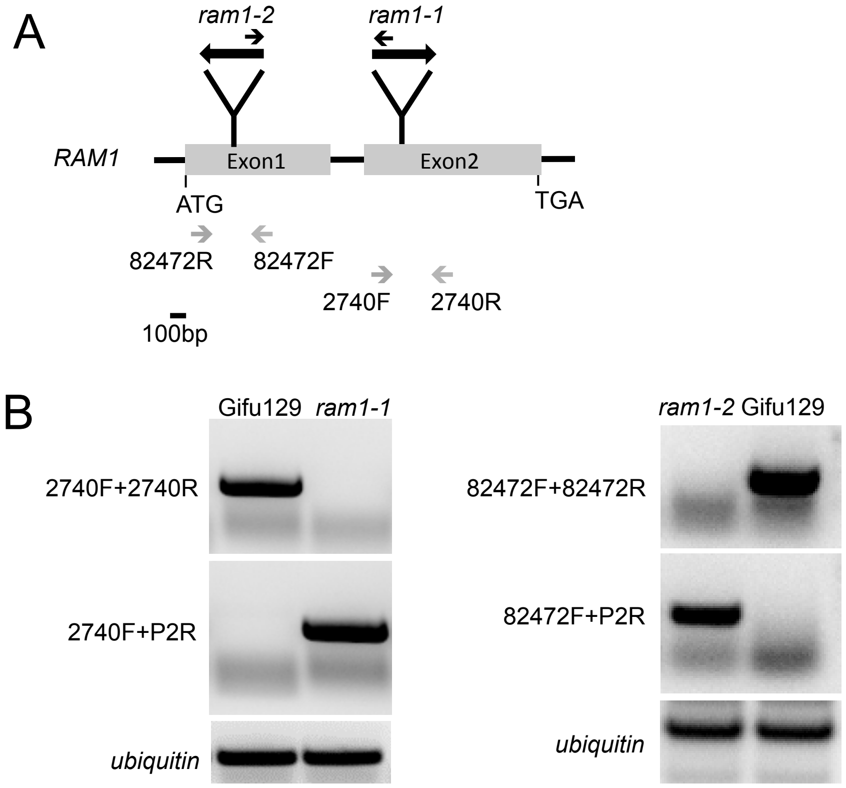
Supplemental Fig. S8. Nuclear localization of RAD1.

(A) Nuclear localization of RAD1 in onion epidermal cells after biolistic bombardment with *35Spro:GFP* or *35Spro:GFP:RAD1*, respectively. Images with green fluorescence (GFP) alone and merged with bright-field image (merge) are shown as indicated. Scale bar is 100 µm.

(B) Nuclear localization of RAD1 in *Lotus* hairy roots expressing *35Spro:GFP* or *35Spro:GFP:RAD1*, respectively. Scale bar is 20 µm.



Supplemental Fig. S9. Interaction between RAM1, RAD1 and LjNSP2 by BiFC assay in *N. benthamiana*. Leaves were infiltrated with mixtures of *A. tumefaciens* strain GV3101 to coexpress RAD1 and RAM1 or LjNSP2 fused to half of split YFP, respectively as indicated at left. Three days after infiltration, images were captured from five different leaves for each plasmid combination. Scale bars are 50 µm.



Supplemental Fig. S10. Gene structure of *RAM1* in wild type *Lotus* and *ram1* mutant.
(A) Gene structure of *RAM1* in *Lotus* and the positions of the *LORE1a* insertions in *ram1-1* and *ram1-2*. Gray arrows indicate the primers matching with the genome sequence used for genotyping. Black arrows indicate P2R primer on the left border of the *LORE1a* transposon.
(B) Identification of homozygous *ram1-1* and *ram1-2* through genomic DNA amplification. Primer pairs as described in (A) were used for PCR and are indicated at left. *Ubiquitin* gene was used as a control.

Supplemental Table S1: List of mycorrhiza up-regulated transcription factors generated by DESeq.

Seq. Name (*Lotus*CDSv2.5)	**Putative annotation of transcription factor**	**RNA-seq data**			
		fold change (+*R.i.* /-*R.i.*)	***P*adj**	**resVarA**	**resVarB**
chr6.CM0679.550.r2.d	MADS-box	M specific	7.0e-04	6.09	0.00
chr6.CM1613.300.r2.m	C2H2 Zinc finger	M specific	5.1e-03	0.24	0.00
chr1.LjB18K24.70.r2.a	MYB	M specific	1.42e-102	17.63	0.00
chr3.CM0111.130.r2.d	NAC	M specific	5.10e-17	0.82	0.00
chr3.CM2163.240.r2.m	GRAS	970.83	6.73e-89	8.54	5.1e-04
chr2.CM0608.1100.r2.m	AP2	586.44	1.18e-06	12.14	1.3e-03
chr3.CM2163.230.r2.m	GRAS	462.42	3.11e-111	12.07	8.84e-06
chr1.CM1852.30.r2.m	GRAS	438.56	8.73e-109	2.33	9.84e-06
chr5.CM0239.240.r2.m	GRAS	170.03	1.89e-40	0.64	0.01
chr2.CM0021.530.r2.m	C2H2 Zinc finger	165.30	5.66e-65	0.18	6.40e-05
chr4.CM0075.50.r2.m	ERF	164.31	3.43e-23	4.27e-04	0.01
chr2.CM0081.1990.r2.m	AP2	150.70	5.7e-69	10.70	3.88e-04
chr1.LjB18K24.100.r2.a	MYB	120.88	1.56e-80	15.0	2.46e-03
chr6.CM0041.30.r2.a	AP2	78.55	4.72e-62	0.08	1.72e-04
LjB17L21.50.r2.d	MADS-box	44.10	7.51e-07	2.13e-03	0.09
chr1.CM0029.1590.r2.a	ARF	43.89	5.02e-48	0.1	8.24e-04
chr1.CM1413.480.r2.d	WRKY	38.12	5.42e-26	0.49	0.08
LjSGA_032180.1	C2H2 Zinc finger	24.65	2.11e-30	0.76	0.02
chr5.CM1667.220.r2.a	GRAS	19.68	6.69e-15	0.63	0.28
LjSGA_122341.1	GRAS	13.04	9.07e-19	1.46	0.14
chr6.CM0139.1440.r2.d	GRAS	11.74	4.35e-35	0.74	4.56e-05
chr2.CM1835.10.r2.m	NAC	8.96	3.65e-09	1.05	1.5e-03
LjSGA_020219.1	GRAS	8.93	5.03e-03	0.60	0.10
LjSGA_012098.1	AP2	6.80	3.26e-09	0.04	0.25
chr4.CM0680.320.r2.m	GRAS	6.02	9.55e-17	11.47	0.10
LjSGA_029723.1	AP2	6.00	8.98e-14	1.73	0.10
chr1.CM0012.840.r2. m	NAC	6.04	2.71e-23	4.12	0.70
chr3.CM0127.880.r2.m	GRAS	5.77	6.12e-13	6.14	0.01

LjSGA_045015.1	GRAS	5.74	2.84e-22	4.13	3.60e-03
LjSGA_073109.1	GRAS	5.34	1.93e-22	5.29	0.15
LjSGA_026563.1	MYB	5.31	4.30e-03	0.95	0.01
chr6.CM0118.1050.r2.a.	ERF	4.98	6.92e-19	0.29	0.13
chr1.cm0122.2130.r2.d	GRAS	4.81	2.83e-13	1.06	0.22
chr3.CM0106.470.r2.d	GRAS	3.57	1.50e-13	2.51	0.12
chr1.CM0375.530.r2.a	C2H2 Zinc finger	3.54	9.86e-12	1.44e-03	1.05e-03
chr2.CM0018.140.r2.m	GRAS	3.20	8.07e-10	0.01	0.71
LjSGA_055804.0.1	C2H2 Zinc finger	3.17	9.21e-04	0.01	0.07
chr3.CM0106.760.r2.m	NIN-like	3.13	7.69e-08	1.24	1.93
chr4.CM1864.540.r2.m	GRAS	2.93	2.01e-10	40.77	1.24
LjT15C06.70.r2.m	GRAS	2.51	4.23e-04	11.13	1.06e-03
chr5.CM0200.2670.r2.d	LOB domain	2.46	6.97e-06	0.21	0.06
chr1.CM0284.80.r2.d	AP2-ERF	2.31	1.60e-03	1.21	2.98e-03
chr6.CM0314.840.r2.d	bZIP	2.30	3.35e-03	0.46	2.86e-03
chr3.CM0176.150.r2.m	NAC	2.27	3.43e-03	0.40	5.15e-05
chr3.CM0460.10.r2.d	GRAS	2.11	2.85e-04	1.50	0.19

"resVar" values indicate the degree of the variance among the biological samples. If resVarA or resVarB are <15, the fold changes are robust. “M specific” indicates mycorrhiza specific induction of gene expression.

Supplemental Table S2: Fourty-five mycorrhiza up-regulated transcription factors under different treatments.

Overall highly expressed genes					
Gene ID	Low P+M	High P+M	shift	Low P	High P
chr1.CM0375.530.r2.a	292.749139	65.0446598	61.1996559	82.7275152	63.6899635
chr3.CM0176.150.r2.m	91.522928	38.2383685	53.9730872	40.394509	26.0924937
chr1.CM0012.840.r2.m	349.15635	32.9624048	39.6685867	57.8409834	34.5023455
chr5.CM0200.2670.r2.d	231.955327	107.937444	170.9069145	94.3299251	179.063813
chr3.CM0460.10.r2.d	244.052245	42.2776687	73.5944351	115.4822037	79.2501851
chr2.CM0018.140.r2.m	255.58247	48.2073169	51.6239006	79.9909595	56.886244
chr3.CM0106.470.r2.d	491.400977	70.7606984	115.6729524	137.4609898	97.676021
chr1.CM0122.2130.r2.d	165.672537	30.088316	31.2386641	34.408076	25.3193636
chr6.CM0139.1440.r2.d	319.955846	31.5771559	33.8243808	27.2527556	26.925496
chr6.CM0314.840.r2.d	83.713264	51.1785688	48.5987186	36.375022	52.2947531
chr3.CM0106.760.r2.m	183.9800069	65.5690423	74.88890491	58.78141048	62.03393774

Genes which were only responsive to AM under low P conditions

Gene ID	Low P+M	High P+M	shift	Low P	High P
chr2.CM0021.530.r2.m	301.577336	0.3172006	3.7890705	1.8244533	1.2694607
LjSGA_032180.1	144.082462	6.8876778	9.0619715	5.8439403	7.6067857
chr1.CM1413.480.r2.d	99.969242	6.2532766	4.9788498	2.6226361	9.6793125
chr2.CM1835.10.r2.m	44.693256	8.3700899	15.0244424	4.9887356	5.4743866
chr3.CM0111.130.r2.d	43.419953	0.5308103	0	0	0
LjSGA_026563.1	16.807706	6.9976965	2.8933424	3.1642823	7.1603485
chr1.LjB18K24.100.r2.a	485.867106	3.812835	5.8745779	4.019487	2.9554226
chr1.LjB18K24.70.r2.a	601.412944	0	2.5113982	0	0.4264798
LjB17L21.50.r2.d	18.859199	0	0.4258908	0.4276024	0.4264798
chr6.CM0679.550.r2.d	8.276469	0.3172006	0.8517816	0	0
chr3.CM0127.880.r2.m	117.540714	12.0600506	19.4895084	20.3825447	12.1882979
chr5.CM1667.220.r2.a	56.110791	1.4824121	1.2337258	2.8507238	1.2694607
chr5.CM0239.240.r2.m	145.41238	2.226832	0.8517816	0.8552047	0
chr1.CM1852.30.r2.m	800.135821	1.7996127	2.5113982	1.8244533	1.2694607
chr3.CM2163.230.r2.m	843.667924	1.1652115	0.8517816	1.8244533	0.4165011
chr3.CM2163.240.r2.m	470.488665	0.3172006	3.3631797	0.4846243	1.259482
chr1.CM0029.1590.r2.a	240.24622	5.5152845	5.4047406	5.4733599	4.6513631
LjSGA_029723.1	123.780115	11.8592965	14.5985516	20.6106325	10.0958136
LjSGA_012098.1	56.634212	4.7644368	5.0227963	8.3240837	6.7737834
chr6.CM0041.30.r2.a	320.224498	2.7512145	3.3192332	4.0765089	6.3173675
chr2.CM0081.1990.r2.m	339.365693	3.5992253	4.6408521	2.2520557	2.9554226
chr2.CM0608.1100.r2.m	284.202937	0	0	0.4846243	0
chr4.CM0075.50.r2.m	70.258661	0	0	0.4276024	0.4264798

Low Pi induced genes regardless of AM status

Gene ID	Low P+M	High P+M	shift	Low P	High P
LjT15C06.70.r2.m	103.902922	2.9712519	5.1106894	41.3637576	6.3173675
chr4.CM1864.540.r2.m	1502.546805	18.216164	41.9738268	513.4417307	32.7066181
LjSGA_073109.1	475.682844	23.3235125	25.1443536	88.3434921	16.8496397
LjSGA_045015.1	380.155738	14.3125937	10.0016461	66.2504137	14.2807822
chr4.CM0680.320.r2.m	176.496151	5.295247	0.807835	29.3052966	3.3719237
LjSGA_020219.1	376.56175	5.6124476	13.3208793	42.1905135	8.4198305
LjSGA_122341.1	96.630563	0	0	7.411857	0.4264798
chr1.CM0284.80.r2.d	103.803575	7.1012875	6.9764641	45.0126642	4.2248833
chr6.CM0118.1050.r2.a	343.723987	19.4006588	24.5123047	69.0726886	23.563551

Intermediates

Gene ID	Low P+M	High P+M	shift	Low P	High P
chr6.CM1613.300.r2.m	6.53636	1.6960217	0.4258908	0	0
LjSGA_055804.0.1	44.863102	16.0993508	16.0823821	14.168024	15.1736565

Normalized counts per transcript from different treatments are shown above.

Supplemental Table S3: Sequences of oligonucleotide primer pairs used.

Primers for RT-PCR:

Gene ID	**primer name and primer sequence**
Lj*NIN*	qLjNIN-F: TGGATCAGCTAGCATGGAAT
	qLjNIN-R: TCTGCTTCTGCTGTTGTCAC
SbtM1	qSbtM1-F: TGTATGCTGCTGCTGAAAAAAACAACT
	qSbtM1-R: CTTCTTGACCTTTTGCAATAAATGGGATTC
STR	qSTR-F: CTGGACAAGATCACCGTCCT
	qSTR-R: GTGGCCATCAAGCTGGTATT
RAD1	q-F1: CCGAGGCTCATGCCTAGGTCCACT
	q-R1: CCCCAATGGGTTTCCATGCCTATCC
	q-F2: ATGGTGGAGCAGGATTCAAG
	q-R2: TTAACATTTCCAGCAAGAAG
RAM1	qRAM1-F: GGAGGTTTCTTGAGGCACTG
	qRAM1-R: CCTTCCATGATCTTCCTCCA
RAM2	qRAM2-F: GGGATGGACCCGTTTTACTT
	qRAM2-R: GACTTTGTTTGCGGCCTTAG
Lj*PT4*	qLjPT4-F: TCCAAGCGGAGCAAGACAAG
	qLjPT4-R: TTCTGTGTGAGGTTCTGGCTGTAG
Ubiquitin	qUbiquitin-F: TTCACCTTGTGCTCCGTCTTC
	qUbiquitin-R: AACAACAGCACACACAGACAATCC
R.irregularis large subunit rRNA (RiLSU)	LR1 : GCATATCAATAAGCGGAGGA
	8.22: AACTCCTCACGCTCCACAGA
chr3.CM0106.760.r2.m	q30-F: AGGATGCTGCAAAGAGCATT
	q30-R: CTCCCTGGACAGAGTCAAGC
chr3.CM0127.880.r2.m	q21-F: GAAGCCATGGGACAAGAAAA
	q21-R: TCCAAAGTGCTCACAACAGC

chr2.CM0608.1100.r2.m	q3-F: AGAGGAGTAGCAAGGCACCA
	q3-R: TGGGGTTTGCTCTTGAGAAG
chr3.CM2163.230.r2.m	q-8-F: TGCCTGTGAAGACAGGTGAA
	q-8-R: GCCACTCAGCAAAAGTCCTC
chr3.CM2163.240.r2.m	q-5-F: TCTTCACCCAAGTGGAAGG
	q-5-R: CCAGCATGATTCTCTCCACA
chr2.CM1835.10.r2.m	q17F: GCAACTGATTCTTGGGCAAT
	q17R: GAATGGCTGATGGTGTTGTG
chr3.CM0106.470.r2.d	q26-F: TCCTCACTTGAAACTCACTG
	q26-R: CATTCGTTGACCAACCTCCT

Primers for genotyping:

LORE1a mutant	**primer name and primer sequence**
rad1-1	1039F: GGAATGCTATTCACACTCACACTC
	1039R: GAACGAGGAACTCAACACATC
rad1-2	576F: CCGAGGCTCATGCCTAGGTCCACT
	576R: CCCCAATGGGTTTCCATGCCTATCC
rad1-3	2260F: AGTCGTGGCGCTTTGAATTCGGTG
	2260R: TGAGTGGTTTCGAATCACCAGAGCG
ram1-1	2740F: GCTTGTGCTGAAGCAGTGGCCAAA
	2740R: TGCGGAGGGAGTGTGCTAATTCGG
ram1-2	82472F: TCAATTGCTGGCAGGGCAAGGTTC
	82472R: TGCAGTCCAAGAAAAATGCCACTCA
Ubiquitin	GS-UB-F:CGTGAAGGCTAAGATCCAGGATAAG
	GS-UB-R:CGATACTACTTGTTCAAGAGGGGC
	P2R: CCATGGCGGTTCCGTGAATCTTAGG

Note: Endogenous fragment: F+R, LORE1a insertion fragment: F+P2R

Primers for Y2H:

plasmids	**primer name and primer sequence**
pAS-RAD1/ pACT-RAD1	RAD1-attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGTCCCCTCCTCTTTATAGTG
	RAD1-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAACATTTCCAGCAAGAAGCTG
pACT-RAM1	RAM1-attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGATCAATTCAATGTGTGG
	RAM1-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAGCATCTCCATGCAGAGG
pACT-AP2	AP2-attB1:

	GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGGAGTTTGCTTCTGTAAAATC
	AP2-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCTCATTCCATAGGGGGAAACATG
pACT-Chr3.CM2163.230.r2.m	Chr3.CM2163.230.r2.m-attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGAGGGAGCTGAGATATGAC
	Chr3.CM2163.230.r2.m-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCTCATTTCCTGAAACTCCATGCTG
pACT-Chr3.CM0127.880.r2	Chr3.CM0127.880.r2.m-attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGGAAGACAAGGGCTTAAAAC
	Chr3.CM0127.880.r2.m-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAAAACTTCCAGGCTGATATAG
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	Chr3.CM0106.470.r2.d-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAAGTAGCTAGCTGGGGCT
pACT-LjT15C06.70.r2.m	LjT15C06.70.r2.m-attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGGACATGGAGATTGACATTG
	LjT15C06.70.r2.m-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACAAATTAGAGTCATCTG
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	LjNSP2-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATGCACAATCTGATTCTG
pAS- RAD1ΔN	RAD1-121-attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCTCTatgGTTGGAGCTGAAGAAGATG
	RAD1-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAACATTTCCAGCAAGAAGCTG
pAS- RAD1ΔPS	RAD1-attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGTCCCCTCCTCTTTATAGTG

	RAD1-340-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAAAGAGCTTCATCATCATTC
pAS- RAD1 ΔS	RAD1-attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGTCCCCTCCTCTTTATAGTG
	RAD1-420-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCTTACATCTTTGCCCTCTTAGTG
pAS- RAD1 ΔNL	RAD1-200-attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCTCTatgTCCATGATGAACATCATG
	RAD1-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAACATTTCCAGCAAGAAGCTG

Primers for BiFC:

pBatTL-RAD1-YFPn/ pBatTL-RAD1-YFPc	RAD1-attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGTCCCCTCCTCTTTATAGTG
	RAD1withoutTGA-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCacatttccagcaagaagctg
pBatTL-RAM1-YFPn/ pBatTL-RAM1-YFPc	RAM1-attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGATCAATTCAATGTGTGG
	RAM1withoutTGA-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCGCATCTCCATGCAGAGG
pBatTL-LjNSP2-YFPn	LjNSP2-attB1: GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGGAAATGGATATAGATTG
	LjNSP2withoutTGA-attB2: GGGGACCACTTTGTACAAGAAAGCTGGGTCTGCACAATCTGATTCTG

Supplemental Text S1. Synteny analysis of potential symbiotic transcription factors. A ~200kb sized region in the *Medicago truncatula* genome containing transcription factors identified by RNAseq was compared to the syntenic region in *Arabidopsis thaliana* (col-0, pink) and *Populus trichocarpa* (green) using CoGe:GEvo (https://genomevolution.org/CoGe/GEvo.pl) as described in Delaux et al. 2014. *Medicago* was used as a proxy for *Lotus* given that this tool is not available for *Lotus*. The target gene in *Medicago* is red-boxed together with orthologs from *Populus* and *Arabidopsis* when present. The vertical orange stripes are regions that contain unknown nucleotides that were inserted during the scaffolding of assembled contigs. However, the position and relative size of the gap (orange stripe) is known due to the size of the genomic library and/or a genomic map.

Examples of genes conserved in host and non-host species

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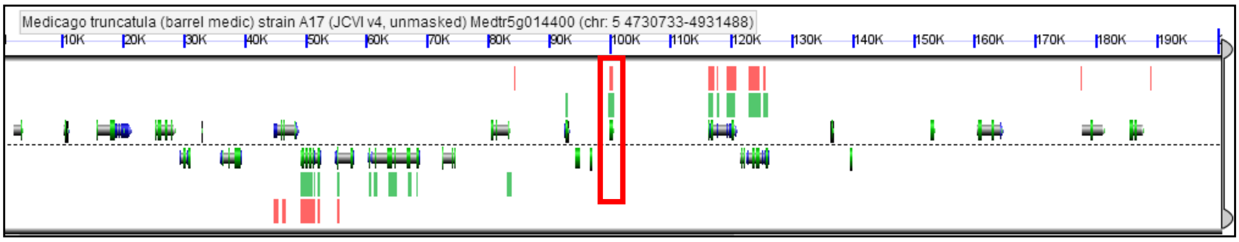
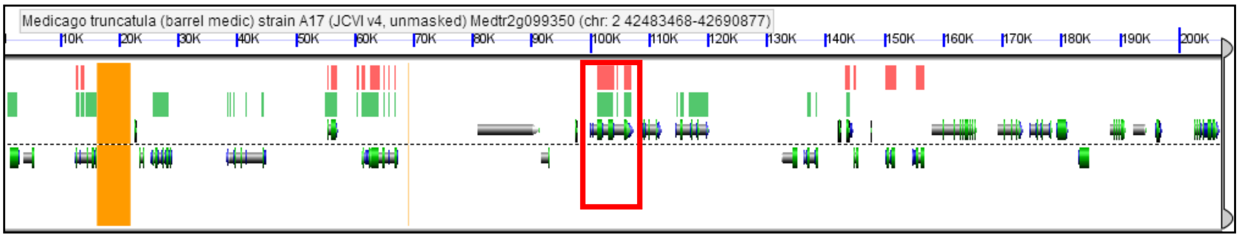


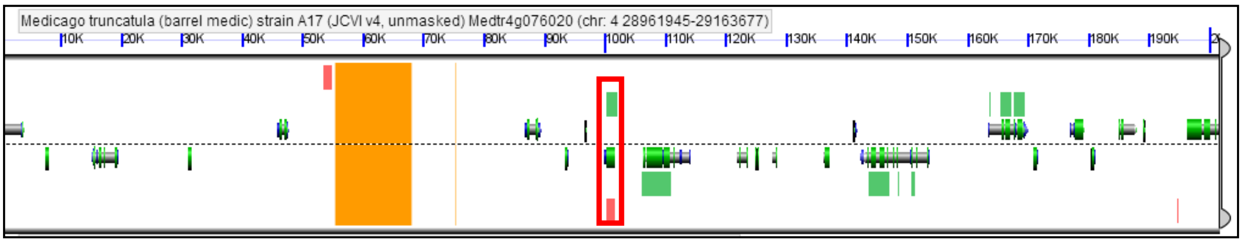
Strong syntenic support for both *Arabidopsis* and *Populus*.

chr3.CM0106.760.r2.m



Strong syntenic support for both *Arabidopsis* and *Populus*.

chr3.CM0460.10.r2.d

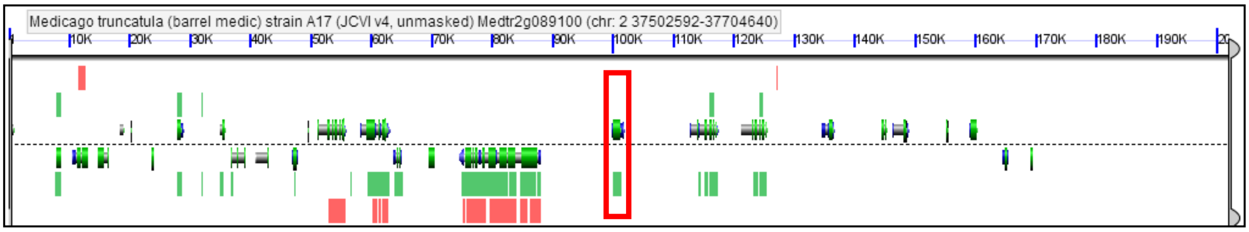


Strong syntenic support for *Populus* but low support for *Arabidopsis*.

Genes missing in in non-host species

Present in Lupinus (potential common symbioses transcription factors)

LjSGA_045015.1



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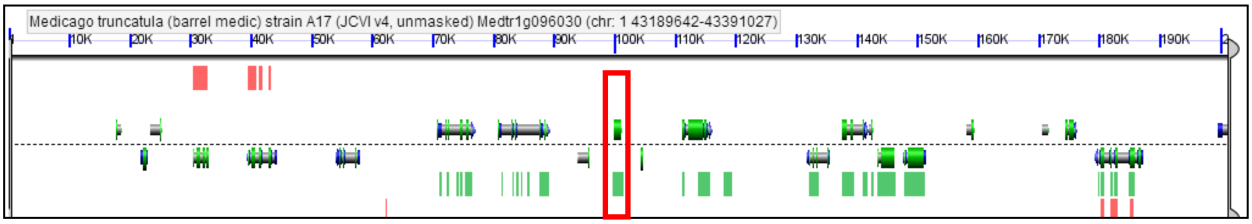

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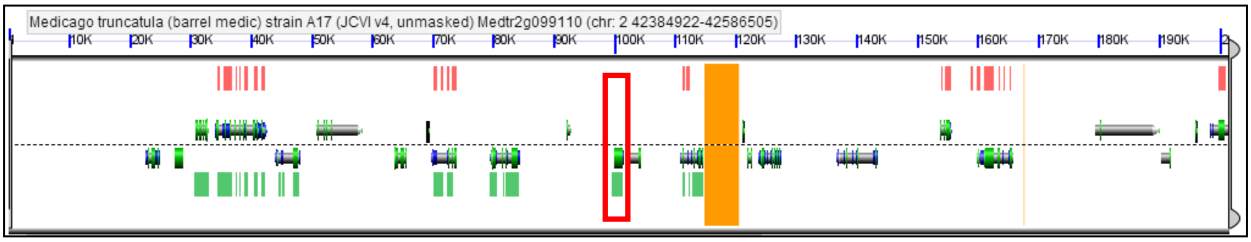

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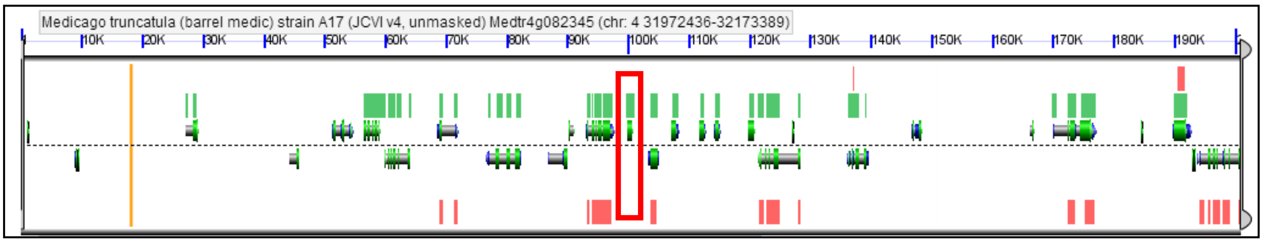

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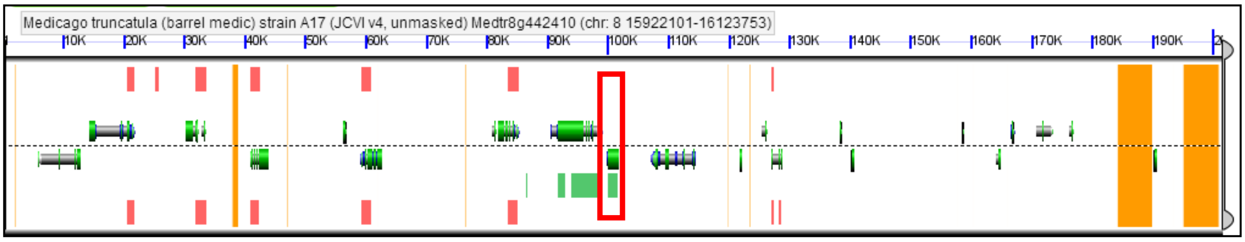
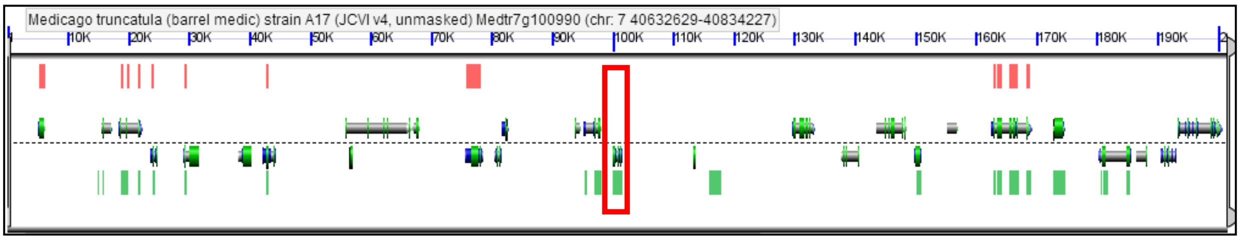


__Absent in Lupinus (potential AMS specific transcription factors)__

chr1.CM0012.840.r2.m



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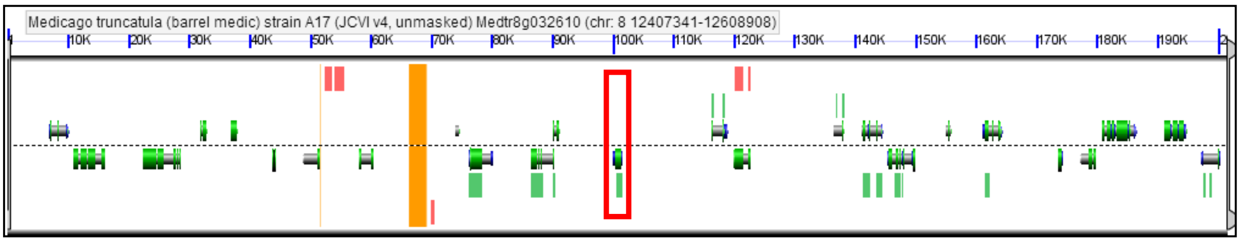

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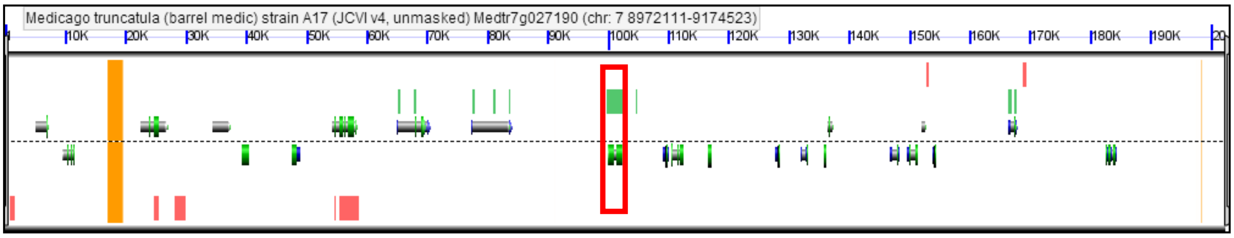
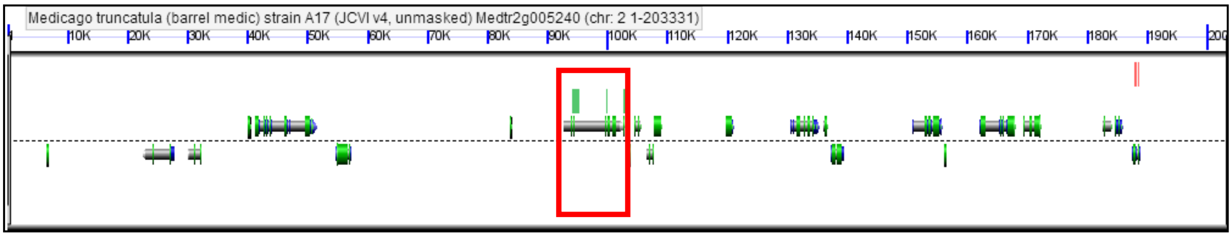
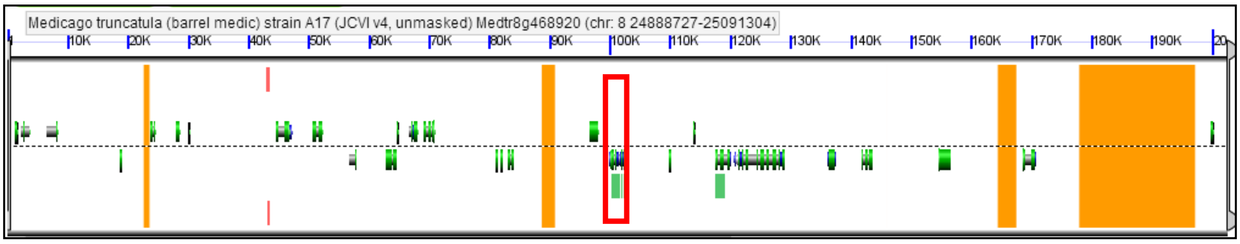


chr1.CM0029.1590.r2.a



Low syntenic support even for *Populus.*

chr2.CM0608.1100.r2.m



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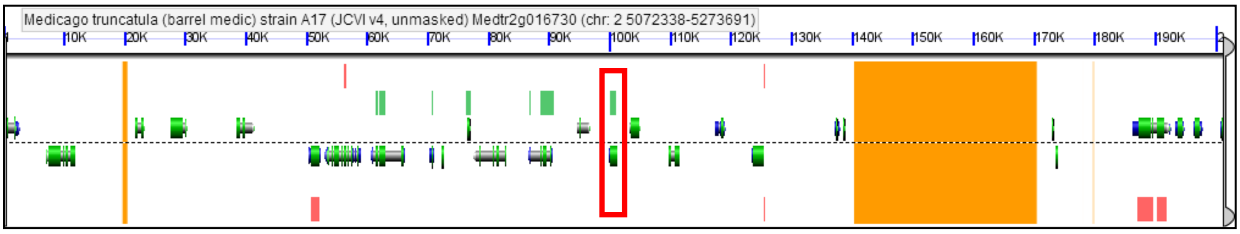

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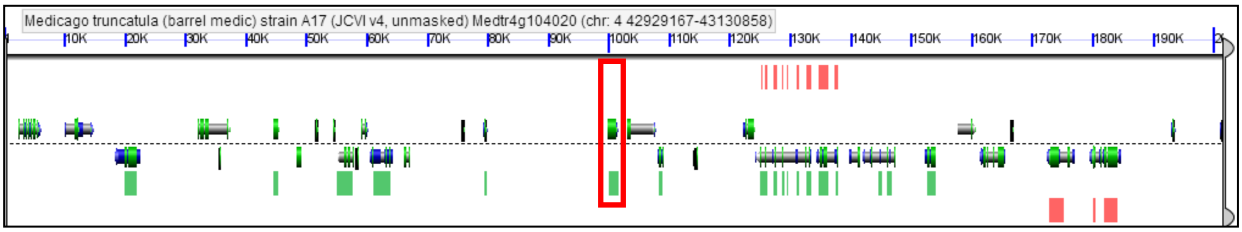
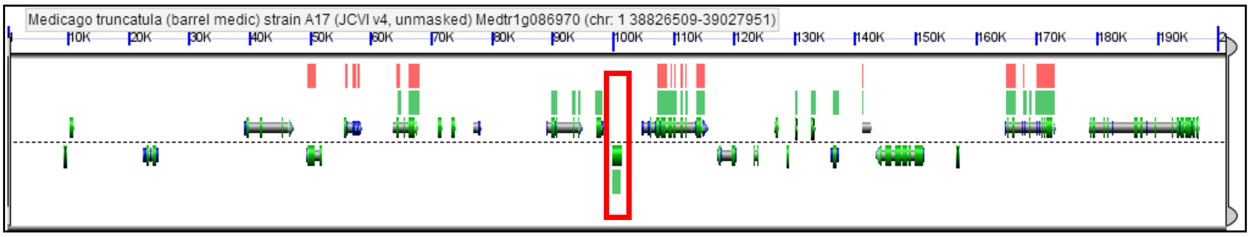


chr5.CM0239.240.r2.m



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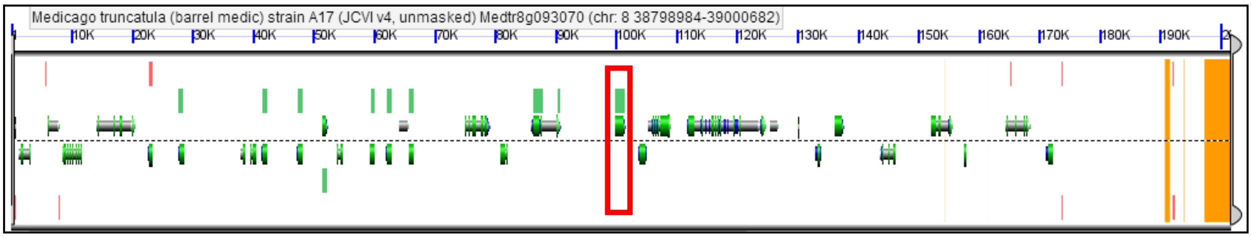

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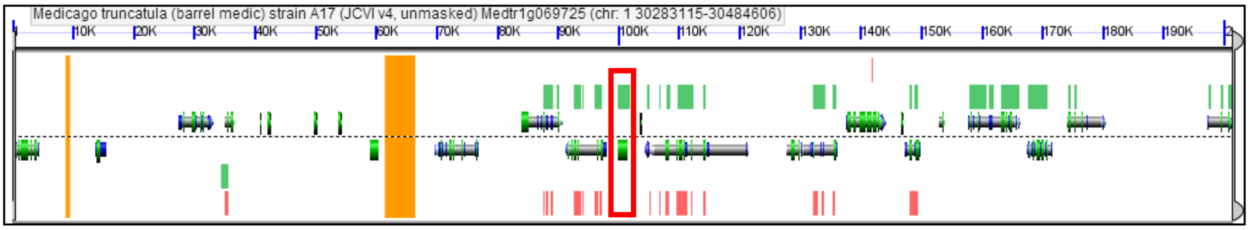

Supplemental Text S2: Sequences used for phylogenetic tree.

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Arabidopsis thaliana
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Lunularia cruciata

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Medicago truncatula

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Prunus persica

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Ricinus communis

> Rc 29929.t000249
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Setaria italica

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Sorghum bicolor

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Theobroma cacao

> Tc Thecc1EG014574
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Vitis vinifera

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Zea mays

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