

Insights into the complex role of GRAS transcription factors in the arbuscular mycorrhiza symbiosis

Hartmann, Rico M.¹; Schaepe, Sieke¹; Nübel, Daniel¹; Petersen, Arne C.¹; Bertolini, Martina^{1,2}; Vasilev, Jana¹; Küster, Helge^{1,#}; Hohnjec, Natalija^{1,#}

¹ Unit IV-Plant Genomics, Institute of Plant Genetics, Leibniz Universität Hannover,
Herrenhäuser Str. 2, D-30419 Hannover, Germany

² Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Via
Mangiagalli 25, 20133 Milano, Italy

These senior authors contributed equally to the article

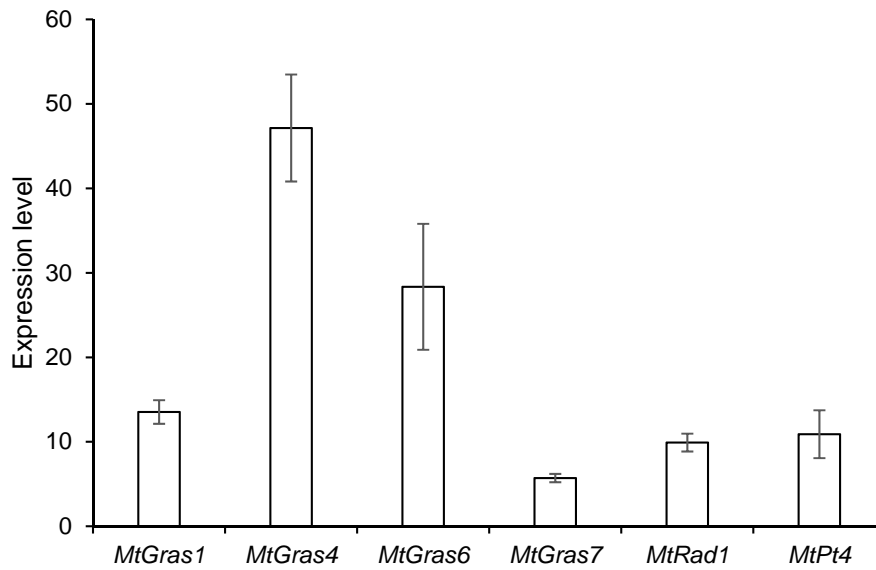


Figure S1: Expression level of selected *MtGras* genes, *MtRad1*, and *MtPt4* in non-mycorrhizal roots under phosphate limitation. Expression levels base on *Medicago* GeneChip hybridizations reported by Hoge Kamp *et al.* (2011) and are accessible via the *Medicago* Gene Expression Atlas (<https://mtgea.noble.org/v3/>) (Benedito *et al.*, 2008). Robust Multiarray Average (RMA) normalized expression levels of three biological replicates derived from plants grown under phosphate limiting conditions (20 μ M P) were averaged. n=3, the standard deviation is indicated.

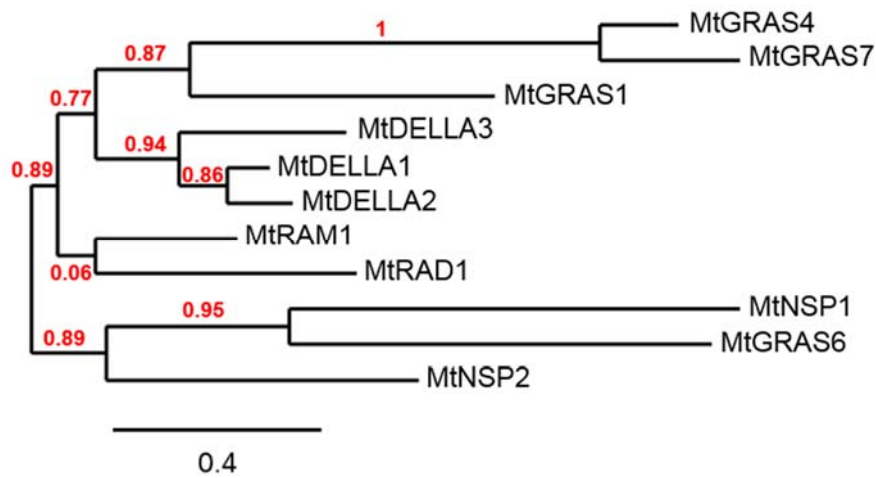


Figure S2: Phylogenetic analysis of selected GRAS TF sequences.

Analyses were performed on the phylogeny.fr platform (Dereeper *et al.*, 2008 and 2010). Sequences were aligned using MUSCLE (v3.8.31) (Edgar, 2004), gaps were removed by Gblocks (v0.91b) (Castresana, 2000), and trees were constructed using the maximum likelihood method in PhyML (v3.1/3.0 aLRT) (Guindon *et al.*, 2010). Bootstrap values are indicated. Except of the MtGras proteins listed in table 1, the GRAS TF sequences included MtRAM1 (Park *et al.*, 2015), MtRAD1 (Rey *et al.*, 2017), MtNSP1 (Delaux *et al.*, 2013), MtNSP2 (Maillet *et al.*, 2011), and the three MtDELLA TFs MtDELLA1, MtDELLA2, and MtDELLA3 (Floss *et al.*, 2013).

	Expression		Promoter activity	
	<i>ram1-1</i>	<i>pt4-2</i>	<i>ram1-1</i>	<i>pt4-2</i>
<i>MtGras1</i>	White	White	White	White
<i>MtGras4</i>	Grey	Grey	Blue	Blue
<i>MtGras6</i>	Grey	Grey	Blue	Blue
<i>MtGras7</i>	White	White	White	White
<i>MtRad1</i>	Grey	Grey	Blue	Blue

Figure S3: Summarized data on gene expression and promoter activities of GRAS TF genes in *M. truncatula ram1-1* and *pt4-2* mutants.

Significantly reduced (white) or unaffected (grey) transcript levels after *GiTefa*-normalization (Figures 2 and 4) as well as absent (white) or still detectable (blue) promoter activities (Figures 3 and 5) in *ram1-1* and *pt4-2* roots displaying a premature arbuscule degeneration phenotype.

WT

Tnt1

sWT

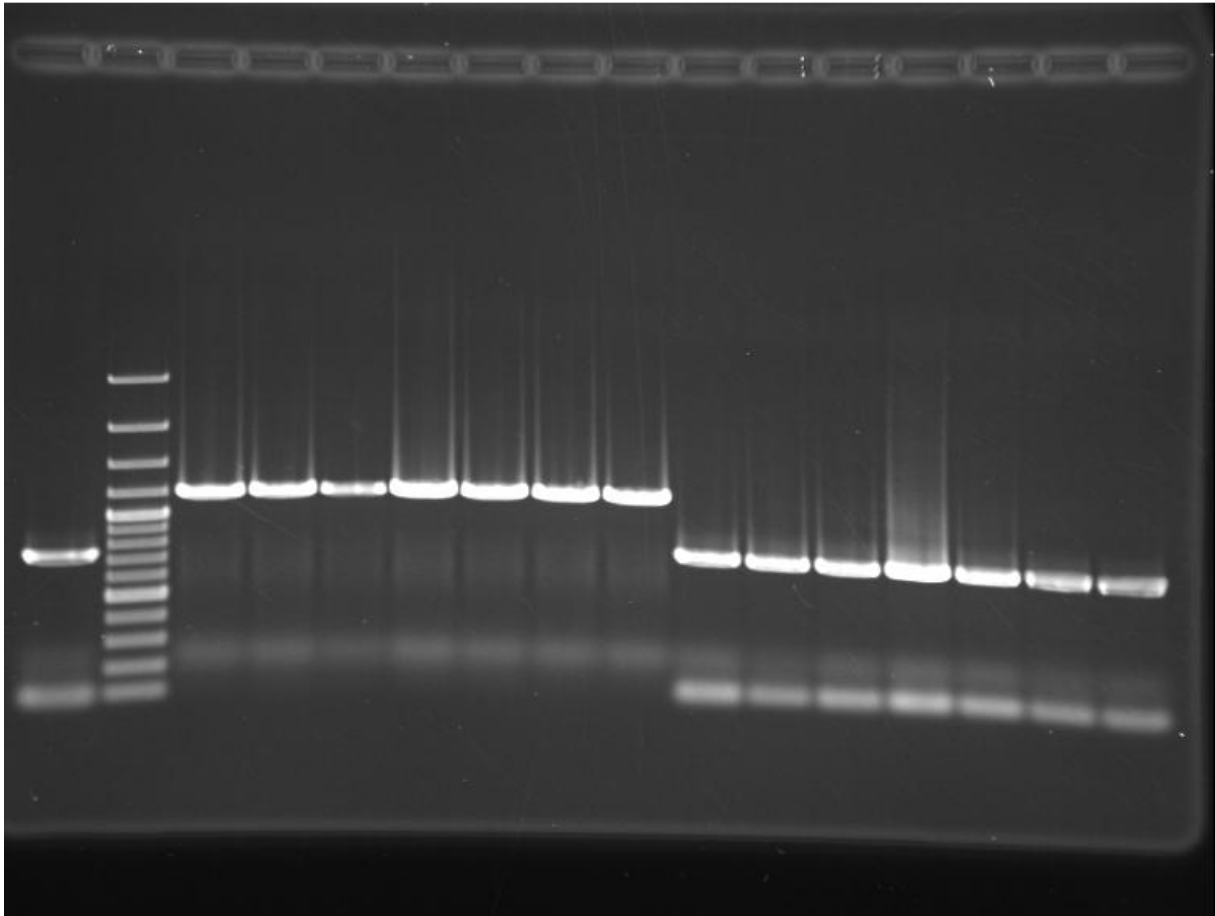


Figure S4: Original, uncropped image of the gel shown in Figure 6B.

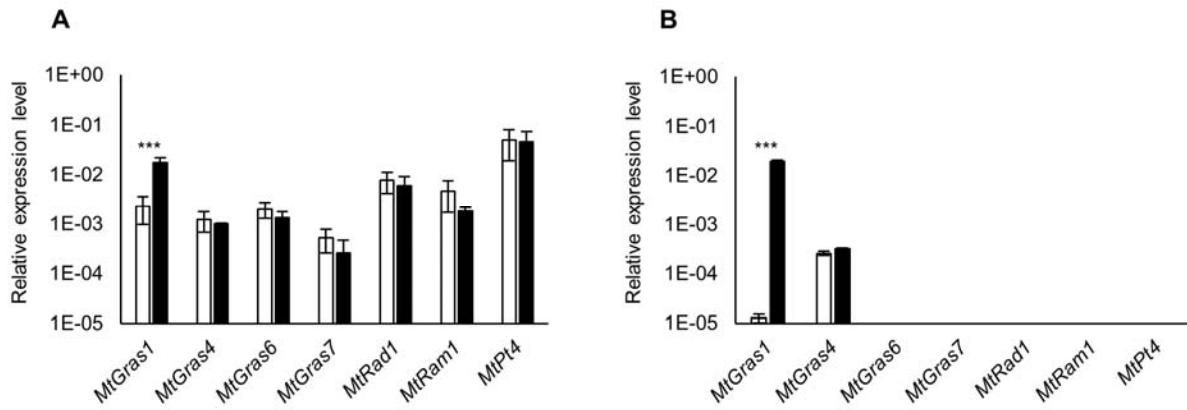


Figure S5: Relative expression of *MtGras1* and selected AM marker genes.

A Mycorrhizal (35 dpi) pPt4:*MtGras1* (black) vs. pPt4:*gusAint* (white) roots. **B** Uninoculated pUbi3:*MtGras1* (black) vs. pUbi3:*gusAint* (white) roots. Transcript amounts are shown relative to *MtTefα*. Roots were harvested at 35 days post inoculation with *R. irregularis*. n=4 biological replicates, error bars represent standard deviations.

Supplemental Table S1
Nomenclature of AM-related genes encoding GRAS TFs.

Gene	Genome ID	MtGEA ID	GRAS subgroup	Alternative IDs
<i>MtGras1</i>	Medtr3g022830	Mtr.7264.1.S1_at	GRASM1*/SCL3**	TF80***/MtGRAS24**
<i>MtGras4</i>	Medtr7g109580	Mtr.1484.1.S1_at	GRASM6*/MIG1****	MtGRAS59**
<i>MtGras6</i>	Medtr2g089100	Mtr.47463.1.S1_at	SHR**/****	MtGRAS12**
<i>MtGras7</i>	Medtr1g086970	Mtr.24642.1.S1_at	GRASM6*/MIG1/SCR**	MtGRAS4**
<i>MtRad1</i>	Medtr4g104020	Mtr.36004.1.S1_at	GRASM5*/Os4****/DELLA**	MtSymSCI3/MtGRAS43**
<i>MtRam1</i>	Medtr7g027190	Not represented	GRASM5*/DELLA**	MtGRAS54**

Identifiers (IDs) in the *Medicago truncatula* genome (Tang *et al.*, 2014), the Medicago Gene Expression Atlas (MtGEA; Benedito *et al.*, 2008), as well as subgroups and alternative identifiers from the literature are listed. *, Song *et al.* (2010); **, Zhang *et al.* (2017); ***, Park *et al.* (2015); ****, Heck *et al.* (2016).

Supplemental Table S4

Expression of AM marker genes in mycorrhizal *M. truncatula* A17 RNAi: *MtGras1* or *ram1-1* roots in comparison to control roots.

Transcript Cluster ID	Mt gene name	Mt genome 4.0 ID	Annotation	Wild type log2 intensity	ram1-1 log2 intensity	Fold change (wild type vs. ram1-1)	p-value (wild type vs. ram1-1)	gusAint control log2 intensity	RNAi: <i>MtGras1</i> log2 intensity	Fold Change (gusAint control vs. RNAi: <i>MtGras1</i>)	p-value (gusAint control vs. RNAi: <i>MtGras1</i>)
TC0100003992.mtr.1	<i>MtPt4</i>	Medtr1g028600.1	Phosphate transporter	12,1	2,6	688,8	0,000	12,3	11,4	1,9	0,108
TC0400004806.mtr.1	<i>MtGlp1</i>	Medtr4g052770.1	Germin-like protein	10,9	2,7	288,6	0,000	11,7	10,5	2,3	0,062
TC0700001334.mtr.1	<i>MtMyb1</i>	Medtr7g068600.1	Myb transcription factor	10,2	2,9	163,4	0,000	8,0	8,4	-1,3	0,818
TC0500000972.mtr.1	<i>MtLec5</i>	Medtr5g031030.1	Lectin	9,0	2,5	91,7	0,000	9,9	7,2	6,7	0,030
TC0100001222.mtr.1	<i>MtRam2</i>	Medtr1g040500.1	Glycerol-3-phosphate acyltransferase	10,3	4,0	78,6	0,000	10,3	9,0	2,4	0,099
TC0800000282.mtr.1	<i>MtDef2</i>	Medtr8g012835.1	Defensin	8,4	2,3	65,9	0,000	8,7	7,3	2,7	0,348
TC0800004220.mtr.1	<i>MtT1</i>	Medtr8g059790.1	Kunitz type trypsin inhibitor	8,9	2,9	64,1	0,000	8,4	8,1	1,3	0,363
TC0500005070.mtr.1	<i>MtGst1</i>	Medtr5g076900.1	Glutathione S-transferase	9,5	3,5	62,1	0,000	9,9	8,6	2,5	0,096
TC0800000278.mtr.1	<i>MtDef1</i>	Medtr8g012805.1	Defensin	8,8	2,9	59,3	0,000	8,8	7,0	3,3	0,240
TC0800002929.mtr.1	<i>MtHa1</i>	Medtr8g006790.1	Plasma membrane H+ATPase	9,6	4,5	34,9	0,000	10,1	8,6	2,9	0,038
TC0800002245.mtr.1	<i>MtNip1</i>	Medtr8g087710.1	Major intrinsic protein (MIP) transporter	9,5	4,5	31,5	0,000	8,5	8,3	1,1	0,950
TC0500003253.mtr.1	<i>MtSbtM1</i>	Medtr5g011320.1	Subtilisin-like serine protease	9,2	4,2	30,8	0,000	10,5	9,0	2,8	0,044
TC0500000966.mtr.1	<i>MtStr2</i>	Medtr5g030910.1	ABC transporter	8,9	4,0	29,1	0,000	9,6	7,7	3,7	0,059
TC0700000122.mtr.1	<i>MtErf1</i>	Medtr7g009410.1	ERF transcription factor	7,6	2,7	28,4	0,000	7,7	6,9	1,8	0,109
TC0800001689.mtr.1	<i>MtLec7</i>	Medtr8g068030.1	Lectin	9,7	5,3	20,0	0,000	11,1	9,4	3,2	0,028
TC0500004795.mtr.1	<i>MtPt8</i>	Medtr5g068140.1	Phosphate transporter	6,2	2,2	15,6	0,000	5,0	3,2	3,4	0,028
TC0700005059.mtr.1	<i>MtBcp1</i>	Medtr7g086190.1	Blue copper protein	7,8	4,6	8,9	0,001	9,3	7,7	3,0	0,033
TC0600000634.mtr.1	<i>MtVapyrin</i>	Medtr6g027840.1	Ankyrin repeat protein	9,0	5,9	8,1	0,000	9,4	7,7	3,1	0,080
TC0300005385.mtr.1	<i>MtScp1</i>	Medtr3g079590.1	Serine carboxypeptidase	10,6	7,9	6,8	0,001	11,8	10,3	2,9	0,032
TC0800003834.mtr.1	<i>MtAnn2</i>	Medtr8g038220.2	Annexin	7,6	5,2	5,4	0,000	8,5	7,2	2,5	0,059
TC0800003833.mtr.1	<i>MtAnn1</i>	Medtr8g038210.2	Annexin	6,8	4,7	4,2	0,000	7,6	7,0	1,5	0,203
TC0700002896.mtr.1	<i>MtAmt1</i>	Medtr7g115050.1	Ammonium transporter	4,8	3,0	3,4	0,002	6,4	4,4	3,9	0,022
TC0200005086.mtr.1	<i>MtCbf1</i>	Medtr2g081600.1	Nuclear transcription factor Y	4,6	2,9	3,2	0,001	6,4	4,2	4,6	0,119
TC0200005087.mtr.1	<i>MtCbf2</i>	Medtr2g081630.1	Nuclear transcription factor Y	4,8	3,3	2,8	0,007	6,6	4,2	5,3	0,160
TC0700003611.mtr.1	<i>MtRam1</i>	Medtr7g027190.1	GRAS transcription factor	8,1	2,7	43,2	0,000	8,9	6,9	4,2	0,042
TC0300003722.mtr.1	<i>MtGras1</i>	Medtr3g022830.1	GRAS transcription factor	6,9	2,7	17,4	0,000	6,5	4,7	3,4	0,004
TC0400002717.mtr.1	<i>MtRad1</i>	Medtr4g104020.1	GRAS transcription factor	7,2	4,4	6,7	0,001	7,9	4,9	8,0	0,035
TC0100005775.mtr.1	<i>MtGras7</i>	Medtr1g086970.1	GRAS transcription factor	4,6	2,6	3,9	0,002	4,2	3,3	1,9	0,044
TC0800003959.mtr.1	<i>MtTF124</i>	Medtr8g442410.1	GRAS transcription factor	6,2	5,4	1,8	0,002	7,2	5,4	3,5	0,027
TC0200002452.mtr.1	<i>MtGras6</i>	Medtr2g089100.1	GRAS transcription factor	5,7	4,4	2,6	0,002	6,8	4,7	4,3	0,041
TC0700005897.mtr.1	<i>MtGras4</i>	Medtr7g109580.1	GRAS transcription factor	4,5	3,3	2,3	0,004	4,9	4,9	1,0	0,653

Supplemental Table S5**Primers for cloning reporter gene, RNAi, overexpression fusions, and genotyping a *Tnt1* line.****Reporter gene fusions**

pMtGras1_for	AAAGCATGCAAGCGATGAAAGGGTGTGAA
pMtGras1_rev	AAACCCGGGGGTGATTTATCTTGAAGAATT
pMtGras4_for	AAAGAATTCCTTGAACCATTATTGGATTA
pMtGras4_rev	AAAAAGCTTATGACGGATTACAAGAGAAA
pMtGras6_for	AAAGCATGCCATTGTACGTAGAGTTCTGA
pMtGras6_rev	AAAAAGCTTGGTTGAAGGAGATTGGAAAA
pMtGras7_XhoI_for	AAACTCGAGCGAATATGCAAAATTGATCC
pMtGras7_HindIII_rev	AAAAGCTTGTTGTTATTGCAAAGAACTG
pMtRad1_for	AAAGAATTCTCATGAAATTGTTATACAAA
pMtRad1_rev	AAAAAGCTTTGACATATTAATTTCTCAATT

MtGras1 overexpression

MtGras1oex_for	AAAACCCGGGGATGGATTCAGGTTCCGCATAT
MtGras1oex_rev	AAAACCCGGGTCACCTAAATTTCCAGGCCGA

MtGras1 RNA interference

RNAi_Gras1_for	GGGGACAAGTTTGTACAAAAAAGCAGGCTGCTGAGGGAATTGAGATATG
RNAi_Gras1_rev	GGGGACCACTTTGTACAAGAAAGCTGGGTATCAATAATATGCACCACCT

Tnt1 genotyping

NF4813_16_for	AAGTGGGGTCCAGTGCATAG
NF4813_16_rev	TACATTGGAGTCCCCTTCCA
Tnt1-F	GCATTCAAAGTAGAAGACAGTGCTACC

Supplemental Table S6**Primers used in real-time RT-PCR experiments.**

Primer	NCBI accession	Sequence
MtGras1_for	XM_003598826	TTTAGGCCCTTACAAGATTAC
MtGras1_rev		CTTCACTGAAATAAGTGACCA
MtGras1_oex_for	XM_003598826	TGGATGTAATTTAACGGAGAG
MtGras1_oex_rev		CTTCACTGAAATAAGTGACCA
MtGras4_for	XM_003625927	AGGGTTAAGAAATATTGTTGC
MtGras4_rev		TAAACACATGCCTCACTAAAC
MtGras4_Tnt1_3'_for	XM_003625927	ATAGAATGGTGGAACTAGGT
MtGras4_Tnt1_3'_rev		CCAAGCTGAGATTGAATACAT
MtGras6_for	XM_003597004	AAGCTTTGGTTATAAATTGCC
MtGras6_rev		GTCCTATGTCAGATTCAAAC
MtGras7_for	XM_003591347	AAACCATAATTCACCTTTGTT
MtGras7_rev		CAGATAATATTGCTTCCATCA
MtRad1_for	XM_003608835	GTGAGGTAGATGAAAATCCAT
MtRad1_rev		GGATAGTTACGGTTGATCTTT
MtMyb1_for	XM_01359369	TACTGCCAAATTTCTGTTCTA
MtMyb1_rev		GGATTGTGTTTTAAAGGATTC
MtPt4_for	AY116211	TCGCGCGCCATGTTTGTTGT
MtPt4_rev		GCGAAGAAGAATGTTAGCCC
GiTefa_for	XM_003588773	ATCCCAAATTTGTTAAGTCTG
GiTefa_rev		ACAGCAACAGTCTGTCTCATA
Gi α -Tub_for	GW088233	TGTCCAACCGTTTTAAAGT
Gi α -Tub_rev		AAAGCACGTTTGCGTACAT
MtRam1_for	XM_003622047	CATTACTACTCCGCAATTTTC
MtRam1_rev		CAACAAACAACCTTTATCCTC

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