Rich et al., Plant Phys. 168, pp 788-797; Supplemental Materials

Supplemental Figures

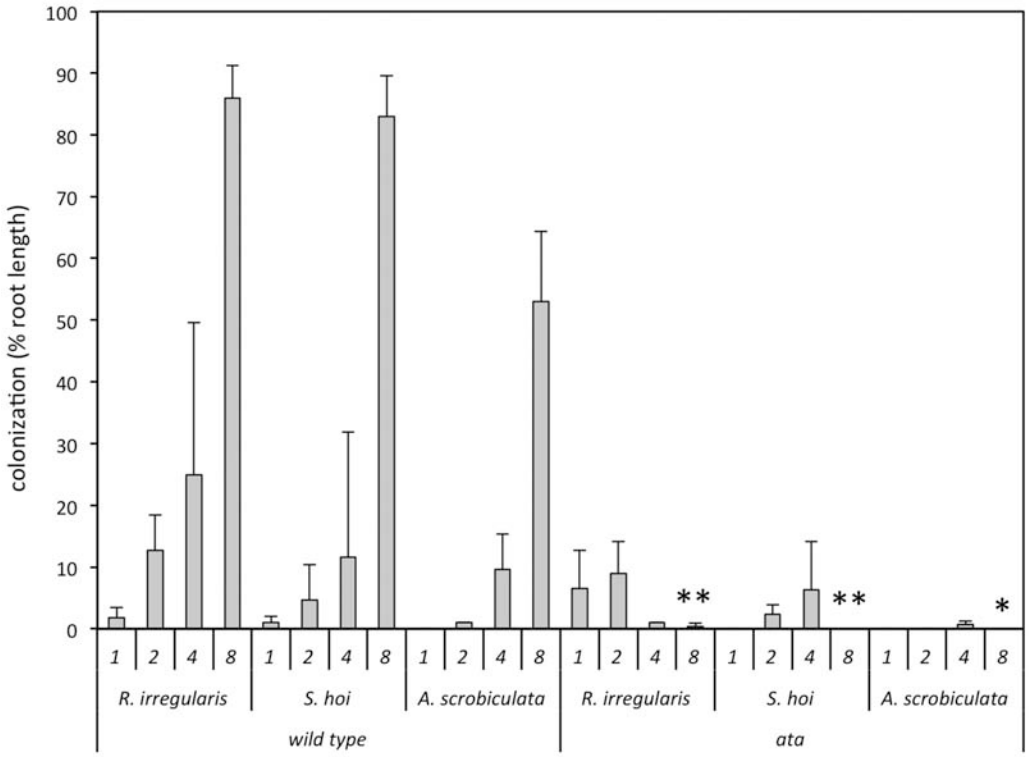

Fig. S1. AM-defective phenotype of *ata* mutant in combination with diverse AM fungi

AM root colonization of wild type (left) and *ata* mutant (right) inoculated with *Rhizophagus irregularis*, *Simiglomus hoi*, and *Acaulospora scrobiculata*. Total root colonization was assessed after 1, 2, 4, and 8 weeks. Columns represent the average of three biological replicates ± standard deviation. Significant differences (Student's t-test) between the mutant and the wild type are indicated with asterisk (*: $p<0.05$; **: $p<0.01$.

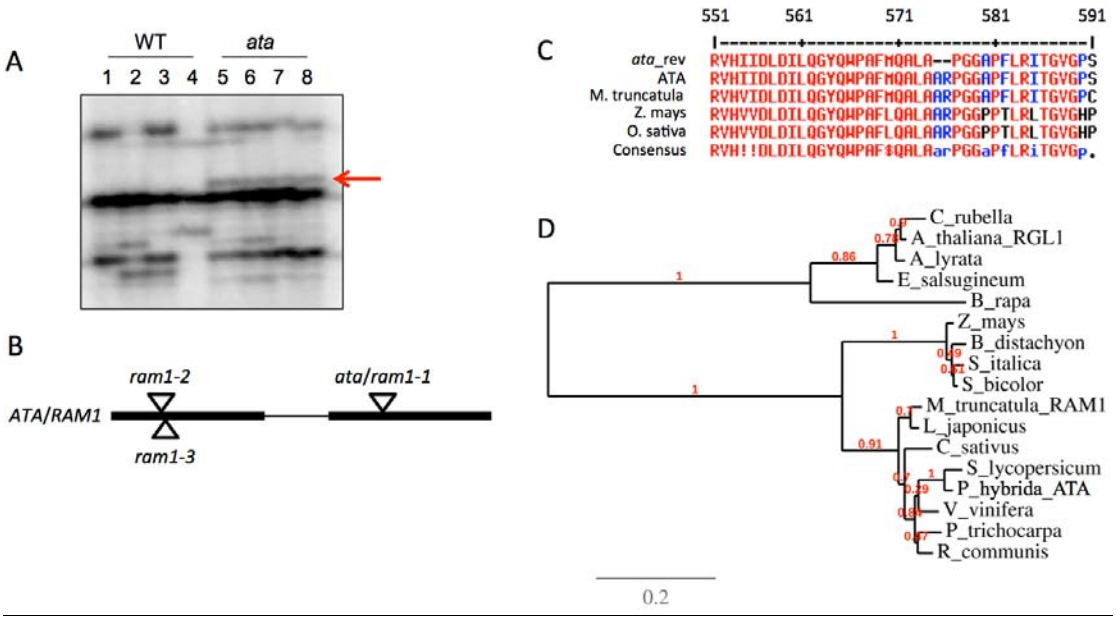

Fig. S2. *ATA* encodes a conserved GRAS transcription factor

(A) Identification of the *ata* mutant locus by transposon display. Red arrow indicates an amplicon that segregated perfectly with the mutant phenotype.

B) Map of the wild type *ATA*/*RAM1* locus and the three *ram1* insertion alleles. Thick lines represent exons, the thin line represents the single intron. Arrows indicate the insertion sites of *dTph1* in the alleles *ata*/*ram1-1*, *ram1-2*, and *ram1-3*, respectively.

C) Analysis of the revertant allele of *ata*/*ram1-1* in comparison with the respective sequence stretch of the wild type allele, as well as the closest homologues from *M. truncatula*, rice (*Oryza sativa*), and mayze (*Zea mays*) (compare with **Figure S3**). Note a deletion of 2 amino acid residues in the revertant allele (*ata*_rev) relative to the wild type allele (ATA).

D) Phylogram of the predicted ATA protein from *P. hybrida* and RAM1 from *M. truncatula* together with the closest homologs from various monocot (*Zea mays*, *Brachypodium distachyon*, *Setaria italica*, *Sorghum bicolor*), and dicot species (*Capsella rubella*, *Arabidopsis thaliana*, *Arabidopsis lyrata*, *Lotus japonicus*, *Cucumis sativus*, *Solanum lycopersicum*, *Vitis vinifera*, *Populus trichocarpa*, and *Ricinus communis*). The distance bar indicates the number of substitutions per site.

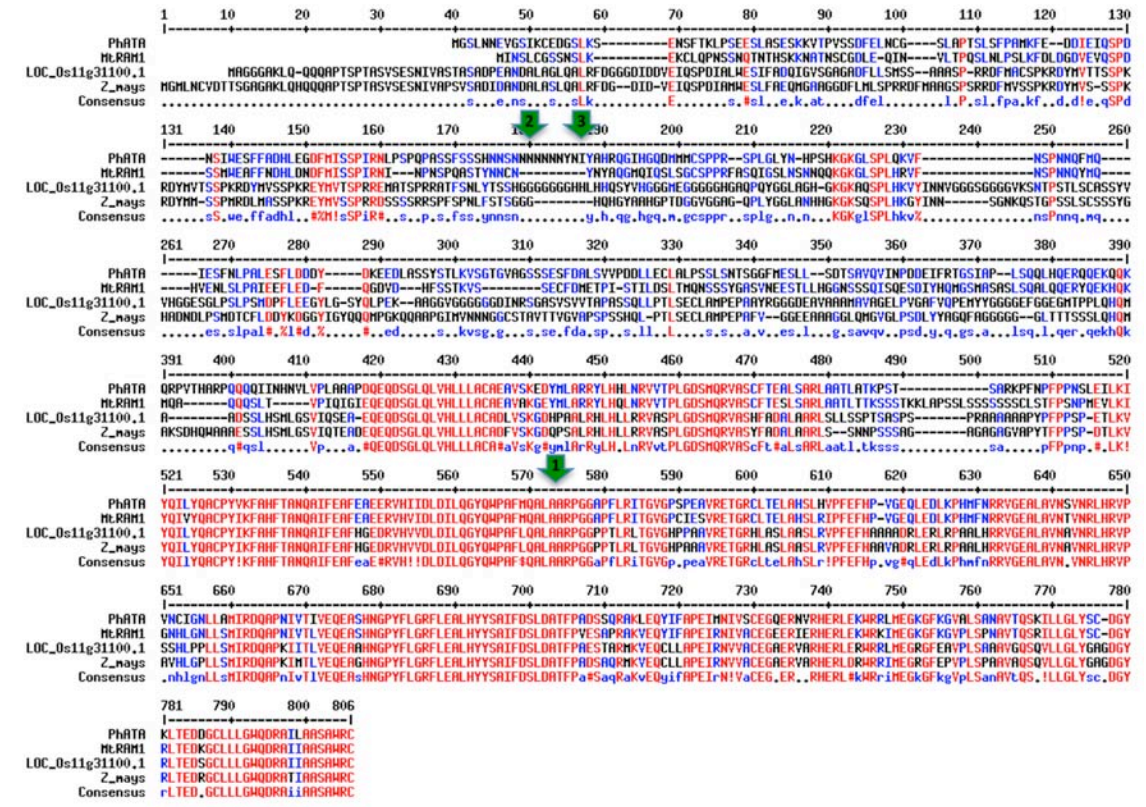

Fig. S3. The GRAS transcription factor encoded by *ATA*/*RAM1* and its closest homologs in *M. truncatula*, rice (*Oryza sativa*), and maize (*Zea mays*).

The predicted petunia ATA/RAM1 protein sequence was aligned with MtRAM1 and the closest homologs of rice (*Oryza sativa*), and mayze (*Zea mays*) using multalin (http://multalin.toulouse.inra.fr). Arrows indicate the insertion sites of *dTph1* in the alleles *ata*/*ram1-1* (arrow 1), *ram1-2* (arrow 2), and *ram1-3* (arrow 3), respectively.

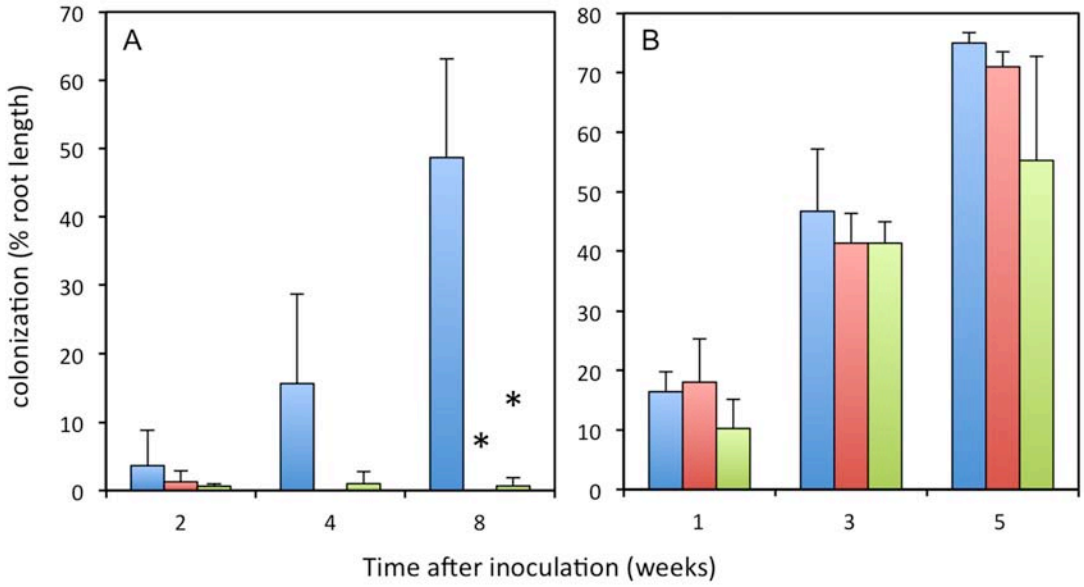

Fig. S4. Colonization of *ram1-2* and *ram1-3* by *R. irregularis*

A) Total root colonization in wild type *Petunia hybrida* (blue), *ram1-2* (red), and *ram1-3* (green), inoculated with *R. irregularis* spore inoculum.

B) Total root colonization in the wild type (blue), *ram1-2* (red), and *ram1-3* (green), colonized from nurse-plants with *R. irregularis*.

Columns represent the average of five biological replicates ± standard deviation. Significant differences (Student's t-test) between the mutant and the wild type are indicated with asterisk (*: $p<0.05$)

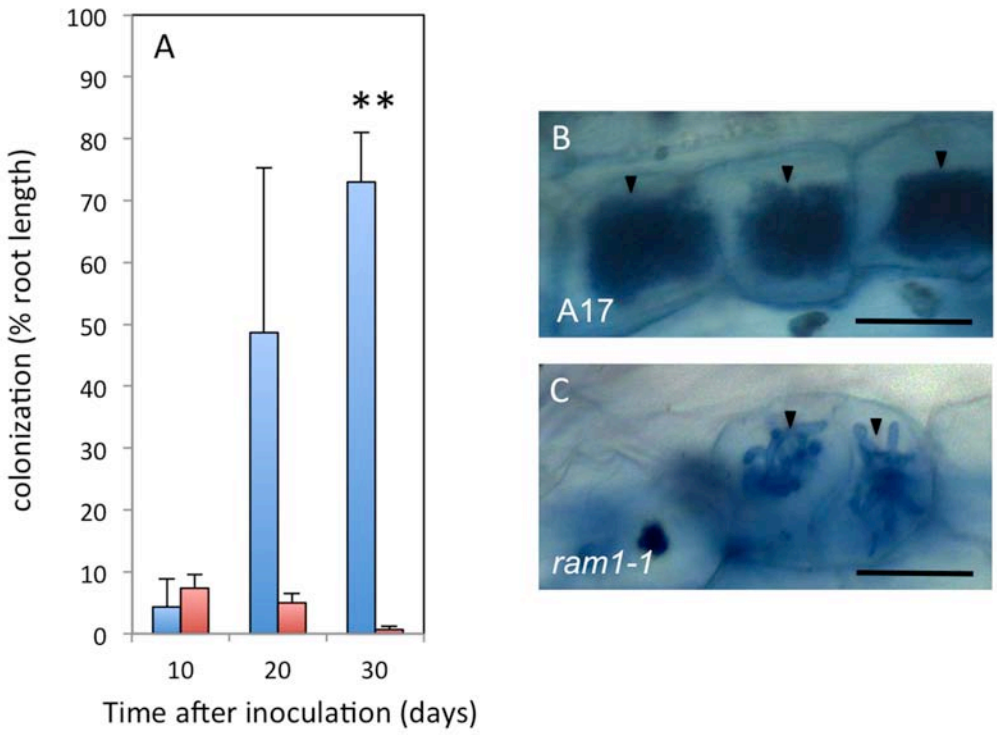

Fig. S5. AM-defective phenotype of *M. truncatula ram1-1*

A) Total root colonization of *R. irregularis* in *M. truncatula* wild type (Line A17; blue) and in *ram1-1* (red).

B) Wild type *M. truncatula* root (line A17) colonized by *R. irregularis*. Arbuscules are indicated by arrowheads.

C) Root of *M. truncatula ram1-1* mutant inoculated with *R. irregularis*. Note the aberrant arbuscules (arrowheads). Size bars: 50 μm in B,C.

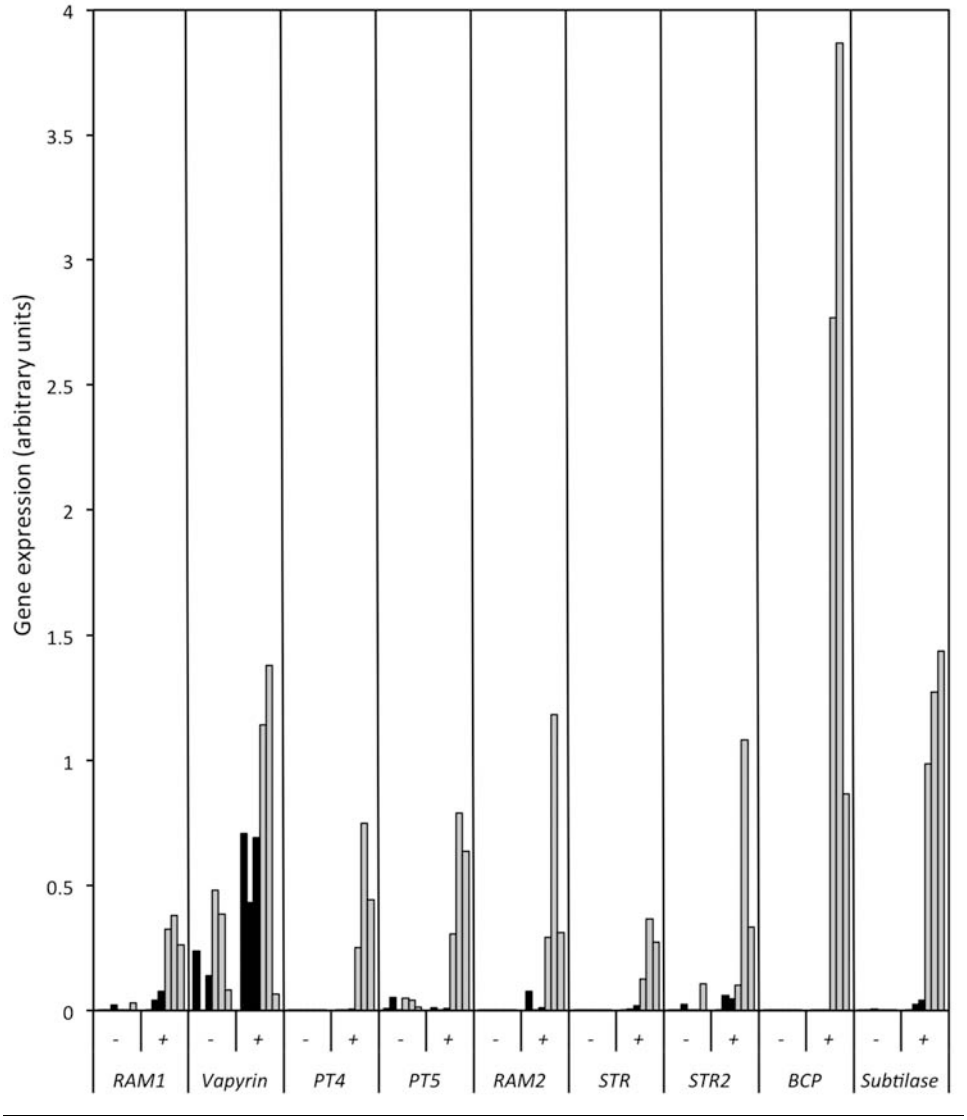

Fig. S6. Expression of AM marker genes in the *ata* mutant

Gene expression was determined by real time quantitative RT-PCR in non-mycorrhizal control roots (-), and in colonized roots (+) of the wild type (grey bars), and in *ata* mutants (black bars). Each bar represents an individual replicate plant.

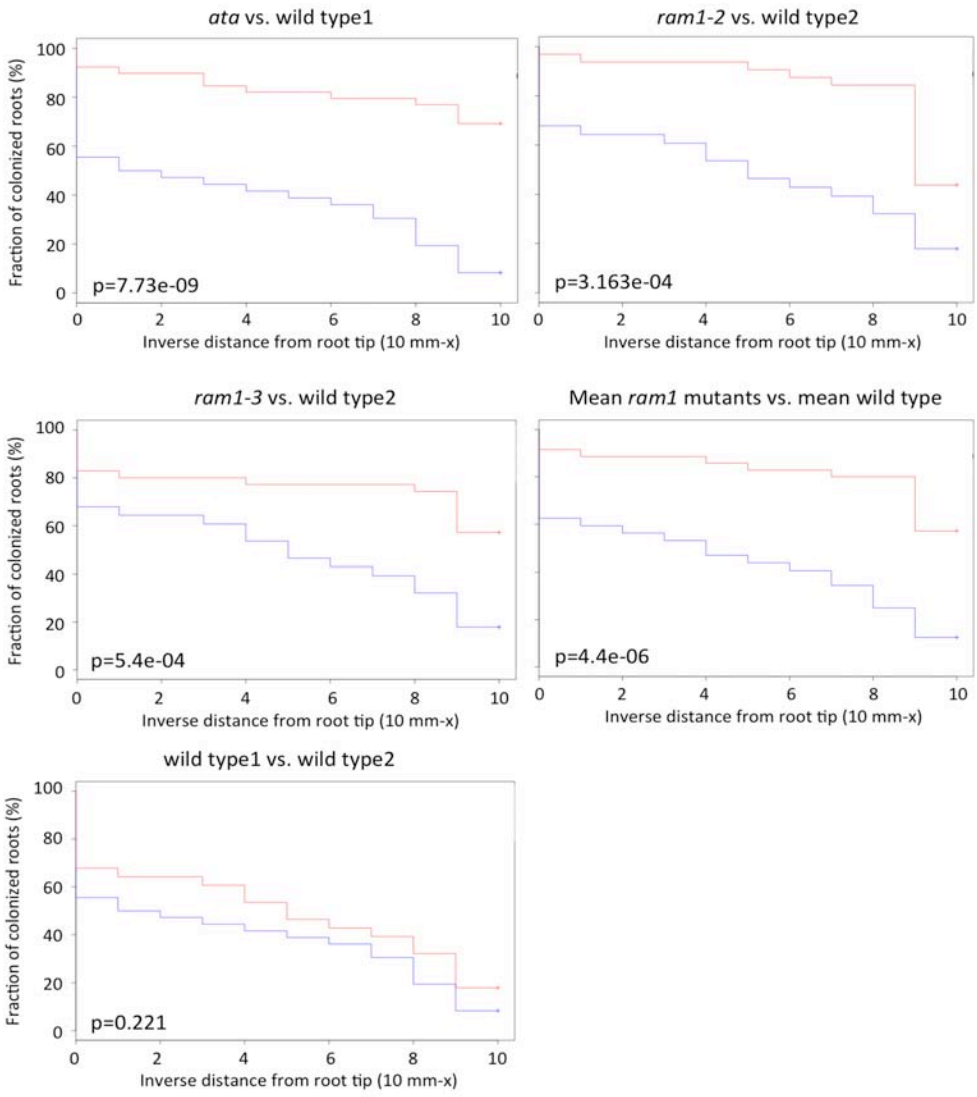

Fig. S7. Statistical analysis of fungal colonization of the root tip

The data shown in Figure 4D,E was transformed into survival curves. Log-rank test was performed to assess the significance of the difference between the curves. A p-value <0.05 is considered statistically significant. From top left to bottom right: *ata*/*ram1-1* vs. wt1, *ram1-2* vs. wt2, *ram1-3* vs. wt2, mean mutants vs. mean wt, wt1 vs wt2. Blue curves represent the wt, red curves represent the mutants. In (E), the blue curve represents wt1, the red curve wt2

	W115-a	*ata/ram1-1*			W115-b	*ram1-2*			*ram1-3*		
	expr.	expr.	fold less	p-value	expr.	expr.	fold less	p-value	expr.	fold less	p-value
RAM1	321,851	39,087	8	0.0022	373,127	75,857	5	0.0220	22,255	17	0.0081
Vapyrin	1,146,643	609,847	2	0.0290	1,327,435	652,040	2	0.2606	885,166	1	0.4145
PT4	481,117	2,078	231	0.0302	979,081	16,216	60	0.0042	2,009	487	0.0040
PT5	577,052	6,933	83	0.0161	706,846	43,167	16	0.0036	19,208	37	0.0032
RAM2	597,126	3,554	168	0.1136	683,175	11,277	61	0.0126	1,410	485	0.0119
STR	255,319	8,981	28	0.0247	161,650	10,121	16	0.0315	8,422	19	0.0298
STR2	505,049	35,359	14	0.1887	232,375	63	3718	0.0148	4,083	57	0.0158
BCP	2,501,280	593	4215	0.0463	2,800,029	67,416	42	0.0247	71,418	39	0.0248
Sbt	1,232,482	22,039	56	0.0008	4,642,693	42,972	108	0.0207	980	4,737	0.0201

Table S1. Expression of AM marker genes in the *ata*/*ram1* mutants

Expression of the indicated genes was analyzed in plants inoculated for 1 week with *R. irregularis* nurse-plant inoculum. Gene expression was analyzed in the wild type and the three petunia *ram1* alleles relative to GAPDH expression. Gene expression values represent the mean of three independent biological replicates. Comparison between the mutants and the respective wild type plants revealed to which degree gene expression was reduced due to the respective *ram1* mutation (fold less), and p-values (t-test) indicate significant differences.

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Gene	Primer name	Primer sequence
GAPDH	PhGAPDH-F3	GGAATCAACGGTTTTGGAAGAATTGGGCG
	PhGAPDH-R4	GGCCGTGGACACTGTCATACTTGAACA
RAM1	PhRAM1-F	ACTGGTGCACCTTCTCCTTG
	PhRAM1-R	GGGTCACGACACGATTCAG
RAM2	PhRAM2-F	GCCCATGAGCCAAGACAAG
	PhRAM2-R	CATGCCAAGAGGAAACCAAC
PT4	PhPT4-F	ATCTTTGCAGGGCTGGTTTC
	PhPT4-R	ATCTTTGCAGGGCTGGTTTC
PT5	PhPT5-F	ACATTTGTGCTCCCTGCTGA
	PhPT5-R	CTAAGAGCGTGGCATGTTGA
STR	PhSTR-F	CCAACGCGAAAGCTAATTCC
	PhSTR-R	TCACCTCTCAAGGCTTGTCC
STR2	PhSTR2-F	TGGTGAACGTCGTAGGGTCT
	PhSTR2-R	ACACTGTGAGCACTGGTGGA
Vapyrin	PhVapyrin-F	CATTGTGCAGTGGAATCTGG
	PhVapyrin-R	CTTTTGTTGCACCACCTTGC
Subtilase	Sbt_CL206	TTGGCAAGTCCAGGCATAGT
	Sbt_CL206	ATGTCAGATCGGTGCCAGTT
BCP	BCP_CL85_F	TGGAGGAGGAGCTATGCAGTT
	BCP_CL85-R	GGCTGGACCATTGATGTTGA

Table S2: Primers used for real time qPCR

R script for statistical analysis of fungal root tip colonization:
(see Figure 4 and Figure S7)

```
library(survival)
#small function to invert the results of counts into a survival curve
invert_curve<-function(a) {
j=a[1];
d=0;
e=1;
x=0;
b=0;
for (i in 1:length(a)) {
  for (k in e:j) {
    b[k]=length(a)-i;
    x[k]=d;
  }
  d=1;
  e=j+1;
  j=a[i+1];
}
return(list(time=b,status=x));
}

#mutant 566
wtdata=c(5,9,11,12,13,15,17,19,19,19,28)
wt=invert_curve(wtdata)
mutdata=c(14,27,28,28,30,30,31,31,31,31,32)
mut=invert_curve(mutdata)

fit1 <- survfit(Surv(wt$time, wt$status) ~ 1)
fit2 <- survfit(Surv(mut$time, mut$status) ~ 1)
plot(fit1,conf.int="none", col ='blue', xlab = 'Inverse root tip distance (10mm-x)', ylab
= '% presence of mycorrhize')
lines(fit2, conf.int="none",col = 'red')
```

```
legend(8,1,c('Group 1 (WT)', 'Group 2 (Mutant)'), col = c('blue','red'), lty = 1)
title(main='KM-Curves for Mycorrhize Data mutant 566')

wt$group = 1
mut$group = 2
wt.df=data.frame(wt)
mut.df=data.frame(mut)
data = rbind(wt.df, mut.df)
survdiff(Surv(time,status) ~ group, data=data)

#mutant 568
mutdata2=c(20,26,27,28,28,28,28,29,29,29,35)
mut=invert_curve(mutdata2)

fit1 <- survfit(Surv(wt$time, wt$status) ~ 1)
fit2 <- survfit(Surv(mut$time, mut$status) ~ 1)
plot(fit1,conf.int="none", col ='blue', xlab = 'Inverse root tip distance (10mm-x)', ylab
= '% presence of mycorrhize')
lines(fit2, conf.int="none",col = 'red')
legend(8,1,c('Group 1 (WT)', 'Group 2 (Mutant)'), col = c('blue','red'), lty = 1)
title(main='KM-Curves for Mycorrhize Data mutant 568')

wt$group = 1
mut$group = 2
wt.df=data.frame(wt)
mut.df=data.frame(mut)
data = rbind(wt.df, mut.df)
survdiff(Surv(time,status) ~ group, data=data)


#mutant ata
wtdata=c(3,7,11,13,14,15,16,17,18,20,36)
wt=invert_curve(wtdata)
mutdata3=c(27,31,31,32,32,33,33,35,36,36,39)
```

```
mut=invert_curve(mutdata3)

fit1 <- survfit(Surv(wt$time, wt$status) ~ 1)
fit2 <- survfit(Surv(mut$time, mut$status) ~ 1)
plot(fit1,conf.int="none", col ='blue', xlab = 'Inverse root tip distance (10mm-x)', ylab
= '% presence of mycorrhize')
lines(fit2, conf.int="none",col = 'red')
legend(8,1,c('Group 1 (WT)', 'Group 2 (Mutant)'), col = c('blue','red'), lty = 1)
title(main='KM-Curves for Mycorrhize Data mutant ata')

wt$group = 1
mut$group = 2
wt.df=data.frame(wt)
mut.df=data.frame(mut)
data = rbind(wt.df, mut.df)
survdiff(Surv(time,status) ~ group, data=data)

#mean values (3 mutants, 2 wt)
wtmean=c(4,8,11,13,14,15,17,18,19,20,32)
wt=invert_curve(wtmean)
mutmean=c(20,28,29,29,30,30,31,32,32,32,35)
mut=invert_curve(mutmean)

fit1 <- survfit(Surv(wt$time, wt$status) ~ 1)
fit2 <- survfit(Surv(mut$time, mut$status) ~ 1)
plot(fit1,conf.int="none", col ='blue', xlab = 'Inverse root tip distance (10mm-x)', ylab
= '% presence of mycorrhize')
lines(fit2, conf.int="none",col = 'red')
legend(8,1,c('Group 1 (WT)', 'Group 2 (Mutant)'), col = c('blue','red'), lty = 1)
title(main='KM-Curves for Mycorrhize Data mutant Mean values')

wt$group = 1
mut$group = 2
wt.df=data.frame(wt)
```

```
mut.df=data.frame(mut)
data = rbind(wt.df, mut.df)
survdiff(Surv(time,status) ~ group, data=data)


wtdata1=c(3,7,11,13,14,15,16,17,18,20,36)
wt=invert_curve(wtdata1)
wtdata2=c(5,9,11,12,13,15,17,19,19,19,28)
mut=invert_curve(wtdata2)

fit1 <- survfit(Surv(wt$time, wt$status) ~ 1)
fit2 <- survfit(Surv(mut$time, mut$status) ~ 1)
plot(fit1,conf.int="none", col ='blue', xlab = 'Inverse root tip distance (10mm-x)', ylab
= '% presence of mycorrhize')
lines(fit2, conf.int="none",col = 'red')
legend(8,1,c('Group 1 (WT)', 'Group 2 (Mutant)'), col = c('blue','red'), lty = 1)
title(main='KM-Curves for Mycorrhize Data wt1 vs wt2 comparison')

wt$group = 1
mut$group = 2
wt.df=data.frame(wt)
mut.df=data.frame(mut)
data = rbind(wt.df, mut.df)
survdiff(Surv(time,status) ~ group, data=data)
```