Supporting Information

Ivanov et al. 10.1073/pnas.1200407109

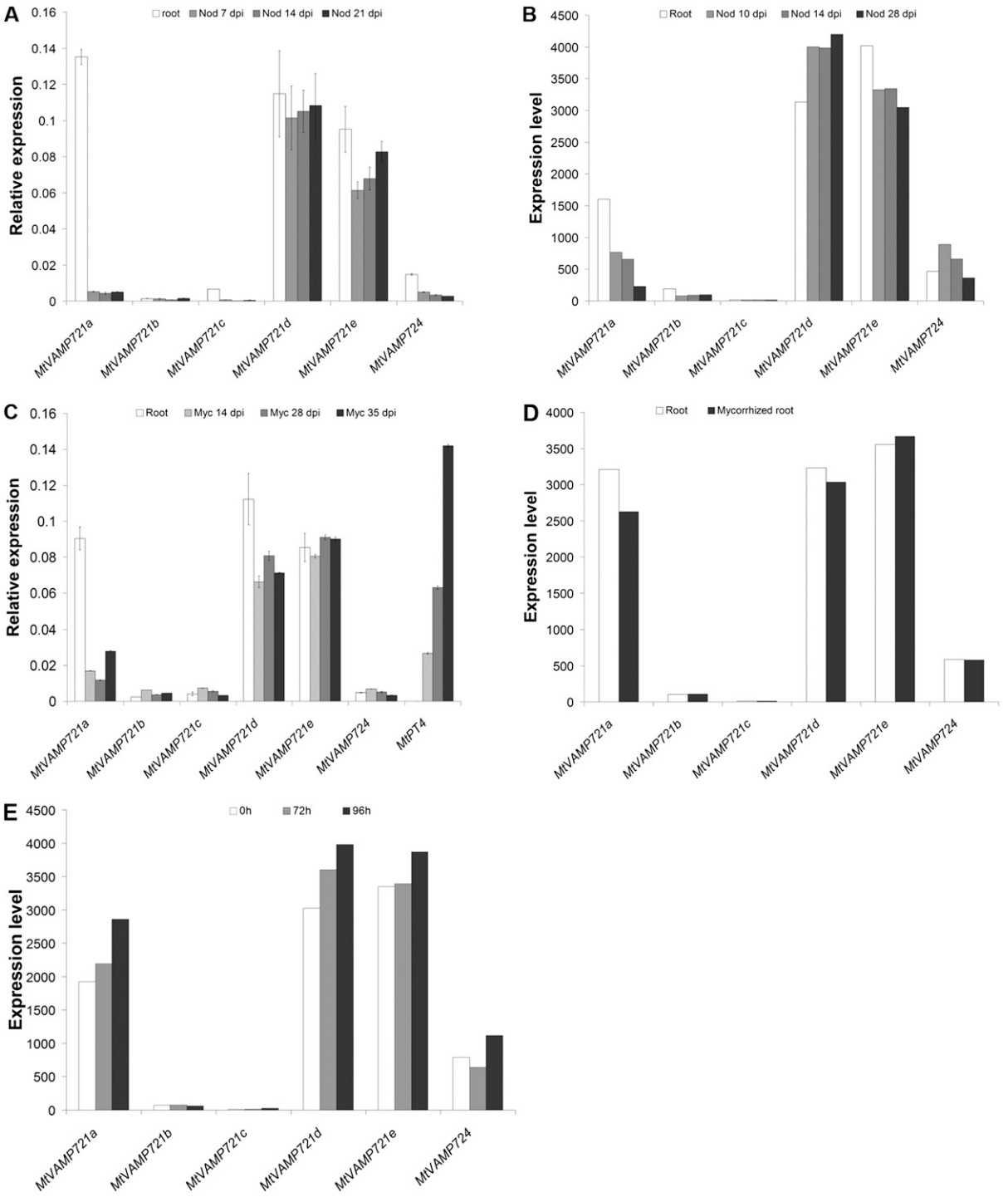

Fig. S1. Expression profile of *MtVAMP72s*. (*A and C*) Quantitative RT-PCR (qRT-PCR) profile of *MtVAMP72s*. qRT-PCR was conducted on RNA isolated from roots and nodules (Nod) 7, 14, and 21 d postinoculation (dpi) with *Sinorhizobium meliloti* 2011 (*A*) and roots colonized by *Glomus intraradices* 14, 28, and 35 dpi. *MtVAMP72s* gene-expression profiles were normalized against transcription level of reference gene *MtUBQ10*. Values represent means of triplicate runs on two independent biological samples. Error bars indicate SDs. This shows that *MtVAMP721d* and *MtVAMP721e* are highly expressed in root nodules. (*B, D*, and *E*) Gene-expression profile of *MtVAMP72s* based on *Medicago truncatula* Gene Expression Atlas data (http://mtgea.noble.org/v2/) in nodules 10, 14, and 28 dpi (*B*), mycorrhized roots (*D*) and roots 72 and 96 h after infection by pathogenic fungus *Phymatotrichopsis omnivore* (Phymatotrichum) (*E*). Note, that none of the MtVAMP721 homologs show a striking transcriptional regulation upon infection by *P. omnivore*, although the nonsymbiotic *MtVAMP721a* appears to be slightly induced.

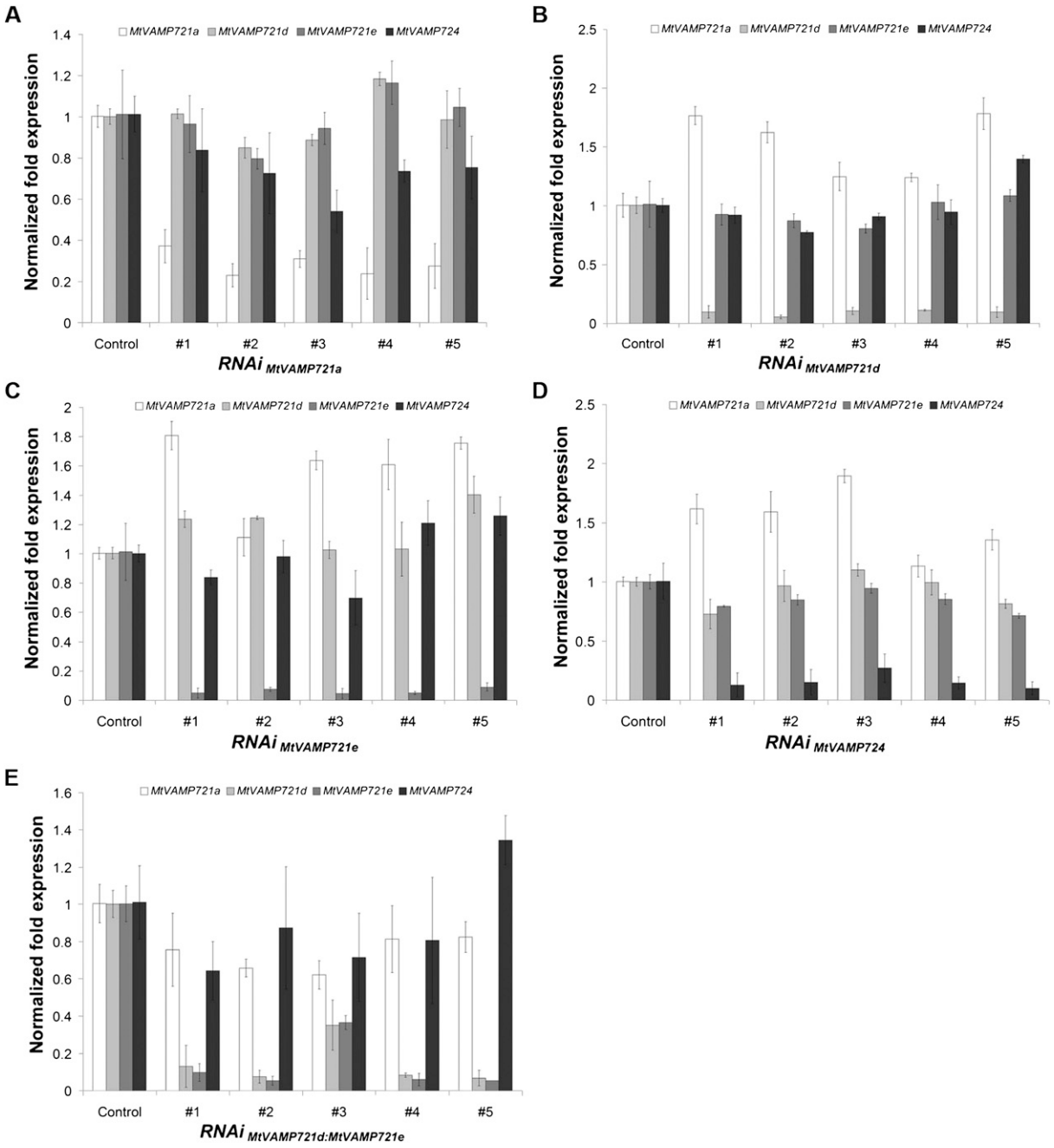

Fig. S2. qRT-PCR of gene-specific RNA interference. Total RNA was extracted independently from five transgenic roots expressing $RNAi_{MtVAMP721a}$ (*A*), $RNAi_{MtVAMP721d}$ (*B*), $RNAi_{MtVAMP721e}$ (*C*), $RNAi_{MtVAMP724}$ (*D*), $RNAi_{VAMP721d:VAMP721e}$ (*E*). Equal amounts of total RNA was subjected to qRT-PCR using gene-specific primers and the expression level of corresponding genes was measured relative to their expression in roots transformed with the empty control vector. *MtUBQ10* transcript level was used as reference. The mean of three independent control roots transformed with an empty vector is shown. Error bars indicate SDs.

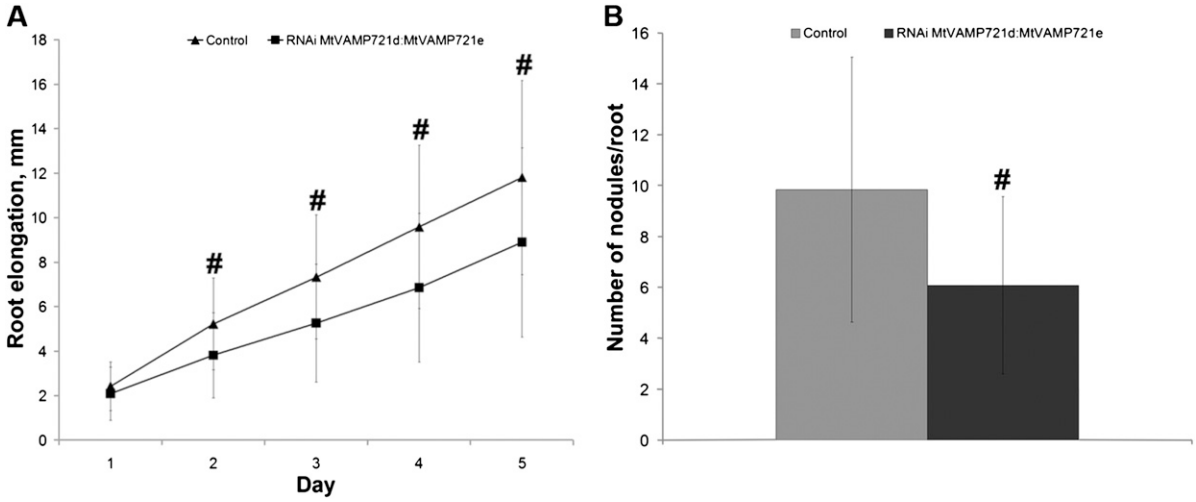

Fig. S3. Effect of $RNAi_{VAMP721d:VAMP721e}$ on root growth and number of nodules. (*A*) Root elongation of $RNAi_{VAMP721d:VAMP721e}$ transgenic roots (n = 20) was measured in 24-h intervals during 5 d and compared with root elongation of transgenic roots expressing an empty vector control (n = 20). "#" indicates statistically significant difference (day 1, P = 0.222; day 2, P = 0.008; day 3, P = 0.005; day 4, P = 0.005; day 5, P = 0.016). (*B*) Nodule number was counted on each $RNAi_{VAMP721d:VAMP721e}$ transgenic root (n = 15) and compared with transgenic control roots (n = 15). "#" indicates statistically significant difference (P = 0.05). ANOVA test was used for statistical analysis.

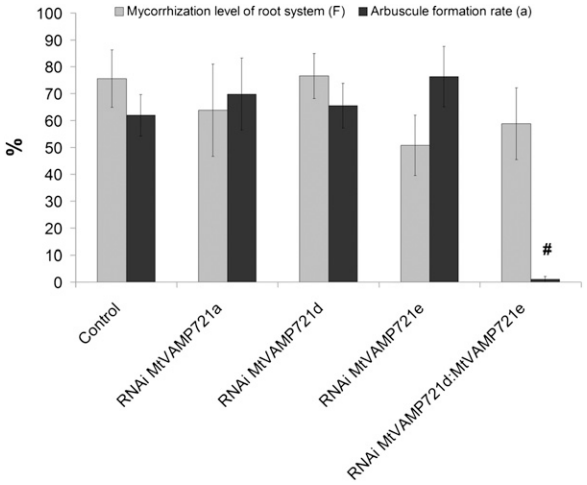

Fig. S4. Arbuscule formation is compromised in $RNAi_{MtVAMP721d:MtVAMP721e}$ transgenic roots. The *G. intraradices* mycorrhization level of the root system (parameter *M*) is equal in control and $RNAi_{MtVAMP721d:MtVAMP721e}$ roots (n = 5, P = 0.06). However, mature arbuscule abundance (parameter *a*) in $RNAi_{MtVAMP721d:MtVAMP721e}$ roots is significantly decreased (n = 5, P = <0.001, "#" indicates statistically significant difference). There was no difference in parameter *a* between control and $RNAi_{MtVAMP721a}$ (n = 5, P = 0.249 and P = 0.302), $RNAi_{MtVAMP721d}$ (n = 5, P = 0.883 and P = 0.498), or $RNAi_{MtVAMP721e}$ (n = 5, P = 0.529 and P = 0.629). Transgenic roots were harvested from composite plants 4 wk after *G. intraradices* inoculation, stained by Trypan blue, and analyzed by light microscopy. Seventy-five centimeters of each transgenic root system was analyzed. Error bars indicate SD. ANOVA test was used for statistical analysis.

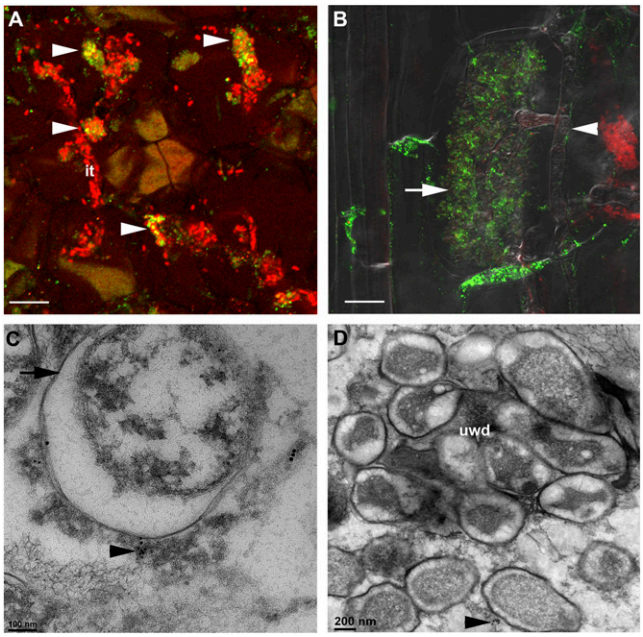

Fig. S5. Localization of MtVAMP721d and MtVAMP721e vesicles at the site of bacterial release and near the symbiosome and periarbuscular membrane. (*A*) MtVAMP721d-positive vesicles accumulate at local regions (arrowheads) of infection threads (*it*). Composite plants with transgenic roots expressing *VAMP721d::GFP-VAMP721d* were inoculated with *S. meliloti* 2011 constitutively expressing RFP. Root nodules were hand-sectioned, exposed to anti-GFP antibodies and secondary antibodies coupled with Alexa488 and analyzed by confocal microscopy. (*B*) Confocal immunolocalization of VAMP721d/e using the anti-VAMP721d/VAMP721e antibody on *Medicago* wild-type root infected by *G. intraradices*. Signal from anti-VAMP721d/VAMP721e antibodies is localized near the fine branches (arrow) of mature arbuscule and absence on intraradical hypha (arrowhead). Root was hand-sectioned and exposed to anti-VAMP721d/VAMP721e antibodies and secondary antibodies coupled with Alexa488. Note the markedly low signal in noninfected cells. (*C* and *D*) GFP-MtVAMP721d and GFP-MtVAMP721e vesicles fuse with the symbiosome membrane (arrow) of young symbiosomes (*C*) and near the site of bacteria release (*D*). GFP-VAMP721e is visualized by EM immunogold detection on nodules expressing either *VAMP721e::GFP-VAMP721e* (*C*) or *VAMP721d::GFP-VAMP721d* (*D*) using anti-GFP antibodies (10 nm) and anti-VAMP721d/VAMP721e antibodies (15 nm). White arrowhead, gold-labeled vesicle in contact with the symbiosome membrane. This shows that VAMP721d-positive vesicles accumulate at the region of the infection thread where an unwalled droplet (*uwd*) is formed and bacteria are released (*D*). (Scale bars, 10 μm in *A* and *B*, 100 nm in *C*, 200 nm in *D*.)

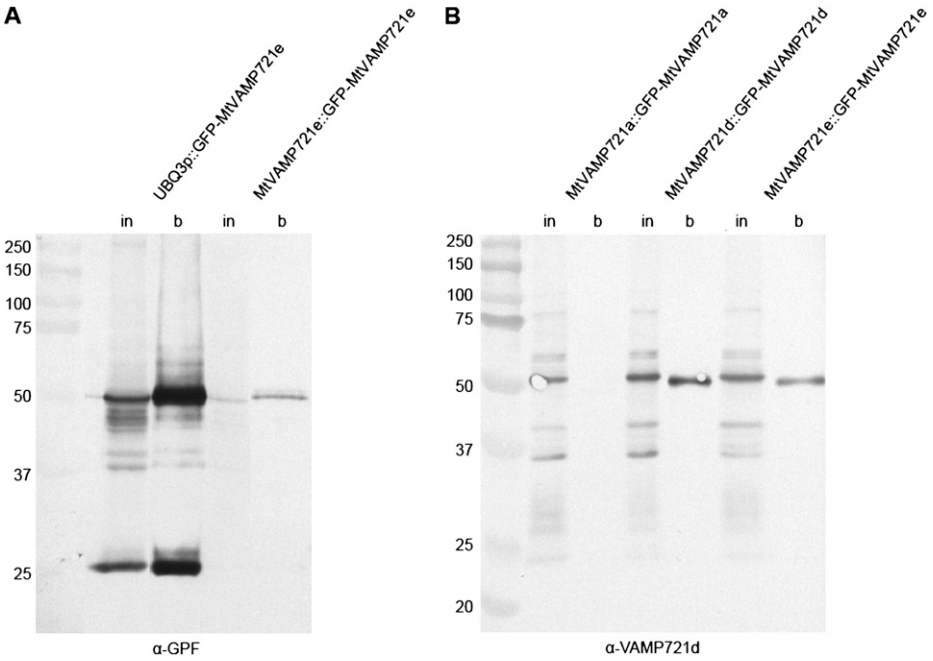

Fig. S6. (*A*) MtVAMP721e is present at low level in plant tissue. Total protein fractions were extracted from transgenic roots expressing GFP fusions of *VAMP721e* under control of the constitutive *Arabidopsis UBQ3* or native 2.5-kb promoters and used for immunoprecipitation using anti-GFP coated agarose beads. Equal amount of crude extract (*in*) and bound fraction (*b*) were subjected to immunoblot and detected using anti-GFP antibody. The fusion proteins of the predicted size (53.5 kDa) are detected. Note the presence of free GFP (27 kDa) in *UBQ3::GFP-MtVAMP721e*-expressing lines. (*B*) Specific antibodies raised against MtVAMP721d cross-react with VAMP721e. Total protein fractions were extracted from transgenic roots expressing GFP fusions of *VAMP721a*, *VAMP721d*, or *VAMP721e* under control of the constitutive *Arabidopsis UBQ3* promoter and used for immunoprecipitation using anti-GFP coated agarose beads. Precipitated proteins were subjected to immunoblot and detected using anti-MtVAMP721d antibody. The antibody against MtVAMP721d cross-reacts with VAMP721e; however, it does not react with VAMP721a.

Table S1. Medicago *VAMP72* genes

Gene name	Corresponding gene locus or TC number
MtVAMP721a	Medtr4g023810*, TC95333
MtVAMP721b	Medtr7g064880
MtVAMP721c	Medtr7g064860, TC171206
MtVAMP721d	Medtr2g034380, TC106930
MtVAMP721e	Medtr4g114750, TC106931
MtVAMP724	TC110430
MtVAMP727	AC233577_38

*Accession numbers are presented according to Phytozome database (http://www.phytozome.net/).

Table S2. Gene sequences used in phylogenetic analysis

Gene name	Corresponding gene locus
GmVAMP72a	Glyma07g34900*
GmVAMP72b	Glyma07g37760
GmVAMP72c	Glyma08g47040
GmVAMP72d	Glyma09g02310
GmVAMP72e	Glyma09g05070
GmVAMP72f	Glyma10g24430
GmVAMP72g	Glyma10g42480
GmVAMP72h	Glyma15g13220
GmVAMP72i	Glyma15g15760
GmVAMP72j	Glyma17g02870
GmVAMP72k	Glyma18g37970
GmVAMP72l	Glyma18g38010
GmVAMP72m	Glyma20g02720
GmVAMP72n	Glyma20g18860
GmVAMP72o	Glyma20g24540
VvVAMP72a	GSVIVG01000524001
VvVAMP72b	GSVIVG01010710001
VvVAMP72c	GSVIVG01012100001
VvVAMP72d	GSVIVG01017751001
VvVAMP72e	GSVIVG01028579001
PtVAMP72a	POPTR_1s14430
PtVAMP72b	POPTR_2s24200
PtVAMP72c	POPTR_3s17620
PtVAMP72d	POPTR_8s02030
PtVAMP72e	POPTR_10s24630
PtVAMP72f	POPTR_12s12000
PtVAMP72g	POPTR_15s15690
PtVAMP72i	POPTR_34s00330
AtVAMP721	AT1G04750†
AtVAMP722	AT2G33120
AtVAMP723	AT2G33110
AtVAMP724	AT4G15780
AtVAMP725	AT2G32670
AtVAMP726	AT1G04760
AtVAMP727	AT3G54300
SlVAMP72a	SGN-U572420‡
SlVAMP72b	SGN-U572423
SlVAMP72c	SGN-U572424

*Accession numbers are presented according to Phytozome database (http://www.phytozome.net/).

†Accession numbers are presented according to *Arabidopsis* Information Resource (TAIR, http://www.arabidopsis.org/).

‡Accession numbers are presented according to Sol Genomics Network (http://solgenomics.net/organism/1/view).

Table S3. Oligonucleotides used in a study

Name	Sequence (5′→3′)
For quantitative PCR	
MtVAMP721a-F	CTGTGTGCCATGGCTTCAGTTGTTA
MtVAMP721a-R	GCATCCTACCACACCTTATTCACTTCC
MtVAMP721b-F	CGCTGAATACACCGAGTTCA
MtVAMP721b-R	GTCCAGCAGACTCAACAGCA
MtVAMP721c-F	CGCCCATGATGGATTTACTT
MtVAMP721c-R	CTCCTTCAATTTCGGTCCAA
MtVAMP721d-F	TGTGGCTGCAAAACATGAAGGTAAA
MtVAMP721d-R	TGGAATAACAATAAAGGCCACAGAGAA
MtVAMP721e-F	GATCACCCGGAGGAGGTGAGTAAG
MtVAMP721e-R	GCCACATTTTTCTGCGGATTTTG
MtVAMP724-F	AGATAGATGCAAAACAACAACACGAAGC
MtVAMP724-R	GAGCTGCAATGGCAGGGGAAGTTAC
MtVAMP727-F	GATCGTGGGGAGAAGATTGA
MtVAMP727-R	AACATTTGAAACCCCCACAA
To generate DNA fragments for RNA interference	
MtVAMP721a-F	**CACC**GTGTGCCATGGCTTCAGTTGTTA
MtVAMP721a-R	ACATTATGCATCCTACCACACCTTATTCA
MtVAMP721d-F	**CACC**GACTCGGGGATAATAAGCACCATTC
MtVAMP721d-R	GAATGGAAACCAAACTTCAAACAGACA
MtVAMP721e-F	**CACC**CCTTAAGAATAAATAAACGCCACTCTCG
MtVAMP721e-R	TAGAAGCATTAGTATATCATCATCACCATCA
MtVAMP724-F	**CACC**TGCGGTGGATTTAACTGTTCAA
MtVAMP724-R	CATCCAATCATACTTTCACCATCTTCA
MtVAMP721d,e -F	AGTTTGGTTTCCATTCCCTTAAGAATAAATA
MtVAMP721d,e -R	TATTTATTCTTAAGGGAATGGAAACCAAACT
To generate DNA fragments of promoter regions	
MtVAMP721a-F	**CACC**AAGCTTTCCAGTGCAAGCTGGTCA
MtVAMP721a-R	ACTAGTGAATGATCACAATTCACAACTCTC
MtVAMP721d-F	**CACC**AAGCTTTTTATGCCAAACAAGAGCATC
MtVAMP721d-R	ACTAGTTGAAGAAGAGATCTGAGAATGGT
MtVAMP721e-F	**CACC**ATATGATCACAAAGACACAACCACA
MtVAMP721e-R	CTTCTTCTCCACAGATCTATCGAAC
To generate coding sequences of gene of interest	
MtVAMP721a-F	**CACC**ATGGGACAACAATCATTGATCTATAGCTTTG
MtVAMP721a-R	TCCTACCACACCTTATTCACTTCCCTTCC
MtVAMP721d-F	**CACC**ATGGCGAACAACCAGAATCAGAAG
MtVAMP721d-R	GATAATCACAAGGTTGGAATAACAATAAAG
MtVAMP721e-F	**CACC**ATGGGACAGAACCAAAAATCTCTGA
MtVAMP721e-R	CCTCATCATCATCATCATATAATAATCACA

Sequences designated in boldface are added to forward primer for TOPO cloning.