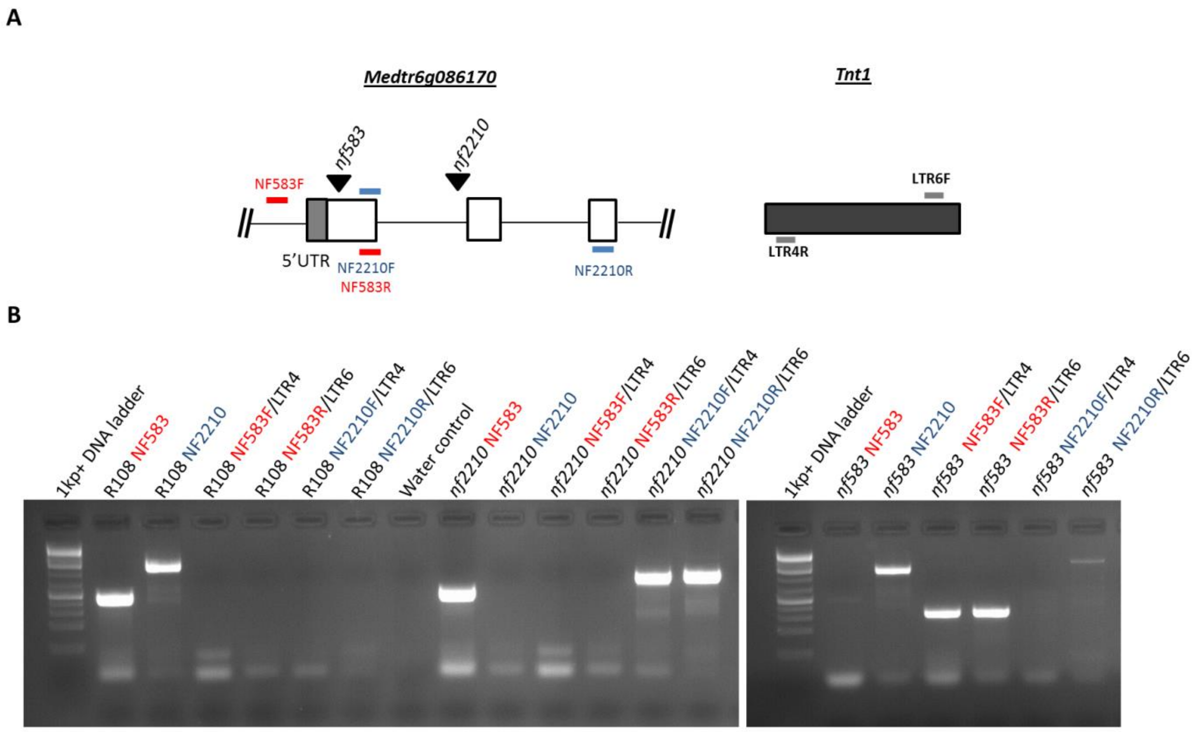
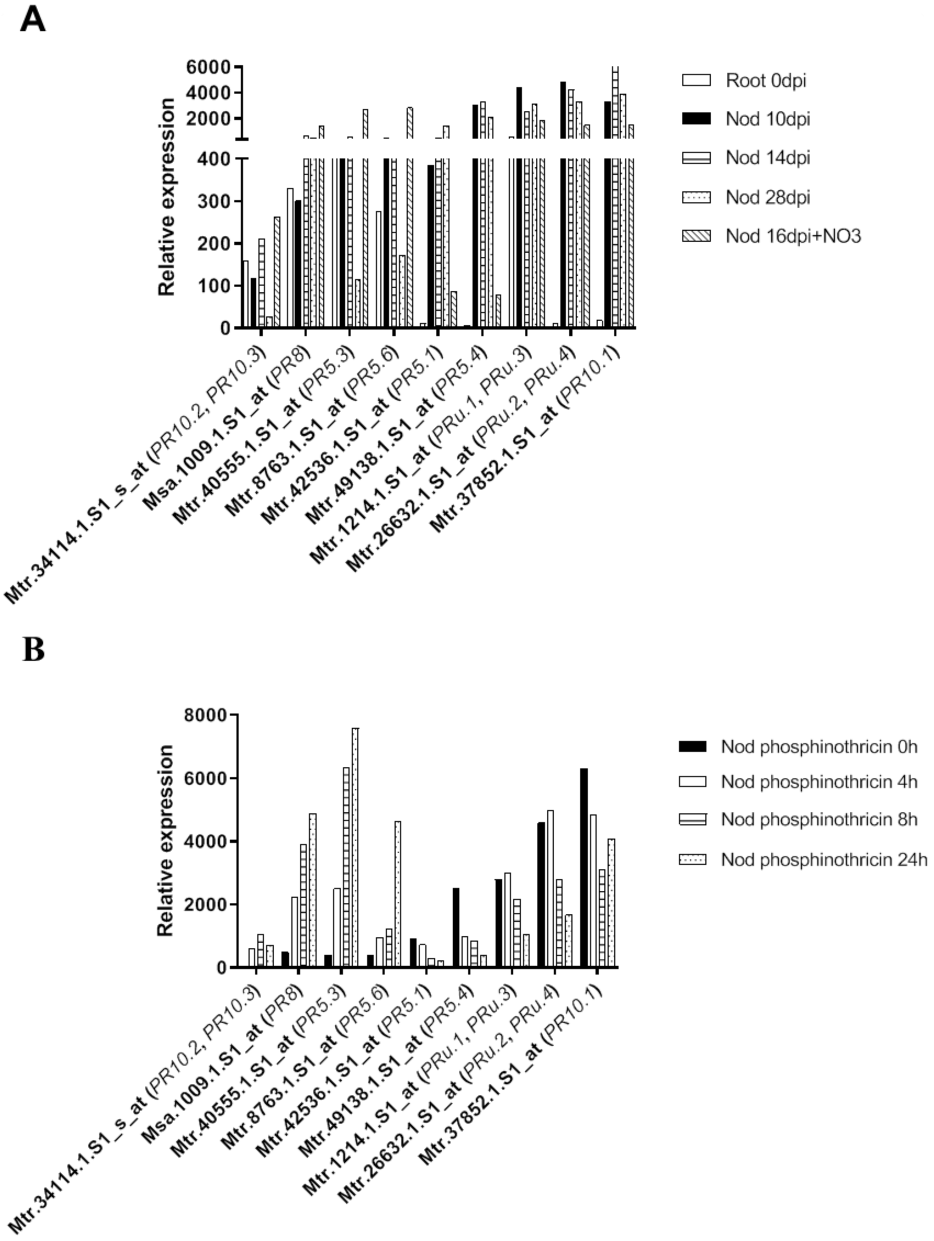


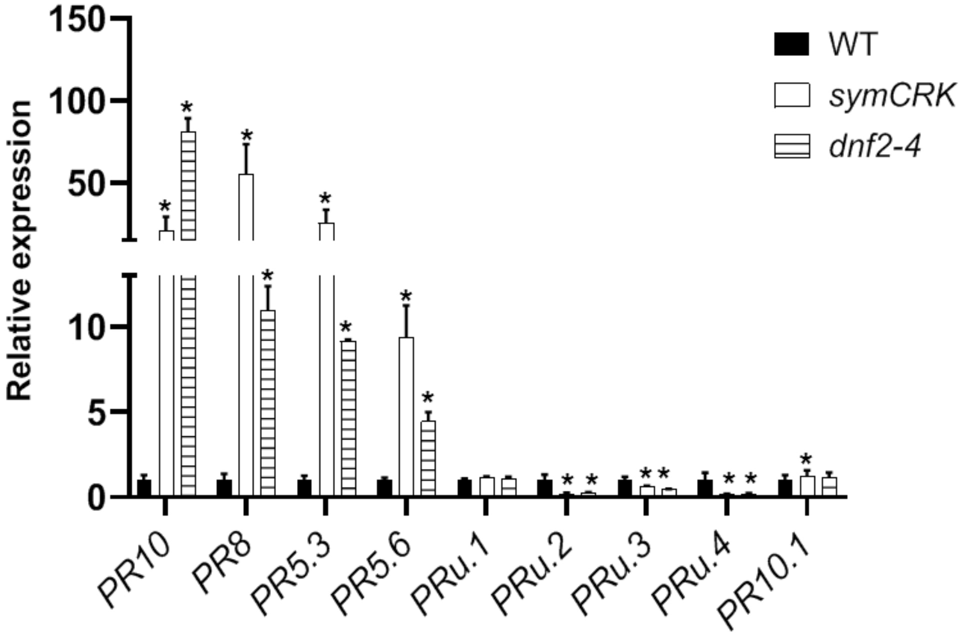
Supplemental Figure S1. PCR genotyping of the *Tnt1* insertion in *nf583* and *nf2210*

(A) Location of the primers used for the genotyping of *Medtr6g086170* and *Tnt1* sequences. (B) PCR products generated in WT (R108), *nf583*, *nf2210* using different combination of primers. The primers colored in blue and red are respectively used for the genotyping of the *Tnt1* insertion in *nf2210* and *nf583* background. The primers colored in grey recognize the *Tnt1* sequence.



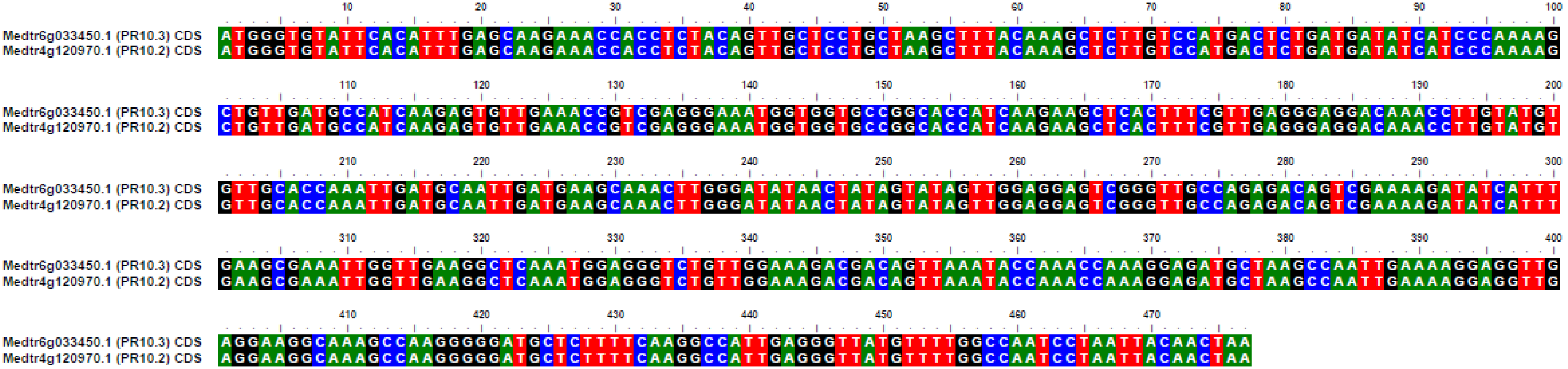
Supplemental Figure S2. Expression patterns of *PR* candidate genes in wild-type nodules in response to nitrate and phosphinothricin.

(A) Expression patterns of *PR* candidate genes for defense studies in Medicago roots (0-dpi (day post-inoculation) or nodules (10, 14 or 28-dpi) inoculated with *S. meliloti* or in 16-dpi nodules treated with nitrate (KNO_3, Benedito et al., 2008). (B) Expression of *PR* candidates in nodules of plants at 0, 4, 8 and 24 h after treatment with inhibitor of glutamine synthase , the phosphinothricin [0.25 mM] (Seabra et al., 2012). Expression profiles are based on data available on the *MtGEA* database (https://medicago.toulouse.inrae.fr/MtExpress, Noble Research Institute). Relative expression corresponds to mean signal of cDNA hybridization on the microarray for three independent experiments.



Supplemental Figure S3. Validation by RT-qPCR analysis of 10 *PR* genes selected for defense monitoring in the *Medicago* nodules.

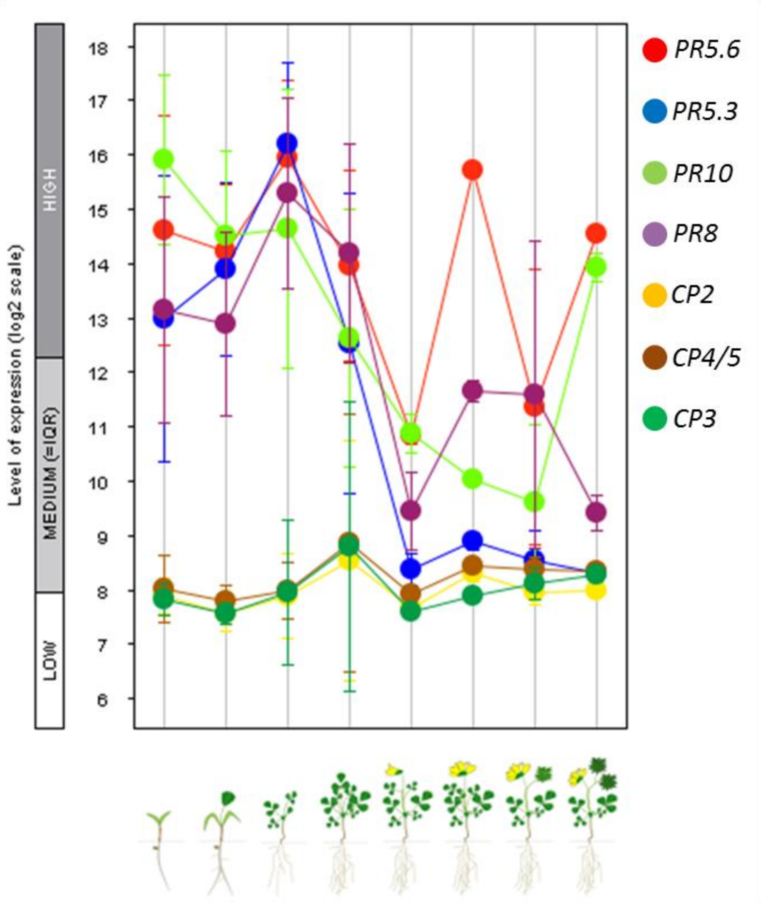
Expression analysis in WT, *symCRK* and *dnf2-4* was done on 21-dpi (day post-inoculation) nodules from plants cultivated *in vitro*. *PRu*: unclassified in a PR group. Error bars show the standard error (SE) and the asterisks represent significant variation (p-value < 2.5%) compared to the WT using Man & Whitney statistical test. The RT-qPCR analyses were made on plants from three biological repetitions (16 plants per repetition) with two technical replicates.



```
                                      10        20        30        40        50        60        70        80        90       100
Medtr6g033450.1 (PR10.3) CDS ATGGGTGTATTCACATTTGAGCAAGAAACCACCTCTACAGTTGCTCCTGCTAAGCTTTACAAAGCTCTTGTCCATGACTCTGATGATATCATCCCAAAAG
Medtr4g120970.1 (PR10.2) CDS ATGGGTGTATTCACATTTGAGCAAGAAACCACCTCTACAGTTGCTCCTGCTAAGCTTTACAAAGCTCTTGTCCATGACTCTGATGATATCATCCCAAAAG

                                     110       120       130       140       150       160       170       180       190       200
Medtr6g033450.1 (PR10.3) CDS CTGTTGATGCCATCAAGAGTGTTGAAACCGTCGAGGGAAATGGTGGTGCCGGCACCATCAAGAAGCTCACTTTCGTTGAGGGAGGACAAACCTTGTATGT
Medtr4g120970.1 (PR10.2) CDS CTGTTGATGCCATCAAGAGTGTTGAAACCGTCGAGGGAAATGGTGGTGCCGGCACCATCAAGAAGCTCACTTTCGTTGAGGGAGGACAAACCTTGTATGT

                                     210       220       230       240       250       260       270       280       290       300
Medtr6g033450.1 (PR10.3) CDS GTTGCACCAAATTGATGCAATTGATGAAGCAAACTTGGGATATAACTATAGTATAGTTGGAGGAGTCGGGTTGCCAGAGACAGTCGAAAAGATATCATTT
Medtr4g120970.1 (PR10.2) CDS GTTGCACCAAATTGATGCAATTGATGAAGCAAACTTGGGATATAACTATAGTATAGTTGGAGGAGTCGGGTTGCCAGAGACAGTCGAAAAGATATCATTT

                                     310       320       330       340       350       360       370       380       390       400
Medtr6g033450.1 (PR10.3) CDS GAAGCGAAATTGGTTGAAGGCTCAAATGGAGGGTCTGTTGGAAAGACGACAGTTAAATACCAAACCAAAGGAGATGCTAAGCCAATTGAAAAGGAGGTTG
Medtr4g120970.1 (PR10.2) CDS GAAGCGAAATTGGTTGAAGGCTCAAATGGAGGGTCTGTTGGAAAGACGACAGTTAAATACCAAACCAAAGGAGATGCTAAGCCAATTGAAAAGGAGGTTG

                                     410       420       430       440       450       460       470
Medtr6g033450.1 (PR10.3) CDS AGGAAGGCAAAGCCAAGGGGGATGCTCTTTTCAAGGCCATTGAGGGTTATGTTTTGGCCAATCCTAATTACAACTAA
Medtr4g120970.1 (PR10.2) CDS AGGAAGGCAAAGCCAAGGGGGATGCTCTTTTCAAGGCCATTGAGGGTTATGTTTTGGCCAATCCTAATTACAACTAA
```

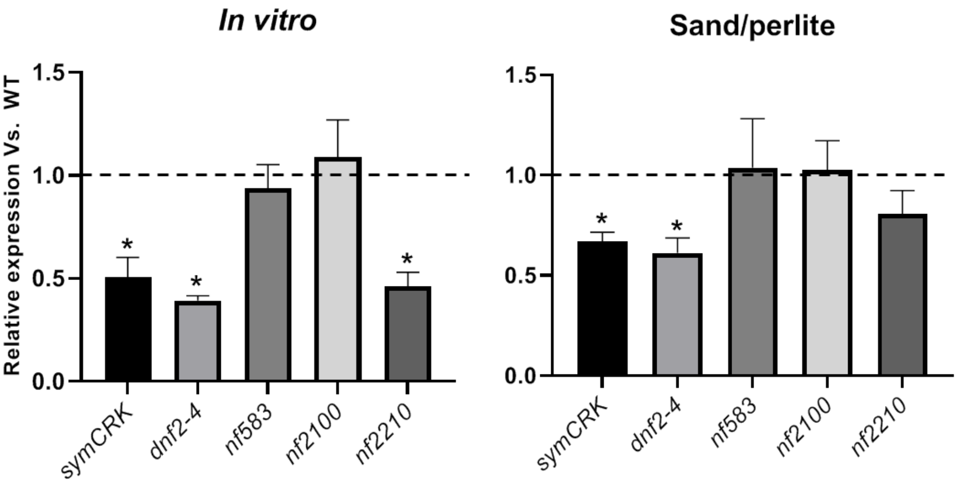
Supplemental Figure S4. Comparison of CDS sequences between *PR10.2* (*Medtr4g120970.1*) and *PR10.3* (*Medtr6g033450.1*).

Sequence alignment was realized using ClusteralW method on Bioedit tool (https://bioedit.software.informer.com).



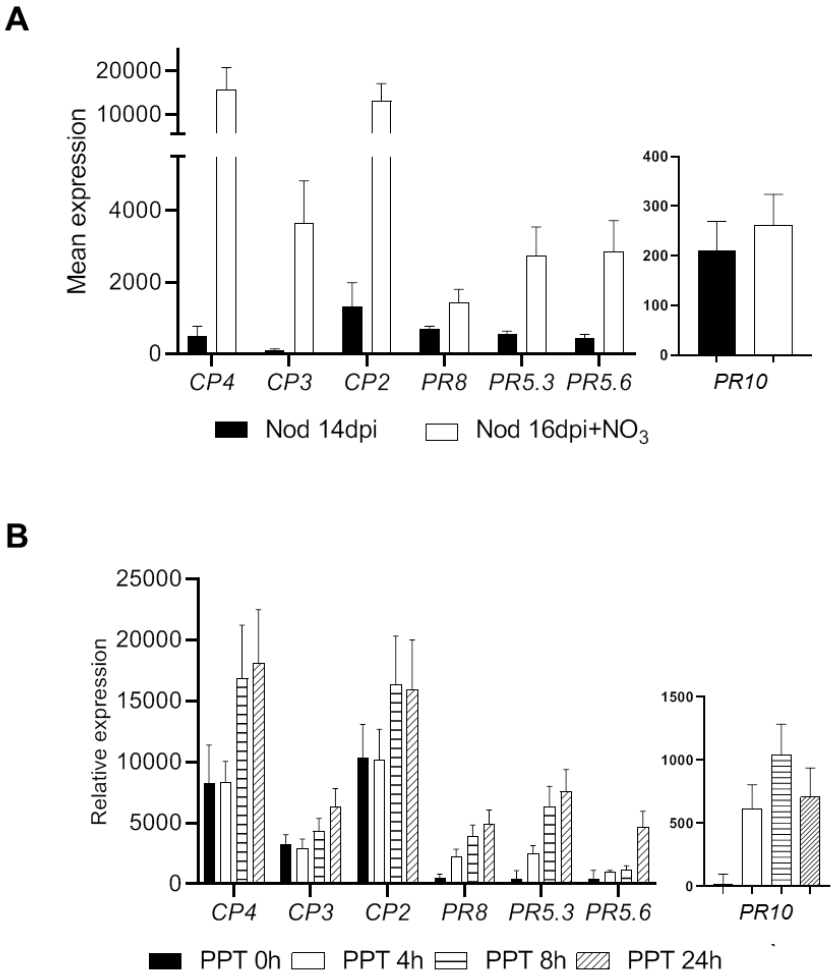
Supplemental Figure S5. Expression analyses of *PRs* and *CPs* during the development of Medicago.

The results show log2 of the expression level in eight developmental stages. The results were generated using the Genevestigator database (https://genevestigator.com/). Error bars show the SE of three independent experiments.

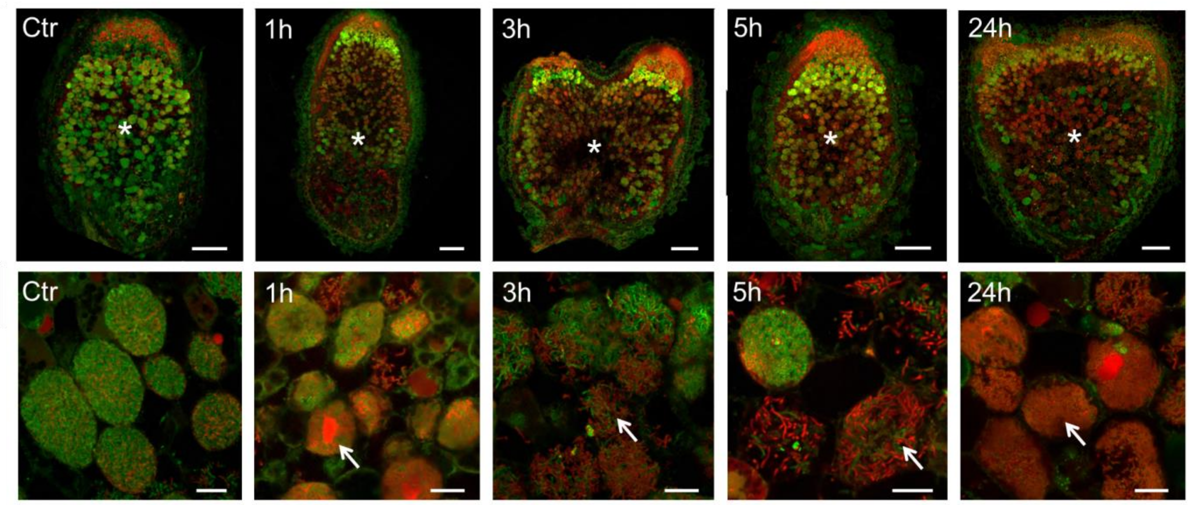


Supplemental Figure S6. Expression pattern of *PHYCYST5* in Medicago fix- nodules of mutants in response to different environmental conditions.

Expression analysis of the *PHYCYST5* in fix- nodules mutants cultivated *in vitro* and in sand/perlite. The expression was measured using RT-qPCR and the results show mean variation in mutants compared to the WT. The RT-qPCR analyses were made on plants from three biological repetitions (16 plants per repetition) with two technical replicates. Error bars show the SE and the asterisks represent significant variation (p-value < 2.5%) compared to the WT using Man & Whitney statistical test. The dotted lines represent the expression level of the WT.

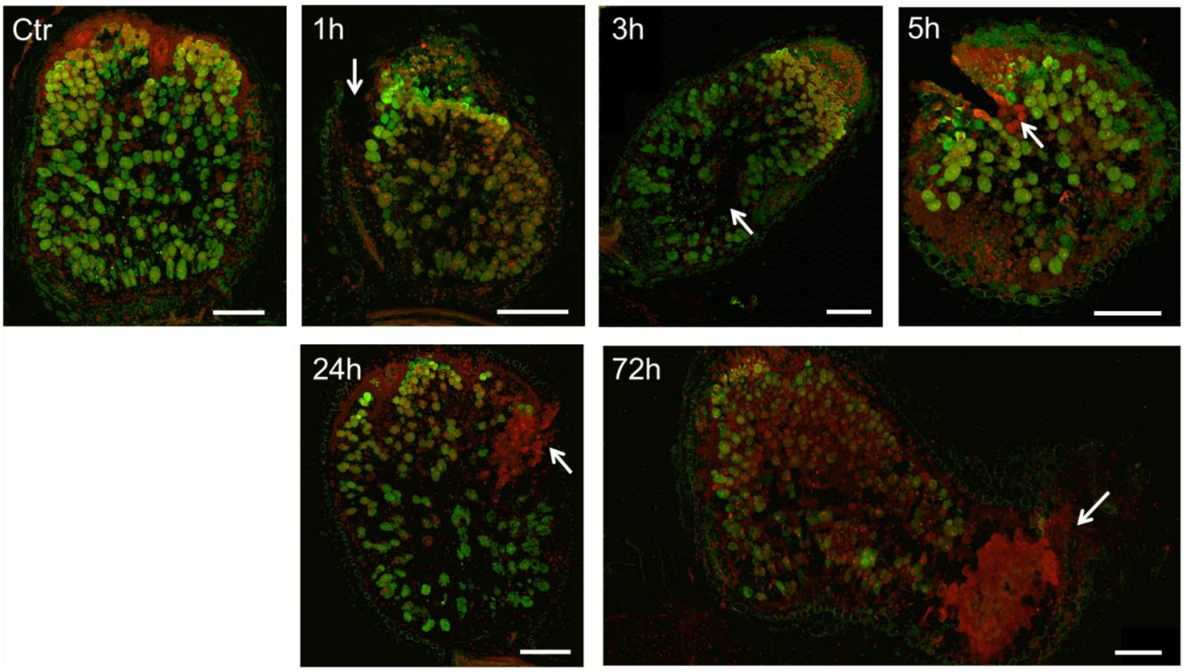


Supplemental Figure S7. Expression pattern of senescence and defense markers in Medicago wild-type nodules in response to nitrate and phosphinothricin treatments.
Expression pattern of senescence (*CP4, CP3* and *CP2*) and defense (*PR8, PR10, PR5.3* and *PR5.6*) markers in untreated (14-dpi) or treated (16-dpi) nodules with nitrate (A, Benedito et al., 2008) or nodules from plants incubated 0, 4, 8, 24h with the inhibitor of glutamine synthase, the phosphinothricin at [0.25 mM] (B, Seabra et al., 2012). Error bars represent SE of three independent experiments. Expression data are provided by *MtGEA* database (https://medicago.toulouse.inrae.fr/MtExpress, Noble Research Institute). Relative expression corresponds to mean signal of cDNA hybridization on the microarray.



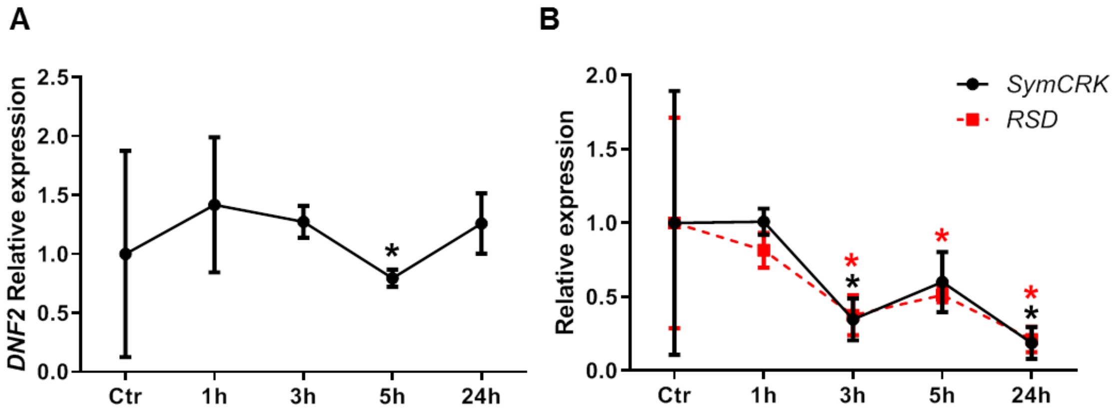
Supplemental Figure S8. Live and dead staining of wild-type inoculated nodules separated from the roots.

Live and dead staining of 21-dpi nodule sections obtained from the WT inoculated with *S. medicae WSM419*. The nodules were separated from the root and incubated 0 (Ctr), 1, 3, 5 and 24h. Top panel displays the nodule sections (scale bars = 200 µm) and bottom panel shows the bacteroids in the fixation zone III (scale bars = 20 µm). Asterisk indicates the zone III and the arrows show dead bacteroids. This figure shows the complete image panel corresponding to the experiment of the figure 5D.



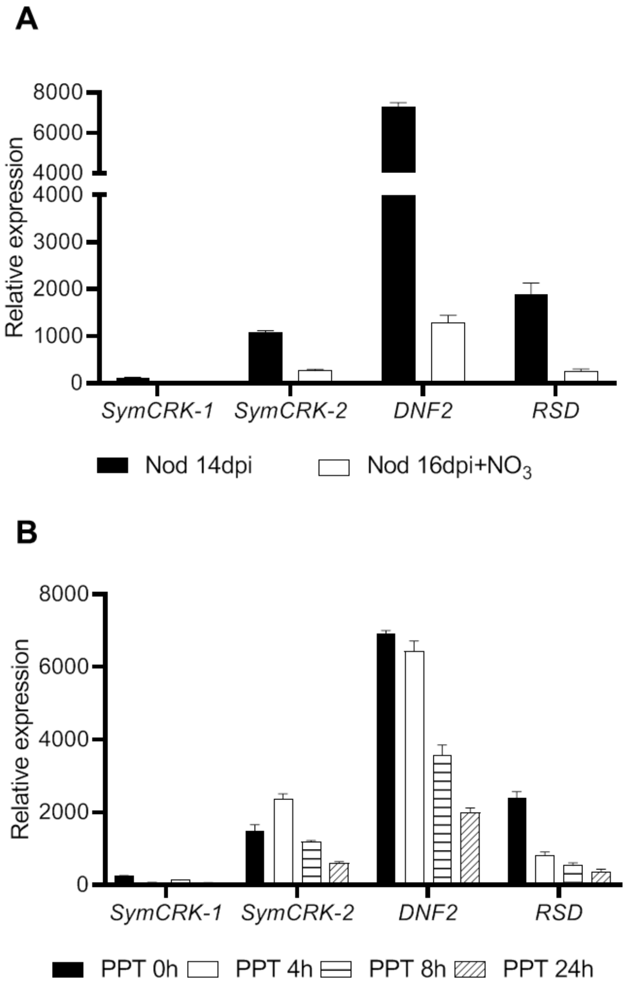
Supplemental Figure S9. Live and dead staining of Medicago wild-type inoculated nodules attached to the roots.

Live and dead staining of 21-dpi nodule sections obtained from the WT inoculated with *S. medicae WSM419*. The nodules tethered to roots were snipped and the nodulated plants were incubated 0 (Ctr), 1, 3, 5 24 and 72h. Scale bars = 250 µm. The arrows show the snipped zone of the nodules. This figure shows the complete image panel corresponding to the experiment of the figure 5H.



Supplemental Figure S10. Expression pattern of *DNF2, SymCRK* and *RSD* in Medicago wild-type nodules in response to wounding.

(A) *DNF2*, (B) *SymCRK*/*RSD* expression 0 (Ctr), 1, 3, 5 and 24h after wounding evaluated using RT-qPCR in 21-dpi WT nodules isolated from plants cultivated *in vitro* and inoculated with *S. medicae WSM419* . The results show mean expression of three independents experiments (16 plants per experiment) with two technical replicates. Error bars show SE and the asterisks represent significant variation using Mann & Whitney statistical test (p-value < 2.5%).



Supplemental Figure S11. Expression pattern of *DNF2*, *SymCRK* and *RSD* in Medicago wild-type nodules in response to nitrate and phosphinothricin treatments.

Expression patterns of *DNF2*, *SymCRK* and *RSD* in 16-dpi nodules treated or not with nitrate (A, Benedito et al., 2008) or from nodulated plants incubated 0, 4, 8 and 24 hours with the inhibitor of glutamine synthase, the phosphinothricin at [0.25 mM] (B, Seabra et al., 2012). Expression data are provided by the *MtGEA* database (https://medicago.toulouse.inrae.fr/MtExpress, Noble Research Institute, Benedito et al., 2008). Two probsets annotated *SymCRK-1*, *SymCRK-2* recognized *SymCRK* in the database. Relative expression corresponds to mean signal of cDNA hybridization on the microarray. Error bars show SE for three independent experiments.

Supplemental Table S1. List of *nf583*. *nf2210* and *nf2100* genes showing FSTs.

The genes with high confidence FST located in the ORFs were selected. For each mutant line. the tagged genes, the protein annotation, the probset ID on MtAffymV4 and the expression of the genes in the roots and the nodules of *M. truncatula*, are listed. FSTs and expression data are provided respectively by the Medicago *Tnt1* mutant database and Genevestigator. ND: not determined.

FST NF2210

Data download from Genvestigator		Number of samples	roots 242	nodules 40
GENE ID Mt4.0V1	Protein annotation	Probeset ID	Mean Expression roots	Mean expression nodules
Medtr6g086170	**SULFATE TRANSPORTER 3.5-RELATED**	**Mtr.37708.1.S1_at**	**15000.42**	**106081.77**
Medtr4g023030	AXI 1 PROTEIN-LIKE PROTEIN	Mtr.14950.1.S1_s_at/ Mtr.10701.1.S1_at	14257.89	14615.44
Medtr5g080390	PUMILIO HOMOLOG 1-RELATED	Mtr.49129.1.S1_s_at	10683.95	13478.64
Medtr5g081960	TRANSFERASE FAMILY (TRANSFERASE)	Mtr.34637.1.S1_at	24149.3	12771
Medtr7g013100	DIHYDROLIPOAMIDE ACETYL/SUCCINYL-TRANSFERASE-RELATED	Mtr.48448.1.S1_at	6924.38	4105.86
Medtr3g079310	BCDNA.GH11111	Mtr.20789.1.S1_at/ Mtr.20787.1.S1_at	4200.64	3629.64
Medtr1g026910	ATP-DEPENDENT PROTEASE CEREBLON	Mtr.39899.1.S1_at	6445.42	2737.87
Medtr1g099290	CHITINASE	Mtr.5384.1.S1_at	3473.9	2621.77
Medtr1g098580	UNKOWN	Mtr.15307.1.S1_at	7399.5	1810.58
Medtr4g066170	PROTEIN C13C4.8	Mtr.37362.1.S1_at/ Msa.1690.1.S1_at	2439.52	1302.47
Medtr1g076720	PANTOTHENATE KINASE	Mtr.6707.1.S1_s_at	1441.21	655.64
Medtr3g080190	ENDOGLUCANASE 11	ND	903.39	594.58
Medtr3g071860	SNF2 DOMAIN-CONTAINING PROTEIN CLASSY 1-RELATED	Mtr.21259.1.S1_at	192.46	234.85
Medtr2g008100	HISTIDINE DECARBOXYLASE / L-HISTIDINE CARBOXY-LYASE	Mtr.22597.1.S1_s_at	204.64	198.11
Medtr1g021520	TRANSCRIPTION REPRESSOR KAN1-RELATED	ND	ND	ND
Medtr1g021965	UNKOWN	ND	ND	ND
Medtr2g015660	UNKOWN	ND	ND	ND
Medtr3g031400	GLUCOSYL/GLUCURONOSYL TRANSFERASES	ND	ND	ND
Medtr3g108080	EMBRYO DEFECTIVE 2410	ND	ND	ND

	PROTEIN			
Medtr3g115650	COILED-COIL DOMAIN-CONTAINING PROTEIN 115	ND	ND	ND
Medtr4g008600	F-BOX/LEUCINE RICH REPEAT PROTEIN	ND	ND	ND
Medtr4g074200	DNA REPAIR PROTEIN XRCC2 HOMOLOG	ND	ND	ND
Medtr5g034370	PLANT PROTEIN OF UNKNOWN FUNCTION (DUF936) (DUF936)	ND	ND	ND
Medtr5g064800	F10B6.4	ND	ND	ND
Medtr5g094810	XENOBIOTIC-TRANSPORTING ATPASE / STEROID-TRANSPORTING ATPASE	ND	ND	ND
Medtr6g035310	UNKOWN	ND	ND	ND
Medtr6g075460	CYCLIC NUCLEOTIDE-GATED ION CHANNEL 19-RELATED	ND	ND	ND
Medtr6g078200	GLUCAN ENDO-1,3-BETA-D-GLUCOSIDASE / LAMINARINASE	ND	ND	ND

FST NF2210

Data download from Genvestigator		Number of samples	roots 242	nodules 40
GENE ID Mt4.0V1	Protein annotation	Probeset ID	Mean Expression roots	Mean expression nodules
Medtr6g086170	SULFATE TRANSPORTER 3.5-RELATED	Mtr.37708.1.S1_at	15000.42	106081.77
Medtr4g050480	PROTEIN IQ-DOMAIN 15-RELATED	Mtr.9374.1.S1_at	34073.8	23460.03
Medtr2g078730	D-AMINO-ACID TRANSAMINASE / D-ASPARTIC AMINOTRANSFERASE	Mtr.37617.1.S1_at	42933.99	22789.97
Medtr2g097670	PUMILIO HOMOLOG 1-RELATED	Mtr.41554.1.S1_at	32940.93	22371.37
Medtr3g074930	ACID PHOSPHATASE RELATED	Mtr.37882.1.S1_at	55444.41	20026.37
Medtr7g029105	UNKOWN	Mtr.45095.1.S1_at	4669.28	4064.76
Medtr6g005390	CBIX (CBIX)	Mtr.5349.1.S1_s_at	4996.46	2072.69
Medtr1g100627	ARM REPEAT SUPERFAMILY PROTEIN-RELATED	Mtr.11503.1.S1_at	28521.97	1712.62
Medtr5g019050	LYSM DOMAIN RECEPTOR-LIKE KINASE 4	Mtr.15787.1.S1_at	3488.11	1449.09
Medtr8g018280	NB-ARC DOMAIN (NB-ARC) // LEUCINE RICH REPEAT	Mtr.46816.1.S1_at	1938.7	981.27
Medtr4g127420	CCT MOTIF (CCT) PROTEIN	Mtr.13254.1.S1_at	882.13	783.93
Medtr5g096200	OLIGOPEPTIDE TRANSPORTER 1-RELATED	Mtr.29264.1.S1_at	694.87	709.75
Medtr5g015170	SF7 - ACR1	Mtr.5494.1.S1_at	428.1	665.88
Medtr8g013610	G-TYPE LECTIN S-RECEPTOR-LIKE SERINE/THREONINE-PROTEIN KINASE SD1-13	Mtr.50504.1.S1_at	2181.7	620.83

Medtr1g069325	KETOHEXOKINASE / HEPATIC FRUCTOKINASE	Mtr.13302.1.S1_at	793.98	477.78
Medtr4g130580	UNKOWN	Mtr.26057.1.S1_at	447.7	463.91
Medtr5g016830	FILAMENT-LIKE PLANT PROTEIN 7	Mtr.11295.1.S1_at	1768.18	431.89
Medtr5g034180	UNKOWN	Mtr.2095.1.S1_at	451.99	401.15
Medtr2g067440	PEROXIDASE / LACTOPEROXIDASE	Mtr.32452.1.S1_at	353.79	289.18
Medtr8g068050	PROTEIN KINASE DOMAIN (PKINASE) // LEGUME LECTIN DOMAIN	Mtr.46816.1.S1_at	1652.28	272.83
Medtr8g018450	LINOLEATE 9S-LIPOXYGENASE / LINOLEATE 9-LIPOXYGENASE	Mtr.24264.1.S1_at	558.91	238.26
Medtr4g102310	CYTOCHROME P450 - LIKE PROTEIN-RELATED	Mtr.38814.1.S1_at	5680.79	209.27
Medtr3g114920	HIGH MOBILITY GROUP B PROTEIN 10-RELATED	Mtr.27853.1.S1_at	207.47	202.83
Medtr5g020900	UNKOWN	Mtr.25509.1.S1_at	193.11	196.59
Medtr4g005270	**BETA-AMYRIN SYNTHASE / 2,3-OXIDOSQUALENE BETA-AMYRIN CYCLASE**	**Mtr.31948.1.S1_at**	**485.49**	**195.08**
Medtr8g093920	NUCLEAR TRANSCRIPTION FACTOR Y SUBUNIT B-7	Mtr.46490.1.S1_at	187.93	193.04
Medtr1g017790	UNKOWN	ND	ND	ND
Medtr1g017795	UNKOWN	ND	ND	ND
Medtr1g036430	OLYADENYLATE-BINDING PROTEIN (RRM SUPERFAMILY) // SPLICING FACTOR 3B, SUBUNIT 4	ND	ND	ND
Medtr1g106975	PUMILIO HOMOLOG 14-RELATED	ND	ND	ND
Medtr2g062310	DOMAIN OF UNKNOWN FUNCTION (DUF966) (DUF966)	ND	ND	ND
Medtr2g067360	UNKOWN	ND	ND	ND
Medtr2g067450	PEROXIDASE / LACTOPEROXIDASE	ND	ND	ND
Medtr2g083030	OXIDOREDUCTASE, 2OG-FE II OXYGENASE FAMILY	ND	ND	ND
Medtr2g089755	TRANSFERASE FAMILY (TRANSFERASE)	ND	ND	ND
Medtr4g081490	ORGANIC CATION/CARNITINE TRANSPORTER 4	ND	ND	ND
Medtr4g087920	STEROL REGULATORY ELEMENT-BINDING PROTEIN	ND	ND	ND
Medtr4g104690	CDP-GLYCEROL DIPHOSPHATASE / CDP-GLYCEROL PYROPHOSPHATASE	ND	ND	ND
Medtr5g076060	UNCHARACTERIZED CONSERVED PROTEIN	ND	ND	ND
Medtr5g083890	F-BOX DOMAIN (F-BOX) // LEUCINE RICH REPEAT (LRR_2)	ND	ND	ND
Medtr6g015000	UDP-GLUCOSE/GDP-MANNOSE DEHYDROGENASE FAMILY, NAD BINDING DOMAIN	ND	ND	ND

Medtr6g032965	3-KETOACYL-COA SYNTHASE 17-RELATED	ND	ND	ND
Medtr6g043850	SF16 - F14O23.23 PROTEIN	ND	ND	ND
Medtr6g061110	GPI16 SUBUNIT, GPI TRANSAMIDASE COMPONENT (GPI16)	ND	ND	ND
Medtr6g065190	PPR REPEAT (PPR) // PPR REPEAT (PPR_1)	ND	ND	ND
Medtr6g082770	UNKOWN	ND	ND	ND
Medtr6g088240	ELONGATION FACTOR TS	ND	ND	ND
Medtr7g066100	F-BOX DOMAIN (F-BOX) // F-BOX ASSOCIATED (FBA_1)	ND	ND	ND
Medtr7g073980	BTB/POZ DOMAIN (BTB) // NPH3 FAMILY (NPH3)	ND	ND	ND
Medtr8g006470	DUO POLLEN 1	ND	ND	ND
Medtr8g008550	UNKOWN	ND	ND	ND
Medtr8g042520	PEPTIDE EXPORTER, ABC SUPERFAMILY	ND	ND	ND

FST NF2100

Data download from Genvestigator		Number of samples	roots 242	nodules 40
GENE ID Mt4.0V1	Protein annotation	Probeset ID	Mean Expression roots	Mean expression nodules
Medtr7g050980	PECTINESTERASE-RELATED PROTEIN-RELATED	Mtr.8508.1.S1_at	85331,41	11474,21
Medtr4g005730	SERINE/THREONINE-PROTEIN KINASE OSR1	Mtr.31949.1.S1_at/ Mtr.28731.1.S1_at	3003,75	1124,01
Medtr3g014060	14-3-3-Like Protein Gf14 Lambda	Mtr.15400.1.S1_at	350,33	302,48
Medtr4g005270	**BETA-AMYRIN SYNTHASE / 2,3-OXIDOSQUALENE BETA-AMYRIN CYCLASE**	**Mtr.31948.1.S1_at**	**485,59**	**195,08**
Medtr5g021920	F-BOX DOMAIN (F-BOX)	ND	ND	ND

Supplemental Table S2. List of the identified *PR* genes in the *M. truncatula* genome.

Unclassified *PR* corresponds to PR without defined classes.

V4 Medicago Genome ID	Probeset V4 affymetrix	Predicted domain	Classe
Medtr2g010670.1	Mtr.34477.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr2g435490.1	Mtr.31096.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr2g010600.1	Mtr.8977.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr2g012370.1	Mtr.8977.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr2g010650.1	Msa.3171.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr5g018755.1	Mtr.25125.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr8g078770.1	Mtr.5901.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr5g018770.1	Mtr.82.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr4g050762.1	Mtr.8977.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr2g010610.1	Mtr.8977.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr2g010630.1	Mtr.8977.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr2g010690.1	Mtr.34477.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr2g010700.1	Mtr.34477.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr2g010640.1	Mtr.34477.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr2g010590.1	Msa.3171.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr5g018750.1	Mtr.81.1.S1_at	CAP, CYSTEINE-RICH SECRETORY PROTEIN, ANTIGEN 5	PR1
Medtr8g045490.1	Mtr.10361.1.S1_at	BET_V_1	PR10
Medtr8g045640.1	Mtr.10363.1.S1_x_at	BET_V_1	PR10
Medtr8g045400.1	Mtr.10364.1.S1_at	BET_V_1	PR10
Medtr4g120760.1	Mtr.12615.1.S1_at	BET_V1-LIKE	PR10
Medtr4g120970.1	Mtr.34114.1.S1_s_at	BET_V1-LIKE	PR10
Medtr6g033450.1	Mtr.34114.1.S1_s_	BET_V1-LIKE	PR10

	at		
Medtr1g030810.1	Mtr.3416.1.S1_at	BET_V1-LIKE	PR10
Medtr4g120950.1	Mtr.37852.1.S1_at	BET_V1-LIKE	PR10
Medtr8g045570.1	Mtr.38110.1.S1_at	BET_V1-LIKE	PR10
Medtr8g045665.1	Mtr.40102.1.S1_at	BET_V1-LIKE	PR10
Medtr8g045520.1	Mtr.40106.1.S1_s_at	BET_V1-LIKE	PR10
Medtr3g055120.1	Mtr.43078.1.S1_at/ Mtr.43078.1.S1_s_at	BET_V1-LIKE	PR10
Medtr2g435310.1	Mtr.12615.1.S1_at	BET_V1-LIKE	PR10
Medtr8g045560.1	Msa.1635.1.S1_at	BET_V1-LIKE	PR10
Medtr4g120940	Not determined	SRPBCC SUPER FAMILY	PR10
Medtr8g045555	Mtr.40109.1.S1_at	SRPBCC SUPERFAMILY	PR10
Medtr1g031640	Mtr.45999.1.S1_at	SRPBCC SUPERFAMILY	PR10
Medtr8g045555.1	Mtr.40109.1.S1_at	BET_V1-LIKE	PR10
Medtr8g045735.1	Msa.1635.1.S1_at	BET_V1-LIKE	PR10
Medtr8g045300.1	Mtr.45935.1.S1_at	BET_V1-LIKE	PR10
Medtr8g045695.1	Msa.1635.1.S1_at	BET_V1-LIKE	PR10
Medtr4g120940.1	Mtr.34114.1.S1_s_at	BET_V1-LIKE	PR10
Medtr2g035220.1	Mtr.10317.1.S1_at/ Msa.3122.1.S1_at	BET_V1-LIKE	PR10
Medtr2g035210.1	Msa.3122.1.S1_at	BET_V1-LIKE	PR10
Medtr2g035190.1	Msa.3122.1.S1_at	BET_V1-LIKE	PR10
Medtr2g035320.1	Msa.3122.1.S1_at	BET_V1-LIKE	PR10
Medtr2g035320.2	Msa.3122.1.S1_at	BET_V1-LIKE	PR10
Medtr1g030840.1	Mtr.29236.1.S1_at	BET_V1-LIKE	PR10
Medtr1g030820.1	Mtr.36367.1.S1_at	BET_V1-LIKE	PR10
Medtr2g035150.1	Mtr.42966.1.S1_at	BET_V1-LIKE	PR10
Medtr2g035120.1	Mtr.42968.1.S1_at	BET_V1-LIKE	PR10
Medtr2g035130.1	Mtr.12277.1.S1_at	BET_V1-LIKE	PR10
Medtr2g035100.1	Mtr.40147.1.S1_s_at/Msa.2942.1.S1_s_at	BET_V1-LIKE	PR10
Medtr2g035105.1	Mtr.40147.1.S1_s_at	BET_V1-LIKE	PR10
Medtr3g055130	Mtr.6516.1.S1_at	BET_V1-LIKE	PR10
Medtr2g034480	Mtr.18650.1.S1_at/ Msa.1736.1.S1_at	GLYCOSYL HYDROLASES FAMILY 17	PR2
Medtr2g034470.1	Mtr.18649.1.S1_s_at	GLYCOSYL HYDROLASES FAMILY 17	PR2
Medtr2g034440.1	Mtr.18649.1.S1_s_at	GLYCOSYL HYDROLASES FAMILY 17	PR2

Medtr3g118390	Mtr.331.1.S1_at	GLYCO_HYDRO_19 (CHITINASE CLASSE I)+ CHITIN_BIND_1 (CHITNIASE BINDING PROT)	PR3
Medtr7g115220	Mtr.42872.1.S1_at/ Mtr.12237.1.S1_at	BARWIN+CHITIN_BIND_1	PR4
Medtr5g022310.2	Msa.1526.1.S1_at	TLP-PA	PR5
Medtr5g022310.1	Msa.1526.1.S1_at/ Mtr.17914.1.S1_at	G64-TLP-SF	PR5
Medtr8g096900.1	Mtr.10968.1.S1_at	G64-TLP-SF	PR5
Medtr8g088960.1	Mtr.11885.1.S1_at	GH64-TLP-SF	PR5
Medtr8g075550.1	Mtr.15054.1.S1_at	GH64-TLP-SF	PR5
Medtr3g114030.1	Mtr.17199.1.S1_at/ Mtr.33394.1.S1_at	GH64-TLP-SF	PR5
Medtr8g107140.1	Mtr.17268.1.S1_at	TLP-PA	PR5
Medtr8g056820.1	Mtr.19129.1.S1_at	GH64-TLP-SF	PR5
Medtr6g009480.1	Mtr.19465.1.S1_at	TLP-PA	PR5
Medtr2g063160.1	Mtr.19470.1.S1_at	GH64-TLP-SF	PR5
Medtr7g076360.1	Mtr.26405.1.S1_at	GH64-TLP-SF	PR5
Medtr2g069660.1	Mtr.26405.1.S1_at	TLP-PA	PR5
Medtr5g059200.1	Mtr.28302.1.S1_at	TLP-PA	PR5
Medtr2g067980.1	Mtr.29368.1.S1_at	TLP-PA	PR5
Medtr2g068030.1	Mtr.32260.1.S1_at	GH64-TLP-SF	PR5
Medtr7g102380.1	Mtr.33691.1.S1_at	TLP-PA	PR5
Medtr8g075510.1	Mtr.35231.1.S1_at	GH64-TLP-SF	PR5
Medtr8g075510.2	Mtr.35231.1.S1_at	TLP-PA	PR5
Medtr4g063630.1	Mtr.37482.1.S1_at	TLP-PA	PR5
Medtr5g010640.1	Mtr.40555.1.S1_at	GH64-TLP-SF	PR5
Medtr4g073730.1	Mtr.42529.1.S1_at	GH64-TLP-SF	PR5
Medtr2g068655.1	Mtr.42536.1.S1_at	GH64-TLP-SF	PR5
Medtr8g096920.1	Mtr.42775.1.S1_at	TLP-P	PR5
Medtr5g010635.1	Mtr.42989.1.S1_at	GH64-TLP-SF	PR5
Medtr4g073720.1	Mtr.43370.1.S1_at	TLP-PA	PR5
Medtr7g062610.1	Mtr.49138.1.S1_at	GH64-TLP-SF SUPER FAMILY	PR5
Medtr1g062640.1	Mtr.6757.1.S1_at	GH64-TLP-SF SUPER FAMILY	PR5
Medtr5g022350.2	Mtr.7850.1.S1_s_at	TLP-PA	PR5
Medtr5g022350.1	Mtr.7850.1.S1_s_at /Mtr.9418.1.S1_s_at/	GH64-TLP-SF SUPER FAMILY	PR5
Medtr8g096910.1	Mtr.8763.1.S1_at	TLP-P	PR5
Medtr1g025420.1	Mtr.9391.1.S1_at	GH64-TLP-SF SUPER FAMILY	PR5
Medtr1g025420.2	Mtr.9391.1.S1_at	TLP-PA	PR5
Medtr1g021945.1	Not determined	GH64-TLP-SF SUPER FAMILY	PR5
Medtr1g062390.1	Not determined	GH64-TLP-SF SUPER FAMILY	PR5
Medtr2g063150.1	Not determined	GH64-TLP-SF SUPER FAMILY	PR5

Medtr3g068015.1	Not determined	GH64-TLP-SF SUPER FAMILY	PR5
Medtr3g081550.1	Not determined	GH64-TLP-SF SUPER FAMILY	PR5
Medtr5g023850.1	Not determined	GH64-TLP-SF SUPER FAMILY	PR5
Medtr5g068670.1	Not determined	GH64-TLP-SF SUPER FAMILY	PR5
Medtr8g037890.1	Not determined	GH64-TLP-SF SUPER FAMILY	PR5
Medtr8g089020.1	Not determined	GH64-TLP-SF SUPER FAMILY	PR5
Medtr3g111620.1	Not determined	TLP-PA	PR5
Medtr6g079580.1	Not determined	TLP-PA	PR5
Medtr8g036215.1	Not determined	TLP-PA	PR5
Medtr1g099310.1	Msa.1009.1.S1_at/ Msa.2848.1.S1_at/ Mtr.12525.1.S1_at	GH18_HEVAMINE_XIPI_CLASS_III+CHITINASE CLASSE III	PR8
Medtr2g076070.1 /Medtr2g076070.2	Mtr.1214.1.S1_at	PUTATIVE NTF2-LIKE PROTEIN SUPER FAMILY	Unclassified
Medtr2g076010.1 /Medtr2g076010.2	Mtr.26632.1.S1_at/ Mtr.26632.1.S1_at	PUTATIVE NTF2-LIKE PROTEIN SUPER FAMILY	Unclassified
Medtr8g058350	Mtr.51369.1.S1_at	PUTATIVE NTF2-LIKE PROTEIN SUPER FAMILY	Unclassified
Medtr8g058700	Not determined	NOT DETERMINED	Unclassified
Medtr2g038000	Mtr.15743.1.S1_at/ Mtr.51386.1.S1_at	PHD_PRHA_LIKE+HOX	Unclassified

Supplemental Table S3. List of *PR* genes validated by RT-qPCR for the study.

Melting curve corresponds to the temperature at which 50% of DNA is denatured. Only primers producing amplification product showing one melting curve were selected for the study. The efficiency corresponds to DNA polymerase efficiency. The primers producing an efficiency less than 80% or superior than 120% were avoided.

Gene ID	Melting curve (specificity)	efficiency =2 (+/- 0,2)	ID Affymetrix	PR classes	PR annotation in the figures
Medtr1g099310.1	Specific	Yes	Msa.1009.1.S1_at	PR8	PR8
Medtr2g076070.1	Specific	Yes	Mtr.1214.1.S1_at	Unkown	PRuk.1
Medtr2g076010.2	Specific	Yes	Mtr.26632.1.S1_at	Unkown	PRuk.2
Medtr2g076070.2	Specific	Yes	Mtr.1214.1.S1_at	Unkown	PRuk.3
Medtr2g076010.1	Specific	Yes	Mtr.26632.1.S1_at	Unkown	PRuk.4
Medtr2g068655.1	Not detected	Not detected	Mtr.42536.1.S1_at	PR5	PR5.1
Medtr4g120950.1	Specific	Yes	Mtr.37852.1.S1_at	PR10	PR10.1
Medtr4g120970.1	Specific	Yes	Mtr.34114.1.S1_s_at	PR10	PR10.2 (**PR10**)
Medtr5g010640.1	Specific	Yes	Mtr.40555.1.S1_at	PR5	PR5.3
Medtr6g033450.1	Specific	Yes	Mtr.34114.1.S1_s_at	PR10	PR10.3 (**PR10**)
Medtr7g062610.1	Non specific	No	Mtr.49138.1.S1_at	PR5	PR5.4
Medtr8g096910.1	Specific	Yes	Mtr.8763.1.S1_at	PR5	PR5.6

Supplemental Table S4. Pearson correlation analysis of the *PRs*. *CPs* and *PRs* vs. *CPs* expression.

Expression data of Medicago response to perturbations were downloaded from Genevestigator database (https://genevestigator.com/) and Pearson correlation was calculated using Excel software.

Pearson corr	***CP5/CP4***	***CP3***	***CP2***	***PR5.6***	***PR10***	***PR5.3***	***PR8***
CP5/CP4	1.00	0.93	0.91	-0.10	-0.07	-0.06	-0.10
CP3	0.93	1.00	0.97	-0.08	-0.08	-0.07	-0.10
CP2	0.91	0.97	1.00	-0.09	-0.11	-0.10	-0.10
PR5.6	-0.10	-0.08	-0.09	1.00	0.54	0.64	0.41
PR10	-0.07	-0.08	-0.11	0.54	1.00	0.65	0.37
PR5.3	-0.06	-0.07	-0.10	0.64	0.65	1.00	0.58
PR8	-0.10	-0.10	-0.10	0.41	0.37	0.58	1.00

Supplemental Table S5. Co-expressed *PHYTOCYSTATIN* genes with the studied *PRs*. *PHYTOCYSTATIN* co-expressed with one or multiple *PRs* were isolated using the Phytomine tools of the Phytozome database (https://phytozome.jgi.doe.gov/phytomine/begin.do). The table shows the two identified *PHYTOCYSTATINS* (*Medtr2g026040*; *PHYTOCYST5* and *Medtr5g088770*; *PHYTOCYST32*) and the corresponding Pearson correlation value.

	PR5.3	*PR5.6*	*PR10.2*	*PR10.3*	*PHYTOCYST32*
Medtr2g026040 (PHYTOCYST5)	0.909	0.85922	0.9168	0.97454	0.95816
Medtr5g088770 (PHYTOCYST32)	0.92549	X	X	0.93896	X

Table S6. List of primers used in this study.

	Gene ID	Gene Name	Primer L	Primer R	Tm	Ref
RT-qPCR primers	*Medtr4g107930*	*CP3*	AGTGGATGCCGCTGAAGG	TCAATCACAGTTTTGCTCAAATTAC	60	Pérez Guerra JC et al., 2010
	Medtr4g079770	*CP4*	TGGAAGCATCTTACCCTACTG	ATATACATAAATCGCAAATCACATTC	60	Pérez Guerra JC et al., 2010
	Medtr5g022560	*CP2*	CATCTTACCCTACTGCTTAAATGC	AACTAGAAACCATGATGAATGTAGC	60	Pérez Guerra JC et al., 2010
	Medtr4g079470	*CP5*	GTTGACGGAACTTGCAGTGC	CACCCCAATCAGTTCCCCAT	60	In this study
	TC106667	*Actine*	TGGCATCACTCAGTACCTTTCAACAG	ACCCAAAGCATCAAATAATAAGTCAACC	60	Berrabah el al., 2015
	Medtr1g099310.1	*PR8*	CCTCAATGTCCTTTCCCTGA	TGGAGCAGCAGCATCATTAG	60	In this study
	Medtr2g076070.1	*PR unkown (PRuk.1)*	ATGGGAGATGGAGCTGACAC	GCAATTTCAGGTGGTCCTGT	60	In this study
	Medtr2g076010.2	*PR unkown (PRuk.2)*	GCAATTTCAGGTGGTCCTGT	GCAATTTCAGGTGGTCCTGT	60	In this study
	Medtr2g076070.2	*PR unkown (PRuk.3)*	GTTAATGGCAGGGAGGGATT	GCAATTTCAGGTGGTCCTGT	60	In this study
	Medtr2g076010.1	*PR unkown (Pruk.4)*	CAAGATCCGGTTGCAAGATT	GCAATTTCAGGTGGTCCTGT	60	In this study
	Medtr4g120950.1	*PR10.1*	CACGATTCATCGAGAAAGCA	GGGTTGGAACCAATTTGAAC	60	In this study
	Medtr4g120970.1	*PR10*	TTGAGGGAGGACAAACCTTG	CCTCAATGGCCTTGAAAAGA	60	In this study
	Medtr6g033450.1					
	Medtr2g068655.1	*PR5.1*	GTTCAAGAGGGCTTGTCCTG	GGGCAGGCCTTACAATTACA	60	In this study
	Medtr5g010640.1	*PR5.3*	GGCCATCATCATGAAAACAA	GACCCCAGATTCTTGCGTTA	60	In this study
	Medtr8g096910.1	*PR5.6*	TACACAAGCAGCAAGGTTCG	CTACCGGATACGCTGCAACT	60	In this study
	Medtr5g088770.1	*PHYTOCYST32*	GGCGGCTCTAGGTGGTAGTA	ACACCTTTTGCTTCCCACCA	60	In this study
	Medtr2g026040.1	*PHYTOCYST5*	AAGGATGCTCTTGTCGGTGG	CAACTTTCGCAGCCAACACA	60	In this study
	Medtr3g079850	*SymCRK*	GATTTCTGTGTTGAAGCTTGGCT	ACATCAGAAGTGAACTCTCTGCAA	60	Berrabah et al., 2014
	Medtr4g085800	*DNF2*	AGGCAATGCGTTCAGAAGGCCT	CGACACCGAACTGAGATAGTCA	60	Bourcy et al., 2013
	MtrunA17_Chr7g0239441	*RSD*	GAAAGATGGAATACACCCAAAACC	AACTTGACCTGGGTCGTCAGA	60	Sinharoy et al., 2013
Genotyping Primers		NF583F	ACTATTGTGTCAACCACACGTG		65	In this study
		NF583R	GGCATTAGTTATGCCAAACTTGC		65	In this study
		NF2210F	GCAAGTTTGGCATAACTAATGCC		65	In this study
		NF2210R	GGCTTGGGATATTGGTTGATTTC		65	In this study
		LTR4F	TACCGTATCTCGGTGCTACA		66	Ratet et al., 2010
		LTR6R	GCTACCAACCAAACCAAGTCAA		66	Ratet et al., 2010