Fig. S1

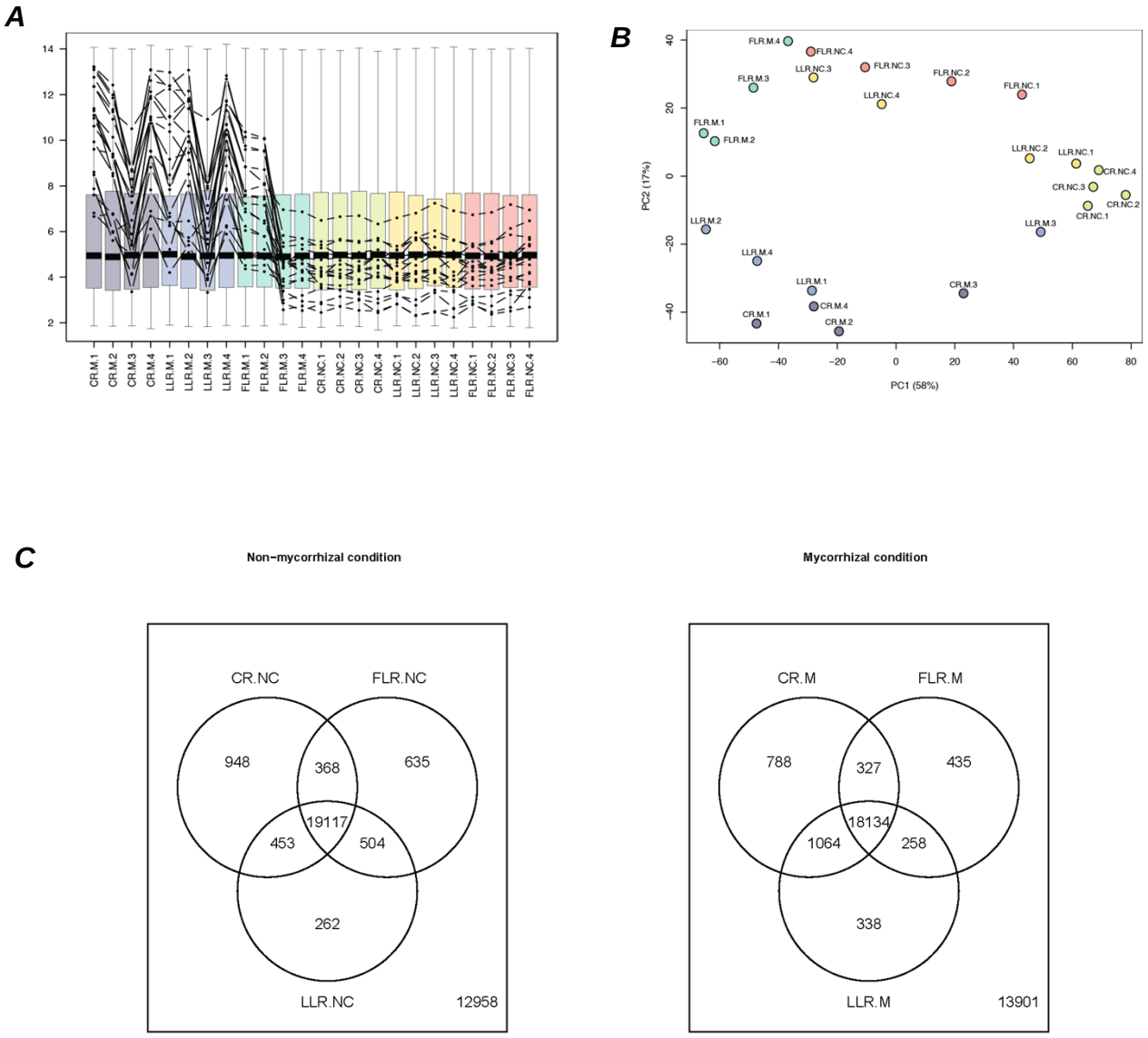

Fig. S1. Gene expression analysis and validation. (*A* and *B*) Exploratory analysis of gene expression in all samples. (*A*) Normalized expression of 19 AM marker genes across all samples (black dotted-lines). Box plot in background shows the expression distribution of all genes: boxes represent the 1st/2nd/3rd quartiles, whiskers the minimum/maximum values. (*B*) Sample separation according to a Principal Component Analysis using the 1000 most variable genes across all samples; axis labels indicate % of variance explained by each component. Samples are coloured by type (see below). (*C*) Number of genes detected as expressed in each root-type. The number of genes detected as "present" by the Affymetrix "MAS5" Present/Absent calls in non-colonized (NC, left Venn diagram) and in mycorrhizal root types (M, right Venn diagram). Numbers in the lower right corner of each diagram represent the genes that were called as "absent". CR, crown roots; LLR, large lateral roots; FLR, fine lateral roots.

Fig. S2

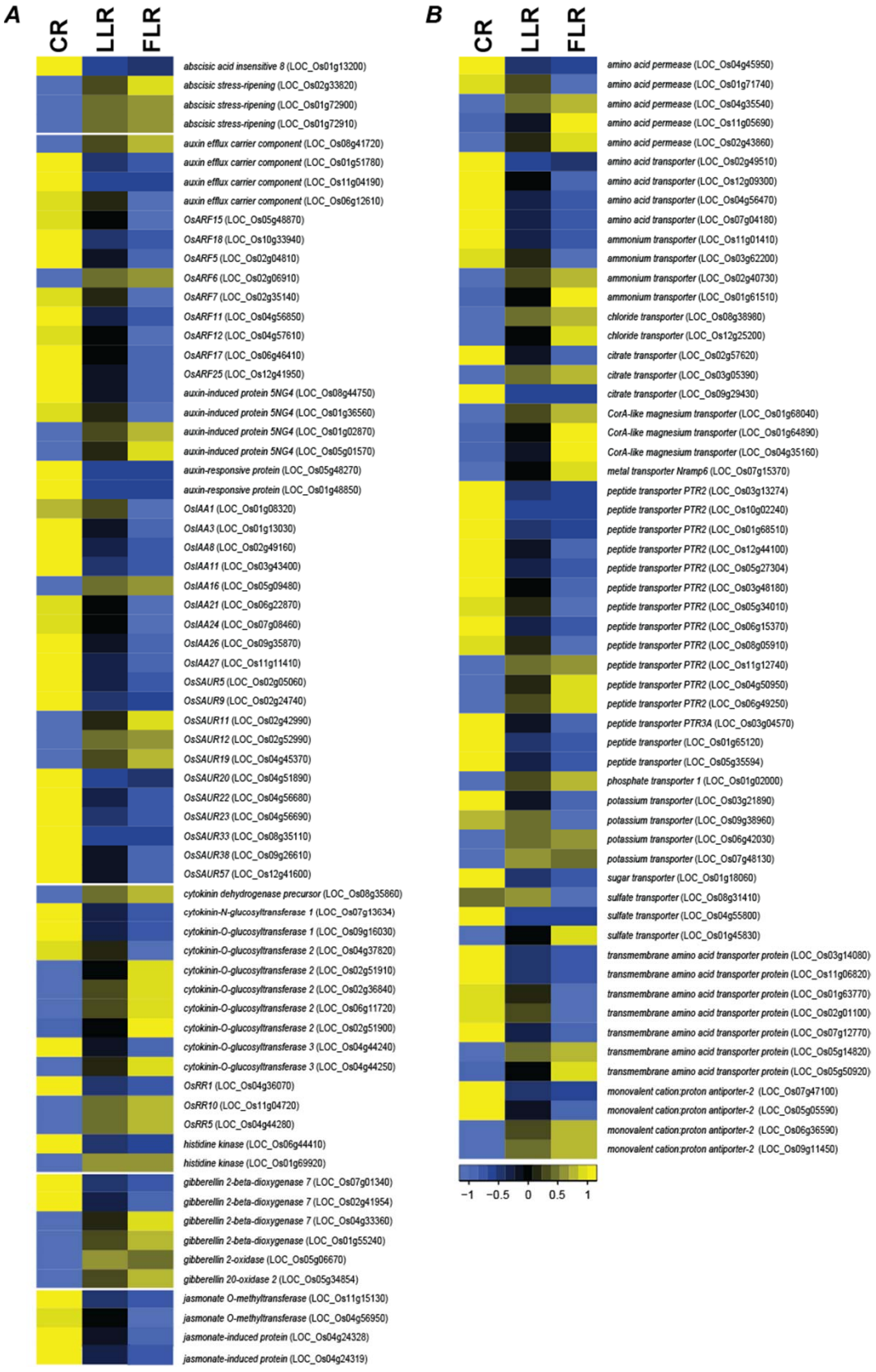

Fig. S2. Hormone signalling and transport related gene expression in rice root types. Relative expression (scale bar, Z-scale of SDs from the cross-sample mean; blue, below the mean; yellow, above the mean) of genes that were significantly differently expressed among at least two conditions related to (*A*) hormone metabolism and signaling and to (*B*) transport.

Fig. S3

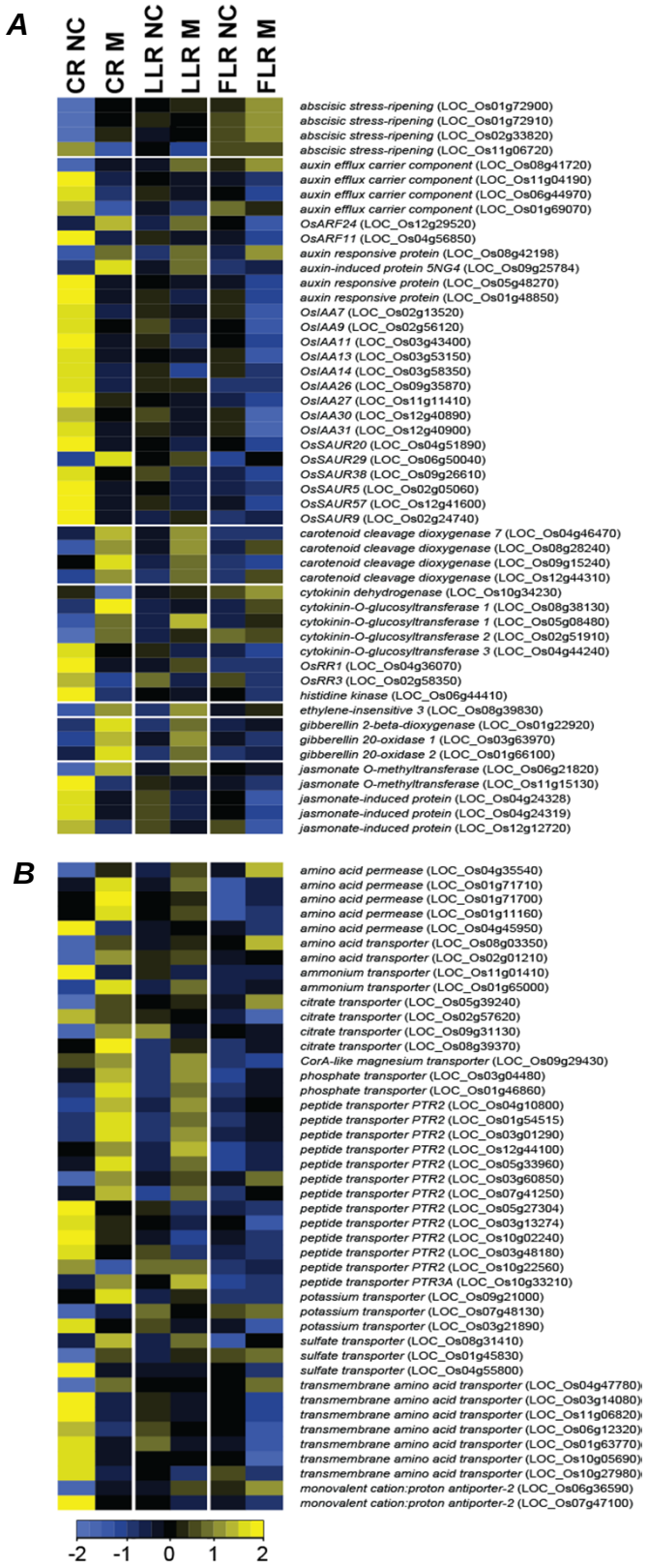

Fig. S3. Hormone signalling and transport related gene expression of rice root-types in response to AM colonization. Relative expression (scale bar, Z-scale of SDs from the cross-sample mean; blue, below the mean; yellow, above the mean) of genes that were significantly differently expressed between non-colonized (NC) and mycorrhizal (M) conditions related to (*A*) hormone metabolism and signaling and to (*B*) transport; crown roots (CR), large lateral roots (LLR) and fine lateral roots (FLR).

Fig. S4

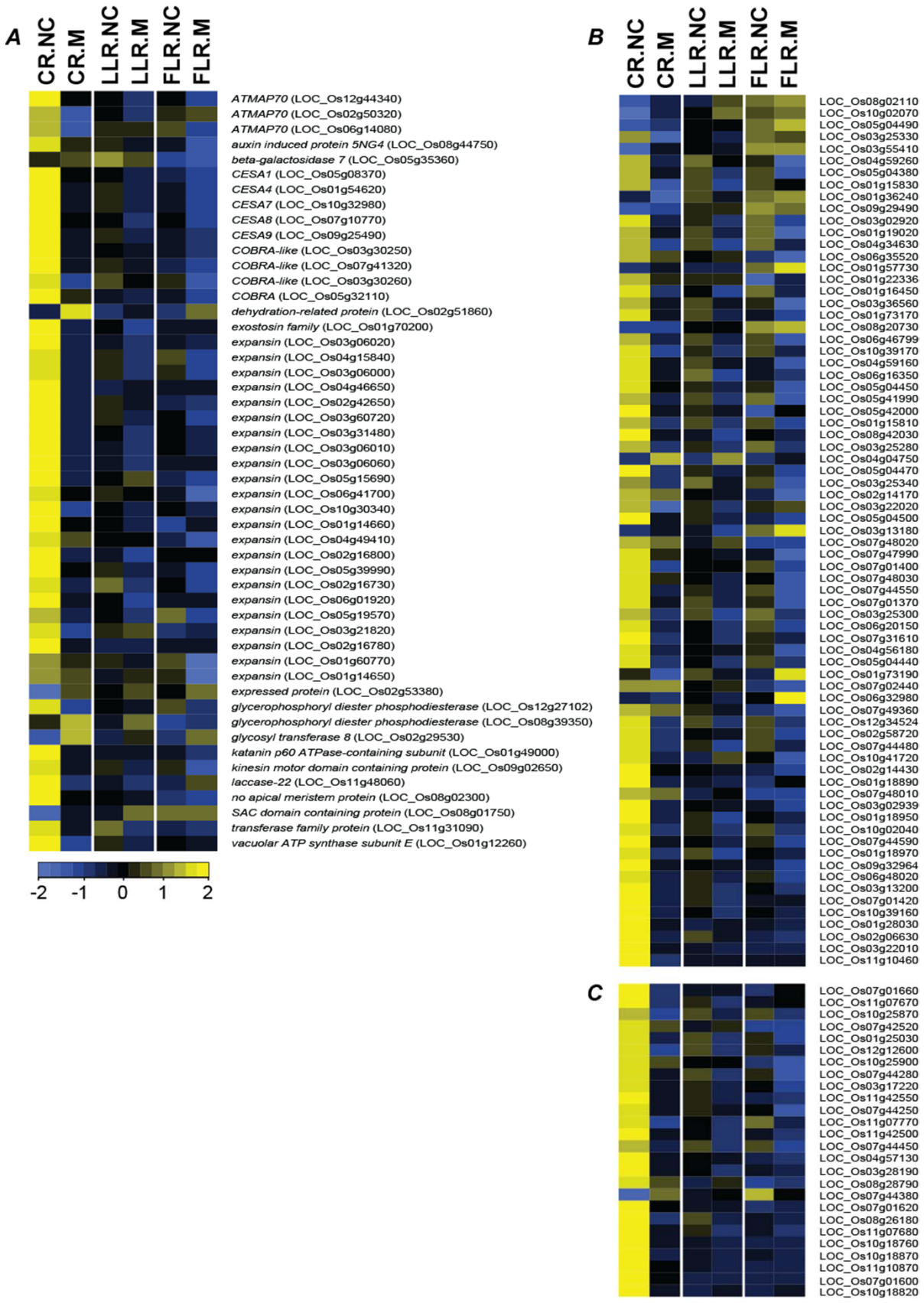

Fig. S4. AM responsive expression changes of “SCW genes”. Microarray-based relative expression (scale bar, Z- scale of SDs from the cross-sample mean; blue, below the mean; yellow, above the mean) of genes that were significantly differently expressed among a minimum of two conditions. (*A*) Genes encoding proteins classified within the GO-term cell wall metabolism; (*B*) genes encoding type III peroxidases and (C) dirigents in non-colonized (NC) and mycorrhizal (M) crown roots (CR) large lateral roots (LLR) and fine lateral roots (FLR).

Fig. S5

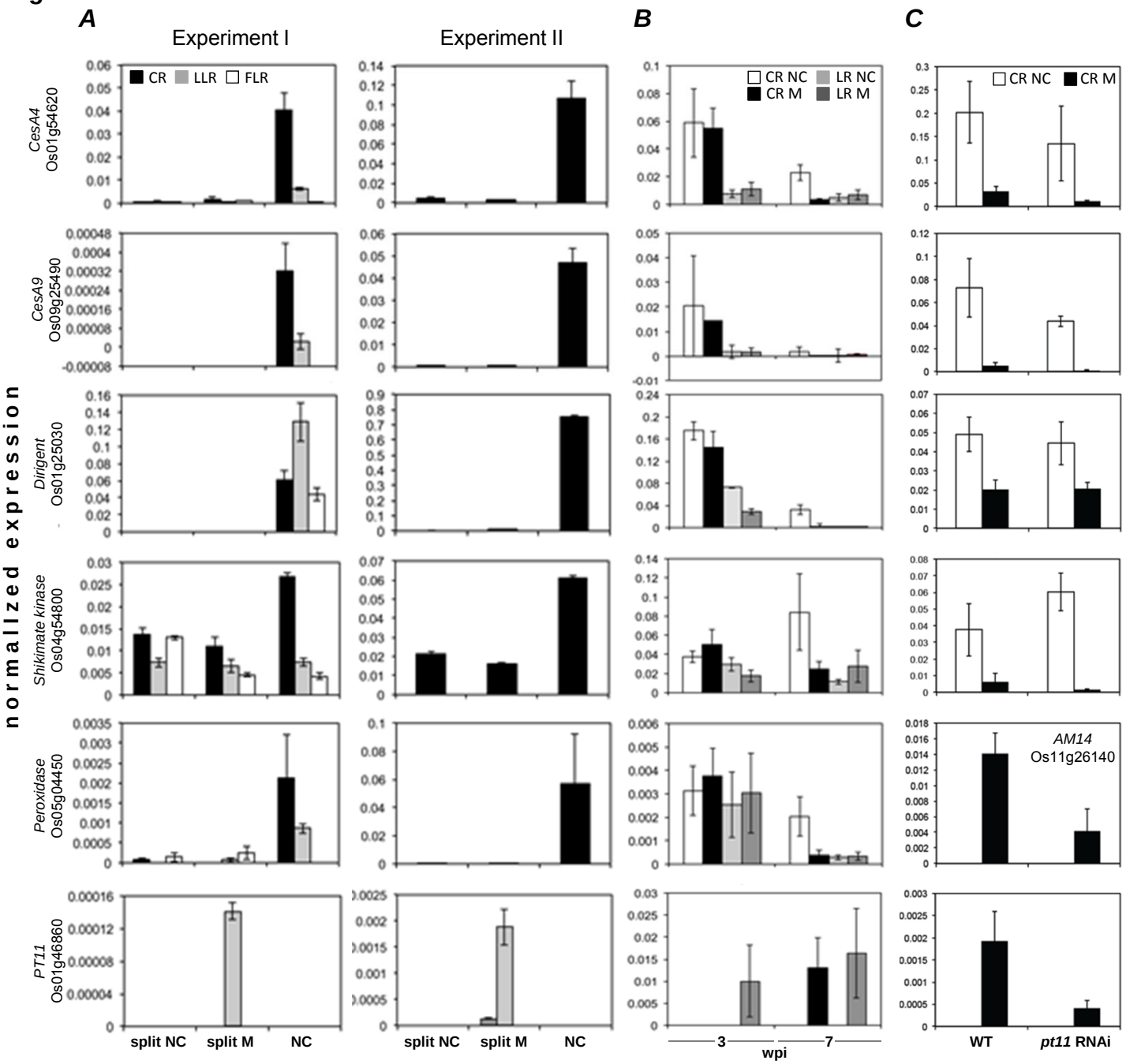

Fig. S5. Real time RT-PCR-based assessment of the regulation of the expression decrease of secondary cell wall (SCW)-related marker genes. (*A*) Systemic regulation: transcript accumulation was measured in mycorrhizal (split M) and non mycorrhizal halves (split NC) of a split root and a mock inoculated control. Means ± SD are shown for three technical replicates and two independent experiments. For each experiment roots of five plants were pooled. In experiment II gene expression was assayed only in CRs for simplicity. AM colonization is indicated by *PT11* expression. CR, crown roots (black bars); LLR, large lateral roots (grey bars); FLR, fine lateral roots (white bars). (*B*) Temporal regulation: Expression of SCW-related marker genes at 3 and 7 weeks post inoculation in crown roots (CR) and lateral roots (LR) of non-colonized (NC) and mycorrhizal (M) root systems. AM colonization is indicated by *PT11* expression. Means ± SE for three biological replicates are shown. Each biological replicate contained a pool of roots from five plants. CR NC, non-colonized crown roots (white bars); CR M, mycorrhizal crown roots (black bars); LR NC, non-colonized lateral roots (bright grey bars); LR M, mycorrhizal lateral roots (dark grey bars). (*C*) Regulation by symbiotic Pi-uptake: examined in crown roots of non-colonized (NC) and mycorrhizal (M) rice root systems of WT and *pt11* RNAi lines at 7 wpi. The expression of *PT11* and an AM specific marker gene *AM14* were also assessed. Each value represents the mean of three biological replicates ± SE (except for *pt11* NC for which two biological replicates were available). For each biological replicate crown roots of five plants were pooled.

Table S1. Description, function and literature reference of genes that are indicators of secondary cell wall (SCW) metabolism.

Gene(s)	Function	Reference
CesA4, *CesA7*, *CesA9*	Cellulose synthesis at SCW	(1)
BRITTLE CULM 1 (*COBRA*)	Cellulose deposition at SCW	(2)
Shikimate kinase	Phenylalanine biosynthesis	(3)
Phenylalanin ammonia lyase	Monolignol biosynthesis	(4)
Type III peroxidases	Monolignol polymerization	(5)
Dirigents	Monolignol polymerization	(6)
OsMYB103 (LOC_Os08g05520)	Transcription factor	(7)
OsMYB55/61 (LOC_Os01g18240)	Transcription factor	(7)

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Tab. S2. Cell wall phenolic acid (lignin precursor) composition of total non-colonized (NC) and mycorrhizal (M) root systems, root tips (one third of root length from apex of CRs, tip), and the remainder of the CRs (top) was determined by HPLC-MRM after alkaline hydrolysis followed by acid hydrolysis. Means ± SE of eight biological replicates each consisting of a pool of five root systems are shown in µg/g of cell wall residues (CWR). Significantly different values according to a t-test ($p \leq 0.05$) are shown in bold. S, H, and G refer to the phenolic acid precursors of S, H, and G lignin. S refers to the sum of syringic and sinapic acid; H refers to the sum of 4-OH-benzaldehyde, 4-OH-benzoic acid, p-coumaric acid, and caffeic acid; G refers to the sum of vanillic acid, ferulic acid, and coniferylaldehyde.

Class	**NC**			**M**		
Root	*whole*	*tip*	*top*	*whole*	*tip*	*top*
S	1.72 ± 0.49	**3.53 ± 0.15**	2.42 ± 0.24	2.10 ± 0.13	**2.11 ± 0.19**	2.78 ± 0.23
H	146.46 ± 16.38	235.56 ± 9.96	189.40 ± 22.13	156.7 ± 12.07	231.6 ± 12.25	218.22 ± 11.47
G	732.12 ± 41.06	1057.43 ± 41.06	911.23 ± 75.25	820.5 ± 65.44	1131.9 ± 67.7	1063.3 ± 65.38

Tab. S3*A*: Primers used in this study for real time RT-PCR.

Gene	**Forward**	**Reverse**
Os01g54620 (*CesA4*)	CATGCACTTGTACCCCTTCC	GTCGATCTTGACCCAGAGGA
Os09g25490 (*CesA9*)	TTCACTTCTCTCCCCCTCCT	AGCGCGATGAAGAACAAACT
Os01g25030 (*Dirigent*)	GATGGCGAGTGGAGCATAAT	TGCATGGGAGTGTAGAATGC
Os05g04450 (*Prx*)	GTGAGCAACAACCAGGTGAC	GTCTGGTCCGACGTGAACAT
Os04g54800 (*Shikimate kinase*)	CCAGGGTTTCACTCGAACAT	GCCTCAATAGCAATGGCACT

Tab. S3*B*: MRM conditions and retention times of detected compounds in hydrolysis extracts for cell-wall-bound phenolic compounds.

Compound	Transition	Collision energy (eV)	Retention time (min)
4-OH-benzaldehyde	120.86 → 91.81	25	6.65
4-OH-benzoic acid	136.87 → 92.88	11	5.55
p-coumaric acid	162.93 → 118.88	13	8.33
Vanillic acid	166.93 → 107.82	19	6.61
Coniferylaldehyde	176.99 → 133.82	19	9.45
Caffeic acid	178.96 → 134.87	15	6.88
Ferulic acid	192.99 → 133.95	15	8.76
Syringic acid	196.99 → 152.90	13	7.13
Sinapic acid	223.16 → 163.81	17	8.84

Supporting Information online

Material & Methods

Plant and fungal material and growth conditions

Oryza sativa ssp. Japonica cv. Nipponbare plants were grown and inoculated with *Rhizophagus irregularis* (formerly called *Glomus intraradices*) as described (1, 2). The plants were fertilized with half Hoagland solution containing 25 µM phosphate as described (3). The *pCesA4-GUS* line was provided by Yihua Zhu (CAS Beijing, China) (4). For comparison of control and *pt11* RNAi line (3) mutants were inoculated and surrounded by WT plants as described (2).

RNA extraction, cDNA synthesis and real time RT-PCR

RNA extraction, cDNA synthesis and real time RT-PCR were performed as described (1). Primers not described in (1) are shown in Tab. S3*A*.

Microarray hybridization, processing and differential expression analysis

For microarray hybridizations RNA was extracted with the Ambion RNaequeous micro-kit (Ambion, Austin, TX, USA) and amplified using the Nugen-ovation Pico kit (Nugen, USA) according to manufacturer's instructions. Biotin-labeled cDNA was hybridized to Affymetrix rice Genechips as described (5).

The CEL files from the 24 microarrays were imported into R/Bioconductor (6, 7) using the *affy* package (8) and normalized with the Robust Microarray Average algorithm (9). All annotation was obtained from *Ensembl Plants* (http://plants.ensembl.org/index.html). Affymetrix probes that did not map to any Ensembl Gene ID were excluded, and if more than one probe mapped to the same Gene ID, only the one with the highest Inter Quartile Range (across all samples) was kept. Presence or absence of expression for each gene was called using Affymetrix' MAS5 algorithm (`mas5calls`), which uses the control (mismatch) probes to calculate a *p*-value for a probeset being expressed above background. A single *p*-value was obtained for each gene, by combining the p-values for each condition (`pVals`) with the following R code:

```
pnorm(sum(qnorm(pVals)) / sqrt(length(pVals)))
```

All genes with a final significance of $p < 0.01$ for above-background expression were called present.

For differential expression (DE) analysis, the samples were first assigned to one of 6 values (3 tissues x 2 treatments). We then used *limma* to fit linear models to the gene expression data; using an empirical Bayes approach to reduce the standard errors towards a common value (10). DE *p*-values were adjusted for multiple testing by the Benjamini-Hochberg method, applying a cut off for a FDR of 0.1 to the output of all tests (11). The resulting numbers of DE genes were loaded into Cytoscape to build a network of the relationship between conditions. Node colour was assigned according to mycorrhizal infection status and edge weight proportional to the inverse of the DE genes. Node placement was manually adjusted to keep nodes with thicker lines (less DE genes) together and nodes with thinner lines (more DE genes) further apart.

Gene set enrichment tests

All contrasts tested for differential expression, were also tested for enrichment of gene sets. These tests were carried out using the *romer* and *mroast* functions from the *limma* package (10, 12). For statistical significance, we used the *romer* function that tests a similar hypothesis to the Gene Set Enrichment Analysis (GSEA) program (13) and the *mroast* function to find the fraction of genes in the set that are differentially expressed. Both functions use a rotation method that is suited for working with linear models (14). The gene sets that were tested were *Oryza sativa* GO terms, downloaded from the *Ensembl Plants* database (http://plants.ensembl.org/index.html). In the rotation test we used the "floormean" statistic and 9999 rotations to estimate statistical significance. Only gene sets with at most 500 genes and at least 15 genes were tested. Enrichment *p*-values were adjusted for multiple testing by the Benjamini-Hochberg method (11).

Extraction of cell wall bound phenolic compounds

Cell wall bound phenolic compounds were extracted according to the method reported with minor modifications (15). Fresh roots were ground and the frozen powder was weighed (300 mg ± 2 mg) and 1.5 ml of cold 80% aqueous ethanol solution was added for organic soluble metabolites extraction. Samples were vortexed, sonicated in a bath at room temperature (5200 Bransonic, Danbury, CT, USA) for 20 minutes, vortexed again and centrifuged at 7000 g for 2 minutes (Hettich mikrolitter D 7200, Buford, USA). The supernatant was removed and the extraction procedure was repeated once with aqueous ethanol. Then, residues were washed twice with methanol and twice with acetone following the same extraction procedure as above. The resulting purified cell wall residues (CWR) were dried for two hours at 80°C and weighed before hydrolysis, according to standard protocols. To recover the ester-linked phenolic acids, CWR were subjected to an alkaline hydrolysis. Briefly, CWR were transferred to screw-cap vials, suspended under N_2 in 5 mL of 2 M NaOH and stirred overnight at room temperature. The solution was then adjusted to a pH of 2, and phenolic acids were extracted three times with 12 mL of a mixture of cold diethyl ether (DE) and ethyl acetate (EA, 1:1 v/v). The organic layers were combined, evaporated to dryness and stored at 20°C until analysis. Then, the remaining CWR were subjected to an acid hydrolysis to recover the ether-linked phenolic acids. Two mL of concentrated HCl were added to the test tube and incubated in a water bath (80°C) for 45 minutes. The samples were allowed to cool, then liquid-liquid extraction with cold DE/EA was performed as described above.

Quantification of phenolic acids by LC-MS/MS

Residues from each hydrolysis sub-fraction were dissolved in 1 mL of 85 % aqueous methanol (HPLC grade, VWR, USA) and subjected to HPLC-MS/MS analysis. Quantification of phenolic acids was carried out using a Quattro Micro triple quadrupole mass spectrometer (Waters, Manchester, UK) interfaced with an Agilent 1100 series chromatograph (Agilent Technologies, Waldbronn, Germany) equipped with a Kinetex (Phenomenex, Torrance, CA, USA) C18 column (100 × 3 mm i.d., 2.6 µm). Separation of phenolic acids was achieved using a gradient program of 95 % of water + 0.1 % of formic acid (FA, solvent A) and 5 % of acetonitrile + 0.1 % of FA (solvent B) to 50 % A in 11.0 minutes, followed by a wash step with 95 % B for 5 minutes and a reconditioning step with 95 % A for 5 minutes for a total of 22 minutes per analysis. The flow rate was 0.5 mL/minutes, the column temperature was kept at 30° C, samples were maintained at 10°C during all analyses and injection volume was set to 10 µl. The quantification of phenolic acids was carried out in the electrospray (ESI) negative-ion (NI) mode. Tandem mass spectrometry (MS/MS) was performed in the multiple reaction monitoring (MRM) mode with the following parameters: an ESI-MS capillary voltage of 2.0kV, an extractor cone voltage of 1V and a sample cone voltage of 20V. Cone gas flow was set at 30 L/h nitrogen and desolvation gas flow was maintained at 700 L/h nitrogen. Source and desolvation temperature were set at 120 and 350°C, respectively. The argon collision gas pressure was set at 3.10^{-3} mBar. The collision parameters and reaction channels were optimized by injection of each standard solution dissolved in 50 % aqueous MeOH at a concentration of 10 µg/mL and a flow rate of 10 µL/min (Tab. S3*B*). The quantification of each compound in extracts was ensured with external calibration curves using five data points. Within the range measured (0.01-1000 µg/mL) good linear relationships were obtained for all monitored compounds (R^2 > 0.98). The dwell time for each monitored transition was 0.2 s with 0.01 s interchannel and interscan delays.

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