

Supplementary information, Data S1 Materials and Methods

Plant Growth and Mycorrhizal Inoculum

The rice genotype used was *O. sativa* ssp. *japonica* cv. *Zhonghua11*. *slr1* mutants were maintained as heterozygotes. Rice plants were inoculated with *R. irregularis* as described previously [1]. For rice germination, seeds were imbibed in water at 28 °C. Seedlings were placed in a 1:1 mixture of Terragreen (Oil-Dri Company, UK) and sand and inoculated with 250 spores per plant, harvested from the *R. irregularis* /carrot co-culture. Rice was grown at 28 °C with 12 hours light about 20000 lux and 24 °C with 12 hours dark cycles. *R. irregularis* was used in most experiments and was maintained on plates co-cultivated with carrot hairy root cultures. Colonised roots of rice were treated with 10% KOH for 15 minutes at 95 °C, followed by 6 minutes incubation in ink as previously described with some modifications [2]. Root length colonization in rice was quantified using the grid line intersect method [3] using a Nikon Eclipse 800 light microscope. For confocal microscopy, roots were stained with WGA-Alexafluor 488 and imaged with a Zeiss LSM 510 confocal microscope.

The *M. truncatula* genotype A17 was used in this study. The seeds were scarified on sand paper, surface sterilised using 10% bleach and imbibed on water agar plates at 4 °C. The seeds were germinated on the water agar plates by transferring them to room temperature in the dark and then planted in pots for mycorrhizal analysis or immediately treated with *Agrobacterium rhizogenes* for hairy root transformation [4]. Plants were grown at 22 °C with 16 hours light / 8 hours dark cycles. To test mycorrhizal colonisation roots were treated with 10% KOH for 6 minutes at 95 °C, followed by 3 minutes in ink. Different concentrations of GA3 indicated were added when plants were transferred into the soil, and the treatment of GA3 was applied every day until plants were harvested for ink staining or RNA extraction.

RNA Extraction and Gene Expression

To monitor expression of the AM induced genes, plants were inoculated with *R. irregularis* spores (250 spores/ plant), roots collected at 10, 20, 30 and 40 dpi and RNA extracted with the Plant RNAEasy Kit (Qiagen). RNAs were treated with TURBO DNA-FREE kit (Ambion) and 1 µg of each RNA was retro transcribed using SuperscriptII reverse transcriptase

(Invitrogen). Gene expression was monitored by SYBR® Green based qPCR on a Biorad thermocycler using gene specific primers (Supplementary information, Table S1). For all reactions *CYCLOPHILIN2* expression was used for normalization [1]. The analysis of Real-time PCR was as follows: after completion of 40 PCR cycles, the Ct of each sample was obtained. For each sample, the ΔCt was calculated by subtracting the Ct of cyclophilin 2 from the corresponding Ct of gene analysed. Then the $\Delta\Delta Ct$ was calculated by subtracting ΔCt of the wild type (reference sample) from the ΔCt of each sample. Thereafter $2^{-\Delta\Delta Ct}$ of each sample was obtained and was considered as the fold change. Each sample included three replicates and each experiment was repeated three times [5].

Yeast Two Hybrid and BiFC Analysis

A cDNA of 1248 bp for the *DIP1* coding DNA sequence was amplified using Gateway ® compatible primers, cloned into a pDONR207 vector by BP reaction. Full length *SLR1*, *MtRAM1* and *OsRAM1* were amplified using the primers in (Supplementary information, Table S1) and cloned into the pDONOR 207 vector by BP reaction. The resulting pENTR vectors were used in LR reactions with the pDEST-GBKT7 and pDEST-GADT7 modified vectors for Y2H and with the pGPTVII.Bar.GW-YN, and pGPTVII.Hyg.GW-YC, for BiFC.

A. tumefaciens strain C58C1 carrying the helper plasmid pCH32 was transformed with the BiFC constructs by electroporation. The strains of interest and the C58C1 strain carrying the p19 silencing suppressor plasmid [6] were brought to an optical density (OD600) of 0.2 to 0.5 with 10 mM MgCl₂ and 150 mM acetosyringone (Sigma-Aldrich). The strains were mixed and incubated at room temperature for 2 h. The *A. tumefaciens* mixture was infiltrated into *N. benthamiana* leaves as described previously [6]. The fluorescence was assayed 3-4 days after infiltration using an inverted Zeiss 510 confocal microscope. For monitoring YFP signals, an argon laser at 514 nm wave length was used for excitation.

The yeast strain AH109 was co-transformed with the destination vectors pDEST-GBKT7 and pDEST-GADT7 containing *DELLA/SLR1*, *DIP1* or *OsRAM1* and *MtRAM1* according to the LiAc transformation method [7]. The expressed proteins were then tested for interaction by dropping 7 μ L of yeast suspension on minimal synthetic dropout (SD) agar medium

containing the dropout supplement (DO) SD/-Leu-Trp, SD/-Leu-Trp-His-Ade and 30 mM 3-amino-1,2,4-triazole (Clontech).

Pull down assays

The coding sequence of *SLRI* and *OsRAM1* was cloned into pCold-TF (Takara) for the production of a His-DELLA, His-OsRAM1 fusion protein in *E.coli*. The coding sequence of *DIP1* was cloned into pMAL-C2X (NEB) for the production of a MBP-DIP1 fusion protein in *E. coli*. The His-DELLA and His-OsRAM1 fusion proteins were purified using Ni-NTA Agarose (QIAGEN). The MBP-DIP1 fusion protein was bound to the amylose resin (NEB), the resultant resin was used to capture the purified His-DELLA or His-OsRAM1 fusion protein. The monoclonal anti-MBP (Ambmart) or anti-HIS (CW Biotech) antibody was used for western blotting analysis.

Generation of Constructs for RNAi Rice Lines

For generating the double RNAi construct, the 456 bp coding region of *DIP1* was cloned into the pENTR/SD/D-TOPO vector. Afterwards, the fragment containing 456 bp coding region of *DIP1* gene was transferred into the pH7GWIIWG2 vector. The construct was introduced into rice *ZHONGHUA11* plants by *A. tumefaciens*-mediated transformation using immature embryos as material [8]. Transgenic plants were genotyped by PCR. 15 independent transformants were identified and the lines exhibiting the strongest down regulation were selected for further analysis.

Phylogenetic analyses

DIP1 homologous proteins were identified with *BLASTp*. Highly conserved DIP1 homology genes in *M. truncatula*, poplar, vine, *Arabidopsis*, *Glycine max*, *Zea mays*, *Brachypodium distachyon*, *Selaginella moellendorffii*, *Physcomitrella patens* and rice proteins were aligned and gaps were removed. Phylogenetic trees were constructed using maximum likelihood with MEGA version 4 [9].

References

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Primers	Sequence
cyclophilin2-F	AGCTCTCCTAGATCTGTGCTG
cyclophilin2-R	GCGATATCATAGAACGAGCGAC
<i>DIP1</i> -Realtime-F	CAGTGGTCGTCGTCATGTC
<i>DIP1</i> -Realtime-R	TGTTTCGTCGTCGTCGTCGTGT
<i>DIP1</i> -RNAi-F	CTCCCTTCTCATTCCCTTCTCCAC
<i>DIP1</i> -RNAi-R	TGCCCTGCCACTGCTAATCTCT
<i>DIP1</i> -F	ATGGAGACCATGTCATACCCT
<i>DIP1</i> -R	CTACTTGGGACACCAAAGAG
SLR1-F	ATGAAGCGCGAGTACCAAGAAGC
SLR1-R	TCACGCCGCGGCGACGCGCCA
SLR1-Realtime-F	GGAGGCCGTGCAGCAGGAGAACT
SLR1-Realtime-R	GCGGCGTCGAGGAGGGTGCTGTC
PT11-F	GAGAAGTTCCTGCTTCAAGCA
PT11-R	CATATCCCAGATGAGCGTATCATG
AM1-F	ACCTCGCCAAAATATATGTATGCTATT
AM1-R	TTTGCTTGCCACACGTTTTAA
AM3-F	CTGTTGTTACATCTACGAATAAGGAGAAG
AM3-R	CAACTCTGGCCGGCAAGT
AM10-F	AGAACACTTGTGGCCGTACTATAAGA
AM10-R	CCTCTCGACGAAAGTACGGACTA
AM14-F	CCAACACCGTTGCAAGTACAATAC
AM14-R	GCACTTTGAAATTGGACTGTAAGAAA
AM15-F	TCCGGCGCCACATAGTG
AM15-R	TCCGTCGCACACGAGAAG
AM34-F	TTGCCAAAAATAGAAGCATCACA
AM34-R	CATAGTACTTAAAGTGAAAGGGCAAGGT
OsRAM1-F	ATGGCCGGCGGCGGCGCGAAG
OsRAM1-R	TCAGCACCGCCACGCGGAGGC