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#### **Supplementary Figure 1. The phylogeny tree of SMAX-like (SMXL)s family**

The tree represents the phylogenetic relationship among SMXL sequences from Arabidopsis, rice, *Brachypodium distachyon*, maize, barley, *Medicago truncatula* and *Lotus japonicus*. Rice SMAX1 is marked with red circle. Arabidopsis Heat Shock Protein 101 was used as an outgroup.



## **Supplementary Figure 2. SMAX1 protein structure and sequences**

The diagram represents the SMAX1 protein structure with known domains indicated as grey boxes and their corresponding protein sequences. The potential nuclear localisation signal is highlighted in green and the potential transcript truncation site of *smax1-1* is marked between asterisks in red.



### **Supplementary Figure 3. Subcellular localisation of OsD14L, D3 and SMAX1**

Subcellular localisation of D14L, D3 and SMAX1 in rice leaf protoplast was visualised by confocal microscopy. Coding regions of each gene were fused with a fluorescent protein, either Venus or GFP as indicated, and transiently expressed under the control of the promoter of the rice *Actin1* housekeeping gene. Three protoplasts per construct were microscopically analysed. scale =  $25 \mu m$ .



#### **Supplementary Figure 4. Mutant analysis of** *SMAX1* **T-DNA insertion lines**

**a**. The diagram indicates the sites of the T-DNA insertions and primers used for reverse transcription (RT) **(b)** and quantitative RT-PCR **(c);** RB: right border.

**b**. RT-PCR amplicons indicate the presence and absence of *SMAX1* transcript in two *smax1* mutants compared to wild-type.

**c**. Quantitative RT-PCR quantified the expression levels of *SMAX1* shortly before and after T-DNA insertion sites. Relative gene expression values were normalised to the geometric mean of the three housekeeping genes, *Cyclophillin2, Ubiquitin and Glyceraldehyde 3-phosphate dehydrogenase (GAPDH),* and shown relative to *Cyclophilin2*. Each dot and red bar indicate individual plant and mean values of three replicates. Kruskal-Wallis test (p<0.05) was used, and followed by the *post hoc* tests according to the Agricolae package in the R software. Different letters represent significant difference (p-value <0.05). Degrees of freedom =2, n=3 per genotype, *SMAX1* (ED'): <sup>2</sup>=7.2, p=0.03; *SMAX1* (FF'):  $\chi^2$ =7.2, p=0.03.



#### **Supplementary Figure 5. Characterisation of symbiosis phenotype of** *smax1* **alleles**

**a.** Quantitative RT-PCR shows the expression level of *SMAX1* and the AM marker *Phosphate transporter 11 (PT11)*, in colonised wild-type and the two *smax1* mutant alleles. Kruskal-Wallis test (p<0.05) was used, and followed by the *post hoc* tests according to the Agricolae package in the R software. Different letters represent significant difference (p-value <0.05). Each dot and red bar indicate values from an individual plant and the mean value of each genotype. Degrees of freedom =2, wild-type (n=5), *smax1-1* (n=4), *smax1-2* (n=4), *SMAX1*: <sup>2</sup>=9.69, p=0.008; *PT11*: <sup>2</sup>=6.49, p=0.04.

**b.** Arbuscule (A) structures were visualised by observing colonised roots stained with Wheat Germ Agglutinin (WGA, green) fluorescent dye conjugated with Alexa-488 by confocal microscopy (i,iv). Propidium iodide (PPI, magenta) staining of the same roots for plant cell walls (ii, v) were merged (iii, vi). Images represent arbuscule structures from at least three independent plants of each genotype. Scale bar 10  $\mu$ m.



**Supplementary Figure 6. AM marker gene expression analysis of** *the smax1-1 SMAX1* **complementation line.**

Quantitative RT-PCR based analysis of the AM marker genes and the *R. irregularis* housekeeping gene *Elongation Factor1a* (*RiEF1a*) in roots of *smax1-1* mutant and the *smax1-1 SMAX1* complemented line inoculated with R. irregularis. Kruskal-Wallis test (p<0.05) was used, followed by the *post hoc* tests according to the Agricolae package of the R software. Different letters represent significant difference (p-value <0.05). Each dot and red bar indicate values from an individual plant and the mean value of each genotype. Degrees of freedom =3, wild-type (n= 4), *smax1* (n=4), *smax1-1 SMAX1* (F3, n= 6), *smax1-1* (F3, n=4), *RiEF1a*: <sup>2</sup>=4.78, p=0.19; *AM1*: <sup>2</sup>=12.50, p=0.006; *AM3*: <sup>2</sup>=15.07, p=0.002; *PT11*: <sup>2</sup>=13.18, p=0.004; *AM14*: 2=10.61, p=0.014.



**Supplementary Figure 7. Mutant analysis of** *dwarf14-like (d14l)* **CRISPR/Cas9 edited lines**

**a.** The diagram shows the site of sgRNA complementarity, gene editing event and primer sets used for *D14L* gene expression analysis. **b.** Detail of *d14l* CRISPR/Cas9 edited sequences. **c**. Quantitative RT-PCR based analysis of the expression levels of *D14L* before and after CRISPR/Cas9 editing. Kruskal-Wallis test (p<0.05) was used, followed by the *post hoc* tests according to the Agricolae package of the R software. Different letters represent significant difference (p-value <0.05). Each dot and red bar indicate values from an individual plant and the mean value of each genotype (n=3). Degrees of freedom =5, n=3 for each genotype, *D14L* (F1R1):  $\chi^2$ =16.02, p=0.007; *D14L* (F2R2):  $\chi^2$ =16.02, p=0.007.



#### **Supplementary Figure 8. AM colonisation of** *dwarf14-like (d14l)* **CRISPR/Cas9 edited lines**

**a.** AM colonization levels of previously published *d14l* mutant (*hebiba AOC*) was compared with new *d14l* CRISPR lines at 7 wpi with 300 spores. Each bar indicates the average percentage value of the respective fungal structure. Total, total colonization, H, hyphopodia, IH, intraradical hyphae, A, arbuscules. Kruskal-Wallis test (p<0.05) was used, followed by the *post hoc* tests according to the Agricolae package of the R software. Different letters represent significant difference (p-value <0.05). Degrees of freedom = 5, n=3 per genotype, Total:  $\chi^2$ =14.87, p=0.01; H:  $\chi^2$ =12.24, p=0.03; IH:  $\chi^2$ =14.89, p=0.01; A:  $\chi^2$ =16.54, p=0.005.

**b.** Micrographs of trypan blue stained roots for fungal structures. V, vesicles, A, arbuscule, EH, extraradical hyphae; scale 200 µm. Images represent at least three independent plants per genotype.



**Supplementary Figure 9. AM marker gene expression analysis of** *dwarf14-like (d14l)* **CRISPR/Cas9 edited lines**

Quantitative RT-PCR based analysis of the expression levels of *R. irregularis* housekeeping gene *Elongation Factor1a* (*RiEF1a*), AM marker genes (*AM1, AM3, PT11 and AM14*) and *SMAX1* in roots inoculated with *R. irregularis* (compare Supplementary Figure 8). Each dot and red bar indicate values from an individual plant and the mean value of each genotype. Relative gene expression values were normalised against the geometric mean of the rice housekeeping genes, *Cyclophillin2, Ubiquitin* and *GAPDH,* and are shown relative to *Cyclophillin2*. One-way ANOVA (p<0.05) was used, followed by the *post hoc* tests according to the Agricolae package of the R software. Different letters represent significant difference (p-value <0.05), n=3 per genotype, F (*RiEF1a*)<sub>5,12</sub> =71.35; F (*AM1*) <sub>5,12</sub> = 499.39; F(*AM3*)5,12=1134.7; F(*PT11*)5,12=347.26; F(*AM14*)5,12=280.26; F(*SMAX1*)5,12=22.34, p<00001).



# **Supplementary Figure 10. AM marker gene expression analysis of** *d14l/smax1* **double knockout mutant**

Quantitative RT-PCR based analysis of the expression levels of *R. irregularis* housekeeping gene *Elongation Factor1a* (*RiEF1a*), AM marker genes (*AM1, AM3, PT11 and AM14*), *SMAX1* and *D14L* in roots inoculated with *R. irregularis* (compare Figure 2). Each dot and red bar indicate values from an individual plant and the mean value of each genotype. Relative gene expression values were normalised against the geometric mean of the rice housekeeping genes, *Cyclophillin2, Ubiquitin* and *GAPDH,* and are shown relative to *Cyclophillin2*. Kruskal-Wallis test (p<0.05) was used, followed by the *post hoc* tests according to the Agricolae package of the R software. Different letters represent significant difference (p-value <0.05). Degrees of freedom =5, Dongjin wild-type (n=4), *smax1*(n=4), Nipponbare wild-type (n=4), *d14l*

(n=4), *d14l/smax1* (n=5), *d14l*/*SMAX1* (n=8), *SMAX1*: <sup>2</sup>=25.56, p=0.0001; *D14L*: <sup>2</sup>=13.07, p=0.02; *RiEF1a*: <sup>2</sup>=24.92, p=0.0001; *AM1*: <sup>2</sup>=24.8, p=0.0002; *AM3*: <sup>2</sup>=24.18, p=0.0002; *PT11*: <sup>2</sup>=23.54, p=0.0003.



**Supplementary Figure 11. AM marker gene expression analysis of** *d3/smax1* **double knockout mutant**

Quantitative RT-PCR based analysis of the expression levels of *R. irregularis* housekeeping gene *Elongation Factor1a* (*RiEF1a*), AM marker genes (*AM1, AM3, PT11 and AM14*), *SMAX1* and *D3* in roots inoculated with *R. irregularis* (compare Figure 2). Each dot and red bar indicate values from an individual plant and the mean value of each genotype. Relative gene expression values were normalised against the geometric mean of the rice housekeeping genes, *Cyclophillin2, Ubiquitin* and *GAPDH,* and are shown relative to *Cyclophillin2*. Kruskal-Wallis test (p<0.05) was used, followed by the *post hoc* tests according to the Agricolae package of the R software. Different letters represent significant difference (p-value <0.05). Degrees of freedom =5, Dongjin wild-type (n=5), *smax1* (n=5), Shiokari wild-type (n=5), *d3* (n=4), *d3/smax1* (n=5), *d3*/SMAX1 (n=8), *SMAX1*: <sup>2</sup>=26.01, p=0.0001; *D3*: <sup>2</sup>=24.09, p=0.0002; *RiEF1a*: <sup>2</sup>=24.81, p=0.0002; *AM1*: <sup>2</sup>=24.61, p=0.0002; *AM3*: <sup>2</sup>=25.49, p=0.0001; *PT11*: 2=23.1, p=0.0003.



## **Supplementary Figure 12. SMAX1-GFP protein level in** *d14l* **and** *d3* **protoplasts**

Western blot showing protein levels of SMAX1-GFP in leaf protoplasts of *d14* and *d3* mutant and corresponding wild-type cultivars Nipponbare and Shiokari, respectively. Actin served as a loading control and was detected by an anti-Actin antibody.



## **Supplementary Figure 13. Mesocotyl length of** *smax1, d3* **and d3/***smax1* **double knockout**

**a**. Mesocotyl length of 7-day old seedlings. The center line of the box plot indicates median; box limits are upper and lower quartiles; whiskers are 1.5x interquartile range; open circles represent outliers. Kruskal-Wallis test (p<0.05) was used followed by the *post hoc* tests according to the Agricolae package in R software. Different letters represent significant difference (p-value <0.05), degree of freedom =5, Dongjin wild-type (n=13), *smax1 (*n=10), Shiokari wild-type (n=14), *d3 (*n=9), *d3/smax1 (*n=10), *d3*/*SMAX1* (n=10), <sup>2</sup>=38.25, p=3.4E-7. **b.** Representative images of the mesocotyl for each genotype as indicated. WT, wild-type, Dongjin and Shiokari are the corresponding cultivars of the respective mutants. F5 indicates the 5th generation progeny from the initial crossing. Scale bar 4 mm. Images represent mesocotyls of all plants analysed per genotype.



## **Supplementary Figure 14. Validation of RNAseq data analysis by quantitative RT- PCR and gene induction in response to AM symbiosis development in the wild-type**

**a**. Analysis of the expression levels representative differentially expressed genes corresponding to the karrikin marker *DLK2a*, the LysM RLKs *NFR5* and *LYK1*, the kinase *KIN6* and the symbiosis phosphate transporter *PT11*. The qRT-PCR experiments were performed with cDNAs made from the RNA used for RNAseq sample library production (experiment I) and another independent experiment (experiment II). Each dot and red bar indicate values from an individual plant and the mean value of each genotype (n=3). Relative gene expression values were normalised against the geometric mean of the rice housekeeping genes*, Cyclophillin2, Ubiquitin* and *GAPDH,* and are shown as fold-change (log2 scale) compared to wild-type (Dongjin). One-way ANOVA (p<0.05) was used followed by the *post hoc* Tukey tests according to the Agricolae package of the R software. Different letters represent significant

difference (p-value <0.05). For Experiment I, n=3 per genotype,  $F(DLK2)_{5,12}$  =143.46;  $F(NFR5)_{5,12}$  = 30.07; F(*LYK1*)5,12=5.84; F(*KIN6*)5,12=7.79; F(*PT11*)5,12=12.3, p<0.01). For Experiment II, n=3 per genotype, F(*DLK2*)5,12 =165.62; F(*NFR5*) 5, 12 = 64.19; F(*LYK1*)5,12=40.66; F(*KIN6*)5,12=28.33; F(*PT11*)5,12=7.16, p<0.003). **b** and **c.** Microscopic observation (b) and qRT-PCR based expression analysis of marker genes showing significant gene induction in wild-type cultivar Dongjin colonised by *R.irregularis* (c)*.* Total, total colonization, H, hyphopodia, IH, intraradical hyphae and A, arbuscule. Expression levels of the *R. irregularis* housekeeping gene, *Elongation Factor 1a* (*RiEF1a*), AM marker genes, *PT11* and four genes that were induced in *smax1* determined by qRT-PCR. Mock, non-inoculated control; myc, colonised by *R. irregularis*. Each dot and red bar indicate values from an individual plant and the mean value of each genotype (n=4). Mann-Whitney U-test (p<0.05) was used and asterisks above each bar represent statistically different groups compared to mock **(c)**.



# **Supplementary Figure 15.** *SMAX1***-regulated gene expression analyses in the** *smax1SMAX1* **complementation line.**

Four genes that were induced in *smax1* are quantified by qRT-PCR. Wild-type (Dongjin), *smax1* mutant and the complementation line (*smax1SMAX1*). Each dot and red bar indicate values from an individual plant and the mean value of each genotype (n=4-8). Relative gene expression values were normalised against the geometric mean of the rice housekeeping genes, *Cyclophillin2, Ubiquitin* and *GAPDH,* and are shown relative to *Cyclophillin2*. Kruskal-Wallis test (p<0.05) was used, followed by the *post hoc* tests according to the Agricolae package of the R software. Different letters represent significant difference (p-value <0.05). Degrees of freedom =2, wild-type (n= 3), *smax1* (n=4), *smax1-1 SMAX1* (n= 5), *DLK2a*: <sup>2</sup>=9.69, p=0.008; *NFR5*: <sup>2</sup>=9.69, p=0.008; *LYK1*: <sup>2</sup>=6.89, p=0.032; *KIN6*: 2=9.69, p=0.008.





Analysis of the expression levels six SMAX1-regulated genes in *d14l* including three strigolactone biosynthesis genes *DXS2, D27* and *CYP11A2 (Os01g0700900)*. The qRT-PCR experiment was performed with cDNA from mock-inoculated control roots. Each dot and red bar indicate values from an individual plant and the mean value of each genotype (n=3). Relative gene expression values were normalised against the geometric mean of the rice housekeeping genes*, Cyclophillin2, Ubiquitin* and *GAPDH,* and is shown relative to *Cyclophillin2*. Two-tailed student t-test (p<0.05) was used according to the Agricolae package of the R software. Asterisk represents significant difference (p-value \*<0.05, \*\*<0.01, NS, not significant).



**Supplementary Figure 17. Strigolactone biosynthesis gene expression by quantitative RT-PCR** Analysis of the expression levels three strigolactone biosynthesis genes *DXS2, D27* and *CYP711A2 (Os01g0700900)*. The qRT-PCR experiment was performed with cDNA from RNA of mock-inoculated control roots from two independent experiments, from the same RNA used for RNAseq sample library production (experiment I) and from a second experiment (experiment II). Each dot and red bar indicate values from an individual plant and the mean value of each genotype. Relative gene expression values were normalised against the geometric mean of the rice housekeeping genes*, Cyclophillin2, Ubiquitin*  and *GAPDH,* and are shown as fold-change (log2 scale) relative to wild-type (Dongjin cultivar) . One-way ANOVA (p<0.05) was used followed by the *post hoc* Tukey tests according to the Agricolae package of the R software. Different letters represent significant difference (p-value <0.05). For Experiment I, n=3 per genotype, F(*DXS2*)5,12 =44.22; F(*D27*) 5, 12 = 17.28; F(*CYP711A2*)5,12=45.96, p<4.1E-05). For Experiment II, n=3 per genotype, F(*DXS2*)<sub>5,12</sub> =21.24; F(*D27*) <sub>5,12</sub> = 36.59; F(*CYP711A2*)<sub>5,12</sub>=46.8, p<1.4E-05).





The level of two SLs, 4-DO (a) and MeO-S-DS is1 (b), were measured in root exdudates of 4-week old rice grown under low phosphate (40 uM) conditions. Each dot represents one biological replicate consisting of 2-5 plants. For statistical analysis, Kruskal-Wallis test (p<0.05) was used, followed by the *post hoc* tests according to the Agricolae package of the R software. Different letters represent significant difference (p-value <0.05). ND not-detected. For 4-DO, degree of freedom=3, n=3-4 per genotype,  $\chi^2$ =8.7, p=0.034. For MeO-S-DS is1, degree of freedom=3; n=3-4 per genotype,  $\chi^2$ =11.83, p=0.008.



**Supplementary Figure 19. Gene ontology enrichment analysis of genes down-regulated by smax1 mutation.**

**a.** Gene Ontology (GO) enrichment analysis of the *smax1*-DOWN gene list. The gene enrichment rate is shown in *p*-value scale (<0.05), where lower values indicate a more significant enrichment. **b.** Relative gene expression of *smax1*-DOWN genes belonging to the GO-term 'cell wall organisation' was visualised relative to mean median-value (bar, z-score 0). The decreased gene expression after AM colonization is shown in fold-change (FDR adjusted p<0.1) relative to non-colonised crown roots  $^1$ .

## **Supplementary Table 1. Mapping summary of the RNAseq reads**





### **Supplementary Table 2. Functionally characterised** *smax1-***UP genes in AM symbiosis**

<sup>a</sup> Group corresponds to Fig.4a.Group I; induced in *smax1* and by AM symbiosis, Group II; AM conserved genes induced in smax1, Group III; AM conserved genes induced in *smax1* and by AM symbiosis, Group IV; induced in *smax1*

<sup>b</sup> AM conserved genes <sup>13</sup>.

<sup>c</sup> Rice crown roots, fold-change (mock vs inoculated), FDR P<0.1 <sup>1</sup>

d References characterized in rice were provided only.

### Supplementary Table 3. Primers used in this study





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