**INTRODUCTION**

Strigolactones (SLs) are carotenoid-derived hormones characterized by an enol-ether bridge connecting a lactone ring (D-ring; fig. S1) (1) in R configuration to a structurally variable second moiety that consists of a tricyclic lactone ring (ABC-ring) in canonical SLs, while noncanonical SLs have variable structures based on a β-ionone ring (A-ring) (fig. S1) (2). SLs are a major determinant of plant architecture and are involved in many other biological processes. Among other phenotypes, mutants affected in SL biosynthesis are characterized by increased branching/tillering, shorter shoot (dwarf), and decreased primary root length (2–4).

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In addition, when exposed to nutrients deficiency, in particular phosphate, plant roots release SLs to attract arbuscular mycorrhiza fungi (AMF) for establishing the plant-AMF symbiosis, the most common type of plant symbiosis that significantly increases the uptake of nutrients and water from the soil (5–7). However, canonical SLs, the first found type of these signaling molecules, were identified as the host-derived signal that stimulates seed germination in root parasitic weeds, such as Orobanche and Striga spp. (8). These weeds are obligate parasites that developed the ability to sense SLs as a seed germination signal enabling the coordination of their life cycle with the presence of an available host in the close vicinity (9). Infestation by root parasitic plants, such as Striga hermonthica, is a severe problem for agriculture and a major threat for global food security, in particular in Africa where it causes more than U.S. $7 billion annual losses in cereal production (10, 11).

The availability of high-branching mutants of monocot and dicot plant species (12–16) paved the way for finding the hormonal function of SLs and enabled later the elucidation of their biosynthesis (fig. S2). SL biosynthesis starts with the reversible isomerization of all-trans- into 9-cis-β-carotene, catalyzed by DWF27 (17–18). This step is followed by cleavage and rearrangement reactions mediated by the CAROTENOID CLEAVAGE DIOXGENASE 7 and 8 (CCD7 and CCD8), which yield carlactone (CL), the core intermediate of SL biosynthesis (18, 19). The discovery of CL unraveled the presence of the noncanonical SLs that were unknown before. Different modifications of CL, which are catalyzed by cytochrome P450 monoxygenases (CYP), in particular MORE AXILLARY GROWTH1 (MAX1) from the CYP711A clade, and other enzymes, give rise to the structural diversity of the more than 30 natural canonical and noncanonical SLs (16, 20–22).

Rice contains five MAX1 homologs: Os01g0700900 (OsMAX1-900), Os01g0701400 (OsMAX1-1400), Os01g0701500 (OsMAX1-1500), Os02g0221900 (OsMAX1-1900), and Os06g0565100 (OsMAX1-5100) (23, 24), with a truncated OsMAX1-1500 in the Nipponbare cv. (24).
In vitro studies and transient expression in *Nicotiana benthamiana* showed that all functional Nipponbare OsMAX1 enzymes (OsMAX1-900, OsMAX1-1400, OsMAX-1900, and OsMAX1-5100) can convert CL into carlactonic acid (CLA) that is transformed into the canonical SLs 4-deoxyorobanchol (4DO) and then orobanchol by sequential action of OsMAX1-900 and OsMAX1-1400 (Fig. 1A) (25, 26). In this work, we investigated the biological function of canonical SLs in rice.

**RESULTS AND DISCUSSION**

For this purpose, we generated two biallelic homozygote OsMAX1-900 knockout (KO) lines (Os900-KO: Os900-32 and Os900-34) disrupted in the biosynthesis of 4DO and orobanchol, the only rice canonical SLs (1), through introducing CRISPR-Cas9–induced deletion, point mutation, and frameshift mutations (Fig. 1B). We first quantified 4DO and orobanchol in roots and root exudates of hydroponically grown and phosphate-starved mutants by liquid chromatography–tandem mass spectrometry (LC-MS/MS) (Fig. 1C and fig. S3, A and B). 4DO and orobanchol were undetectable in both lines, confirming in planta the role of OsMAX1-900 as the rice 4DO synthase (25, 26) and that 4DO is the exclusive precursor of orobanchol in rice. Besides the absence of 4DO and orobanchol, exudates of the mutant lines showed around 96% decrease in the content of a non-canonical SL tentatively identified as methyl 4-oxo-carlactonoate (4-oxo-MeCLA) (fig. S3C). This noncanonical SL, previously described as methoxy-5-deoxystrigol isomer (27), was also not detectable in the Os900-KO roots (fig. S3C). On the basis of the ion peak characteristic of the D-ring at 97.028, we also identified a novel SL “CL + 30” with a molecular formula C_{19}H_{24}O_{5} [mass/charge ratio (m/z) 333.16989 as positive ion [M + H]^+), calculated for m/z 333.16965], which was present at high levels in the Os900 mutants (Fig. 1D and fig. S3C). Feeding Os900-34 seedlings with [^{13}C]3-labeled CL confirmed that CL + 30 is a downstream product of CL (fig. S4); however, the enzyme responsible for the production of this metabolite remains elusive. To check the possibility that rice plants may compensate for the absence of functional MAX1-900 by increasing the expression of other MAX1 homologs and such increase is the reason for the accumulation of CL + 30, we determined the level of their transcripts in mutant roots. However, we did not detect significant changes in level of these transcripts, compared to wild type (WT; fig. S5). Hence, we do not have any hint for the involvement of MAX1 enzymes in the formation of CL + 30. The higher accumulation of CL + 30 in Os900-KO lines (fig. S3B) indicated that it might be a substrate of OsMAX1-900. We confirmed this assumption by expressing OsMAX1-900 in yeast cells and feeding them with a CL + 30–containing fraction. After incubation and LC-MS/MS analysis, we detected a reduction in CL + 30 content and its conversion into a novel metabolite eluting at 6.1 min...
Moreover, our results indicate that noncanonical SLs are like-
cently published study on the role of orobanchol in tomato (

determinant of the tiller number in rice, which is in line with a re-
In conclusion, these data suggest that canonical SLs are not a major

does not depend on canonical SLs in its growth-promoting activity.

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lacking 4DO and orobanchol. Therefore, we set out to identify chemical(s) that inhibit canonical SL biosynthesis in rice.

TIS108 is an inhibitor of SL biosynthesis, which contains a 1H-1,2,4-triazole moiety (Fig. 3A) that can bind to the heme iron of P450s, such as MAX1 enzymes, and potentially impede their function (36). TIS108 inhibited the conversion of CL to CLA to 4DO by OsMAX1-900 [half maximal inhibitory concentration (IC50) = 0.15 μM, for both conversions], and of 4DO to orobanchol by OsMAX1-1400 (IC50 = 0.02 μM) (Fig. 3A), when added to assays with microsomes prepared from yeast cells overexpressing the corresponding MAX1 enzyme. We could not determine whether TIS108 also affects the activity of OsMAX1-5100 and OsMAX1-1900, as we did not detect the reported conversion of CL to CLA, neither with native nor with codon-optimized OsMAX1-5100 and OsMAX1-1900 (fig. S20).

To confirm the effect of TIS108 on the biosynthesis of canonical SLs in planta and to check its impact on plant growth and architecture, we applied the inhibitor to hydroponically grown rice seedlings under phosphate starvation. TIS108 treatment caused a significant decrease of 4DO, orobanchol, and 4-oxo-MeCLA level and an accumulation of CL + 30 (Fig. 3B and fig. S21). Seedlings of the rice d14-1 SL-perception mutant, which contains higher amounts of SLs due to the absence of a negative feedback regulation, showed similar responses to TIS108 treatment, i.e., a decrease of canonical SLs in roots and root exudates and an enhancement in CL + 30 level (fig. S22). The application of TIS108 to 2-week-old rice WT seedlings grown in hydroponics (fig. S23) or soil (Fig. 3, C and D) did not cause phenotypic alterations, compared to the mock. We also investigated the effect of TIS108 on rice transcriptome, using RNA sequencing (data file S1). None of the identified 174 up-regulated and 107 down-regulated differentially expressed genes in TIS108-treated rice (tables S1 and S2) was related to tillering or SL biosynthesis. Although the effect of TIS-108 on meristematic area might be plausible, this result is in line with the absence of significant morphological changes upon TIS108 treatment (table S3). Furthermore, we investigated the impact of TIS108 on AM symbiosis. Application of this inhibitor at a 10 μM concentration to plants grown in sand containing R. irregularis unraveled a colonization pattern similar to that observed with the Os900-KO mutants, reported through the expression level of OsPT11 transcript. TIS108 caused a delay in mycorrhization at 10 dpi, which was recovered at 20 dpi. However, by the end of the experiment, TIS108-treated plants showed a tendency toward reduction of OsPT11 transcript level, compared to WT (fig. S24). Next, we investigated whether TIS108 can be used for reducing Striga infestation. For this purpose, we exposed rice grown in Striga-infested soil to TIS108 at concentrations of 0, 0.0782, 0.235, and 0.782 mg/liter (total

Fig. 2. Evaluation of rhizospheric interactions. Effect of Os900-KO lines on the arbuscule formation (A and B) and the germination of root parasitic weeds [(C) Striga and (D) Phelipanche]. The values are represented as the means ± SD for the number of biological replicates [(A) and (B), n = 4; (C) 2 < n < 4; and (D) n = 3]. Scale bars, 10 μm. The statistical significance is determined by one-way analysis of variance (ANOVA) and Tukey’s multiple comparison test. Arbuscule formation of R. irregularis was quantified by measuring the expression of marker gene (OsPT11) (A). (B) Arbuscule formation at 35 dpi. Arb, arbuscule-containing cells; Erm, extraradical mycelium.
amounts) over a 7-week time period. Results obtained showed a reduction of Striga emergence in a dose-dependent manner (Fig. 4, A to E, and figs. S25 and S26). We did not observe this decrease when we added the SL analog MP1 (29) to the TIS108 treatment, suggesting that the lower Striga emergence detected with TIS108 alone is a result of lower level of germination stimulants in the root exudates. Lower infestation protected the rice plants from Striga-induced growth inhibition (Fig. 4A), leading to number of tillers and spikes, plant height, grain yield, and grain number similar to those of WT rice grown in Striga-free soil and without TIS108 treatment.

Fig. 3. TIS108 is an OsMAX1 inhibitor. (A) Structure of TIS108 and inhibition of the activity of rice MAX1s by TIS108. Different substrates (CL, CLA, and 4DO) and concentrations of TIS108 were incubated with MAX1-containing yeast microsomes. Assay extracts and authentic standard controls were analyzed by liquid chromatography–tandem mass spectrometry. (B) TIS effect on canonical SLs, 4DO and orobanchol, in root exudates of WT grown under constant low-Pi conditions. The data are presented as means ± SD of five biological replicates. Asterisk indicates significant difference without (mock) and with 10 μM TIS108 treatment (TIS108) (**P < 0.001 and ****P ≤ 0.0001, Student’s t test). (C) Three-month-old rice plants treated with TIS108. Scale bar, 10 cm. (D) Tiller number and plant height of plants from (C).
(Fig. 4, B to E, and figs. S24 and S25). We also tested the effect of TIS108 on Indica rice and sorghum, major crops in Striga-infested regions in Africa. Here again, we observed lower Striga germination—inducing activity of the exudates isolated from TIS108-treated plant rice (fig. S27). Overall, the application of TIS108 mimics the effect of knocking out MAXI-900 in the Os900 mutants (fig. S28), with respect to the level of canonical SLs and biological activity of root exudates, suggesting that rice canonical SLs are rhizospheric signals rather than the tillering-inhibitory hormone.

Together, we used genetic and chemical strategies to manipulate rice SL compositions, which allowed us to distinguish the biological functions of canonical and noncanonical SLs in rice. Our findings unraveled the possibility of reducing Striga infestation by gene editing or chemical treatment without significantly affecting host's morphology and growth or its ability to establish AM symbiosis. Direct application of TIS108 and using gene editing represent promising strategies for alleviating the threat posed by Striga and other root parasitic plants to global food security.

**MATERIALS AND METHODS**

**Plant and fungi materials**

Rice (Oryza sativa, cv. Nipponbare) mutant lines were generated in previous studies: $d17$ (dl-2) (28) and $d10$ (cv. Shioikari) (36). Their respective WT backgrounds were used in the experiments.

*R. irregularis* DAOM 197198 was obtained from Agronutrition, in Labège, France. *S. hermonthica* seeds were collected from sorghum fields during the 2012 rainy season in Sudan and were provided by A. Gabbar Babiker. *P. ramosa* seeds were provided by P. Semier, Université de Nantes, France.

**Generation of Os900-KO plants**

Rice (O. sativa L. ssp. japonica cv. Nipponbare) OsMAX1-900 (Os01g0700900/JX235697) gene was targeted by CRISPR-Cas9 guided by two guide RNAs [gRNAs; single gRNA1 (sgRNA1), 5′-ggagtaatg-ggcctcatcctc-3′ and sgRNA2, 5′-aattctcctgttcatcagaa-3′], designed using the CRISPR-PLANT database (28). The construction of the tRNA-gRNA-Cas9 cassette was accomplished through Golden Gate assembly into rGEB32 binary vector containing hygromycin resistance gene (29). Induced Nipponbare calli, from mature seeds, were transformed with Agrobacterium tumefaciens EHA105 culture containing the plasmid of interest, selected and regenerated in the EHA105 culture before further analysis (seedling phenotyping).

**Hydropenic culture of rice seedlings**

Seeds from T$_3$ homozygous plants were first surface-sterilized in 2% sodium hypochlorite ($v/v$) with 0.01% Tween 20 for 20 min under gentle agitation before being rinsed generously with sterile water and germinated overnight in the dark (30°C). The pregerminated seeds were then transferred to round petri dishes containing two sheets of sterile Whatman filter paper and 5 ml of half-strength Murashige and Skoog (MS) (PhytoTechnology Laboratories, catalog no. M519) solution (pH 5.7) and incubated for another 2 days in the dark at 30°C. Last, 15 ml of modified Hoagland nutrient solution adjusted to pH 5.8 (0.4 mM KH$_2$PO$_4$·3H$_2$O, 5.6 mM NH$_4$NO$_3$, 0.8 mM MgSO$_4$·7H$_2$O, 0.8 mM K$_2$SO$_4$, 0.18 mM FeSO$_4$·7H$_2$O, 0.18 mM Na$_2$EDTA·H$_2$O, 1.6 mM CaCl$_2$, 0.8 mM KNO$_3$, and micronutrients (0.023 mM H$_2$BO$_3$, 4.5 μM MnCl$_2$·4H$_2$O, 0.3 μM CuSO$_4$·5H$_2$O, 0.1 μM ZnCl$_2$, and 0.1 μM Na$_2$MoO$_4$·2H$_2$O) was added, and seedlings were incubated in a Percival for 5 days (day/night temperature of 28°C/22°C and a 12-hour photoperiod and 200 μmol photons m$^{-2}$ s$^{-1}$).

The setup of the hydropenic culture consisted of 50-ml black tubes with a perforated cap containing in its center a 1.5-ml bottomless Eppendorf tube, into which the 1-week-old seedlings were transferred. The nutrient solution provided with (+Pi) or without (−Pi) 0.4 mM K$_2$HPO$_4$·3H$_2$O. −Pi conditions were achieved by feeding the seedlings with +Pi for 2 weeks, followed by 1 week of −Pi treatment before further analysis (seedling phenotyping). The solutions were changed every 3 days.

For low-Pi conditions, the same procedure was performed but with replacing the half-strength MS solution by 5 ml of modified Hoagland nutrient solution adjusted to pH 5.8 and containing 0.004 mM K$_2$HPO$_4$·3H$_2$O (low Pi), right after the sterilization step. Plants were kept for 3 weeks in low-Pi solution.

**Phenotyping in pots and rhizotron**

For phenotyping of Os900 mutants, seedlings were transferred into pots filled with soil containing half-strength modified Hoagland nutrient solution. The nutrient solution consisted of 5.6 mM NH$_4$NO$_3$, 0.8 mM MgSO$_4$·7H$_2$O, 0.8 mM K$_2$SO$_4$, 0.18 mM FeSO$_4$·7H$_2$O, 0.18 mM Na$_2$EDTA·2H$_2$O, 1.6 mM CaCl$_2$·2H$_2$O, 0.8 mM KNO$_3$, 0.023 mM H$_2$BO$_3$, 0.0045 mM MnCl$_2$·4H$_2$O, 0.0003 mM CuSO$_4$·5H$_2$O, 0.0015 mM ZnCl$_2$, 0.0001 mM Na$_2$MoO$_4$·2H$_2$O, and with or without 0.4 mM K$_2$HPO$_4$·2H$_2$O, resulting in the +Pi and −Pi medium, respectively. The pH of the solution was adjusted to 5.8, and the solution was applied every third day. On day 56, phenotypic data were recorded. The plants were grown in a greenhouse from February to April 2020, in Thuwal (Saudi Arabia).

For observing the root phenotypes of the Os900 mutants in the rhizotron system (48 cm by 24 cm by 5 cm), 3-day-old seedlings were grown in soil with Hoagland nutrient solution containing 0.4 mM K$_2$HPO$_4$·2H$_2$O (+Pi) for 2 weeks. The solution was changed every other day with fresh nutrient solution. Root length, angle, and surface area were analyzed with the ImageJ software.

**Qualitative and quantitative analysis of SLs in root exudates, root tissues, leaves, and shoot base**

Analysis of SLs in rice root exudates and root tissues was performed following the protocol described by Wang et al. (30, 37). Briefly, collected 50-ml root exudates of two seedlings grown together in one tube and spiked with 0.672 ng of D$_6$-5DS were brought on a C$_{18}$−Fast Reversed-Phase SPE column (500 mg/3 ml; GracePure) pre-conditioned with 3 ml of methanol and 3 ml of water. After washing...
with 3 ml of water, SLs were eluted with 5 ml of acetone. The SL fraction was concentrated to SL aqueous solution (~1 ml), followed by 1 ml of ethyl acetate extraction. SL-enriched organic phase (750 μl) was dried under vacuum. For analysis of root tissues, around 25 mg of lyophilized and ground root tissues spiked with 0.672 ng of D<sub>5</sub>-5DS was extracted twice with 2 ml of ethyl acetate in an ultrasonic bath (Branson 3510 ultrasonic bath) for 15 min, followed by centrifugation for 8 min at 3800 rpm at 4°C. The two supernatants were combined and dried under vacuum. The residue was dissolved in 50 μl of ethyl acetate and 2 ml of hexane, followed by a Silica Cartridges SPE column (500 mg/3 ml; HyperSep) purification. After washing with 3 ml of hexane, SLs were eluted in 3 ml of ethyl acetate and evaporated to dryness under vacuum. The same procedure was used for shoot base (shoot-root junction) extraction, except for the tissue powder preparation: 12 fresh shoot bases were pulled together and manually grinded while preserved in liquid nitrogen. The entire sample was used during the extraction. The final extract was redissolved in 100 μl of acetonitrile:water [25:75 (v/v)] and filtered through a 0.22-μm filter for LC-MS/MS analysis.

SLs were identified by using the UHPLC-Orbitrap ID-X Tribrid Mass Spectrometer (Thermo Scientific Altis) with a heated-electrospray ionization source (H-ESI). Chromatographic separation was achieved on the Hypersil GOLD C<sub>18</sub> Selectivity HPLC Column (150 mm by 4.6 mm; 3 μm; Thermo Fisher Scientific) with mobile phases consisting of water (A) and acetonitrile (B), both containing 0.1% formic acid, and the following linear gradient (flow rate, 0.5 ml/min): 0 to 15 min, 25 to 100% B, followed by washing with 100% B and equilibration with 25% B for 3 min. The injection volume was 10 μl, and the column temperature was maintained at 30°C for each run. The MS conditions were as follows: positive mode, ion source of H-ESI; spray voltage of 3500 V; sheath gas flow rate of 60 arbitrary units; auxiliary gas flow rate of 15 arbitrary units; ion transfer tube temperature of 350°C; vaporizer temperature of 400°C; S-lens radio-frequency level of 60; resolution of 30,000 for MS/MS. The MS accuracy [accurate mass ± 5 parts per million (ppm) mass tolerance] of identified compounds and their MS spectra (accurate mass ± 5 ppm mass tolerance) were acquired using Xcalibur software version 4.1.

SLs were quantified by LC-MS/MS using a high-performance liquid chromatography (HPLC)—triple quadrupole/linear ion trap instrument (QTRAP5500; AB Sciei) and UHPLC-Triple-Stage Quadrupole Mass Spectrometer (Thermo Scientific Altis). Chromatographic separation was achieved on a ZORBAX Eclipse plus C<sub>18</sub> column (150 mm by 2.1 mm; 3.5 μm; Agilent) with mobile phases consisting of water:acetonitrile [95:5 (v/v); A] and acetonitrile (B), both containing 0.1% formic acid, and the following linear gradient (flow rate, 0.5 ml/min): 0 to 15 min, 25 to 100% B, followed by washing with 100% B and equilibration with 25% B for 3 min. The injection volume was 10 μl, and the column temperature was maintained at 35°C for each run. The MS parameters of QTRAP5500 were as follows: positive ion mode, ion source of turbo spray, ion spray voltage of 5500 V, curtain gas of 40 psi, collision gas of medium, gas 1 of 60 psi, gas 2 of 50 psi, turbo gas temperature of 400°C, declustering potential of 60 V, entrance potential of 10 V, collision energy of 16 eV, and collision cell exit potential of 10 V. The MS parameters of Thermo Scientific Altis were as follows: positive ion mode, ion source of H-ESI, ion spray voltage of 5000 V, sheath gas of 40 arbitrary units, auxiliary gas of 15 arbitrary units, sweep gas of 2 arbitrary units, ion transfer tube gas temperature of 350°C, vaporizer temperature of 350°C, collision energy of 17 eV, collision-induced dissociation (CID) gas of 2 mtorr, and full width at half-maximum 0.2 Da of Q1/Q3 mass. The characteristic multiple reaction monitoring transitions (precursor ion → product ion) were 331.15 → 216.0, 331.15 → 234.1, and 331.15 → 97.02 for 4DO; 347.14 → 329.14, 347.14 → 233.12, 347.14 → 205.12, and 347.14 → 97.02 for orobanchol; 361.16 → 247.12, 361.16 → 177.05, 361.16 → 208.07, and 361.16 → 97.02 for 4-oxo-MeCLA; 333.17 → 219.2, 333.17 → 173.2, 333.17 → 201.2, and 333.17 → 97.02 for putative 4-oxo-hydroxyl-CL (CL + 30); and 337.19 → 222.15, 337.19 → 240.16, and 337.19 → 97.02 for D<sub>5</sub>-5-deoxyxystigol.

**Feeding with <sup>13</sup>C-labeled CL**

<sup>13</sup>C-CL was prepared by following the protocol described by Bruno et al. (19). Briefly, the OsCCD8 complementary DNA (cDNA), controlled by an arabinose-inducible promoter, was expressed as thioedoxin-fusion in BL21 Rosetta Escherichia coli cells. A single colony of the transformed E. coli was cultured overnight, from which 0.5 ml was inoculated into 50 ml of media and grown at 28°C. When the optical density at 600 nm = 0.5, we induced the protein production with 0.2% (w/v) arabinose and incubated under agitation for 4 hours at 28°C. The harvested cells (centrifugation) were resuspended in lysis buffer [sodium phosphate buffer pH 8 containing 1% Triton X-100 and 10 mM dithiothreitol (DTT)], and lysozyme (1 mg/ml]) and incubated on ice for 30 min. The crude lysate was then sonicated and centrifuged at 12,000 rpm and 4°C for 10 min. The protein was collected (supernatant) to be used for the in vitro incubation with <sup>13</sup>C-labeled 9-cis-β-apo-10-carotenol, from Buchem B. V. (Apeldoorn, the Netherlands). The substrate (<sup>13</sup>C-labeled 9-cis-β-apo-10-carotenol) was quantified spectrophotometrically, dried, and resuspended in ethanolic detergent mixture 0.4% (v/v) Triton X-100. The mixture was then dried using a vacuum centrifuge to produce a carotenoid-containing gel, which was resuspended in incubation buffer [2 mM tris-2-carboxyethylphosphine, 0.4 mM FeSO<sub>4</sub>, and catalase (2 mg/ml; Sigma-Aldrich, Deisenhofen, Germany) in 200 mM Hepes/NaOH, pH 8]. OsCCD8 crude cell lystate, i.e., 50 μl of the soluble fraction of overexpressing cells, was added to the assay, and the whole mix was incubated 4 hours under shaking at 140 rpm at 28°C in the dark. The reaction was stopped by adding two volumes of acetone, and the lipophilic compounds were separated by partition extraction with petroleum ether:diethyl ether 1:4 (v/v), dried, and resuspended in methanol for HPLC analysis.

<sup>13</sup>C-CL was preparatively purified using a YMC-Pack C30-reversed-phase column [250 mm by 4.6 mm inner diameter (i.d.), 5 μm] in Agilent 1260 HPLC. The following separation systems were used. The column was developed at a flow rate of 1 ml/min with a gradient from 100% B to 80% B [MeOH:water:tert-butylmethyl ether (30:10:1, v/v/v)] within 15 min, then to 100% A [methanol:tert-butylmethyl ether (1:1, v/v)] within 0.5 min, and last to 100% A and a flow rate of 2 ml/min within 0.5 min, maintaining the final conditions for another 14 min. The collected fractions were dried under nitrogen gas, dissolved in dichloromethane, and kept at −80°C. Around 20 ng of <sup>13</sup>C-CL was fed to 2-week-old Os900 rice seedlings for 6 hours, and then 500 ml of root exudates was collected for LC-MS/MS analysis.

**Gene expression analysis**

For transcript analysis, total RNA was extracted from rice roots, leaves, and shoot base using the Direct-zol RNA Miniprep Plus kit.
For investigating the effect of zaxinone (customized synthesis from Agronutrition, Labège, France). Seeds of WT plants and Os900 mutants cv Nipponbare were germinated in pots containing sand and incubated for 10 days in a growth chamber under a 14-hour light (23°C)/10-hour dark (21°C). Plants used for mycorrhization were inoculated with ~1000 sterile spores of R. irregularis DAOM 197198 (Agronutrition, Labège, France). A set of WT mycorrhizal plants were treated with TIS108 (10 μM), once per week, by applying the compound once a week directly in the nutrient solution. Nonmycorrhizal and mycorrhizal plants were grown in sterile quartz sand and watered with a modified Long-Ashton (LA) solution containing 3.2 μM Na2HPO4·12H2O (39). Mycorrhizal level was monitored during a time course experiment from 10 to 35 dpi. In the last time point (35 dpi), WT and Os900 mycorrhizal roots were stained with 0.1% cotton blue in lactic acid and the estimation of mycorrhizal parameters was performed by the Trouvelot method (40). Four parameters were considered: F%, percentage of segments showing internal colonization (frequency of mycorrhization); M%, average percentage of colonization of root segments (intensity of mycorrhization); a%, percentage of arbuscules within infected areas; and A%.

For the phenotype evaluation of nonmycorrhizal and mycorrhizal plants, we considered the following parameters: crown root number, root and shoot length, leaves number, and root and shoot fresh weight. Data are means ± SE (n ≤ 10). To analyze the fungal intraradical morphology, root apparatus was stained in cotton blue [0.1% (w/v)] in lactic acid, cut in pieces 1 cm long, and observed under an optical microscope.

**Investigation of TIS108 effect on WT plants and R. irregularis root colonization**

Seeds of WT plants and Os900 mutants cv Nipponbare were germinated in pots containing sand and incubated for 10 days in a growth chamber under a 14-hour light (23°C)/10-hour dark (21°C). Plants used for mycorrhization were inoculated with ~1000 sterile spores of R. irregularis DAOM 197198 (Agronutrition, Labège, France). A set of WT mycorrhizal plants were treated with TIS108 (10 μM) by applying the compound once a week directly in the nutrient solution. Plants were grown in sterile quartz sand and were watered with a modified LA solution containing 3.2 μM Na2HPO4·12H2O. Mycorrhizal level was monitored during a time course experiment at 10, 20, and 35 dpi.

**Gene expression analysis of mycorrhizal plants**

Total RNA was extracted from rice roots using the Plant RNeasy Kit (Qiagen), according to the manufacturer’s instructions. The RNA samples were routinely checked for DNA contamination by means of PCR analysis, using primers for OsRubQ1 (32). For single-strand cDNA synthesis, about 1000 ng of total RNA was denatured at 65°C for 5 min and then reverse-transcribed at 25°C for 10 min, 42°C for 50 min, and 70°C for 15 min. The reaction was carried out in a final volume of 20 μl containing 10 μM random primers, 0.5 mM deoxynucleotides (dNTPs) 4 μl 5x buffer, 2 μl 0.1 M DTT, and 1 μl SuperScript II (Invitrogen). qRT-PCR was performed using a Rotor-Gene Q 5plex HRM Platform (Qiagen). Each PCR was carried out in a final volume of 15 μl containing 2 μl of diluted cDNA (about 10 ng), 7.5 μl of 2× SYBR Green Reaction Mix, and 2.75 μl of each primer (3 μM). The following PCR program was used: 95°C for 90 s, 40 cycles of 95°C for 15 s, and 60°C for 30 s. A melting curve (80 steps with a heating rate of 0.5°C per 10 s and a continuous fluorescence measurement) was recorded at the end of each run to exclude the generation of nonspecific PCR products. All reactions were performed on at least three biological and three technical replicates. Baseline range and take-off values were automatically calculated using Rotor-Gene Q 5plex software. Transcript level of OsPT11 (32) was normalized using OsRubQ1 housekeeping gene (32). Only take-off values leading to a Ct mean with a SD below of 0.5 were considered.

**Exogenous applications of 4DO and zaxinone**

For investigating the effect of zaxinone (customized synthesis from Buchem B.V.; Apeldoorn, the Netherlands) on different genotypes, 1-week-old seedlings were grown hydroponically in half-strength Hoagland nutrient solution containing 0.4 mM K2HPO4·2H2O (+Pi), 2.5 μM zaxinone (dissolved in 0.1% acetone), 1 μM rac-GR24 (purchased from StrigoLab, Turin, Italy), or the corresponding volume of the solvent (mock; acetone) for 2 weeks. The solution was changed twice per week, adding the chemical at each renewal.

**Plant material and growth conditions for R. irregularis root colonization**

Seeds of WT plants and Osmax1-independent lines (Os900-32 and Os900-34) were germinated in pots containing sand and incubated for 10 days in a growth chamber under a 14-hour light (23°C)/10-hour dark (21°C). Plants used for mycorrhization were inoculated with ~1000 sterile spores of R. irregularis DAOM 197198 (Agronutrition, Labège, France). A set of WT mycorrhizal plants were treated with TIS108 (10 μM), once per week, by applying the compound once a week directly in the nutrient solution. Nonmycorrhizal and mycorrhizal plants were grown in sterile quartz sand and watered with a modified Long-Ashton (LA) solution containing 3.2 μM Na2HPO4·12H2O (39). Mycorrhizal level was monitored during a time course experiment from 10 to 35 dpi. In the last time point (35 dpi), WT and Os900 mycorrhizal roots were stained with 0.1% cotton blue in lactic acid, cut in pieces 1 cm long, and observed under an optical microscope.

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products. All reactions were performed on at least three biological and three technical replicates. Baseline range and take-off values were automatically calculated using Rotor-Gene Q Splex software. Transcript level of OsPT11 (32) were normalized using OsRubQ1 housekeeping gene (32). Only take-off values leading to a C\textit{i} mean with an SD below 0.5 were considered. Statistical tests were carried out through one-way analysis of variance (ANOVA) and Tukey’s post hoc test, using a probability level of \(P < 0.05\). All statistical elaborations were performed using PAST statistical [version 2.16; (41)].

**S. hermonthica** and **P. ramosa** germination bioassays

*S. hermonthica* and *P. ramosa* seeds were first sterilized and preconditioned as described before (42). The preconditioned seeds were spread on a 9-mm-diameter Whatman filter paper disc. Each disc contains from 40 up to 100 seeds. Then, five discs were moved to a plastic petri dish, where each disc was used as a technical replicate. Eluted in acetone (the same procedure was followed for root exudates’ extracts to be used for bioassay experiments, omitting the addition of labeled SL as the internal standard), the root exudates were added to water (300 \(\mu\text{L}\) of sample in 300 \(\mu\text{L}\) of \(H_2O\)) to allow acetone evaporation under vacuum centrifugation while minimizing sample degradation and was applied on each disc containing pregerminated seeds. Corresponding volumes of rac-GR24 (purchased from StrigoLab, Turin, Italy) and sterile MilliQ water were included as positive and negative control, respectively, knowing that seeds would germinate only in the presence of SL-like compounds. The petri dishes were sealed with parafilm, enfolded with aluminum foil and incubated for 24 hours at 30°C for *Striga* and 3 days at 28°C for *Phelipanche*. The germinated and nongerminated *Striga* seeds–containing discs were photographed using a Leica LED3000 R binocular microscope, adjusted to 50% medium light, mounted with a charge-coupled device camera (Leica Microsystems). The germination percentage of the acquired images was assessed by the seed counter software SeedQuant (43).

**Heterologous expression of SL biosynthetic genes in yeast**

Heterologous expressions of Os900 and Os1400 in yeast (*Saccharomyces cerevisiae*) was carried out as described previously (26). Yeast microsomes (approximately 100 \(\mu\text{g}\) of proteins/100 \(\mu\text{L}\)) were incubated with 0.001 to 1000 \(\mu\text{M}\) TIS108 [1 \(\mu\text{M}\) substrate rac-CL and 500 \(\mu\text{M}\) NADPH (reduced form of nicotinamide adenine dinucleotide phosphate)] at 28°C for 1 min. The reaction was stopped with the addition of 1 \(\mu\text{L}\) of ethyl acetate. The ethyl acetate phase was thoroughly mixed in 1.5 liters of sand and soil mixture (1:1) and added to a 3-liter perforated plastic pot containing 0.5-liter clean soil in the bottom. The pots were kept in greenhouse-controlled conditions at 30°C in moist conditions for 10 days to precondition the *Striga* seeds. On the 11th day, 5-day-old rice seedlings (*O. sativa* L. IAC-165) were planted in the middle of each pot. After 3 days, each pot was treated with 250 \(\mu\text{L}\) of TIS108 at 10, 20, or 40 \(\mu\text{M}\) as an irrigation application. The compound was applied once a week for 3 weeks. The total amount of TIS108 applied was 0.0782, 0.235, and 0.782 mg/liter soil. After 8 weeks, the number of *S. hermonthica* plants that emerged from each pot was counted and photographically recorded.

**For S. hermonthica** pot test, seeds of *S. hermonthica* (3 mg) were sown in plastic pots (70 mm i.d. and 84 mm in height) containing 150 ml of soil [Bonsol No.2 (Sumitomo Chemical, Osaka, Japan) and river sand = 1:1 (v/v)] and 60 ml of distilled water. Seeds in the pots were conditioned in the dark at 30°C for 7 days. On the eighth day, one 5-day-old rice seedling (*O. sativa* L. IAC-165) was then planted in each *S. hermonthica* condition pot and grown in growth chamber (NK System, Tokyo, Japan) at 30°C under light-emitting diode light (500 \(\mu\text{mol} \text{ m}^{-2} \text{s}^{-1}\)) with long-day conditions (16-hour light/8-hour dark). Each pot was treated with 50 ml of 0.1, 0.3, or 1 \(\mu\text{M}\) TIS108 and MP1 (29, 47, 48) once a week for 7 weeks. The total amount of TIS108 applied was 0.0782, 0.235, and 0.782 mg/liter soil. After 8 weeks, the number of *S. hermonthica* plants that emerged from each pot was counted and photographically recorded.
**Statistical analysis**

Data are represented as means and their variations as SD. The statistical significance was determined by one-way ANOVA and Tukey’s multiple comparison test, using a probability level of P < 0.05. All statistical elaborations were performed using GraphPad Prism 8 for Mac OS, version 8.3.0.

**SUPPLEMENTARY MATERIALS**

Supplementary material for this article is available at https://doi.org/10.1126/sciadv.add1278

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Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. The RNA-seq data are deposited in the DDBJ Read Archive under accession no. DEA009250. RPKM values of all genes in the RNA-seq analysis are presented in data file S1. The rice OsMAX1-900 KO lines can be provided by KAUST pending scientific review and a completed material transfer agreement. Requests for the rice OsMAX1-900 KO lines used in this study should be submitted to S.A.-B.

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