Supporting Information

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SI Materials and Methods

Complementation of *Arabidopsis* max1-1 Mutants. ORFs were amplified from cDNA by using primers containing restriction sites or the Gateway 5′–CACC sequence in the forward cloning primer for directional cloning, as described in Table S4. The products were cloned either by restriction digest directly into the pART7 binary vector (1) containing the 35S promoter (*Os01g0700900*) or into Gateway entry vector pENTR/D-TOPO (Invitrogen) (*Os01g0701400* and *AtMAX1*). The pENTR vectors carrying *Os1g0701400* and *AtMAX1* were each combined with pENTR–p4p1r containing a CaMV 35S promoter and pENTR–p2rp3 containing a NOS terminator [offered by the Department of Molecular Biology of Wageningen University & Research Centre (Wageningen UR)] into the destination expression vector pHm43GW (2) by a multisite Gateway LR reaction (Invitrogen), resulting in 35S::Os01g0701400 and 35S::AtMAX1 in pHm43GW.

Constructs were transformed into max1-1 plants by floral dip (3). Single insertion lines were selected by antibiotic resistance and brought to homozygosity. For assessment of branching phenotype, 35S::Os01g0700900 plants were sown on F2 compost treated with Intercept 70WG (both Levington Horticultures) and stratified for 2 d at 4 °C before moving to plant growth rooms or greenhouses with temperatures between 18 °C and 24 °C and light period defined by the decapitation method experiment (4). The 35S::Os01g0701400 and 35S::MAX1 plants were grown in *Arabidopsis* soil pots in the climate room of Wageningen UR greenhouse with temperatures between 18 °C and 22 °C and 60% humidity. Branching was scored by using the decapitation method (4).

Rice Bala Variety Complementation. Mature calli were prepared from mature seeds of Bala surface sterilized with 30-s treatment of 75% (vol/vol) ethanol followed by 30 min 2.5% (wt/vol) sodium hypochlorite. Calli were induced on N6B5 basal medium (N6 medium basal salt mixture and B5 vitamins) (5, 6), supplemented with 30 g/L sucrose, 500 mg/L L-proline, 2 mg/L glycine, 500 mg/L L-glutamine, 300 mg/L casein acid hydrolysate, 2 mg/L 2,4-dichlorophenoxyacetic acid and 4 g/L phytagel (pH 5.8) in the dark at 28 °C, transferring to fresh medium every week. Embryogenic calli were selected after 5–6 wk to subculture on the same medium for 4 d before infection with agrobacterium. Calli were transformed as describe in Ozawa et al. (7) with modifications. The constructs p35S:Os01g0700900, p35S:Os01g0701400, and empty pHm43GW with the CaMV35 promoter and nos-terminator elements were introduced separately by electroporation into *Agrobacterium* strains EHA105. After 3-d coculitvation, calli were cultured for 3 wk on N6B5 medium containing 400 mg/L carbenicillin and 50 mg/L hygromycin, with transferring to fresh medium every week. For regeneration, the actively growing calli were transferred on Murashige and Skoog basal medium supplemented with 500 mg/L L-glutamine, 500 mg/L L-proline, 30 g/L sucrose, 30 g/L sorbitol, 0.5 mg/L kinetin, 2 mg/L 6-benzylaminopurine, 0.2 mg/L 1-naphthaleneacetic acid, 30 mg/L hygromycin B, and 4 g/L phytagel (pH 5.8) for 3 wk at 28 °C, with a 16-h photoperiod. Shoots grown from the calli were transferred to rooting medium (1/2 MS medium, 30 g/L sucrose, 50 mg/L hygromycin, pH 5.8) to obtain T0 seedlings.

Detection and Quantification of Strigolactones by Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS). Chromatographic separation was obtained on an Acquity UPLC BEH C18 column (100 mm, 2.1 mm, 1.7 mm; Waters) by applying a water/acetonitrile gradient to the column starting from 5% (vol/vol) of acetonitrile for 0.33 min and rising to 27% (vol/vol) of acetonitrile in 0.67 min, followed by a 4.33-min gradient to 40% (vol/vol) acetonitrile, followed by a 4.0-min gradient to 65% (vol/vol) acetonitrile, which was maintained for 0.67 min, followed by a 0.2 min gradient to 90% (vol/vol) acetonitrile, which was maintained for 0.46 min before going back to 5% (vol/vol) acetonitrile using a 0.2 min gradient, before the next run. Finally, the column was equilibrated for 2.47 min by using this solvent composition. Total run was 12 min. The column was operated at 50 °C with a flow rate of 0.5 $mL{\cdot}min^{-1}$. Sample injection volume was 20 μL. The mass spectrometer was operated in positive electrospray ionization mode. Cone and desolvation gas flows were set to 50 and 1,000 $L{\cdot}h^{-1}$, respectively. The capillary voltage was set at 3.0 kV, the source temperature at 150 °C and the desolvation temperature at 650 °C. The cone voltage was optimized for each standard compound by using the IntelliStart MS Console (Waters). Argon was used for fragmentation by collision-induced dissociation. Multiple reaction monitoring (MRM) was used for strigolactone identification and quantification. Parent–daughter transitions for the standards, strigolactones and $[^2H]_6$-2′-*epi*-5-deoxystrigol, deuterium labeled strigolactones (used as internal standards), were set by using the IntelliStart MS Console. MRM transitions selected for identification of strigolactones in rice were as follows: for orobanchol mass-to-charge ratio (*m*/*z*) 347.20 > 97.00 at a collision energy of 22 eV, 347.20 > 205.20 at 18 eV, and 347.20 > 233.15 at 12 eV; for *ent*-2′-*epi*-5-deoxystrigol, *m*/*z* 331.20 > 97.00 at 22eV, 331.20 > 216.15 at 15 eV, and 331.20 > 234.15 at 10 eV; for methoxy-5-deoxystrigol isomers *m*/*z* 361.20 > 96.96 at 20 eV and 361.20 > 247.00 at 10 eV; for $[^2H]_6$-2′-epi-5-deoxystrigol *m*/*z* 337.16 > 97.02 at 22eV and 337.16 > 240.19 at 10eV. Cone voltage was set to 18, 15, 20, and 16 eV, correspondingly.

Strigolactones were quantified by using a calibration curve with known amount of standards and based on the ratio of the peak areas of the MRM transition for strigolactone standards (*m*/*z* 347.20 > 97.00; 331.20 > 97.00) to the MRM transition for $[^2H]_6$-2′-epi-5-deoxystrigol (*m*/*z* 337.16 > 97.02). Methoxy-5-deoxystrigol isomers were quantified by using only peak areas for the transition *m*/*z* 361.20 > 247.00. Data acquisition and analysis were performed by using MassLynx 4.1 (TargetLynx) software (Waters).

1. Gleave AP (1992) A versatile binary vector system with a T-DNA organisational structure conducive to efficient integration of cloned DNA into the plant genome. *Plant Mol Biol* 20(6):1203–1207.
2. Ovchinnikova E, et al. (2011) IPD3 controls the formation of nitrogen-fixing symbiosomes in pea and *Medicago* spp. *Mol Plant Microbe Interact* 24(11):1333–1344.
3. Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16(6):735–743.
4. Greb T, et al. (2003) Molecular analysis of the *LATERAL SUPPRESSOR* gene in *Arabidopsis* reveals a conserved control mechanism for axillary meristem formation. *Genes Dev* 17(9):1175–1187.
5. Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. *Exp Cell Res* 50(1):151–158.
6. Chu CC (1975) Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. *Sci Sin* 18:659–668.
7. Ozawa K (2009) Establishment of a high efficiency *Agrobacterium*-mediated transformation system of rice (*Oryza sativa* L.). *Plant Sci* 176:522–527.

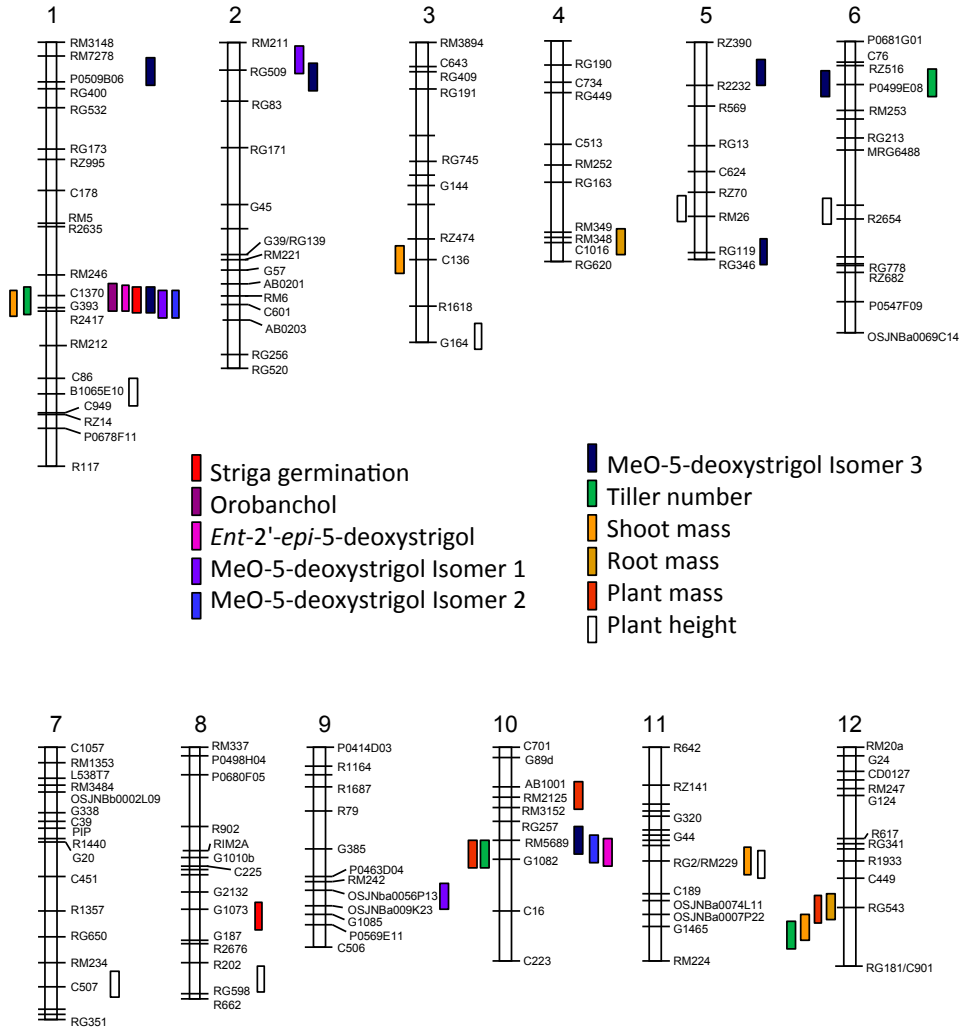

Fig. S1. Molecular map of quantitative trait loci (QTLs) for traits analyzed in a Bala × Azucena mapping population. The indicated markers are restriction fragment length polymorphism and microsatellite markers (amplified fragment length polymorphism marker names omitted unless mentioned in the text). All indicated QTLs have a logarithm of the odds ratio (LOD) score of >2.5. QTLs indicated on the right of a chromosome indicate a positive contribution by the Azucena allele; QTLs on the left indicate a positive contribution of the Bala allele. See Table S1 for details.

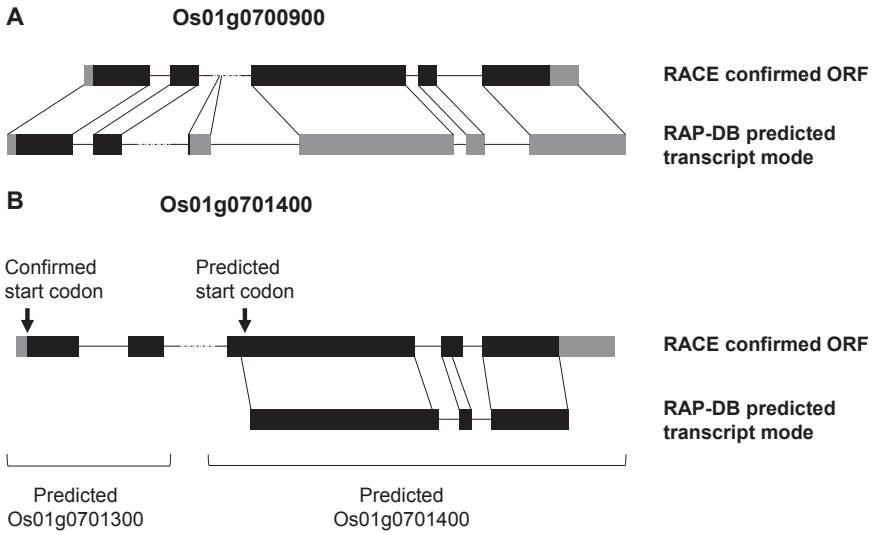

Fig. S2. Os01g0700900 (*A*) and Os01g0701400 (*B*) mRNA sequence analyses. Comparison between the gene model predicted by the International Rice Genome Sequencing Project (IRGSP) and the ORF identified with RACE-PCR. Gray shaded bars represent untranslated regions, and black shaded bars indicate the coding region between START and STOP codons. The IRGSP predicts Os01g0701300 and Os01g0701400 genes that were confirmed to be one single ORF.

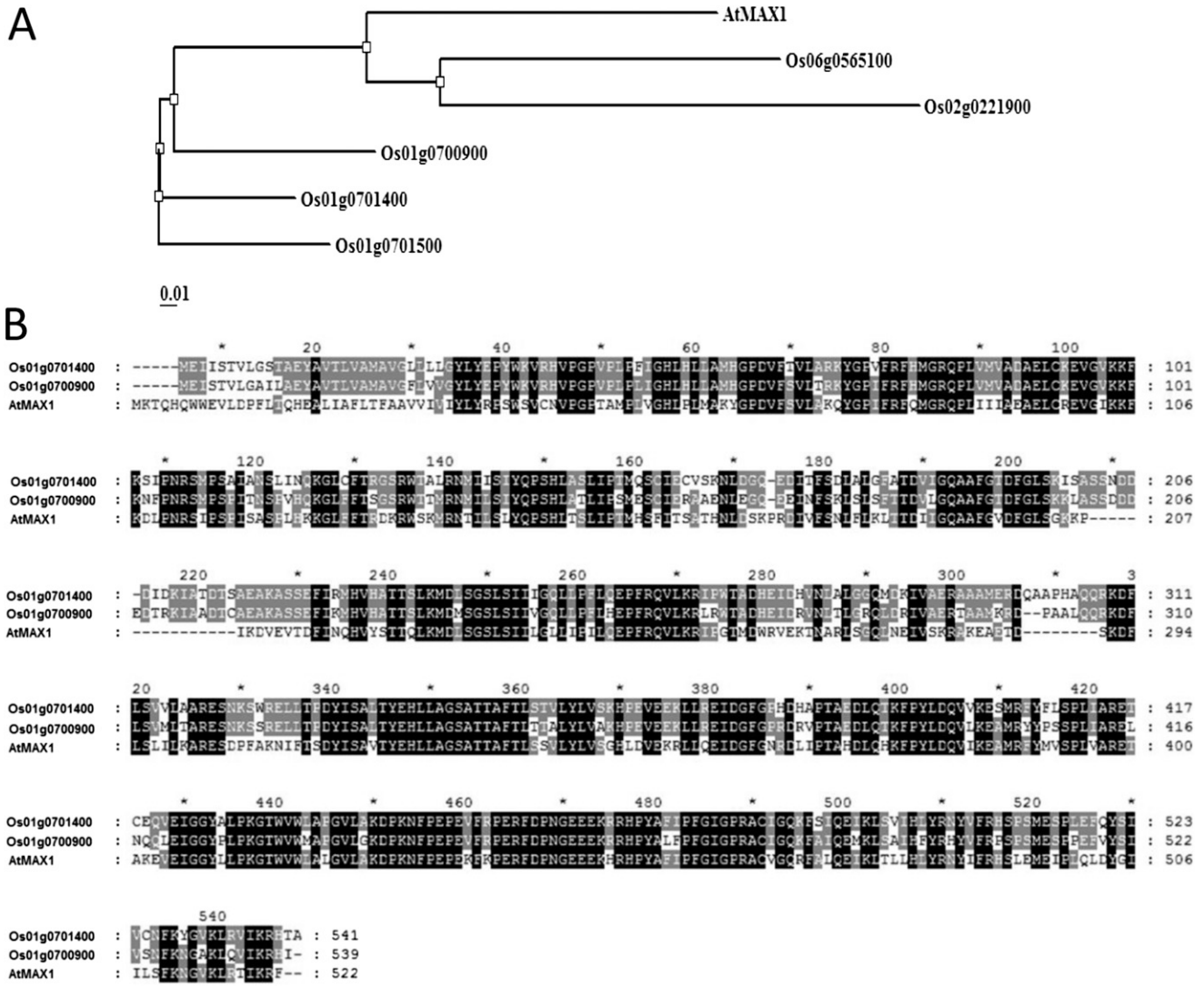

Fig. S3. MAX1 orthologs sequence analyses. (*A*) Similarity tree of the CYP711A1 found in the rice genome and AtMAX1 (NP_565617) protein sequences using the sequences confirmed by RACE-PCR of Os01g0700900 and Os01g0701400 and the predicted sequences of Os01g0701500, Os02g0221900, and Os06g0565100. The tree was built by using clustalW2 (www.ebi.ac.uk/Tools/msa/clustalw2). (*B*) Sequence alignment of the amino acid sequences of AtMAX1 (NP_565617), and the confirmed sequences of Os01g0700900 and Os01g0701400.

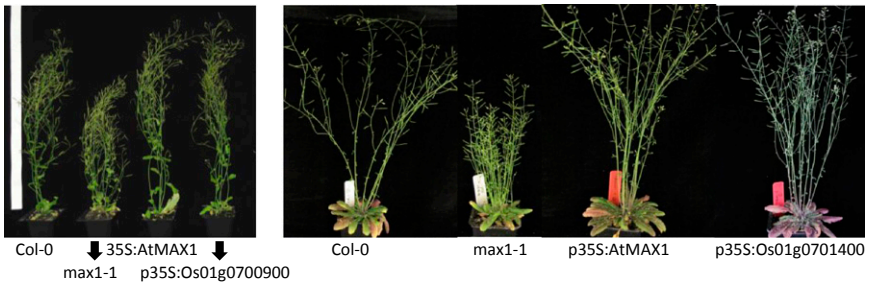

Fig. S4. Effect of complementation of *Arabidopsis max1-1* with p35S:AtMAX1, p35S:Os01g0700900, and p35S:Os01g0901400 on branching phenotype and comparison with wild-type Col-0 and *max1-1*.

Table S1. Main-effect quantitative trait loci for traits measured on the Bala × Azucena recombinant inbred line population

Trait	Chromosome	QTL position in cM	QTL position in Mbp (approx.)	LOD	R^2, %	Donor of positive allele
Striga Germination	1	147.3	29.3	29.4	66.0	Azucena
	8	98.4	21.1	3.3	6.1	Azucena
Orobanchol	1	145.3	29.0	29.2	70.2	Azucena
Ent-2′-*epi*-5-deoxystrigol	1	145.3	29.0	32.7	71.2	Azucena
	10	61.7	13.7	2.5	3.3	Azucena
MeO-5-DS isomer 1	1	148.5	29.4	2.8	6.7	Azucena
	2	10.0	3.0	2.7	10.3	Azucena
	9	84.4	18.8	3.1	7.8	Azucena
MeO-5-DS isomer 2	1	148.5	29.4	20.8	49.2	Azucena
	10	59.7	13.6	3.0	5.8	Azucena
MeO-5-DS isomer 3	1	18.1	3.3	3.0	4.6	Azucena
	1	147.3	29.3	29.1	52.8	Azucena
	2	20.4	4.0	2.7	4.2	Azucena
	5	18.0	2.7	3.5	5.7	Azucena
	5	121.7	28.8	2.6	2.7	Azucena
	6	24.3	3.9	3.0	3.8	Bala
	10	53.7	13.2	4.6	5.1	Azucena
Tiller number	1	147.3	29.3	12.4	28.8	Bala
	6	24.3	3.9	3.4	7.2	Azucena
	10	61.7	13.7	3.0	6.1	Bala
	12	117.5	25.9	2.9	8.8	Bala
Root fresh weight	4	111.8	32.5	3.3	8.3	Azucena
	12	100.7	23.4	4.6	14.6	Bala
Shoot fresh weight	1	148.5	29.4	6.6	14.6	Bala
	3	126.0	26.7	2.6	5.6	Bala
	11	65.2	17.5	3.8	8.8	Azucena
	12	115.5	25.6	2.7	9.8	Bala
Total plant fresh weight	10	29.5	4.7	3.8	9.2	Azucena
	10	61.7	13.7	3.6	10.9	Bala
	12	103.5	23.8	3.7	9.8	Bala
Plant height	1	205.0	38.4	8.7	19.4	Azucena
	3	170.3	33.6	10.6	27.1	Azucena
	5	97.9	26.6	4.8	13.0	Bala
	6	97.7	16.2	5.5	12.0	Bala
	7	138.9	26.6	3.0	9.4	Azucena
	8	133.1	25.7	2.6	8.0	Azucena

MeO-5-DS, methoxy-5-deoxystrigol.

Table S2. SL levels in root exudates and root tissue, number of tillers, and stimulation of Striga germination by root exudates of rice lines varying for the presence and absence of the rearrangement

				Impact of the presence of the rearrangement on strigolactone levels and number of tillers in different rice lines							
				Exudate, pmol/mL		Root fresh weight, pmol/g		LC-MS peak areas exudate			
Line	Allele	Sub-group*	Country of origin	Orobanchol	*Ent*-2′-epi-5-DS	Orobanchol	*Ent*-2′-epi-5-DS	MeO-5DS isomer 2	MeO-5DS isomer 3	Striga germination, %	No. of tillers
Azucena	A	TRJ	Phi	152.08 ± 19.20	22.27 ± 4.16	30.08 ± 4.33	4.41 ± 0.88	1,656.97 ± 319.75	3,942.09 ± 775.65	47.42 ± 2.32	4 ± 0
DZ-78	A	AUS	Ban	102.45 ± 16.49	9.71 ± 4.68	19.09 ± 3.22	1.81 ± 0.88	2,063.75 ± 283.89	7,592.69 ± 1196.57	42.67 ± 3.06	4.67 ± 0.067
Aswina-330	A	AUS	Ban	26.39 ± 6.56	4.80 ± 2.26	5.35 ± 1.29	0.97 ± 0.46	479.12 ± 48.17	1,835.51 ± 347.77	33.94 ± 2.59	5 ± 0.2
Kun-Min-TH	A	IND	Chi	29.52 ± 2.97	5.16 ± 0.69	5.48 ± 0.74	0.96 ± 0.16	547.84 ± 111.01	1,767.85 ± 255.44	30.84 ± 3.69	4.27 ± 1.05
Ta-Mao-T	A	TEJ	Chi	32.98 ± 10.84	18.86 ± 2.64	6.20 ± 1.66	3.62 ± 0.31	577.09 ± 197.51	2,456.21 ± 597.66	33.13 ± 2.52	4.2 ± 0.11
Sinaguing	A	TRJ	Phi	8.35 ± 2.63	3.29 ± 1.53	1.56 ± 0.46	0.60 ± 0.28	187.78 ± 66.00	874.90 ± 236.34	44.5 ± 4.4	5.93 ± 0.13
Bala	B	IND	Ind	0 ± 0	0.34 ± 0.27	0 ± 0	0.06 ± 0.05	12.95 ± 8.04	89.87 ± 4.64	13.61 ± 1.45	10 ± 0.23
Dhala-S	B	AUS	Ban	6.024 ± 1.16	0.05 ± 0.04	1.15 ± 0.24	0.01 ± 0.01	61.28 ± 59.5	198.00 ± 159.13	2.67 ± 0.42	7.93 ± 0.35
Kachilon	B	AUS	Ban	0 ± 0	0.02 ± 0.01	0 ± 0	0.00 ± 0.00	12.65 ± 3.48	34.80 ± 1.18	10.88 ± 1.28	8.07 ± 0.48
Guan-Yin-T	B	IND	Chi	4.78 ± 0.73	0.015 ± 0.01	0.96 ± 0.14	0.00 ± 0.00	13.42 ± 5.85	63.051 ± 5.27	3.63 ± 0.52	6.8 ± 0.2
Sung-Liao-2	B	TEJ	Chi	0 ± 0	2.58 ± 0.01	0 ± 0	0.51 ± 0.00	15.46 ± 6.95	76.83 ± 6.62	17.03 ± 2.01	6.8 ± 0.11
Asse Y Pung	B	TRJ	Phi	0 ± 0	0.30 ± 0.08	0 ± 0	0.06 ± 0.01	12.07 ± 4.95	34.86 ± 13.11	12.9 ± 2.65	8.73 ± 0.18
Kinastano	B	TRJ	Phi	0 ± 0	0.30 ± 0.03	0 ± 0	0.05 ± 0	2.75 ± 2.43	88.24 ± 8.11	8.98 ± 2.14	7.6 ± 0.23

Orobanchol, 5-deoxystrigol and methoxy-5-deoxystrigol isomers 2 and 3 were measured in plants grown after 1 wk/10 d of Pi starvation ($n = 3$). Striga germination induced by root exudates ($n = 3$) was measured in a germination bioassay. A maximum of 58.41 ± 2.68% germination was obtained with the synthetic strigolactone control GR24 at 3.3 μM concentration. The table shows the mean values ± SE.A, Azucena allele; B, Bala allele; Ban, Bangladesh; Chi, China; *Ent*-2′-epi-5-DS, *Ent*-2′-epi-5-deoxystrigol; Ind, India; MeO-5DS, methoxy-5-deoxystrigol; Phi, Philippines.
*Ref. 1.

1. Zhao K, et al. (2010) Genomic diversity and introgression in *O. sativa* reveal the impact of domestication and breeding on the rice genome. *PLoS ONE* 5(5):e10780.

Table S3. Primers used for quantitative RT-PCR

Primers for quantitative RT-PCR		
Primer pair		Locus/gene
GGCTTCTCTGCTTGCTGCTTC	ACCAACGGTTGCCTTCCC	Os01g0700900
ACGACGGCGTTCACTCTCTC	TCCGAACCCGTCAATCTCC	Os01g0701400
AGCGAAGGTATCCACTAGGC	CCGGGGATATAGACACTC	OsPI1 - Os01g0838350
CTTGTCGCCTGGTACGAC	GTCGATGACACGGTTGCTGTA	GADPH used as internal control

Table S4. Primers used for RACE-PCR and cloning PCR

Primer for RACE-PCR and cloning PCR	
Primers	Sequence
Os01g0700900 5RACE primer	GTGGATCGCTGACAATTTCATCTC
Os01g0700900 5RACE Nested primer	GCAGCTCCCTCGAGGATTTGTTCG
Os01g0700900 3RACE primer	GCAAGATCGCGGCCGATACCTG
Os01g0700900 3RACE Nested primer	CGGACGGCTGCAATGAAGCGAG
Os01g0700900 Forward Cloning primer (with EcoRI site)	gggggaattcATGGAGATCAGCACAGTG
Os01g0700900 Reverse Cloning primer (with ClaI site)	ggggatcgatTTATATATGCCTCTTGATGACCTG
Os01g0701400 5RACE primer	CATATCCTCCAATTTCAACCTGTTCGCAC
Os01g0701400 5RACE nested primer	GCGTAAGAAGCTCCCGCCAGG
Os01g0701400 3RACE primer	CGGGAGCTTCTTACGCCGGAC
Os01g0701400 3RACE nested primer	CGTGCGAACAGGTTGAAATTGGAGGATATG
Os01g0701400 cloning forward primer(with pENTR/D-TOPO cloning site)	caccATGGAGATCATCAGCACAGTGCTG
Os01g0701400 cloning reverse primer	CTATGCAGTGTGCCTCTTGATGACCCG
AtMAX1 cloning forward primer(with pENTR/D-TOPO cloning site)	caccATGAAGACGCAACATCAAT
AtMAX1 cloning reverse primer	TCAGAATCTTTTGATGGTTCTGAGC

Table S5. Primers used for assessing allelic diversity

Primers for assessing allelic diversity			
Primer pair		Locus/gene	Product size, bp
GGACGTGTTCTCAGTGCTCA	TCCTTGCACAACGCATC	Os01g0700900	200
ACAGTGCTGGGCTCAACG	GGCTCGTACAAGTAGCCCAAC	Os01g0701400	100
TCAAAGCTGCCAGTCACACC	ATCCGGACAGGTCCATCTTG	Os01g0701500	150