New Phytologist

New Phytologist Supporting Information

Article title:

Strigolactone deficiency induces jasmonate, sugar and flavonoid phytoalexin accumulation enhancing rice defense against the blast fungus *Pyricularia oryzae*

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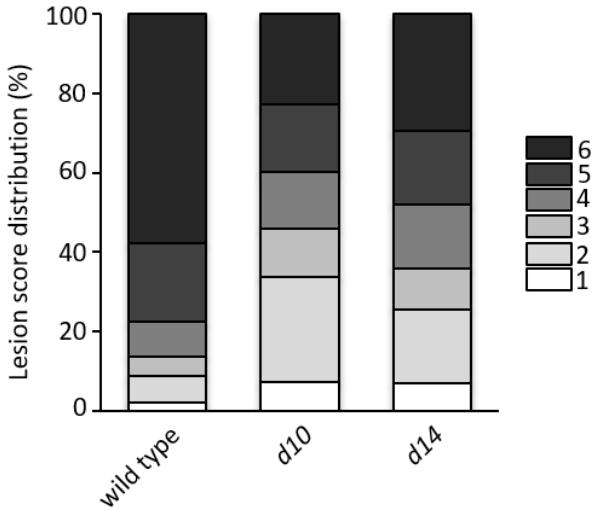

Fig. S1 Lesion score distribution in rice strigolactone-mutants and wild-type (WT) plants infected by *Pyricularia oryzae.* Two-wk-old rice seedlings were inoculated with the *P. oryzae* strain VT5M1 by spraying a spore suspension, and the disease was scored at 6 d post inoculation. Lesion types 1-3 are non-sporulating lesions, and 4-6 are sporulating lesions.

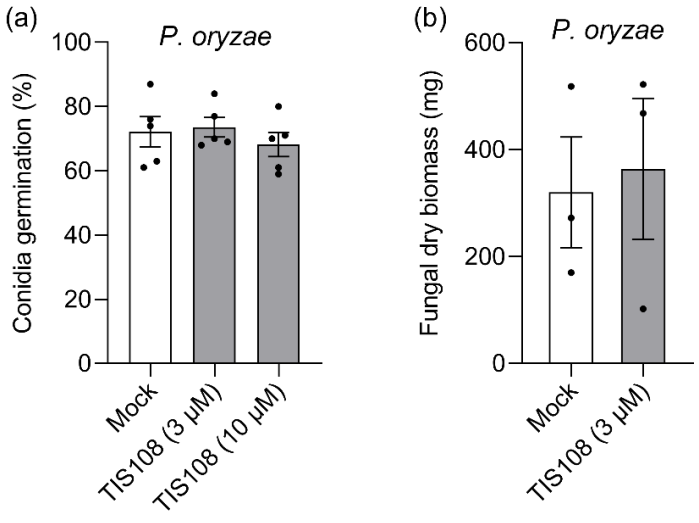

Fig. S2 *In vitro* bioassay with *Pyricularia oryzae* using the strigolactone inhibitor TIS108. (a) Conidial germination in potato dextrose agar (PDA) medium supplemented with or without TIS108 after 24 hours. The germination test using freshly harvested conidia was performed on glass slides following the method described by Ullah *et al.* (2017). Data were analyzed using a one-way ANOVA (non-significant, $P = 0.611$). Bars represent mean ± standard error ($n = 5$). (b) Fungal growth in a liquid culture medium (potato dextrose broth, PDB). Each 250 ml conical flask was filled with 50 ml PDB supplemented with TIS108 (3 µM final concentration). An agar plug (5 mm diameter) with the actively grown culture of *P. oryzae* was inoculated in the liquid culture, followed by incubation at 25 °C for 3 days under continuous shaking (150 rpm). The PDB medium with densely grown *P. oryzae* was centrifuged at high speed, the supernatant was discarded, and fungal biomass was freeze-dried for 2 d to calculate the dry weight. Data were analyzed using a two-tailed Student's t-test (non-significant, $P = 0.806$). Bars represent mean ± SE ($n = 3$).

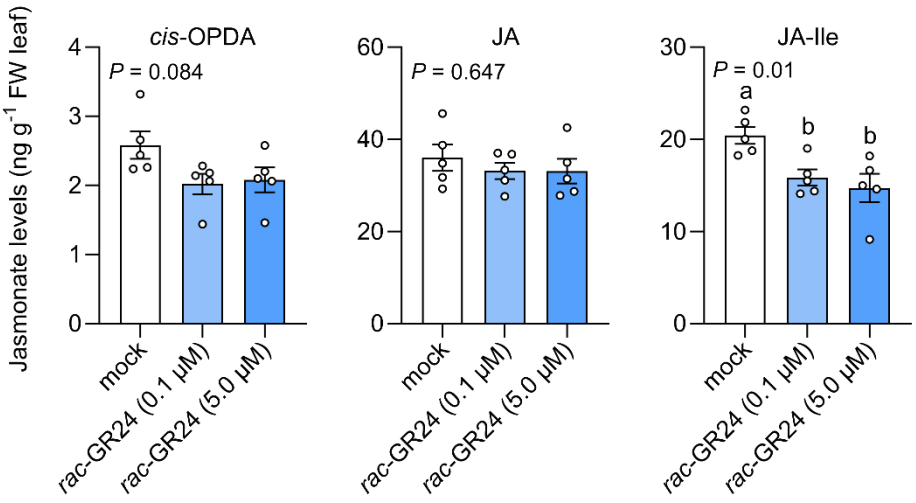

Fig. S3 Concentrations of jasmonates in rice leaves after treatment with the strigolactone (SL) analog *rac*-GR24. Two-wk-old transplanted rice seedlings were sprayed with *rac*-GR24 or solvent only as the mock. After 24 hours, shoot samples were harvested, and jasmonate levels were determined using a triple quadrupole mass spectrometer. Data were analyzed using a one-way ANOVA, followed by Tukey's multiple comparison test with a 95% confidence interval. Different letters above bars indicate groups that are significantly different ($P < 0.05$). Data are presented using bar plots as the mean ± SE, and all data points are shown on the graph as circles ($n = 5$, each replicate consisting of 3-4 seedlings grown in separate pots). *cis*-OPDA, *cis*-(+)-12-oxo-phytodienoic acid; JA, jasmonic acid; JA–Ile, jasmonoyl-isoleucine.

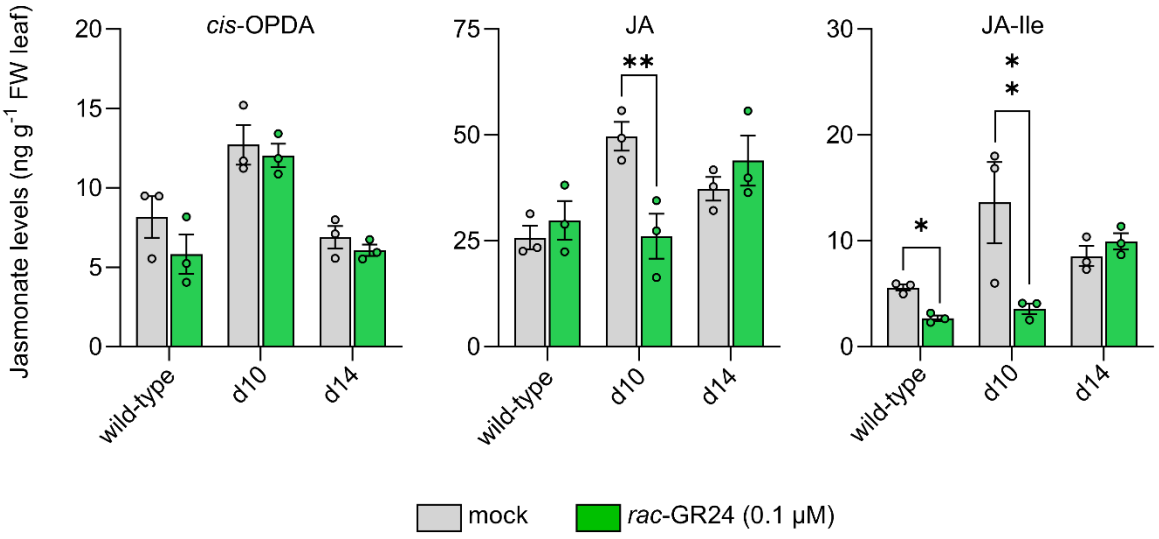

Fig. S4 Complementation of the strigolactone (SL) biosynthetic mutant *d10* by *rac*-GR24 treatment. Transplanted rice seedlings (wild-type Shiokari and SL mutants) were grown for two weeks. The mutant *d10* is deficient in SL biosynthesis, while *d14* is a SL perception or signaling mutant. Half of the seedlings from each line were sprayed with 0.1 µM *rac*-GR24, and the remaining seedlings were treated with the solvent only as a mock. After 24 h, shoot samples were harvested, and jasmonate concentrations were determined using a triple quadrupole mass spectrometer. Data were analyzed using a two-way ANOVA, followed by Tukey's multiple comparison test with a 95% confidence interval. Asterisks (*, $P < 0.05$; **, $P < 0.01$) indicate pairwise significance between mock vs *rac*-GR24 treated means of each plant line. Data are presented as the mean ± SE, and all data points are shown on the graph as circles ($n = 3$, each replicate consisting of 4-6 seedlings). *cis*-OPDA, *cis*-(+)-12-oxo-phytodienoic acid; JA, jasmonic acid; JA–Ile, jasmonoyl-isoleucine.

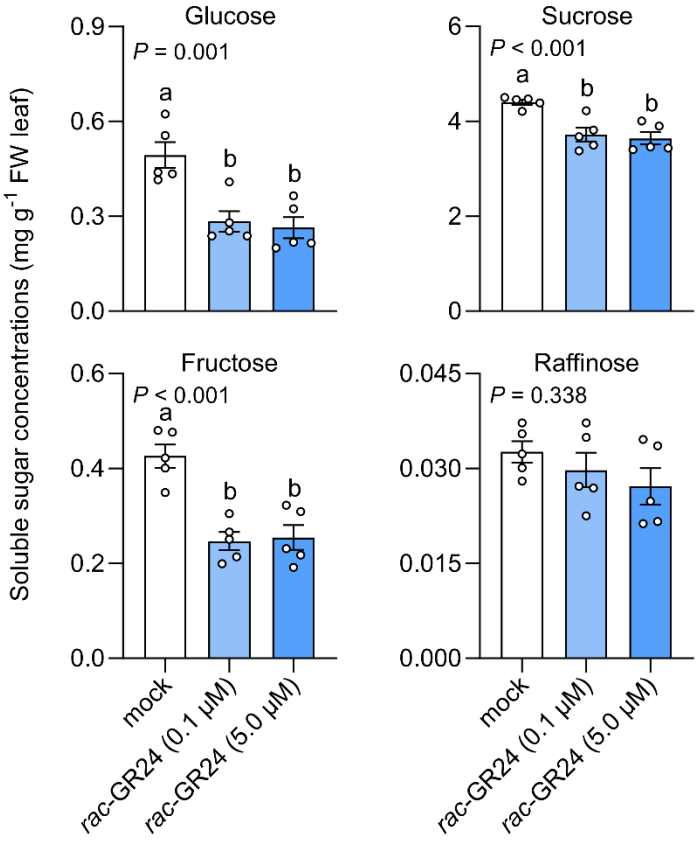

Fig. S5 Soluble sugar content in rice leaves after *rac*-GR24 treatment. Two-wk-old transplanted rice seedlings were sprayed with the strigolactone analog *rac*-GR24 or solvent only as the mock. After 24 h, shoot samples were harvested, and soluble sugars were quantified using a triple quadrupole mass spectrometer. Data were analyzed using a one-way ANOVA, followed by Tukey's multiple comparison test with a 95% confidence interval. Different letters above bars indicate groups that are significantly different ($P < 0.05$). Data are presented using bar plots as the mean ± SE, and all data points are shown on the graph as circles ($n = 5$, each replicate consisting of 3-4 seedlings).

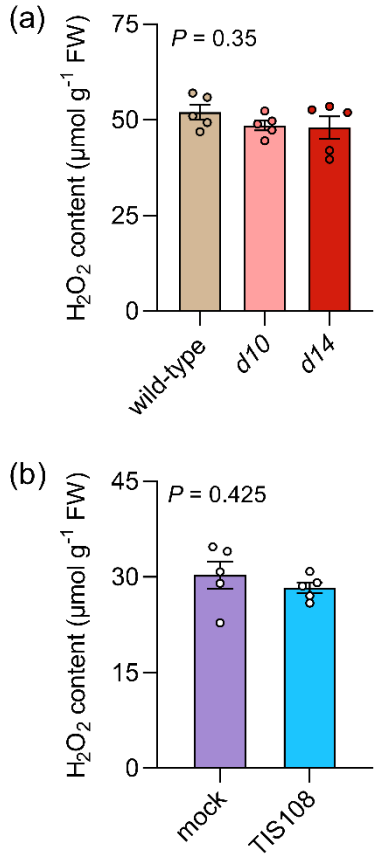

Fig. S6 Concentrations of reactive oxygen species (ROS) in rice strigolactone-mutants and TIS108-treated plants. **(a)** H_2O_2 contents in the leaves of rice strigolactone (SL)-mutants and the wild-type (*cv*. Shiokari). The mutant *d10* is deficient in SL biosynthesis, while *d14* is a SL perception or signaling mutant. Leaf samples were collected two weeks after transplantation. Data were analyzed using a one-way ANOVA. **(b)** H_2O_2 contents in wild-type rice leaf after spraying with the SL inhibitor TIS108 (3 µM). Two-wk-old rice seedlings were sprayed with the inhibitor or solvent only (mock), and samples were harvested one day after spraying. Data were analyzed using a two-tailed Student's t-test. Bars represent mean ± SE (n = 5, each replicate consisting of 3-4 seedlings). All data points are shown on the graph as circles.

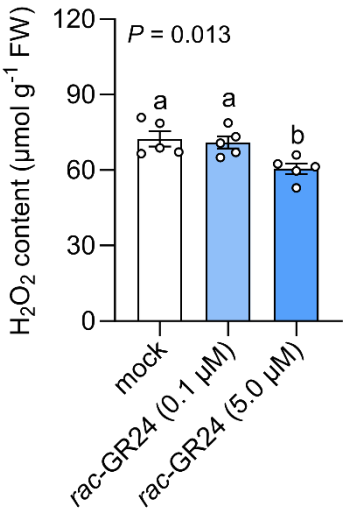

Fig. S7 Concentrations of ROS in rice seedlings after treatment with the strigolactone analog *rac*-GR24. Two-wk-old wild-type (WT) rice seedlings were sprayed with the strigolactone analog GR24 or with solvent only as the mock. Leaf samples were harvested after 24 h of treatment, ground in liquid nitrogen, and H_2O_2 content was determined from the fresh tissues. Data were analyzed using a one-way ANOVA, followed by Tukey's multiple comparisons test with a 95% confidence interval. Different letters above bar plots indicate groups that are significantly different ($P < 0.05$). Data are presented as the mean ± SE, and all data points are shown as circles ($n = 5$, each replicate consisting of 3-4 seedlings).

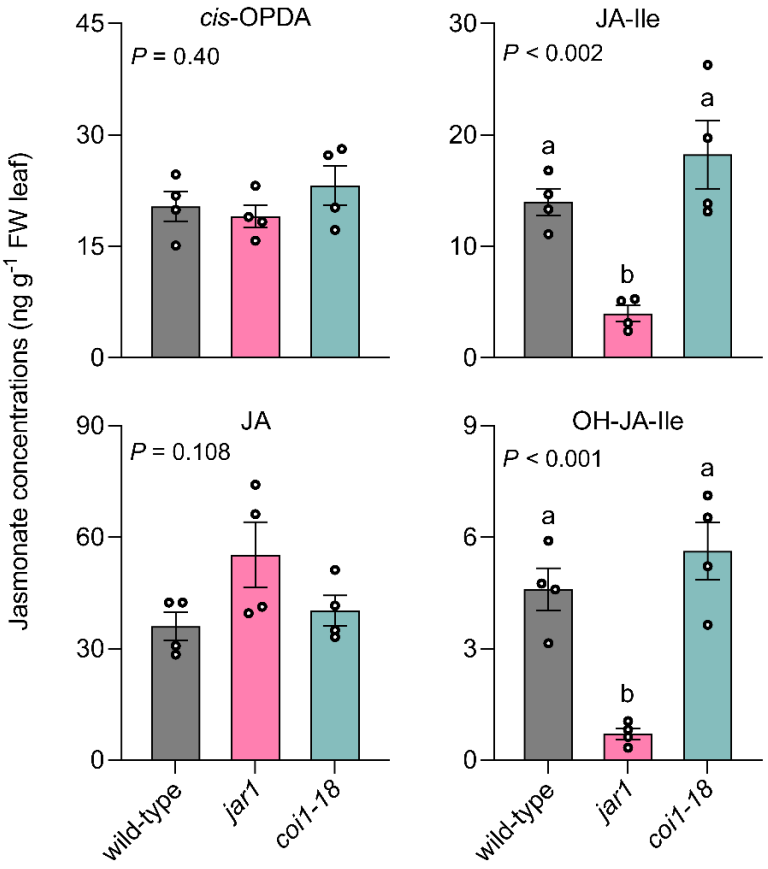

Fig. S8 Jasmonate levels in rice mutants defective in JA-Ile synthesis (*jar1*) and jasmonate perception (*coi1-18* RNAi). Leaf samples were harvested from three-week-old rice seedlings, and hormones were quantified using a triple quadrupole mass spectrometer. Data were analyzed using a one-way ANOVA, followed by Tukey's multiple comparison test with a 95% confidence interval. Different letters above bars indicate groups that are significantly different ($P < 0.05$). Data are presented using bar plots as the mean ± SE, and all data points are shown on the graph as open circles ($n = 4$, each replicate consisting of 3-4 seedlings).

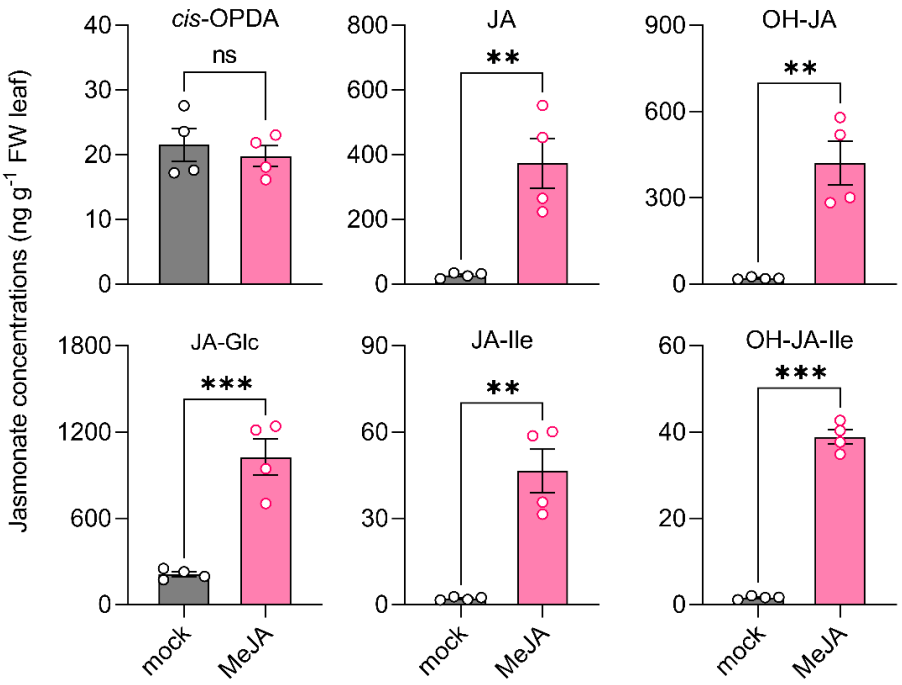

Fig. S9 Accumulation of jasmonates in rice leaves after treatment with methyl jasmonate (MeJA). Three-wk-old rice seedlings were sprayed with 200 µM MeJA or water only as a mock. Leaf samples were harvested 24 h after treatment, and JA metabolites were quantified using a triple quadrupole mass spectrometer. Data were analyzed using a two-tailed Student's t-test (**, $P < 0.01$; ***, $P < 0.001$; ns, non-significant). Data are presented using bar plots as the mean ± SE, and all data points are shown on the graph as dots (n = 4, each replicate consisting of 4-6 seedlings). *cis*-OPDA, *cis*-(+)-12-oxo-phytodienoic acid; JA, jasmonic acid; OH-JA, hydroxy-jasmonic acid; JA-Glc, jasmonic acid-glucoside; JA–Ile, jasmonoyl-isoleucine; OH-JA-Ile, hydroxy- jasmonoyl-isoleucine.

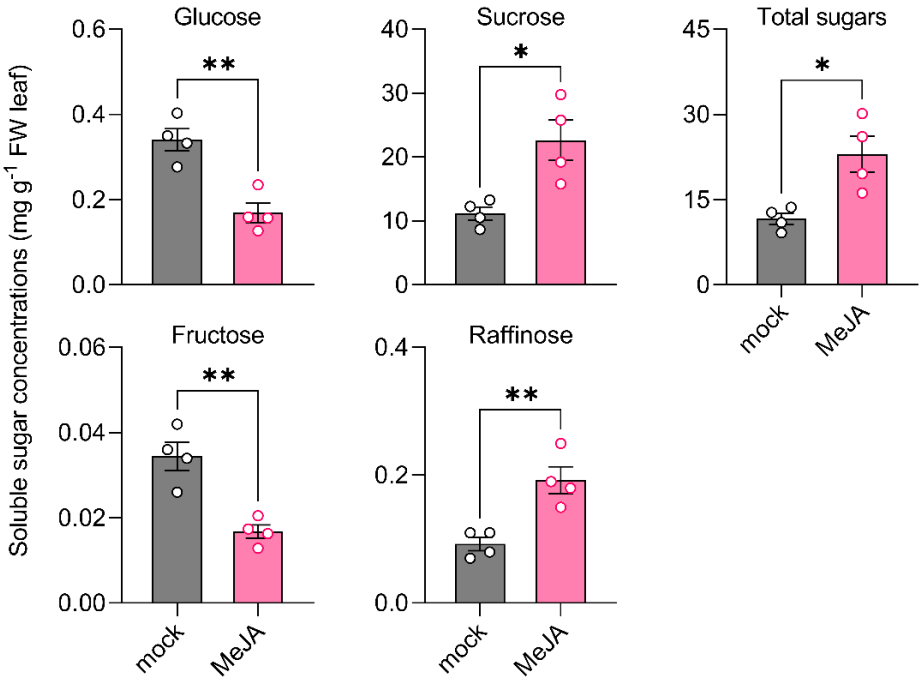

Fig. S10 Effect of methyl jasmonate (MeJA) treatment on the accumulation of soluble sugars in rice. Three-wk-old rice seedlings were sprayed with 200 µM MeJA or water only as a mock. Leaf samples were harvested 24 h after treatment, and soluble sugar levels were determined using a triple quadrupole mass spectrometer. Data were analyzed using a two-tailed Student's t-test (*, $P < 0.05$; **, $P < 0.01$). Data are presented using bar plots as the mean ± SE, and all data points are shown on the graph as dots ($n = 4$, each replicate consisting of 4-6 seedlings).

Table S1 Chromatographic gradient for analysis of jasmonates by LC-MS/MS.

Time (min)	Flow rate (ml min^{-1})	% A (0.05% formic acid)	% B (acetonitrile)
0.00	1.1	90	10
0.50	1.1	90	10
4.00	1.1	10	90
4.02	1.1	0	100
4.50	1.1	0	100
4.51	1.1	90	10
7.00	1.1	90	10

Table S2 Details of analysis of jasmonates by LC-MS/MS.

The LC-MS/MS system [Agilent 1260 HPLC (Agilent Technologies)- QTRAP 6500 tandem mass spectrometry (SCIEX)] was operated in negative ionization mode. Q1, Quadrupole 1, Q3, Quadrupole 3; RT, Retention time; RF, Response Factor; DP, Declustering Potential; CE, Collision Energy.

Q1 (m/z)	Q3 (m/z)	RT (min)	Analyte name	Internal standard	RF	DP (V)	CE (V)
209.07	59.00	3.6	JA	D_5-JA+D_6-JA	1.0	-20	-24
322.19	130.10	3.9	JA-Ile	D_5+D_6-JA-Ile	1.0	-50	-30
290.90	165.10	4.6	*cis*-OPDA	D_5-JA+D_6-JA	1.0	-20	-24
338.10	130.10	3.o	OH-JA-Ile	D_5+D_6-JA-Ile	1.0	-50	-30
225.10	59.00	2.6	OH-JA	D_5-JA+D_6-JA	1.0	-20	-24
352.10	130.10	3.0	COOH-JA-Ile	D_5+D_6-JA-Ile	1.0	-50	-30
387.10	207.00	2.4	JA-glucoside	D_5-JA+D_6-JA	-	-20	-28
214.00	59.00	3.6	D_5-JA	-	-	-20	-24
215.00	59.00	3.6	D_6-JA	-	-	-20	-24
327.19	130.10	3.9	D_5-JA-Ile	-	-	-50	-30
328.19	130.10	3.9	D_6-JA-Ile	-	-	-50	-30

Table S3 Chromatographic gradient for analysis of flavonoid phytoalexins by LC-MS/MS.

Time (min)	Flow rate (ml min^{-1})	% A (0.05% formic acid)	% B (acetonitrile)
0.00	1.1	95	5
0.50	1.1	95	5
6.00	1.1	62.6	37.4
6.02	1.1	20	80
7.50	1.1	0	100
9.50	1.1	0	100
9.52	1.1	95	5
12.0	1.1	95	5

Table S4 Details of analysis of flavonoid phytoalexins by LC-MS/MS.
The LC-MS/MS system [Agilent 1260 HPLC (Agilent Technologies)- QTRAP 6500 tandem mass spectrometry (SCIEX)] was operated in positive ionization mode. Q1, Quadrupole 1; Q3, Quadrupole 3; RT, Retention time; RF, Response Factor; DP, Declustering Potential; CE, Collision Energy.

Q1 (m/z)	Q3 (m/z)	RT (min)	Analyte name	Internal standard	RF	DP (V)	CE (V)
273.0	153.0	7.18	Naringenin	D_6-ABA	0.77	-30	-31
287.0	167.0	7.43	Sakuranetin	D_6-ABA	0.51	-30	-31
271.0	253.0	6.39	D6-ABA	-	-	-30	-11

Table S5 Chromatographic gradient for analysis of soluble sugars by LC-MS/MS.

Time (min)	Flow rate (ml min^{-1})	% A (water)	%B (acetonitrile)
0.0	1.0	20	80
0.5	1.0	20	80
13.0	1.0	45	55
14.0	1.0	20	80
18.0	1.0	20	80

Table S6 Details of analysis of soluble sugars by LC-MS/MS. The LC-MS/MS system [HPLC 1200 (Agilent Technologies)-API3200 (AB SCIEX)] was operated in negative ionization mode. Q1, Quadrupole 1; Q3, Quadrupole 3; RT, Retention time; DP, Declustering Potential; CE, Collision Energy.

Q1	Q3	RT (min)	Compound	DP	CE
178.8	89.0	7.0	Glucose	-25	-10
178.801	89.0	6.0	Fructose	-25	-12
340.9	59.0	8.5	Sucrose	-45	-46
503.1	179.0	10.5	Raffinose	-75	-28
185.0	92.0	7.0	^{13}C-glucose	-25	-10
185.01	92.0	6.0	^{13}C-fructose	-25	-12

Table S7 Primer sequences used in this study for RT-qPCR.

Primer name	Sequence 5'-3'	Reference
OsD27-for	TCTGGGCTAAAGAATGAAAAGGA	Ito *et al.,* 2017
OsD27-rev	AGAGCTTGGGTCACAATCTCG	Ito *et al.,* 2017
OsD10-for	AGATTGTGGCGAGCGTGGAG	Sun *et al.,* 2014
OsD10-rev	AGGAGCGGAGGTTGTGGAGG	Sun *et al.,* 2014
OsD14-for	TTGAACGACAGCGACTACCACG	Sun *et al.,* 2014
OsD14-rev	GAAGAGGGTGCGGCTGAACT	Sun *et al.,* 2014
OsExp-for	TGTGAGCAGCTTCTCGTTTG	Ji *et al.,* 2015
OsExp-rev	TGTTGTTGCCTGTGAGATCG	Ji *et al.,* 2015
OsActin-for	CTCTCAGCACATTCCAGCAG	Sun *et al.,* 2014
OsActin-rev	AGGAGGACGGCGATAACAG	Sun *et al.,* 2014

Table S8 Statistical results of a two-way ANOVA for the levels of hormone metabolites in rice leaves infected with the blast fungus *Pyricularia oryzae*.

JA metabolites	**Factor**	**Degrees of Freedom (df)**	**F ratio (DFn, DFd)**	***P* value**
cis-OPDA	Interaction	2	F (2, 18) = 22.1	$P < 0.001$
	P. oryzae infection	1	F (1, 18) = 64.0	$P < 0.001$
	Time points	2	F (2, 18) = 17.0	$P < 0.001$
JA	Interaction	2	F (2, 18) = 12.3	$P < 0.001$
	P. oryzae infection	1	F (1, 18) = 44.3	$P < 0.001$
	Time points	2	F (2, 18) = 35.1	$P < 0.001$
OH-JA	Interaction	2	F (2, 18) = 36.7	$P < 0.001$
	P. oryzae infection	1	F (1, 18) = 113	$P < 0.001$
	Time points	2	F (2, 18) = 15.0	$P < 0.001$
JA-Ile	Interaction	2	F (2, 18) = 42.2	$P < 0.001$
	P. oryzae infection	1	F (1, 18) = 157	$P < 0.001$
	Time points	2	F (2, 18) = 45.4	$P < 0.001$
COOH-JA-Ile	Interaction	2	F (2, 18) = 16.8	$P < 0.001$
	P. oryzae infection	1	F (1, 18) = 55.8	$P < 0.001$
	Time points	2	F (2, 18) = 4.78	$P = 0.02$
OH-JA-Ile	Interaction	2	F (2, 18) = 51.0	$P < 0.001$
	P. oryzae infection	1	F (1, 18) = 212	$P < 0.001$
	Time points	2	F (2, 18) = 51.5	$P < 0.001$

Table S9 Statistical results of a two-way ANOVA for the levels of sugar metabolites in *Pyricularia oryzae*-infected rice leaves.

Sugar metabolites	Factor	Degrees of Freedom (df)	F ratio (DFn, DFd)	*P* value
Glucose	Interaction	2	F (2, 18) = 21.8	*P* < 0.001
	P. oryzae infection	1	F (1, 18) = 80.6	*P* < 0.001
	Time points	2	F (2, 18) = 29.3	*P* < 0.001
Fructose	Interaction	2	F (2, 18) = 27.3	*P* < 0.001
	P. oryzae infection	1	F (1, 18) = 99.8	*P* < 0.001
	Time points	2	F (2, 18) = 37.8	*P* < 0.001
Sucrose	Interaction	2	F (2, 18) = 4.52	*P* = 0.03
	P. oryzae infection	1	F (1, 18) = 18.7	*P* < 0.001
	Time points	2	F (2, 18) = 3.94	*P* = 0.04
Raffinose	Interaction	2	F (2, 18) = 7.94	*P* = 0.003
	P. oryzae infection	1	F (1, 18) = 21.1	*P* < 0.001
	Time points	2	F (2, 18) = 70.2	*P* < 0.001

Table S10 Statistical results of a two-way ANOVA for the transcripts of strigolactone genes.

Gene	Factor	Degrees of Freedom (df)	F ratio (DFn, DFd)	*P* value
d27	Interaction	2	F (2, 18) = 1.40	*P* = 0.27
	P. oryzae infection	1	F (1, 18) = 42.1	*P* < 0.001
	Time points	2	F (2, 18) = 29.3	*P* < 0.001
d10	Interaction	2	F (2, 18) = 3.95	*P* = 0.04
	P. oryzae infection	1	F (1, 18) = 28.7	*P* < 0.001
	Time points	2	F (2, 18) = 28.5	*P* < 0.001
d14	Interaction	2	F (2, 18) = 2.28	*P* = 0.13
	P. oryzae infection	1	F (1, 18) = 47.3	*P* < 0.001
	Time points	2	F (2, 18) = 29.9	*P* = 0.04

Table S11 Statistical results of a two-way ANOVA for the levels of flavonoid metabolites in rice leaves infected with the blast fungus *Pyricularia oryzae*.

Flavonoid phytoalexins	Factor	Degrees of Freedom (df)	F ratio (DFn, DFd)	*P* value
Naringenin	Interaction	2	F (2, 18) = 23.8	*P* < 0.001
	P. oryzae infection	1	F (1, 18) = 137	*P* < 0.001
	Time points	2	F (2, 18) = 27.1	*P* < 0.001
Sakuranetin	Interaction	2	F (2, 18) = 11.9	*P* < 0.001
	P. oryzae infection	1	F (1, 18) = 64.2	*P* < 0.001
	Time points	2	F (2, 18) = 12.0	*P* < 0.001

Table S12 Statistical results of a two-way ANOVA for *Pyricularia oryzae*-infected strigolactone-mutant lines with and without *rac*-GR24 pre-treatment.

Lesion type	Factor	Degrees of Freedom (df)	F ratio (DFn, DFd)	*P* value
Sporulating	Interaction	2	F (2, 30) = 1.687	*P* = 0.202
	Plant lines	2	F (2, 30) = 29.76	*P* < 0.0001
	rac-GR24 treatment	1	F (1, 30) = 16.11	*P* = 0.0004
Non-sporulating	Interaction	2	F (2, 30) = 0.388	*P* = 0.682
	Plant lines	2	F (2, 30) = 2.025	*P* = 0.149
	rac-GR24 treatment	1	F (1, 30) = 0.516	*P* = 0.478

Table S13 Statistical results of a two-way ANOVA for *Pyricularia oryzae*-infected JA-mutant lines with and without strigolactone inhibition.

Lesion type	Factor	Degrees of Freedom (df)	F ratio (DFn, DFd)	*P* value
Sporulating	Interaction	2	F (2, 42) = 5.065	*P* = 0.011
	Plant lines	2	F (2, 42) = 455.8	*P* < 0.001
	TIS108 treatment	1	F (1, 42) = 2.731	*P* = 0.106
Non-sporulating	Interaction	2	F (2, 42) = 0.663	*P* = 0.521
	Plant lines	2	F (2, 42) = 14.39	*P* < 0.001
	TIS108 treatment	1	F (1, 42) = 0.204	*P* = 0.654

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