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Supporting Information

Lack of antagonism between salicylic acid and jasmonate signalling pathways in poplar

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The following Supporting Information is available for this article:

Fig. S1 Soluble sugars in leaves of transgenic high salicylic acid vs wild-type black poplar lines.

Fig. S2 Content of free amino acids in leaves of transgenic high salicylic acid vs wild-type black poplar lines.

Fig. S3 Relative concentrations of shikimic acid and quinic acid in leaves of transgenic high salicylic acid vs wild-type black poplar lines.

Fig. S4 Levels of phenolic acids in leaves of transgenic high salicylic acid vs wild-type black poplar lines.

Fig S5. Relative levels of salicinoids in leaves of transgenic high salicylic acid vs wild-type black poplar lines.

Fig. S6 Accumulation of benzoic acid and its derivatives in leaves of transgenic high salicylic acid vs wild-type black poplar lines.

Fig. S7 Levels of flavonoids in leaves of transgenic high salicylic acid vs wild-type black poplar lines.

Fig. S8 Accumulation of flavan-3-ols in leaves of transgenic high salicylic acid vs wild-type black poplar lines.

Fig. S9 Growth of transgenic high salicylic acid vs wild-type black poplar lines.

Fig. S10 Correlation between rust colonization with PR gene expression, salicylic acid and flavan-3-ol contents in leaves of black poplar lines.

Fig. S11 Induction of PR genes in transgenic high salicylic acid and wild-type black poplar lines after rust infection.

Notes S1 Quantification of soluble sugars using liquid chromatography-tandem mass spectrometry.

Notes S2 Quantification of free amino acids using liquid chromatography-tandem mass spectrometry.

Table S1 Chromatographic gradient for analysis of phytohormones by liquid chromatography-tandem mass spectrometry.

Table S2 Details of the analysis of phytohormones by liquid chromatography-tandem mass spectrometry.

Table S3 List of RT-qPCR primers used in this study.

Table S4 Chromatographic gradient for analysis of flavonoids by liquid chromatography-tandem mass spectrometry.

Table S5 Details of the analysis of flavonoids by liquid chromatography-tandem mass spectrometry.

Table S6 Chromatographic gradient for analysis of salicinoids and other metabolites.

Table S7 Details of the analysis of salicinoids and other phenolic metabolites by liquid chromatography-tandem mass spectrometry.

Table S8 Chromatographic gradient for analysis of soluble sugars by liquid chromatography-tandem mass spectrometry.

Table S9 Details of the analysis of soluble sugars.

Table S10 Chromatographic gradient for analysis of free amino acids by liquid chromatography-tandem mass spectrometry.

Table S11 Details of the analysis of amino acids by liquid chromatography-tandem mass spectrometry.

Table S12 Statistical results of a two-way ANOVA for the levels of hormone metabolites in poplar leaves infected with the rust fungus *Melampsora larici-populina*.

Table S13 Statistical results of a two-way ANOVA for the expression of PR and WRKY transcription factor genes in poplar leaves infected with *Melampsora larici-populina*.

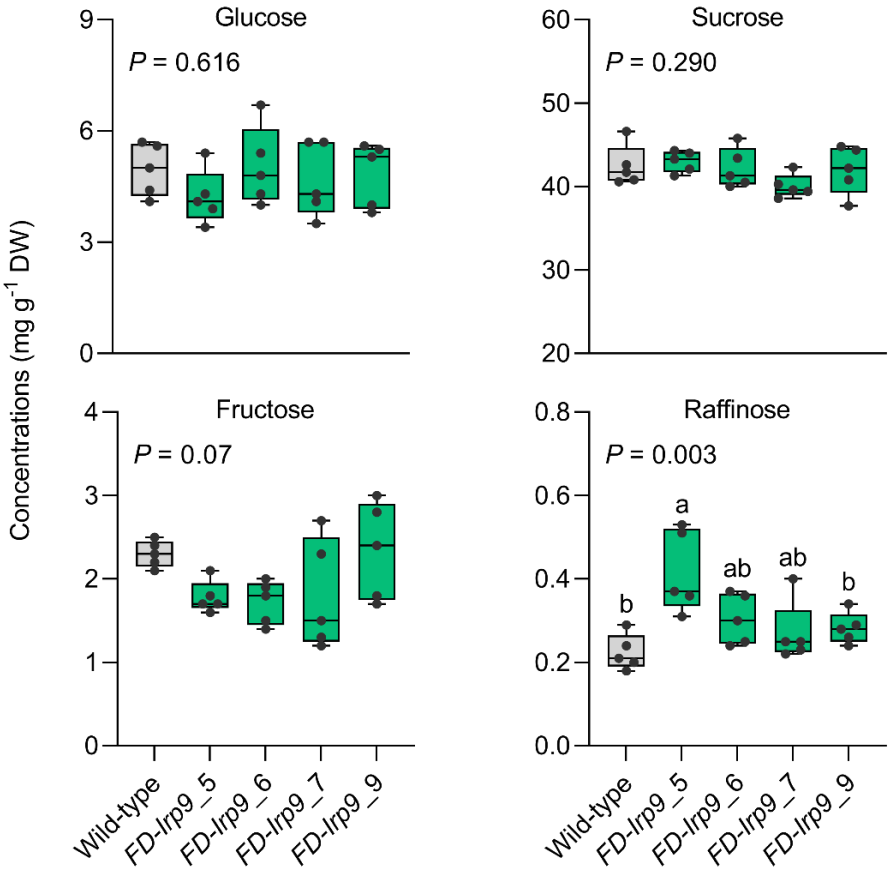

Fig. S1 Soluble sugars in leaves of transgenic high salicylic acid vs wild-type black poplar lines. Data were analysed using a one-way ANOVA, followed by Tukey's multiple comparison test with a 95% confidence interval. Different letters above boxes are shown in panels where lines were significantly different ($P < 0.05$). Each box extends from the 25th to 75th percentiles, and the horizontal line inside the box represents the median. Whiskers were plotted down to the minimum and up to the maximum value. All data points are plotted on the graph as black dots ($n = 5$).

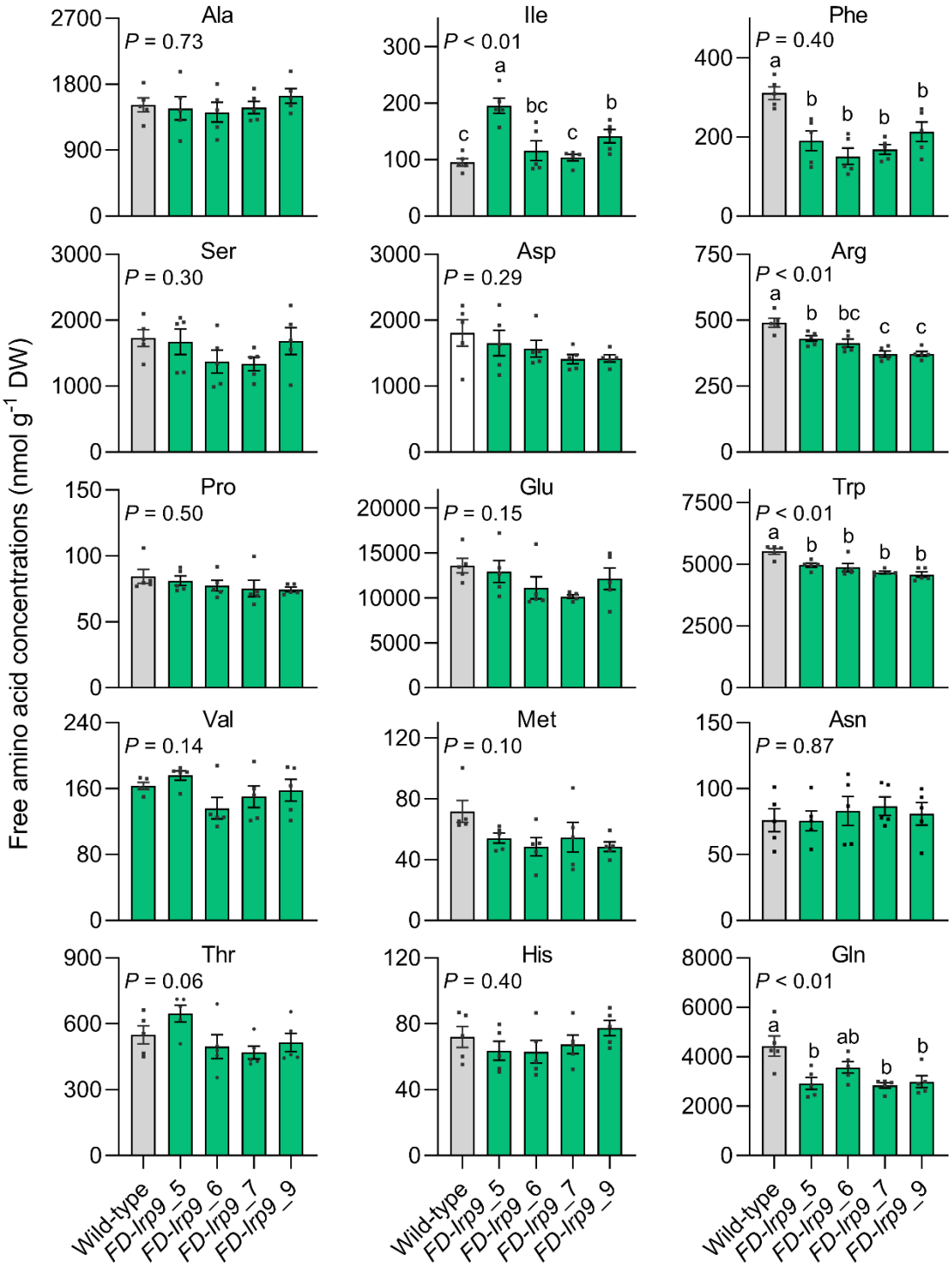

Fig. S2 Content of free amino acids in leaves of transgenic high salicylic acid vs wild-type black poplar lines. Data were analysed using a one-way ANOVA, followed by Tukey's multiple comparison test with a 95% confidence interval. Different letters above bar plots are shown in panels where lines were significantly

different ($P < 0.05$). Bars represent the mean with standard error ($n = 5$). All data points are plotted on the graph as black dots.

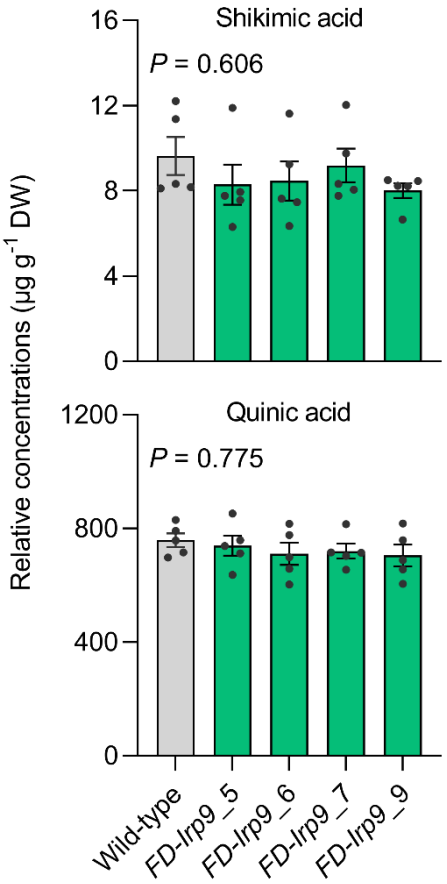

Fig. S3 Relative concentrations of shikimic acid and quinic acid in leaves of transgenic high salicylic acid vs wild-type black poplar lines. Data were analysed using a one-way ANOVA. Bars represent the mean with standard error ($n = 5$). All data points are plotted on the graph as black dots. Concentrations of shikimic acid and quinic acid are presented as equivalent to Trifluoro-methyl-cinnamic acid (TFCA).

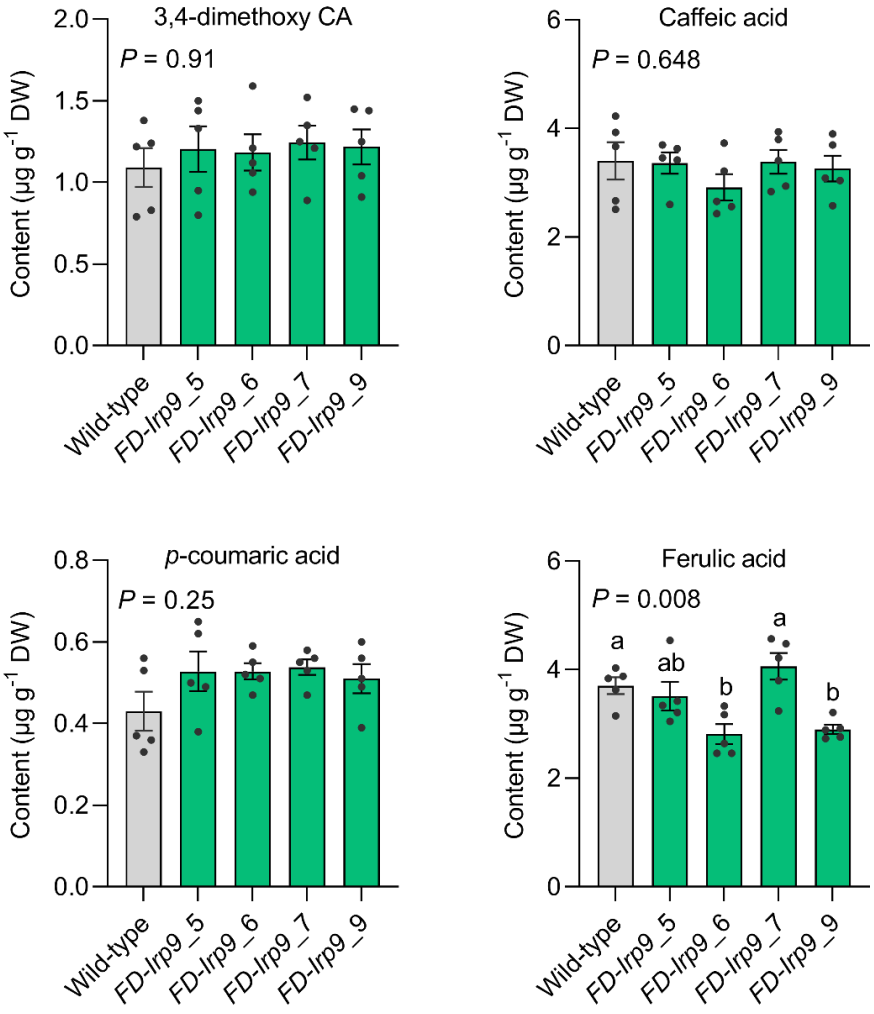

Fig. S4 Levels of phenolic acids in leaves of transgenic high salicylic acid vs wild-type black poplar lines. Data were analysed using a one-way ANOVA, followed by Tukey's multiple comparison test with a 95% confidence interval. Different letters above bar plots are shown in panels where lines were significantly different ($P < 0.05$). Bars represent the mean with standard error ($n = 5$). All data points are plotted on the graph as black dots. CA, cinnamic acid.

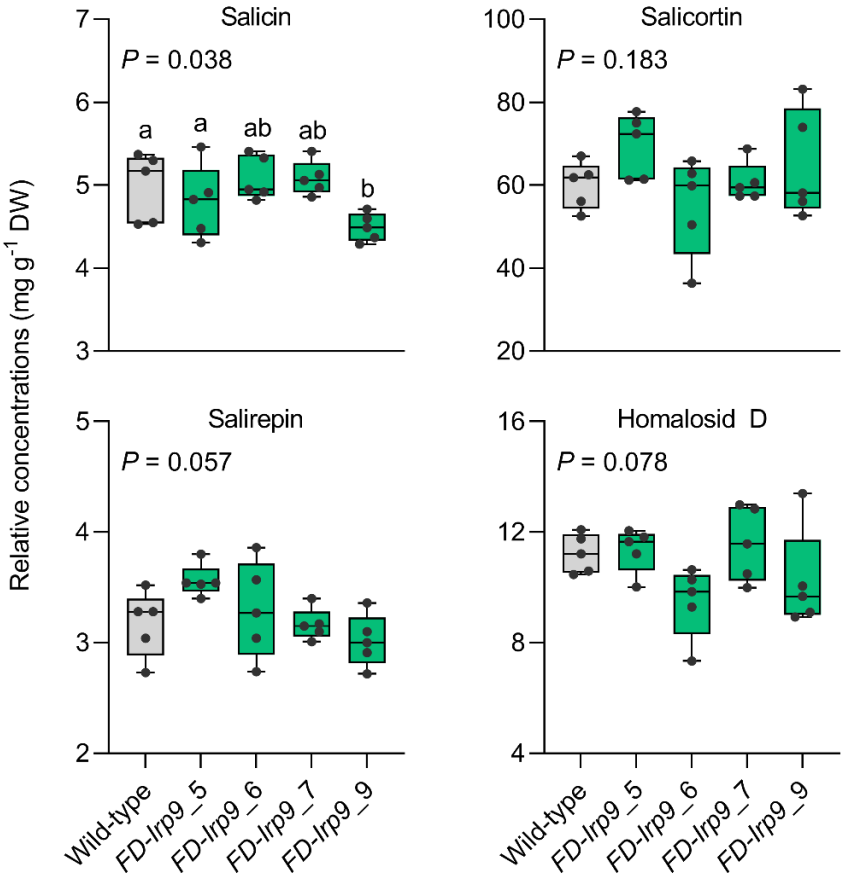

Fig. S5 Relative levels of salicinoids in leaves of transgenic high salicylic acid vs wild-type black poplar lines. Data were analysed using a one-way ANOVA, followed by Tukey's multiple comparison test with a 95% confidence interval. Different letters above boxes are shown in panels where lines were significantly different ($P < 0.05$). Each box extends from the 25th to 75th percentiles, and the horizontal line inside the box represents the median. Whiskers were plotted down to the minimum and up to the maximum value. All data points are plotted on the graph as black dots ($n = 5$).

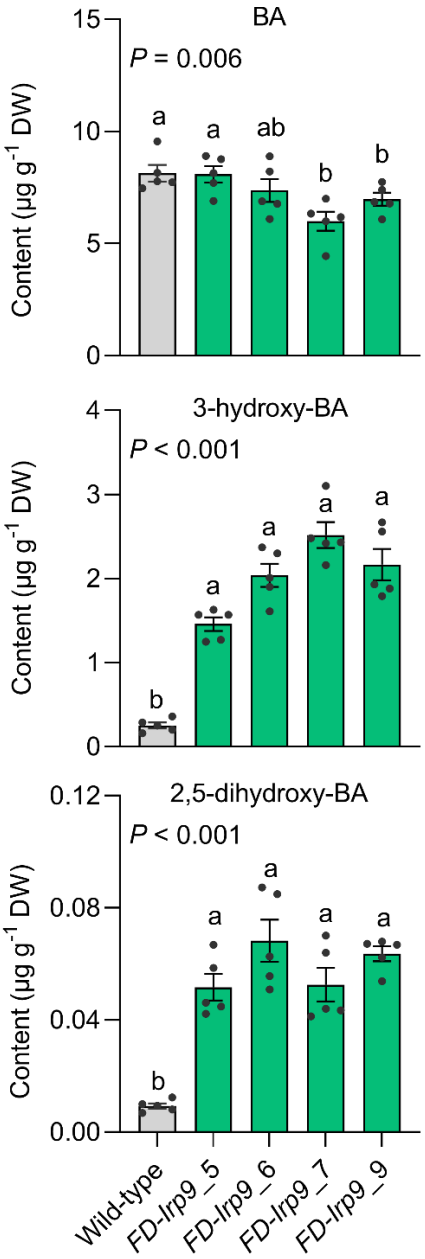

Fig. S6 Accumulation of benzoic acid and its derivatives in leaves of transgenic high salicylic acid vs wild-type black poplar lines. Data were analysed using a one-way ANOVA, followed by Tukey's multiple comparison test with a 95% confidence interval. Different letters above bar plots indicate that lines were significantly different ($P < 0.05$). Bars represent the mean with standard error ($n = 5$). All data points are plotted on the graph as black dots. BA, benzoic acid.

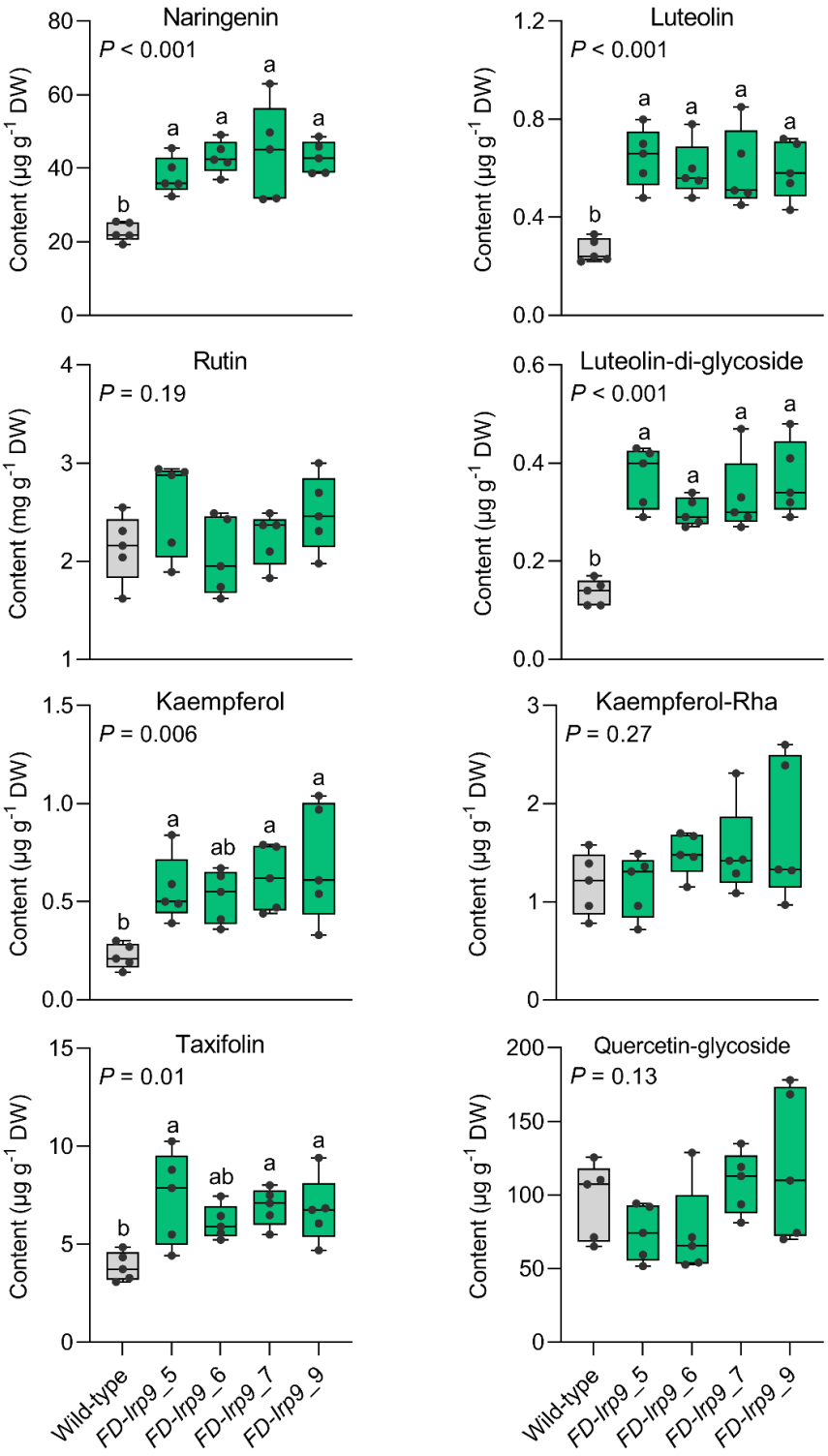

Fig. S7 Levels of flavonoids in leaves of transgenic high salicylic acid vs wild-type black poplar lines. Data were analysed using a one-way ANOVA, followed by Tukey's multiple comparison test with a 95%

confidence interval. Different letters above boxes are shown in panels where lines were significantly different ($P < 0.05$). Each box extends from the 25th to 75th percentiles, and the horizontal line inside the box represents the median. Whiskers were plotted down to the minimum and up to the maximum value. All data points are plotted on the graph as black dots ($n = 5$). Rha, rhamnose.

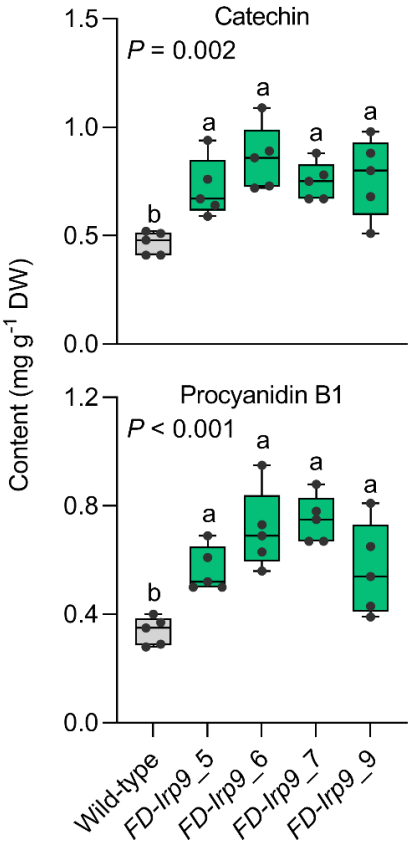

Fig. S8 Accumulation of flavan-3-ols in leaves of transgenic high salicylic acid vs wild-type black poplar lines. Data were analysed using a one-way ANOVA, followed by Tukey's multiple comparison test with a 95% confidence interval. Different letters above boxes are shown in panels where lines were significantly different ($P < 0.05$). Each box extends from the 25th to 75th percentiles, and the horizontal line inside the box represents the median. Whiskers were plotted down to the minimum and up to the maximum value. All data points are plotted on the graph as black dots ($n = 5$).

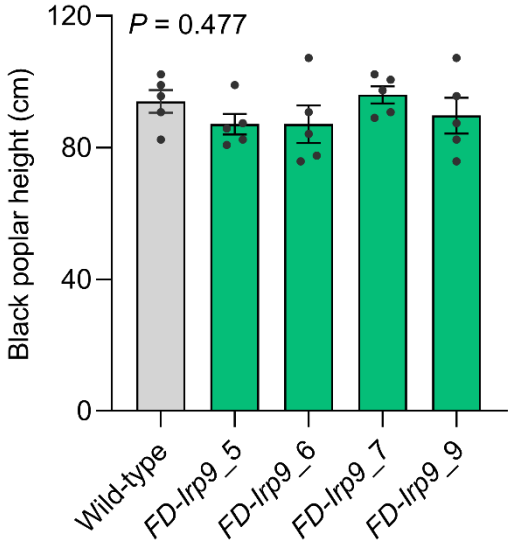

Fig. S9 Growth of transgenic high salicylic acid vs wild-type black poplar lines. Data were analysed using a one-way ANOVA. Bars represent the mean with standard error (n = 5). All data points are plotted on the graph as black dots.

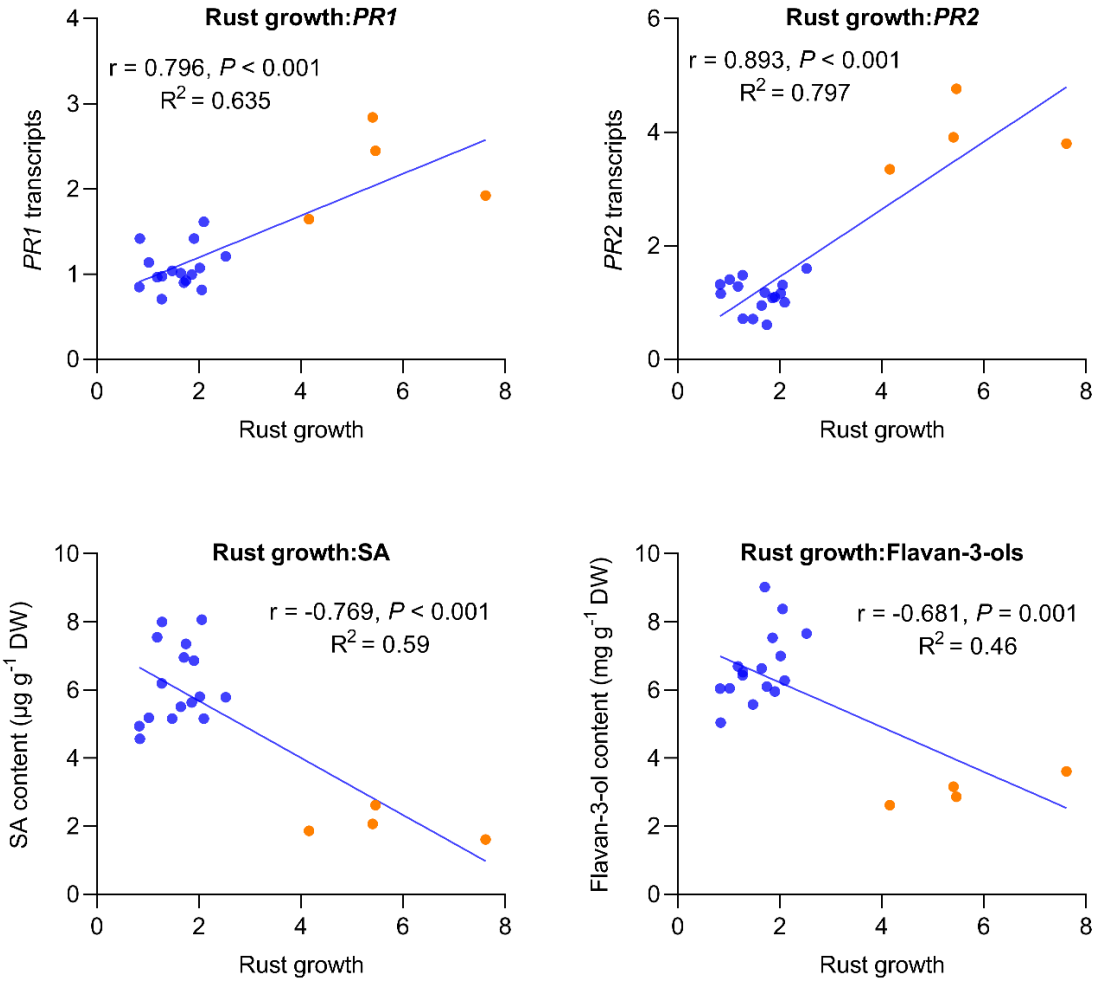

Fig. S10 Correlation between rust colonization with PR gene expression, salicylic acid and flavan-3-ol contents in leaves of black poplar lines. Blue dots represent salicylic acid hyperaccumulating trees, and orange dots are wild-type trees. Data were analysed using Pearson's correlation coefficient (two-sided test). r, Pearson r; R^2, percentage of the variance shared between two variables (X,Y).

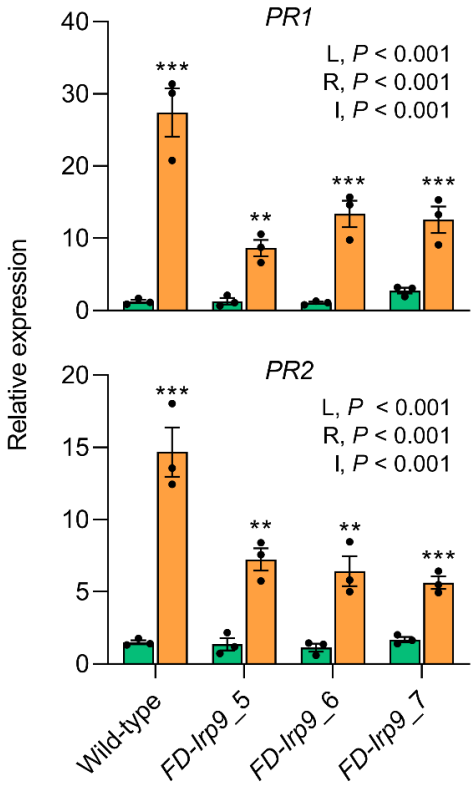

Fig. S11 Induction of PR genes in transgenic high salicylic acid and wild-type black poplar lines after rust infection. Data were analysed using a two-way ANOVA (factors: transgenic line, rust treatment) followed by Tukey's multiple comparison test with a 95% confidence interval. Asterisks (**P < 0.01, ***P < 0.001) indicate pairwise significance. Bars represent mean ± standard error (n = 3). All data points are plotted on the graph as black dots. L, transgenic line; R, rust treatment, I, interaction effect.

Notes S1 Quantification of soluble sugars using liquid chromatography-tandem mass spectrometry.

The HPLC was equipped with a hydrophilic interaction liquid chromatography (HILIC) column (apHera-NH2 Polymer; Supelco, Bellefonte, PA, USA), and chromatographic separation was performed using water and acetonitrile as mobile phases A and B, respectively with a flow rate of 1.1 ml min^{-1}. The column temperature was maintained at 20°C. The mass spectrometer equipped with a turbo spray ion source was operated in the negative ionization mode (Table S8). The ion spray voltage was maintained at −4,500 eV and the turbo gas temperature was set at 700°C. Nebulizing gas was set at 60 psi, curtain gas at 40 psi, heating gas at 60 psi, and collision gas at a medium level. MRM was used to monitor analyte parent ion to product ion formation (MRM, Table S9). Data were acquired using the software Analyst 1.5.1 and

quantification was performed using the software MultiQuant 3.0.3 (Sciex, Framingham, MA, USA). The concentrations of glucose and fructose were determined relative to the internal standards of ^{13}C-glucose and ^{13}C-fructose, respectively. The contents of sucrose (Sigma-Aldrich) and raffinose (Fluka) were calculated based on external standard curves.

Notes S2 Quantification of free amino acids using liquid chromatography-tandem mass spectrometry.

The HPLC was equipped with a Zorbax Eclipse XDB-C18 column (50 × 4.6 mm, 1.8 µm), and chromatographic separation was performed using 0.05% formic acid (v/v) and acetonitrile as mobile phases A and B, respectively. The elution profile is provided in Table S10. After chromatographic separation, the mass spectrometer equipped with a turbo spray ion source was operated in the negative ionization mode to monitor analyte parent ion to product ion formation (MRM, Table S11). Data acquisition and quantification were performed using the software MultiQuant 3.0.3 (Sciex, Framingham, MA, USA). Concentrations of each amino acid were calculated relative to their corresponding labelled amino acids (Table S11).

Table S1 Chromatographic gradient for analysis of phytohormones by liquid chromatography-tandem mass spectrometry.

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 phytohormones by liquid chromatography-tandem mass spectrometry. 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)
136.93	93.00	3.3	SA	D4-SA	1.0	-20	-24
209.07	59.00	3.6	JA	D6-JA	1.0	-20	-24
322.19	130.10	3.9	JA-Ile	D6-JA-Ile	1.0	-50	-30
290.90	165.10	4.6	*cis*-OPDA	D6-JA	1.0	-20	-24
338.10	130.10	3.o	OH-JA-Ile	D6-JA-Ile	1.0	-50	-30
225.10	59.00	2.6	OH-JA	D6-JA	1.0	-20	-24
352.10	130.10	3.0	COOH-JA-Ile	D6-JA-Ile	1.0	-50	-30
299.1	136.9	1.8	SAG	D4-SA	-	-20	-18
387.1	207.0	2.4	JA-glucoside	D6-JA	-	-20	-28
140.93	97.00	3.3	D4-SA			-20	-24
215.00	59.00	3.6	D6-JA			-20	-24
214.00	59.00	3.6	D5-JA			-20	-24
328.19	130.10	3.9	D6-JA-Ile			-50	-30

Table S3 List of RT-qPCR primers used in this study.

Primer	Primer sequence (5’ → 3’)
PnUbiquitin-for	GTTGATTTTTGCTGGGAAGC
PnUbiquitin-rev	GATCTTGGCCTTCACGTTGT
PnActin-for	CCCATTGAGCACGGTATTGT
PnActin-rev	TACGACCACTGGCATACAGG
PnPR1-for	TGGGTTGATGAGAAACCAAAGTATG
PnPR1-rev	GCTGCACCTTGCTTTAGCAC
PnPR2-for	CAAAGGATTGCTTCCAGTCAAGC
PnPR2-rev	TCAAGAAGGGCATCGAAGAGG
PnWRKY18-for	TTATGAAGGAGAGCACAACC
PnWRKY18-rev	TTCTGATGGATGATGGACTG
Irp9-for	ATGCGTTTACCGTGCTGTTTCCGT
Irp9-rev	AGGGCGCAATGCTCGCTAATTTCT
MlpActin-for	GACTGAGGCACCTCTTAATCCAAAAGTC
MlpActin-rev	GTGAGTAACACCGTCACCAGAATCC

Table S4 Chromatographic gradient for analysis of flavonoids by liquid chromatography-tandem mass spectrometry.

Time (min)	Flow rate (ml min^{-1})	% A (0.05% formic acid)	% B (acetonitrile)
0.00	1.1	100	0
1.00	1.1	100	0
7.00	1.1	35	65
7.01	1.1	0	100
8.00	1.1	0	100
8.01	1.1	100	0
10.00	1.1	100	0

Table S5 Details of analysis of flavonoids by liquid chromatography-tandem mass spectrometry. 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	RF	DP (V)	CE (V)
288.9	109.1	4.1	Catechin	8.6	-30	-34
304.9	125.0	3.5	Gallocatechin	8.6	-30	-28
576.9	289.1	4.0	Procyanidin B1	14.2	-50	-30
430.8	268	5.0	Apigenin-7-glucoside	IS	-80	-46
462.91	301	4.5	Quercetin-glycoside	1	-55	-40
271	151	6.5	Naringenin	1	-55	-28

Table S6 Chromatographic gradient for analysis of salicinoids and other metabolites.

Time (min)	Flow rate (ml min^{-1})	% A (0.05% formic acid)	% B (acetonitrile)
0.0	1.1	95.0	5.0
0.5	1.1	95.0	5.0
6.0	1.1	62.6	37.4
6.0	1.1	20.0	80.0
7.5	1.1	0.0	100.0
9.5	1.1	0.0	100.0
9.5	1.1	95.0	5.0
12.0	1.1	95.0	5.0

Table S7 Details of analysis of salicinoids and other phenolic metabolites by liquid chromatography-tandem mass spectrometry. Q1, quadrupole 1; Q3, quadrupole 3; RT, retention time; RF, response factor; DP, declustering potential; CE, collision energy; IS, internal standard.

Q1 (m/z)	Q3 (m/z)	RT (min)	Analyte name	DP (V)	CE (V)	IS	RF
121.0	121.0	5.6	benzoic acid	-20	-5	TFCA	28.3
137.0	93.0	4.1	3-hydroxy-benzoic acid	-20	-16		
153.0	108.0	3.6	2.5-Di-hydroxy-benzoic acid	-20	-28		
173.0	93.0	0.5	shikimic acid	-20	-18		
191.0	85.0	0.5	quinic acid	-20	-28		
179.0	134.9	4.0	caffeic acid	-20	-22	TFCA	1.62
163.0	118.9	4.7	*p*-coumaric acid	-20	-20	TFCA	2.56
193.1	133.9	5.0	ferulic acid	-20	-22	TFCA	7.77
207.0	103.0	6.1	3,4-dimethoxycinnamic acid	-20	-20	TFCA	21.24
215.1	171.1	7.3	Trifluoro-methyl-cinnamic acid (TFCA)	-20	-18		
255.0	161.0	3.2	phenyl β-D-glucopyranoside	-20	-13		
284.9	122.9	2.9	salicin	-20	-18		
301.0	139.0	0.9	salirepin	-20	-18		
422.8	123.1	4.7	salicortin	-20	-30		
543.0	139.0	6.4	homalosid-D	-20	-30		
285.0	93.0	7.2	kaempferol	-20	-46		
285	133	6.82	luteolin	-20	-44		
303.0	125.0	5.4	Taxifolin	-20	-31		
431.0	268.0	5.6	Apigenin-7-glucoside	-20	-44		
431.0	285.0	6.0	Kaempferol-rhamnoside	-20	-45		
447.0	285.0	5.1	luteolin-glucoside	-20	-40		
609.0	447.0	4.7	luteolin-di-glucoside	-20	-34		
609.0	300.0	4.9	rutin	-20	-50		

Table S8 Chromatographic gradient for analysis of soluble sugars by liquid chromatography-tandem mass spectrometry.

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 S9 Details of analysis of soluble sugars. Q1, quadrupole 1, Q3, quadrupole 3; RT, retention time; DP, declustering potential; CE, collision energy.

Q1 (m/z)	Q3 (m/z)	RT (min)	Analyte name	DP (V)	CE (V)
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-C6-Glucose	-25	-10
185.01	92.0	6.0	13-C6-Fructose	-25	-12

Table S10 Chromatographic gradient for analysis of free amino acids by liquid chromatography-tandem mass spectrometry.

Time (min)	Flow rate (ml min^{-1})	% A (0.05% formic acid)	% B (acetonitrile)
0.0	1.1	97	3
1.0	1.1	97	3
2.7	1.1	0	100
3.0	1.1	0	100
3.1	1.1	97	3
6.0	1.1	97	3

Table S11 Details of analysis of amino acids by liquid chromatography-tandem mass spectrometry. Q1, quadrupole 1, Q3, quadrupole 3; RT, retention time; DP, declustering potential; CE, collision energy, IS, internal standard.

Analyte	Q1 (m/z)	Q3 (m/z)	RT (min)	Internal standard	IS Q1 (m/z)	IS Q3 (m/z)	DP (V)	CE (V)
Ala	90.1	44.1	0.5	$^{13}C,^{15}N$-Ala	94.1	47.1	20	17
Ser	106.0	60.1	0.5	$^{13}C,^{15}N$-Ser	110.0	63.1	20	15
Pro	116.1	70	0.5	$^{13}C,^{15}N$-Pro	122.1	75.0	20	19
Val	118.1	72.2	0.5	$^{13}C,^{15}N$-Val	124.1	77.2	20	13
Thr	120.1	74.2	0.5	$^{13}C,^{15}N$-Thr	125.1	78.2	20	13
Ile	132.2	86.1	1.1	$^{13}C,^{15}N$-Ile	139.2	92.1	20	13
Leu	132.2	86.1	1.3	$^{13}C,^{15}N$-Leu	139.2	92.1	20	13
Asp	134.1	74.1	0.5	$^{13}C,^{15}N$-Asp	139.1	77.1	20	19
Glu	148.1	102.1	0.5	$^{13}C,^{15}N$-Glu	154.1	107.1	20	15
Met	150.2	104.1	0.7	$^{13}C,^{15}N$-Met	156.2	109.1	20	13
His	156.2	110.1	0.4	$^{13}C,^{15}N$-His	165.2	118.1	20	17
Phe	166.2	120.2	2.6	$^{13}C,^{15}N$-Phe	176.2	129.2	20	17
Arg	175.1	70.1	0.4	$^{13}C,^{15}N$-Arg	185.1	75.1	20	31
Tyr	182.1	136.2	1.4	$^{13}C,^{15}N$-Tyr	192.1	145.2	20	17
Asn	133.1	74.1	0.5	$^{13}C,^{15}N$-Asp			20	21
Gln	147.1	130	0.5	$^{13}C,^{15}N$-Gln	154.1	136.0	20	13
Trp	205.2	188.1	3.2	D_5-Trp	210.0	193.0	20	13

Table S12 Statistical results of a two-way ANOVA for the levels of hormone metabolites in poplar leaves infected with the rust fungus *Melampsora larici-populina*.

Hormone metabolites	Factor	Degrees of Freedom (df)	F ratio (DFn, DFd)	*P* value
Salicylic acid (SA)	Interaction	4	F (4, 40) = 3.41	$P = 0.02$
	Genotype	4	F (4, 40) = 3.80	$P = 0.01$
	Rust	1	F (1, 40) = 359	$P < 0.001$
SA-glucoside (SAG)	Interaction	4	F (4, 40) = 7.87	$P < 0.001$
	Genotype	4	F (4, 40) = 12.4	$P < 0.001$
	Rust	1	F (1, 40) = 250	$P < 0.001$
cis-OPDA	Interaction	4	F (4, 40) = 7.48	$P < 0.001$
	Genotype	4	F (4, 40) = 22.8	$P < 0.001$
	Rust	1	F (1, 40) = 78.5	$P < 0.001$
Jasmonic acid (JA)	Interaction	4	F (4, 40) = 4.08	$P = 0.007$
	Genotype	4	F (4, 40) = 9.37	$P < 0.001$
	Rust	1	F (1, 40) = 155	$P < 0.001$
JA-glucoside	Interaction	4	F (4, 40) = 3.19	$P = 0.02$
	Genotype	4	F (4, 40) = 113	$P < 0.001$
	Rust	1	F (1, 40) = 151	$P < 0.001$
Jasmonoyl-L-isoleucine (JA-Ile)	Interaction	4	F (4, 40) = 4.20	$P = 0.006$
	Genotype	4	F (4, 40) = 9.19	$P < 0.001$
	Rust	1	F (1, 40) = 120	$P < 0.001$

Table S13 Statistical results of a two-way ANOVA for the expression of PR and WRKY transcription factor genes in poplar leaves infected with *Melampsora larici-populina*.

Genes	Factor	Degrees of Freedom (df)	F ratio (DFn, DFd)	*P* value
PR1	Interaction	4	F (4, 40) = 43.6	$P < 0.001$
	Genotype	4	F (4, 40) = 40.7	$P < 0.001$
	Rust	1	F (1, 40) = 307	$P < 0.001$
PR2	Interaction	4	F (4, 40) = 147	$P < 0.001$
	Genotype	4	F (4, 40) = 148	$P < 0.001$
	Rust	1	F (1, 40) = 424	$P < 0.001$
WRKY89	Interaction	4	F (4, 40) = 4.84	$P = 0.003$
	Genotype	4	F (4, 40) = 8.71	$P < 0.001$
	Rust	1	F (1, 40) = 287	$P < 0.001$
WRKY18	Interaction	4	F (4, 40) = 15.8	$P < 0.001$
	Genotype	4	F (4, 40) = 16.4	$P < 0.001$
	Rust	1	F (1, 40) = 190	$P < 0.001$