

Supporting information

Quantification of stress- and resistance-related metabolites in barley leaves (*Hordeum vulgare* L.) infected with *Bipolaris sorokiniana* via UHPLC-MS/MS_{MRM}

Lisa Kurzweil,¹ Timo D. Stark,² Karina Hille,² Felix Hoheneder,³ Jana Mrtva,² Hans Hausladen,⁴
Miriam Lenk,⁵ Mohammed Saddik Motawie,⁶ Corina Vlot-Schuster,^{5,7} Klaus Pillen,⁸ Mette
Sørensen,^{6,9} Birger Lindberg Møller,⁶ Ralph Hüchelhoven,³ Corinna Dawid,^{1,2,10,11*}

¹ Professorship for Functional Phytometabolomics, TUM School of Life Sciences, Technical University of Munich, Lise-Meitner-Str. 34, 85354 Freising, Germany.

² Chair of Food Chemistry and Molecular Sensory Science, TUM School of Life Sciences, Technical University of Munich, Lise-Meitner-Str. 34, 85354 Freising, Germany.

³ Chair of Phytopathology, TUM School of Life Sciences, Technical University of Munich, Emil-Ramann-Str. 2, 85354 Freising, Germany.

⁴ Plant Technology Center, TUM School of Life Sciences, Technical University of Munich, Dürnast 9, 85354 Freising, Germany.

⁵ Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany.

⁶ Plant Biochemistry Laboratory, Department of Plant and Environmental Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Copenhagen, Denmark.

⁷ Chair of Crop Plant Genetics, Faculty of Life Sciences: Food, Nutrition and Health, University of Bayreuth, Fritz-Hornschuch-Straße 13, 95326 Kulmbach, Germany.

⁸ Chair of Plant Breeding, Martin-Luther-University Halle-Wittenberg, Betty-Heimann-Str. 3, 06120 Halle (Saale), Germany.

⁹ LEO Pharma, Industriparken 55, 5750 Ballerup, Copenhagen, Denmark.

¹⁰ Professorship for Chemosensory Food Systems, TUM School of Life Sciences, Technical University of Munich, Lise-Meitner-Str. 34, 85354 Freising, Germany.

¹¹ Leibniz Institute for Food Systems Biology at the Technical University of Munich, Lise-Meitner-Str. 34, 85354 Freising, Germany.

* **Corresponding author:**

Phone: +49 8161 712902

Fax: +49 8161 712949

E-mail: corinna.dawid@tum.de

Table S1. Optimized MS parameters of the UHPLC-MS/MS-sMRM method for the quantification of marker metabolites in barley leaves. The first mass transition of each substance served as a quantifier, the remaining mass transitions as qualifiers. The system was operated in polarity switch mode, in which ESI⁻ and ESI⁺ are measured simultaneously. IS internal standard, t_R retention time, EP entrance potential, CE collision energy, CXP collision exit potential.

No.	Substance name	mass transition Q1/Q3	t_R (min)	t_R window (s)	EP (V)	CE (V)	CXP (V)
1	hordatine A	276.1/131.0	7.8	120	11	25	20
1	hordatine A	276.1/265.1	7.8	120	11	27	24
2	hordatine B	291.1/131.1	7.7	100	51	25	16
2	hordatine B	291.1/277.9	7.7	100	51	41	24
3	hordatine C	306.2/131.1	8.1	90	1	25	16
3	hordatine C	306.2/157.0	8.1	90	1	25	18
4	hordatine A glucoside	357.2/276.2	7.8	60	61	23	24
4	hordatine A glucoside	357.2/290.9	7.8	60	61	35	32
5	hordatine B glucoside	372.2/291.2	7.7	60	26	25	24
5	hordatine B glucoside	372.2/295.0	7.7	60	26	37	24
6	hordatine C glucoside	387.2/306.1	8.0	60	61	25	16
6	hordatine C glucoside	387.2/350.9	8.0	60	61	37	40
7	<i>p</i> -CA	277.0/147.0	5.6	60	66	33	18
7	<i>p</i> -CA	277.0/91.0	5.6	60	66	63	10
8	feruloylagmatine	307.0/177.0	6.2	60	61	29	20
8	feruloylagmatine	307.0/144.9	6.2	60	61	43	16
9	sinapoylagmatine	337.0/207.0	6.5	60	76	29	24
9	sinapoylagmatine	337.0/175.0	6.5	60	76	39	20
10	<i>p</i> -CHA	293.0/147.0	4.8	90	111	31	26
10	<i>p</i> -CHA	293.0/275.1	4.8	90	111	21	26
11	<i>p</i> -CHDA	273.0/254.8	4.8	90	136	21	22
11	<i>p</i> -CHDA	273.0/146.9	4.8	90	136	31	16
11	<i>p</i> -CHDA	273.0/126.9	4.8	90	136	25	14
12	<i>p</i> -coumaroylputrescine	235.0/147.0	4.5	60	36	25	16
12	<i>p</i> -coumaroylputrescine	235.0/91.0	4.5	60	36	51	10
13	feruloylputrescine	365.1/177.0	9.5	60	36	25	20
13	feruloylputrescine	365.1/145.0	9.5	60	36	43	18
14	sinapoylputrescine	295.0/207.0	5.7	60	46	21	24
14	sinapoylputrescine	295.0/175.0	5.7	60	46	31	20
15	isovitexin	430.9/310.9	10.0	60	-45	-30	-39
15	isovitexin	430.9/341.0	10.0	60	-45	-32	-19
16	saponarin	593.1/310.9	8.6	60	-240	-50	-17
16	saponarin	593.1/296.9	8.6	60	-240	-60	-25
17	meloside A	593.0/293.0	9.4	60	-160	-46	-17
17	meloside A	593.0/412.8	9.4	60	-160	-34	-35
18	schaftoside	563.1/353.1	8.8	60	-130	-48	-19
18	schaftoside	563.1/382.9	8.8	60	-130	-50	-27

19	isoschaftoside	563.0/353.1	9.4	60	-65	-50	-23
19	isoschaftoside	563.0/382.9	9.4	60	-65	-50	-45
20	epiheteroendrin	283.9/284.0	5.0	90	126	9	22
20	epiheteroendrin	261.9/262.0	5.0	90	121	5	16
21	sutherlandin	297.9/297.9	1.5	90	41	9	18
21	sutherlandin	275.8/275.9	1.5	90	121	7	22
22	osmaronin	281.9/282.0	3.9	90	121	9	32
22	osmaronin	260.0/259.9	3.9	90	111	9	14
23	dihydroosmaronin	283.9/283.8	4.1	90	51	11	22
23	dihydroosmaronin	262.0/262.0	4.1	90	116	9	18
24	epidermin	283.9/284.1	3.2	90	141	9	18
24	epidermin	261.9/262.1	3.2	90	111	5	16
24	epidermin	261.9/203.9	3.2	90	111	19	24
25	<i>p</i> -coumaric acid	162.9/118.9	7.9	60	-75	-18	-17
25	<i>p</i> -coumaric acid	162.9/93.2	7.9	60	-75	-34	-15
26	ferulic acid	192.9/134.0	8.9	60	-40	-20	-15
26	ferulic acid	192.9/178.0	8.9	60	-40	-18	-19
27	sinapic acid	222.9/163.9	9.3	60	-10	-20	-17
27	sinapic acid	222.9/207.9	9.3	60	-10	-18	-9
28	tryptophan	205.0/187.9	4.6	90	1	13	22
28	tryptophan	205.0/145.8	4.6	90	1	25	18
28	tryptophan	205.0/117.9	4.6	90	1	33	14
29	tryptamine	160.9/143.9	5.1	90	86	15	16
29	tryptamine	160.9/116.9	5.1	90	86	27	14
30	oxyglutathione	611.0/305.9	1.6	90	-120	-34	-21
30	Oxyglutathione	611.0/271.8	1.6	90	-120	-36	-15
31	5-carboxyblumenol C glucoside	401.0/100.9	9.3	90	-100	-34	-9
31	5-carboxyblumenol C glucoside	401.0/221.0	9.3	90	-100	-30	-19
32	5-carboxydidehydroblumenol C glucoside	399.0/219.0	9.6	90	-60	-24	-19
32	5-carboxydidehydroblumenol C glucoside	399.0/174.9	9.6	90	-60	-32	-19
33	grashopper ketone sulfate	302.9/96.8	7.0	120	-25	-30	-11
33	grashopper ketone sulfate	302.9/79.9	7.0	120	-25	-102	-37
34	unknown (C ₁₃ H ₂₁ SO ₆)	304.9/96.9	6.5	90	-65	-28	-13
34	unknown (C ₁₃ H ₂₁ SO ₆)	304.9/79.9	6.5	90	-65	-106	-9
IS1	phenformin	206.0/60.0	5.4	90	26	21	14
IS1	phenformin	206.0/105.1	5.4	90	26	35	12
IS2	puerarin	415.0/267.1	7.4	60	-50	-48	-23
IS2	puerarin	415.0/294.8	7.4	60	-50	-32	-19
IS3	vicenin 2	593.0/353.0	8.0	60	-5	-48	-33
IS3	vicenin 2	593.0/383.1	8.0	60	-5	-50	-43
IS4	linamarin	269.9/270.3	2.7	90	111	13	10
IS4	linamarin	248.1/248.1	2.7	90	111	5	16
IS5	<i>o</i> -coumaric acid	162.9/118.9	9.9	60	-75	-18	-17
IS5	<i>o</i> -coumaric acid	162.9/93.2	9.9	60	-75	-34	-15
IS6	¹³ C ₄ ¹⁵ N ₂ -oxyglutathione	617.0/308.8	1.6	90	-125	-34	-33
IS6	¹³ C ₄ ¹⁵ N ₂ -oxyglutathione	617.0/274.9	1.6	90	-125	-38	-17

IS7	tryptophan- <i>d</i> ₅	210.0/210.1	4.6	60	11	5	12
IS7	tryptophan- <i>d</i> ₅	210.0/192.1	4.6	60	11	15	12
IS8	abscisic acid- <i>d</i> ₆	268.9/159.1	13.2	60	-35	-16	-13
IS8	abscisic acid- <i>d</i> ₆	268.9/225.0	13.2	60	-35	-20	-15

Table S2. Results of the validation of the UHPLC-MS/MS-sMRM method for the quantification of marker metabolites in barley leaves ($n = 3$). Recovery rate (mean values of three spiked concentrations), C_v coefficient of variation, LOD limit of detection, LOQ limit of quantification.

No.	Analyte	Internal standard (IS)	Recovery rate (%)	C_v (%) interday	C_v (%) intraday	LOD ($\mu\text{mol/L}$)	LOQ ($\mu\text{mol/L}$)
1	hordatine A	phenformin (IS1)	113	3.4	2.9	0.119	0.398
2	hordatine B	phenformin (IS1)	131	15.4	6.0	0.053	0.178
3	hordatine C	phenformin (IS1)	93	11.0	2.1	0.056	0.188
4	hordatine A glucoside	phenformin (IS1)	98	19.4	3.6	0.002	0.006
5	hordatine B glucoside	phenformin (IS1)	100	16.8	3.5	0.021	0.069
6	hordatine C glucoside	phenformin (IS1)	90	9.1	5.4	0.012	0.039
7	<i>p</i> -CA	phenformin (IS1)	88	0.2	5.0	0.056	0.185
8	feruloylagmatine	phenformin (IS1)	105	8.6	6.6	0.030	0.102
9	sinapoylagmatine	phenformin (IS1)	93	5.5	4.1	0.056	0.186
10	<i>p</i> -CHA	phenformin (IS1)	93	1.6	4.5	0.051	0.171
12	<i>p</i> -coumaroylputrescine	phenformin (IS1)	93	7.9	5.1	0.013	0.042
13	feruloylputrescine	phenformin (IS1)	117	11.3	4.3	0.137	0.456
14	sinapoylputrescine	phenformin (IS1)	80	17.0	5.0	0.108	0.360
15	isovitexin	puerarin (IS2)	110	13.4	5.1	0.075	0.250
16	saponarin	vicenin 2 (IS3)	102	8.9	6.8	0.011	0.038
18	schaftoside	puerarin (IS2)	123	11.3	9.2	0.078	0.101
19	isoschaftoside	puerarin (IS2)	100	9.3	6.7	0.053	0.061
20	epiheteroendrin	linamarin (IS4)	123	20.5	7.0	0.366	1.220
21	sutherlandin	linamarin (IS4)	112	12.2	3.8	0.361	1.205
22	osmaronin	linamarin (IS4)	115	9.7	1.4	0.311	1.037
23	dihydroosmaronin	linamarin (IS4)	108	26.2	5.9	0.178	0.594
24	epidermin	linamarin (IS4)	117	7.9	4.9	0.356	1.186
25	<i>p</i> -coumaric acid	<i>o</i> -coumaric acid (IS5)	112	12.6	4.2	0.014	0.045
26	ferulic acid	<i>o</i> -coumaric acid (IS5)	114	5.5	4.2	0.009	0.030
27	sinapic acid	<i>o</i> -coumaric acid (IS5)	97	14.7	6.3	0.049	0.163
28	tryptophan	tryptophan- <i>d</i> ₅ (IS7)	99	8.8	2.0	0.001	0.004
29	tryptamine	tryptophan- <i>d</i> ₅ (IS7)	112	14.4	6.7	0.003	0.011
30	oxyglutathione	¹³ C ₄ ¹⁵ N ₂ -oxyglutathione (IS6)	99	15.3	2.9	0.005	0.018
31	5-carboxyblumenol C glucoside	abscisic acid- <i>d</i> ₆ (IS8)	88	9.5	6.3	0.011	0.037

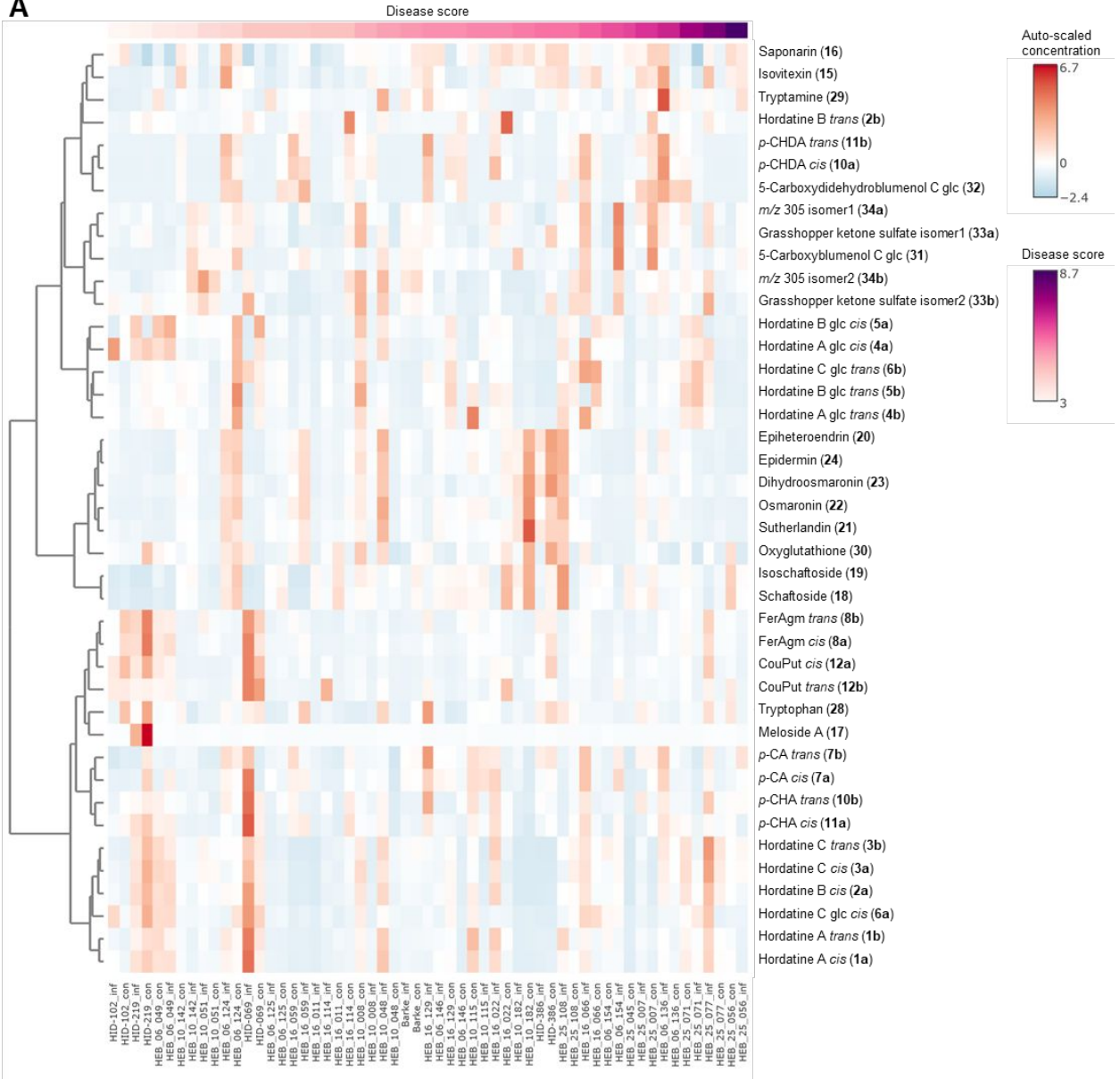
Table S3. Calibration functions and coefficient of determination (R^2) of the quantitation of marker metabolites in barley leaves using UHPLC-MS/MS.

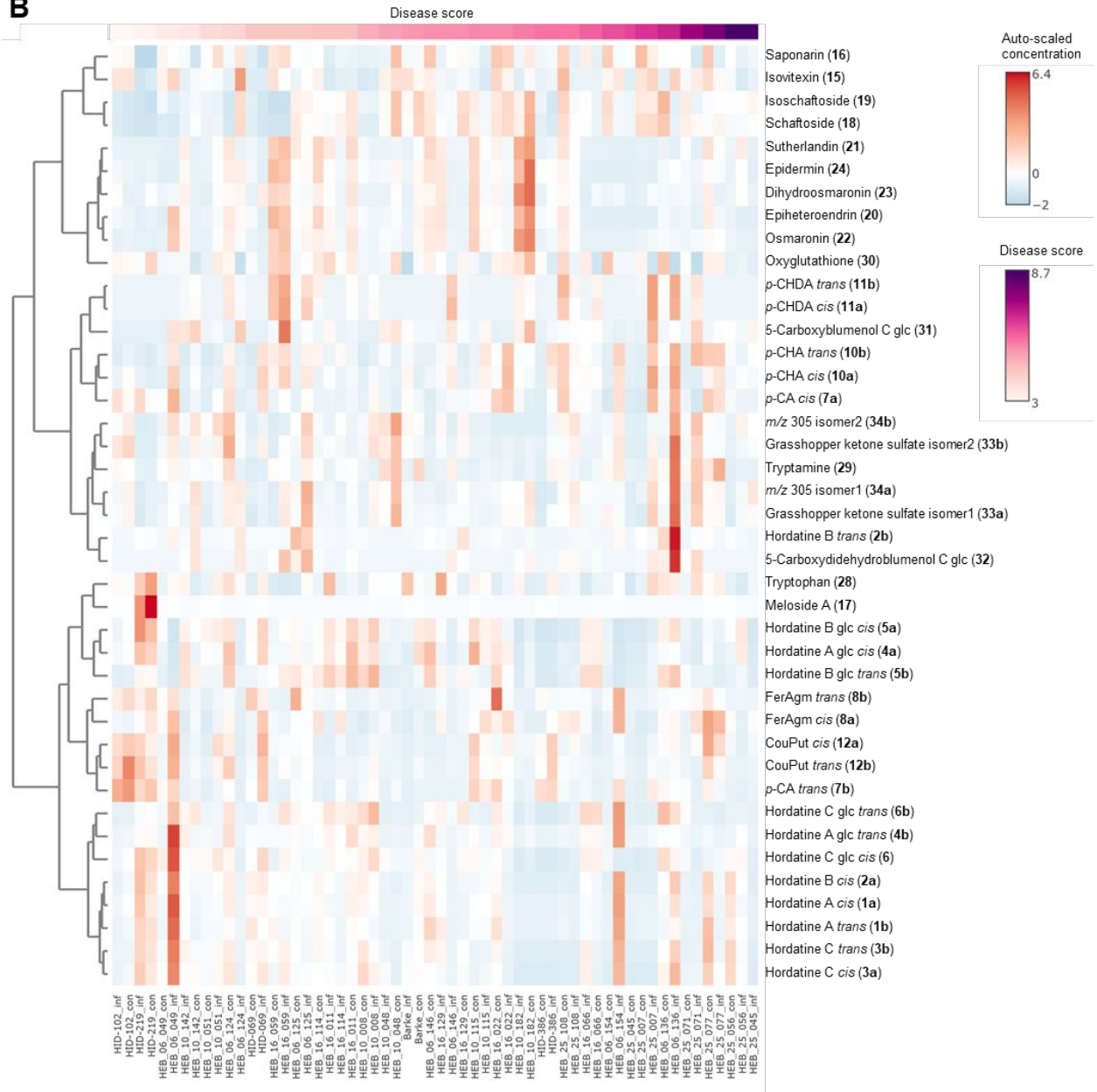
No.	Analyte	Internal standard (IS)	Calibration function	R^2
1	hordatine A	phenformin (IS1)	$y = 0.1401x$	0.9926
2	hordatine B	phenformin (IS1)	$y = 0.0559x$	0.9913
3	hordatine C	phenformin (IS1)	$y = 0.0327x$	0.9919
4	hordatine A glucoside	phenformin (IS1)	$y = -0.0548x^2 + 0.6513x - 0.0141$	0.9971
5	hordatine B glucoside	phenformin (IS1)	$y = -0.0960x^2 + 1.007x - 0.0304$	0.9972
6	hordatine C glucoside	phenformin (IS1)	$y = -0.1191x^2 + 0.9009x - 0.0234$	0.9987
7	<i>p</i> -CA	phenformin (IS1)	$y = 0.9399x$	0.9961
8	feruloylagmatine	phenformin (IS1)	$y = 0.0447x$	0.9973
10	<i>p</i> -CHA	phenformin (IS1)	$y = 0.2893x$	0.9991
12	<i>p</i> -coumaroylputrescine	phenformin (IS1)	$y = 0.1550x$	0.9953
13	feruloylputrescine	phenformin (IS1)	$y = 0.0019x$	0.9969
14	sinapoylputrescine	phenformin (IS1)	$y = 0.0270x$	0.9975
15	isovitexin	puerarin (IS2)	$y = 0.0992x$	0.9956
16	saponarin	vicenin 2 (IS3)	$y = 0.3737x$	0.9977
18	schaftoside	puerarin (IS2)	$y = 0.0501x$	0.9979
19	isoschaftoside	puerarin (IS2)	$y = 0.0638x$	0.9985
20	epiheteroendrin	linamarin (IS4)	$y = 0.5280x$	0.9985
21	sutherlandin	linamarin (IS4)	$y = 0.6656x$	0.9952
22	osmaronin	linamarin (IS4)	$y = 0.5017x$	0.9977
23	dihydroosmaronin	linamarin (IS4)	$y = 1.64365x$	0.9924
24	epidermin	linamarin (IS4)	$y = 0.5064x$	0.9941
25	tryptophan	<i>o</i> -coumaric acid (IS5)	$y = 0.3828x$	0.9942
26	tryptamine	<i>o</i> -coumaric acid (IS5)	$y = 0.1254x$	0.9995
27	<i>p</i> -coumaric acid	<i>o</i> -coumaric acid (IS5)	$y = 1.2875x$	0.9997
28	ferulic acid	tryptophan- <i>d</i> ₅ (IS7)	$y = 0.4118x$	0.9997
29	sinapic acid	tryptophan- <i>d</i> ₅ (IS7)	$y = 0.2917x$	0.9994
30	oxyglutathione	¹³ C ₄ ¹⁵ N ₂ -oxyglutathione (IS6)	$y = 1.5783x$	0.9994
31	5-carboxyblumenol C glucoside	abscisic acid- <i>d</i> ₆ (IS8)	$y = 0.0352x$	0.9996

Table S4. Measured concentrations of marker metabolites in barley leaves of the HEB-25 NAM population (1).

No.	Analyte	Concentration ($\mu\text{mol/g FW}$)	
		Average	Natural range
1a	hordatine A <i>cis</i>	0.044	< LOD – 0.95
1b	hordatine A <i>trans</i>	0.015	< LOD – 0.35
2a	hordatine B <i>cis</i>	0.21	< LOD – 2.72
2b	hordatine B <i>trans</i>	0.053	< LOD – 1.00
3a	hordatine C <i>cis</i>	0.093	< LOD – 0.84
3b	hordatine C <i>trans</i>	0.042	< LOD – 0.52
4a	hordatine A glucoside <i>cis</i>	0.0044	0.00040 – 0.073
4b	hordatine A glucoside <i>trans</i>	0.0034	0.00040 – 0.085
5a	hordatine B glucoside <i>cis</i>	0.011	< LOQ – 0.082
5b	hordatine B glucoside <i>trans</i>	0.0077	< LOQ – 0.075
6a	hordatine C glucoside <i>cis</i>	0.0041	< LOD – 0.056
6b	hordatine C glucoside <i>trans</i>	0.0022	< LOD – 0.035
7a	<i>p</i> -CA <i>cis</i>	0.012	< LOD – 0.14
7b	<i>p</i> -CA <i>trans</i>	0.0073	< LOD – 0.11
8a	feruloylagmatine <i>cis</i>	0.065	< LOD – 1.78
8b	feruloylagmatine <i>trans</i>	0.041	< LOD – 0.83
10a	<i>p</i> -CHA <i>cis</i>	0.013	< LOD – 0.17
10b	<i>p</i> -CHA <i>trans</i>	0.014	< LOD – 0.099
11a	<i>p</i> -CHDA <i>cis</i>	0.0030	< LOD – 0.062
11b	<i>p</i> -CHDA <i>trans</i>	0.0050	< LOD – 0.055
12a	<i>p</i> -coumaroylputrescine <i>cis</i>	0.0047	< LOD – 0.080
12b	<i>p</i> -coumaroylputrescine <i>trans</i>	0.00060	< LOD – 0.014
15	isovitexin	0.0080	< LOD – 0.085
16	saponarin	1.66	0.055 – 4.46
17	meloside A	0.10	< LOD – 4.12
18	schaftoside	0.041	< LOQ – 0.33
19	isoschaftoside	0.00078	< LOQ – 0.0075
20	epiheteroendrin	0.12	< LOD – 1.09
21	sutherlandin	0.080	< LOD – 1.47
22	osmaronin	0.13	< LOD – 1.52
23	dihydroosmaronin	0.0092	< LOD – 0.086
24	epidermin	0.080	< LOD – 4.09
25	<i>p</i> -coumaric acid	0.0021	< LOD – 0.025
26	ferulic acid	0.0091	< LOQ – 0.074
27	sinapic acid	0.00045	< LOD – 0.016
28	tryptophan	0.079	0.019 – 1.04
29	tryptamine	0.040	0.0040 – 0.94
30	oxyglutathione	0.059	0.0016 – 0.35
31	5-carboxyblumenol C glucoside	0.012	< LOD – 0.15

A



B

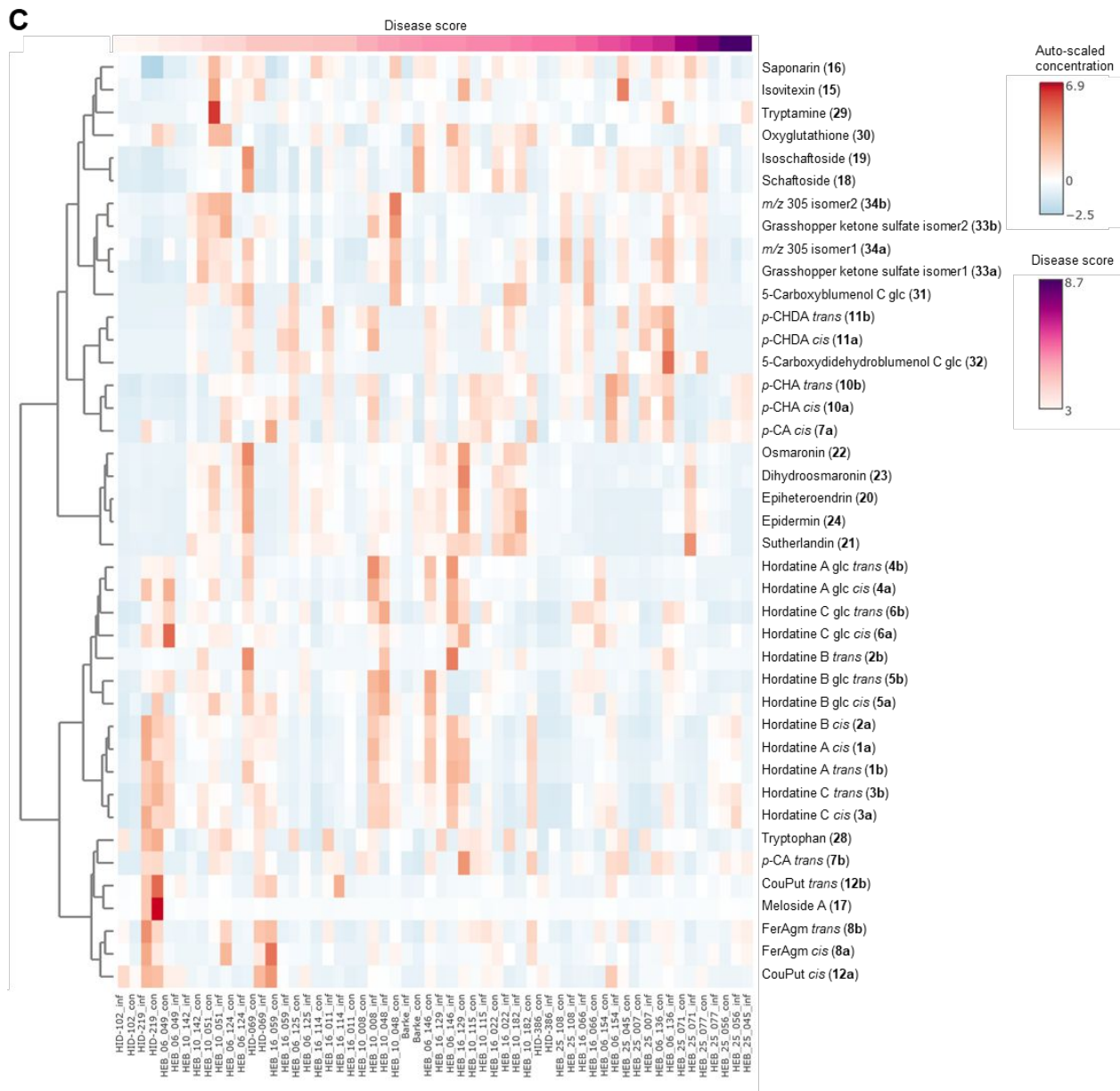


Figure S1. Hierarchical cluster analyses (HCA) of the quantitation of marker metabolites in barley leaves of 29 selected genotypes of the HEB-25 NAM population (1). Sampling of infected (inf) and non-infected control (con) plants was performed (A) 10, (B) 14 and (C) 17 days after inoculation with *B. sorokiniana*.

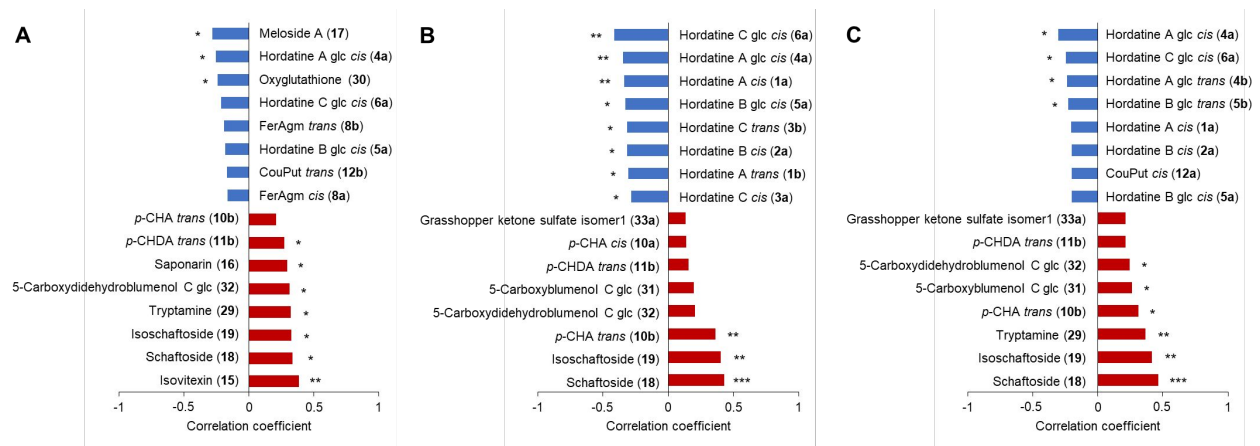


Figure S2. Spearman rank correlation coefficients of the metabolites that most positively (red) and negatively (blue) correlate with the disease score of the *B. sorokiniana* infection. Sampling of infected and non-infected control plants was carried out (A) 10, (B) 14, and (C) 17 days after inoculation with *B. sorokiniana*. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.1$ ($n = 4$).

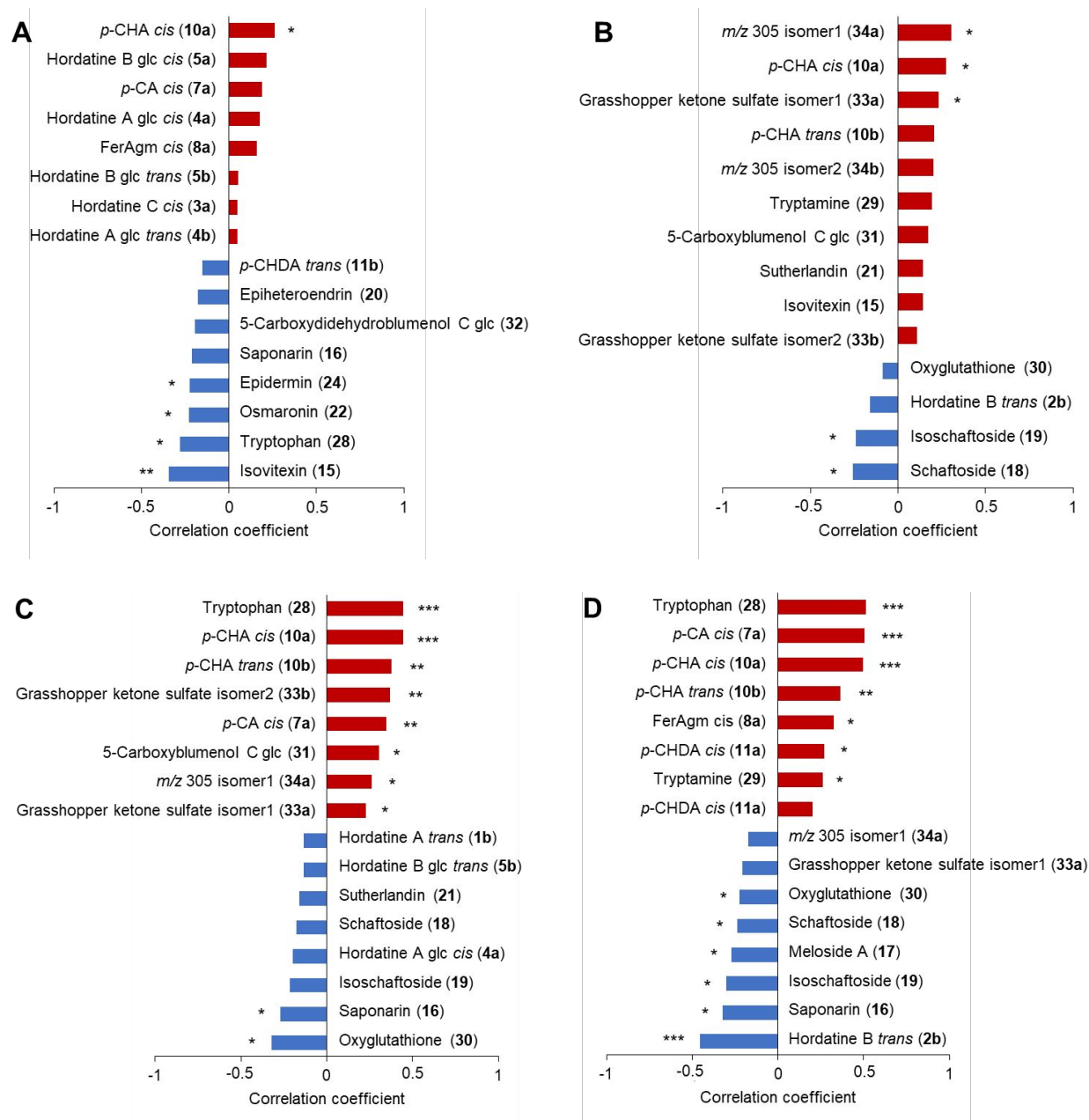


Figure S3. Spearman rank correlation coefficients of the metabolites that are correlated with the infected (red) and non-infected (blue) state. Sampling of infected and non-infected control plants was carried out (A) 7, (B) 10, (C) 14, and (D) 17 days after inoculation with *B. sorokiniana*. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.1$ ($n = 29$).

References

(1) Maurer, A.; Draba, V.; Jiang, Y.; Schnaithmann, F.; Sharma, R.; Schumann, E.; Kilian, B.; Reif, J. C.; Pillen, K. Modelling the genetic architecture of flowering time control in barley through nested association mapping. *BMC Genom.* **2015**, *16*, 290.