Research article – Supplementary data

Concurrent metabolic profiling and quantification of aromatic amino acids and phytohormones in *Solanum lycopersicum* plants responding to *Phytophthora capsici*.

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Fig. S1: Symptom development in tomato leaves harvested at different time points following inoculation with *Phytophthora capsici* zoospores. Leaf wilting started on day 2 and progressed over time, and at day 8 the tomato leaves were completely wilted.



Figure S2: Eight days post-infection comparison between *P. capsici* -infected and control tomato plants. (A): Zoospore inoculated plant with arrow indicating infection and necrosis at the inoculation site and (B): Control plant with arrow pointing to a Ventti filter wrapped around the inoculation site. (Section 4.1).



Figure S3: Representative BPI MS chromatograms of extracts from tomato plants infected with *Phytophthora capsici;* **leaf tissue.** Base peak mass chromatograms displaying comparative chromatographic differences in different time points: non-treated (NT, days 2 and 8) and *P. capsici* (PC, days 2, 4, 6 and 8) infected. Visual inspection of the chromatograms evidently shows differential peak populations, for instance in the **4-20 min** chromatographic region. (A): ESI negative mode and (B): ESI positive mode.



Figure S4: Representative BPI MS chromatograms of extracts from tomato plants infected with *Phytophthora capsici;* **stem tissue.** Base peak mass chromatograms displaying comparative chromatographic differences in different time points: (i) non-treated (NT, days 2 and 8) and (ii) *P. capsici* (PC, days 2, 4, 6 and 8) infected. Visual inspection of the chromatograms evidently shows differential peak populations, for instance in the **4-20 min** chromatographic region. (A): ESI negative mode and (B): ESI positive mode.



Figure S5: Representative BPI MS chromatograms of extracts from tomato plants infected with *Phytophthora capsici;* **root tissue.** Base peak mass chromatograms displaying comparative chromatographic differences in different time points: (i) non-treated (NT, days 2 and 8) and (ii) *P. capsici* (PC, days 2, 4, 6 and 8) infected. Visual inspection of the chromatograms evidently shows differential peak populations, for instance in the **4-20 min** chromatographic region. (A): ESI negative mode and (B): ESI positive mode.



Figure S6: Unsupervised statistical analysis of extracts from tomato plants infected with *Phytophthora capsici;* leaf data acquired in ES1⁺ mode. (A): A PCA scores scatter plot of all the samples, including the QC samples, colored according to time points. The PCA model presented here was a 7-component model, with R^2 of 0.697 and Q^2 of 0.645. (B): The HCA dendrogram corresponding to (A). Unsupervised statistical analysis is used to generate subgrouping of samples based on similar observations in (A) while the HCA dendrogram shows the hierarchical relationship between samples (B).

3



Figure S7: Unsupervised statistical analysis of extracts from tomato plants infected with *Phytophthora capsici;* stem data acquired in EST mode. (A): A PCA scores scatter plot of all the samples, including the QC samples, colored according to time points. The PCA model presented here was a 7-component model, with R^2 of 0.804 and Q^2 of 0.752. (B): The HCA dendrogram corresponding to (A). Unsupervised statistical analysis is used to generate subgrouping of samples based on similar observations in (A) while the HCA dendrogram shows the hierarchical relationship between samples (B).



Figure S8: Unsupervised statistical analysis of extracts from tomato plants infected with *Phytophthora capsici*; stem data acquired in ESI⁺ mode. (A): A PCA scores scatter plot of all the samples, including the QC samples, colored according to time points. The PCA model presented here was a 7-component model, with R^2 of 0.835 and Q^2 of 0.783. (B): The HCA dendrogram corresponding to (A). Unsupervised statistical analysis is used to generate subgrouping of samples based on similar observations in (A) while the HCA dendrogram shows the hierarchical relationship between samples (B).



Figure S9: Unsupervised statistical analysis of extracts from tomato plants infected with *Phytophthora capsici*; root data acquired in EST mode. (A): A PCA scores scatter plot of all the samples, including the QC samples, colored according to time points. The PCA model presented here was a 7-component model, with R^2 of 0.759 and Q^2 of 0.640. (B): The HCA dendrogram corresponding to (A). Unsupervised statistical analysis is used to generate subgrouping of samples based on similar observations in (A) while the HCA dendrogram shows the hierarchical relationship between samples (B).



Figure S10: Unsupervised statistical analysis of extracts from tomato plants infected with *Phytophthora capsici;* root data acquired in ESI⁺ mode. (A): A PCA scores scatter plot of all the samples, including the QC samples, colored according to time points. The PCA model presented here was a 7-component model, with R^2 of 0.788 and Q^2 of 0.692. (B): The HCA dendrogram corresponding to (A). Unsupervised statistical analysis is used to generate subgrouping of samples based on similar observations in (A) while the HCA dendrogram shows the hierarchical relationship between samples (B).



Figure S11: OPLS-DA modeling and variable/feature selection of extracts from tomato plants infected with *Phytophthora capsici;* leaf data acquired on ESI⁺ mode. (A): A typical OPLS-DA score plot separating nontreated (NT) day 8 plants *vs. P. capsici* (PC)-treated day 6 plants $(1 + 1 + 0 \text{ components}, \mathbb{R}^2 X = 0.741, \mathbb{Q}^2 = 0.998$, CV-ANOVA *p*-value = 7.96×10^{-15}). (B): An OPLS-DA loadings S-plot for the same model in (A); only variables with the correlation $[(p(corr)] \ge |0.6|$ and covariance $(p1) \ge |0.5|$ were chosen as discriminating variables and identified using the *m*/*z* to generate elemental composition. (C): A variable importance for the projection (VIP) plot for the same model, pointing mathematically to the importance of each variable in contributing to group separation in the OPLS-DA model. (D): A typical variable trend plot (of the selected variable in VIP and S-plots), displaying the changes of the selected variables across the samples (NT day 8 *vs.* PC day 6). This shows that the selected features significantly discriminate the treated from the control samples.



Figure S12: OPLS-DA modeling and variable/feature selection of extracts from tomato plants infected with *Phytophthora capsici;* stem data acquired on EST mode. (A): A typical OPLS-DA score plot separating non-treated (NT) day 8 plants *vs. P. capsici* (PC)-treated day 6 plants $(1 + 1 + 0 \text{ components}, \mathbb{R}^2 X = 0.817, \mathbb{Q}^2 = 0.999$, CV-ANOVA *p*-value = 3.19×10^{-20}). (B): An OPLS-DA loadings S-plot for the same model in (A); only variables with the correlation $[(p(corr)] \ge |0.6|$ and covariance $(p1) \ge |0.5|$ were chosen as discriminating variables and identified using the *m*/*z* to generate elemental composition. (C): A variable importance for the projection (VIP) plot for the same model, pointing mathematically to the importance of each variable in contributing to group separation in the OPLS-DA model. (D): A typical variable trend plot (of the selected variable in VIP and S-plots), displaying the changes of the selected variables across the samples (NT day 8 *vs.* PC day 6). This shows that the selected features significantly discriminate the treated from the control samples.



Figure S13: OPLS-DA modeling and variable/feature selection of extracts from tomato plants infected with *Phytophthora capsici;* stem data acquired on ESI⁺ mode. (A): A typical OPLS-DA score plot separating nontreated (NT) day 8 plants *vs. P. capsici* (PC)-treated day 6 plants $(1 + 1 + 0 \text{ components}, R^2X = 0.855, Q^2 = 0.995, CV-ANOVA$ *p* $-value = <math>3.9 \times 10^{-18}$). (B): An OPLS-DA loadings S-plot for the same model in (A); only variables with the correlation $[(p(corr)] \ge |0.6|$ and covariance $(p1) \ge |0.5|$ were chosen as discriminating variables and identified using the *m/z* to generate an elemental composition. (C): A variable importance for the projection (VIP) plot for the same model, pointing mathematically to the importance of each variable in contributing to group separation in the OPLS-DA model. (D): A typical variable trend plot (of the selected variable in VIP and S-plots), displaying the changes of the selected variables across the samples (NT day 8 *vs.* PC day 6). This shows that the selected features significantly discriminate the treated from the control samples.



Figure S14: OPLS-DA modeling and variable/feature selection of extracts from tomato plants infected with *Phytophthora capsici;* root data acquired on ESI mode. (A): A typical OPLS-DA score plot separating non-treated (NT) day 8 plants *vs. P. capsici* (PC)-treated day 6 plants $(1 + 1 + 0 \text{ components}, \mathbb{R}^2 X = 0.677, \mathbb{Q}^2 = 0.998$, CV-ANOVA *p*-value = 9.48×10^{-16}). (B): An OPLS-DA loadings S-plot for the same model in (A); only variables with the correlation $[(p(corr)] \ge |0.6|$ and covariance $(p1) \ge |0.5|$ were chosen as discriminating variables and identified using the *m*/*z* to generate an elemental composition. (C): A variable importance for the projection (VIP) plot for the same model, pointing mathematically to the importance of each variable in contributing to group separation in the OPLS-DA model. (D): A typical variable trend plot (of the selected variable in VIP and S-plots), displaying the changes of the selected variables across the samples (NT day 8 *vs.* PC day 6). This shows that the selected features significantly discriminate the treated from the control samples.



Figure S15: OPLS-DA modeling and variable/feature selection of extracts from tomato plants infected with *Phytophthora capsici;* root data acquired on ESI⁺ mode. (A): A typical OPLS-DA score plot separating non-treated (NT) day 8 plants *vs. P. capsici* (PC)-treated day 6 plants $(1 + 1 + 0 \text{ components}, \mathbb{R}^2 X = 0.742, \mathbb{Q}^2 = 0.996$, CV-ANOVA *p*-value = 1.08×10^{-15}). (B): An OPLS-DA loadings S-plot for the same model in (A); only variables with the correlation $[(p(corr)] \ge |0.6|$ and covariance $(p1) \ge |0.5|$ were chosen as discriminating variables and identified using the *m*/*z* to generate an elemental composition. (C): A variable importance for the projection (VIP) plot for the same model, pointing mathematically to the importance of each variable in contributing to group separation in the OPLS-DA model. (D): A typical variable trend plot (of the selected variable in VIP and S-plots), displaying the changes of the selected variables across the samples (NT day 8 *vs.* PC day 6). This shows that the selected features significantly discriminate the treated from the control samples.

Table S1: Summary of annotated (MSI-level 2) metabolites that contributed to the discriminating variability in the altered metabolomes of root -, stem - and leaf tissue of tomato plants infected with *Phytophthora capsici* (as described by chemometric models). These discriminating metabolites were identified based on OPLS-DA S-plots, with a rigorous statistical validation (as explained in the text – Figure 4). These reported metabolites had VIP scores > 1.0.

No.	Rt	Ionization	m/z	Compound name	Abbreviation	Chemical	Fragments
	(min)					formula	(<i>m</i> / <i>z</i>)
1	0.87	[M-H]-	191.018	Citric acid I	C-acid I	C6H8O7	173, 115, 111
2	0.87	[M+H]+	116.063	L-Proline	L-Pro	C5H9NO2	70
3	0.92	[M-H]-	191.017	Citric acid II	C-acid II	C6H8O7	173, 111
4	0.95	[M-H]-	133.007	Malic acid	M-Acid	C4H6O5	114, 89, 72
5	1.85	[M+H]+	166.082	L-Phenylalanine	L-Phe	C9H11NO2	120, 103, 91, 77
6	2.19	[M+H]+	220.117	N', N'', N'''-Triferuloylagmatine	tri-F-agmatine	C35H38N4O9	660
7	2.62	[M+H]+	176.105	N-Acetyl-aspartic acid	N-Acetyl-Asp	C6H9NO5	115
8	2.72	[M-H]-	353.084	3-Caffeoylquinic acid	3-CQA	C16H18O9	191, 179, 135
9	2.92	[M-H]-	203.077	Tryptophan	Trp	C11H12N2O2	142, 116
10	3.16	[M-H]-	285.058	Dihydroxybenzoic acid pentose	diHydro-Be acid pent	C12H14O8	153
12	3.96	[M-H]-	343.186	Homovanillic acid glycoside	H-acid glyc	C15H20O9	181
13	4.22	[M-H]-	353.083	5-Caffeoylquinic acid	5-CQA	C16H18O9	191, 179, 173, 135
14	4.35	[M-H]-	367.099	4-Feruloylquinic acid	4-FQA	C17H20O9	193
15	4.39	[M+H]+	323.154	N-Feruloylspermidine I	F-spe I	C17H27N3O3	321, 177, 145, 117
16	4.4	[M-H]-	353.082	4-Caffeoylquinic acid	4-CQA	C17H20O9	191, 179, 173, 135
17	4.9	[M-H]-	355.098	Feruloylglycoside I	F-glyco I	C16H20O9	193
18	4.94	[M+H]+	307.17	N-Feruloylagmatine I	F-agm I	C15H22N4O3	177, 145, 114
19	5.06	[M-H]-	385.107	Sinapoylglycoside II	S-glyc I	C17H20O10	223
20	5.27	[M+H]+	337.184	N-Feruloylagmatine II	F-agm II	C15H22N4O3	177, 145, 114
21	5.42	[M+H]+	322.187	<i>N</i> -Feruloylspermidine II	F-spe II	C17H27N3O3	321, 177, 145, 117
22	6.21	[M-H]-	367.099	5-Feruloylquinic acid	5-FQA	C17H20O9	191
23	7	[M-H]-	741.189	Quercetin-3-O-trisacharide	Qu-3-O-trisach	C31H36O21	300
24	7.34	[M-H]-	245.089	Acetyl tryptophan	Acetyl Trp	C13H14O3	203
25	7.7	[M-H]-	609.145	Rutin	Rutin	C27H30O16	300
26	8.02	[M-H]-	463.083	Quercetin 7-O-glucoside	Qu-7-O-gluc	C15H20O12	300
27	8.63	[M-H]-	593.149	Kaempferol-3-O-B-rutinoside	Ka-3-O-B-rut	C27H30O15	285
28	9.48	[M-H]-	349.094	Azelaic acid-glycoside	Aza-glyc	C15H26O9	187
29	9.97	[M+H]+	1032.54	Dehydrotomatine	De-tomatine	C50H81NO21	588, 576, 414
30	10.07	[M+H]+	738.443	Alpha tomatine I	A-tom I	C50H83NO21	738, 578, 416
31	10.26	[M-H]-	745.27	Delphinidin-coumaroyltyramine glycoside	De-Cotyr glyc	C38H36NO14	282, 162, 119
32	10.7	[M-H]-	312.12	Feruloyltyramine I	F-tyr	C18H19NO4	178, 134
33	10.73	[M+H]+	1032.251	Dehydrotomatine I	De-tomatine I	C9H81NO21	576, 414
34	10.97	[M+H]+	1032.55	Dehydrotomatine II	De-tomatine II	C50H81NO21	576, 414
35	11.1	[M+H]+	344.146	Alpha tomatine II	A-tom II	C50H83NO21	578, 416
36	11.11	[M+H]+	344.147	Filotomatine	Filo	C50H83NO21	578, 416, 207

37	11.13	[M-H]-	299.183	Salicylic acid glycoside I	SA-glyc I	C13H16O8	137
38	11.2	[M+H]+	1092.56	Alpha tomatine III	A-tom III	C50H83NO21	578, 416, 295
39	11.56	[M+H]+	1034.56	Alpha-tomatine IV	A-tom IV	C50H83NO21	578, 416, 416, 295
40	11.66	[M+H]+	1004.55	Alpha tomatine V	A-tom V	C50H83NO21	578, 416, 295
41	11.8	[M-H]-	447.219	Kaempferol-3-glucoside	Ka-3-O-gluc	C21H20O11	285
42	12.06	[M-H]-	299.182	Salicylic acid glycoside II	SA-glyc II	C13H16O8	137
43	13.41	[M+H]+	414.333	Tomatidenol	Tomato I	C27H43NO2	273, 255, 161
44	13.42	[M-H]-	313.197	Methyl salicylate glycoside	MeSA-glyc	C14H18O8	151
45	14.14	[M+H]+	412.318	Tomatid-4-en-3-one	Toma-one	C27H43NO2	325, 271, 161
46	14.75	[M+H]+	414.334	Tomatidenol	Tomato II	C27H43NO2	273, 255, 161
47	14.87	[M-H]-	329.228	Hydroxyoctadecanedioc acid	C27H43NO2	C27H43NO2	171, 139
48	15.28	[M+H]+	416.345	Tomatidine	Tomati I	C27H45NO2	273, 255, 163
49	15.99	[M+H]+	416.347	Tomatidine	Tomati II	C27H45NO2	273, 255, 163
50	16.08	[M-H]-	329.229	Hydroxyoctadecanedioc acid	C27H43NO2	C18H33O5	171, 139
51	16.09	[M+H]+	353.225	Tomatidenol	Tomato II	C27H43NO2	414, 369
52	16.49	[M-H]-	329.229	Hydroxyoctadecanedioc acid	C27H43NO2	C18H33O5	171, 139
53	20.54	[M-H]-	474.258	Feruloytyramine glycoside	F-tyr glyc I	C24H29NO9	312, 178
54	20.99	[M-H]-	474.26	Feruloytyramine glycoside	F-tyr glyc II	C24H29NO9	312, 178
55	22.06	[M-H]-	504.305	Feruloyl-3-methoxytyramine glycoside	F-met-tyr glyc I	C25H31NO10	342, 178
56	22.51	[M-H]-	504.307	Feruloyl-3-methoxytyramine glycoside	F-met-tyr glyc II	C25H31NO10	342, 178

Note: The table only shows the identified metabolites in the various tissue. For differential reprogramming and tissue-specific metabolites, please refer to the correlation analysis (Figure 5) and VIP score-plots (Figure 6).



Figure S16: Correlation matrix among the changes (Δ) within/between extracts from stems of non-treated (NT) day 8 tomato plants and *Phytophthora capsici* (PC)-treated day 6 plants. Metabolite-metabolite correlations among identified molecules were obtained by deriving a Pearson correlation coefficient. Red indicates a positive correlation, and blue indicates a negative correlation. Abbreviations are explained in **Table S1**. Dendrograms are shown on the top and left of the correlation, indicating clustering of positive and negative correlations.



Figure S17: Correlation matrix among the changes (Δ) within/between extracts from roots of non-treated (NT) day 8 tomato plants and *Phytophthora capsici* (PC)-treated day 6 plants. Metabolite-metabolite correlations among identified molecules were obtained by deriving a Pearson correlation coefficient. Red indicates a positive correlation, and blue indicates a negative correlation. Abbreviations are explained in **Table S1**. Dendrograms are shown on the top and left of the correlation, indicating clustering of positive and negative correlations.

Table S2: One-way ANOVA comparing mean values of quantified aromatic amino acids and phytohormones in various tissue of tomato plants infected with *Phytophthora capsici*.

Compound	<i>p</i> -value
Roo	ts
Phe	0.000
Trp	0.000
Tyr	0.000
MeSA	0.000
ACC	0.000
Ster	ns
Phe	0.000
Trp	0.000
Tyr	0.000
MeSA	0.000
ACC	0.000
Leav	ves
Phe	0.000
Trp	0.000
Tyr	0.000
MeSA	0.000
ACC	0.000

Table S3: Post-hoc tests comparing mean values of quantified aromatic amino acids and phytohormones in various tissue of tomato plants infected with *Phytophthora capsici*.

Compound	(I) Treatment	(J) Treatment	<i>p</i> -value
name	<u> </u>		
Phe	NT Day 2	NT Day 8	0.000
		PC Day 2	0.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
	NT Day 8	NT Day 2	0.000
		PC Day 2	0.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000

Trp	NT Day 2	NT Day 8	0.013
		PC Day 2	0.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.005
	NT Day 8	NT Day 2	0.013
		PC Day 2	0.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.999
Tyr	NT Day 2	NT Day 8	0.914
		PC Day 2	0.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
	NT Day 8	NT Day 2	0.914
		PC Day 2	0.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
MeSA	NT Day 2	NT Day 8	0.510
		PC Day 2	0.004
		PC Day 4	0.000
		PC Day 6	0.198
		PC Day 8	0.001
	NT Day 8	NT Day 2	0.510
		PC Day 2	0.000
		PC Day 4	0.000
		PC Day 6	0.002
		PC Day 8	0.122
ACC	NT Day 2	NT Day 8	0.302
		PC Day 2	0.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
	NT Day 8	NT Day 2	0.302
		PC Day 2	0.001
		PC Day 4	0.000
		PC Day 6	0.000

		PC Day 8	0.000
	Ster	ns	
Phe	NT Day 2	NT Day 8	1.000
		PC Day 2	0.010
		PC Day 4	0.000
		PC day 6	0.000
		PC Day 8	0.000
	NT Day 8	NT Day 2	1.000
		PC Day 2	0.022
		PC Day 4	0.000
		PC day 6	0.000
		PC Day 8	0.000
Trp	NT Day 2	NT Day 8	0.999
		PC Day 2	0.666
		PC Day 4	0.000
		PC day 6	0.000
		PC Day 8	0.000
	NT Day 8	NT Day 2	0.999
		PC Day 2	0.862
		PC Day 4	0.000
		PC Day 6	0.000
	_	PC Day 8	0.000
Tyr	NT Day 2	NT Day 8	0.908
		PC Day 2	0.008
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
	NT Day 8	NT Day 2	0.908
		PC Day 2	0.108
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
MeSA	NT Day 2	NT Day 8	0.810
		PC Day 2	0.001
		PC Day 4	0.002
		PC Day 6	0.888
		PC Day 8	0.000
	NT Day 8	NT Day 2	0.810
		PC Day 2	0.025

		PC Day 4	0.059
		PC Day 6	1.000
		PC Day 8	0.001
ACC	NT Day 2	NT Day 8	0.994
		PC Day 2	0.939
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
	NT Day 8	NT Day 2	0.994
		PC Day 2	0.683
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
	Lea	ves	
Phe	NT Day 2	NT Day 8	1.000
		PC Day 2	0.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
	NT Day 8	NT Day 2	1.000
		PC Day 2	0.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
	PC Day 2	NT Day 2	0.000
		NT Day 8	0.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
Trp	NT Day 2	NT Day 8	0.993
		PC Day 2	0.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
	NT Day 8	NT Day 2	0.993
		PC Day 2	0.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000

Tyr	NT Day 2	NT Day 8	1.000
		PC Day 2	0.395
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
	NT Day 8	NT Day 2	1.000
		PC Day 2	0.379
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
MeSA	NT Day 2	NT Day 8	0.946
		PC Day 2	0.747
		PC Day 4	0.390
		PC Day 6	0.449
		PC Day 8	0.110
	NT Day 8	NT Day 2	0.946
		PC Day 2	0.997
		PC Day 4	0.900
		PC Day 6	0.086
		PC Day 8	0.011
ACC	NT Day 2	NT Day 8	0.933
		PC Day 2	1.000
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000
	NT Day 8	NT Day 2	0.933
		PC Day 2	0.856
		PC Day 4	0.000
		PC Day 6	0.000
		PC Day 8	0.000