

Supplementary tables for:

Title: Characterization of Arabidopsis CYP79C1 and CYP79C2 by Glucosinolate Pathway Engineering in *Nicotiana benthamiana* Shows Substrate Specificity Toward a Range of Aliphatic and Aromatic Amino Acids

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Supplementary Table S1. Primers in this study.

Primers	Sequences 5'-3'
CW172-CYP79C1-F	GGCTTAAUATGGATTATTATCTGAATAATGTTATCTTCTC
CW173-CYP79C1-R	GGTTTAAUTCAAGCTGGACCGGTCCAGAG
CW174-CYP79C2-F	GGCTTAAUATGACTATTATCACGAATCCATCC
CW175-CYP79C2-R	GGTTTAAUCTAGATCTGGATTTTCGGATAT
CW176-Seq-F	GAGTCGACCTGCAGGCAT
CW177-Seq-R	GTTTTCCAGTCACGACGTT
CW404-AtCYP79F1	GGCTTAAUATGATGAGCTTTACCACATCA
CW405-AtCYP79F1	GGTTTAAUTTAAGGACGGAACCTTTGGAT
CW368-CYP83A1-F	GGCTTAAUATGGAAGATATCATCATCGGCGT
CW369-CYP83A1-R	GGTTTAAUCTAATACTTGTTCACTTTCTCTGG
CW370-CYP83A1-seq-R	CTTAGGATCAAGCGTCTCATT
CW365 -CYP83B1-F	GGCTTAAUATGGATCTCTTATTGATTATAGC
CW366 -CYP83B1-R	GGTTTAAUTCAGATGTGTTTCGTTGGT
CW367-CYP83B1-seq-R	TTAGGGTCAAGAGTCTCGT
CW373-CYP79D2-F	GGCTTAAUATGGCCATGAACGTCTCCA
CW374-CYP79D2-R	GGTTTAAUTTAATTCAAGGTGAAGTGGGGT
CW376-CYP79D2-seq-R	CGTAATGCCTTGCCGCAAT
CW371-GGP1-F	GGCTTAAUATGGTGGAGCAAAAGAGA
CW372-GGP1-R	GGTTTAAUCTAGTTAGTTGGAACCTCTGCCT

The primers containing uracil (U) were used for USER cloning and the primers labelled 'seq' were used for sequencing.

Supplementary Table S2: MRM transitions for quantification of selected dsGLSs.

Analyte [M+H] ⁺	Retention time [min]	Q1 [m/z]	Q3 [m/z]	CE [eV]
ds1ME GLS	2.02	282.1	120.0 ^{Qt}	6
		282.1	86.2	19
ds2MP GLS	2.36	296.1	134.1 ^{Qt}	7
		296.1	100.1	19
ds1MP GLS	2.50	296.1	134.1 ^{Qt}	7
		296.1	100.1	19
dsBGLS	1.94	330.0	168.0 ^{Qt}	6
		330.0	91.0	23
ds3MB GLS	2.94	310.0	148.0 ^{Qt}	7
		310.0	114.0	19
ds4MP GLS	3.46	324.0	162.0 ^{Qt}	7
		324.0	128.0	19
ds allyl GLS (Internal standard)	1.55	280.0	118.0 ^{Qt}	5
		280.0	145.0	10
		280.0	74.2	8

CE = collision energy; Q = quadrupole; Qt = product ion used for quantification.

Supplementary Table S3. The yield of GLSs produced with the aliphatic core pathway.

	Yield of GLSs (nmol/g fw)					
	1ME	2MP	1MP	BGLS	pOHB	I3M
CYP79C2	n.d.	34.67	1.00	61.40	n.d.	0.21
	n.d.	56.48	2.44	105.65	n.d.	0.27
	n.d.	28.59	1.09	61.20	n.d.	0.09
	n.d.	4.49	0.21	29.30	n.d.	n.d.
	n.d.	42.73	1.72	84.02	n.d.	n.d.
	n.d.	56.30	1.70	78.23	n.d.	0.13
	n.d.	6.50	0.25	31.05	n.d.	0.13
	n.d.	31.42	0.96	100.71	n.d.	n.d.
	n.d.	51.16	1.54	134.13	n.d.	0.15
Mean±stddev	-	34.71±19.47	1.21±0.72	76.19±34.59	-	0.16±0.07
CYP79C1	0.16	0.36	0.17	2.20	n.d.	n.d.
	0.12	0.59	0.15	2.74	n.d.	n.d.
	0.59	0.40	0.33	2.33	n.d.	n.d.
	n.d.	n.d.	n.d.	0.52	n.d.	n.d.
	0.11	0.30	0.10	1.36	n.d.	n.d.
	0.20	0.21	0.16	1.23	n.d.	n.d.
	0.11	0.12	0.13	1.03	n.d.	n.d.
	n.d.	0.93	n.d.	1.81	n.d.	n.d.
	n.d.	1.64	0.31	4.41	n.d.	n.d.
Mean±stddev	0.14±0.19	0.57±0.50	0.15±0.11	1.96±1.15	-	-
CYP79D2	36.05	0.24	16.90	n.d.	n.d.	n.d.
	68.93	1.47	39.40	n.d.	n.d.	n.d.
	72.93	0.62	29.70	n.d.	n.d.	n.d.
	51.83	1.04	30.14	n.d.	n.d.	n.d.
	17.78	0.38	11.59	n.d.	n.d.	n.d.
	54.31	0.77	31.96	n.d.	n.d.	n.d.
	31.47	0.47	19.54	n.d.	n.d.	n.d.
	64.54	1.59	38.91	n.d.	n.d.	n.d.
	87.00	1.26	36.40	n.d.	n.d.	n.d.
Mean±stddev	53.87±22.16	0.87±0.49	28.28±10.04	-	-	-
No CYP79	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.d.	n.d.	n.d.	0.90	n.d.	n.d.
	n.d.	n.d.	n.d.	0.69	n.d.	n.d.
	n.d.	n.d.	n.d.	0.35	n.d.	n.d.
	n.d.	n.d.	n.d.	0.48	n.d.	n.d.
	n.d.	n.d.	n.d.	0.30	n.d.	n.d.
	n.d.	n.d.	n.d.	1.25	n.d.	n.d.
	n.d.	n.d.	n.d.	0.83	n.d.	n.d.
	n.d.	n.d.	n.d.	1.25	n.d.	n.d.
Mean±stddev	-	-	-	0.76±0.37	-	-

n.d. = not detected, In some cases the detection of very low levels of dsGLSs by LC-MS was close to the detection limit. However, the presence of all presented dsGLSs was confirmed by repeated analysis by LC-MS.

Supplementary Table S5. The yield of GLSs produced with the aromatic core pathway.

	Yield of GLSs (nmol/g fw)					
	1ME	2MP	1MP	BGLS	pOHB	I3M
CYP79C2	n.d.	93.62	1.77	136.87	0.62	0.13
	n.d.	175.87	3.83	183.49	1.17	0.22
	n.d.	101.93	2.06	191.37	0.77	0.20
	n.d.	79.34	2.33	83.46	0.64	0.14
	n.d.	246.58	5.15	250.77	1.72	0.31
	n.d.	328.72	n.d.	179.17	n.d.	0.40
	n.d.	145.24	2.32	150.16	0.84	0.22
	n.d.	295.02	6.52	206.90	1.03	0.33
	n.d.	154.91	3.96	139.21	0.95	0.19
Mean±stddev	-	180.14±90.36	3.49±1.69	169.04±48.13	0.97±0.36	0.24±0.09
CYP79C1	n.d.	4.11	0.82	4.90	n.d.	n.d.
	n.d.	14.93	1.47	3.01	n.d.	n.d.
	n.d.	6.99	1.17	2.50	n.d.	n.d.
	n.d.	2.38	0.48	2.45	n.d.	n.d.
	n.d.	10.08	1.33	2.95	n.d.	n.d.
	n.d.	17.38	1.51	4.84	n.d.	n.d.
	n.d.	2.33	0.28	2.79	n.d.	n.d.
	n.d.	3.83	0.84	3.11	n.d.	n.d.
	n.d.	5.64	1.07	2.80	n.d.	n.d.
Mean±stddev	-	7.52±5.49	1.00±0.43	3.26±0.94	-	-
CYP79A2	n.d.	n.d.	n.d.	231.35	0.18	n.d.
	n.d.	n.d.	n.d.	651.49	0.66	n.d.
	n.d.	n.d.	n.d.	321.57	1.11	n.d.
	n.d.	n.d.	n.d.	346.06	0.31	n.d.
	n.d.	n.d.	n.d.	393.63	0.57	n.d.
	n.d.	n.d.	n.d.	727.56	1.47	n.d.
	n.d.	n.d.	n.d.	256.81	0.32	n.d.
	n.d.	n.d.	n.d.	615.04	1.40	n.d.
	n.d.	n.d.	n.d.	773.88	0.87	n.d.
Mean±stddev	-	-	-	479.71±211.37	0.77±0.48	-
No CYP79	n.d.	0.86	n.d.	1.31	n.d.	n.d.
	n.d.	0.52	n.d.	0.95	n.d.	n.d.
	n.d.	0.29	n.d.	0.93	n.d.	n.d.
	n.d.	0.20	n.d.	0.79	n.d.	n.d.
	n.d.	0.81	n.d.	1.34	n.d.	n.d.
	n.d.	0.74	n.d.	0.90	n.d.	n.d.
	n.d.	1.04	n.d.	2.08	n.d.	n.d.
	n.d.	0.83	n.d.	1.53	n.d.	n.d.
	n.d.	1.08	n.d.	1.25	n.d.	n.d.
Mean±stddev	-	0.71±0.31	-	1.23±0.40	-	-

n.d. = not detected In some cases the detection of very low levels of dsGLS by LC-MS was close to the detection limit. However, the presence of all presented dsGLSs was confirmed by repeated analysis by LC-MS.

Supplementary Table S7. The yield of GLSs produced with the chain elongation pathway and the aliphatic core pathway.

	Yield of chain-elongated GLSs (nmol/g fw)						
	2MP	1MP	3MB	4MP	3MTP	4MTB	4MSB
CYP79C2	1.13	0.00	n.d.	n.d.	n.d.	n.d.	n.d.
	0.88	0.00	n.d.	n.d.	n.d.	n.d.	n.d.
	7.30	0.53	1.83	n.d.	n.d.	n.d.	n.d.
	4.00	0.38	1.94	0.45	n.d.	n.d.	n.d.
	10.10	0.70	3.75	1.25	n.d.	0.38	n.d.
	3.35	0.00	1.69	n.d.	n.d.	n.d.	n.d.
	3.82	0.00	1.43	0.53	n.d.	n.d.	n.d.
	1.92	0.00	1.04	n.d.	n.d.	n.d.	n.d.
	8.06	0.52	2.71	n.d.	n.d.	n.d.	n.d.
	4.00	0.00	2.42	n.d.	n.d.	n.d.	n.d.
	10.06	0.46	3.76	0.96	n.d.	n.d.	0.87
	13.48	1.22	4.45	2.22	n.d.	0.79	n.d.
	Mean±stddev	5.68±4.05	0.32±0.39	2.08±1.42	0.45±0.70	-	0.10±0.24
CYP79C1	n.d.	n.d.	n.d.	1.11	n.d.	3.26	n.d.
	n.d.	n.d.	n.d.	1.67	n.d.	1.60	n.d.
	n.d.	n.d.	n.d.	2.35	n.d.	6.43	n.d.
	n.d.	n.d.	n.d.	2.06	n.d.	5.86	n.d.
	n.d.	n.d.	n.d.	1.86	n.d.	4.13	n.d.
	n.d.	n.d.	n.d.	1.56	n.d.	1.95	n.d.
	n.d.	n.d.	n.d.	3.55	n.d.	2.82	n.d.
	n.d.	n.d.	n.d.	2.40	n.d.	5.14	n.d.
	n.d.	n.d.	n.d.	1.08	n.d.	3.91	n.d.
	n.d.	n.d.	n.d.	1.13	n.d.	2.51	n.d.
	n.d.	n.d.	n.d.	1.41	n.d.	4.63	n.d.
	n.d.	n.d.	n.d.	0.96	n.d.	1.66	n.d.
	Mean±stddev	-	-	-	1.76±0.75	-	3.66±1.63
CYP79F1	n.d.	n.d.	33.26	74.66	11.02	42.87	2.08
	n.d.	n.d.	31.27	56.69	8.83	23.71	1.31
	n.d.	n.d.	73.74	129.75	7.50	21.87	1.36
	n.d.	n.d.	55.78	101.79	7.50	32.63	1.69
	n.d.	n.d.	69.63	138.47	13.22	52.21	2.33
	n.d.	n.d.	61.02	122.19	11.10	38.34	2.04
	n.d.	n.d.	48.16	89.70	11.59	49.78	2.27
	n.d.	n.d.	48.57	113.56	8.37	29.54	1.65
	n.d.	n.d.	64.40	97.47	9.64	28.72	1.67
	n.d.	n.d.	26.63	45.79	6.57	16.22	0.87
	n.d.	n.d.	50.87	109.35	4.10	10.62	0.94
	n.d.	n.d.	47.55	116.65	15.27	49.97	2.50
	Mean±stddev	-	-	50.90±15.05	99.67±28.57	9.56±3.07	33.04±13.78
Negative control	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Only chain elongation pathway	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Mean ±stddev	-	-	-	-	-	-

n.d. = not detected. No other GLSs were detected, including BGLS, pOHB and I3M, In some cases the detection of very low levels of dsGLS by LC-MS was close to the detection limit. However, the presence of all presented dsGLSs was confirmed by repeated analysis by LC-MS.

Supplementary Table S4. *P*-values (Student's *t*-test) used to compare the same type GLS yields between co-expression of *CYP79C1*, *CYP79C2* and no *CYP79* with the aliphatic core pathway.

	<i>P</i> -values (Student's <i>t</i> -test) for comparison of GLS yields by expression of aliphatic core structure pathway									
	1ME		2MP		1MP		BGLS		I3M	
	<i>CYP79C2</i>	<i>CYP79C1</i>	<i>CYP79C2</i>	<i>CYP79C1</i>	<i>CYP79C2</i>	<i>CYP79C1</i>	<i>CYP79C2</i>	<i>CYP79C1</i>	<i>CYP79C2</i>	<i>CYP79C1</i>
No <i>CYP79</i>	-	0.05	< 0.001	0.01	< 0.001	< 0.001	< 0.001	0.01	0.01	-

Supplementary Table S6. *P*-values (Student's *t*-test) used to compare the same type GLS yields between co-expression of *CYP79C1*, *CYP79C2* and no *CYP79* with the aromatic core pathway.

	<i>P</i> -values (Student's <i>t</i> -test) for comparison of GLS yields by expression of aromatic core structure pathway									
	2MP		1MP		BGLS		pOHB		I3M	
	<i>CYP79C2</i>	<i>CYP79C1</i>	<i>CYP79C2</i>	<i>CYP79C1</i>	<i>CYP79C2</i>	<i>CYP79C1</i>	<i>CYP79C2</i>	<i>CYP79C1</i>	<i>CYP79C2</i>	<i>CYP79C1</i>
No <i>CYP79</i>	< 0.001	0.01	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	-	< 0.001	-

Supplementary Table S8. *P*-values (Student's *t*-test) used to compare the GLS yields between co-expression of *CYP79C1* and *CYP79C2* with the chain elongation pathway and the aliphatic core pathway.

	<i>P</i> -values (Student's <i>t</i> -test) for comparison of GLS yields between expression of <i>CYP79C1</i> or <i>CYP79C2</i> with the chain elongation and aliphatic core pathways						
	2MP	1MP	3MB	4MP	3MTP	4MTB	4MSB
<i>P</i> -values	< 0.001	0.02	< 0.001	< 0.001	-	< 0.001	0.34