

Comparative metabolomics and transcriptomics of pistils, stamens and pistiloid stamens reveal key knowledge of pistil and stamen development in wheat

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Electronic Supplementary Material (ESM)

Table S1. Primers used in real-time PCR

Primer	Forward	Reverse	Amplified products (bp)
SAM synthase	AGTAGAAGCAGCGCAAGAGCA	CAAGCAGGCATCCAAGACG	140
ACC synthase	GCCTCCCTGGTGATCCTGAA	GTGCCCACGCCTTACTACCC	113
ACC oxidase	CGCCGATGTTGACGACGAT	TCCTGCTGTTCCAAGACGACC	105
Ubiquitin	AAGGCGAAGATCCAGGACAAG	TGGATGTTGTAGTCCGCCAAG	107
Actin	ACGCTTCCTCATGCTATCCTTC	ATGTCTCTGACAATTCCCGC	121

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Table S2. Annotated metabolites were identified from pistils (P), pistiloid stamens (PS), and stamens (S) by gas chromatography-mass spectrometry (GC-MS)

Name	Retention index (RI)	m/z	Retention time (min)	KEGG entry No.	Mean value of P after normalized	Mean value of PS after normalized	Mean value of S after normalized
Pyruvic acid	1040.9	89.09	6.03	C00022	36.27	37.79	22.01
Lactic acid	1052.2	117.1	6.21	C00186	84.7	71.78	50.23
Glycolic acid	1066.3	66.06	6.43	C00160	14.7	13.84	16.22
Alanine	1094.1	116.1	6.86	C00041	821.06	472.07	11443.07
3-Hydroxypyridine	1128.8	152.06	7.38		6.75	6.69	9.69
Oxalic acid	1132.8	45.08	7.45	C00209	215.69	188.98	147.72
Heptanoic acid	1163.8	144.13	7.91	C17714	27.86	30.12	52.21
Monomethylphosphate	1172.2	241.1	8.05		143.19	152.26	380.83
2-Oxoisocaproic acid	1206.4	99.1	8.59	C00233	10.95	9.97	10.36
1,3-Di-tert-butylbenzene	1244.2	175.2	9.1		81.29	66.93	73.5
Ethanolamine	1259.7	174.1	9.32	C00189	2983.93	3014.39	3066.39
Isoleucine	1287.1	158.2	9.71	C00407	596.91	422.3	3318.95
Glycine	1299.7	174.1	9.9	C00037	694.04	735.11	1463.43
Glyceric acid	1326.9	189.1	10.27	C00258	188.24	188.78	185.99
Uracil	1332.2	241.1	10.34	C00106	70.92	72.64	127.71
Fumaric acid	1335.3	245.1	10.38	C00122	2304.38	2144.78	2980.83
Serine	1356.6	204.2	10.67	C00065	1262.7	957.98	9050.02
Pipecolic acid	1357.4	156.12	10.68	C00408	40.21	26.12	16.65
Threonic acid-1,4-lactone	1370	45.03	10.83		41.06	39.76	56.1
Threonine	1383.7	218.2	11.03	C00188	258.01	202.76	821.18
2,4-Dihydroxybutanoic acid	1407.5	s	11.35		11.06	10.29	61.09
beta-Alanine	1419.7	248.18	11.5	C00099	60.36	61.99	181.11
Homoserine	1445.7	218.17	11.83	C00263	6.16	5.35	42
Putrescine	1452.6	174.12	11.92	C00134	57.74	46.6	61.24
Threitol	1510.1	217.11	12.65	C16884	77.23	77.06	35.88
Methionine	1512.8	176.1	12.68	C00073	94.33	95	1237.09
Pyroglutamic acid	1516.7	157.1	12.73	C01879	358.87	315.48	360.54
4-Hydroxyproline	1523.2	230.2	12.81	C01157	114.43	113.43	564.98
4-Aminobutyric acid	1524.1	174.18	12.81	C00334	12856.91	12723.99	21045.88
2,4,6-Tri-tert.-butylbenzenethiol	1535.9	263.2	12.96		33.8	28.72	32.07
Threonic acid	1563.5	292.19	13.29	C01620	53.35	59.11	99.91
2-Ketoglutaric acid	1570.5	198.1	13.37	C00026	535.78	530.34	3.07
2,3-Dihydroxybutanedioic acid	1604.7	117.1	13.77		25.06	27.54	469.77
Glutamic acid	1614.2	246.2	13.88	C00025	3837.79	3202.84	235.63
4-Hydroxybenzoic acid	1617.2	267.1	13.94	C00156	7.98	6.97	36.39
Asparagine	1670.5	100.09	14.53	C00152	38.49	22.47	40.17
Xylose	1670.6	217.11	14.52	C00181	81.74	79.79	185.72
Ribose	1685.7	217.12	14.69	C00121	109.84	151.77	251.56
2-Aminoadipic acid	1710.5	260.17	14.97	C00956	3.13	3.07	49.79
Ribonic acid	1763.6	103.1	15.54	C01685	209.79	177.83	202.35
Glycerol-3-phosphate	1769.1	299.1	15.58	C00093	297.74	197.3	248.35

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Table S2 to be continued

Name	Retention index (RI)	m/z	Retention time (min)	KEGG entry No.	Mean value of P after normalized	Mean value of PS after normalized	Mean value of S after normalized
Shikimic acid	1808	204.1	16.02	C00493	1586.48	1881.48	997.94
Citric acid	1824	273.14	16.18	C00158	10929.68	9969.59	6477.89
Quinic acid	1872.7	255.2	16.68	C06746	3084.12	3820.9	4810.47
Fructose	1889.3	103.1	16.89	C02336	33514.33	29742.94	30753.33
Sorbose	1897.9	217.2	16.94	C08356	29901.93	26587.33	26509.55
Galactose	1915.8	205.16	17.11	C00124	37514.51	33849.83	33211.5
Glucose	1917.7	319.2	17.11	C00031	39540.12	35067.46	35075.9
Tyrosine	1939	218.2	17.33	C00082	3519.98	3400.59	2498.49
Sorbitol	1954.2	319.2	17.48	C00794	146.33	153.05	64.94
Hexadecanoic acid	2027	132.1	18.19	C00249	177.58	85.72	119.18
Glucaric acid	2035.3	333.2	18.26	C00818	346.8	530.69	21201.52
trans-Ferulic acid	2083.1	338.17	18.72	C01494	16.27	19.37	18.61
myo-Inositol	2109	217.2	18.94	C00137	35592.71	30964.89	2589.1
9,12-(Z,Z)-Octadecadienoic acid	2193.9	81.1	19.71	C01595	39.86	16.38	28.41
9-(Z)-Octadecenoic acid	2198.1	117.1	19.79	C00712	43.21	25.57	77.17
Octadecanoic acid	2222.6	117.1	19.97	C01530	251.14	122.09	195.02
Fructose-6-phosphate	2334.8	299.1	20.93	C00085	66.12	61.1	14.42
Glucose-6-phosphate	2349.5	387.2	21.04	C00092	175.97	178.52	16.74
Sorbitol-6-phosphate	2369	299.1	21.19	C00644	27.59	25.69	9.13
6-P-gluconic acid	2399.5	387.19	21.44	C00345	22.66	31.18	2.23
myo-Inositol-1-phosphate	2445.1	318.2	21.81	C04006	453.62	463.47	66.98
1-Monohexadecanoylglycerol	2575	371.31	22.82		106.47	80.45	107.71
Sucrose	2679.3	361.2	23.59	C00089	19045.14	21894.78	1213.5
Maltose	1964.4	361.2	24.36	C00208	4816.04	5996.36	425.82
5-Methylthioadenosine	2798.9	236.1	24.44	C00170	4.75	5.54	120.01
Maltitol	2832.6	361.2	24.68		115.38	145.25	71.14
Isomaltose	2891.7	361.2	25.1	C00252	98.63	114.53	112.54

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Table S3. Annotated metabolites were identified from pistils (P), pistiloid stamens (PS), and stamens (S) by liquid chromatography-mass spectrometry (LC-MS)

Name	Mass	m/z	Retention time (min)	KEGG entry No.	Mean value of P after normalized	Mean value of PS after normalized	Mean value of S after normalized
Malonic acid	104.06	102.99	1.86	C00383	0.01	0.28	0.36
2-Furoic acid	112.08	111.01	2.58	C01546	0.22	0.75	0.28
Maleic acid	116.01	114.99	3.52		0.09	0.09	0.14
Succinic acid	118.09	117.00	3.99	C00042	0.09	0.09	0.07
Nicotinic acid	123.11	122.03	2.81	C00253	0.04	0.04	0.05
Itaconic acid	130.10	129.02	4.47	C00490	0.48	0.74	1.84
4-Methyl-2-oxopentanoate	130.06	129.03	9.01	C00233	0.05	0.04	0.02
Aspartic acid	133.10	132.03	1.51	C00049	0.10	0.13	3.17
(S)-Malic acid	134.09	132.99	1.80	C00149	0.53	0.47	0.73
3-Hydroxybenzoic acid	138.12	137.02	9.21	C00587	0.01	0.01	0.21
Glutamine	146.14	145.06	1.51	C00064	0.32	0.29	1.06
7H-Xanthine	152.11	151.03	3.95	C00385	0.00	0.00	0.09
Histidine	155.15	154.06	1.46	C00135	0.17	0.33	0.23
Aconitic acid	174.11	172.98	4.48		0.07	0.23	0.02
Pyranoside	180.06	179.02	1.61	C15594	0.09	0.11	0.10
(5-Hydroxyindol-3-yl) acetic acid	191.18	190.05	11.50	C05635	0.00	0.00	1.73
D-Gluconic acid	196.06	195.05	1.57		0.43	0.86	2.92
Pantothenate	219.11	218.10	6.83	C00864	0.24	0.21	0.81
2-(2-Carboxyethyl)-4-methyl-5-propylfuran-3-carboxylic acid	240.25	239.09	11.79		0.00	0.00	0.73
Inosine	268.23	267.07	5.08	C00294	0.01	0.02	0.04
3',5'-Cyclic GMP	345.21	344.04	4.52	C00942	0.00	0.00	0.09
Glutathione disulfide	612.63	611.14	4.43	C00127	0.00	0.00	0.19
Lactamide	89.09	90.05	1.48		1.57	1.61	6.33
Beta-vinyl Acrylic acid	98.04	99.04	15.50		0.31	0.38	0.15
4-Hydroxy-crotonic acid	102.03	103.04	15.52		0.19	0.21	0.07
2-Hydroxy-3-oxopropanoic acid	104.06	104.99	1.24	C01146	0.03	1.94	1.42
Proline	115.13	116.05	1.66	C00148	1.86	2.00	0.27
4-Oxopentanoic acid	116.12	117.05	15.52		0.47	0.50	0.16
Valine	117.15	118.07	1.56	C00183	0.17	0.12	0.21
L-Allothreonine	119.12	120.06	1.50	C05519	0.65	0.61	0.79
Isonicotinic acid	123.11	124.04	2.46	C07446	1.24	0.97	0.68
2S-Amino-3S-methylpentanoic acid	131.09	132.08	3.95		0.07	1.38	0.19
L-2-Amino-5-hydroxypentanoic acid	133.07	134.06	5.12		0.04	0.03	1.04
Adenine	135.13	136.06	1.73	C00147	0.24	0.33	2.87
N-Methylnicotinate	137.14	138.05	1.64	C01004	1.86	1.33	1.96
Triacetic acid	144.13	145.05	15.50	C01757	1.37	1.35	0.66
Lysine	146.19	147.11	3.85	C16440	1.05	0.90	0.00
Guanine	151.13	152.06	4.11	C00242	0.09	0.09	0.42
Beta-alanopine	161.16	162.08	6.24	C02207	0.08	0.05	0.27
trans-3-Coumaric acid	164.16	165.05	3.58	C12621	1.13	1.11	0.41
Phenylalanine	165.19	166.05	4.77	C00079	0.07	0.06	0.15

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Table S3 to be continued

Name	Retention index (RI)	m/z	Retention time (min)	KEGG entry No.	Mean value of P after normalized	Mean value of PS after normalized	Mean value of S after normalized
3R-Aminononanoic acid	173.14	174.15	5.98		0.16	0.14	0.20
(2S)-2-Isopropyl-3-oxosuccinic acid	174.15	175.06	4.19	C04236	0.02	0.02	0.28
Arginine	174.20	175.12	1.42	C00062	0.31	0.34	0.99
9-Hydroxy pelargonic acid	174.13	175.13	8.67		0.41	0.22	0.02
Calystegine B5	175.08	176.06	6.52		1.04	1.52	1.19
2-Amino-2-deoxy-D-mannopyranose	179.17	180.09	15.47	C03570	0.48	0.41	0.26
Isocitric acid	192.12	193.03	2.70	C00311	3.06	2.79	0.98
2,4,8-Trihydroxy-1-tetralone	194.18	195.06	7.85	C09974	0.26	0.27	0.09
Tryptophan	204.23	205.10	5.57	C00078	3.28	2.87	8.82
N-Acetyl-L-phenylalanine	207.23	208.10	7.75	C03519	0.08	0.07	0.01
(+)-Cucurbitic acid	212.29	213.15	10.80	C08482	1.58	1.32	0.83
Uridine	244.20	245.08	3.47	C00299	0.44	0.38	0.95
Glutaminy-l-valine	245.14	246.14	4.97		0.04	0.03	0.17
Octopine	246.13	247.14	4.62		0.76	0.56	0.70
Cordycepin	251.24	252.11	4.08	C08431	0.02	0.05	0.13
N-Acetylvani-lalanine	253.25	254.10	7.76		1.55	1.26	0.11
Adenosine	267.24	268.10	4.03	C00212	7.66	6.96	0.72
18:4(5Z,9Z,12Z,15Z)	276.21	277.21	13.91		0.05	0.03	0.03
Guanosine	283.24	284.10	4.11	C00387	1.08	1.00	3.17
Gamma-glutamylphenylalanine	294.12	295.13	5.97		0.07	0.07	0.02
17-Hydroxy-linolenic acid	294.22	295.23	10.80		0.69	0.60	0.33
Alpha-kamlolenic acid	294.22	295.23	13.82		0.35	0.22	0.01
Avenoleic acid	296.24	297.24	13.38		1.65	1.16	0.02
5'-S-Methyl-5'-thioadenosine	297.33	298.10	5.36	C00170	0.02	0.02	1.05
9,10-12,13-Diepoxyoctadecanoate	312.45	313.24	12.45	C14836	2.67	2.13	0.08
8R,11S-DiHODE	312.23	313.24	13.82		0.48	0.25	0.01
N,N-Dimethyl-Safingol	329.33	330.33	13.37		0.23	0.35	0.32
Succinyladenosine	383.31	384.11	4.83		0.28	0.23	0.02
S-Adenosyl-L-homocysteine	384.41	385.13	3.78	C00021	0.04	0.03	0.06
Di(ethylhexyl) phthalate	390.28	391.28	14.68		0.54	0.56	0.26
(-)-Fusicoplagin A	438.26	439.27	11.64		0.00	0.00	0.19
PA(13:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	678.43	679.43	10.30		0.09	0.05	0.42

Table S4. Different metabolites in stamens (S) vs. pistilloid stamens (PS)

Methionine	Mass	Retention time (min)	P-value	log2FC S vs. PS
(5-Hydroxyindol-3-yl)acetic acid	191.18	11.5	0.01	9.83
Glutathione disulfide	612.63	4.43	0.01	9.23
2-(2-Carboxyethyl)-4-methyl-5-propylfuran-3-carboxylic acid	240.25	11.79	0.01	8.23
(-)-Fusicoplagin A	438.26	11.64	0.01	6.94
5-S-Methyl-5-thioadenosine	297.33	5.36	0.01	5.58
Glucaric acid	2035.30	18.264325	0.01	5.32
Alanine	1094.10	6.858342	0.01	4.60
Aspartic acid	133.10	1.51	0.01	4.59
3,5-Cyclic GMP	345.21	4.52	0.01	4.58
3-Hydroxybenzoic acid	138.12	9.21	0.01	4.48
7H-Xanthine	152.11	3.95	0.01	4.47
5-Methylthioadenosine	2798.90	24.444683	0.01	4.44
2,3-Dihydroxybutanedioic acid	1604.70	13.773983	0.01	4.09
2-Aminoadipic acid	1710.50	14.966892	0.01	4.02
(2S)-2-Isopropyl-3-oxosuccinic acid	174.15	4.19	0.01	3.95
Methionine	1512.80	12.676833	0.01	3.70
Serine	1356.60	10.66655	0.01	3.24
Adenine	135.13	1.73	0.01	3.13
PA(13:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	678.43	10.3	0.01	3.12
Isoleucine	1287.10	9.714533	0.01	2.97
Homoserine	1445.70	11.8273	0.01	2.97
Glutaminyl-valine	245.14	4.97	0.01	2.69
2,4-Dihydroxybutanoic acid	1407.50	11.345867	0.01	2.57
Beta-alanopine	161.16	6.24	0.01	2.54
4-Hydroxybenzoic acid	1617.20	13.940325	0.01	2.38
4-Hydroxyproline	1523.20	12.806	0.01	2.32
Guanine	151.13	4.11	0.01	2.27
Threonine	1383.70	11.030367	0.01	2.02
Lactamide	89.09	1.48	0.01	1.97
Pantothenate	219.11	6.83	0.01	1.93
Glutamine	146.14	1.51	0.01	1.86
D-Gluconic acid	196.06	1.57	0.01	1.76
Guanosine	283.24	4.11	0.01	1.66
Tryptophan	204.23	5.57	0.01	1.62
9-(Z)-Octadecenoic acid	2198.10	19.78545	0.01	1.59
Arginine	174.20	1.42	0.01	1.55
Beta-alanine	1419.70	11.501008	0.01	1.55
Monomethylphosphate	1172.20	8.045808	0.01	1.32
Itaconic acid	130.10	4.47	0.01	1.32
Uridine	244.20	3.47	0.01	1.31
S-Adenosyl-L-homocysteine	384.41	3.78	0.03	1.21
Phenylalanine	165.19	4.77	0.01	1.19
Inosine	268.23	5.08	0.01	1.15
Glycine	1299.70	9.896367	0.01	0.99
Uracil	1332.20	10.340283	0.01	0.81
9,12-(Z,Z)-Octadecadienoic acid	2193.90	19.712383	0.01	0.79

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Table S4 to be continued

Methionine	Mass	Retention time (min)	P-value	log2FC S vs. PS
Heptanoic acid	1163.80	7.912017	0.01	0.79
Valine	117.15	1.56	0.01	0.76
Threonic acid	1563.50	13.288583	0.01	0.76
Ribose	1685.70	14.694117	0.01	0.73
4-Aminobutyric acid	1524.10	12.8061	0.01	0.73
Octadecanoic acid	2222.60	19.965717	0.01	0.68
(S)-Malic acid	134.09	1.8	0.01	0.61
Citric acid	1824.00	16.1757	0.02	-0.62
Pipecolic acid	1357.40	10.677283	0.01	-0.65
2-Amino-2-deoxy-D-mannopyranose	179.17	15.47	0.03	-0.65
Shikimic acid	1808.00	16.015225	0.01	-0.91
Maltitol	2832.60	24.680017	0.01	-1.03
Triacetic acid	144.13	15.5	0.01	-1.03
Threitol	1510.10	12.649783	0.01	-1.10
Di(ethylhexyl) phthalate	390.28	14.68	0.01	-1.10
Sorbitol	1954.20	17.484142	0.01	-1.24
Beta-vinyl acrylic acid	98.04	15.5	0.01	-1.34
2-Furoic acid	112.08	2.58	0.02	-1.40
trans-3-Coumaric acid	164.16	3.58	0.01	-1.42
Sorbitol-6-phosphate	2369.00	21.192667	0.01	-1.49
Isocitric acid	192.12	2.7	0.01	-1.51
4-Oxopentanoic acid	116.12	15.52	0.01	-1.64
4-Hydroxy-crotonic acid	102.03	15.52	0.01	-1.66
Gamma-glutamylphenylalanine	294.12	5.97	0.03	-1.70
Fruuctose-6-phosphate	2334.80	20.930617	0.01	-2.08
myo-Inositol-1-phosphate	2445.10	21.81315	0.01	-2.79
Proline	115.13	1.66	0.01	-2.90
Adenosine	267.24	4.03	0.01	-3.28
9-Hydroxy pelargonic acid	174.13	8.67	0.01	-3.40
Glucose-6-phosphate	2349.50	21.0375	0.01	-3.41
Succinyladenosine	383.31	4.83	0.01	-3.56
N-Acetylvanilalanine	253.25	7.76	0.01	-3.56
myo-Inositol	2109.00	18.943517	0.01	-3.58
Glutamic acid	1614.20	13.881083	0.01	-3.76
6-P-Gluconic acid	2399.50	21.4441	0.01	-3.81
Maltose	2788.30	24.36455	0.01	-3.82
N-Acetyl-L-phenylalanine	207.23	7.75	0.01	-3.82
Alpha-kamlolenic acid	294.22	13.82	0.01	-4.03
Sucrose	2679.30	23.588833	0.01	-4.17
8R,11S-DiHODE	312.23	13.82	0.01	-4.48
9,10-12,13-Diepoxyoctadecanoate	312.45	12.45	0.01	-4.78
Avenoleic acid	296.24	13.38	0.01	-5.72
2-Ketoglutaric acid	1570.50	13.368292	0.01	-7.43
Lysine	146.19	3.85	0.01	-8.24

Table S5. Different metabolites in stamens (S) vs. pistils (P)

Methionine	Mass	Retention time (min)	P-value	log2FC S vs. P
(5-Hydroxyindol-3-yl)acetic acid	191.18	11.50	0.01	9.70
Glutathione disulfide	612.63	4.43	0.01	9.03
2-(2-Carboxyethyl)-4-methyl-5-propylfuran-3-carboxylic acid	240.25	11.79	0.01	8.25
(-)-Fusicoplagin A	438.26	11.64	0.01	8.03
Glucaric acid	2035.30	18.26	0.01	5.89
5-S-Methyl-5-thioadenosine	297.33	5.36	0.01	5.21
3,5-Cyclic GMP	345.21	4.52	0.01	4.89
Aspartic acid	133.10	1.51	0.01	4.80
5-Methylthioadenosine	2798.90	24.44	0.01	4.59
7H-Xanthine	152.11	3.95	0.01	4.33
2,3-Dihydroxybutanedioic acid	1604.70	13.77	0.01	4.21
2-Amino adipic acid	1710.50	14.97	0.01	3.93
3-Hydroxybenzoic acid	138.12	9.21	0.01	3.83
Alanine	1094.10	6.86	0.01	3.69
Methionine	1512.80	12.68	0.01	3.68
(2S)-2-Isopropyl-3-oxosuccinic acid	174.15	4.19	0.01	3.57
Adenine	135.13	1.73	0.01	3.52
Serine	1356.60	10.67	0.01	2.78
Homoserine	1445.70	11.83	0.01	2.70
D-Gluconic acid	196.06	1.57	0.01	2.66
Isoleucine	1287.10	9.71	0.01	2.46
2,4-Dihydroxybutanoic acid	1407.50	11.35	0.01	2.40
4-Hydroxyproline	1523.20	12.81	0.01	2.29
Guanine	151.13	4.11	0.01	2.14
4-Hydroxybenzoic acid	1617.20	13.94	0.01	2.09
Glutaminyl-Valine	245.14	4.97	0.01	2.08
lactamide	89.09	1.48	0.01	1.94
Inosine	268.23	5.08	0.01	1.79
Itaconic acid	130.10	4.47	0.01	1.75
Arginine	174.20	1.42	0.01	1.67
Glutamine	146.14	1.51	0.01	1.61
Threonine	1383.70	11.03	0.01	1.60
Beta-alanopine	161.16	6.24	0.01	1.56
Pantothenate	219.11	6.83	0.01	1.54
2S-Amino-3S-methylpentanoic acid	131.09	3.95	0.01	1.47
Aconitic acid	174.11	4.48	0.01	1.47
Guanosine	283.24	4.11	0.01	1.43
Beta-alanine	1419.70	11.50	0.01	1.43
Monomethylphosphate	1172.20	8.05	0.01	1.41
Tryptophan	204.23	5.57	0.01	1.29
Ribose	1685.70	14.69	0.01	1.15
Glycine	1299.70	9.90	0.01	1.04
Uridine	244.20	3.47	0.01	0.96
Phenylalanine	165.19	4.77	0.01	0.90
Threonic acid	1563.50	13.29	0.01	0.87
Heptanoic acid	1163.80	7.91	0.01	0.83
Uracil	1332.20	10.34	0.01	0.81

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Table S5 to be continued

Methionine	Mass	Retention time (min)	P-value	log2FC S vs. P
9-(Z)-Octadecenoic acid	2198.10	19.79	0.01	0.75
4-Aminobutyric acid	1524.10	12.81	0.01	0.68
Quinic acid	1872.70	16.68	0.01	0.61
Oxalic acid	1132.80	7.45	0.02	-0.63
Hexadecanoic acid	2027.00	18.19	0.01	-0.65
18:4(5Z,9Z,12Z,15Z)	276.21	13.91	0.04	-0.67
Shikimic acid	1808.00	16.02	0.01	-0.74
Maltitol	2832.60	24.68	0.01	-0.74
Lactic acid	1052.20	6.21	0.02	-0.77
Citric acid	1824.00	16.18	0.01	-0.82
Di(ethylhexyl) phthalate	390.28	14.68	0.01	-0.82
Pyruvic acid	1040.90	6.03	0.01	-0.86
Isonicotinic acid	123.11	2.46	0.01	-1.00
(+)-Cucurbitic acid	212.29	10.80	0.01	-1.00
Sorbitol	1954.20	17.48	0.01	-1.13
17-Hydroxy-linolenic acid	294.22	10.80	0.01	-1.18
Threitol	1510.10	12.65	0.01	-1.18
4-Hydroxy-crotonic acid	102.03	15.52	0.04	-1.22
4-Oxopentanoic acid	116.12	15.52	0.01	-1.23
Pipecolic acid	1357.40	10.68	0.01	-1.25
4-Methyl-2-oxopentanoate	130.06	9.01	0.01	-1.28
trans-3-Coumaric acid	164.16	3.58	0.01	-1.57
Sorbitol-6-phosphate	2369.00	21.19	0.01	-1.67
2,4,8-Trihydroxy-1-tetralone	194.18	7.85	0.01	-1.74
Isocitric acid	192.12	2.70	0.01	-1.77
Gamma-glutamylphenylalanine	294.12	5.97	0.01	-1.90
Fructose-6-phosphate	2334.80	20.93	0.01	-2.25
myo-Inositol-1-phosphate	2445.10	21.81	0.01	-2.80
proline	115.13	1.66	0.01	-2.89
6-P-Gluconic acid	2399.50	21.44	0.01	-3.35
Glucose-6-phosphate	2349.50	21.04	0.01	-3.47
Adenosine	267.24	4.03	0.01	-3.54
Maltose	2788.30	24.36	0.01	-3.55
myo-Inositol	2109.00	18.94	0.01	-3.84
Succinyladenosine	383.31	4.83	0.01	-3.95
N-Acetylvanilalanine	253.25	7.76	0.01	-3.95
Glutamic acid	1614.20	13.88	0.01	-4.03
N-Acetyl-L-phenylalanine	207.23	7.75	0.01	-4.09
Sucrose	2679.30	23.59	0.01	-4.10
9-Hydroxy pelargonic acid	174.13	8.67	0.01	-4.40
Alpha-kamlolenic acid	294.22	13.82	0.01	-4.92
9,10-12,13-Diepoxyoctadecanoate	312.45	12.45	0.01	-5.25
8R,11S-DiHODE	312.23	13.82	0.01	-5.66
Avenoleic acid	296.24	13.38	0.01	-6.38
2-Ketoglutaric acid	1570.50	13.37	0.01	-7.46
Lysine	146.19	3.85	0.01	-8.56

Table S6. Different metabolites in pistils (P) vs. pistilloid stamens (PS)

Methionine	Mass	Retention time (min)	P-value	log2FC P vs. PS
9,12-(Z,Z)-Octadecadienoic acid	2193.90	19.71	0.01	1.35
8R,11S-DiHODE	312.23	13.82	0.02	1.18
Hexadecanoic acid	2027.00	18.19	0.01	1.12
Octadecanoic acid	2222.60	19.97	0.01	1.11
9-Hydroxy pelargonic acid	174.13	8.67	0.01	0.99
Alanine	1094.10	6.86	0.01	0.91
Alpha-kamlolenic acid	294.22	13.82	0.01	0.88
9-(Z)-Octadecenoic acid	2198.10	19.79	0.01	0.84
Avenoleic acid	296.24	13.38	0.01	0.66
3-Hydroxybenzoic acid	138.12	9.21	0.04	0.64
N-Methylnicotinate	137.14	1.64	0.01	0.64
Glycerol-3-phosphate	1769.10	15.58	0.01	0.63
Pipecolic acid	1357.40	10.68	0.04	0.60
Valine	117.15	1.56	0.01	0.60
Inosine	268.23	5.08	0.04	-0.64
Histidine	155.15	1.46	0.01	-0.85
D-Gluconic acid	196.06	1.57	0.01	-0.90
2-Furoic acid	112.08	2.58	0.02	-1.55

Table S7. The list of metabolites, correlated gene and KEGG pathways

Gene ID	Gene annotation detail	log2 FC (S vs. PS)	log2 FC (S vs. P)
Pathway: Phenylpropanoid biosynthesis			
Metabolites: phenylalanine			
comp112524_c0	beta-glucosidase 22 (<i>A. tauschii</i>)	6.12	6.55
comp108539_c0	cinnamyl alcohol dehydrogenase 7 [<i>A. tauschii</i>]	-5.83	-5.28
comp108827_c0	peroxidase 1 (<i>A. tauschii</i>)	6.77	9.44
comp111424_c1	peroxidase 44 (<i>A. tauschii</i>)	6.00	9.73
comp110800_c0	peroxidase 2 (<i>H. vulgare</i>)	5.87	5.04
comp119870_c1	serine carboxypeptidase 18 (<i>H. vulgare</i>)	5.11	7.34
Pathway: Cysteine and methionine metabolism			
Metabolites: Alanine, aspartic acid, Serine, Methionine, 5-S-methyl-5-thioadenosine, Homoserine			
comp121488_c1	ACC oxidase (<i>H. vulgare</i>)	5.86	5.25

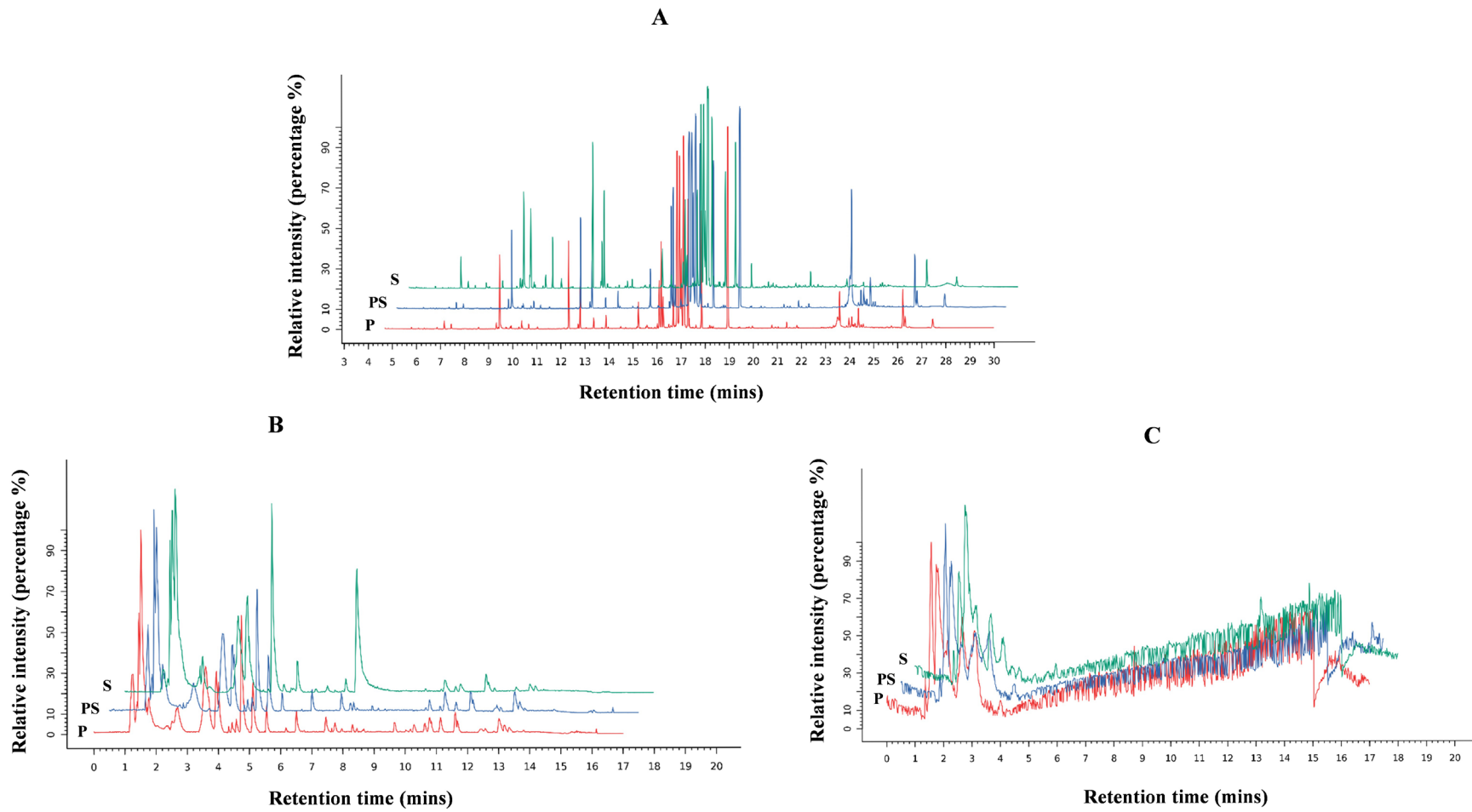


Figure S1. Total ion chromatograms (TICs) and base peak chromatogram of the pistils (P), pistilloid stamens (PS), and stamens (S): (A) TICs of the P, PS and S base GC-MS, (B) BPC in positive ion mode of P, PS and S base LC-MS, (C) BPC in negative ion mode of P, PS and S base LC-MS

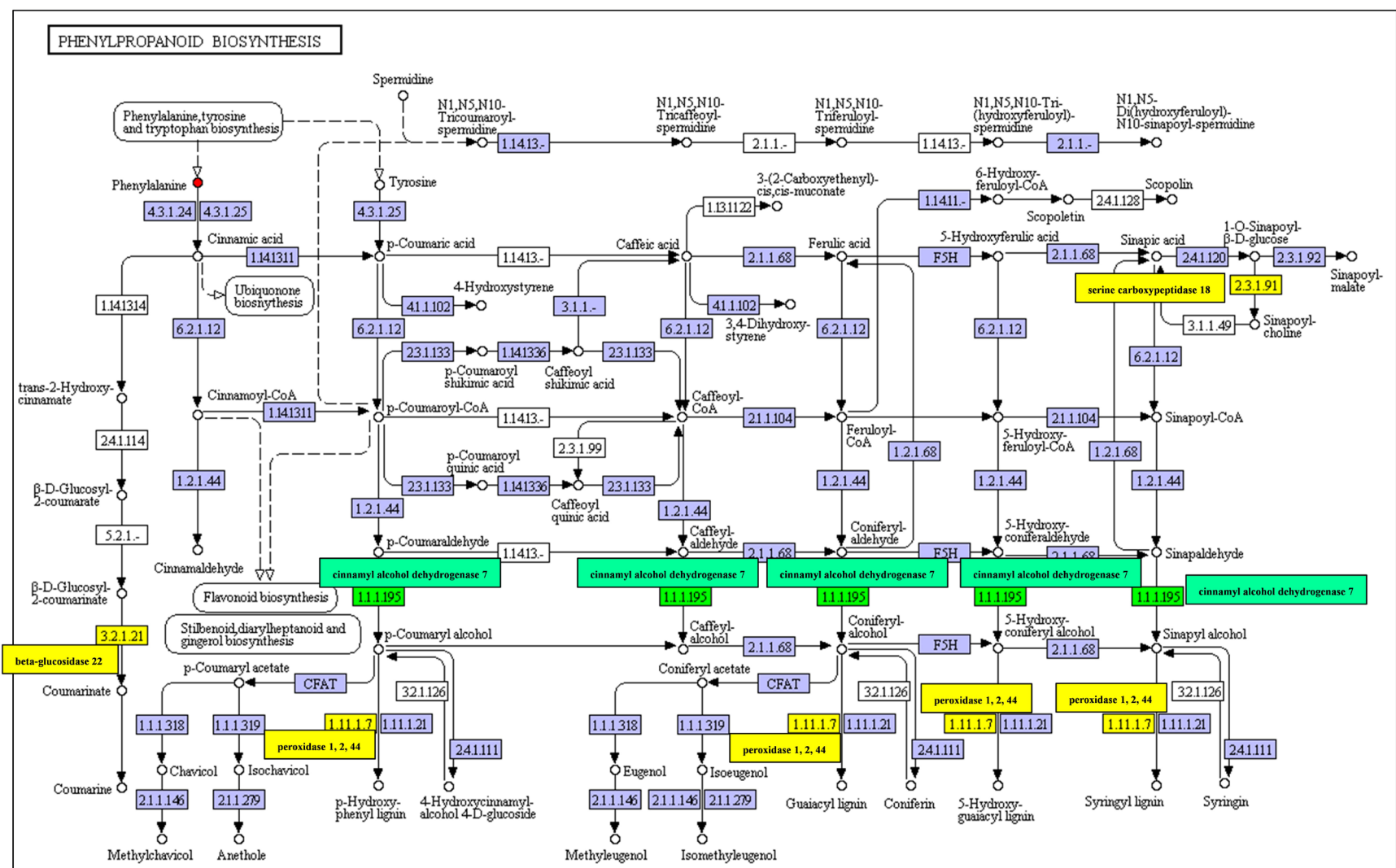


Figure S2. The phenylpropanoid biosynthesis pathway

Red solid circle represents up-regulation metabolites; blue solid circle represents down-regulation metabolites; yellow bottom rectangle represents up-regulation genes and green bottom shape represents down-regulation genes

