High Resolution LC-MS Data Output and Analysis

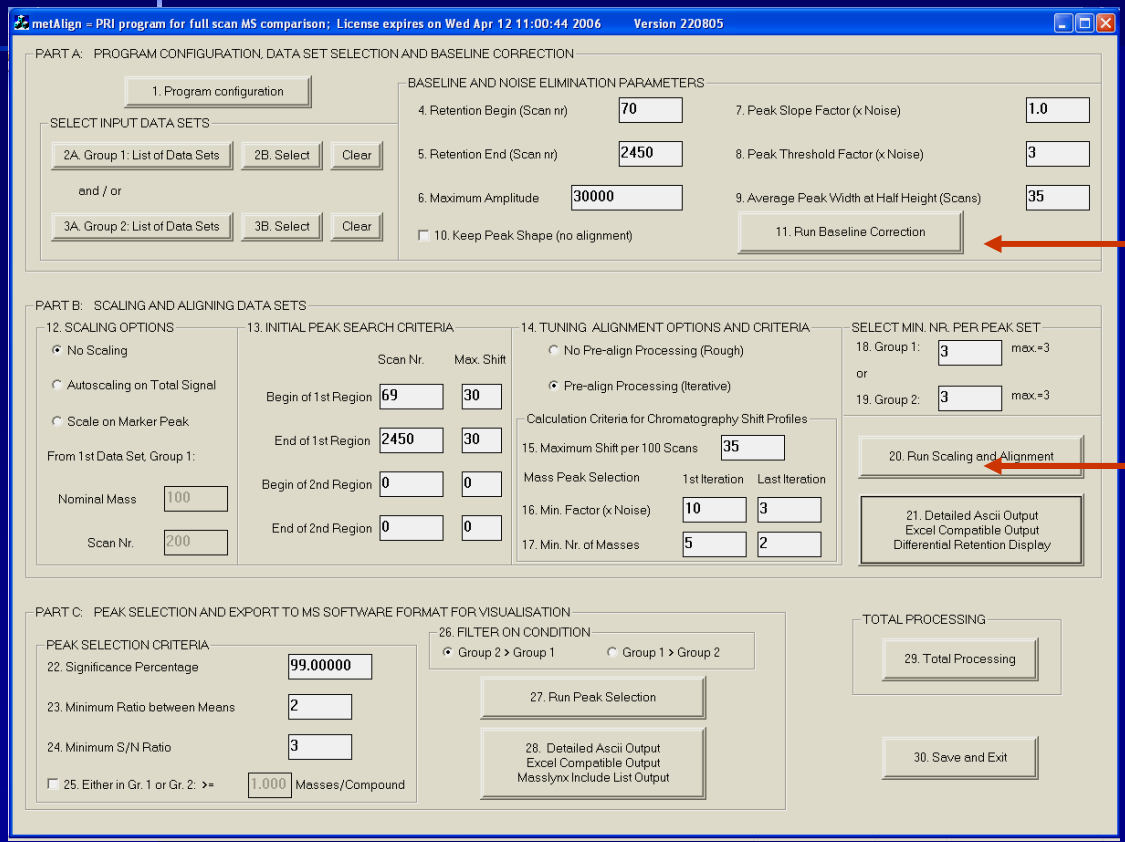
Software for comparing full-scan datasets

MetAlign method

- MetAlign™ (Plant Research International)
  - www.metalign.nl
    - uses nominal masses
    - estimation of local noise as a function of retention time and ion trace
    - alignment on scan basis via “landmark” mass traces
    - for all mass spectrometers

Software for comparing full-scan datasets

MetAlign method

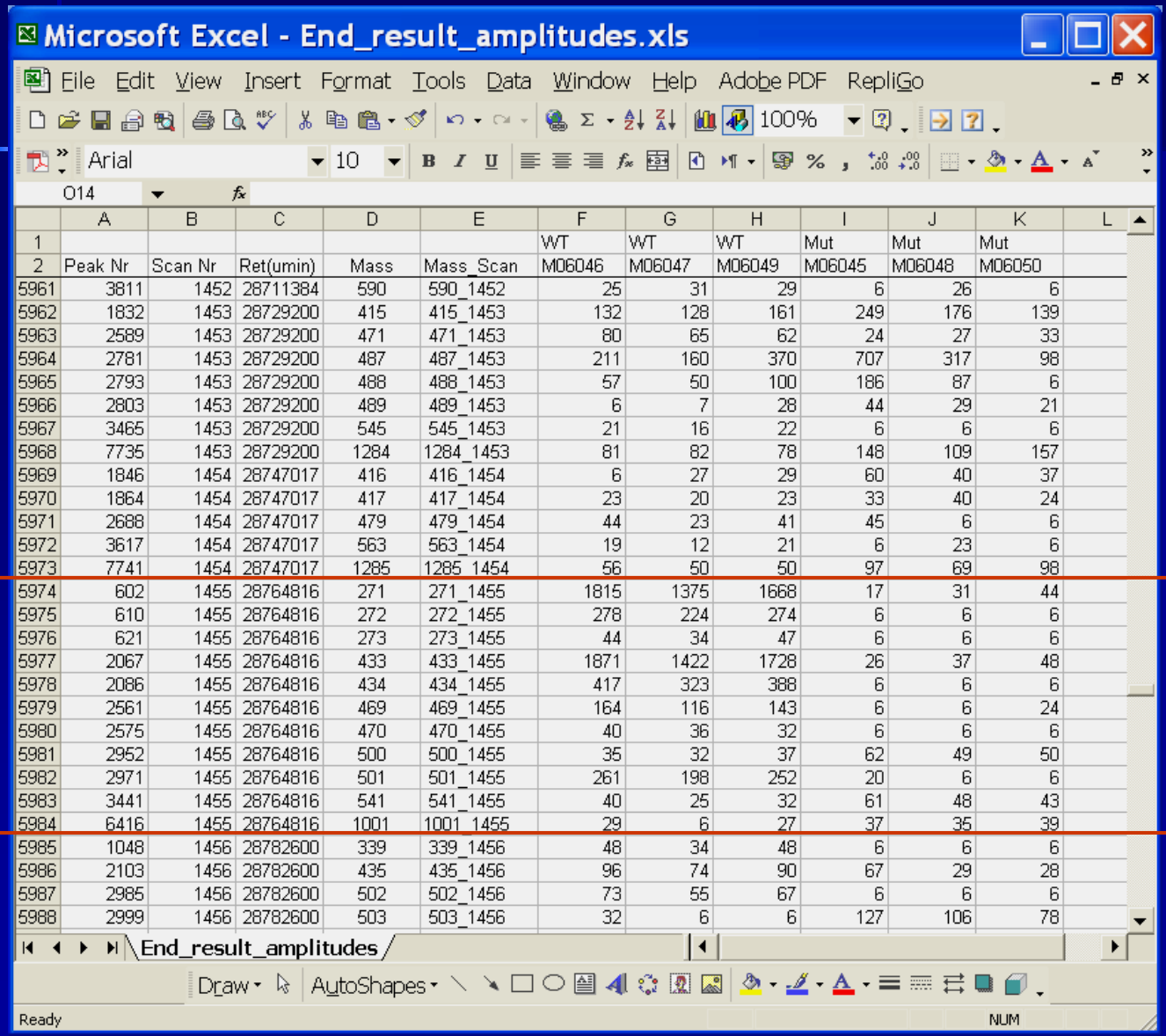


Base line correction and peak picking- new chromatograms with corrected baseline are generated

Chromatogram alignment based on RT and nominal mass

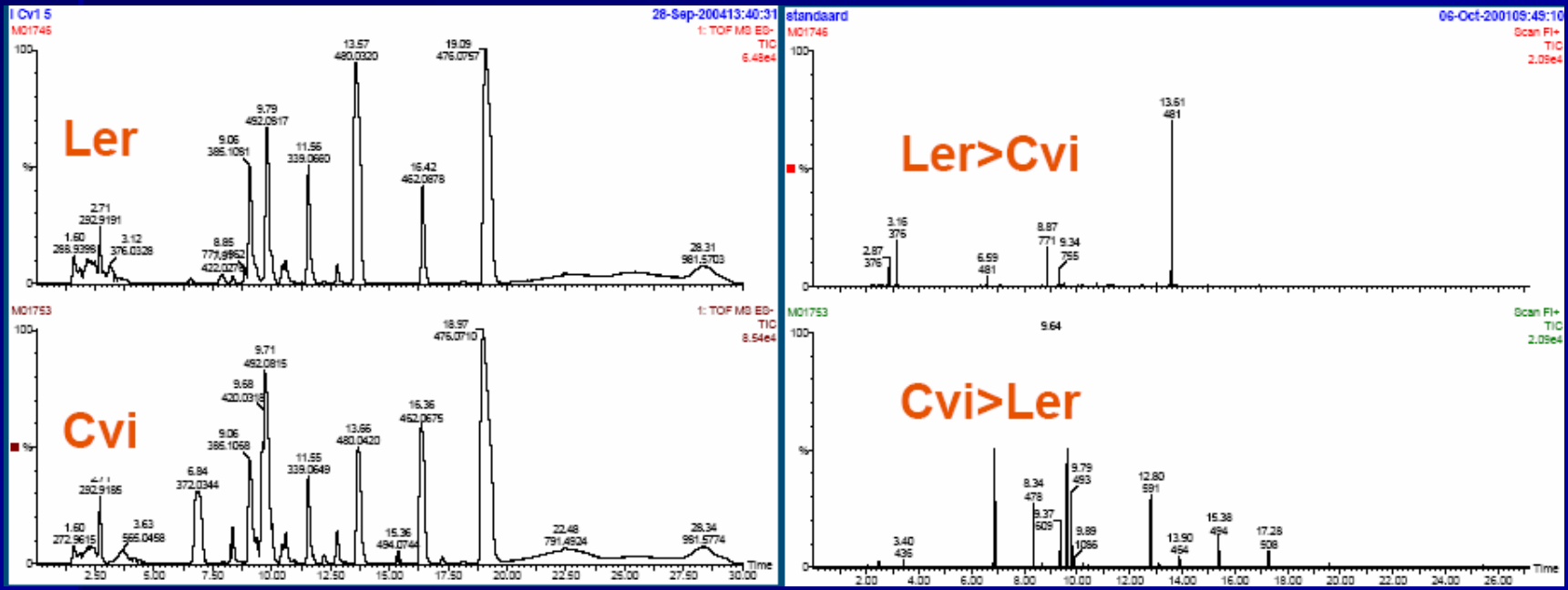
Statistical analysis

MetAlign output, ES(-), Tomato peel, orange, WT vs Mut “Y”



	A	B	C	D	E	F	G	H	I	J	K	L
1						WT	WT	WT	Mut	Mut	Mut	
2	Peak Nr	Scan Nr	Ret(umin)	Mass	Mass_Scan	M06046	M06047	M06049	M06045	M06048	M06050	
5961	3811	1452	28711384	590	590_1452	25	31	29	6	26	6	
5962	1832	1453	28729200	415	415_1453	132	128	161	249	176	139	
5963	2589	1453	28729200	471	471_1453	80	65	62	24	27	33	
5964	2781	1453	28729200	487	487_1453	211	160	370	707	317	98	
5965	2793	1453	28729200	488	488_1453	57	50	100	186	87	6	
5966	2803	1453	28729200	489	489_1453	6	7	28	44	29	21	
5967	3465	1453	28729200	545	545_1453	21	16	22	6	6	6	
5968	7735	1453	28729200	1284	1284_1453	81	82	78	148	109	157	
5969	1846	1454	28747017	416	416_1454	6	27	29	60	40	37	
5970	1864	1454	28747017	417	417_1454	23	20	23	33	40	24	
5971	2688	1454	28747017	479	479_1454	44	23	41	45	6	6	
5972	3617	1454	28747017	563	563_1454	19	12	21	6	23	6	
5973	7741	1454	28747017	1285	1285_1454	56	50	50	97	69	98	
5974	602	1455	28764816	271	271_1455	1815	1375	1668	17	31	44	
5975	610	1455	28764816	272	272_1455	278	224	274	6	6	6	
5976	621	1455	28764816	273	273_1455	44	34	47	6	6	6	
5977	2067	1455	28764816	433	433_1455	1871	1422	1728	26	37	48	
5978	2086	1455	28764816	434	434_1455	417	323	388	6	6	6	
5979	2561	1455	28764816	469	469_1455	164	116	143	6	6	24	
5980	2575	1455	28764816	470	470_1455	40	36	32	6	6	6	
5981	2952	1455	28764816	500	500_1455	35	32	37	62	49	50	
5982	2971	1455	28764816	501	501_1455	261	198	252	20	6	6	
5983	3441	1455	28764816	541	541_1455	40	25	32	61	48	43	
5984	6416	1455	28764816	1001	1001_1455	29	6	27	37	35	39	
5985	1048	1456	28782600	339	339_1456	48	34	48	6	6	6	
5986	2103	1456	28782600	435	435_1456	96	74	90	67	29	28	
5987	2985	1456	28782600	502	502_1456	73	55	67	6	6	6	
5988	2999	1456	28782600	503	503_1456	32	6	6	127	106	78	

MetAlign Output, Visualization of Differential Peaks



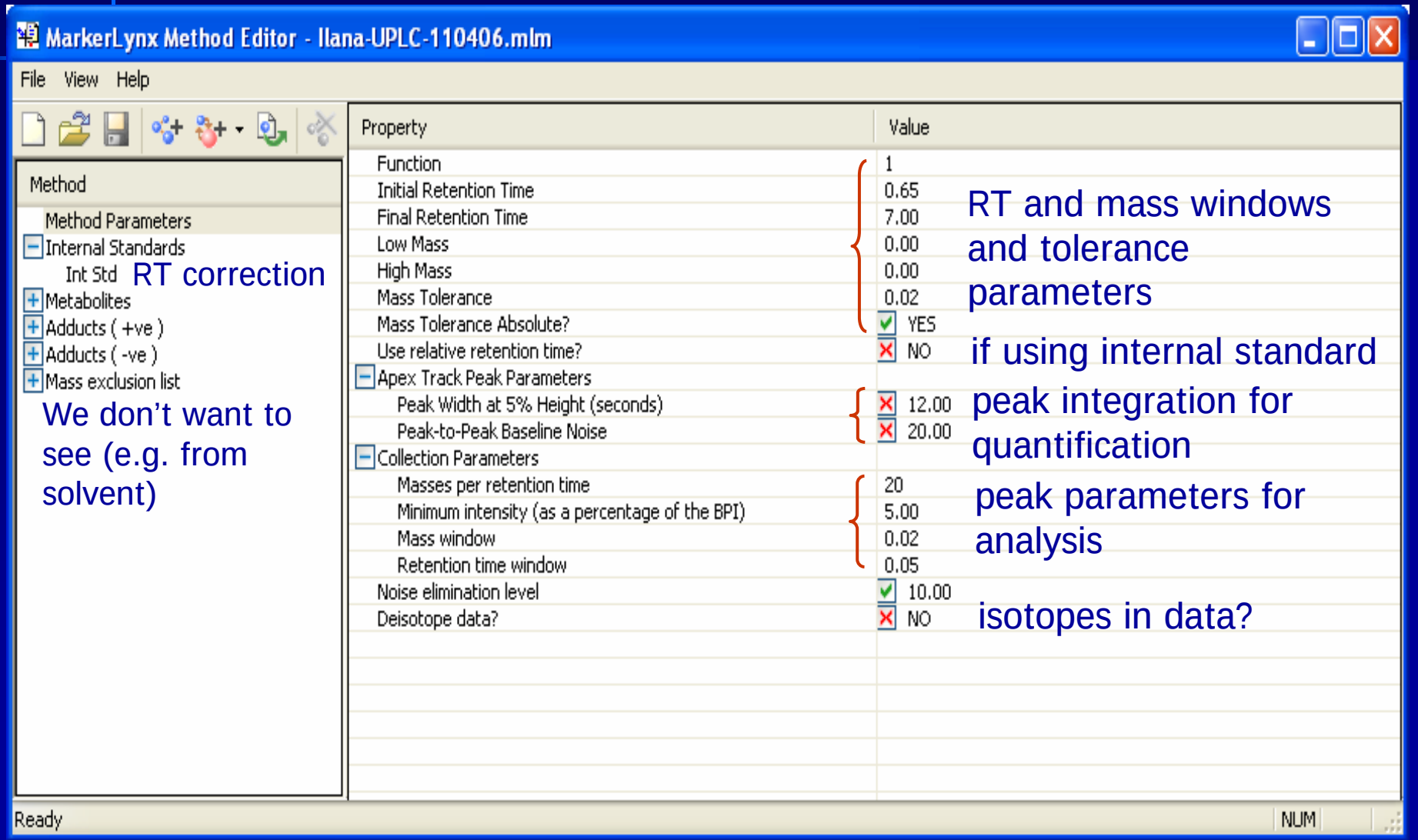
Software for comparing full-scan datasets

MarkerLynx method

- Markerlynx (Waters Corp.)
  - www.waters.com
    - makes use of high mass resolution of TOF MS
    - used-defined windows for mass accuracy, retention time and noise for all samples
    - only for (Waters) TOF-mass spectrometers

Software for comparing full-scan datasets

MarkerLynx Method



RT correction

We don't want to see (e.g. from solvent)

RT and mass windows and tolerance parameters

if using internal standard

peak integration for quantification

peak parameters for analysis

isotopes in data?

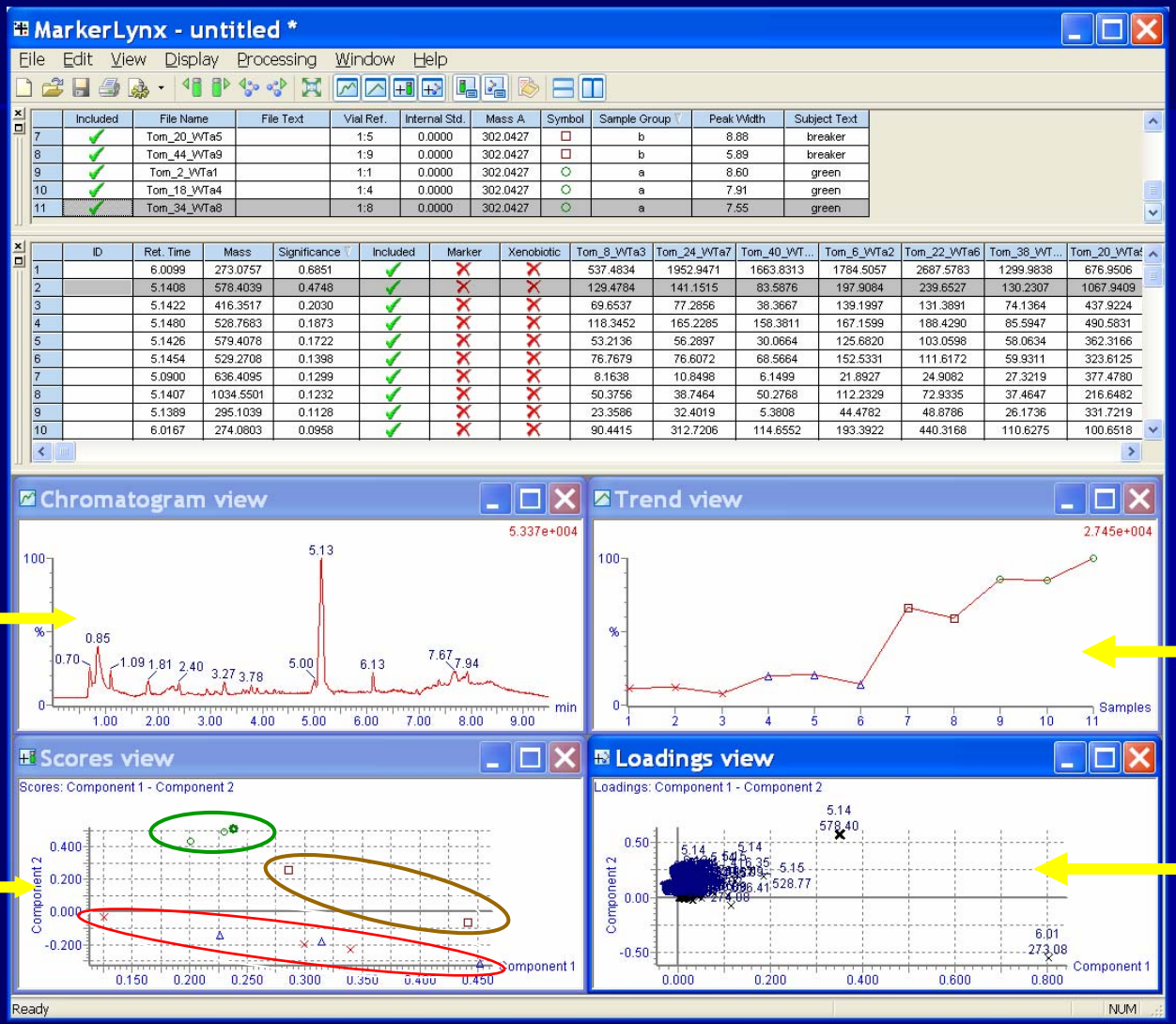
Out-put from the MarkerLynx (Waters) Program : Analyses of Wild-Type Tomato Peel (Different Stages of Development; ES+)

Samples Processed

Markers Detected

TIC of currently selected sample

Principle Components Analysis



Response of a marker across all samples

Markers responsible for clustering (by PCA)

Output Data For High Res. LC-MS Based Metabolomics

scan	rt (umin)	Nominal mass	area	Accurate mass	Accumass database	calc mass	ppm
112	2166433	147	1277	147.0767	glutamine	147.0764	2.2
115	2218350	138	652	138.0544	TRIGONELLINE	138.0550	-4.2
121	2339467	133	117	133.0627	ASPARAGINE	133.0608	14.7
123	2374533	284	100	284.0990	guanosine	284.0989	0.2
124	2391750	182	113	182.0839	TYROSINE	182.0812	14.9
124	2391750	132	64	132.1047	LEUCINE	132.1019	21.5
125	2409000	148	2730	148.0590	GLUTAMIC-ACID	148.0604	-9.7
125	2409000	150	85	150.0631	METHIONINE	150.0583	31.8
128	2477900	343	75	343.1250	SUCROSE	343.1235	4.3
128	2477900	134	434	134.0460	ASPARTIC-ACID	134.0448	8.9
133	2564183	304	22	304.1520	NICOTIANAMINE	304.1503	5.5
170	3273917	193	2774	193.0352	CITRIC-ACID	193.0343	5.0
170	3273917	245	575	245.0794	uridine	245.0768	10.7
171	3291367	308	595	308.0946	glutathione	308.0916	9.8
172	3326083	268	1583	268.1056	ADENOSINE	268.1040	5.8
172	3326083	182	272	182.0827	TYROSINE	182.0812	8.4
174	3360967	132	1111	132.1022	LEUCINE	132.1019	2.0
243	4672433	166	145	166.0883	PHENYLALANINE	166.0863	12.1
244	4706900	118	155	118.0663	PHENYLACETONITRILE	118.0651	9.7
404	7771017	146	417	146.0615	1H-Indole-3-carboxaldehyde, 9CI	146.0600	10.0
634	12210317	265	119	265.1470	ABSCISIC-ACID	265.1434	13.4
707	13614350	147	97	147.0448	coumarin	147.0441	4.8
1107	21309401	465	1031	465.1039	quercetin-glucoside (quercitrin)	465.1028	2.4
1107	21309401	743	502	743.2054	q-trisaccharide	743.2029	3.3
1107	21309401	303	1151	303.0510	Quercetin	303.0499	3.4
1107	21309401	611	568	611.1642	Quercetin 3-O-rutinoside (rutin)	611.1607	5.8
1212	23333666	303	3323	303.0482	Quercetin	303.0499	-5.9
1212	23333666	611	806	611.1646	Quercetin 3-O-rutinoside (rutin)	611.1607	6.5
1224	23557133	449	199	449.1092	QUERCETIN-3-O-RHAMNOSIDE	449.1078	3.1
1225	23591383	595	111	595.1677	Kaempferol-3-O-rutinoside	595.1657	3.3

To MS-MS

A Case Study in Tomato

Small Green | Middle Green | Big Green | Breaker | Orange | Red

Sample Preparation for Tomato Fruit Metabolome Analysis

Harvesting tomato fruit at different developmental stages

Separation of peel and flesh tissues

Immediate freezing in liquid nitrogen

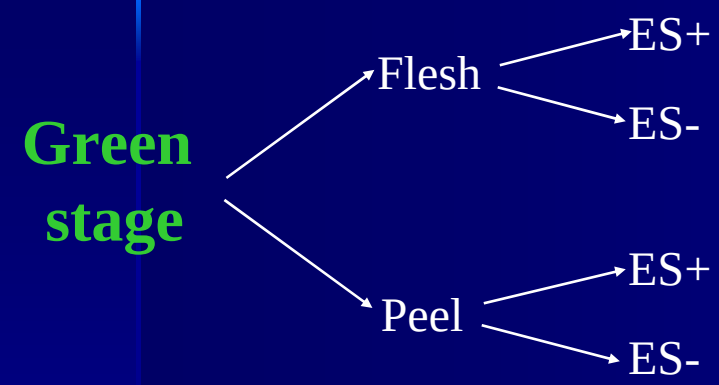
Grinding to fine powder

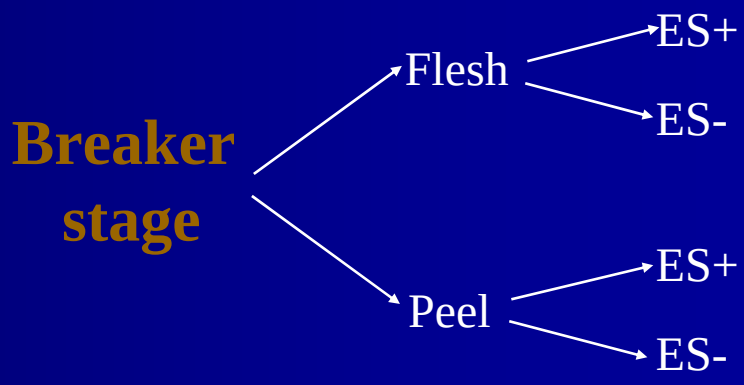
Weighing frozen samples

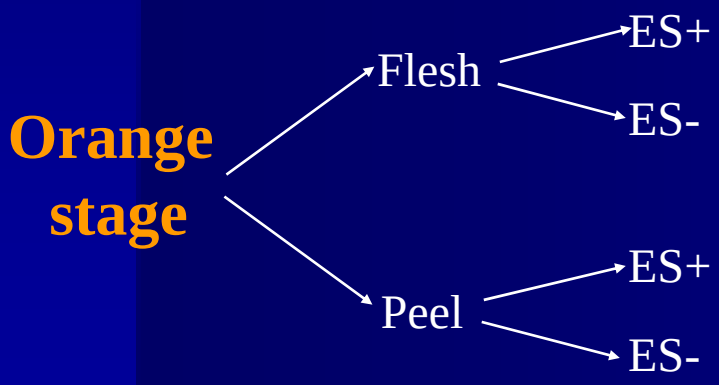
Extraction in 100% MeOH by vortexing and sonication

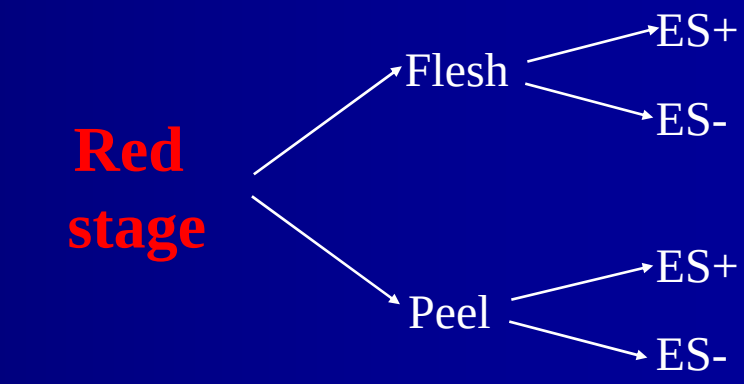
Analyzing with UPLC-MS [ES (+) and ES (-)]

Experimental Set-Up for LC-MS Analysis: PEEL and FLESH during tomato fruit development

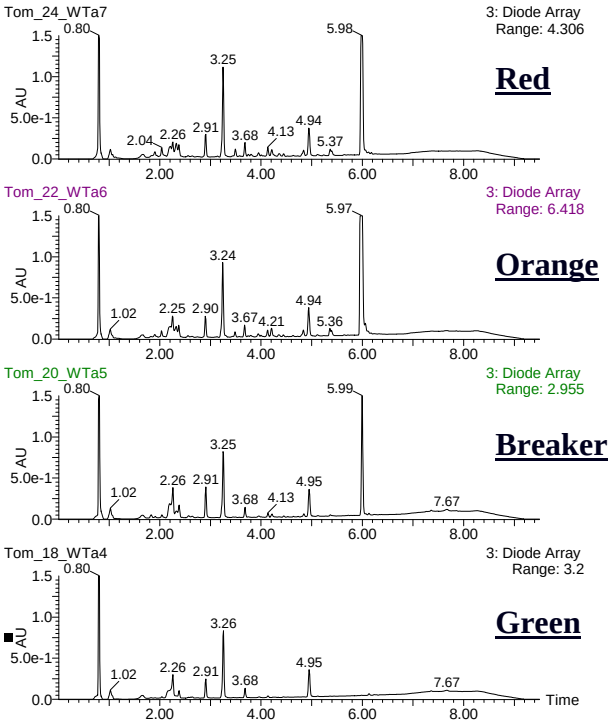




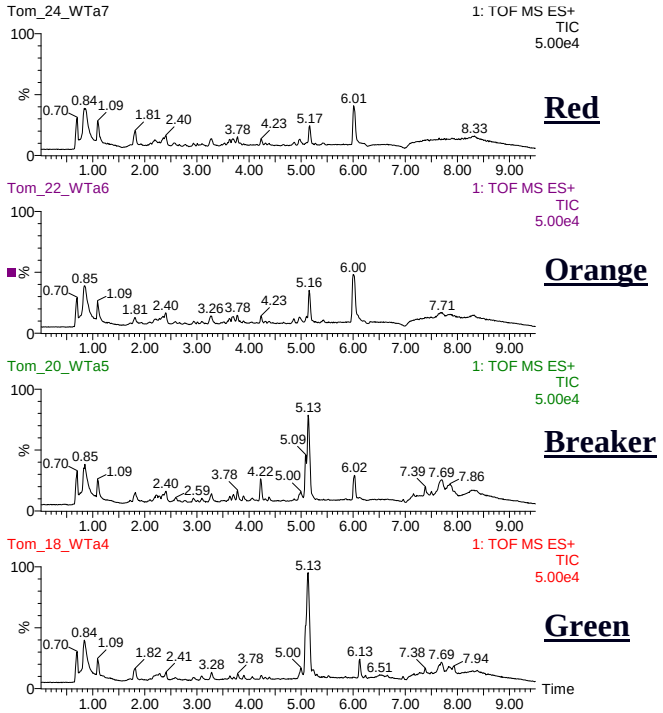




UPLC-Q-Tof-MS Analysis of Four Stages of Tomato Fruit Development

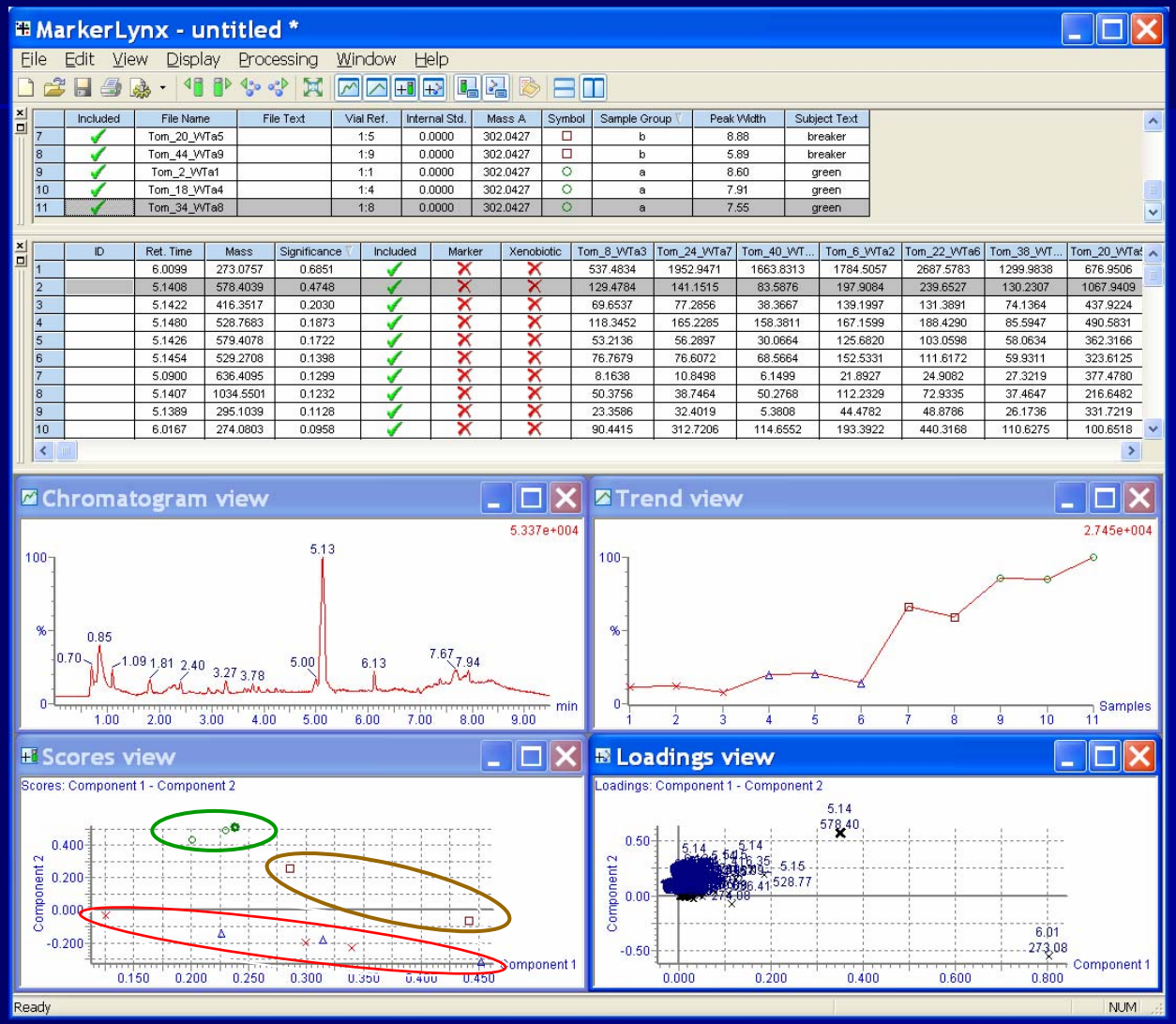


UV (240 nm)

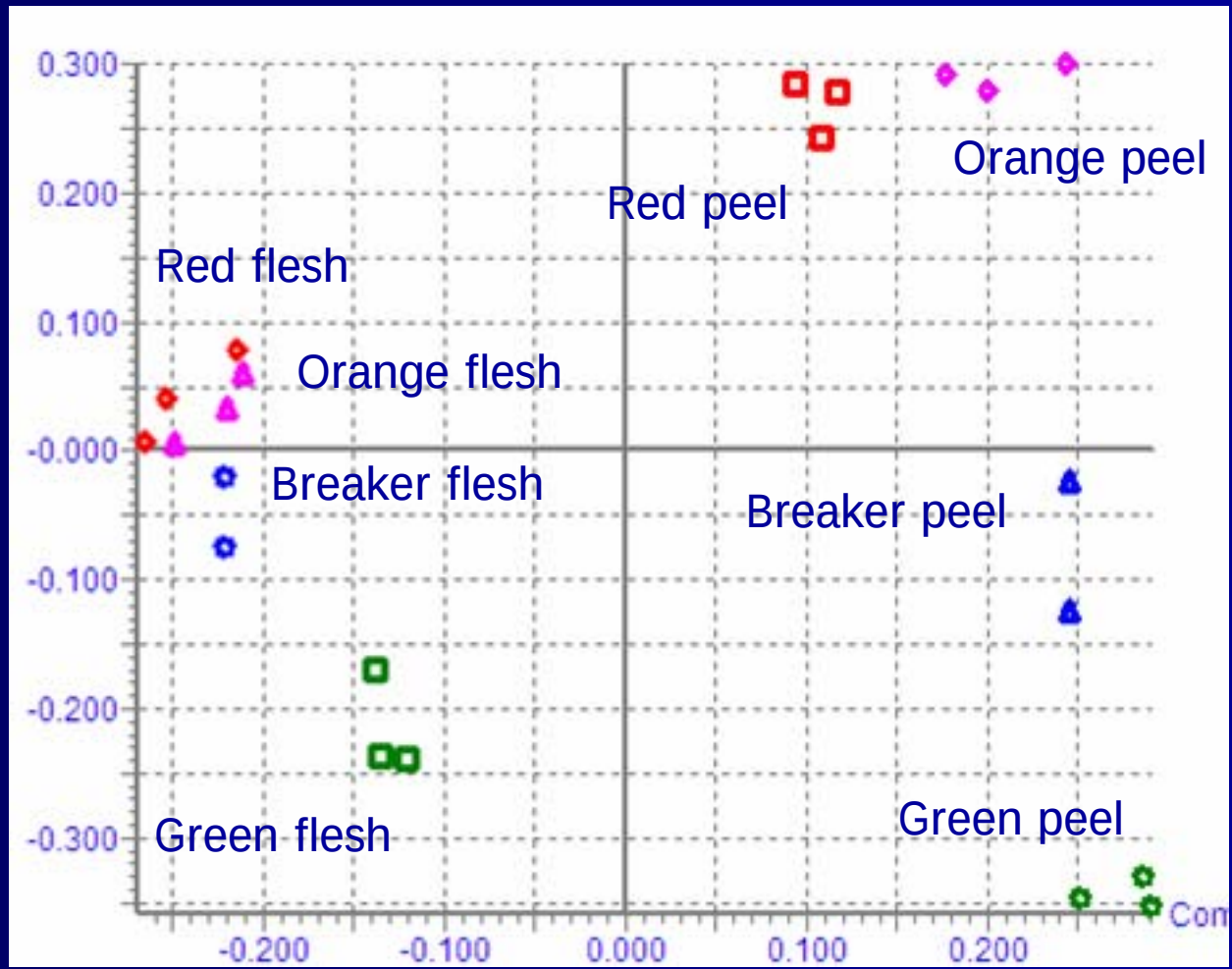


MS (ES(+),TIC)

Out-put from the MarkerLynx (Waters) Program : Analyses of Wild-Type Tomato Peel (Different Stages of Development; ES+)

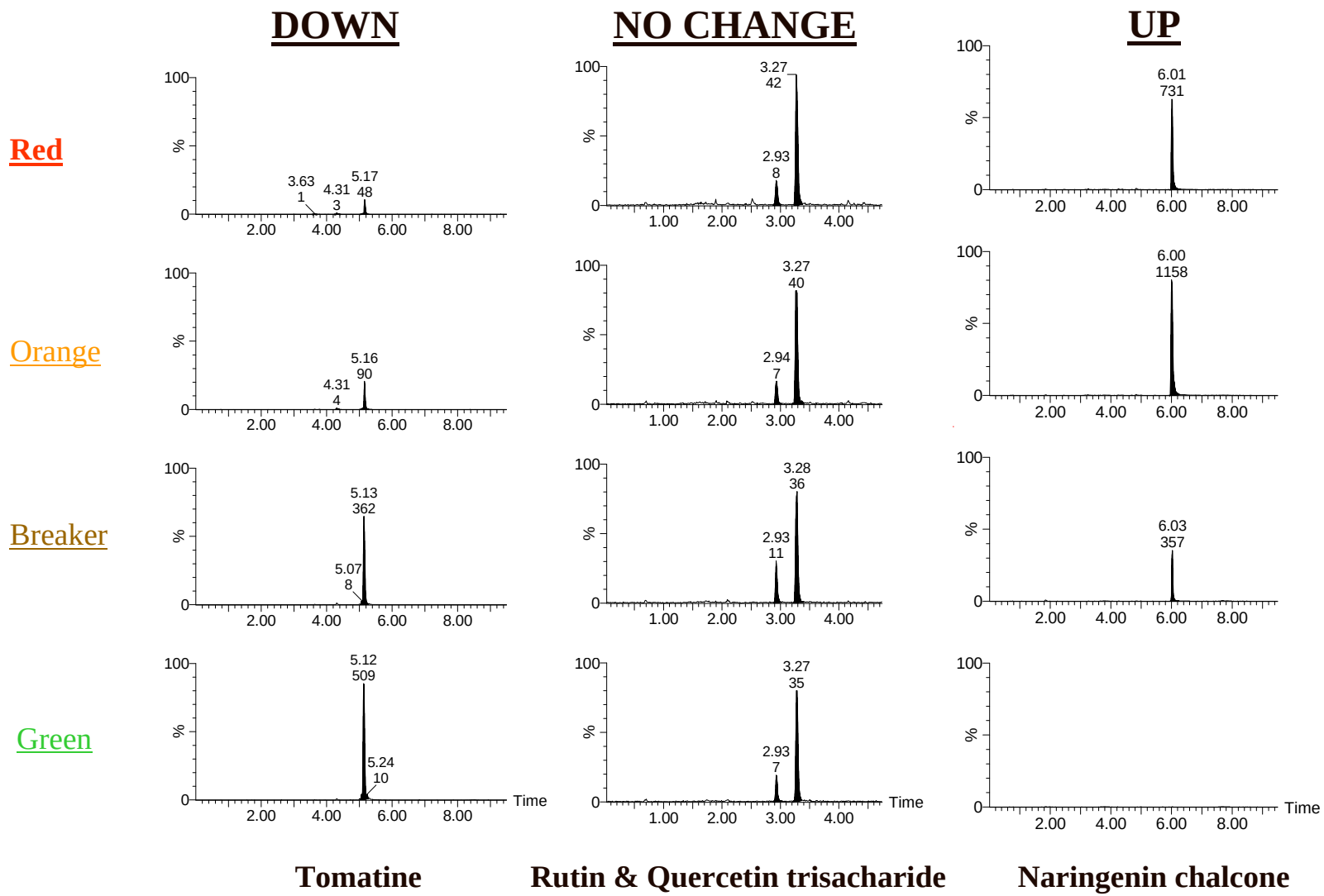


Peel-Flesh Profiles During Fruit Development

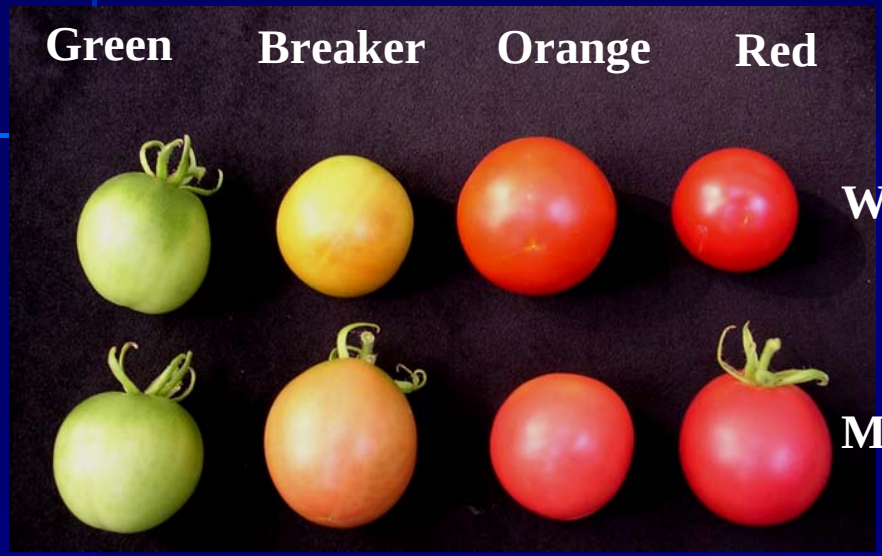


Wild-type Tomato Peel, Different Developmental Stages

Selected Ion Chromatograms

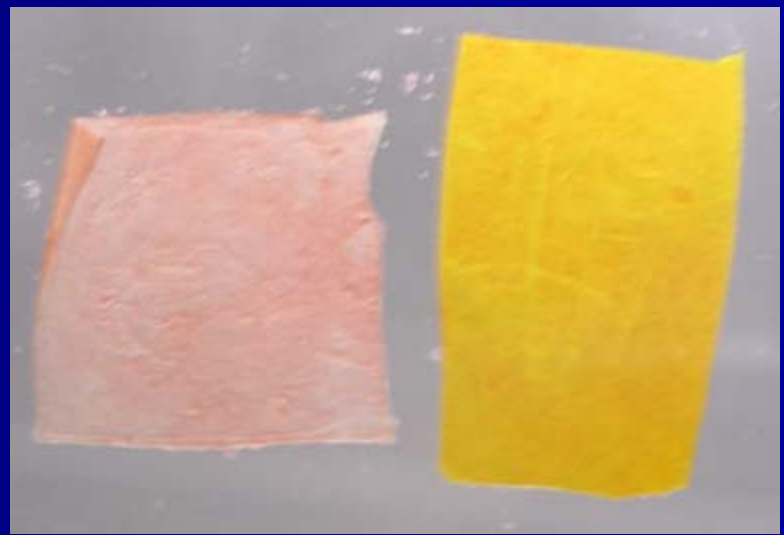


Tomato (var. Ailsa Craig) and the Mutant “Y”, at different ripening stages

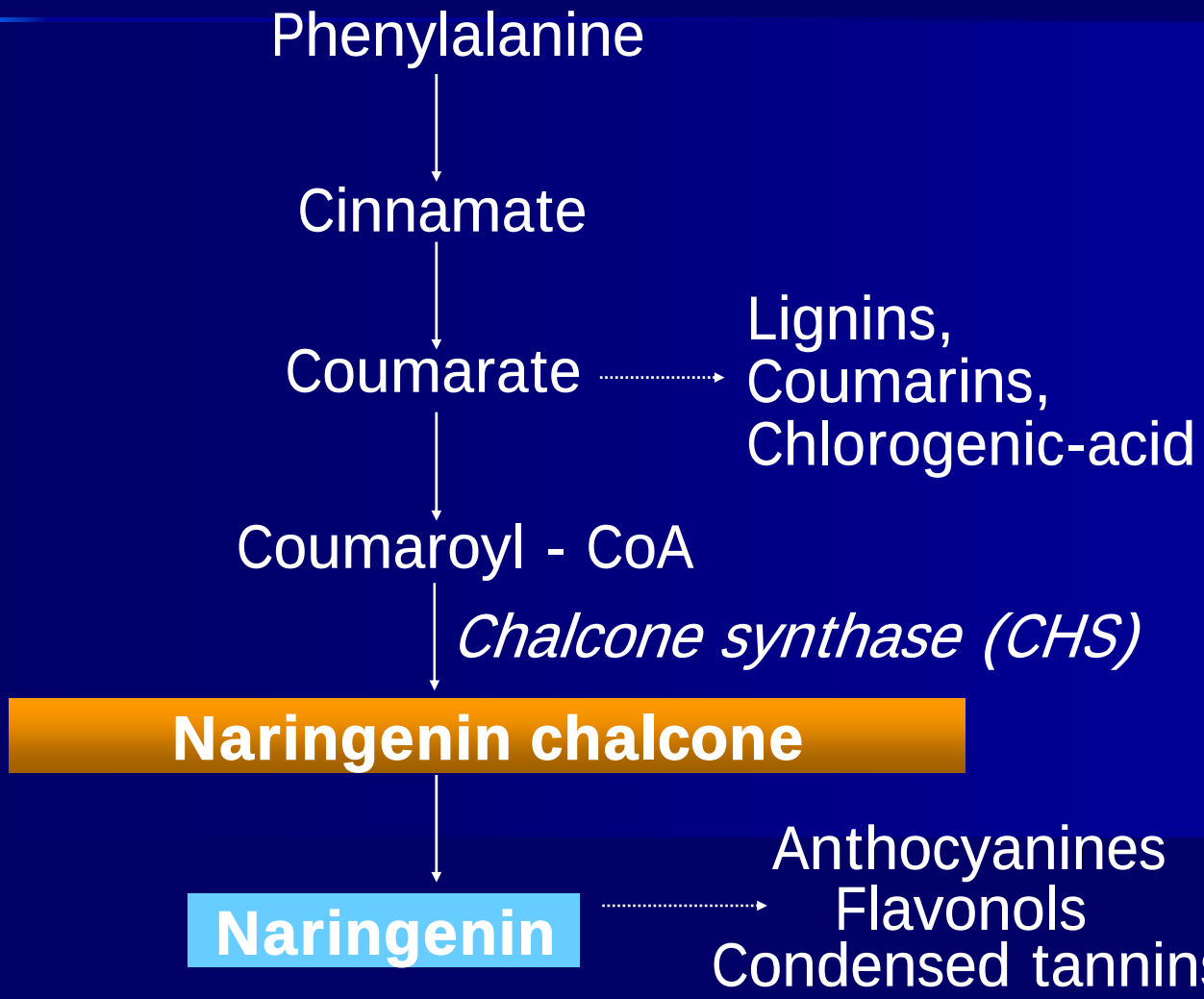


Tomato peel

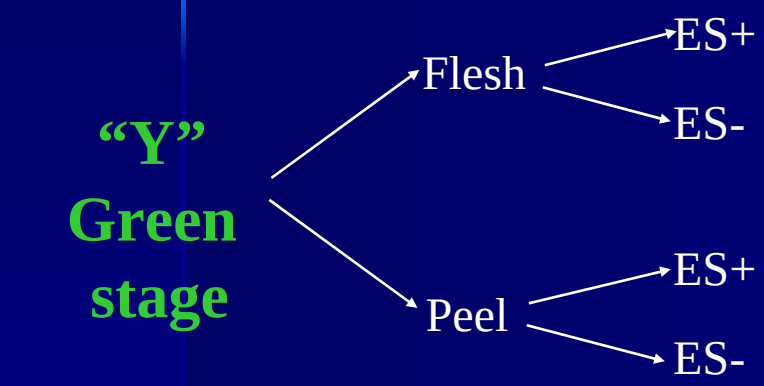
of Mutant “Y” and Wild-type

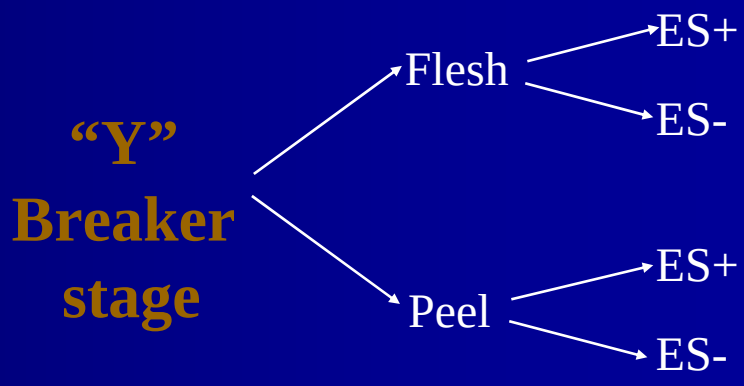


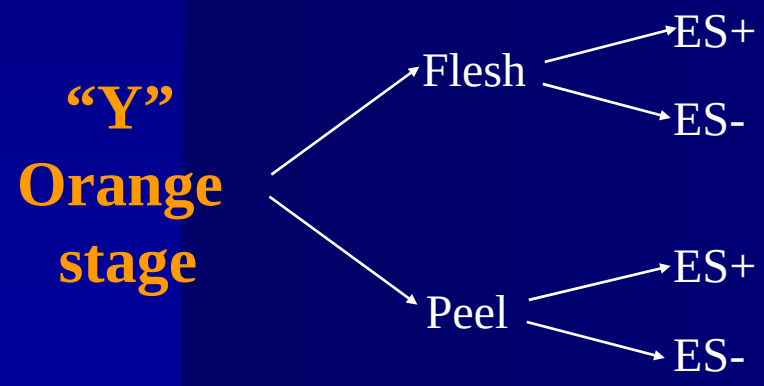
Biosynthesis of Naringenin Chalcone in the Flavonoid Pathway

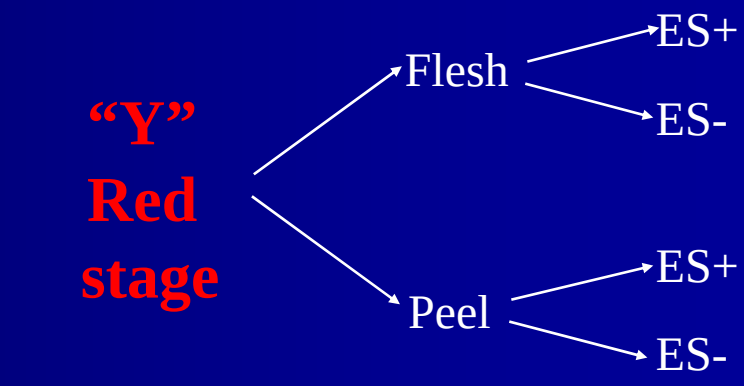


Experimental Set-Up for LC-MS Analysis: PEEL and FLESH during "Y" fruit development

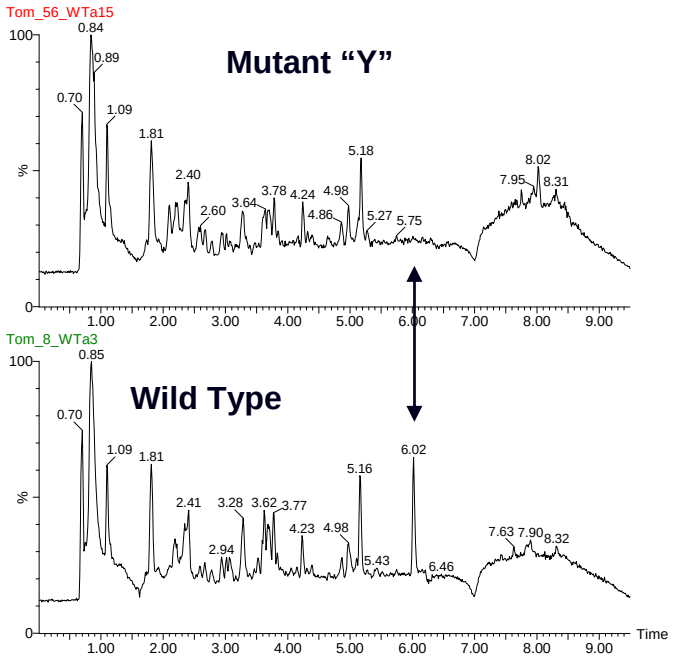




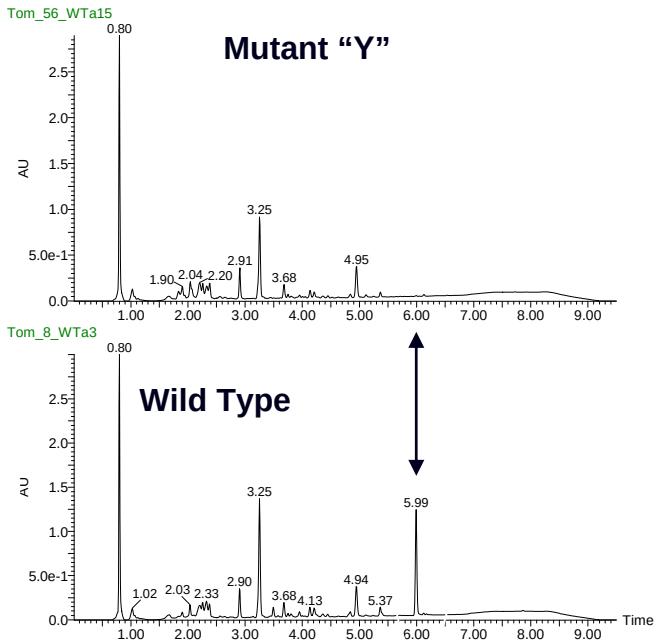




UV and MS Chromatograms of Red Tomato Peel (Wild Type and Mutant “Y”)

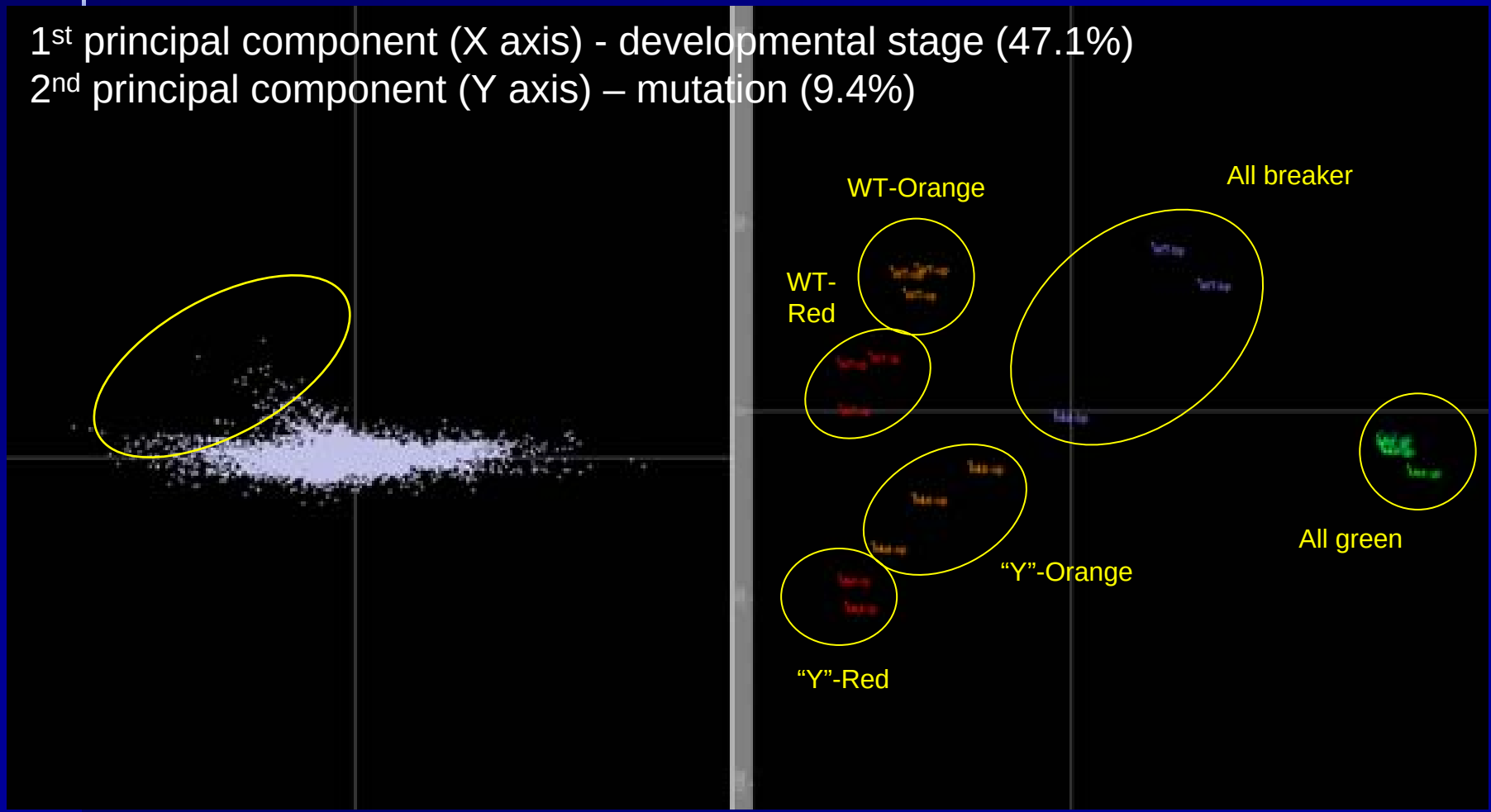


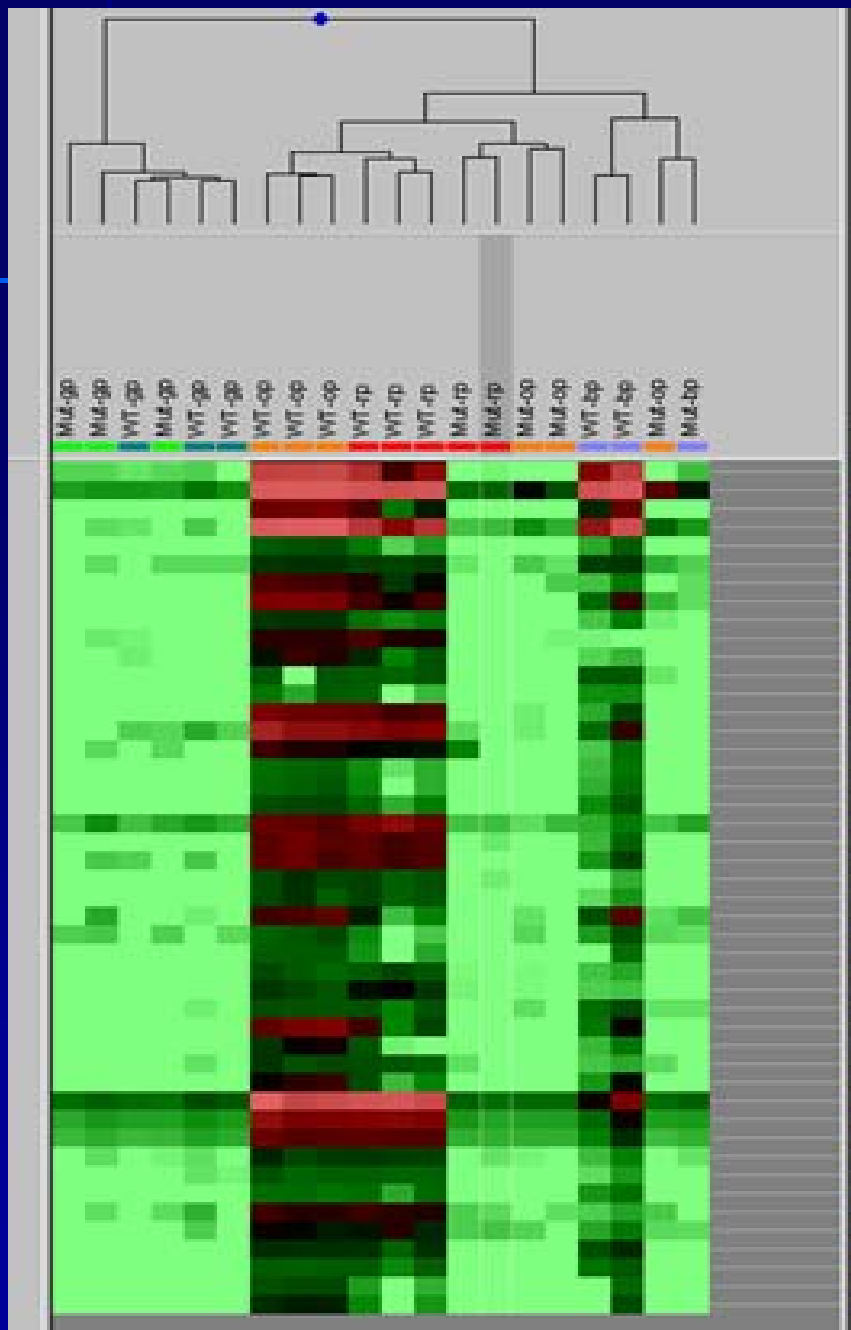
UV (240 nm)



MS (ES(+),TIC)

HPLC-QTOF-MS (Ultima-PRI) - Wild Type and “Y” Peels



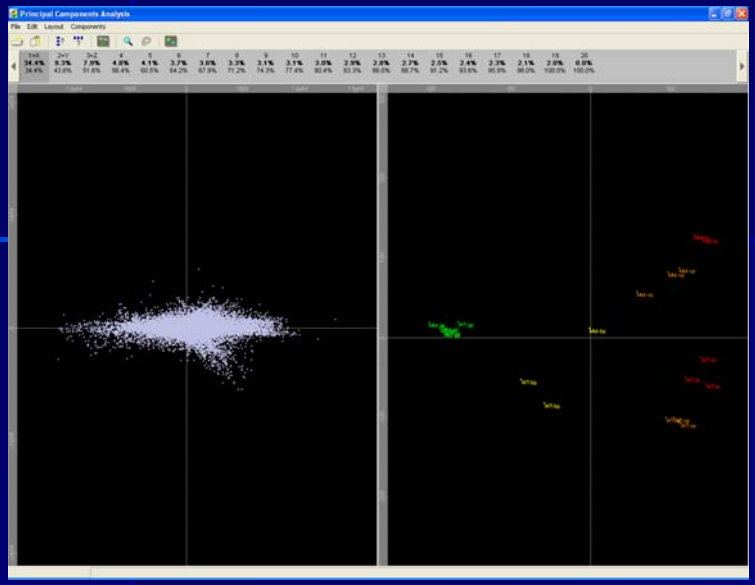


Post Metalign:
all WT > “Y” mass peaks

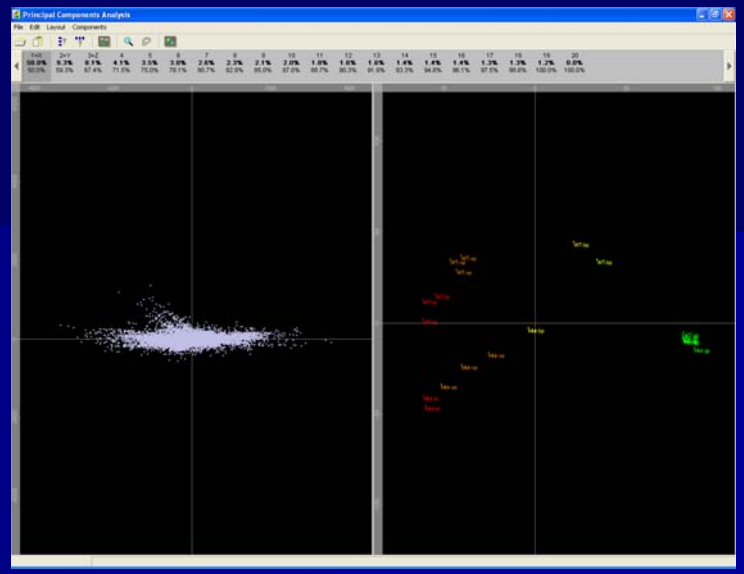
Scan # **RT in umin** **M/z**

	Gene	Label 1	Label 2	Label 3	Label 4
	1524	2136	42123857	273	273_2136
	1525	2164	42672615	273	273_2164
	1543	2135	42106201	274	274_2135
	1544	2164	42672615	274	274_2164
	1558	2136	42123857	275	275_2136
	1559	2163	42654800	275	275_2163
	1737	1816	35813958	289	289_1816
	1739	1879	37052834	289	289_1879
	1768	1880	37870568	290	290_1880
	1883	2202	43417133	303	303_2202
	1884	2232	44004135	303	303_2232
	3209	2163	42654800	368	368_2163
	3689	1322	26078516	388	388_1322
	3869	1969	21092934	394	394_1969
	3869	1995	21608500	394	394_1995
	3897	1995	21608500	395	395_1995
	4053	2135	42106201	409	409_2135
	4305	2136	42123857	411	411_2136
	4378	2136	42123857	413	413_2136
	4999	1455	28705234	425	425_1455
	5002	1548	30531433	425	425_1548
	5005	1695	33441433	425	425_1695
	5035	1567	30921450	426	426_1567
	5039	1696	33459133	426	426_1696
	5377	1722	33972618	447	447_1722
	5411	1721	33955002	448	448_1721
	8282	2160	42583935	546	546_2160
	8631	749	14793000	556	556_749
	8632	795	15697950	556	556_795
	8693	2164	42672615	564	564_2164
	9359	1230	24271433	580	580_1230
	9388	1230	24271433	581	581_1230
	9390	1287	25388149	581	581_1287
	9853	2135	42106201	598	598_2135
	9869	1286	25370234	599	599_1286
	9903	1286	25370234	600	600_1286
	10405	1286	25370234	621	621_1286
	10431	1286	25370234	622	622_1286
	10819	1284	25334957	637	637_1284
	12142	2164	42672615	700	700_2164
	13321	1012	19974051	761	761_1012
	14316	1287	25388149	836	836_1287
	14787	2164	42672615	870	870_2164
	14810	2164	42672615	871	871_2164
	18701	2161	42619465	1362	1362_2161
	19258	2161	42619465	1498	1498_2161

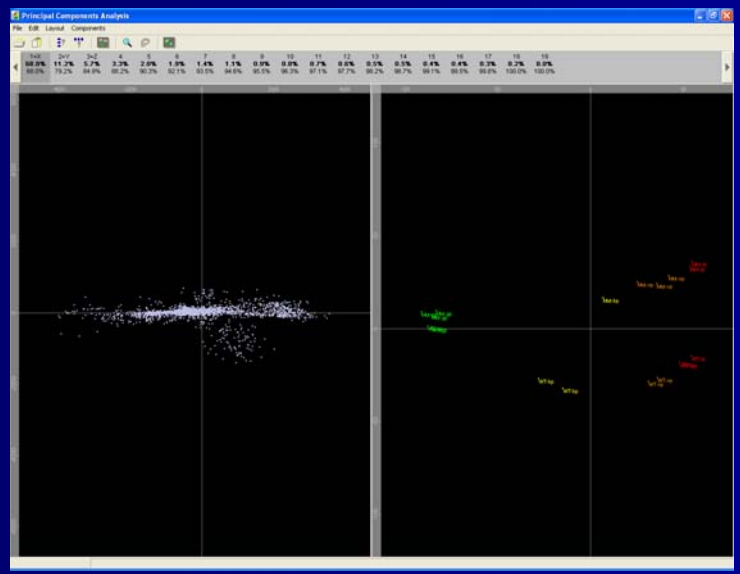
HPLC-qTOF -MarkerLynx



HPLC-qTOF-MetAlign

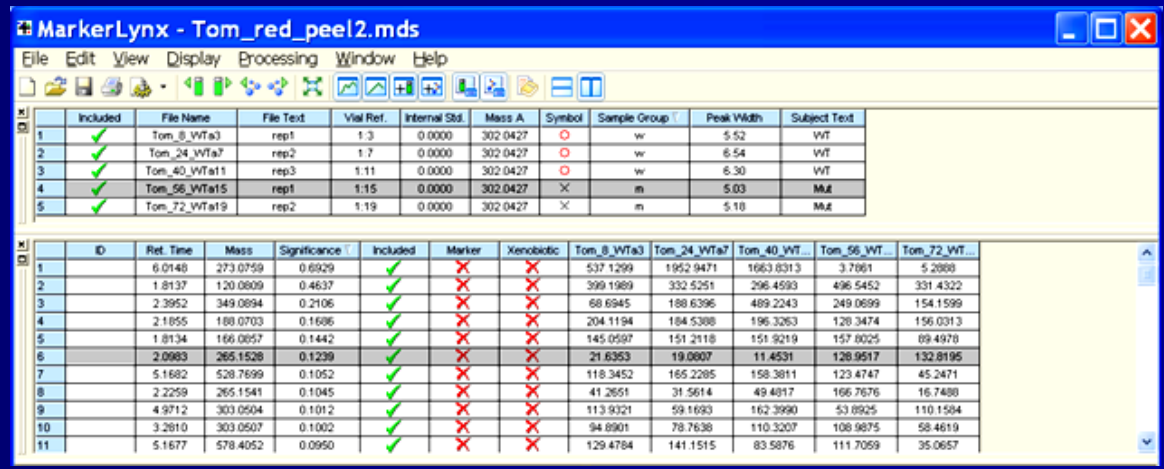


HPLC-qTOF-XCMS



In-depth Analysis of Red Tomato Peel (ES+) Wild-type vs. the “Y” Mutant

MarkerLynx
Differential Markers

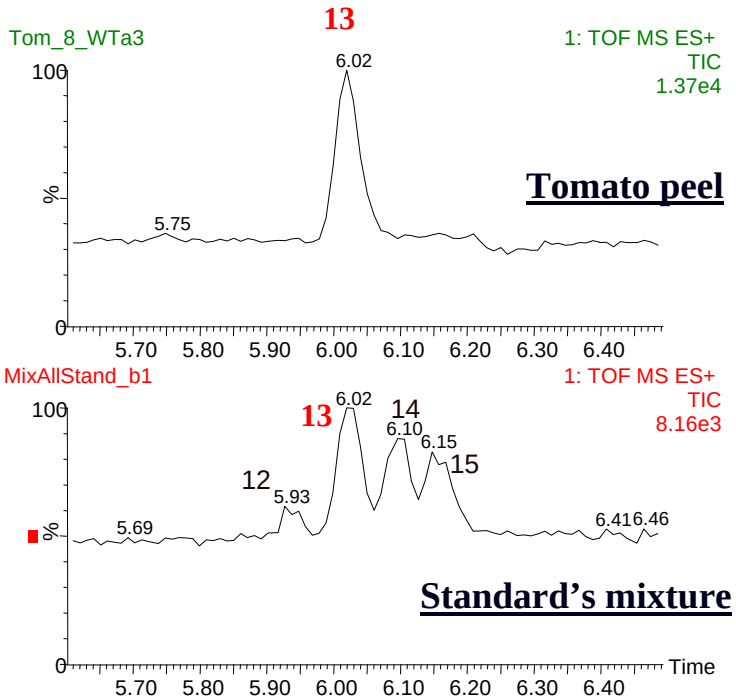


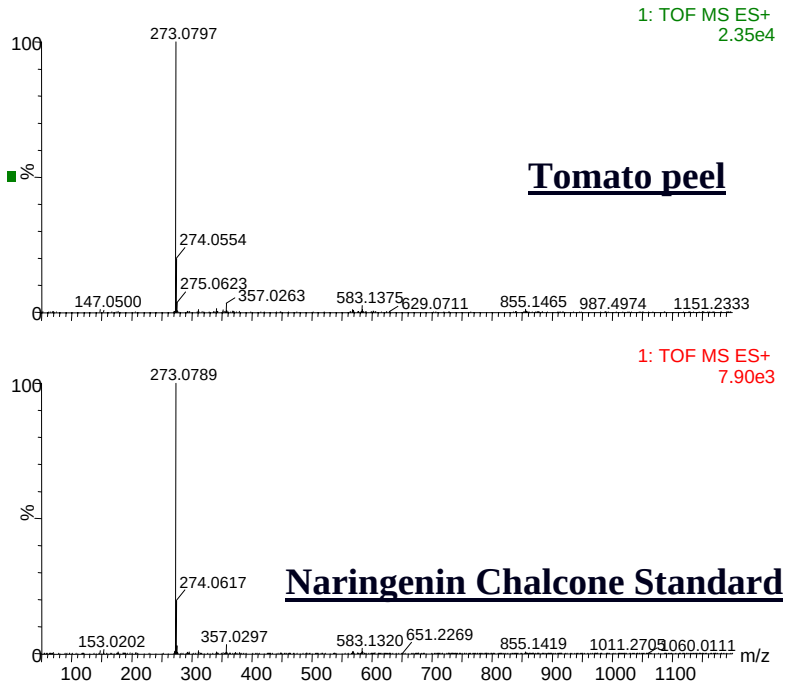
	Included	File Name	File Text	Vial Ref.	Internal Std.	Mass A	Symbol	Sample Group	Peak Width	Subject Text
1	✓	Tom_8_WTa3	rep1	1:3	0.0000	302.0427	○	w	5.52	WT
2	✓	Tom_24_WTa7	rep2	1:7	0.0000	302.0427	○	w	6.54	WT
3	✓	Tom_40_WTa11	rep3	1:11	0.0000	302.0427	○	w	6.30	WT
4	✓	Tom_56_WTa15	rep1	1:15	0.0000	302.0427	×	m	5.03	Mut
5	✓	Tom_72_WTa19	rep2	1:19	0.0000	302.0427	×	m	5.18	Mut

	ID	Ret. Time	Mass	Significance	Included	Marker	Xenobiotic	Tom_8_WTa3	Tom_24_WTa7	Tom_40_WT...	Tom_56_WT...	Tom_72_WT...
1		6.0148	273.0759	0.6929	✓	×	×	537.1299	1952.9471	1663.8313	3.7861	5.2888
2		1.8137	120.0809	0.4637	✓	×	×	399.1989	332.5251	296.4593	496.5452	331.4322
3		2.3952	349.0894	0.2106	✓	×	×	68.6945	188.6396	489.2243	249.0899	154.1599
4		2.1855	188.0703	0.1686	✓	×	×	204.1194	184.5388	196.3263	128.3474	156.0313
5		1.8134	166.0657	0.1442	✓	×	×	145.0597	151.2118	151.9219	157.8025	69.4978
6		2.0983	265.1528	0.1239	✓	×	×	21.6353	19.0807	11.4531	128.9517	132.8195
7		5.1682	528.7699	0.1052	✓	×	×	118.3452	165.2285	158.3811	123.4747	45.2471
8		2.2259	265.1541	0.1045	✓	×	×	41.2651	31.5614	49.4817	166.7676	16.7488
9		4.9712	303.0504	0.1012	✓	×	×	113.9321	59.1693	162.3990	53.8925	110.1584
10		3.2610	303.0507	0.1002	✓	×	×	94.8901	78.7638	110.3207	108.9875	58.4619
11		5.1677	578.4052	0.0950	✓	×	×	129.4784	141.1515	83.5876	111.7059	35.0657

Filtering in MS Excel – Matlab and subsequently peak analysis by eye

The Yellow Pigment Absent in the “Y” Mutant Fruit Peel is Naringenin Chalcone

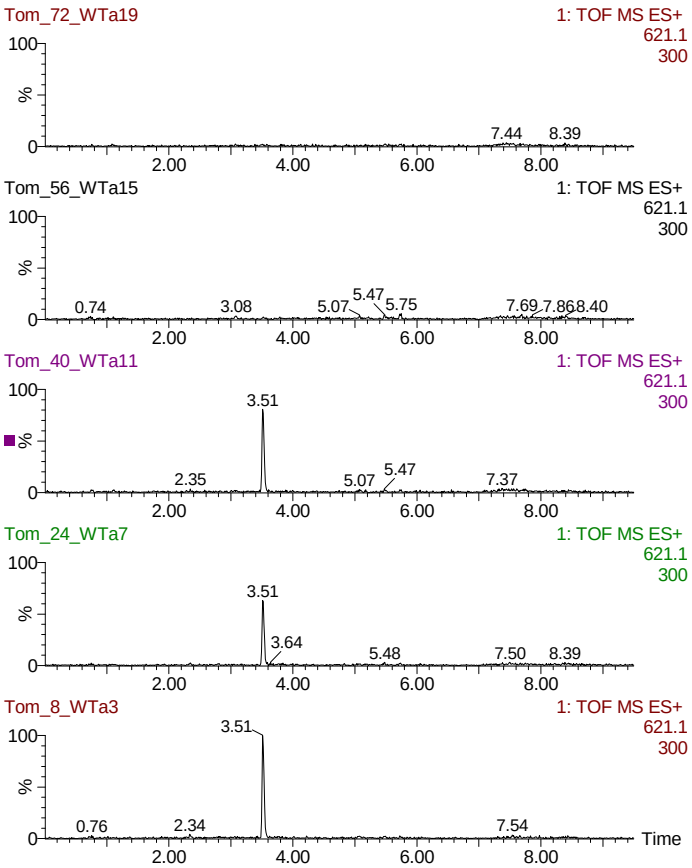




12 – Cinnamic acid
13 – Naringenin Chalcone
14 – Naringenin
15 – Kaempferol

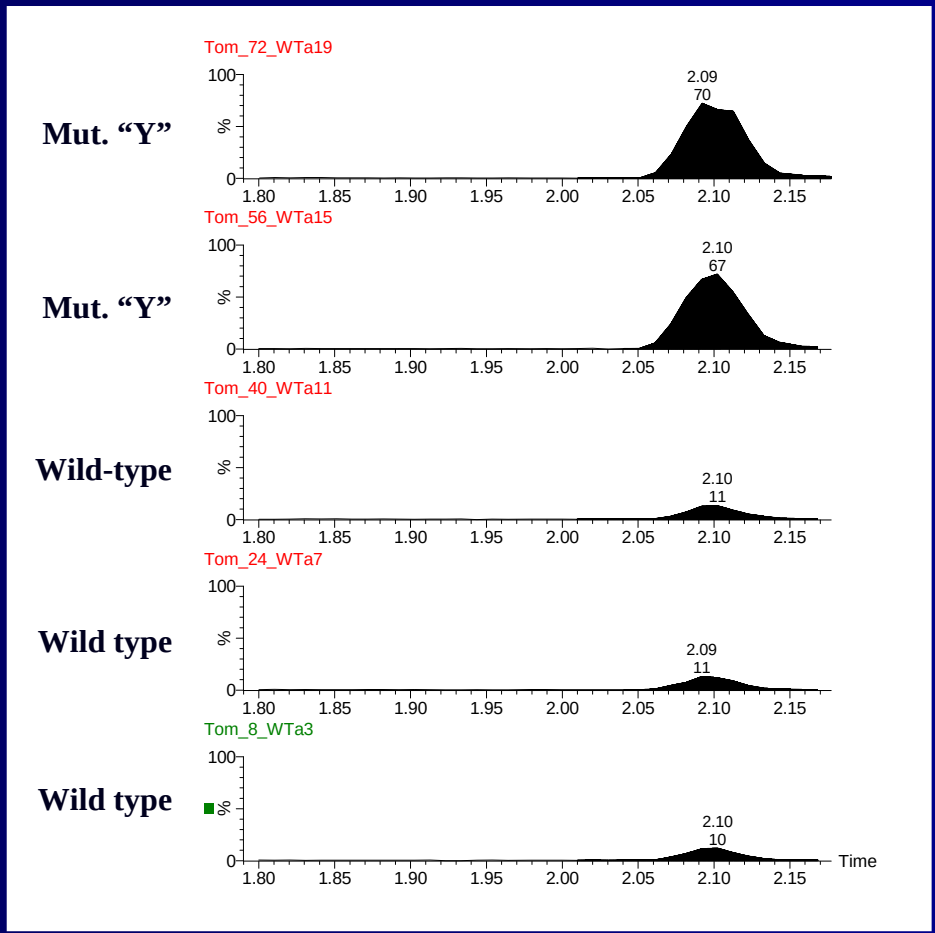
Looking for a known compound

Unknown Mass peak (RT=3.51 min) Detected Only in Wild Type



Extracted mass chromatogram at m/z 621.1 Da

Unknown Mass peak Detected in Higher Levels in “Y”



Extracted mass chromatogram at m/z 265.1 Da

What are the Differentially Expressed Metabolites between WT and “Y” ?

Peak assignment by:

- Accurate mass and elemental composition calculation (natural products database)
- MS-MS fragmentation
- UV spectrum
- Literature
- UV & MS spectra databases
- Isotope pattern

Differential Metabolites between the "Y" and Wild Type Peel UPLC-QTOF-MS in ES- Mode

	Differential metabolites (ES-)	
	WT > "Y"	"Y" > WT
R only	10	11
O only	12	16
Both R & O	26	4
Total	48	31

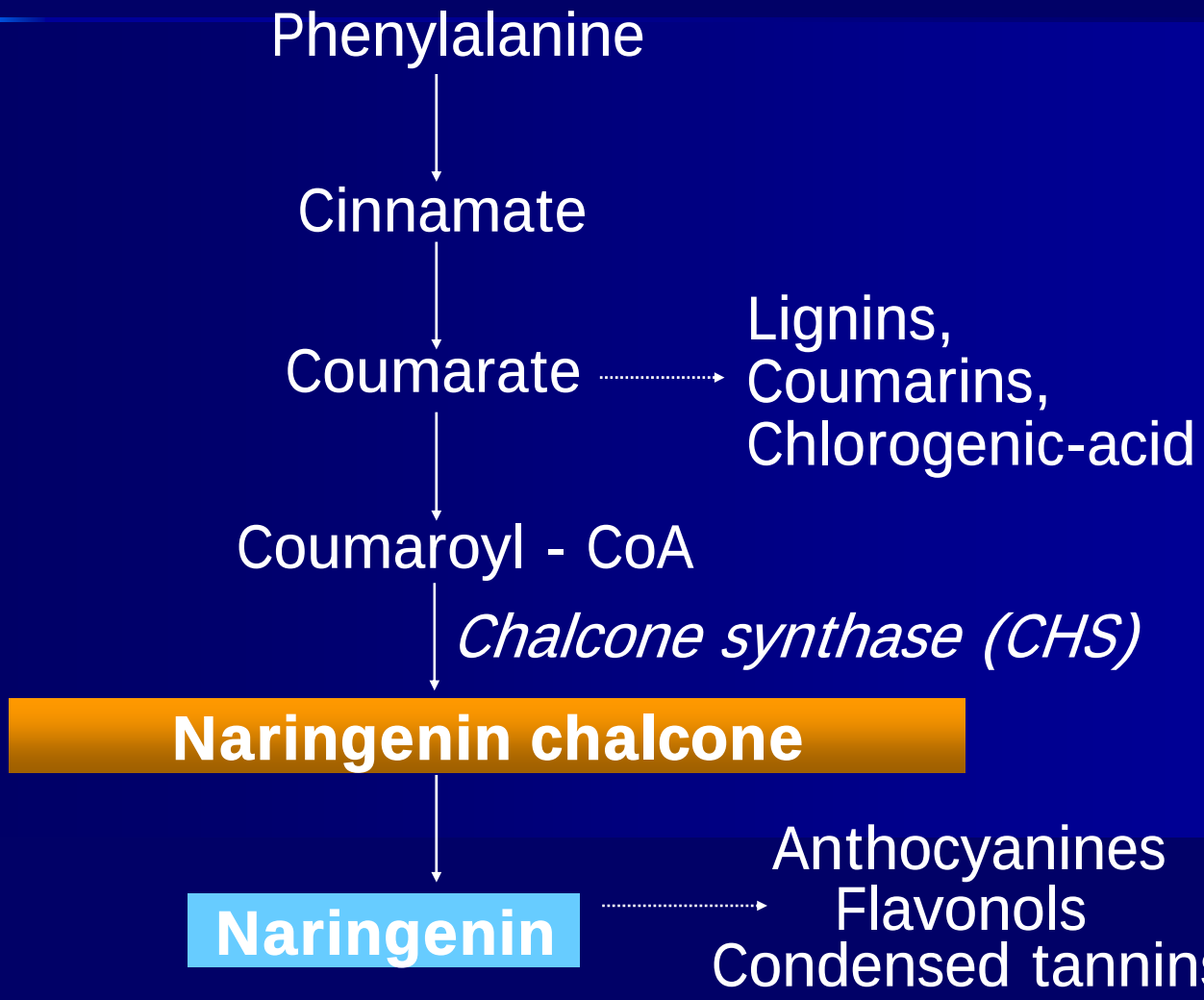
Differential Metabolites between the "Y" and Wild Type Peel UPLC-QTOF-MS in ES+ Mode

	Differential metabolites (ES+)	
	WT >"Y"	"Y" > WT
R only	3	2
O only	0	2
Both R & O	16	8
Total	19	12

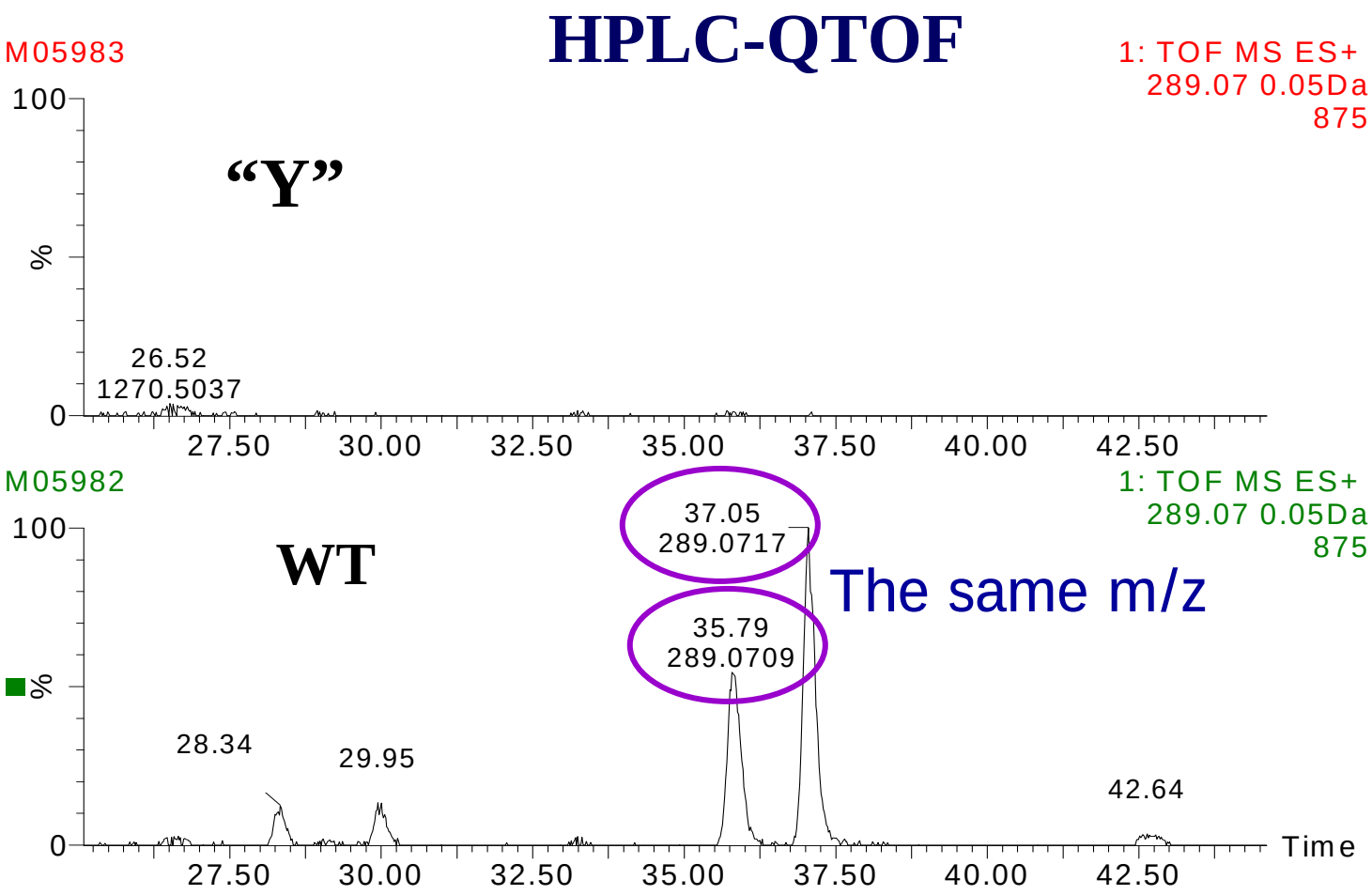
What are the Differentially Expressed Metabolites between WT and “Y” ?

Most mass peaks showing higher levels in WT than in the “Y” mutant are derivatives of Naringenin Chalcone or Naringenin.

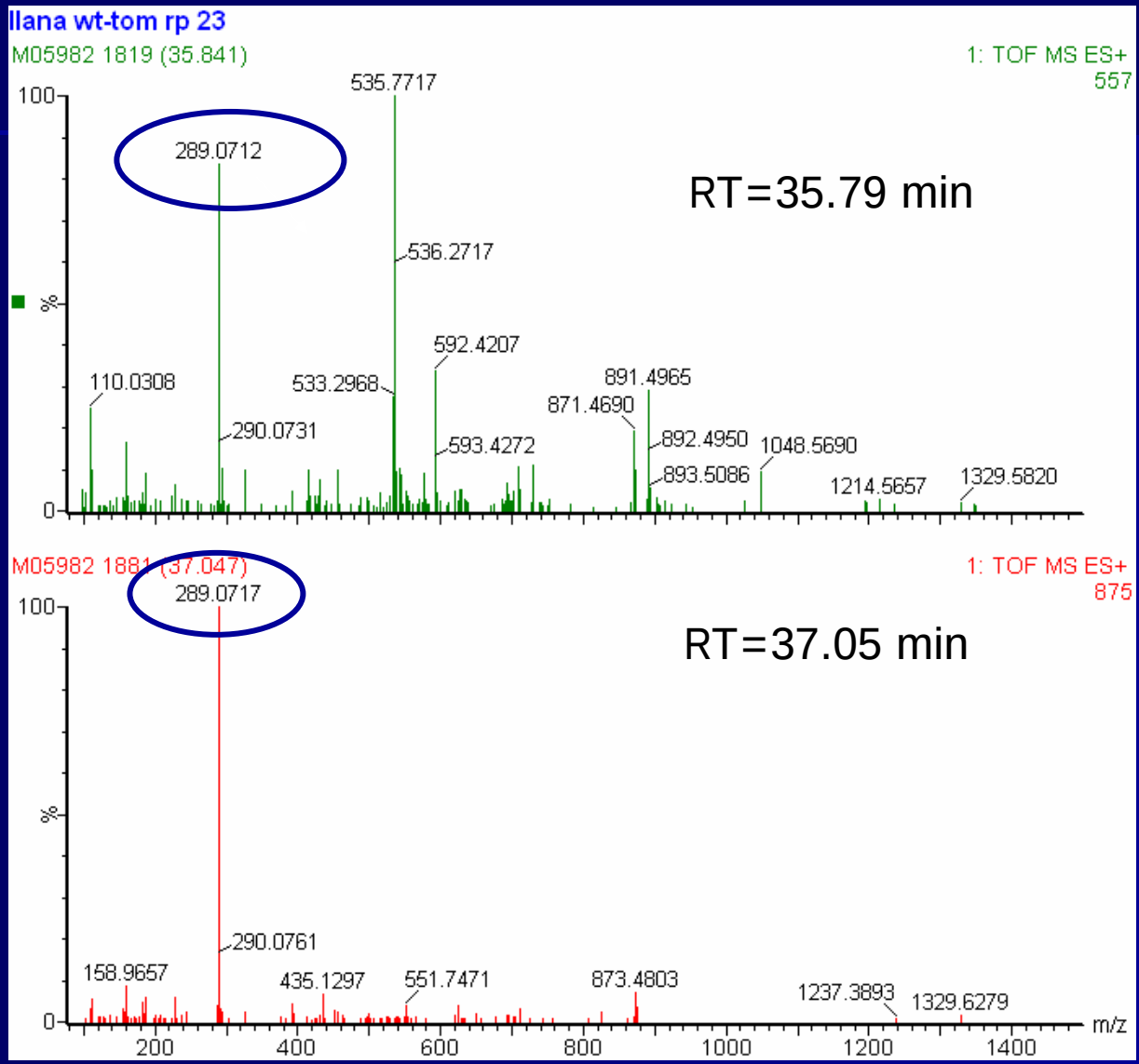
Biosynthesis of Naringenin Chalcone in the Flavonoid Pathway



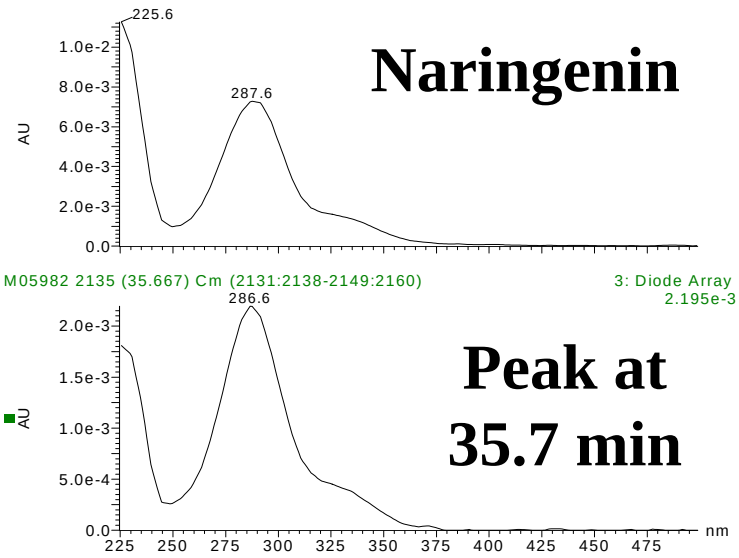
Hydroxylated Naringenin & Naringenin- Chalcone

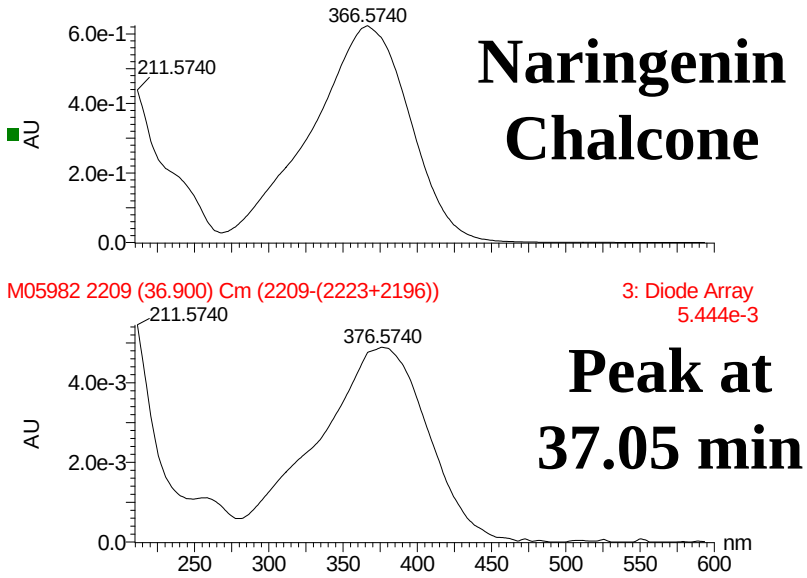


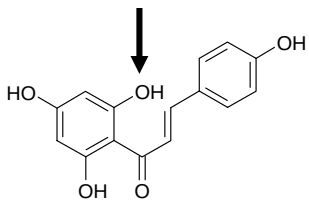
Spectra of differential compounds at RT=35.79 min and 37.05 min



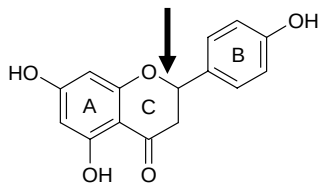
UV Spectra of Differential Peaks (35.7 min. and 37.05 min.)





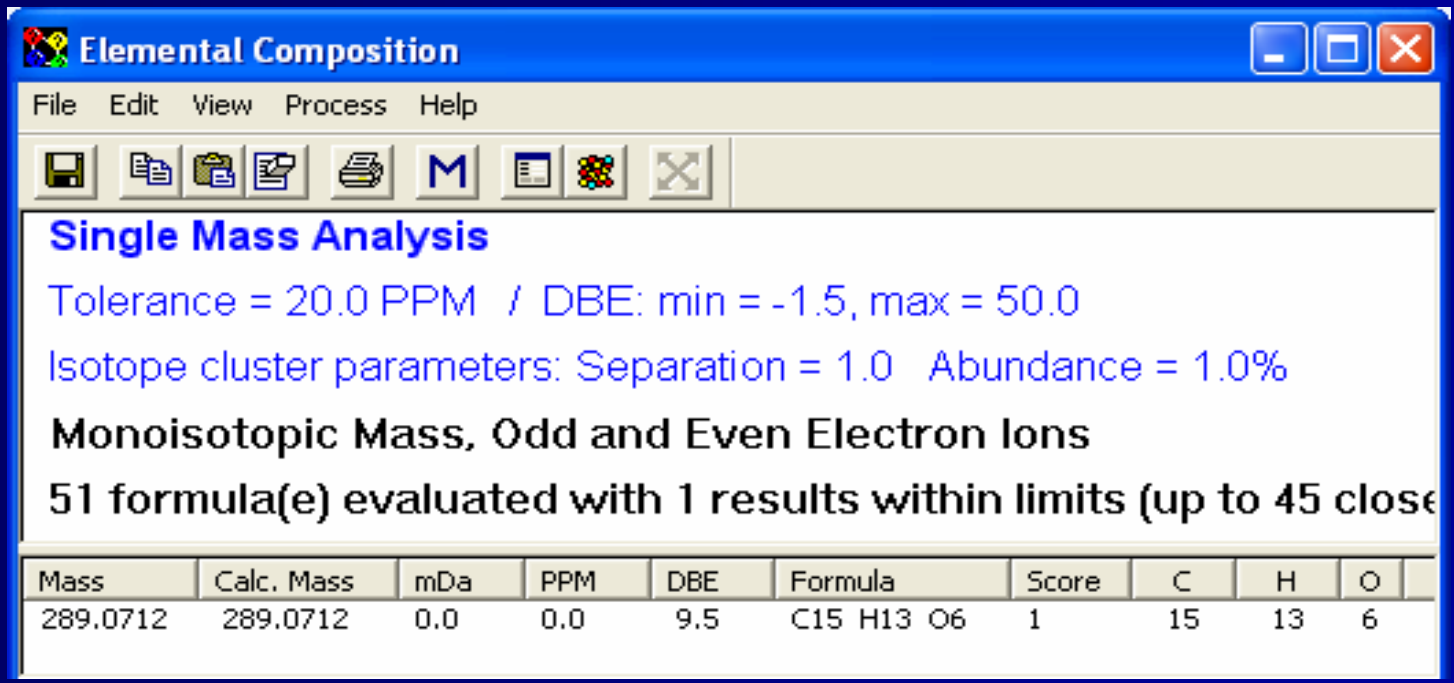


Naringenin Chalcone



Naringenin

Elemental Composition (289.0712)

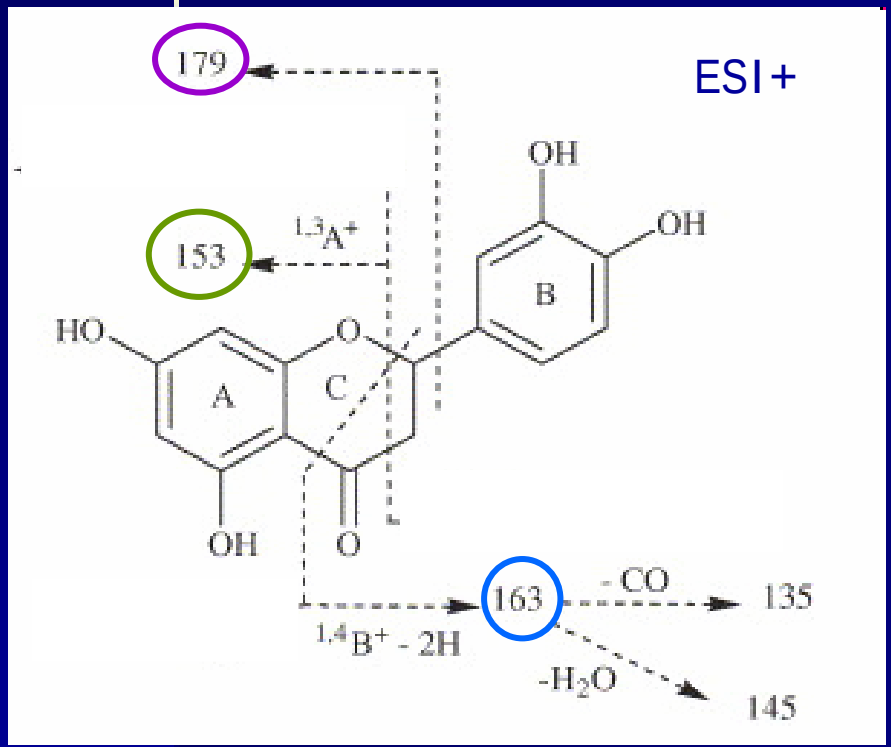


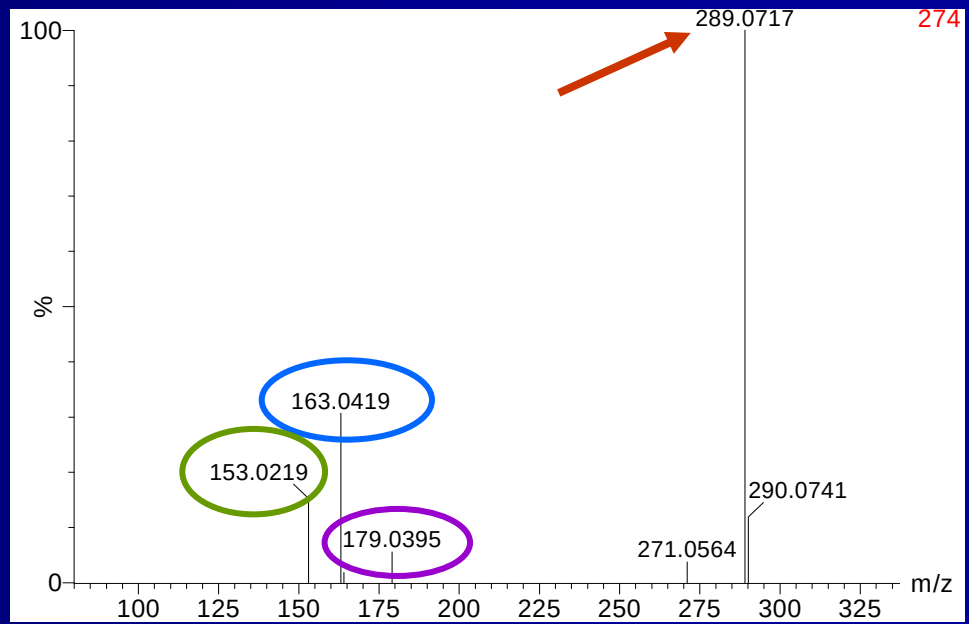
Mass	Calc. Mass	mDa	PPM	DBE	Formula	Score	C	H	O
289.0712	289.0712	0.0	0.0	9.5	C15 H13 O6	1	15	13	6

Elemental composition: $C_{15}H_{12}O_6$ for neutral compound
Elemental composition of Naringenin: $C_{15}H_{12}O_5$
Difference: OH group instead of H

Suggested Metabolite

MS-MS Fragmentation of m/z 289.07





MS-MS (ES+) fragmentation of m/z 289.07

Eriodictyol an Hydroxylated Naringenin ($C_{15}H_{12}O_6$)

Main fragmentations in reference:

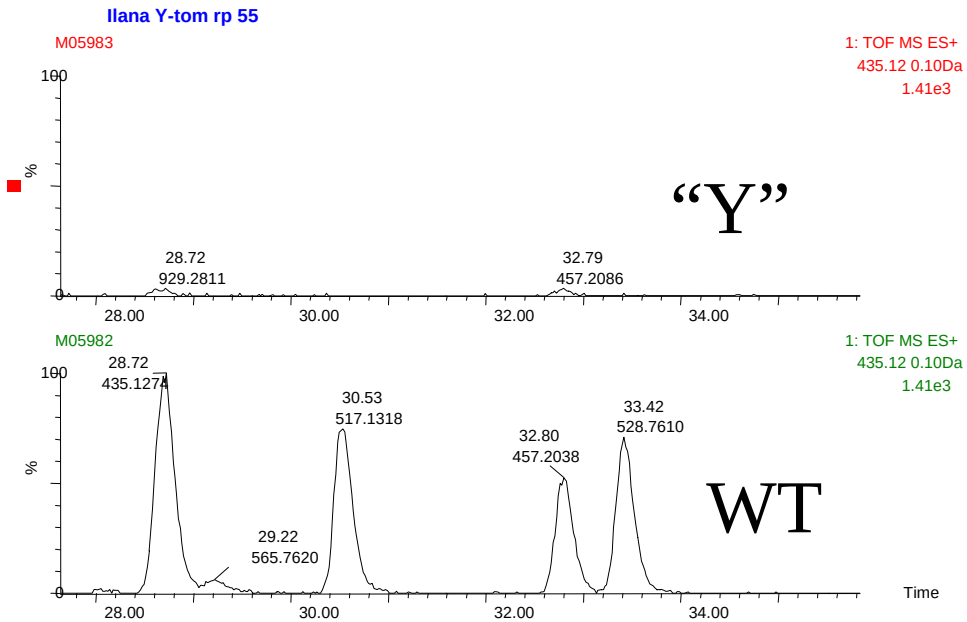
I. J. M. S. 247 (2005) 93–100

Injection of Eriodictyol standard or NMR

Naringenin and Naringenin Chalcone Glycosides

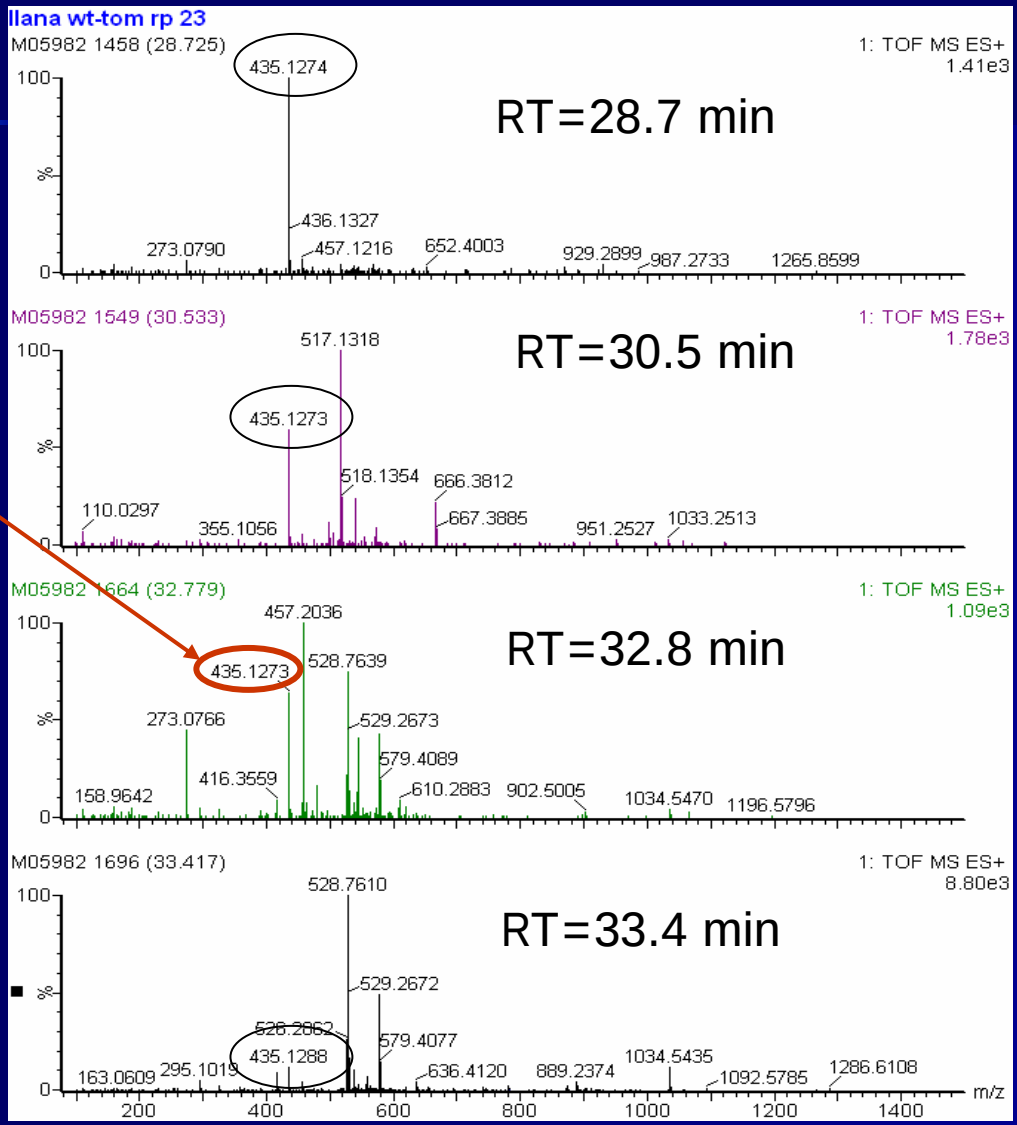
HPLC-QTOF

Extracted Ion Chromatograms at 435.12 Da



MS spectra at RT=28, 30 32 and 33 min

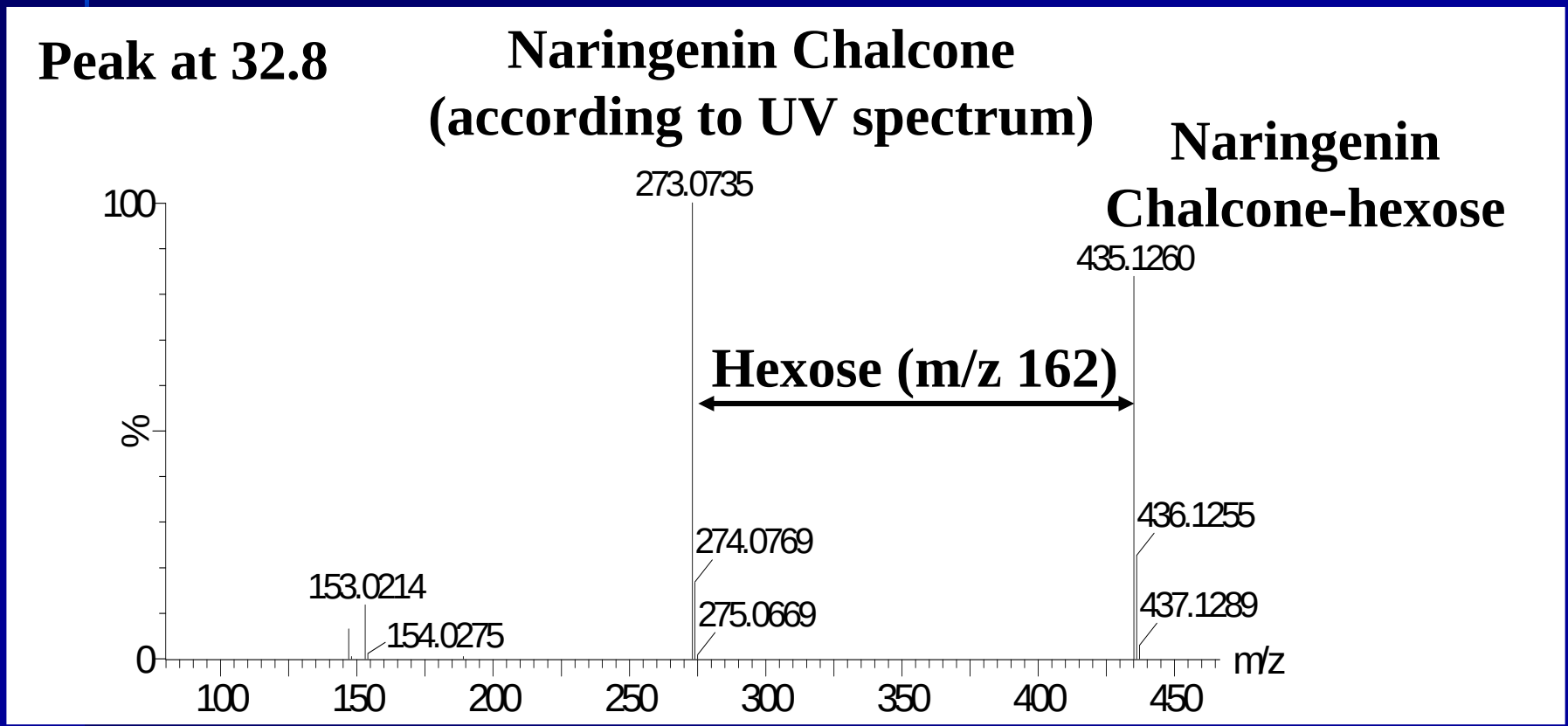
For MS-MS



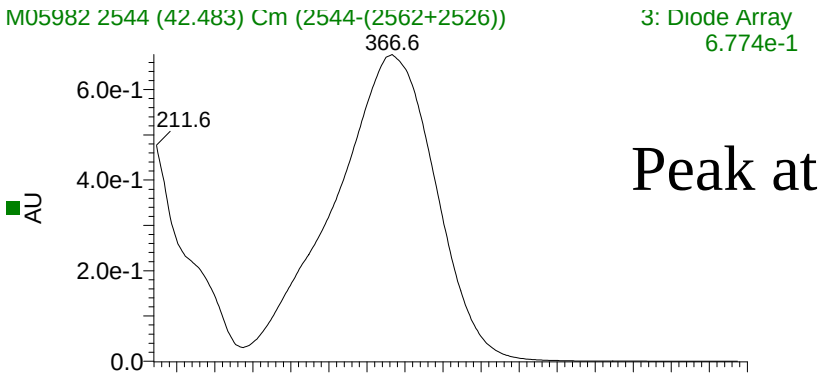
Exact mass of 435.1273 shows elemental composition of $C_{21}H_{22}O_{10}$

m/z 435.1273 is an hexose derivative of Naringenin or NarChalc

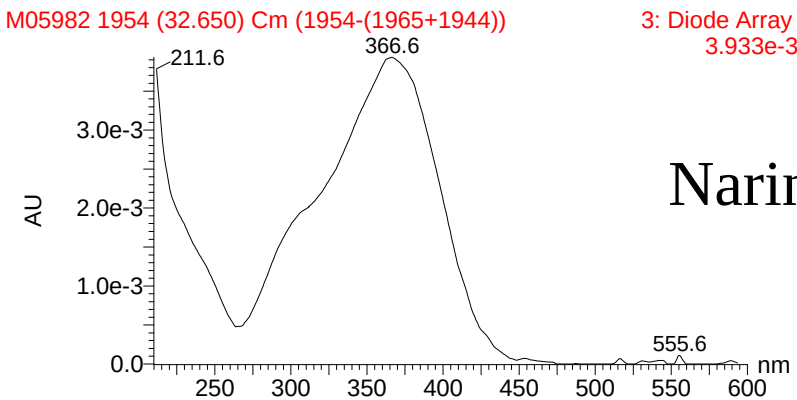
MS-MS Fragmentation of m/z 435.12 for Peak at RT=32.8



UV Spectra of Peak at 32.8 min. and Naringenin Chalcone



Peak at 32.8 min



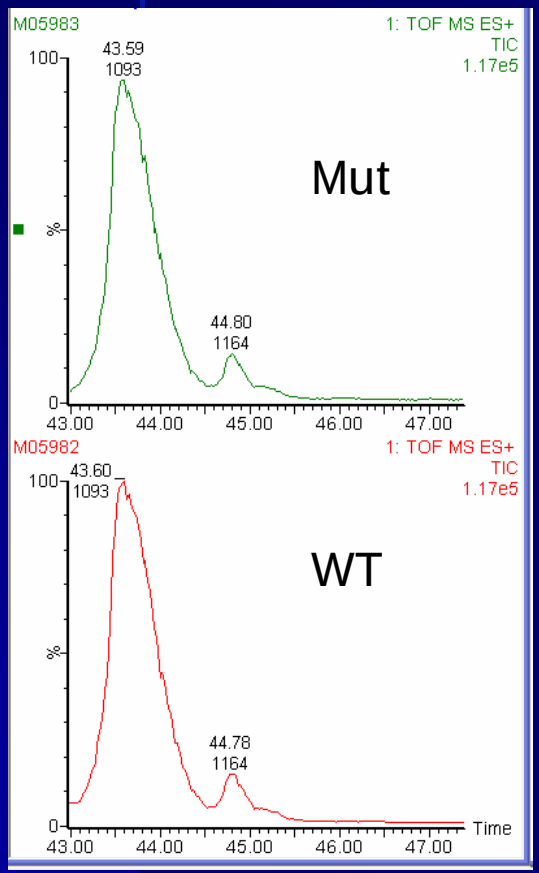
Naringenin Chalcone

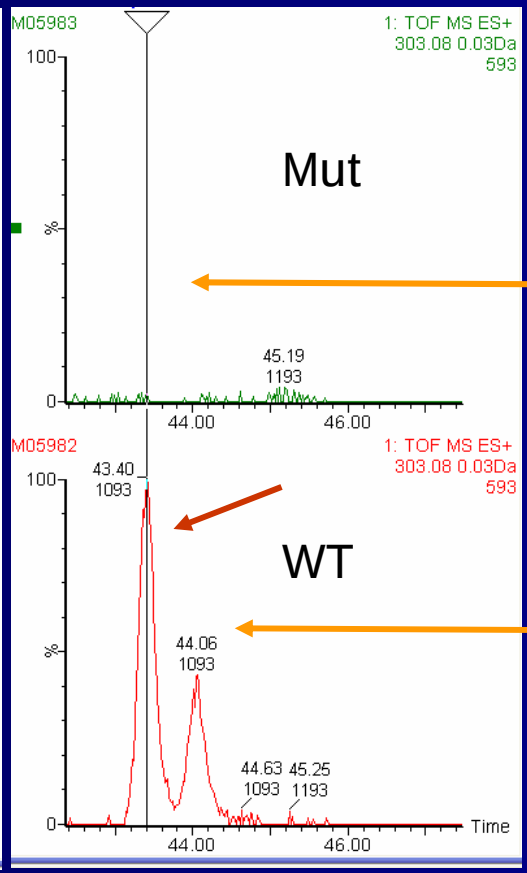
Methyl ether of Hydroxylated Naringenin and Naringenin Chalcone

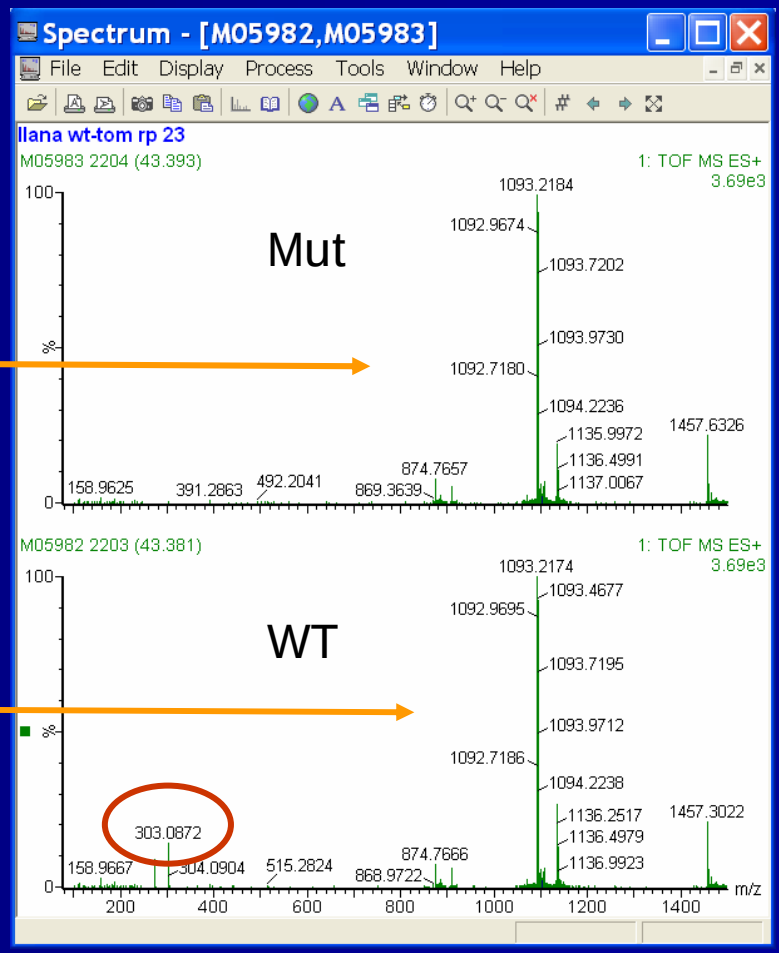
TIC chromatogram

Extracted Ion Chromatogram m/z 303.08 Da

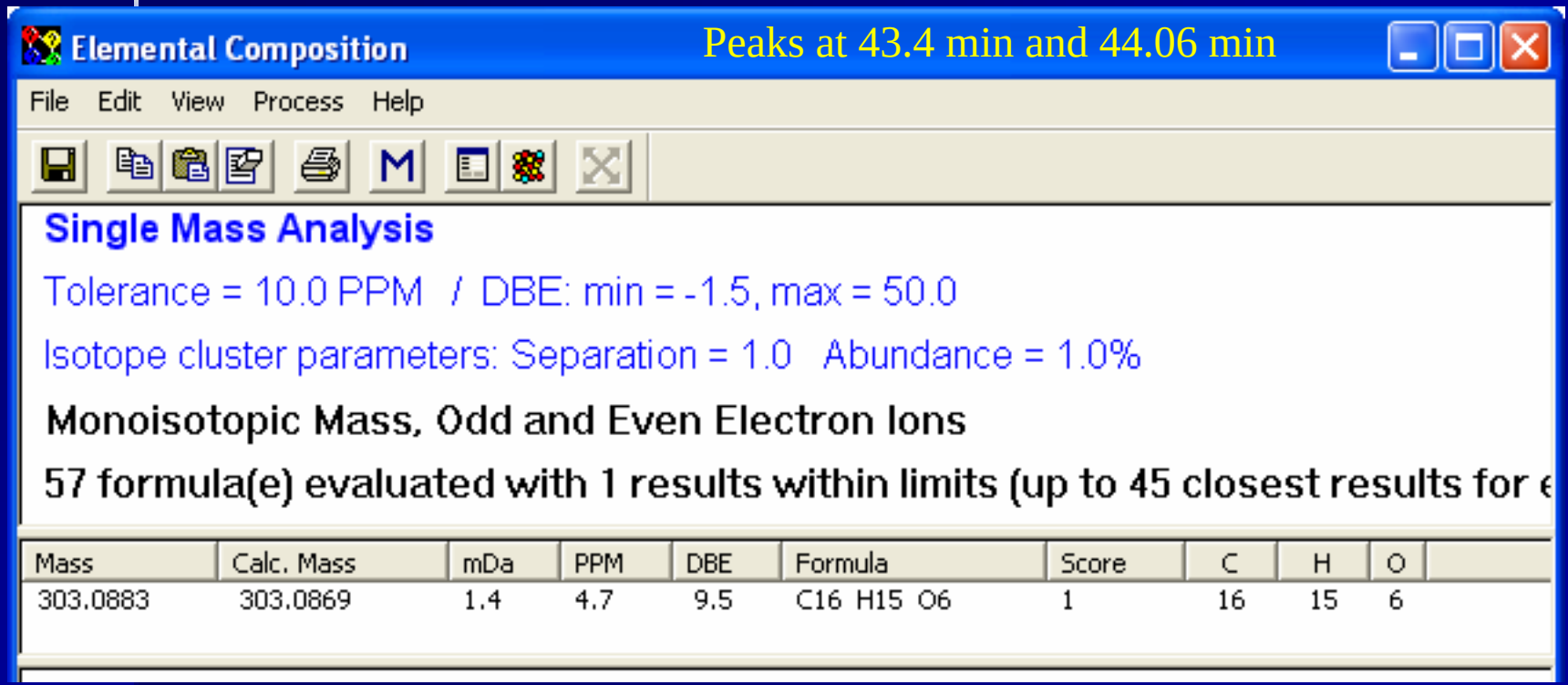
MS spectra at RT=43.40min







Methyl ether of Hydroxylated Naringenin and Naringenin Chalcone

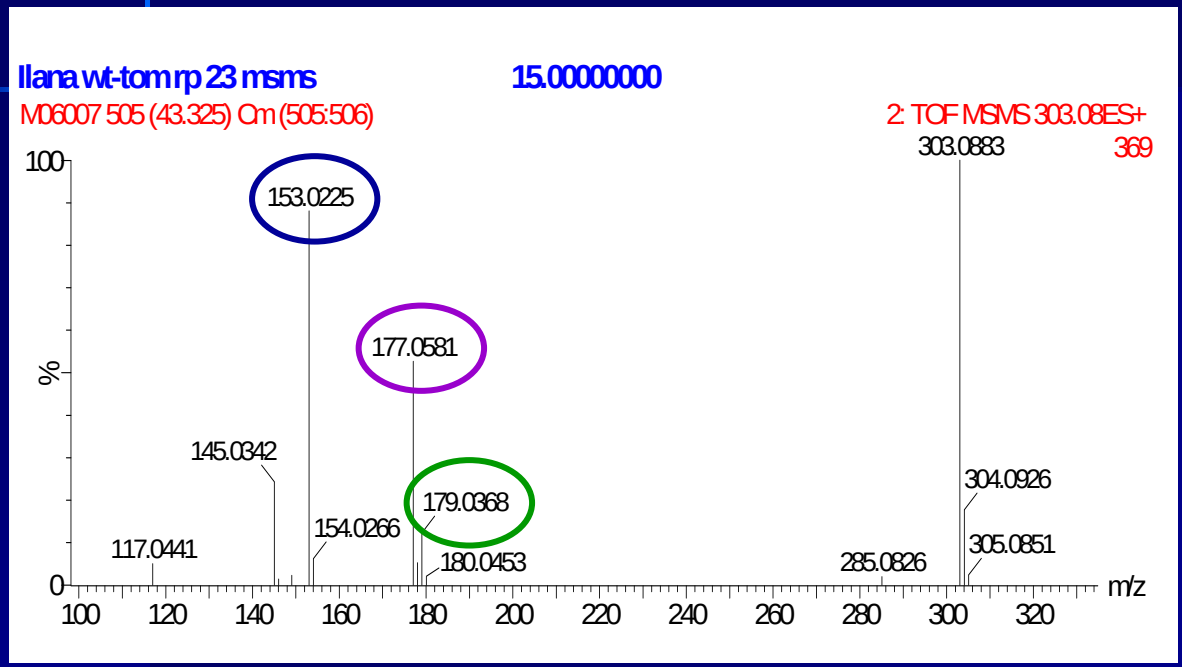


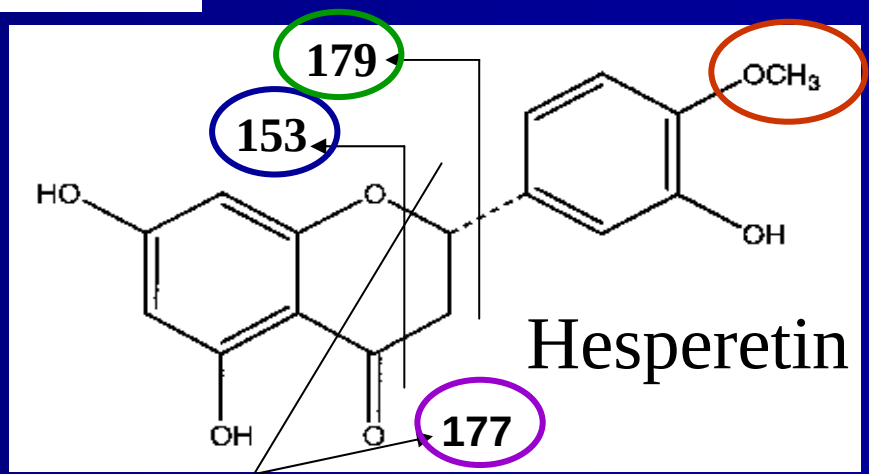
Calculated formula is $C_{16}H_{14}O_6$ based on exact mass

Proposed structure for the peak at 43.4 min: Hesperetin or a B-ring positional isomer

Hesperetin is a Methoxylated Naringnein

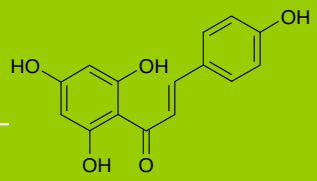
MS-MS Fragmentation of m/z 303.08 (43.4 min.)



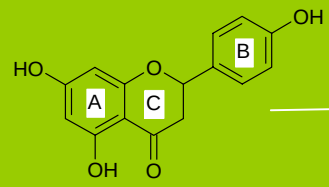


Proposed Scheme of Naringenin & Naringenin Chalcone Hydroxylation, Methylation and Glycosylation

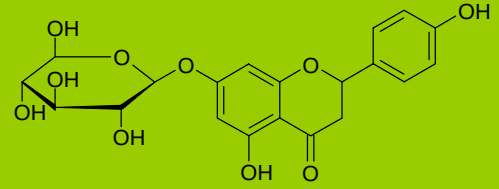
Glycosylation



Naringenin Chalcone

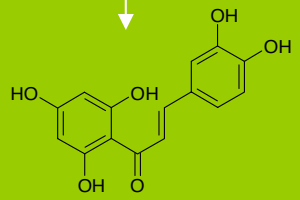


Naringenin

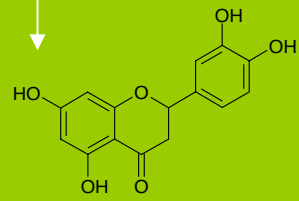


Glycosylation
e.g. Naringenin glucoside

Glycosylation



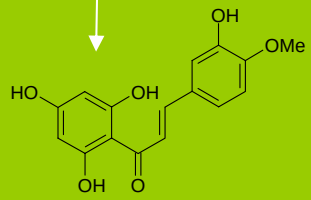
Hydroxylated Naringenin Chalcone



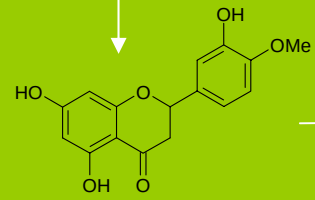
Glycosylation

Hydroxylated Naringenin
e.g. Eriodyctyol or a B-ring isomer

Glycosylation



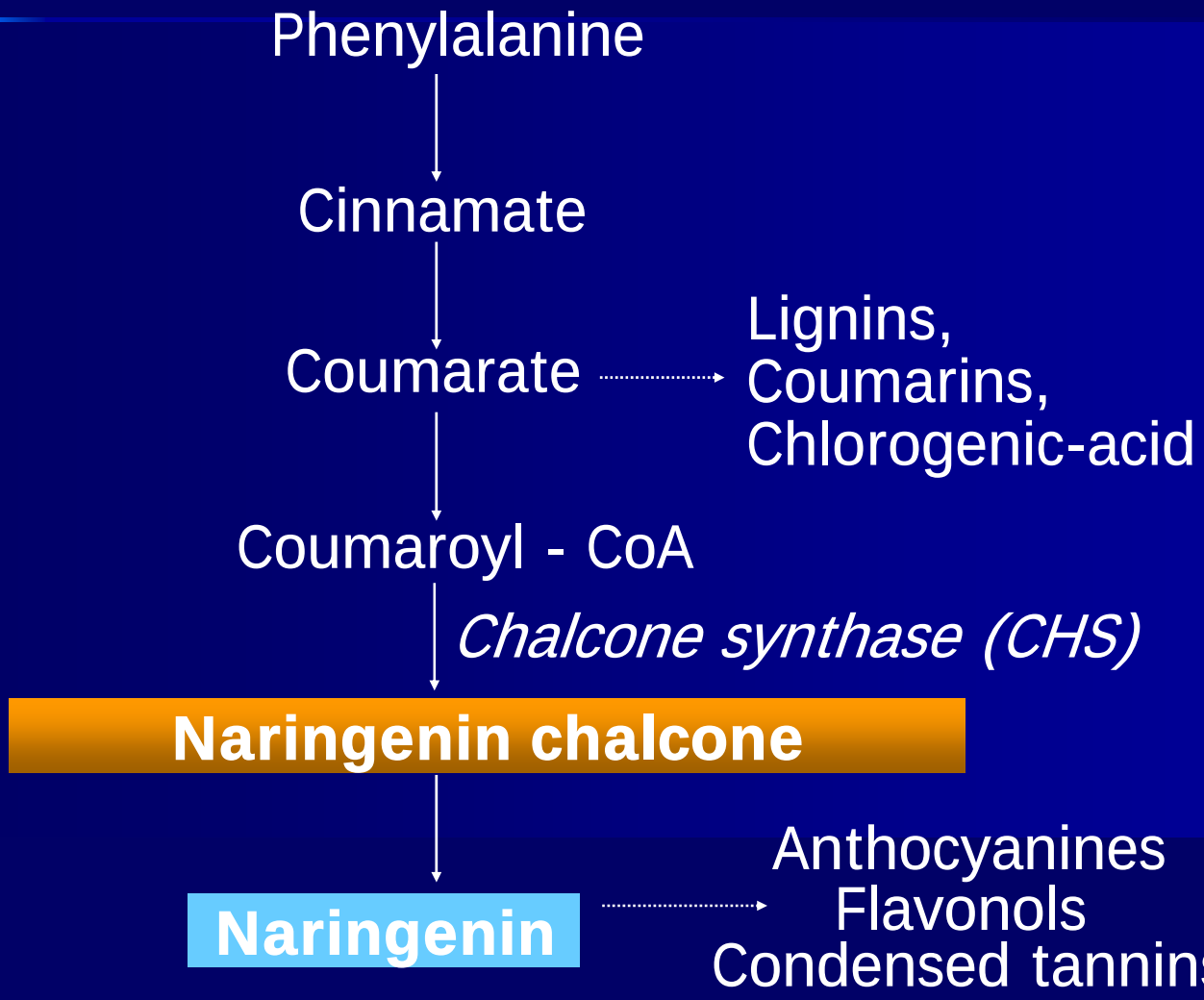
Methyl ether of Hydroxylated Naringenin Chalcone



Glycosylation

Methyl ether of Hydroxylated Naringenin
e.g.Hesperetin or a B-ring isomer

Biosynthesis of Naringenin Chalcone in the Flavonoid Pathway



FTMS metabolic phenotyping

Crude biological extract → Direct injection into FTMS via HPLC autosampler

↓

+/- ESI and +/- APCI ionization

↓

High resolution separation and detection of metabolites

Instrument acquisition time: (2-5 min/sample/mode)

↓

Off-line spectral processing

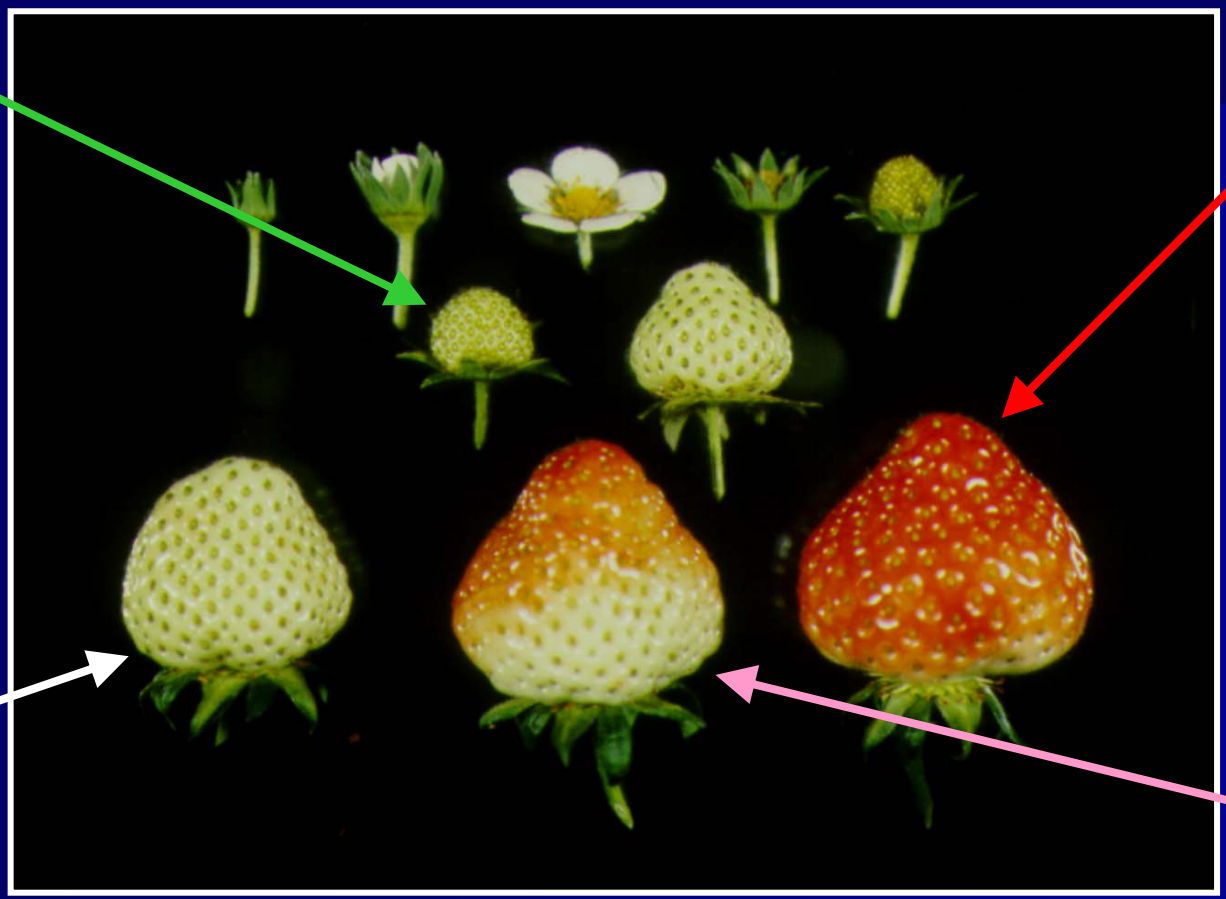
↓

Exportation of processed data to database

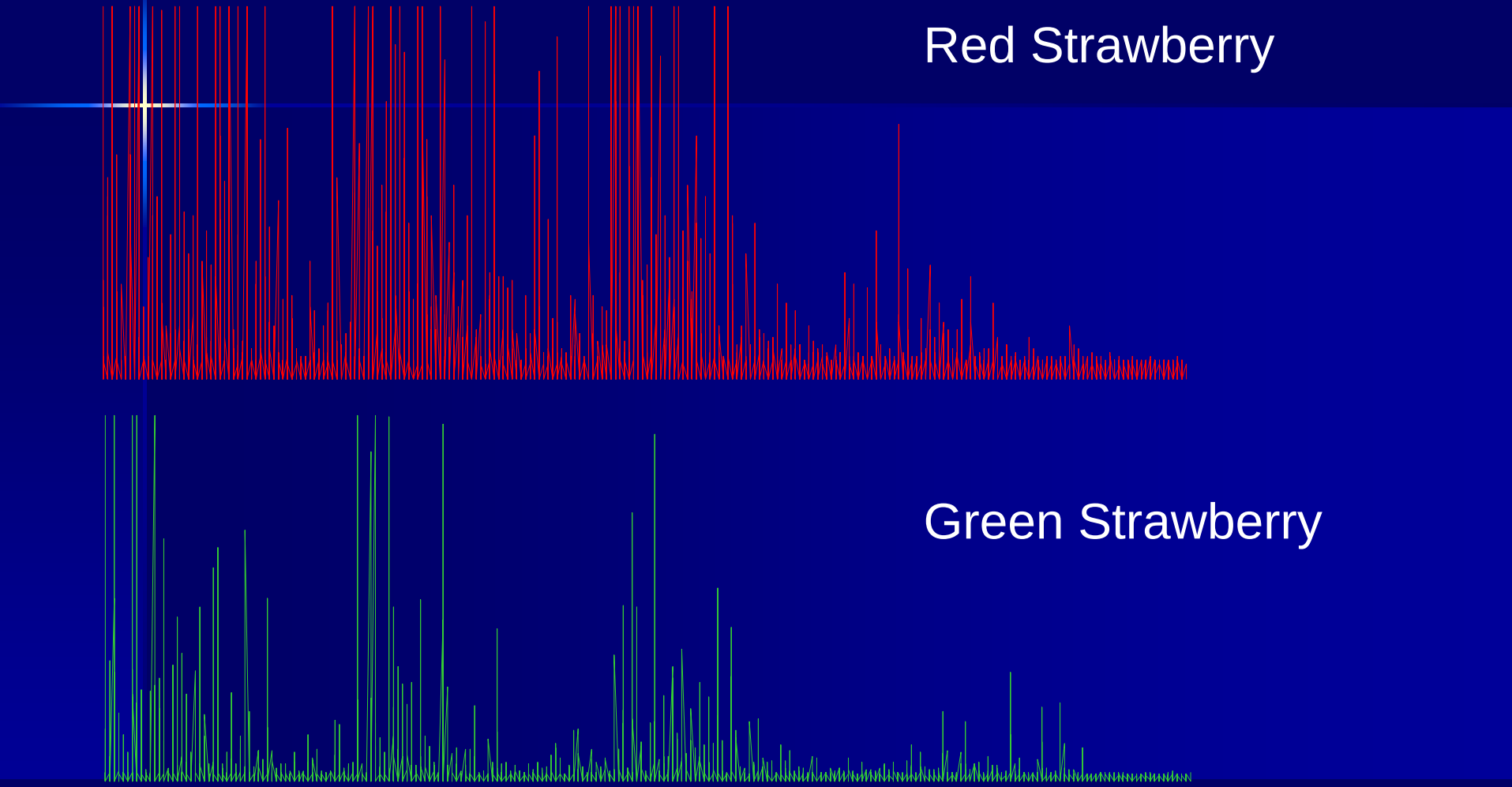
Analytical Instrumentation (FTMS)

Fourier Transform Ion Cyclotron Mass Spectrometry

Non-targeted metabolic profiling in strawberry



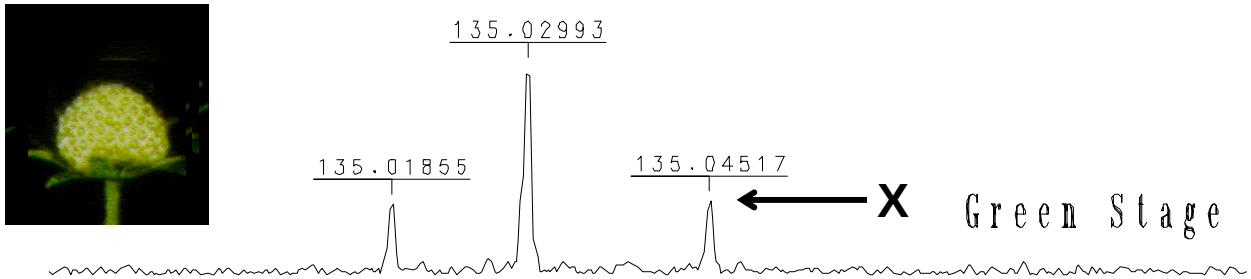
Positive ion ESI of strawberry fruit

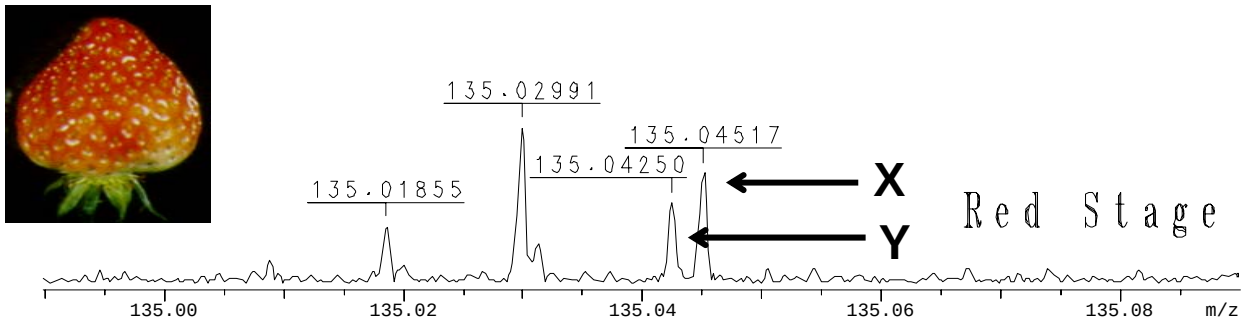


800 amu Window

High resolution separation

X = 135.04517 **Y = 135.04250**





Resolution = 135,000, 0.1 AMU Window

Empirical formula determination

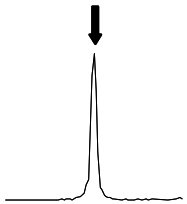
Internal Mass Calibration Of Sample

Calibration Results:

Ref. Masses	Exp. Masses	Diff (ppm)
124.039300	124.039309	0.0718
140.034220	140.034205	0.1064
303.166300	303.166457	0.5191
962.430130	962.430546	0.4319

Calculate Accurate Mass Of Metabolite

433.11270



Calculate Possible Empirical Formulas

Mass Analysis for mass 433.1127000

#	12C	1H	16O	14N	32S	mass	error
1	21	21	10	0	0	433.1129233	5.156e-07
2	13	25	12	2	1	433.1122714	9.896e-07
3	11	13	2	16	1	433.1122608	1.014e-06
4	14	29	7	2	3	433.1131398	1.015e-06
5	11	21	1	12	3	433.1117918	2.097e-06
6	14	21	8	6	1	433.1136088	2.098e-06
7	26	17	1	4	1	433.1117583	2.174e-06
8	22	25	5	0	2	433.1137917	2.521e-06
9	18	13	4	10	0	433.1115754	2.597e-06
10	26	25	0	0	3	433.1112893	3.257e-06
11	22	17	6	4	0	433.1142607	3.603e-06
12	18	21	3	6	2	433.1111064	3.679e-06
13	10	17	6	12	1	433.1109234	4.102e-06
14	15	25	3	6	3	433.1144772	4.103e-06
15	15	17	4	10	1	433.1149462	5.186e-06
16	25	21	5	0	1	433.1104209	5.262e-06
17	23	21	1	4	2	433.1151291	5.609e-06
18	17	17	8	6	0	433.1102380	5.685e-06
19	23	13	2	8	0	433.1155981	6.691e-06
20	17	25	7	2	2	433.1097690	6.767e-06
21	10	29	12	2	2	433.1156422	6.793e-06
22	9	21	10	8	1	433.1095860	7.190e-06
23	10	21	13	6	0	433.1161112	7.876e-06
24	16	13	0	14	1	433.1162836	8.274e-06
25	17	19	5	7	1	433.1162889	8.286e-06
26	18	25	10	0	1	433.1162941	8.298e-06
27	16	21	12	2	0	433.1089006	8.772e-06

Assign Empirical Formula Based Upon Calibration Errors

$433.1127 = C_{21}H_{20}O_{10}[+H]^+$

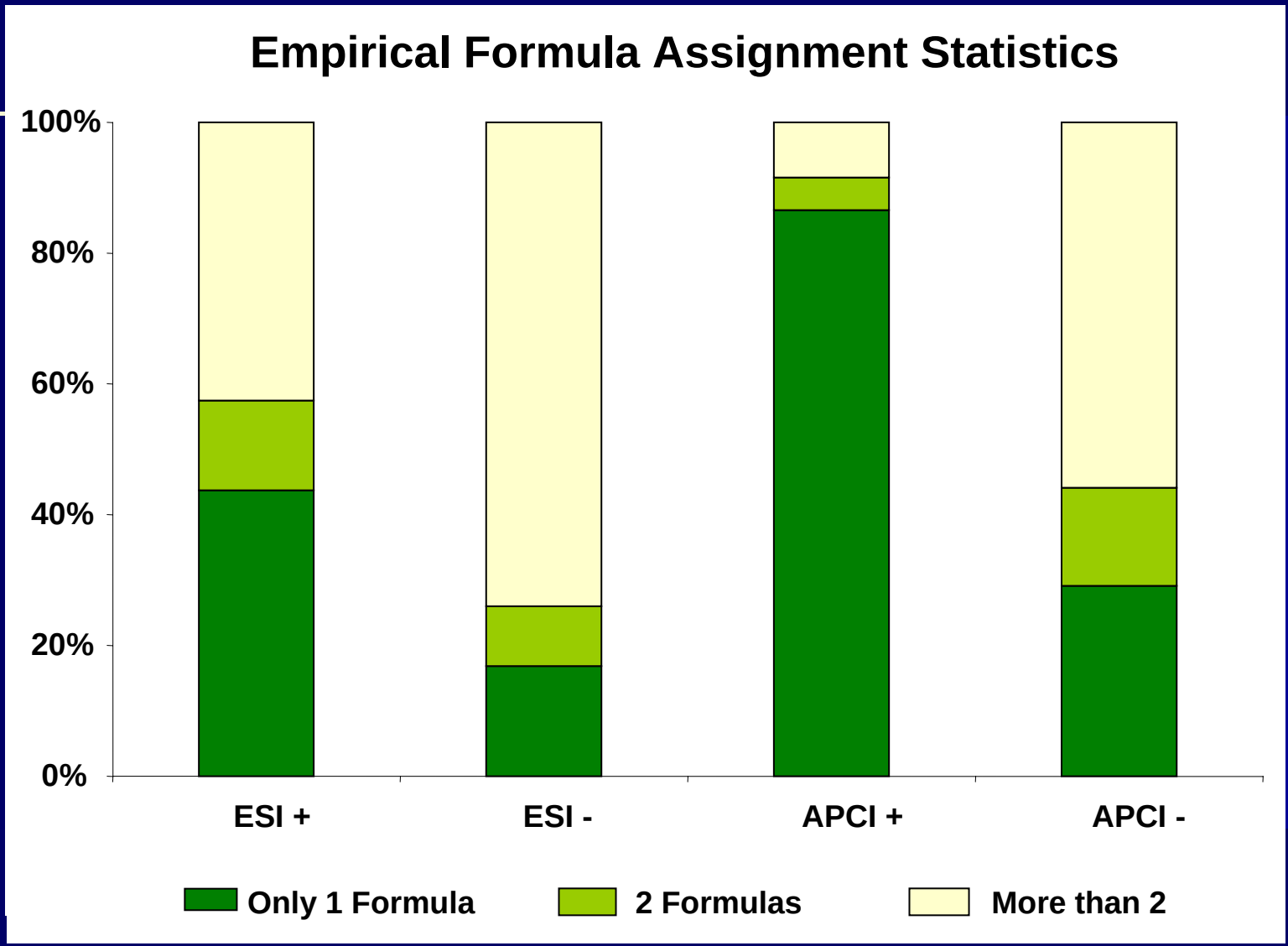
Error = 0.52ppm

Number of mass peaks and metabolites observed

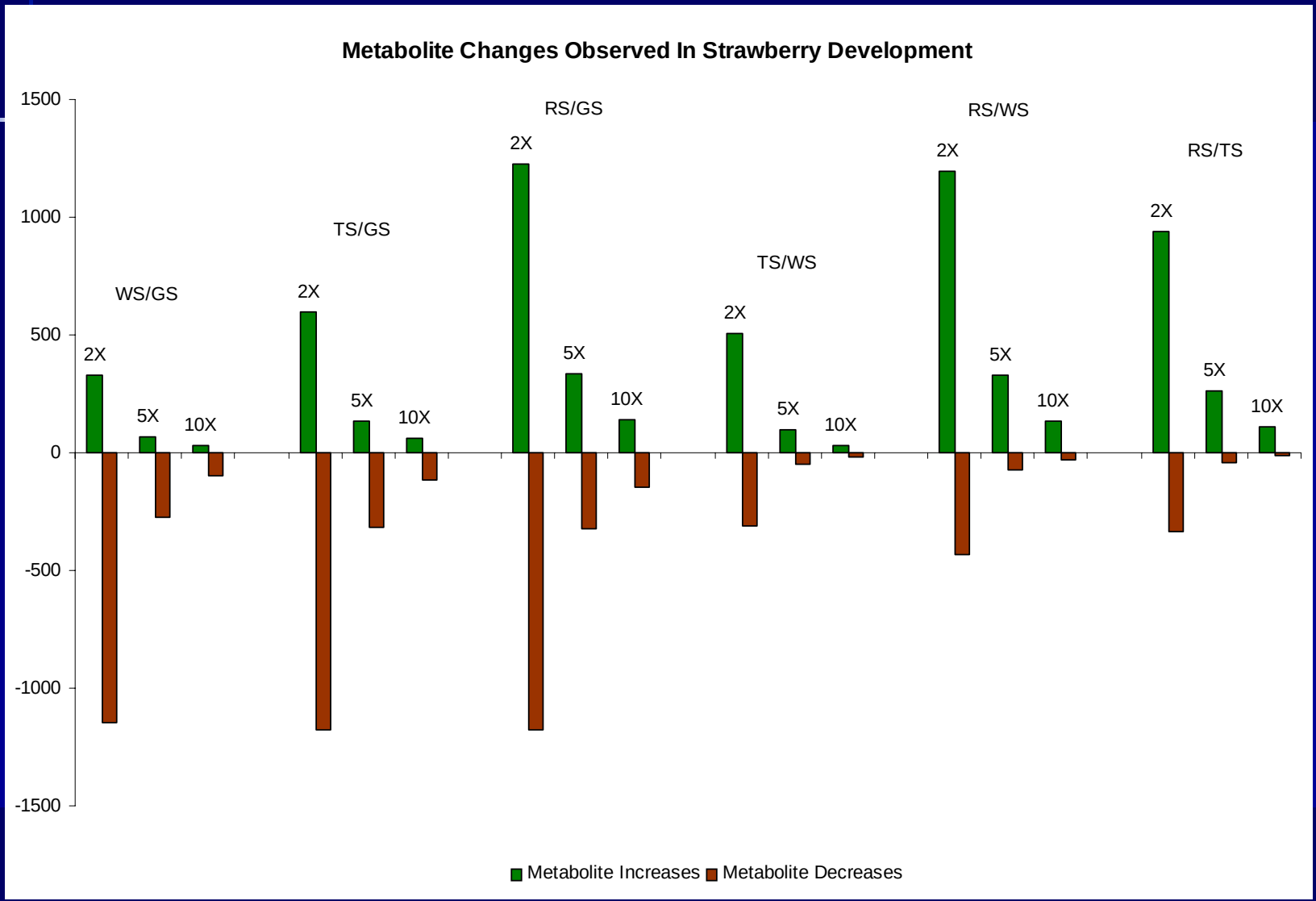
Number of metabolites observed across stages of fruit development, ionization modes and extractions

	Overall mass peaks									Unique mass peaks			
	Green		White		Turning		Red		Total	Only		Both (%)	Total
	50	AN	50	AN	50	AN	50	AN	50 + AN	50	AN	50 + AN	
ESI +	454	471	504	426	513	339	419	493	3619	578	520	374 (25%)	1472
ESI-	513	285	315	247	269	319	348	193	2489	608	454	208 (16%)	1270
APCI+	446	340	672	470	528	399	587	902	4344	368	595	536 (36%)	1499
APCI-	519	546	211	435	260	296	233	460	2960	492	800	311 (19%)	1603
Mean	483	411	426	395	393	338	397	512		512	592	357 (24%)	1461
Total	1932	1642	1702	1578	1570	1353	1587	2048	**13,412**	2046	2369	1429 (24%)	**5844**

Accurate empirical formula determination



Quantitative determination of change between samples



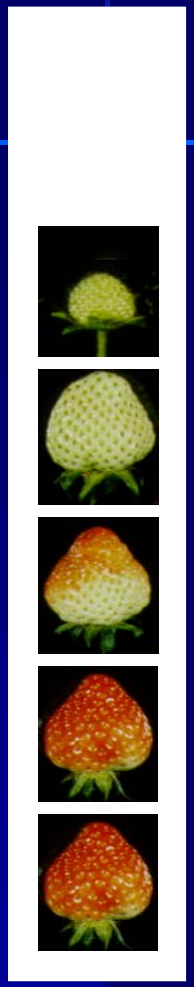
Metabolic profiling of strawberry fruit development

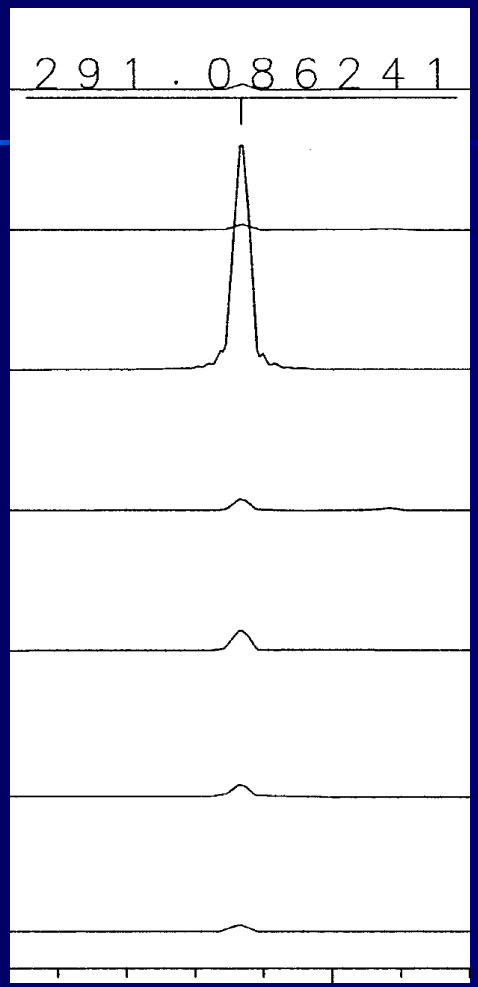
Metabolite	CG	EF	NM	IM	EX	R/G	T/G	W/G
Group 1: Early expression		**Profile A**						
Ellagic acid*	P. Acid	$C_{14}H_6O_8$	302.0063	AP-	AN	0.03	0.03	0.03
Hydroxybenzoic acid*	P. Acid	$C_7H_6O_3$	138.0317	AP-	AN	0.09	0.08	0.09
Arginine	A. acid	$C_6H_{14}N_4O_2$	174.1117	ES+	50	0.06	0.06	0.10
Vanillic acid*	P. Acid	$C_8H_8O_4$	168.0423	AP-	AN	0.08	0.05	0.06
Malic acid	O. acid	$C_4H_6O_5$	134.0215	ES-	AN	0.04	0.04	0.04
Catechin (epi)/leucopelargonidin*	Phenolic	$C_{15}H_{14}O_6$	290.0790	ES-	50	0.04	0.07	0.12
Caffeate*	P. Acid	$C_9H_8O_4$	180.0421	AP-	50	0.16	0.14	0.11
Ethyl cinnamate*	Ester	$C_{11}H_{12}O_2$	176.0837	AP-	AN	0.13	0.13	0.12
Quercitin glucoside*	Flavonoid	$C_{21}H_{20}O_{12}$	464.0955	ES-	50	0.13	0.13	0.13
Methyl cinnamate*	Ester	$C_{10}H_{10}O_2$	162.0681	AP-	AN	0.19	0.1	0.14
Dihydrokaempferol*	Flavonoid	$C_{15}H_{12}O_6$	288.0634	AP+	50	0.11	0.15	0.28
Leucocyanidin*	Flavonoid	$C_{15}H_{14}O_7$	306.0737	AP-	AN	0.37	0.37	0.37
N-Alanylcysteine	A. Acid d.	$C_6H_{12}N_2O_3S$	192.0567	ES+	AN	0.22	0.22	0.22
Gentisic/Protocatechuic acid*	P. Acid	$C_7H_6O_4$	154.0266	AP+	50	0.31	0.26	0.25
Malusfuran	Furan	$C_{12}H_8O_5$	232.0370	AP-	50	0.25	0.25	0.25
(E)2-hexenol / hexanal	Alcohol	$C_6H_{12}O$	100.0888	AP+	AN	0.25	0.25	0.25
Glucogallin*	Phenolic	$C_{13}H_{16}O_{10}$	332.0744	AP-	50	0.23	0.23	0.23
Pantothenic acid	Vitamin	$C_9H_{17}NO_5$	219.1107	ES+	AN	0.27	0.27	0.27
Dihydroxybenzoquinone	Phenolic	$C_6H_4O_4$	140.0109	ES+	AN	0.49	0.48	0.46
Dihydroquercitin*	Flavonoid	$C_{15}H_{12}O_7$	304.0581	AP+	50	0.42	0.42	0.42

Metabolic profiling of strawberry fruit development

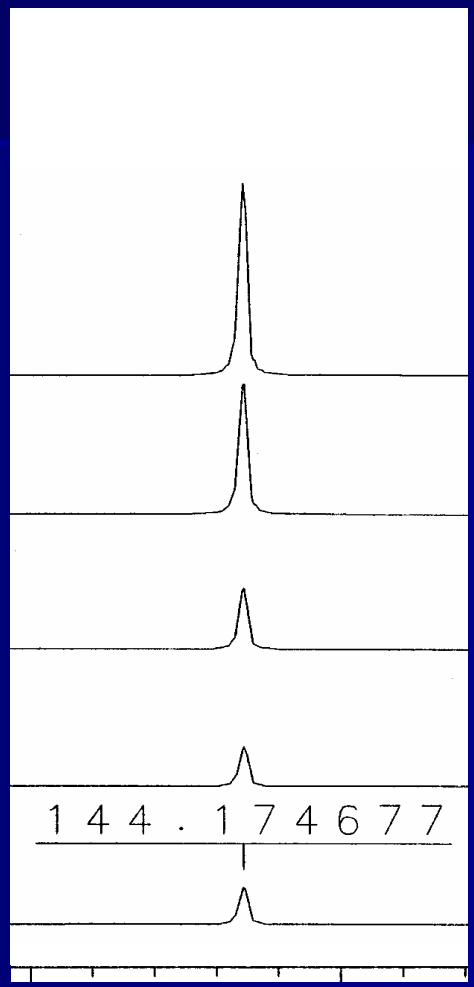
Metabolite	CG	EF	NM	IM	EX	R/G	T/G	W/G
			Profile I					
Linoleic acid	F. Acid	$C_{18}H_{32}O_2$	280.2402	AP+	AN	**20.80**	1.93	0.97
Palmitoleic acid	F. Acid	$C_{16}H_{30}O_2$	254.2246	AP+	AN	**24.30**	1.34	0.85
Oleic acid	F. Acid	$C_{18}H_{34}O_2$	282.2559	AP+	AN	**32.56**	1.50	0.99
Pentadecanoic acid	F. Acid	$C_{15}H_{30}O_2$	242.2246	AP+	AN	**28.80**	0.91	0.62
Palmitic acid	F. Acid	$C_{16}H_{32}O_2$	256.2402	AP+	AN	**40.20**	1.67	0.80
3-methylbutyl nonanoate	Ester	$C_{14}H_{28}O_2$	228.2089	AP+	AN	**29.80**	1.38	1.00
Stearic acid	F. acid	$C_{18}H_{36}O_2$	284.2715	AP+	AN	**18.03**	1.87	1.24
Furaneol (R=D-glucopyranose)	Furan	$C_{12}H_{18}O_8$	290.1002	ES+	AN	**10.04**	1.00	1.00
Furaneol (R=D-glucopyranose, malonyl)	Furan	$C_{15}H_{20}O_{11}$	376.1006	ES+	50	**11.00**	1.00	1.00
Nonanoic acid	F. Acid	$C_9H_{18}O_2$	158.1307	AP+	AN	**5.20**	1.00	1.00
Capric acid	F. Acid	$C_{10}H_{20}O_2$	172.1463	AP+	AN	**5.49**	1.00	1.00
Eicosanoic acid	F. Acid	$C_{20}H_{40}O_2$	312.3028	AP+	AN	**7.85**	1.00	1.00
Linolenic acid	F. Acid	$C_{18}H_{30}O_2$	278.2246	AP+	AN	**8.52**	1.97	1.10
Cinnamate*	P. Acid	$C_9H_8O_2$	148.0524	AP+	50	**5.86**	1.17	0.87
1-octyl butanoate	Ester	$C_{12}H_{24}O_2$	200.1776	AP+	AN	**5.69**	1.00	1.00
1-methyhexyl hexanoate	Ester	$C_{13}H_{26}O_2$	214.1933	AP+	AN	**6.67**	1.00	1.00
Cis-hex-3-enyl octanoate	Ester	$C_{14}H_{26}O_2$	226.1933	AP+	AN	**6.93**	0.91	0.90
Methylstearate	Ester	$C_{19}H_{38}O_2$	298.2872	AP+	AN	**7.91**	0.76	0.68
Sinapyl alcohol*	Phenolic	$C_{11}H_{14}O_4$	210.0891	AP-	AN	**5.8**	1.00	1.00
Scopoletin*	Benzopyranoid	$C_{10}H_8O_4$	192.0421	AP+	50	**5.31**	1.94	0.99
2-Isopropyl-3-methoxypyrazine	Alkaloid	$C_8H_{12}N_2O$	152.0949	AP+	AN	**6.79**	1.00	1.00
Farnesyl acetone	Terpene	$C_{18}H_{30}O$	262.2295	AP+	AN	**6.65**	1.57	0.59
Hydroxycoumarine*	Benzopyranoid	$C_9H_6O_3$	162.0316	AP+	AN	**5.44**	0.73	0.66
Coumarine*	Benzopyranoid	$C_9H_6O_2$	146.0368	AP+	AN	**5.47**	0.96	1.57
Pelargonidin*	Flavonoid	$C_{15}H_{10}O_5$	270.0527	ES-	50	**9.46**	1.00	1.00
Smipine	Alkaloid	$C_{10}H_{16}N_2O$	180.1261	AP+	AN	**5.33**	1.00	1.00

Group 1: Early expression

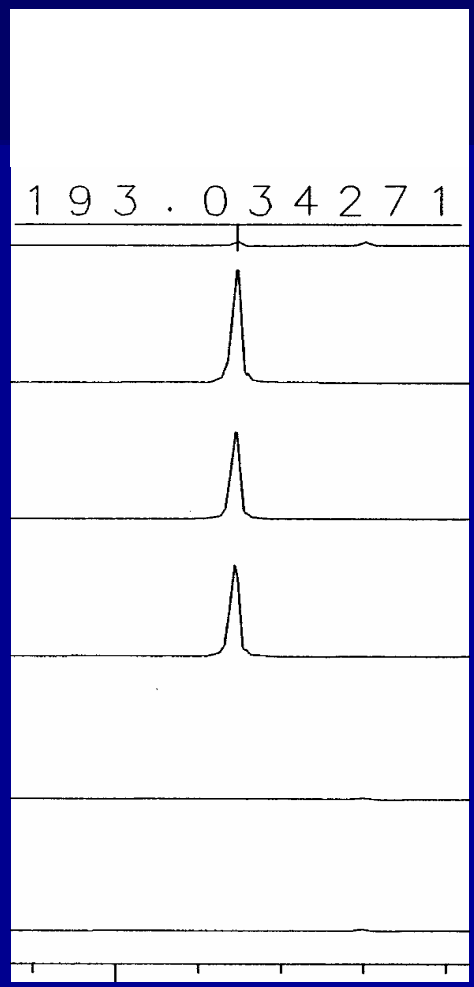




Catechin
(APCI +)

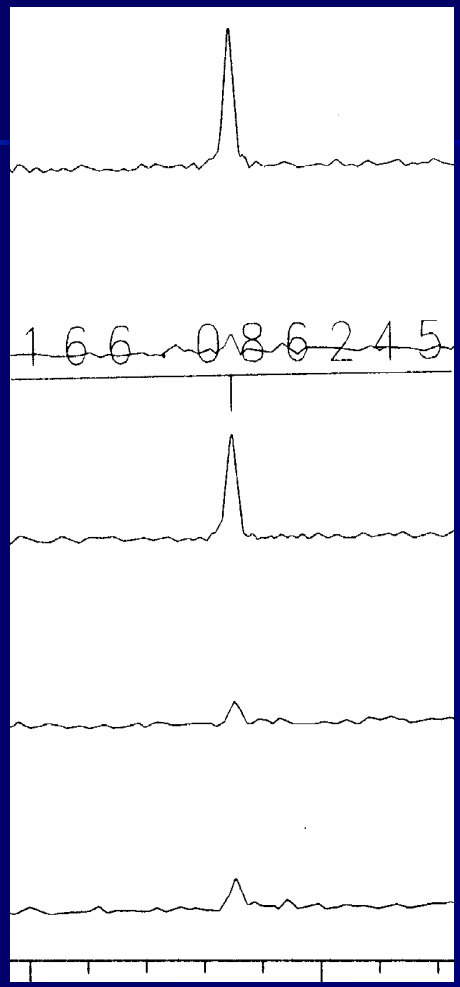


Heptylamine
(ESI+)

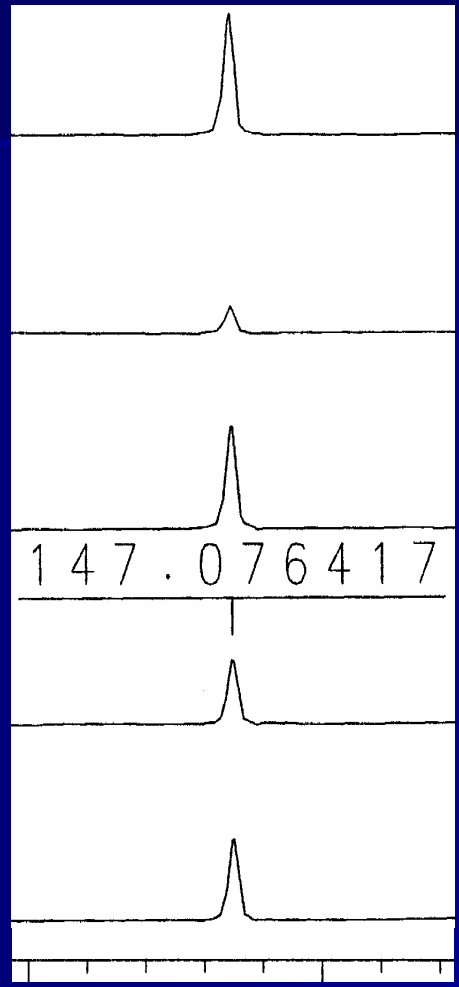


Citrate/Isocitrate
(APCI+)

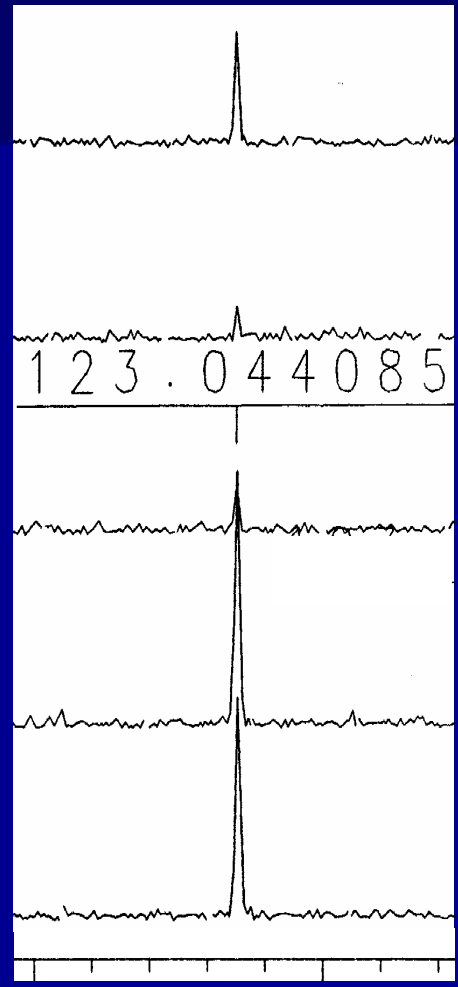
Group 2: Intermediate expression



Phenylalanine
(ESI+)

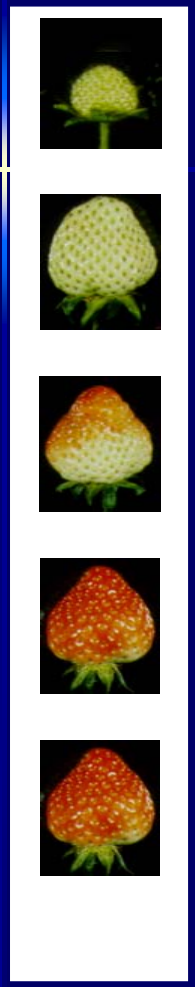


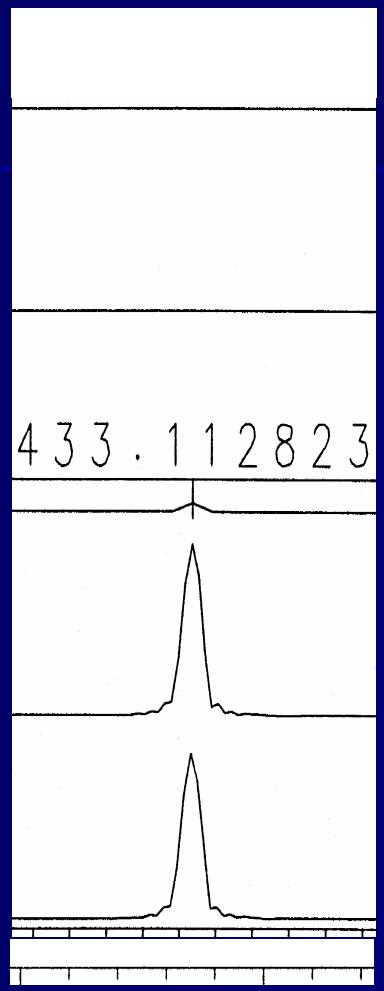
Glutamine
(ESI+)



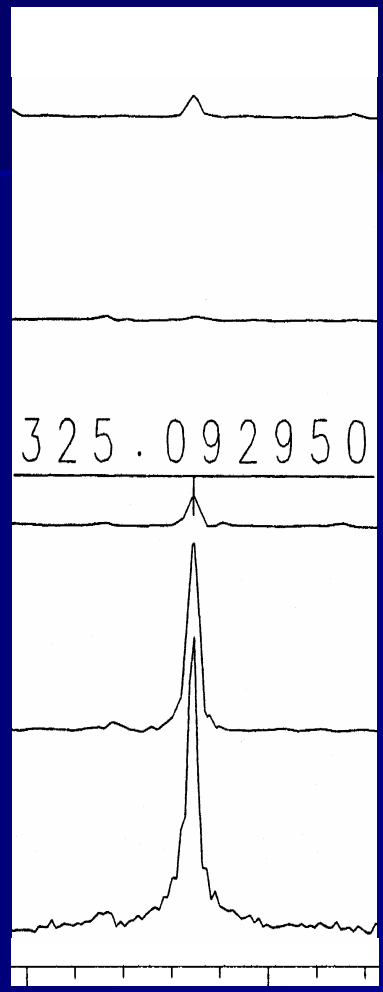
Benzoate
(APCI+)

Group 3: Late expression

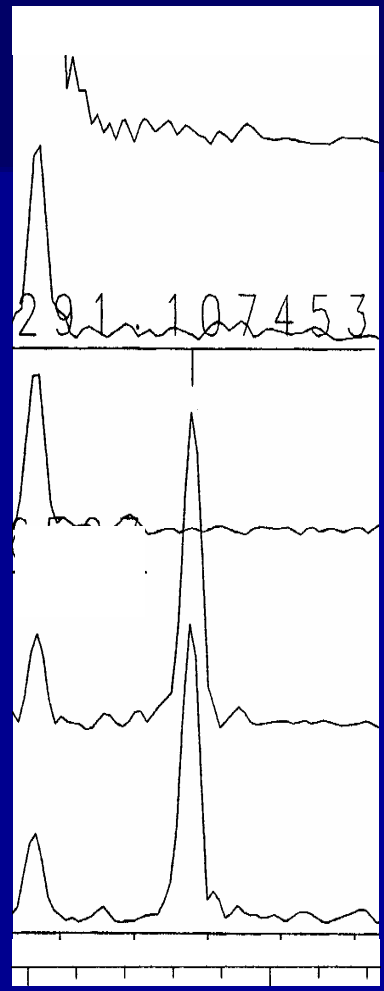




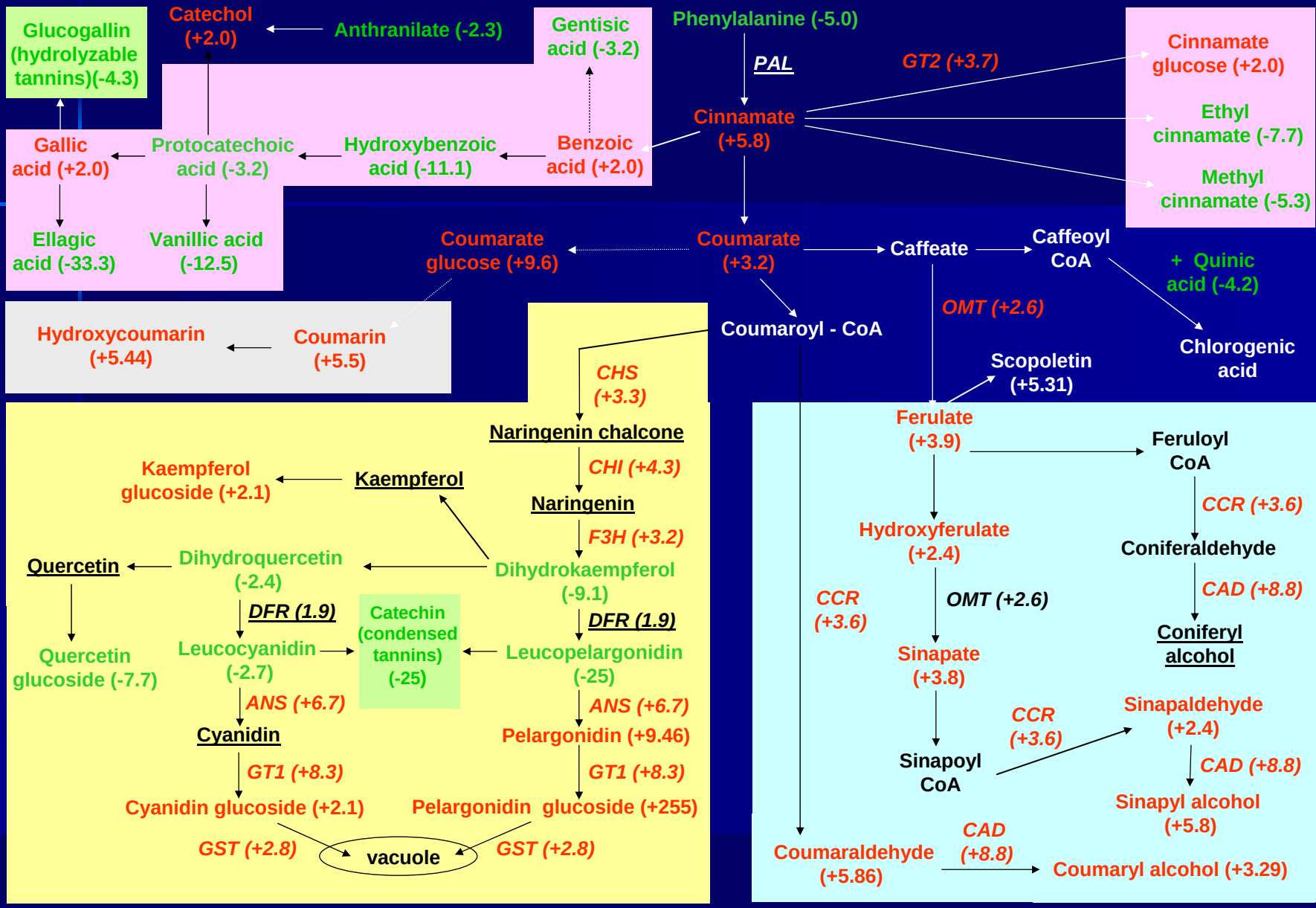
Pelargonidin glucoside (ESI+)



Coumaroyl glucoside (ESI-)



Furaneol glucoside (ESI+)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GT2 (+3.7)
Cinnamate glucose (+2.0)
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Gallic acid (+2.0)
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Vanillic acid (-12.5)
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GST (+2.8)
Ferulate (+3.9)
Feruloyl CoA
CCR (+3.6)
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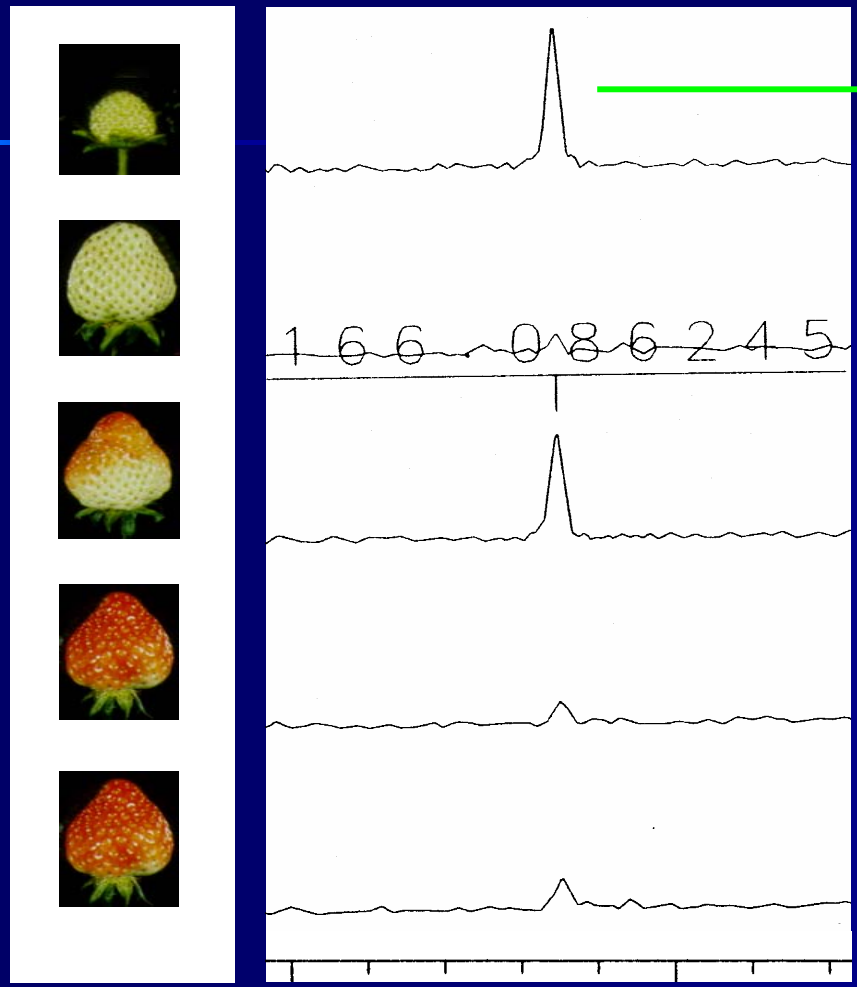
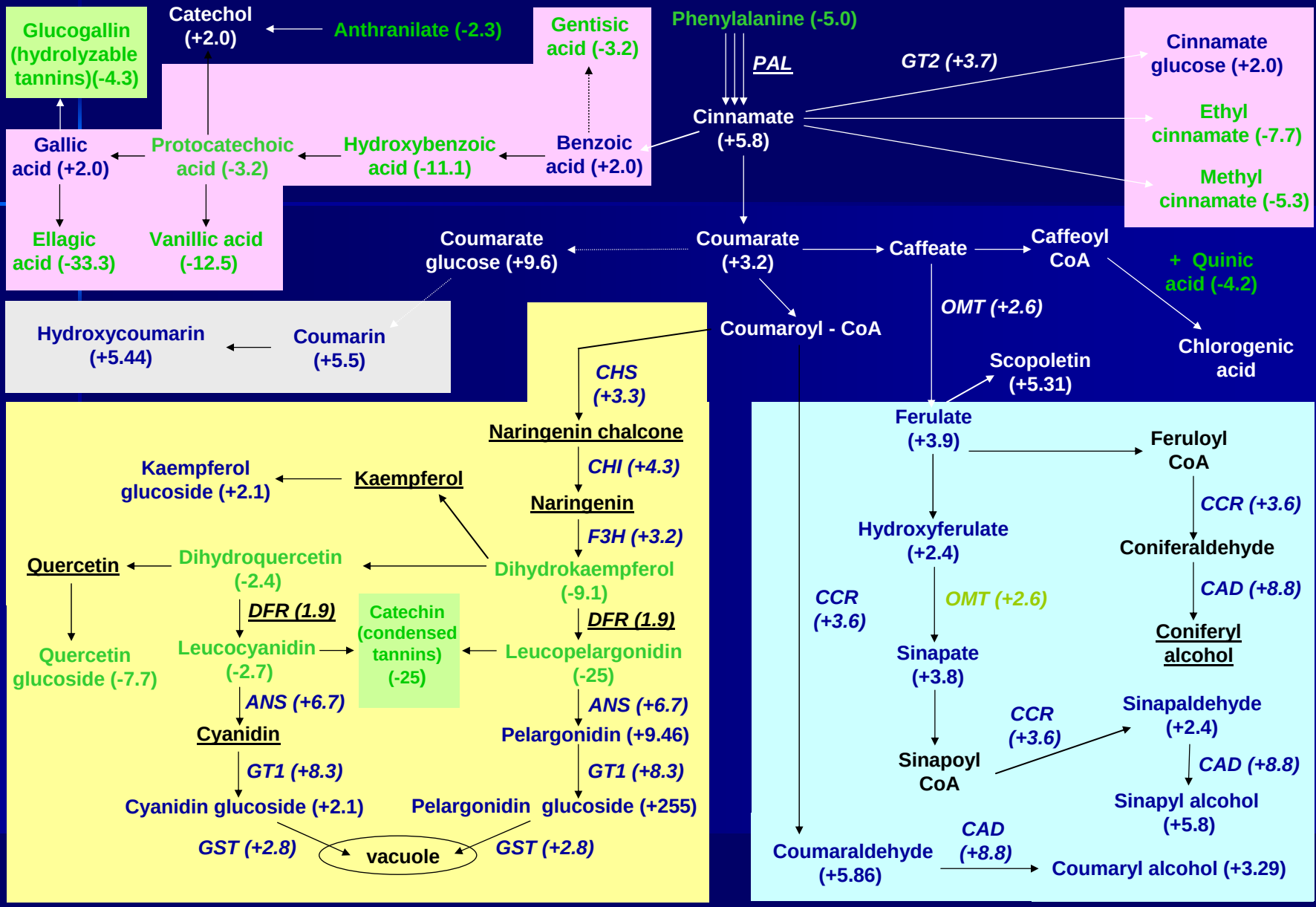
Coumaraldehyde (+5.86)
Coumaryl alcohol (+3.29)

Phenylalanine and changes in gene and metabolite expression

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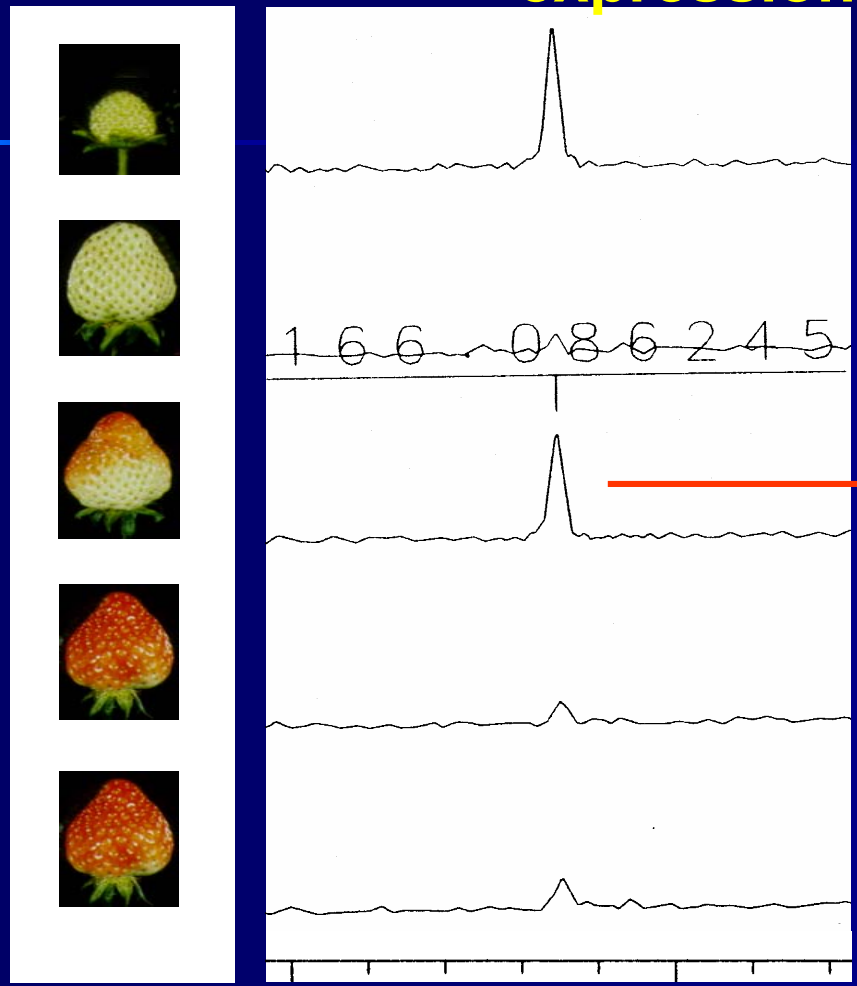
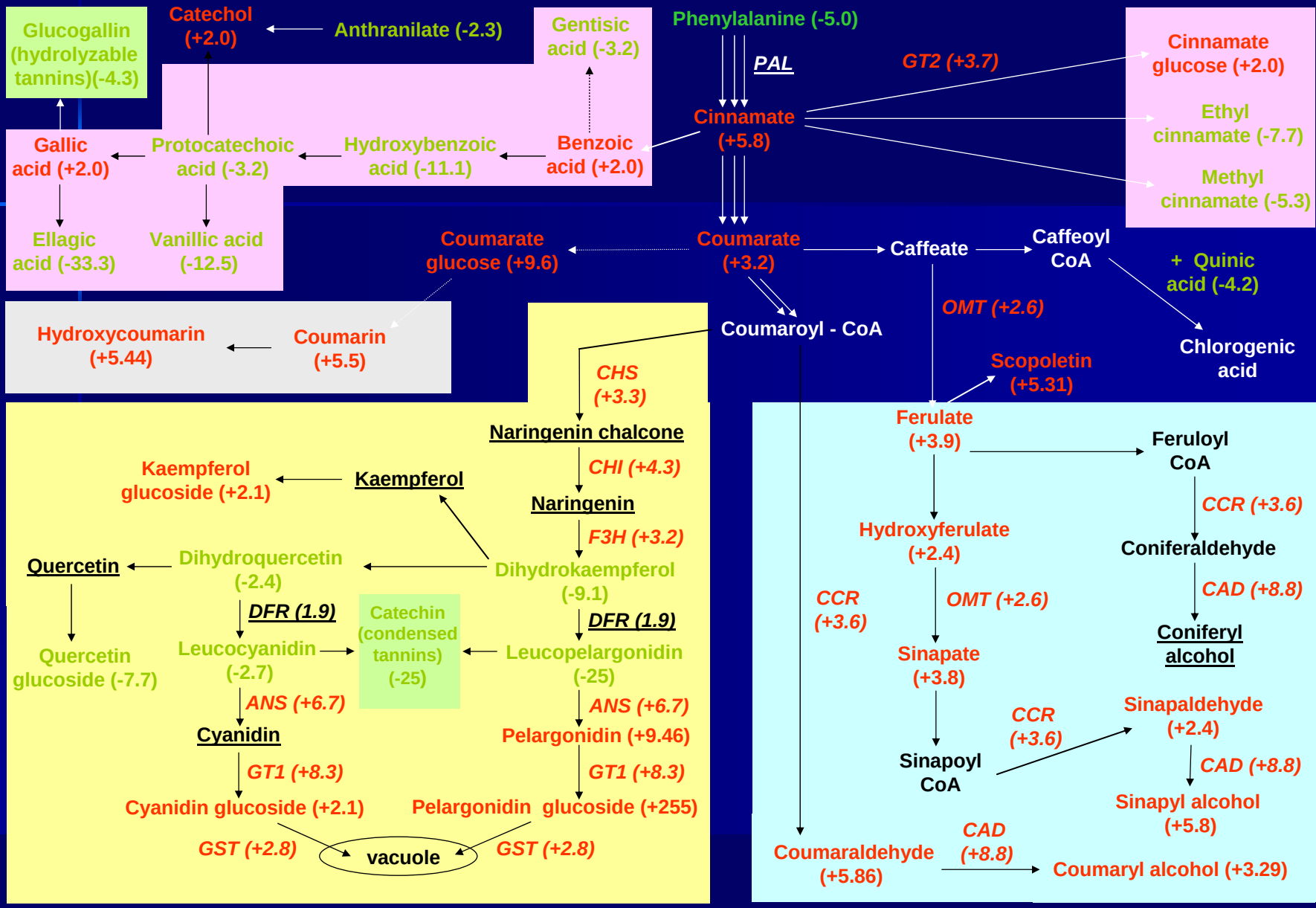
Phenylalanine and changes in gene and metabolite expression

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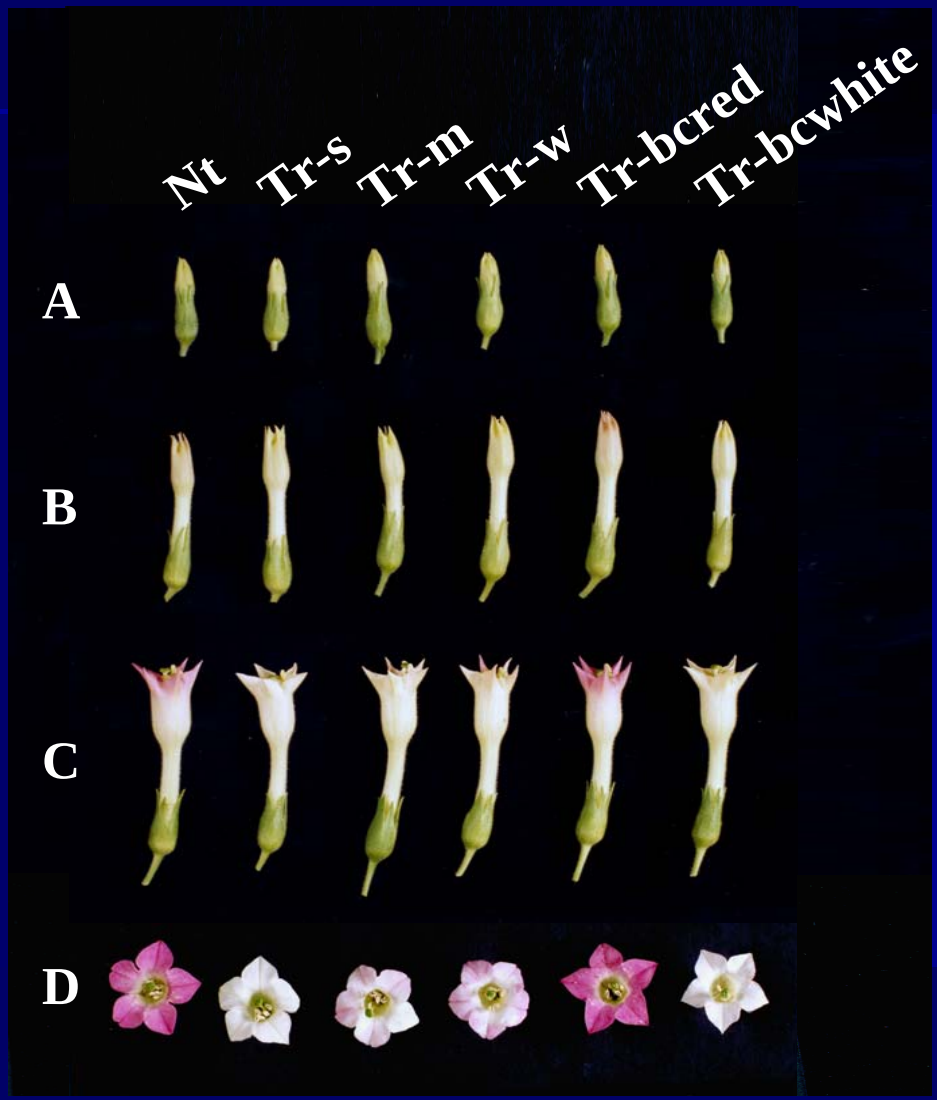
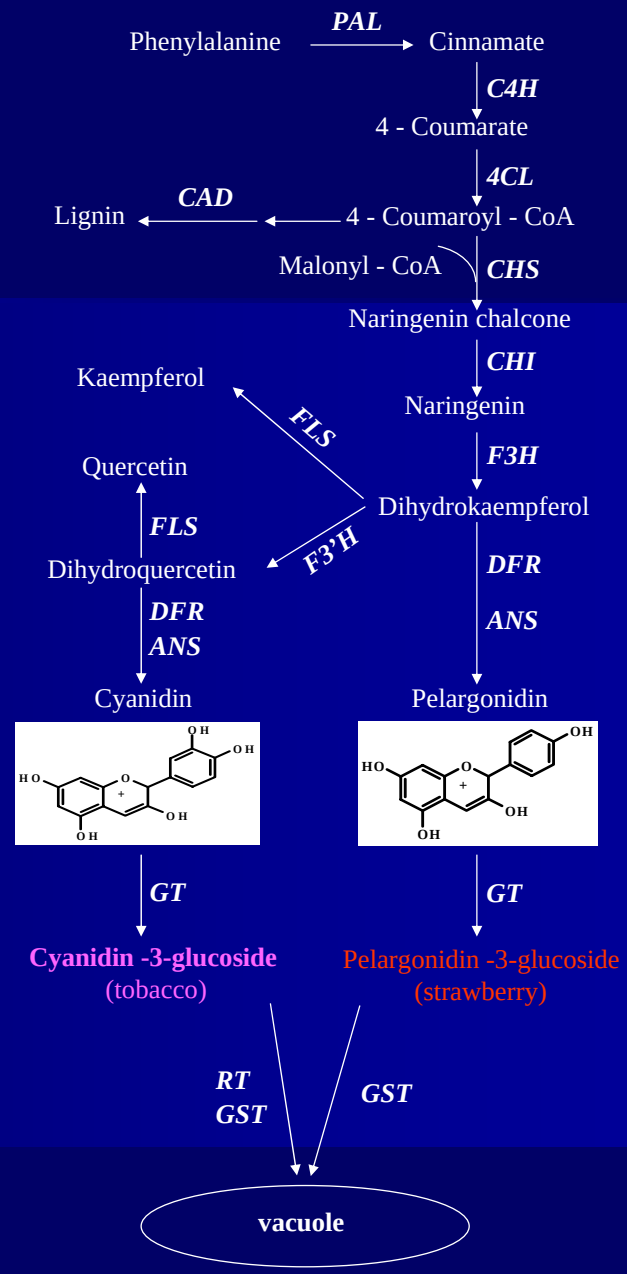
Coumaryl alcohol (+3.29)

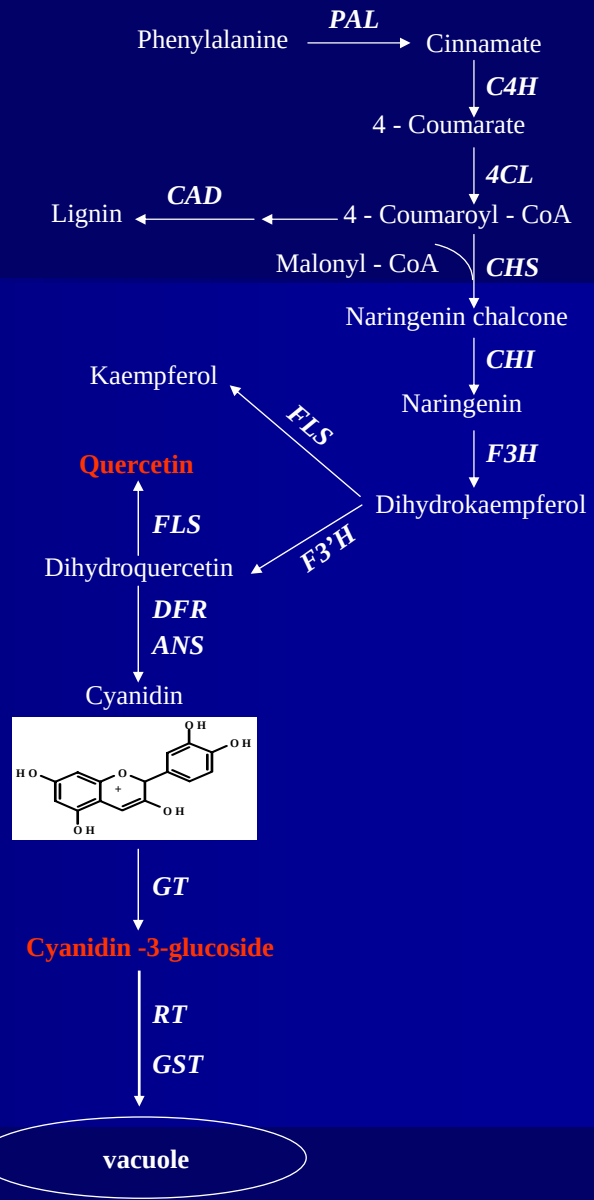
Over-expression of *FaMYB1* in tobacco



The phenylpropanoid pathway in strawberry & tobacco

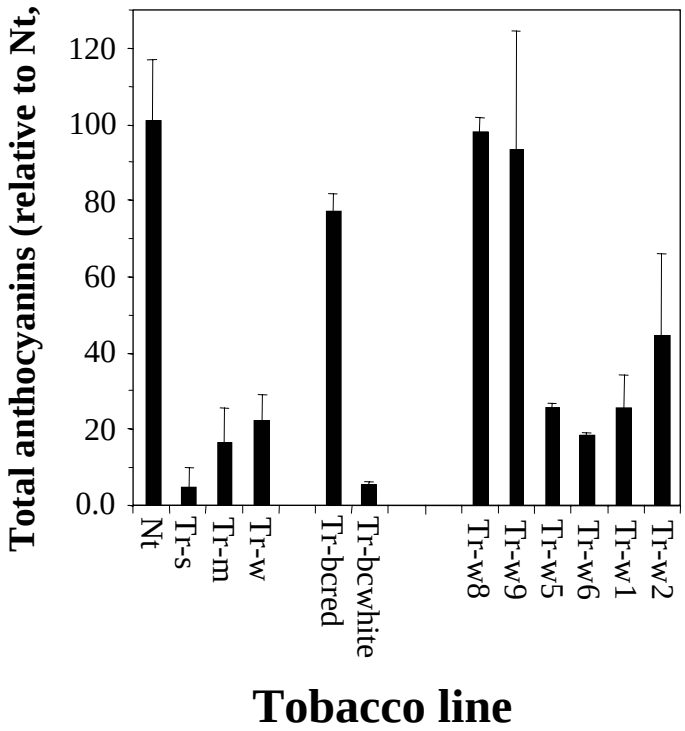


Metabolite changes in the *FaMYB1* overexpressing tobacco lines

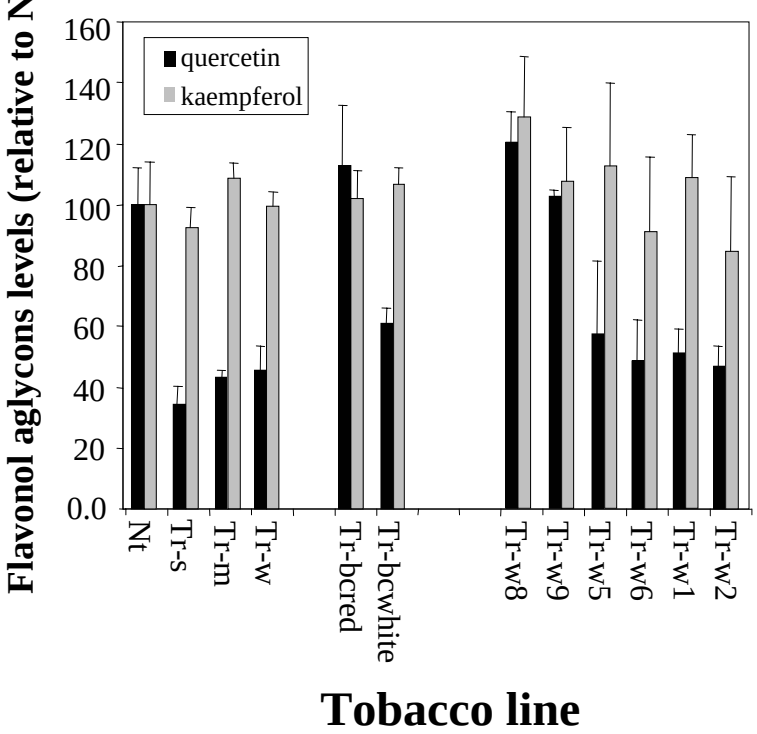


Anthocyanin and flavonol (quercetin) levels are reduced

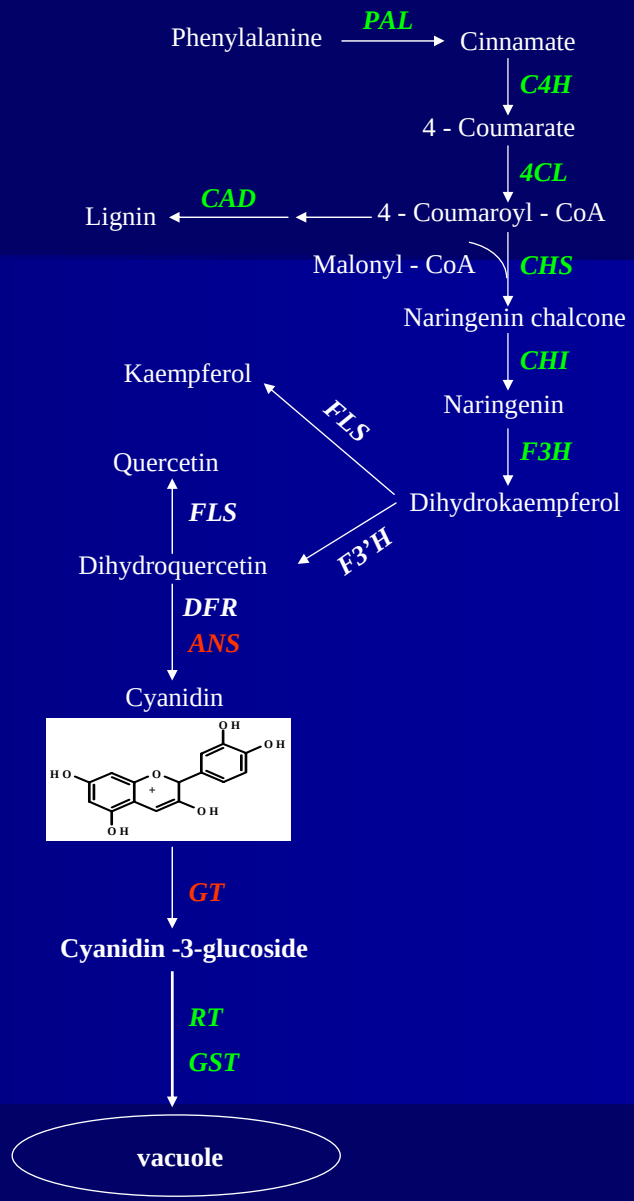
Anthocyanins (total)



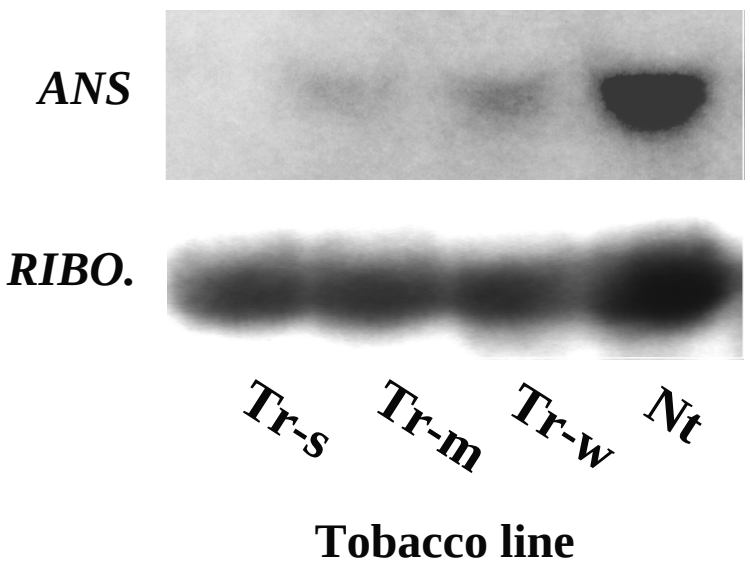
Flavonols (aglycons)

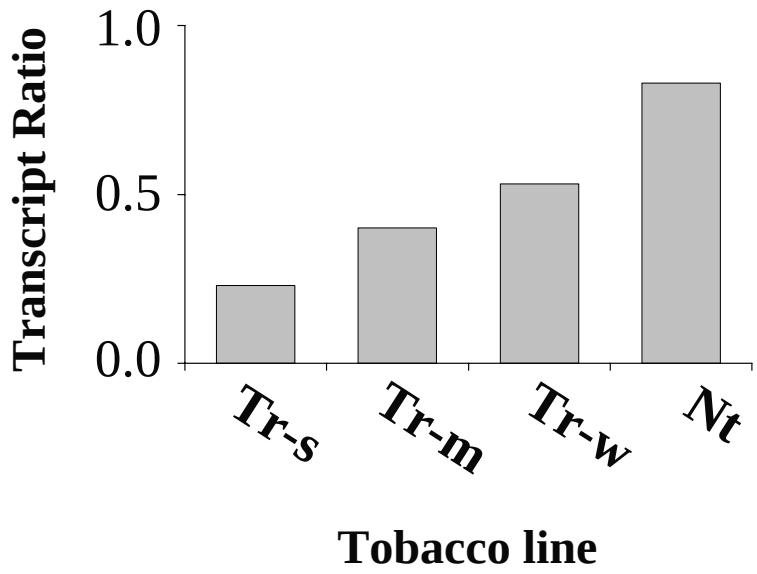


Gene expression and enzymatic activity changes in the *FaMYB1* over-expressing tobacco lines

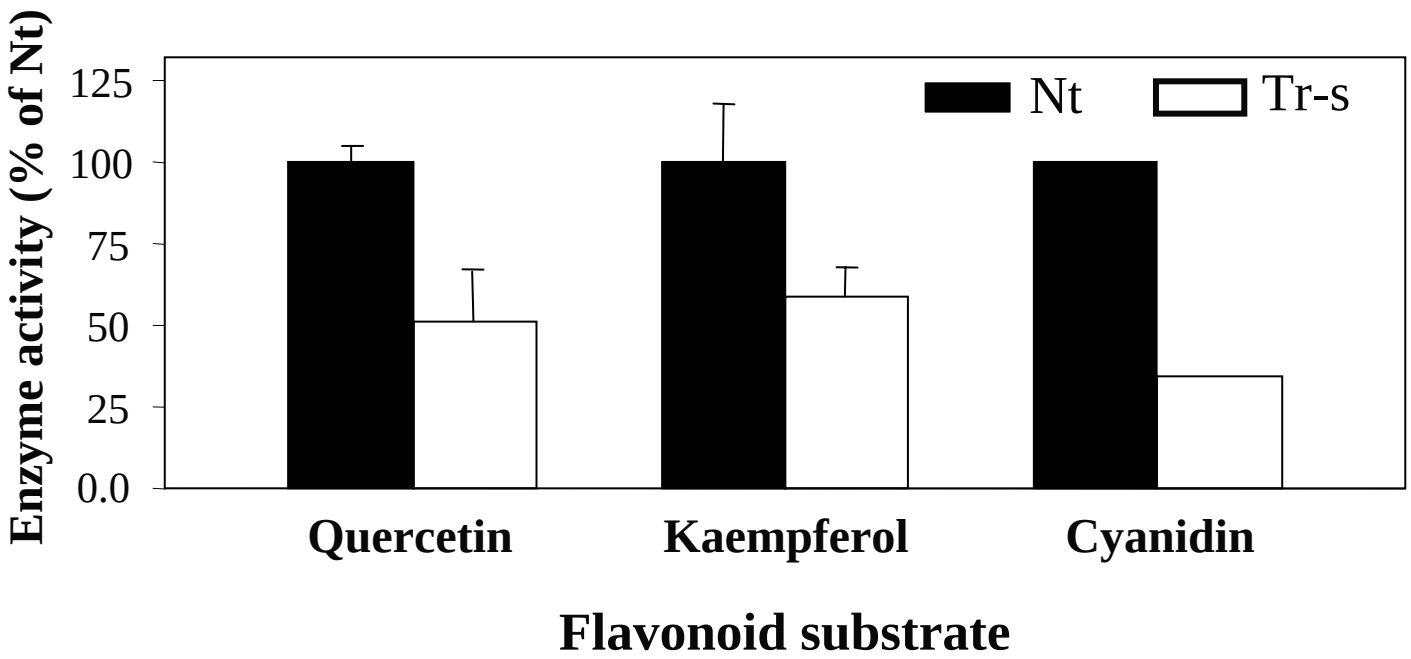


Anthocyanidin synthase (*ANS*) expression is down regulated



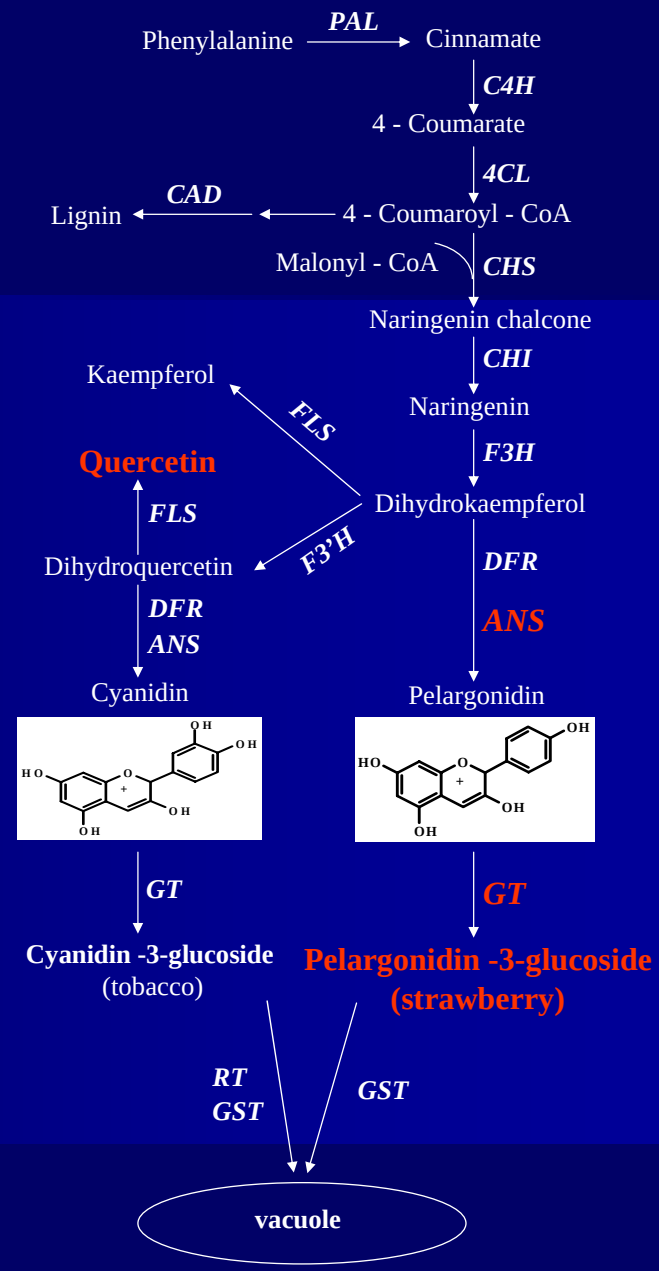


Flavonoid glucosyl transferase enzyme activity is down regulated

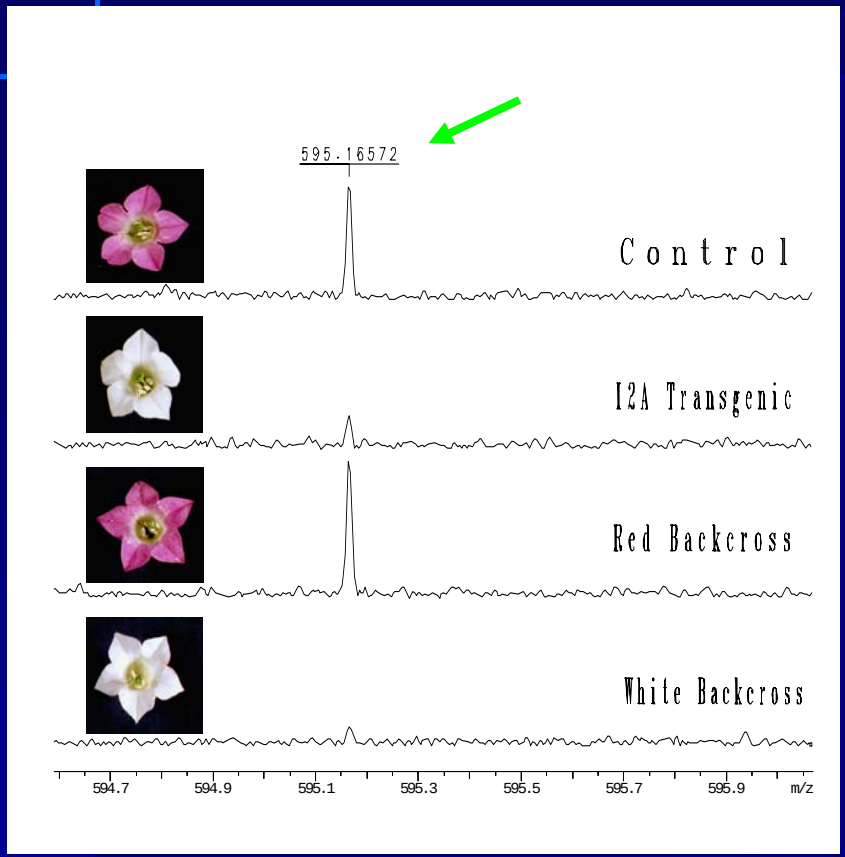


FaMYB1 is possibly involved in the regulation of flavonoid biosynthesis in late strawberry fruit ripening

It may act as a repressor of late flavonoid biosynthesis genes



Wild type vs. white mutant tobacco



Observed mass of unknown:
595.16572

Empirical formula search result:
$C_{27}H_{30}O_{15}$ [+H]+

Mass:
595.16575

Mass error:
0.04 ppm

Proposed metabolite:
Cyanidin – Rhamnoglucoside

See You Next Week