Kazusa DNA Res. Inst.

Workshop Argentina-Japan
"Bioscience and Biotechnology for the promotion of Agricutulure and Food Production – August 3rd to 7th 2009-

Metabolomics approaches for Agro-Biotechnology

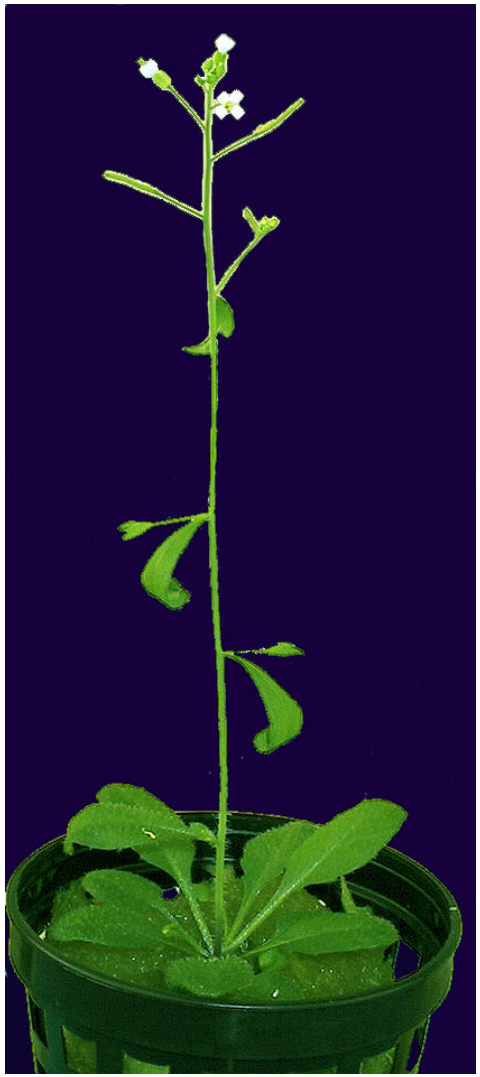
Kazusa DNA Res Inst.
Daisuke Shibata

Chiba Prefecture State Government supports financially KDRI.

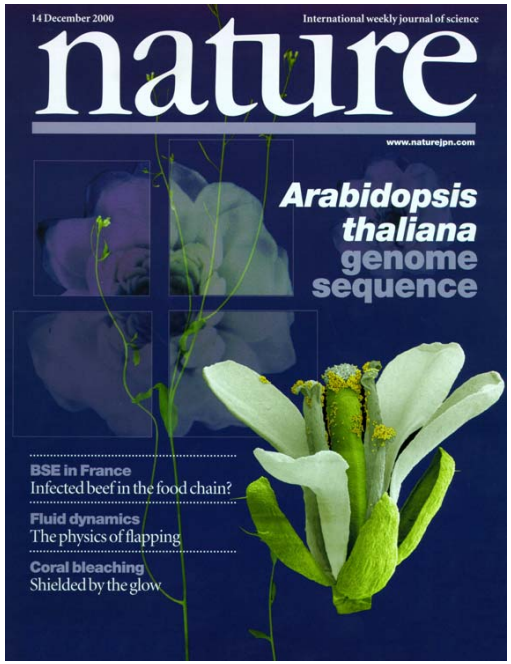
Dep. Plant Genomics
Dep. Human Genomics
Dep. Biotechnology

Kazusa DNA Research Institute (KDRI)

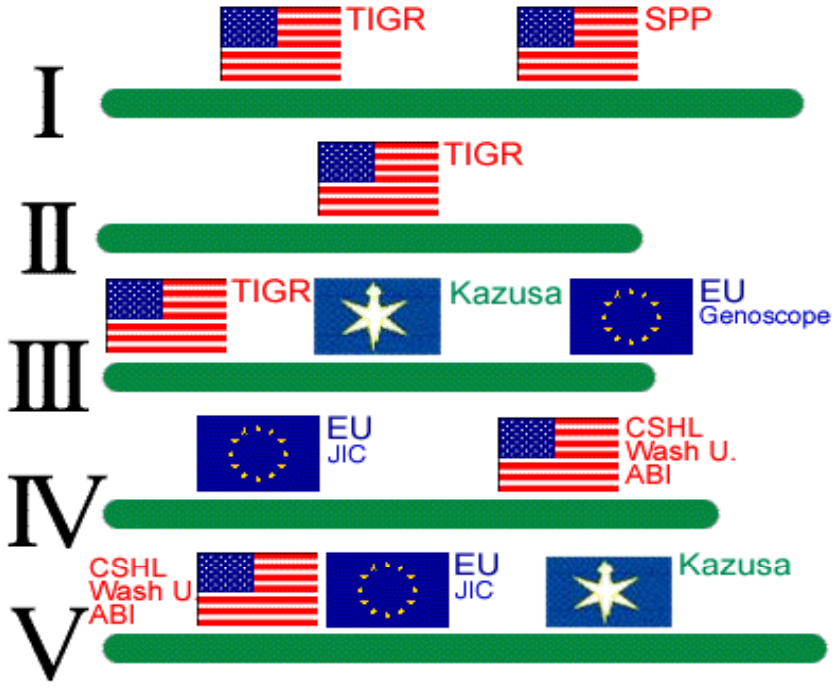
KDRI



Arabidopsis thaliana



December 2000



Kazusa DNA Res. Inst. (Tabata's lab) sequenced about 30Mb (28% contribution) in the Arabidopsis genome sequencing of 116 Mb.

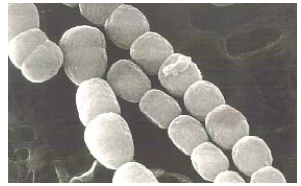
KDRI

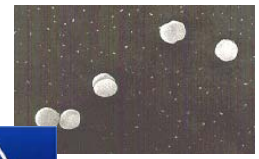
Plant-related Genome Sequencing at KDRI

Photosynthetic Bacteria

- *Synechocystis* sp. strain PCC 6803 (1996)
- *Anabaena* sp. PCC 7120 (2001)
- *Thermosynechococcus elongatus* BP-1 (2002)
- *Gloeobacter violaceus* PCC7421 (2003)

→ Photosynthesis





Nitrogen-fixation bacteria

- *Mesorhizobium loti* MAFF303099 (2000)
- *Bradyrhizobium japonicum* USDA110 (2002)

Plants

- Lotus japonicus (Legume) (2008)

→ Nitrogen fixation

- Arabidopsis (Chromosome 3 &5) (2000)

→ Model plant

- **Eucalyptus (for pulp)** In progress
- **Tomato (Chromosome 8)** (2009)

→ Agricultural and industrial application

Metabolomics approaches in agricultural biotechnology

Researches in the Japan Society for Bioscience, Biotechnology, and Agrochemistry are diverged ;

Cop and vegetable breeding

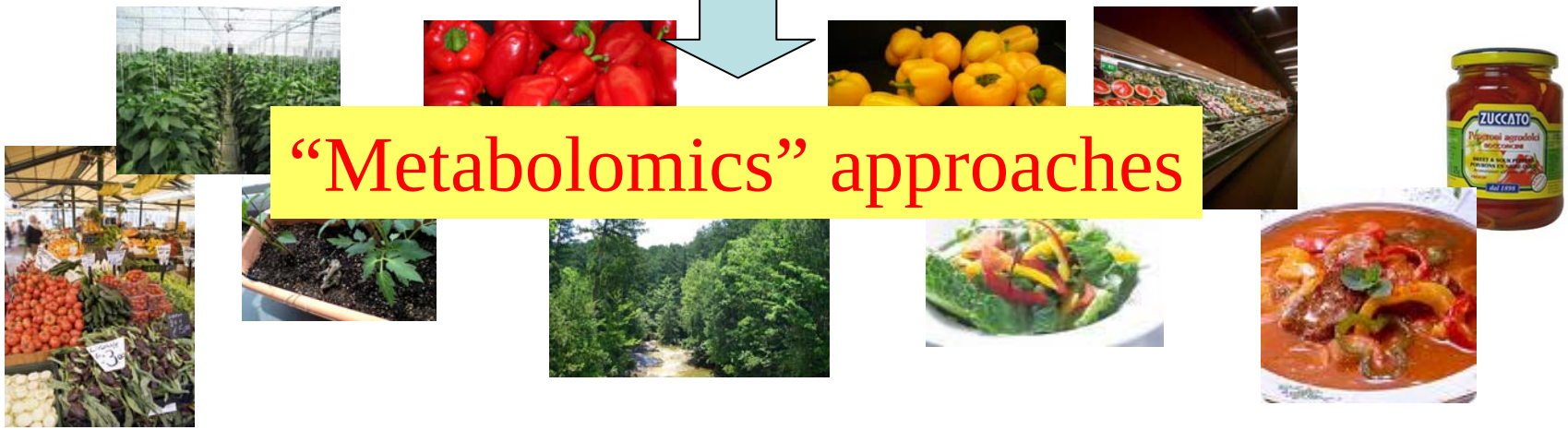
Genetically modified crops

Wood utilization

Fermentation

Food production

More,,,,



"Metabolomics" approaches

High-through-put tools for metabolite analysis

LC-IT-MS

UPLC-Q-TOF-MS

LC-MS

UPLC-TOF-MS

GC-TOF-MS x 2

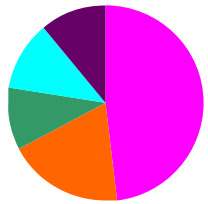
CE-MS x2

FT-IR

LC-FT/ICR-MS

LC-PDA x2

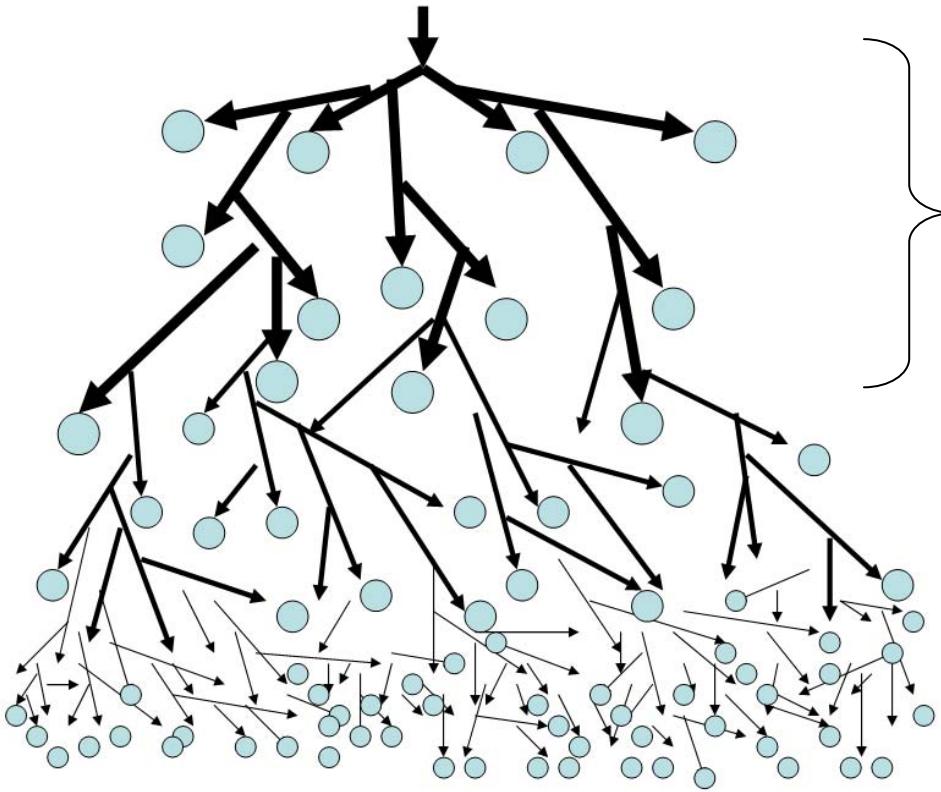
GPC-MALS



# of analyzed：> 80,000 at KDRI

What is the problem?

Carbon oxide, water & nutrients



Primary metabolites
(Common in plants)

Secondary metabolites
(Specific to each plant)

>200,000 metabolites in the plant kingdom

Is it possible to identify all?

Liquid Chromatography Fourier Transform Ion Cyclotron Resonance Mass Spectrometer

LC –FT/ICR-MS

LTQ-FT
(Thermo Electron Co.)
Magnetic field: 7 T

LC-FT-Orbitrap-MS

- **Ultra-high mass accuracy, ~ 1 ppm**
  Using internal standards 0.5~0.2 ppm
- **Coupling LC prior to MS**
  - **Prevents ion suppression**
  - **allows UV/Vis spectrum acquisition**
  - **Separates isomers**
  - **allows acquisition of Retention Time**
  - **RT information is useful**
    to discriminate
    - **isotopic ions**
    - **adduct ions**
    - **spontaneously fragmented ions**
- **Automated MS/MS acquisition**

Ultra-high mass accuracy measurement

For example:

1ppm accuracy: m/z=500.0000±0.0005

$^{12}C = 12$
$^{13}C = 13.003355$
$^{1}H = 1.0078250319$
$^{16}O = 15.9949146223$
$^{14}N = 14.0030740074$
$^{31}P = 30.973763$
$^{32}S = 31.972071$

$[M+H]^+ = 410.0908$

$C_{18}H_{19}O_8NS$

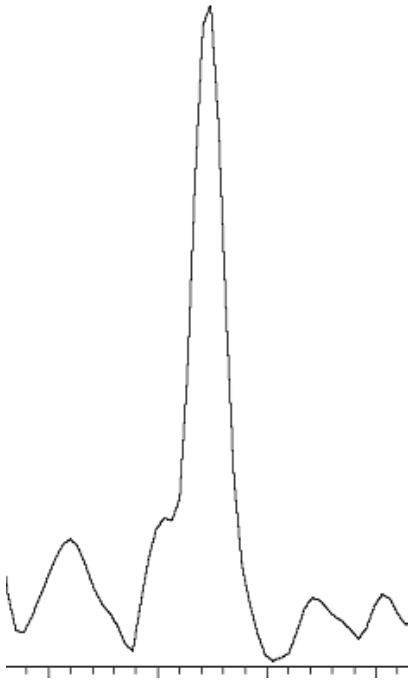
MS/MS

Structure Speculation

Speculation of Molecular Formula by FT-ICR-MS

$^{12}C = 12.000000$ $^{1}H = 1.007825$、$^{14}N = 14.003074$、$^{16}O = 15.994915$

433.11270

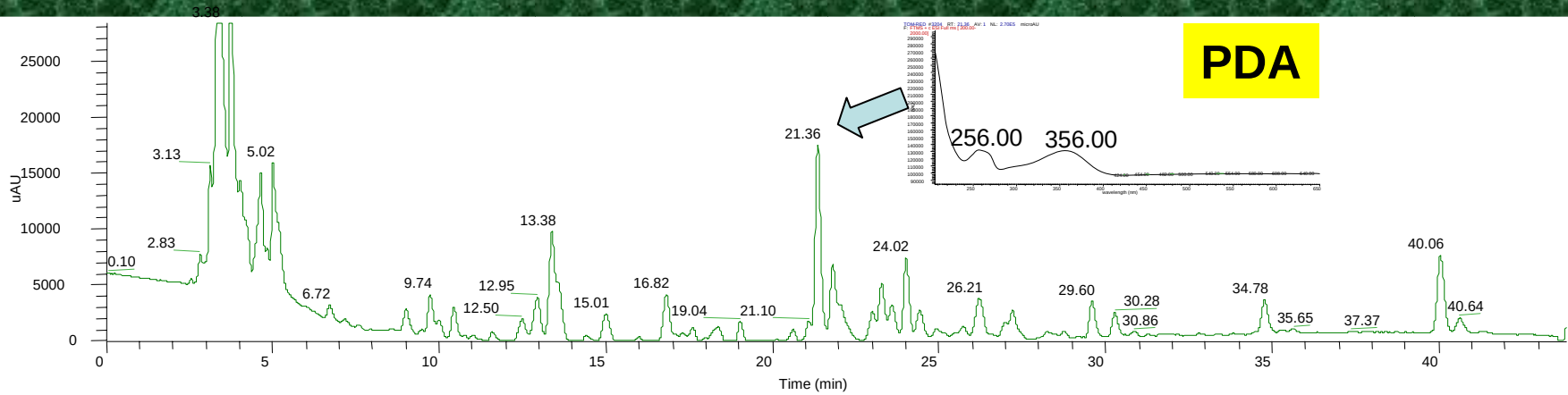


433.11270 → $C_{21}H_{20}O_{10}[+H]^+$

C	H	O	N	S		
21	21	10	0	0	433.112 7	2
13	25	12	2	1	433.112 2	7
11	13	2	16	1	433.112 2	6
14	29	7	2	3	433.113 1	3
11	21	1	12	3	433.111 7	9
.	.	.	.	.	.	
.	.	.	.	.	.	

Milli mass values give a limited number of formulas.

LC-FTICR-MS analysis of tomato fruit

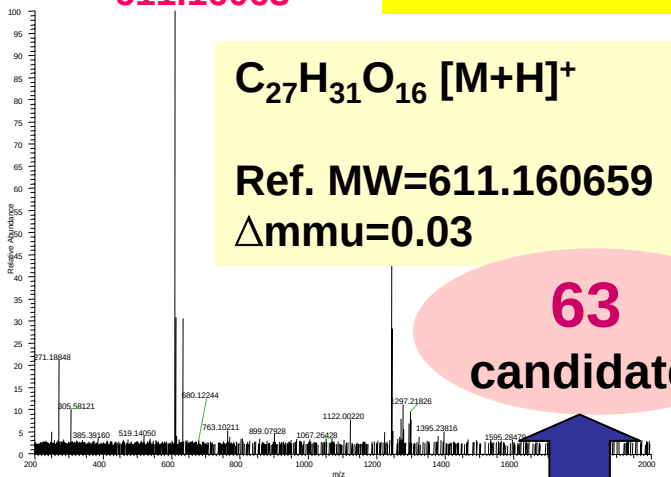


611.16063

FT-full MS

$C_{27}H_{31}O_{16}$ $[M+H]^+$

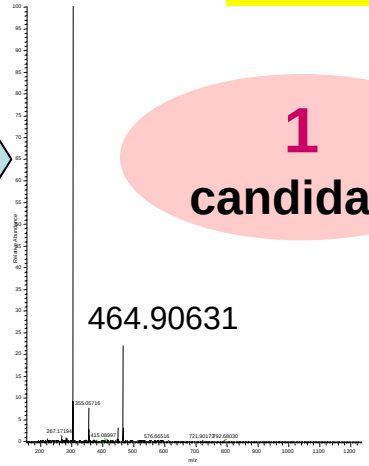
Ref. MW=611.160659
△mmu=0.03



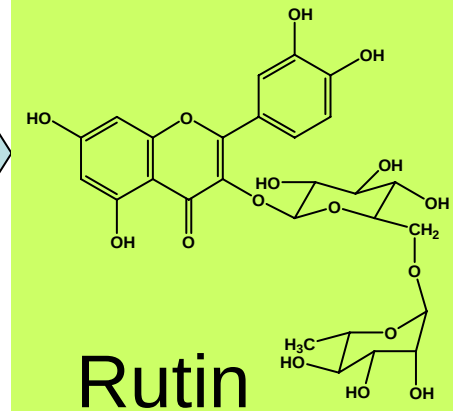
63 candidates

303.09119

$(MS)^n$



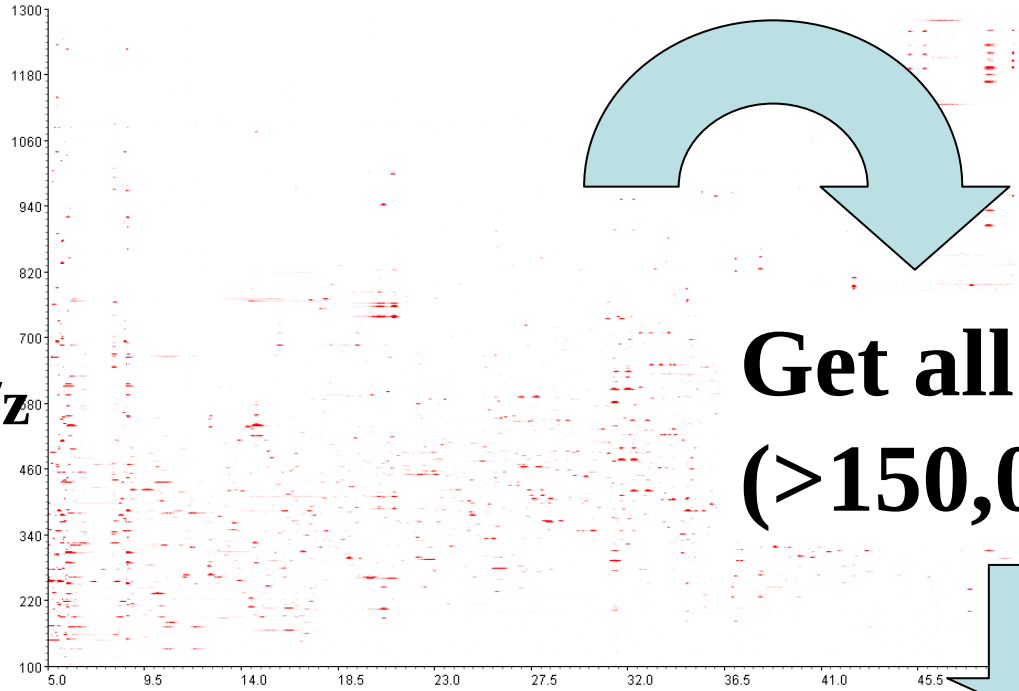
1 candidate



Metabolite Database

MS/MS Fragmentation
UV absorption (PDA)

Annotation, but not identification



Get all peak data (>150,000 peaks)

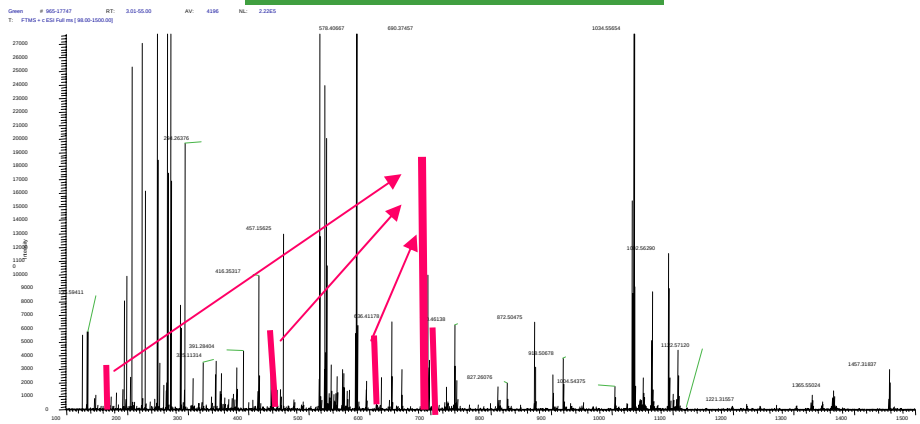
Data processing

Giving chemical information to each metabolite (Metabolite Annotation)

Gene and Metabolite annotation

Genomics

Metabolomics



Identify gene region

Identify molecular ion

DNA sequence
Coding protein

(Structural) Annotation

m/z, MS^n, Rt, λ max
molecular formula

Prediction of function, Homology search
Consistent identification of components across datasets

Expression, GO

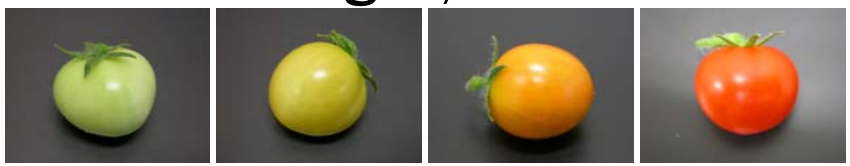
(Functional) Annotation

Accumulation, Pathway

Comprehensive analysis of tomato metabolites

Tissues

Fruit 4 stages, 2 tissues



G Y O R

Peel (P), flesh (F)

Leaf, Root, Flower

Extraction

70% methanol

100% chloroform

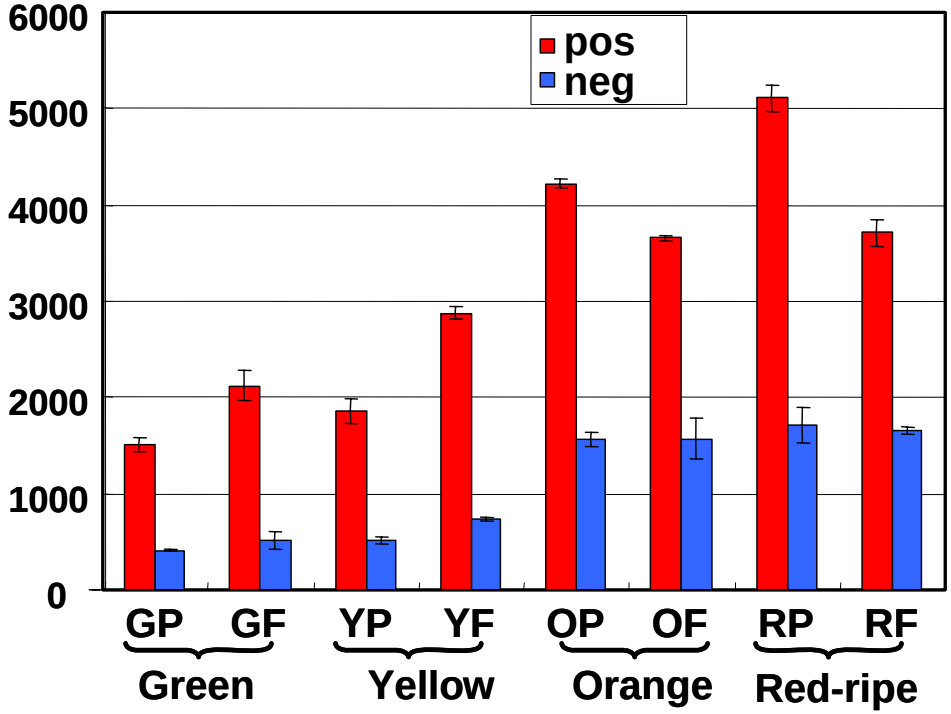
Ionization **LC** C18, C30

ESI (positive, negative)

APPI (positive, negative)

Number of detected peaks

ESI (Methanol fraction)



Iijima et al. (2008) Plant J.

How many metabolites ?

70% MeOH extraction

LC-ESI-based MS

Tissues	Ionization mode	Number of mass signals[a]	Number of peak groups[a]	Number of metabolites[a]	Number of annotated metabolites	Total number of annotated metabolites in each tissue[b]	Annotation grade A	B	C
Mature green									
Flesh	Positive	30 412 ± 3069	1470 ± 155	306 ± 35	154	267			
	Negative	17 292 ± 1483	1673 ± 102	305 ± 22	167				
Peel	Positive	42 734 ± 5067	2311 ± 260	479 ± 69	228	368			
	Negative	20 769 ± 2938	1925 ± 226	397 ± 51	228				
Breaker									
Flesh	Positive	28 782 ± 8835	1729 ± 271	357 ± 96	182	291			
	Negative	15 853 ± 4078	1604 ± 311	308 ± 66	168				
Peel	Positive	43 462 ± 9540	2621 ± 379	636 ± 119	250	440	23	236	181
	Negative	32 675 ± 4440	2733 ± 376	602 ± 85	295				
Turning									
Flesh	Positive	24 353 ± 6111	1680 ± 58	352 ± 26	188	284	15	158	111
	Negative	12 498 ± 4924	1239 ± 460	251 ± 134	156				
Peel	Positive	63 258 ± 6645	3495 ± 348	784 ± 112	358	611	26	329	256
	Negative	39 274 ± 3449	3187 ± 364	676 ± 79	402				
Red									
Flesh	Positive	28 109 ± 1791	1700 ± 132	353 ± 42	179	263	18	147	98
	Negative	13 808 ± 4403	1444 ± 414	266 ± 64	144				
Peel	Positive	70 278 ± 3619	4305 ± 288	1039 ± 77	445	696	29	372	295
	Negative	55 429 ± 2452	4723 ± 301	1026 ± 68	428				

[a]Numbers indicate means ± SD of three measurements.
[b]Total numbers of non-redundant annotated metabolites detected in positive- and negative-ionization modes.

Description of availability of supporting evidence

Total: 869 non-redundant metabolites
Including 494 metabolites not in the databases

Annotation of other metabolites Finished at molecular formula-level

Approx. 5600 non-redundant peaks

Checking candidate formulas, providing Evidences.

Detected	Ionization	Formula	GF	GP	YF	YP	OF	OP	RF	RP	Speculated
104.0704	Ep	C4H9NO2									GABA
130.0498	Ep	C5H7NO3									Pyroglutamic acid
134.0449	Ep	C4H7NO4									Aspartic acid
138.0549	Ep	C7H7NO2									p-Aminobenzoic acid
147.0765	Ep	C5H10N2O3									glutamine
147.1128	Ep	C6H14N2O2									L-Lysine
148.0605	Ep	C5H9NO4									L-Glutamic acid
156.0768	Ep	C6H9N3O2									L-Histidine
166.0863	Ep	C9H11NO2									L-Phenylalanine
175.0966	Ep	C8H14O4									Suberic acid
175.1190	Ep	C6H14N4O2									L-arginine
189.1598	Ep	C9H20N2O2									
191.1026	Ep	C7H14N2O4									meso-2,6-Diaminoheptanedioate
193.0344	Ep	C6H8O7									citric acid
205.0971	Ep	C11H12N2O2									Tryptophan
207.1128	Ep	C11H14N2O2									12-Hydroxycytisine
212.0919	Ep	C10H13NO4									
219.1492	Ep	C13H18N2O									
230.0151	Ep	C5H11NO5S2									
245.0769	Ep	C9H12N2O6									5-Ribosyluracil
251.0696	Ep	C8H14N2O5S									
251.1390	Ep	C13H18N2O3									N-Caffeoylputrescine
261.0370	Ep	C6H13O9P									
263.1392	Ep	C14H18N2O3									

Iijima et al. (2008) Plant J.

Annotation for major secondary metabolite Flavonoids

Rt	Detected	Theoretical	delta ppm	Formula	MS/MS	λ max	GP	GF	YP	YF	OP	OF	RP	RF
27.06	394.0954	394.0955	-0.21	C18H19O7NS	273(100)	290			■		■		■	■
26.56	394.0966	394.0955	-0.54	C18H19O7NS	273(100)	290							■	■
24.02	410.0906	410.0904	0.55	C18H20O8NS	289(100), 375(13)	284							■	
24.48	410.0907	410.0904	0.62	C18H20O8NS	289(100), 375(13)	284					■		■	
33.9	435.1288	435.1286	0.47	C21H22O10	273(100)	284, 336(w)							■	■
34.86	435.1288	435.1286	0.55	C21H22O10	273(100)	362			■		■		■	
37.19	435.1284	435.1286	-0.29	C21H22O10	273(100)	368			■		■		■	■
37.65	435.1288	435.1286	0.55	C21H22O10	273(100)	368	■		■		■	■	■	■
26.16	449.1077	449.1078	-0.31	C21H20O11	287(100)	256,354			■		■		■	
33.57	449.1083	449.1078	1.03	C21H20O11							■		■	
35.81	451.1231	451.1235	-0.86	C21H22O11	289(100)	262,354					■		■	
40.29	451.1236	451.1235	0.18	C21H22O11	289(100)	370					■		■	
29.31	451.1238	451.1235	0.65	C21H22O11	289(100)	284					■		■	
30.63	465.1030	465.1028	0.52	C21H20O12	303(100)	256, 352			■		■		■	
34.52	465.1393	465.1391	0.44	C22H24O11	303(100)	284, 358					■		■	
26.49	495.1860	495.1861	-0.23	C24H30O11	289(100), , 451(24), 452(5), 477(4)		■	■	■	■	■	■	■	■
37.78	521.1286	521.1290	-0.63	C24H24O13	273(100), 274(11), 297(5), 315(9), 339(5), 381(8), 485(7), 503(14), 504(3)	290, 366					■		■	■
41	521.1288	521.1290	-0.32	C24H24O13	273(100), 274(12), 297(3), 315(4), 339(2), 381(3), 485(2)	366							■	
33.42	537.1237	537.1239	-0.34	C24H24O14	289(100), 290(10), 313(7), 331(14), 355(7), 383(4), 397(8), 501(11), 519(19)	284							■	
38.2	551.1395	551.1395	-0.06	C25H26O14	303(100), 304(13), 327(5), 345(10), 369(5), 383(4), 401(3), 411(7), 515(9), 516(2), 533(17), 534(4)	268, 358							■	
20.22	556.1479	556.1481	-0.72	C24H29O12NS	273(100), 274(15), 315(2), 394(2), 435(55), 436(10)	286					■		■	■
20.67	556.1481	556.1483	-0.43	C24H29O12NS	273(100), 274(7), 284(9), 297(1), 394(49), 399(1), 417(2), 435(14)	286			■				■	■
20.96	556.1481	556.1483	-0.35	C24H29O12NS	273(100), 274(11), 315(2), 394(3), 435(85), 436(10)	328?							■	■
21.26	556.1476	556.1483	-0.06	C24H29O12NS	273(100), 274(12), 315(1), 394(2), 417(2), 435(7), 436(1)	286							■	■
33.12	567.1711	567.1708	0.54	C26H30O14	273(100), 315(4), 399(3), 417(11), 435(19)	366					■		■	■
19.97	572.1430	572.1432	-0.48	C24H29O13NS	289(100), 290(14), 451(12)	292							■	
33	581.1864	581.1865	-0.14	C27H32O14	273(100), 314(2), 315(4), 383(4), 401(5), 417(7), 419(13), 435(24), 527(4), 561(5)	370			■		■		■	
31.28	595.1660	595.1657	0.34	C27H30O15	287(100), 433(2), 449(25)	266, 348	■		■		■		■	■
28.53	611.1608	611.1607	0.29	C27H30O16	303(100), 397(1), 448(3), 449(1), 465(27)	256, 354	■	■	■	■	■	■	■	■
20.93	611.1605	611.1607	-0.32	C27H30O16	287(29), 365(5), 368(4), 397(35), 436(4), 448(56), 449(100), 450(10), 577(8)	268, 330							■	■
23.3	614.2082	614.2080	0.40	C27H35O15N	273(100), 435(66), 436(4), 597(7)	286					■		■	
18.8	627.1555	627.1556	-0.12	C27H30O17	303(24), 465(100), 466(9)	258, 356					■		■	
24.56	630.2032	630.2032	0.52	C27H36O16N	289(49), 451(100), 452(3), 586(5), 612(8), 613(29)	284							■	
24.61	642.1517	642.1512	0.70	C23H31O20N	273(57), 274(8), 315(6), 339(2), 381(3), 485(4), 503(8), 504(3), 521(100), 522(21)	284					■		■	
35.34	683.1816	683.1818	-0.28	C30H34O18	273(12), 297(3), 315(7), 381(4), 485(6), 503(28), 521(100), 665(4)	280							■	■
25.92	743.2026	743.2029	-0.44	C32H38O20	303(100), 435(2), 449(3), 465(13), 597(6), 611(38)	256, 354	■	■	■	■	■	■	■	■
28.03	727.2074	727.2080	-0.83	C32H38O19	287(100), 419(3), 433(4), 449(23), 581(6), 595(57)	266, 352	■	■	■	■	■	■	■	■
20.65	757.2185	757.2186	-0.09	C33H40O20	287(48), 433(4), 449(100), 595(36), 611(66), 612(2)	226, 354	■		■		■		■	■
18.64	773.2133	773.2135	-0.24	C33H40O21	303(58), 449(7), 465(100),611(36), 627(59)	258, 356	■		■		■		■	■
23.9	773.2135	773.2135	0.02	C33H40O21	303(66), 431(4), 449(21), 465(100), 610(55), 611(48), 754(3)	284							■	■
40.47	811.2659	811.2655	0.50	C37H46O20	287(100), 345(5), 433(4), 449(11), 503(2), 595(5), 665(6), 666(2), 680(5), 737(11)	weak	■		■		■		■	
35.83	813.2444	813.2448	-0.53	C36H44O21	303(100), 331(4), 449(4), 465(9), 505(3), 611(5), 667(13), 680(15), 681(5), 723(4), 737(32), 752(5), 789(7), 794(3), 802(4), 803(26)	weak	■		■		■		■	
38.33	827.2604	827.2604	-0.04	C37H46O21	303(100), 304(11), 327(5), 345(10), 369(5), 383(4), 401(3), 411(7), 515(9), 516(2), 533(19), 534(4)	256, 356	■	■	■	■	■	■	■	■
38.78	831.2341	831.2342	-0.15	C39H42O20	287(100), 288(3), 365(4), 383(4), 433(3), 449(12), 523(3), 595(6), 669(3), 685(5)	290, 336	■		■		■		■	
24.39	843.2186	843.2190	-0.43	C36H42O23	287(12), 535(100), 681(5), 697(10)	284							■	
36.66	847.2289	847.2291	-0.43	C39H42O21	303(100), 30492), 365(3), 383(3), 449(4), 465(11), 539(4), 563(2), 581(3), 611(7), 683(2), 685(6), 701(11), 801(2), 811(2), 827(2), 828(2), 829(2), 830(2)	268,352					■		■	
35.55	873.2443	873.2448	-0.56	C41H44O21	287(100), 389(5), 407(4), 425(16), 433(4), 441(2), 449(21), 565(4), 579(3), 595(7), 711(4), 726(3), 727(7)	284, 370			■		■		■	
33.59	889.2388	889.2397	-1.01	C41H44O22	303(100), 345(4), 389(3), 407(4), 425(13), 449(7), 465(25), 581(7), 611(8), 693(4), 727(7), 743(13)	288, 394	■		■		■		■	
35.24	903.2552	903.2553	-0.17	C42H46O22	287(100), 291(11), 309(4), 329(4), 419(5), 433(3), 437(2), 449(31), 454(6), 455(14), 471(4), 595(12), 705(2), 721(4), 741(4), 757(9), 883(5), 884(7), 885(3)	328	■		■		■		■	■
17.1	905.2551	905.2557	-0.71	C38H48O25	303(52), 308(20), 309(3), 345(3), 435(10), 449(8), 465(100), 470(3), 580(5), 597(14), 611(37), 613(4), 627(39), 742(35), 743(16), 759(6), 773(87), 775(4)	weak	■		■		■		■	
33.37	919.2495	919.2503	-0.83	C42H46O23	303(100), 309(4), 345(3), 419(4), 449(5), 455(12), 465(30), 471(4), 611(14), 755(4), 756(4), 757(6), 772(2), 773(9), 901(3)	290, 348	■		■		■		■	■
35.71	923.2822	923.2816	0.66	C42H51O23	303(100), 345(3), 423(3), 441(3), 449(5), 456(9), 465(24), 611(4), 615(6), 761(4), 777(8)	282, 384	■		■		■		■	■
33.98	933.2651	933.2659	-0.89	C43H48O23	287(100), 303(4), 321(16), 339(7), 353(3), 433(3), 449(40), 485(21), 501(3), 595(6), 625(5), 771(7), 787(14)	284, 336	■		■		■		■	■
32	949.2604	949.2608	-0.45	C43H48O24	275(3), 303(100), 321(13), 339(5), 345(3), 449(11), 465(28), 485(16), 501(3), 611(8), 641(4), 787(8), 803(14), 870(2), 887(27), 888(8), 929(3)	332	■		■	■	■		■	■
34.72	951.3120	951.3129	-0.91	C44H54O23	287(100), 303(3), 321(5), 339(4), 357(5), 433(5), 449(37), 467(6), 485(13), 499(6), 503(18), 517(5), 595(8), 643(7), 647(3), 789(3), 805(9), 932(3)	282, 378	■		■		■		■	
37.78	955.2550	955.2503	0.85	C45H46O23	273(24), 435(100), 521(29), 907(6), 908(16), 909(4)	366							■	
32.95	967.3139	967.3137	0.26	C37H58O29	303(100), 321(3), 339(3), 357(4), 449(7), 465(25), 467(4), 485(10), 502(3), 503(12), 611(7), 659(5), 805(4), 821(9)	weak	■		■		■		■	■
17.4	977.3071	977.3074	-0.26	C49H52O21	303(5), 465(18), 611(12), 627(11), 773(100)	270, 354							■	

58 flavonoids

More abundant in the peel than in the flesh.

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Annotation for major secondary metabolite Glycoalkaloids

	Rt	Detected	delta mass	Formula	G	Y	O	R	L	S	F	MS/MS
1	32.81	738.44257	738.442	C39H63O12N								414(11), 558(4), 576(60), 577(21), 600(4), 704(2), 720(100), 721(42)
2	35.03	738.44275		C39H63O12N								255(49), 273(9), 386(6), 396(40), 414(100), 576(22)
3	31.64	756.45313	756.453	C39H65O13N								738(100)
4	33.2	756.4538		C39H65O13N								722 (90), 720(100), 576(66), 558(5), 414(10), 255(6)
5	43.17	842.46972	842.469	C46H69O12N								396(3), 414(4), 456(8), 542(17), 680(80), 720(43), 824(100), 825(54)
6	38.78	872.50244	872.5	C44H73O16N								398(2), 416(2), 710(2), 854(100)
7	33.77	906.51062	-0.55	C44H75O18N								415(2), 577(100), 709(2), 739(3), 871(5)
8	42.51	912.47485	912.474	C49H69O15N								394(9), 412(8), 454(8), 540(11), 718(27), 750(69), 880(18), 882(100)
9	38.09	914.4923		C49H71O15N								394(55), 412(29), 466(100), 718(18), 736(11), 750(54), 882(30), 894(66)
10	38.95	914.49188	914.496	C49H71O15N								396(32), 412(12), 414(13), 542(16), 720(49), 734(8), 752(66), 884(72), 896(100)
11	41.7	914.49188	914.496	C49H71O15N								396(18), 414(11), 456(8), 542(14), 720(35), 752(76), 884(63), 896(100)
12	29.06	916.49304	916.49	C45H73O18N					\			412(1), 430(1), 898(100)
13	28.23	918.50903	918.506	C45H75O18N								414(4), 432(2), 576(1), 593(1), 898(2), 900(100)
14	29.75	918.50903	918.506	C45H75O18N								414(0.2), 432(3), 576(1), 593(1), 899(5), 900(100)
15	30.13	918.50897	918.506	C45H75O18N								414(2), 432(4), 576(2), 900(100)
16	31.55	918.50708	918.506	C45H75O18N								414(8), 432(4), 576(2), 594(2), 898(1), 900(100)
17	32.1	918.50635	918.506	C45H75O18N								396(2), 414(5), 432(3), 576(2), 594(1), 738(2), 756(2), 882(12), 900(100), 901(53)
18	41.03	918.50891		C45H75O18N								414(12), 432(17), 576(7), 594(6), 738(9), 756(6),900(100)
19	31.79	920.52399	920.521	C45H77O18N								398(3), 416(5), 560(2), 578(3), 740(2), 884(100), 885(44)
20	31.85	902.51312		C45H75O17N								398(5), 416(6), 578(2), 740(1), 884(100)
21	42.86	940.4715	940.469	C50H69O16N								392(49), 410(48), 539(17), 716(31), 778(100), 908(18), 910(42), 925(40)
22	39.15	940.54865	-0.64	C45H81O19N								289(13), 325(7), 365(3), 383(4), 401(14), 419(23), 487(4), 596(36), 599(100), 760(17), 761(32)
23	42.26	942.48578	942.485	C50H71O16N								394(11), 412(10), 454(4), 540(6), 718(18), 780(44), 910(11), 912(100)
24	37.76	944.50159	944.5	C50H73O16N								396(14), 414(13), 542(20), 720(22), 782(66), 896(18), 914(80), 926(100)
25	40.72	944.50208	944.5	C50H73O16N								396(17), 414(13), 542(9), 718(9), 720(36), 723(17), 780(8), 782(83), 896(16), 912(19), 914(71), 926(100), 927(55)
26	39.34	944.50201		C50H73O16N								396(58), 414(100), 531(16), 641(11), 780(12), 912(11), 928(11)
27	31.5	974.49829	974.496	C47H75O20N								410(2), 470(1), 867(3), 896(4), 914(100), 956(6)
28	32.23	976.51514	976.511	C47H77O20N								412(1), 869(5), 898(10), 916(100), 958(8)
29	37.97	1004.54169	0.13	C49H81O20N								398(5), 416(11), 578(5), 842(4), 872(7), 986(100)
30	38.86	1004.54309	1004.54	C49H81O20N								398(7), 416(14), 530(3), 548(7), 842(4), 854(2), 872(18), 986(100)
31	34.48	1008.5336	1008.5	C47H77O22N								409(20), 427(30), 449(7), 505(100), 589(12), 607(65), 866(6)
32	40.15	1018.55835	-0.66	C50H83O20N								398(5), 416(16), 560(2), 578(6), 856(4), 886(6),1000(100)
33	36.05	1032.53958	1032.54	C50H81O21N								378(6), 396(12), 414(25), 576(26), 870(8), 900(12), 1014(100)
34	30.79	1034.55371	1034.55	C50H83O21N								398(3), 416(6), 578(4), 872(4), 902(13), 1016(100)
35	36.77	1034.55469	1034.55	C50H83O21N								398(5), 416(10), 578(7), 872(2), 884(1), 902(12), 1016(100)
36	33.17	1044.53955	1044.54	C51H81O21N								390(3), 408(2), 426(2), 570(2), 912(5), 1026(100)
37	34.12	1044.53955		C51H81O21N								390(3), 408(2), 426(2), 570(2), 912(5), 1026(100)
38	33.8	1046.5592	-0.66	C51H83O21N								410(2), 572(2), 866(1), 896(1), 914(3), 1028(100)
39	28.97	1048.53088	1048.53	C50H81O22N								374(4), 376(17), 394(43), 412(2), 430(2), 574(2), 1012(3), 1030(100), 1031(27)
40	27.54	1048.5321		C50H81O22N								394(2), 412(12), 430(8), 574(13), 592(10), 868(4), 886(3), 898(3), 916(3), 1030(100)
41	29.64	1048.56848	1048.57	C51H85O21N								412(2), 430(6), 592(4), 886(2), 916(4), 1030(100)
42	28.41	1050.54907	1050.55	C50H83O22N								414(6), 432(4), 576(5), 594(4), 900(2), 918(2), 1032(100), 1033(57)
43	29.9	1050.54895	1050.55	C50H83O22N								414(1), 432(1), 576(1), 594(1), 1032(100)
44	30.24	1050.55273	1050.55	C50H83O22N								414(4), 432(2), 576(3), 594(2), 900(1), 1032(100)
45	31.72	1050.54871	1050.55	C50H83O22N								414(11), 432(6), 576(8), 594(5), 900(2), 1032(100)
46	34.77	1050.54822	1050.55	C50H83O22N								384(5), 296(4), 414(15), 432(49), 576(10), 594(40), 888(11), 918930), 1002(20), 1030(11), 1032(100), 1033(54)
47	40.9	1050.54432	1050.55	C50H83O22N								414(6), 432(14), 594(16), 756(6), 888(14), 900(8), 918(100), 1032(32)
48	32.15	1052.56921	1052.56	C50H85O22N								414(1), 416(1), 432(1), 578(2), 1034(100)
49	35.48	1052.55286	1052.55	C51H83O22N								396(12), 414(19), 558(7), 576(22), 900(19), 1044(100)
50	36.6	1064.5575	1064.56	C51H85O23N								398(10), 416(16), 560(5), 578(16), 902(13), 1046(100)
51	30.44	1066.54468	1066.54	C50H83O23N								430(4), 592(3), 610(2), 995(2), 1038(1), 1046(1), 1048(100)
52	29.65	1068.56079	1068.56	C50H85O23N								414(1), 432(1), 576(2), 594(5), 1032(4), 1050(100)
53	34.930	1068.55872	-0.60	C50H85O23N								413(21), 575(40), 737(100), 869(50), 1031(17), 1033(63)
54	35.49	1090.55347	1090.56	C56H83O20N								412(3), 472(1), 574(3), 634(3), 898(1), 1030(100), 1072(6)
55	36.4	1092.56128	1092.56	C52H85O23N								414(2), 474(1), 636(2), 576(2), 1014(1), 1032(100), 1074(5)
56	35.9	1092.56128		C52H85O23N								414(3), 474(2), 1014(2), 1032(100), 1074(7)
57	35.87	1100.58643	1100.58	C51H89O24N								417(2), 485(2), 579(10) 607(2), 647(3), 665(9), 749(1), 892(2), 903(1), 1043(4), 1065(100), 1073(4), 1083(5)
58	29.7	1108.55188	-0.66	C52H85O24N								384(5), 401(4), 443(6), 444(12), 585(5), 590(5), 606(7), 618(3), 652(3), 1001(3), 1019(3), 1048(100), 1049(21), 1052(4), 1060(3), 1061(7), 1090(48), 1092(15)
59	32.33	1108.55334	1108.55	C52H85O24N								412(1), 430(2), 490(1), 574(1), 1030(8), 1048(100), 1090(9)
60	32.82	1110.57257	1110.57	C52H87O24N								576(2), 636(5), 1032(2), 1048(7), 1092(100)
61	31.68	1122.54823	0.04	C52H86O25N								444(20), 606(13), 930(9), 1062(85), 1064(11), 1104(16), 1105(24)
62	32.27	1122.5321	-0.37	C52H83O25N								426(5), 444(26), 606(14), 930(6), 990(5), 1062(100), 1065(69), 1104(11), 1105(21)
63	35.69	1122.57349	1122.57	C53H87O24N								414(4), 474(3), 576(2), 636(2), 1044(2), 1062(100), 1063(48), 1104(8), 1105(10)
64	45.76	1222.60815		C54H91O25N								325(5), 486(14), 649(5), 994(7), 1037(5), 1105(100), 1105(25)
65	34.04	1136.5863	-0.45	C54H89O24N								414(2), 1003(1), 1014(2), 1032(100), 1118(11)
66	23.2	1228.59375	0.07	C56H93O28N								430(5), 592(28), 754(18), 772(4), 1048(25), 1049(12), 1210(100)
67	29.44	1240.60291	0.24	C57H93O28N								412(1), 592(4), 634(1), 754(3), 1000(6), 1018(8), 1060(4), 1078(2), 1180(100), 1222(3)
68	30.9	1240.59766	0.06	C57H93O28N								412(1), 430(1), 592(4), 754(2), 772(3), 1030(5), 1048(8), 1090(3), 1108(3), 1180(100), 1222(3)
69	40.34	1240.61597	1240.62	C54H97O30N								416(4), 578(2), 766(3), 784(11), 1078(2), 1108(3), 1222(100)
70	44.5	1240.61487	1240.62	C54H97O30N								416(10), 578(3), 766(3), 784(22), 785(6), 946(2), 1078(5), 1108(7), 1222(100), 1223(57)
71	25.95	1242.60805	1242.61	C56H93O29N								414(1), 430(1), 610(2), 1210(8), 1228(100),
72	25.76	1246.6084	-0.87	C56H95O29N								430(1), 592(1), 1210(622), 1228(100)
73	24.89	1268.59326	0.60	C58H93O29N								381(1), 423(1),441(13), 603(9), 632(2), 1088(100), 1250(2)
74	28.26	1268.5955	0.56	C58H93O29N								410(2), 572(3), 590(10), 752(9), 812(2), 1028(3), 1046(10), 1088(3), 1106(4), 1208(100), 1250(2)
75	25.39	1270.61121	-0.41	C58H95O29N								443(8), 545(4), 605(6), 1030(4), 1090(100), 1210(2)
76	26.85	1270.60986	0.04	C58H95O29N								443(10), 605(7), 1030(4), 1061(2), 1072(6), 1090(100), 1108(4), 1210(3), 1252(3)
77	29.04	1270.60925	1270.61	C58H95O29N								412(1), 592(5), 754(4), 1030(6), 1048(8), 1090(2), 1108(3), 1210(100), 1252(2)
78	29.35	1284.58765	-0.43	C58H93O30N								444(2), 504(2), 606(5), 666(2), 768(3), 1062(6), 1122(100), 1123(8), 1203(1), 1122(100), 1123(8), 1224(7), 1225(3),
79	30.21	1298.6394	0.59	C60H99O29N								414(4), 576(4), 577(2), 680(3), 842(2), 843(3), 1014(2), 1032(100), 1033(41), 1136(5), 1137(3), 1280(10), 1281(4)
80	33.54	1298.63928	-0.09	C60H99O29N								394(1), 412(3), 430(5), 574(1), 592(3), 886(2), 916(6), 917(3), 1030(100), 1031(52), 1032(17)
81	33.2	1360.63892	0.59	C61H101O32N								413(93), 575(100), 707(30), 737(28), 869(23), 899(17), 1001(11), 1031(59), 1163(44), 1325(32)
82	32.52	1362.65515	0.06	C61H103O32N								397(17), 415(16), 577(9), 577(28), 595(9), 707(9), 709(100), 727(13), 739(12), 871(50), 1032(6), 1033912), 1164(7), 1165(7), 1195(13), 1325(22), 1326(22), 1327(48)
83	32.9	1362.65686	0.27	C61H103O32N								413(72), 415(39), 547(25), 575(74), 576(35), 577(52), 595(43), 709(83), 726(100), 739(23), 756(25), 870(24), 871(49), 888(49), 1031(32), 1032(53), 1163(26), 1325(32), 1326(29), 1327(60)
84	33.86	1362.66235	0.32	C61H103O32N								415(100), 575(77), 577(83), 708(11), 709(29), 739(21), 841(5), 870(3), 871(32), 901(19), 1003(7), 1033(50), 1164(5), 1165(25), 1194(6), 1195(9), 1325(5), 1226(11), 1327(23)
85	34.62	1362.65405	-0.26	C61H103O32N								397(7), 415(100), 577(1), 709(25), 739(22), 841(3), 870(3), 871(22), 901(20), 1003(5), 1032(11), 1033(40), 1063(5), 1164(13), 1165(12), 1194(22), 1214), 1212(5), 1326(29), 1327(31)
86	29.73	1364.67334	0.91	C61H105O32N								399(8), 417(17), 435(11), 566(10), 578(5), 596(21), 618(5), 711(6), 729(100), 758(10), 860(5), 873(5), 890(7), 891(33), 921(8), 1053(17), 1184(16), 1185(10), 1214(9), 1328(8)
87	33.06	1364.6779	0.64	C61H105O32N								415(71), 416(15), 577(86), 578(14), 597(9), 709(28), 729(63), 871(31), 872(13), 891(11), 1033(39), 1034(9), 1165(15), 1166(10), 1215(9), 1233(42), 1234(26), 1326(96), 1327(61), 1328(100)
88	33.44	1364.6779	-0.37	C61H105O32N								415(12), 416(4), 417(7), 576(4), 577(9), 578(4), 579(10), 597(8), 708(5), 711(11), 728(16), 870(4), 871(4), 891(5), 1033(4), 1165(9), 1326(11), 1327(88), 1328(100), 1329(10)
89	33.88	1364.67639	-0.10	C61H105O32N								415(100), 416(23), 577(75), 578(38), 709(29), 710(17), 739(31), 740(10), 871(32), 872(20), 901(11), 1033(42), 1034(28), 1165(22), 1166(14), 1195(15), 1326(13), 1327(22), 1328(33), 1329(61)
90	27.9	###########	0.08	C68H105O34N								430(1), 592(3), 754(2), 916(2), 1036(7), 1031(2), 1048(14), 1049(7), 1090(3), 1108(4), 1109(2), 1210(7), 1211(4), 1270(2), 1271(2), 1372(100), 1373(61), 1374(2), 1414(3), 1415(2)

90 glycoalkaloids

Mostly unknown.
During ripening, larger compounds increased.

Further glycosylation hydroxylation acetylation of pre-existing alkaloids?

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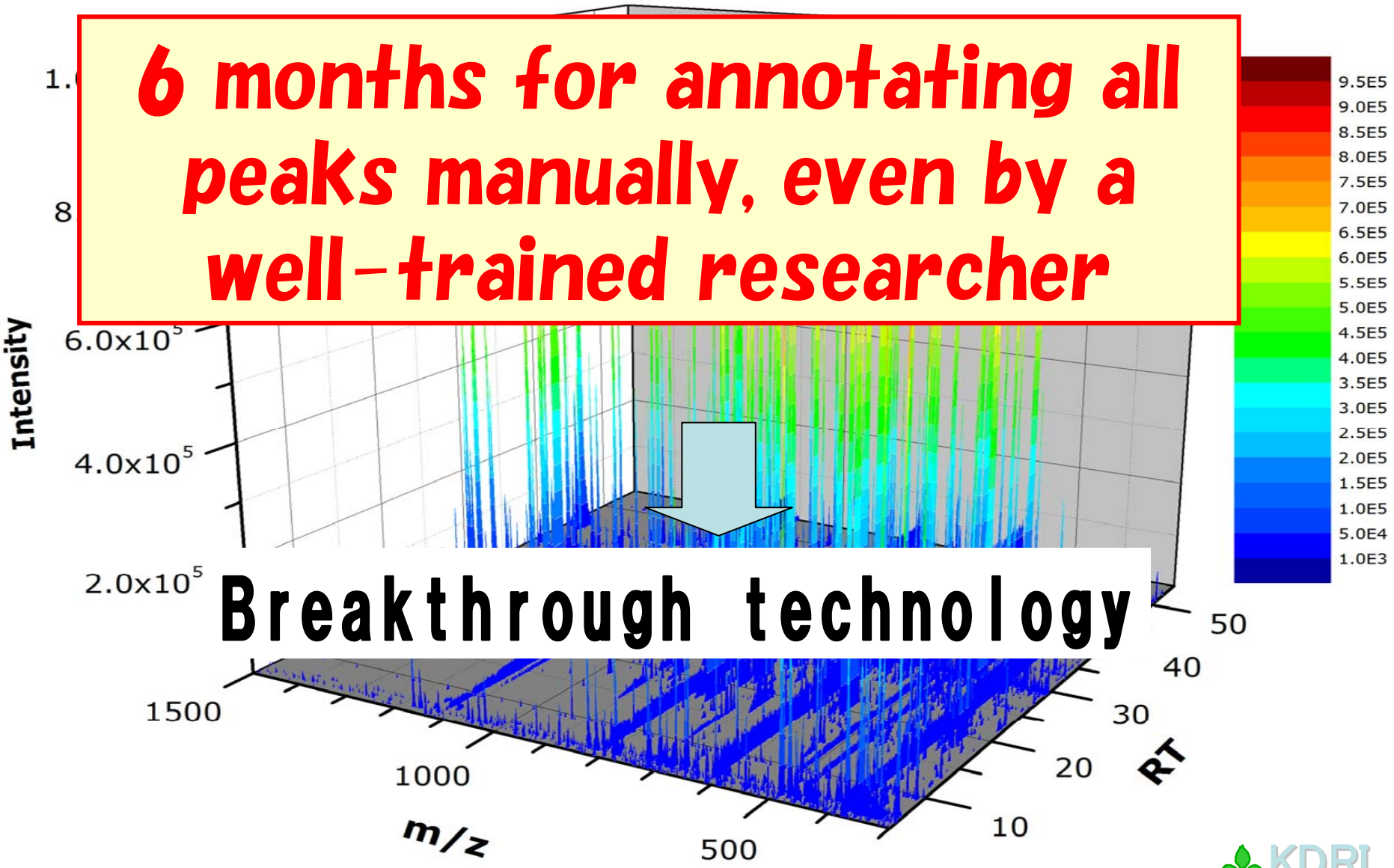
The major problem of this approach

6 months for annotating all peaks manually, even by a well-trained researcher

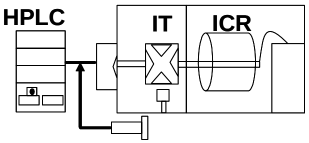


Breakthrough technology

KDRI

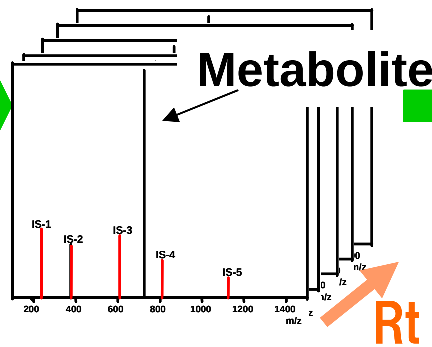
PowerFT: high-through-put pipeline

LC-FTICR/MS analysis



Internal standard mix
(m/z 235-1123, 4-5 compounds)

Raw data acquisition



m/z calibration by I.S.

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45 505.3608537090683^  9080.4599609375
46 563.7856319206811^  11845.8623046875
47 569.7864132550492^  11762.7734375
48 580.1462556075436^  8638.587890625
49 580.1603547001539^  156112.015625
50 581.163587536724^  31624.013671875
51 599.1977307919923^  50878.32421875
52 600.1994377527379^  12416.6328125
53 609.2806572^23903.119140625
54 610.2849098306476^  13073.103515625
55 611.1338920898123^  18487.01171875
56 611.1387137719458^  23682.087890625
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58 611.1607470282763^  1279004.875
59 612.1497411711599^  25184.765625
60 612.1646334552172^  375310.4375
61 613.166994033382^  55208.76953125
62 614.169598747351^  9905.435546875
```

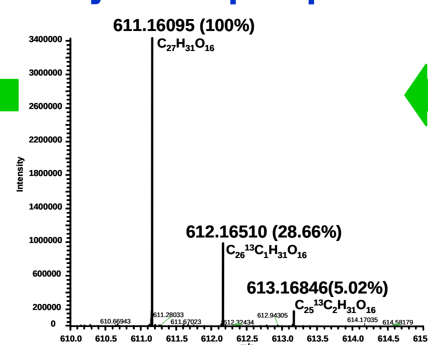
Extract m/z peak groups

Detection of isotopic peak pairs

MF calculation

```
303
304 611.1607470282763^  1279004.875
305 Total=76^   Actual Mw=610.1534719963763
306 C27H30O16      0 0 0 0 0 0 0 0 0^ 610.153384^ 8.708257632861205E-5
307 C25H18N14O6    0 0 0 0 0 0 0 0 0^ 610.153374^ 9.758477631294227E-5
308 C10H14N26O7    0 0 0 0 0 0 0 0 0^ 610.153876^ 4.049987237522146E-4
309 C12H26N12O17   0 0 0 0 0 0 0 0 0^ 610.153887^ 4.155009237365448E-4
310 C40H22N2O5     0 0 0 0 0 0 0 0 0^ 610.152871^ 6.0016827626441 23E-4
311 C41H18N6O1     0 0 0 0 0 0 0 0 0^ 610.154209^ 7.372445237479042E-4
312 C11H30N8O21    0 0 0 0 0 0 0 0 0^ 610.152550^ 9.219118762757716E-4
313 C9H18N22O11    0 0 0 0 0 0 0 0 0^ 610.152539^ 9.324140762601019E-4
314 C7H6N36O1      0 0 0 0 0 0 0 0 0^ 610.152529^ 9.429162762444321E-4
315 C26H14N18O2    0 0 0 0 0 0 0 0 0^ 610.154711^ 0.0012398280235856873
316 C28H26N4O12    0 0 0 0 0 0 0 0 0^ 610.154722^ 0.0012503302236837044
317 C24H22N10O10   0 0 0 0 0 0 0 0 0^ 610.152036^ 0.0014349975763252587
318 C22H10N24      0 0 0 0 0 0 0 0 0^ 610.152026^ 0.001445499776309589
319 C11H10N30O3    0 0 0 0 0 0 0 0 0^ 610.155214^ 0.0017424115236508442
320 C13H22N16O13   0 0 0 0 0 0 0 0 0^ 610.155224^ 0.0017529137237488612
321 C15H34N2O23    0 0 0 0 0 0 0 0 0^ 610.155235^ 0.0017634159237331914
322 C8H22N18O15    0 0 0 0 0 0 0 0 0^ 610.151202^ 0.0022698268762724183
323 C6H10N32O5     0 0 0 0 0 0 0 0 0^ 610.151191^ 0.0022803290762567485
324 C29H22N8O8     0 0 0 0 0 0 0 0 0^ 610.156059^ 0.002587743023696021
325 C23H26N6O14    0 0 0 0 0 0 0 0 0^ 610.150699^ 0.002772410376337575
326 C21H14N20O4    0 0 0 0 0 0 0 0 0^ 610.150689^ 0.0027829125763219054
```

Narrow down candidate formulas by isotopic pattern



MF confirmation

$C_nH_mO_p$

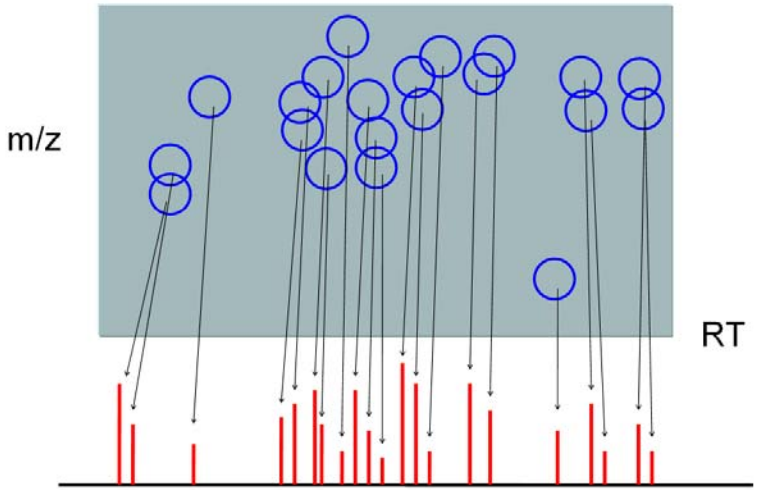
Manual assignment

Metabolite annotations

- MS^n
- PDA
- Database hit
- Literature

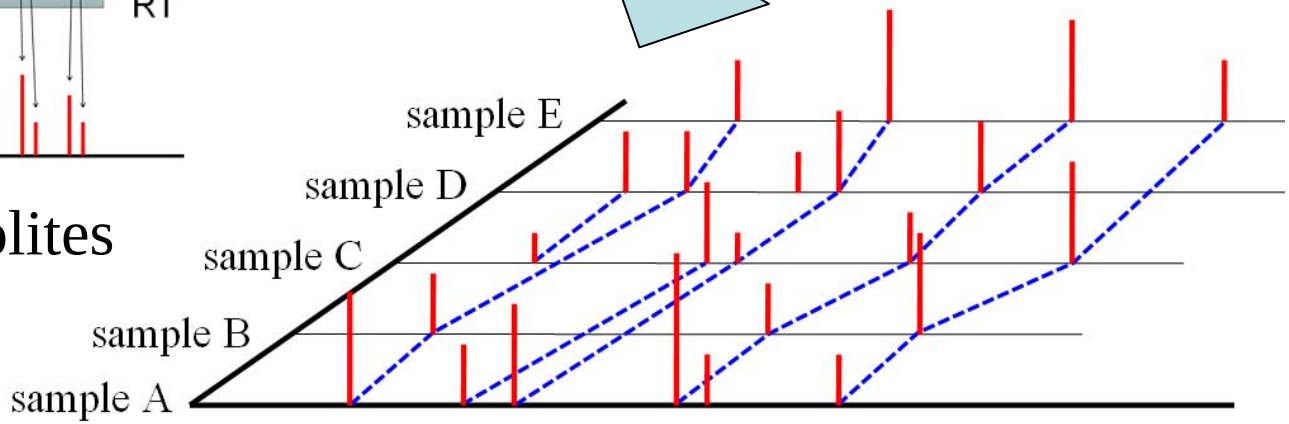
How to compare metabolomes

2-D image of LC-FT/ICR-MS



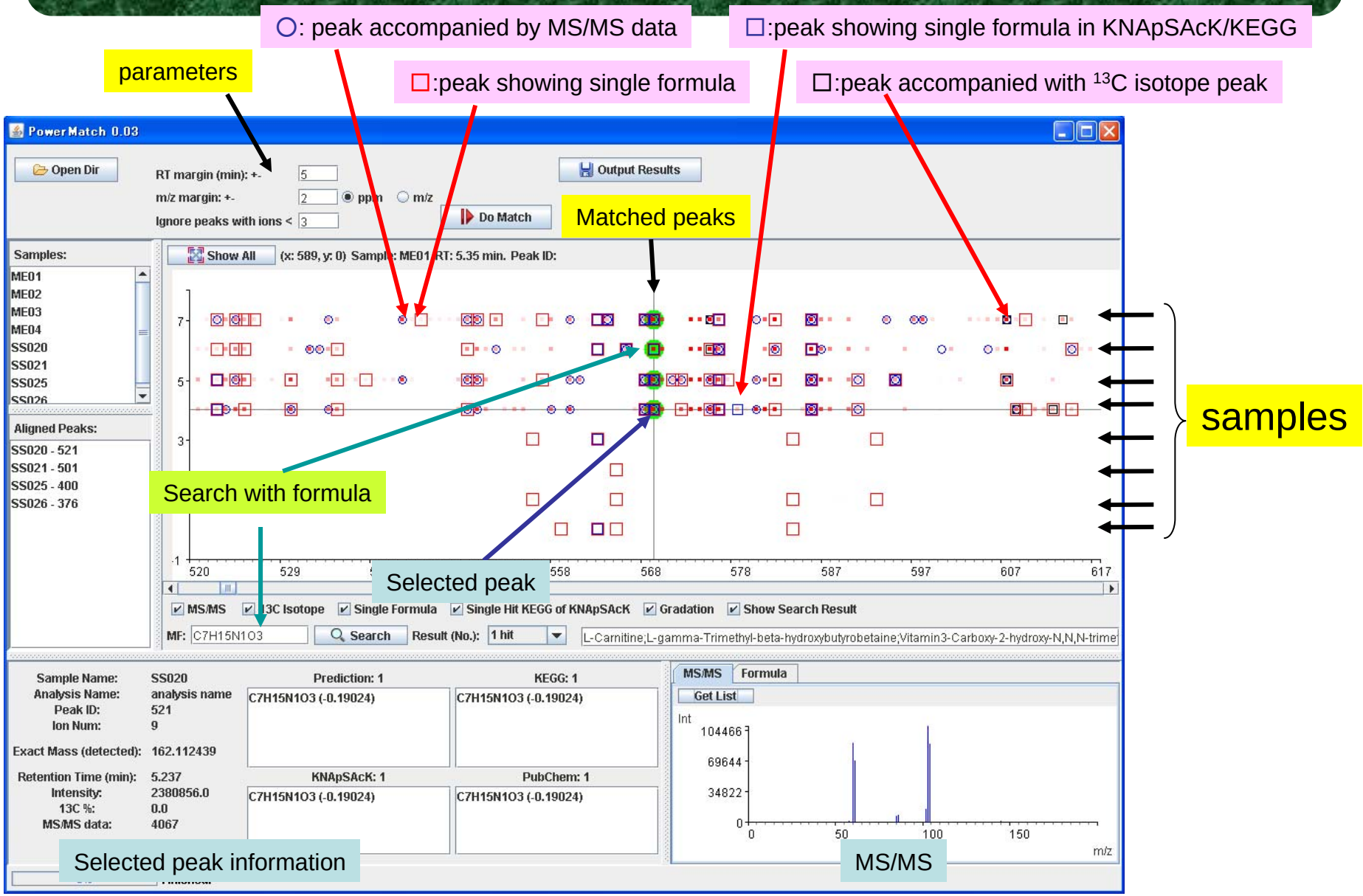
1-D image of metabolites

PowerMatch

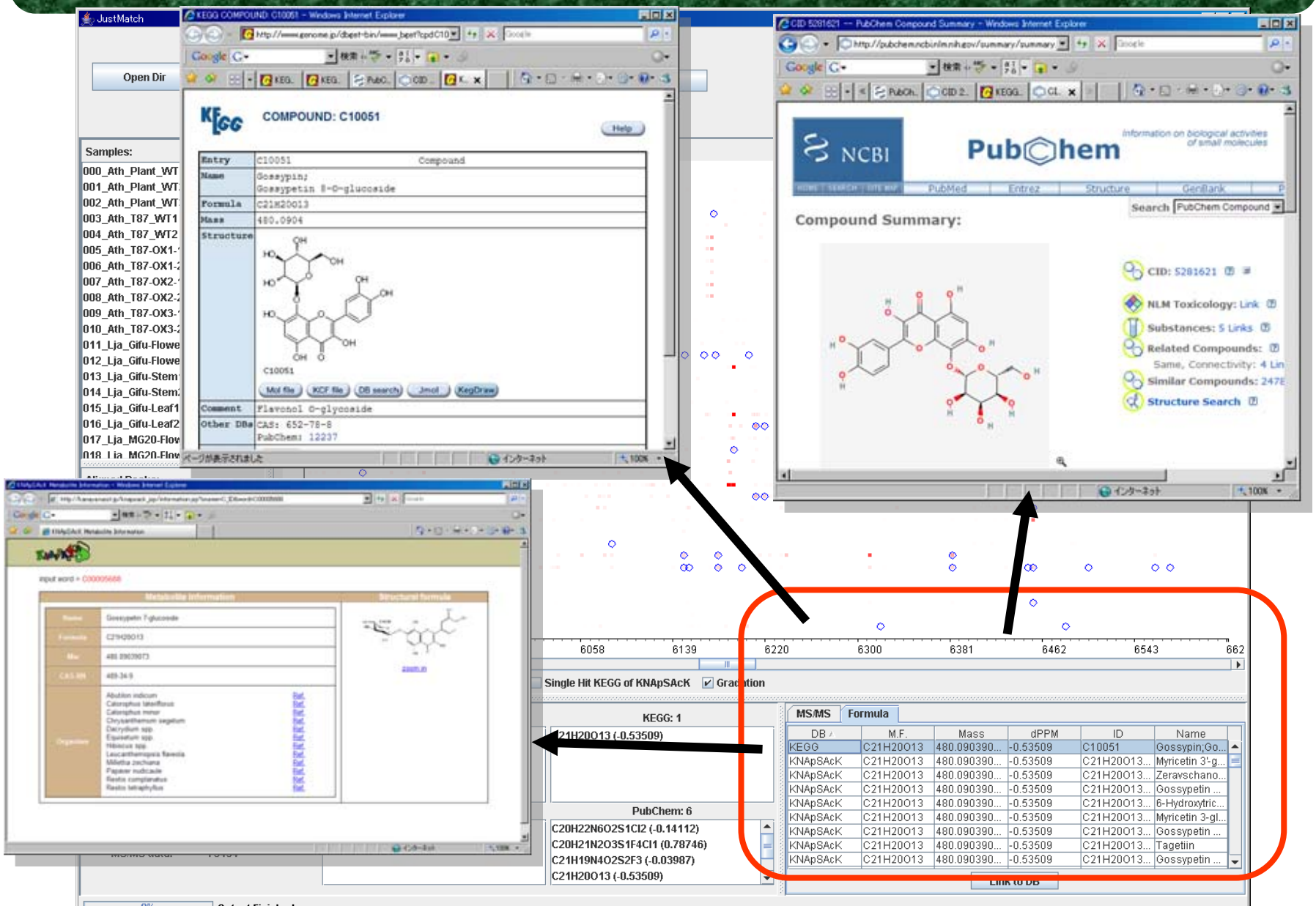


Alignment of metabolites with information of Retention time, mass values, MS/MS patters on 1-D

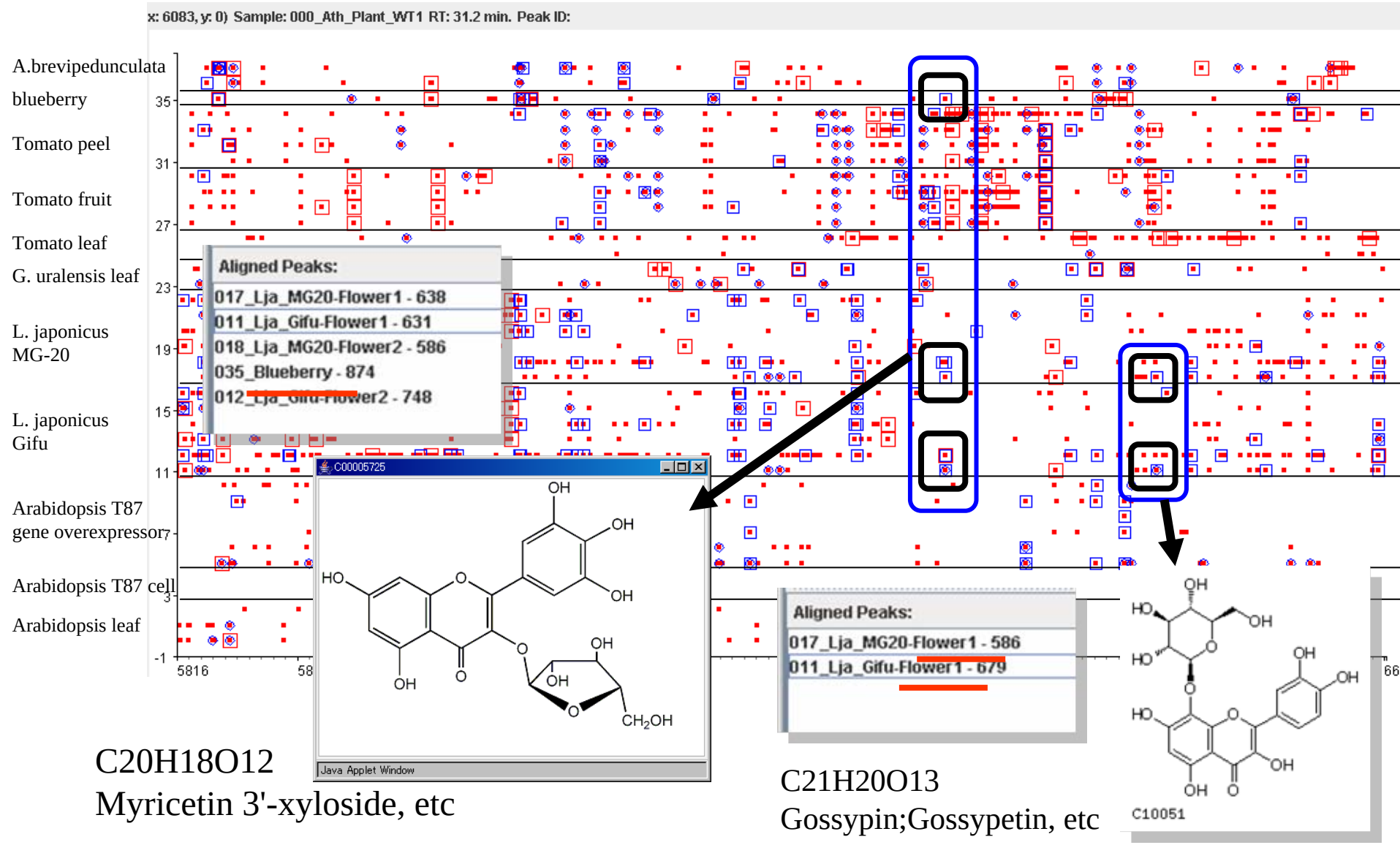
PowerMatch: aligment software



Links to Metabolite Databases



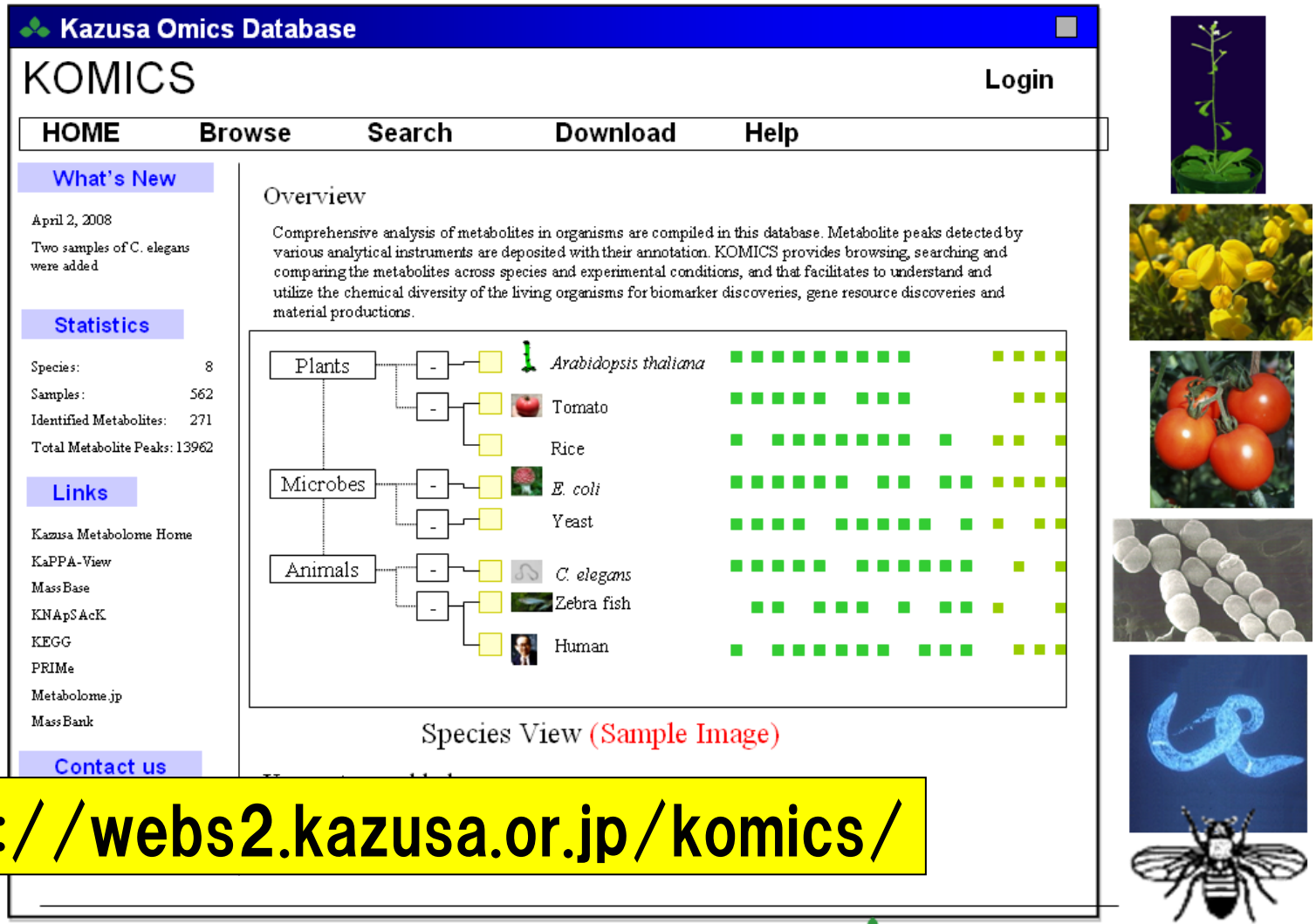
Comparison of 38 plant metabolomes



C20H18O12
Myricetin 3'-xyloside, etc

C21H20O13
Gossypin;Gossypetin, etc

Metabolite annotation database KOMICS



http://webs2.kazusa.or.jp/komics/

KDRI