JD4.2 A catalogue of aromatic molecules from C4 plants; continuous throughout the project sugar cane, maize.

Objectives:

To identify the aromatic metabolites present in sugar cane and maize lignocellulosic material.

Procedure:

Phenolic profiling is used to identify the aromatic molecules that are present in bio-energy grasses. P12 (VIB) has developed a new method to increase the throughput of metabolite identification. This method makes use of high-resolution mass spectral data only and is based on the mass differences between known substrates and products. These differ by a mass that is typical for enzymatic reaction(s), e.g. reduction, methylation, methoxylation, hydroxylation, hexosylation, etc... In addition, the expected shift in retention time during UHPLC-separation is taken into account. The combination of these two parameters (mass difference and retention time) is calculated for each peak pair in the chromatogram. Via in-house software developed by P12 (VIB), this lead to a list of "candidate substrate-product pairs" (CSPP's). This list is further visualized in the open source software Cytoscape and results in the CSPP-network. In addition, information about correlation in abundances of the possible substrate and product can then be added, as two correlated molecules have a higher chance to belong to the same molecular class. Furthermore, also the similarity of the MS2 data of both the possible substrate and the possible product can be added, because similar molecules often lead to similar fragmentations. This whole procedure has been optimized for phenolics in Arabidopsis because the number of known metabolites is higher in that plant species. A publication about this method is in revision.

Results:

The CSPP technique is used to analyze maize and sugarcane. For maize, a set of 44 stem extracts (22 young and 22 mature) were analyzed via high-resolution UHPLC-MS. Doing so, P12 (VIB) could annotate 94 compounds: 12 benzoxazinoids, 3 benzenoids, 15 phenylpropanoids, 11 flavonoids, 14 oligolignols, 22 flavonolignans, 15 aromatic esters and 2 other compounds (see table). As such, a catalogue of aromatic molecules from maize has been created of which the majority was never reported before. This catalogue will be further extended during the course of the SUNLIBB project. For sugar cane, the samples have been send by CeProBIO Proj. 4 coordinator (UFV), they have been analyzed via high-resolution UHPLC-MS, subjected to the CSPP algorithm and the data is currently being in further process.

nr	t_R (min)	m/z	Compound
1	2	299.20533	*p*-benzoic acid Hex
2	2.33	342.08180[a]	DIBOA-Hex
3	2.85	359.09712	syringic acid Hex
4	2.85	203.08235	tryptophane
5	4.18	353.08667	caffeoylquinic acid
6	4.75	326.08714	HBOA-Hex
7	4.98	342.08255[a]	DIBOA-Glc
8	5.74	355.10243	feruloyl hexose
9	5.98	356.09762[a]	HMBOA-Glc
10	6.07	534.1453[a]	DIMBOA-Glc-Pen
11	6.29	784.22711	FAD
12	6.33	372.09337[a]	DIMBOA-Glc
13	6.53	337.05594	*p*-coumaroyl/phenylpyruvoyl isocitric acid
14	6.58	402.10349[a]	Methoxy-DIMBOA-Glc
15	6.75	179.03499	caffeic acid
16	6.75	209.04542	5-hydroxyferulic acid - like
17	6.76	163.04022	*p*-coumaric acid
18	6.95	412.08734[b]	HBOA-Hex-Malon
19	7.15	428.08226[b]	DIBOA-Glc-Malon
20	7.39	371.09719	benzoyl glycerol hexuronate
21	7.48	223.0608	*p*-coumaric acid ethanediol ether or ester
22	7.97	563.13805	caffeoyl p-hydroxybenzoyl Pen-Pen
23	8.35	193.05055	ferulic acid
24	8.37	319.08144	p-coumaroyl shikimic acid
25	8.65	514.14822	methyldihydro-hydroxyferuloyl/trihydroxyphenylpyruvoyl glutathione
26	8.66	498.15362	methoxy-caffeoyl/methyldihydroferuloyl glutathione
27	8.69	482.12238	methoxy-*p*-coumaroyl/feruloyl glutathione
28	9	293.06611	p-coumaroyl 2-hydroxyglutarate
29	9.28	651.11775	chrysoeriol HexuroA-HexuroA
30	9.34	432.11372	HDMBOA-Pen + 76 Da
31	9.38	164.0354	HBOA
32	9.45	221.04539	p-coumaroyl 2-hydroxy acetate
33	9.57	577.15377	caffeoyl benzoyl pen-Pen
34	9.65	681.12692	tricin HexuroA-HexuroA
35	10.69	577.15398	caffeoyl benzoyl Hex-Pen
36	10.77	667.14806	tricin HexuroA-Hex
37	11.34	398.10837	HMBOA-Glc-Ace
38	11.46	547.1432	caffeoyl benzoyl Pen-Pen
39	11.56	307.08152	p-coumaroyl 3-hydroxy-3-methylglutaric acid
40	11.62	711.17357	glycerol tricin HexuroA-Pen
41	12.01	507.11314	syringetin Hex
42	12.12	607.12784	chrysoeriol HexuroA-Pen
43	12.16	389.12354	S(8–O–4)pCA
44	12.18	547.14362	caffeoyl benzoyl Pen-Pen
45	12.28	637.13921	tricin HexuroA-Pen
46	13.01	491.11796	tricin Hex
47	13.75	817.17917	tricin HexuroA-Pen-syringic acid
48	13.95	561.15905	caffeoyl benzoyl Deoxyhex-Pen
49	14.03	687.19038	G(8–O–4)tricin Hex
50	14.07	803.20141	tricin Hex-Pen-syringic acid

nr	t_R (min)	m/z	Compound
51	14.22	833.21077	G(8–O–4)tricin HexuroA-Pen
52	14.3	433.14896	S (8-O-4) S oxy
53	14.32	555.18649	G(8–O–4)G(8–O–4)pCA
54	14.42	787.16863	tricin HexuroA-Pen-vanillic acid
55	14.68	561.15934	caffeoyl benzoyl Deoxyhex-Pen
56	14.75	717.20116	S(8–O–4)tricin Hex
57	14.95	833.21111	G(8–O–4)tricin HexuroA-Pen
58	15.01	577.11809	tricin Hex-Malon
59	15.06	585.19596	G(8–O–4)S(8–O–4)pCA
60	15.26	745.26928	G(8–O–4)S(8–5)G Hex
61	15.74	813.18414	tricin HexuroA-Pen-ferulic acid
62	16.33	1009.25877	tricin HexuroA-Pen-FA(4–O–8)G
63	16.34	403.13886	αMe-S(8–O–4)pCA
64	16.86	373.12832	αMe-G(8–O–4)pCA
65	16.86	983.24185	G(8–O–4)tricin HexuroA-Pen-vanillic acid
66	17.08	583.21627	G(*t*8–O–4)S(8–5)G
67	17.45	403.1022	tricin glycerol
68	17.95	583.21617	G(*e*8–O–4)S(8–5)G
69	18.96	329.06579	tricin
70	19.36	581.20023	G(8–O–4)S(8–5)G´
71	19.79	581.20099	G(8–O–4)S(8–5)G´ ?
72	19.83	541.13322	H (8-O-4) tricin + formic acid
73	19.83	555.14936	S(*t*8–O–4)tricin ?
74	19.84	495.12873	H(*t*8–O–4)tricin
75	20.32	751.22203	G(8–O–4)S(8–O–4)tricin
76	20.42	571.14337	G(*t*8–O–4)tricin + formic acid
77	20.43	585.15917	9-O-acetyl G(8-O-4) tricin + H2O
78	20.43	525.13904	G(*t*8–O–4)tricin
79	20.59	541.13335	H (8-O-4) tricin + formic acid
80	20.59	495.12909	H(*e*8–O–4)tricin
81	20.59	555.14873	S(*e*8–O–4)tricin ?
82	20.67	581.20024	G(8–O–4)S(8–5)G´
83	21.19	585.15916	9-O-acetyl G(8-O-4) tricin + H2O
84	21.19	525.13882	G(*e*8–O–4)tricin
85	21.23	809.30133	G(8–O–4)S(8–8)S(4–O–8)G
86	21.93	809.29955	G(8–O–4)S(8–8)S(4–O–8)G
87	22.18	553.13345	vanilic tricin glycerol
88	24.64	567.14847	9-O-acetyl G(8-0-4)tricin
89	24.97	567.1498	9-O-acetyl G(8-0-4)tricin
90	25.61	701.18314	p-coumaroyl S(8-O-4) tricin
91	25.79	671.1728	p-coumaroyl G(8-O-4) tricin
92	25.81	672.17643	p-coumaroyl G(8-O-4) tricin C13 ?
93	26.89	897.25574	G(8-O-4)S (8-0-4) tricin rhamnoside
94	28.99	939.26667	9-O-acetyl G(8-0-4) S (8-O-4) tricin rhamnoside

discussion/conclusions.

Maize extracts have been analyzed by P12 (VIB) via UHPLC and CSPP. Manual curation resulted in the structural annotation of 94 compounds, many of these were never described before. Sugarcane extracts

have been analyzed by P12 (VIB) via UHPLC and CSPP, but need manual curation. We expect to have a library of the phenolic metabolites in sugarcane in Month 36. Generally, because of the high number of different phenolic compounds in plants, identification of compounds is a never ending task. As we will proceed in identification in WP4, new metabolites will be continuously be add to the library during the Sunlibb/CeProBio project.