QTL and underlying candidate genes controlling agronomical traits, fruit quality and aroma in octoploid strawberry

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Strawberry fruit is an economically important crop worldwide

Growth → Ripening

30-40 days

- Cell division and expansion
- Dismantling of photosynthetic apparatus
- Change in fruit firmness
- Reduction of organic acids
- Accumulation of sugars, phenolic compounds, ascorbic acid anthocyanins
- Changes in volatile profile

↑	glucose fructose sacarose
↑↓	citrate malate quinate Aa
↑↓	Hydroxybenzoic & hydroxycinnamic acids, flavonols, Flavonoids, Anthocyanins, Ellagitannins
↑	L-AA
↑↓	volatiles

Source: Fait et al., Plant Physiol. 2008.

Strawberry aroma

- More than 360 volatile compounds identified.
- Only 15-20 compounds are key for strawberry aroma.

  - Esters

  methyl and ethyl esters of hexanoic and butanoic acid, ethyl-2-methyl butanoate, hexyl and (E)-2-hexenil acetate

  - Terpenes

  Linalool, terpineol, Nerolidol…

  Aldehydes

  hexanal
  (Z)-3-hexenal

  - Ketones

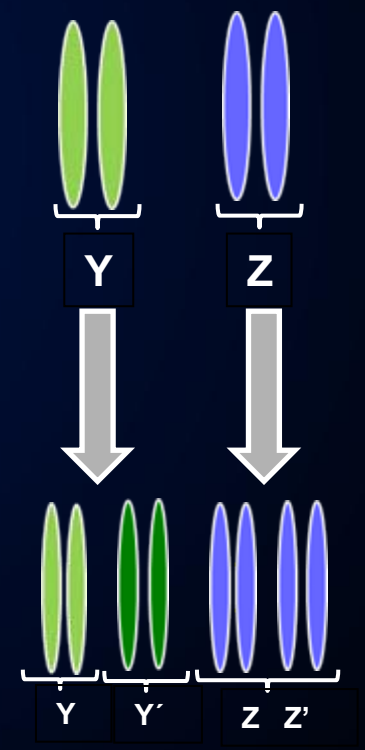
  2-heptanone, γ–decalactone

  - Furanones

  2,5-di-metil-4-hidroxi-3(2H)-furanone (HDMF, Furaneol)
  2,5-di-metil-4-metoxi-3-(2H)-furanone (DMMF, Mesifurane)

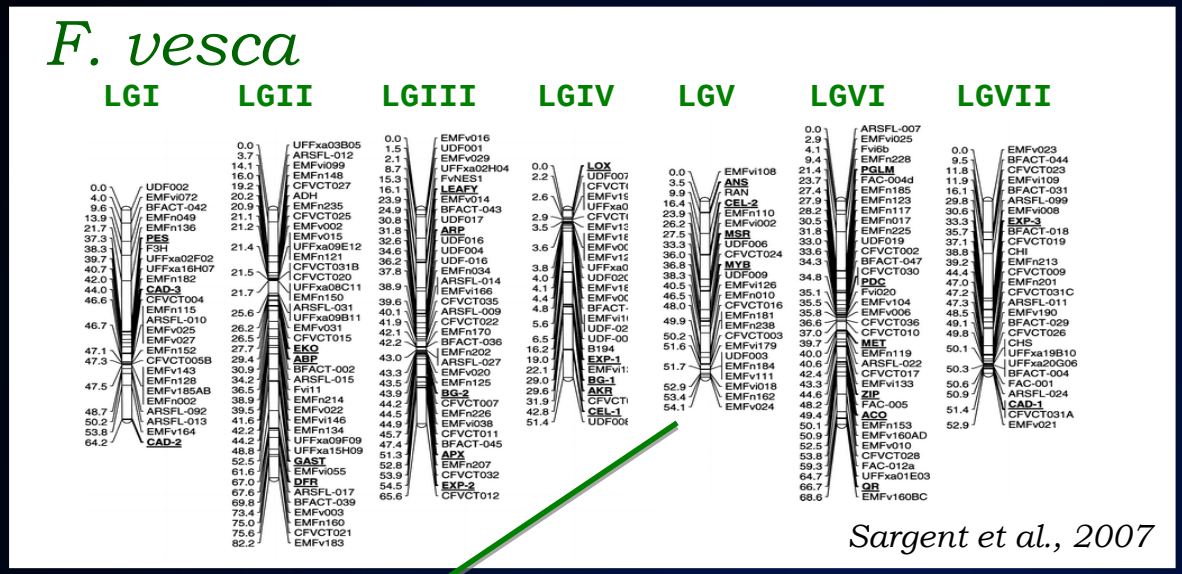
Cultivated strawberry is an octoploid species

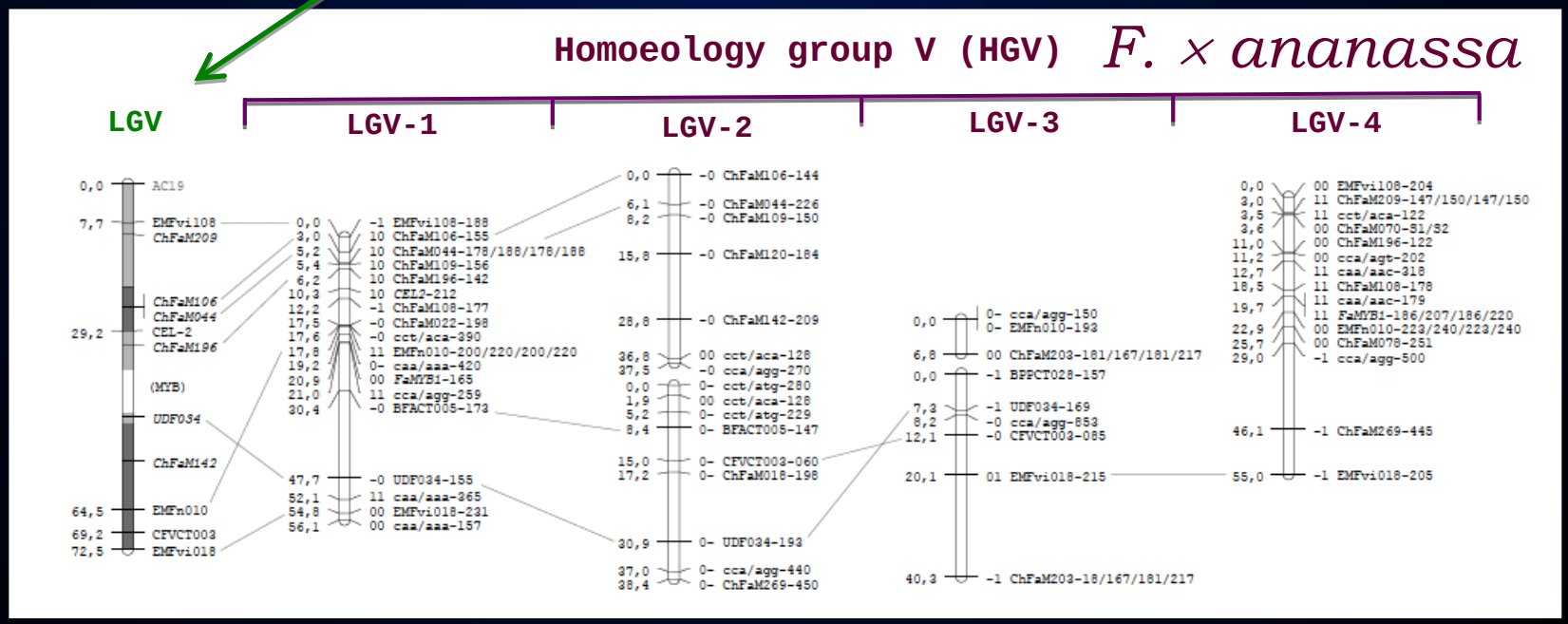
2X	*F. Bucharica*	
	F. daltoniana	
	F. × bifera	
	F. mandshurica	
	F. nilgerrensis	
	F. hayatai	
	F. nipponica	
	F. nubicola	
	F. pentaphylla	
	F. chinensis	
	F. viridis	
	F. vesca	2n=2x=14
	F. iinumae	
4X	*F. corymbosa*	
	F. gracilis	
	F. moupinensis	
	F. orientalis	
	F. tibetica	
6X	*F. moschata*	
8X	*F. chiloensis*	YYY´Y´ZZZ'Z'
	F. virginiana	
	F. × ananassa	2n=8x=56
	F. Cuneifolia	
	F. × bringhurstii	
10X	*F. iturrupensis*	



Rousseau-Gueutin et al. (2009)

The genomes of diploid and octoploid *Fragaria* are highly collinear





Project aims

1. Development of a 'functional' linkage map of octoploid strawberry
2. Localization of novel markers in the diploid *Fragaria* reference map
3. QTL mapping of agronomical and fruit quality traits

F. × *ananassa* SSRs → FREST http://www.fresa.uco.uma.es

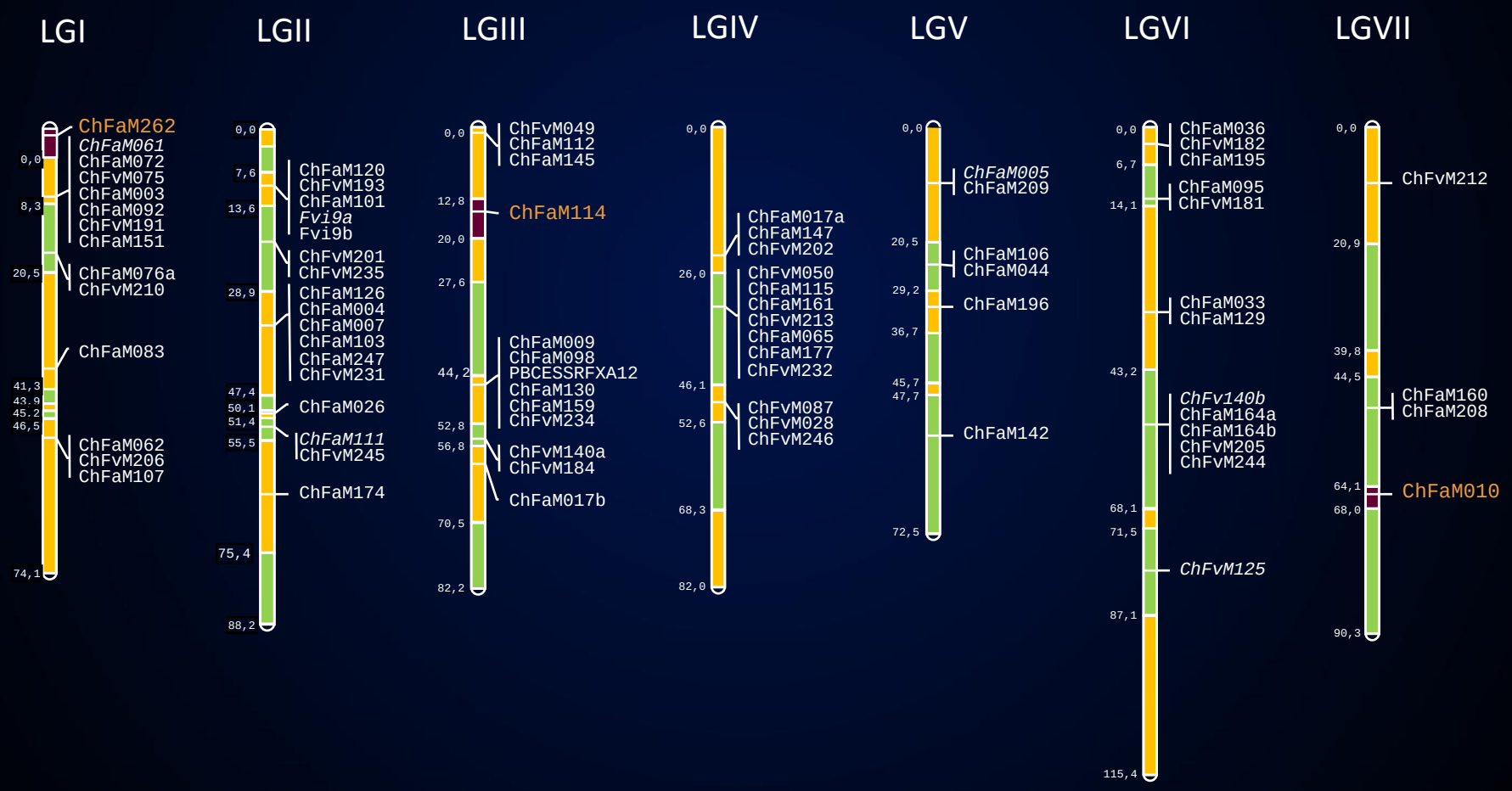
fruit ESTs
(Bombareli et al., 2010)

F. vesca SSRs → Genebank

EST-SSR Markers

Species	SSR
F. × *ananassa*	102
F. vesca	34
Total	136

Selective or bin mapping in diploid *Fragaria*



Octoploid Mapping population

'232'

×

'1392'

F1 (Pseudo-test cross) population of 95 progeny lines

Larger fruits

High yield

'232'

high firmness
Better flavor
Higher L-AA
Darker color

'1392'

Description of the linkage maps	Female map	Male map	Integrated map
SSR	184	157	255
AFLP	53	57	83
STS and SSCP	16	13	25
Total	253	227	363
Total no. of linkage groups	40	36	39
Mean no. of markers per linkage group (± SD)	6.3 (± 3.8)	6.3 (± 4.5)	7.9 (±5.3)
Range of marker no. per linkage group	2-16	2-17	2-20
Average marker spacing (cM)	4.3	4.6	4.4
Mean size per linkage group (cM ± SD)	22.7 (± 20.1)	24.1 (± 18.5)	30.4 (±21.4)
Range size per LG (cM)	0.0 - 84.5	0.0 - 65.1	2.4 - 84.5
Largest gap (cM)	31.7	33.7	33.7
Cumulative Genome Length (cM)	909.5	868.5	1400.1

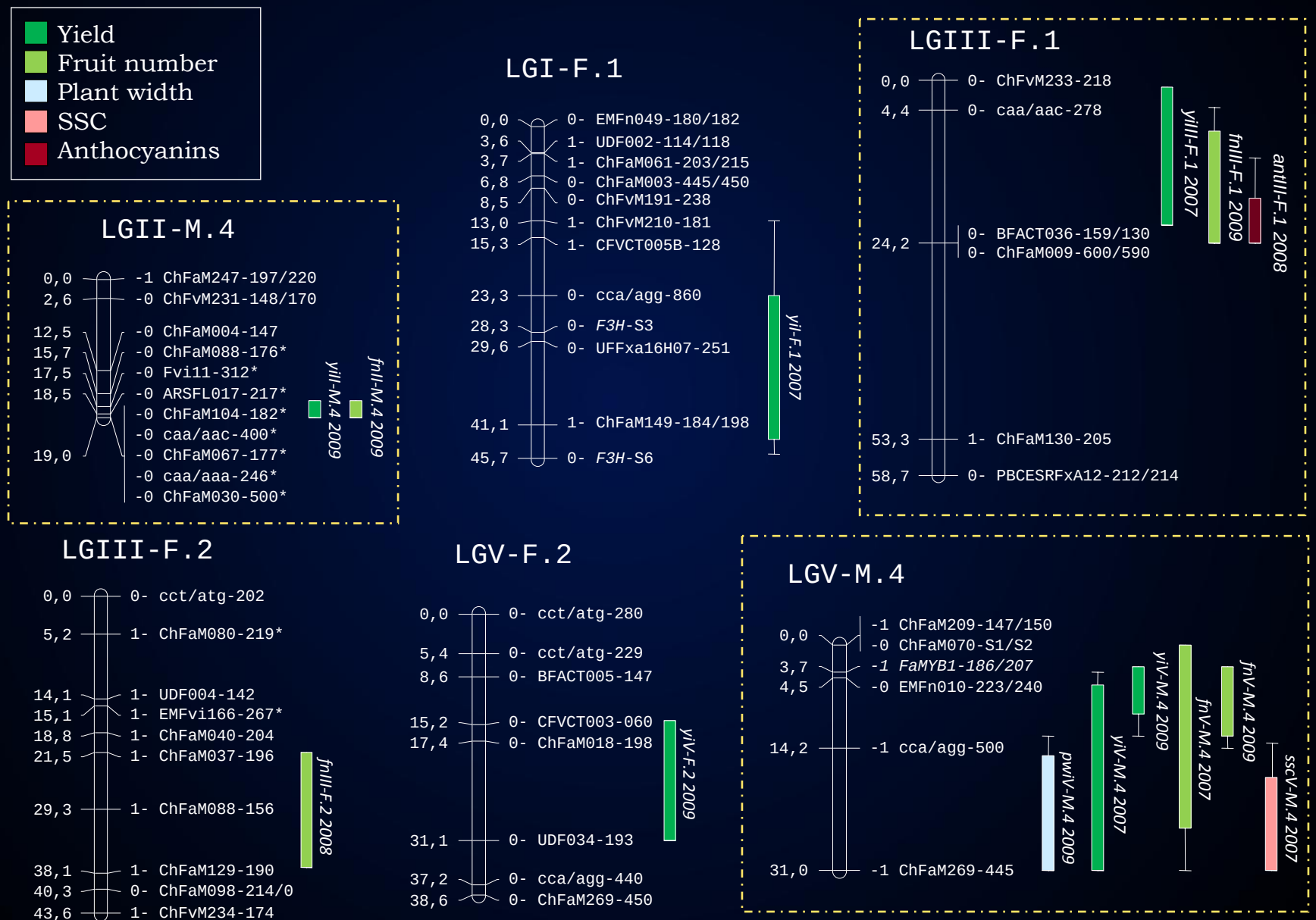
QTL mapping of agronomical and fruit quality traits

Trait	Year	Parental lines		F_1
		232	1392	Range
Flowering time (d)	2008	60	50	35.30 - 91.00
	2009	86	84	57.20 - 98.50
Plant height	2007	2.25	2.87	1.00 - 3.00
	2008	1.38	2.90	1.00 - 3.00
	2009	1.63	2.13	1.00 - 3.00
Plant width(cm)	2007	28.38	26.00	18.83 - 32.25
	2008	22.83	21.61	14.25 - 30.06
	2009	23.75	20.63	14.75 - 30.17
Yield (g)	2007	1727.25	1161.67	650.00 - 1,990.00
	2008	1081.00	902.50	433.33 - 1,572.50
	2009	1136.25	1005.00	463.33 - 1,573.33
fruit number	2007	71.50	58.00	34.67 - 108.67
	2008	52.60	41.75	25.50 - 74.75
	2009	58.50	51.25	28.67 - 94.75
fruit weight(g)	2007	24.16	20.03	13.92 - 26.02
	2008	20.55	21.62	13.16 - 29.66
	2009	19.42	19.61	11.36 - 24.40
Firmness (g)	2007	288.50	353.50	248.00 - 413.12
	2008	299.17	351.67	227.50 - 410.00
	2009	287.50	444.17	270.00 - 435.00
SSC (ºBrix)	2007	5.50	8.24	4.10 - 9.43
	2008	7.58	11.50	6.28 - 11.67
	2009	7.52	12.08	6.56 - 12.66

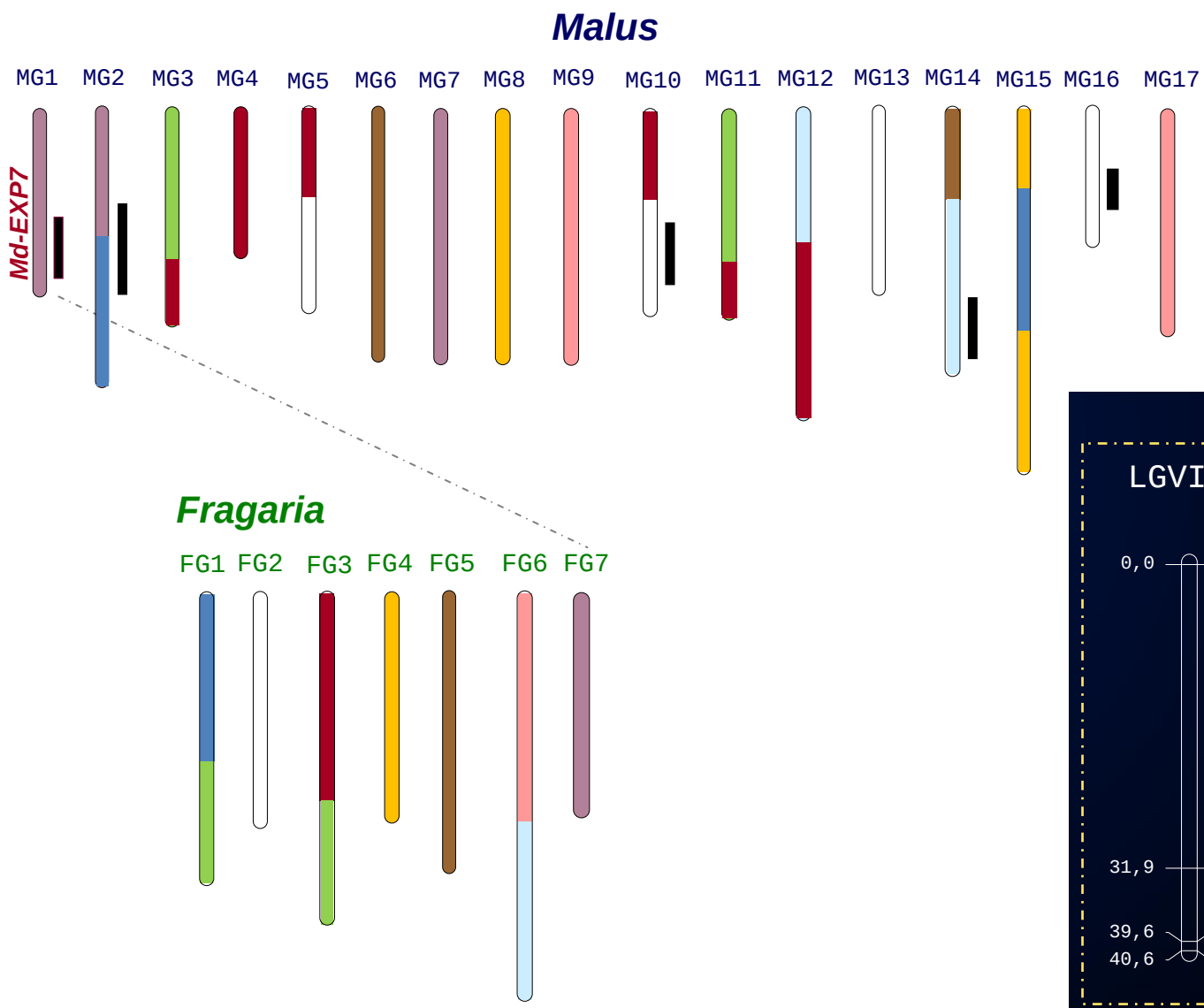
33 QTLs { Stability: 36,4%
R^2: 9,2% - 30,5%

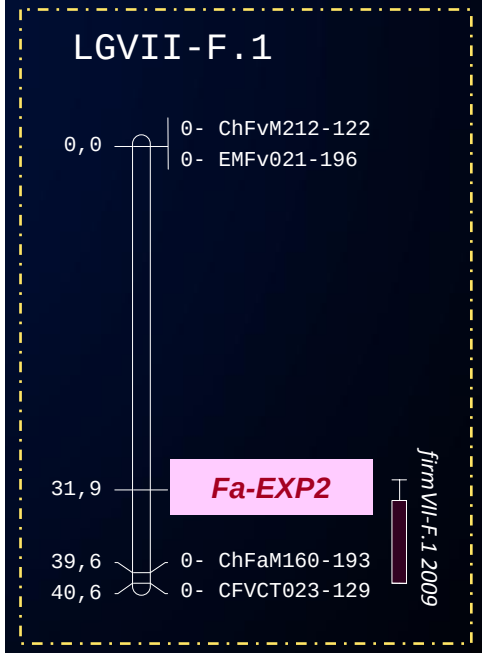
Trait		Year	Parental lines		F_1
			232	1392	Range
Titratable acidity (g/100g FW)		2007	0.43	0.50	0.27 - 0.74
		2008	0.63	0.69	0.43 - 0.89
		2009	0.43	0.69	0.36 - 0.79
pH		2007	4.02	3.95	3.72 - 4.23
		2008	3.52	3.50	3.44 - 4.21
		2009	4.07	3.88	3.69 - 4.29
L-AA (mg/100g FW)		2007	35.76	47.29	23.23 - 54.74
		2008	43.55	52.95	26.48- 53.48
		2009	32.05	44.00	26-08 - 51.25
Anthocyanins(mg/100g FW)		2007	27.39	37.44	12.09 - 41.65
		2008	22.8	34.06	12.84 - 42.60
		2009	24.88	30.18	12.39 - 45.28
*a**	COLOR	2007	46.74	34.72	34.03 - 47.90
		2008	46.13	40.55	34.76 - 46.93
		2009	44.87	40.02	36.22 - 47.20
*b**		2007	18.87	13.46	8.60 - 22.40
		2008	17.44	13.71	10.96 - 20.70
		2009	19.73	15.43	12.03 - 23.28
*L**		2007	36.80	29.80	26.60 - 38.60
		2008	32.90	29.70	26.70 - 38.30
		2009	33.10	28.30	25.30 - 36.80
h^0		2007	24.00	20.40	10.10 - 28.90
		2008	19.70	19.80	15.20 - 26.60
		2009	23.90	20.40	17.00 - 29.2
*C**		2007	50.71	38.55	35.62 - 55.80
		2008	49.10	43.23	34.47 - 53.33
		2009	49.21	42.92	32.69 - 52.30

Examples of QTLs controlling agronomical traits



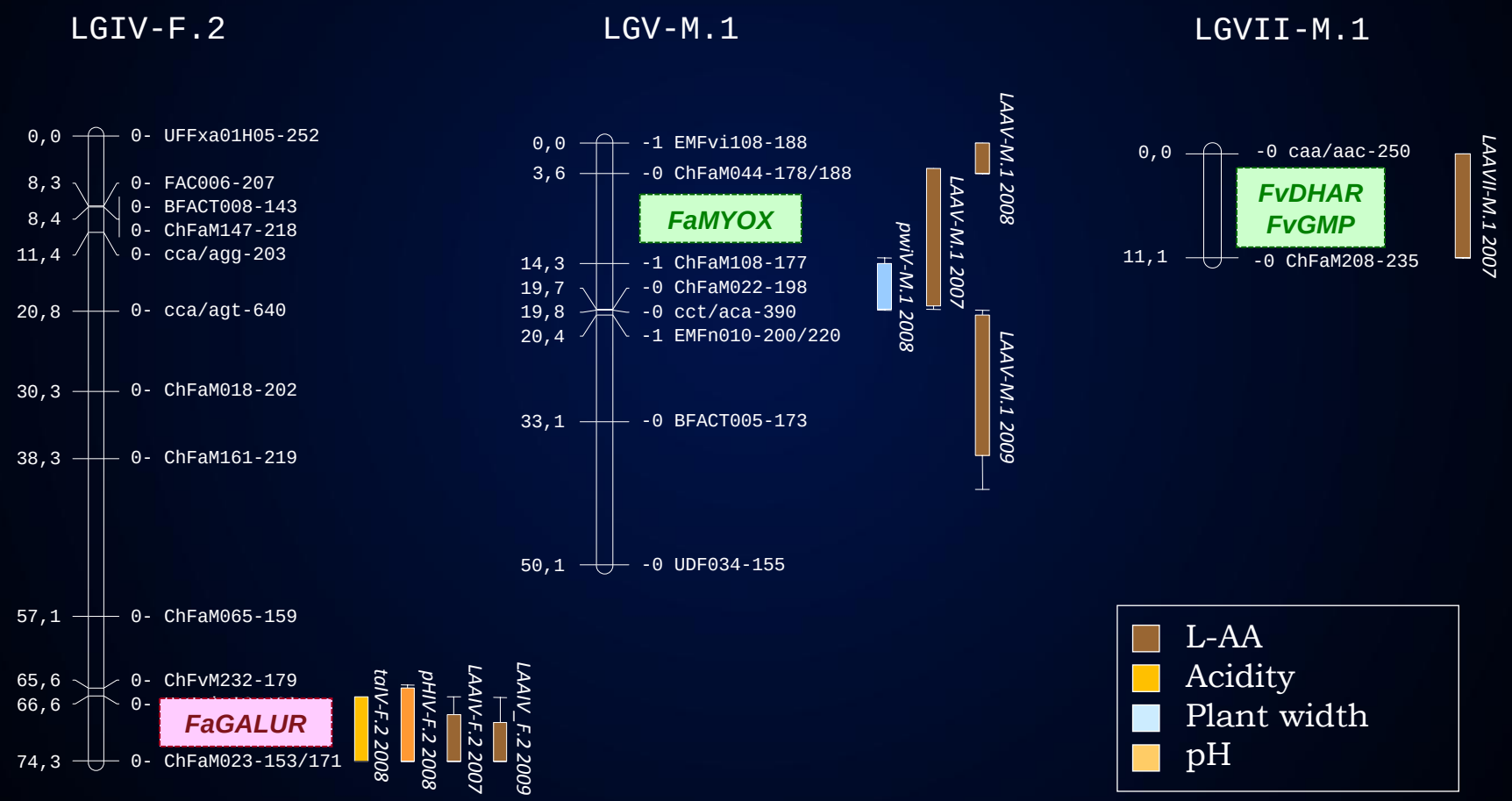
Expansins may partly control fruit firmness in different Rosaceae species





Costa *et al.* (2008; 2010); Illa *et al.* (2011); Kenis *et al.* (2008); King *et al.* (2001)

3 QTLs control aprox. 45% of the variation of L-AA content in strawberry



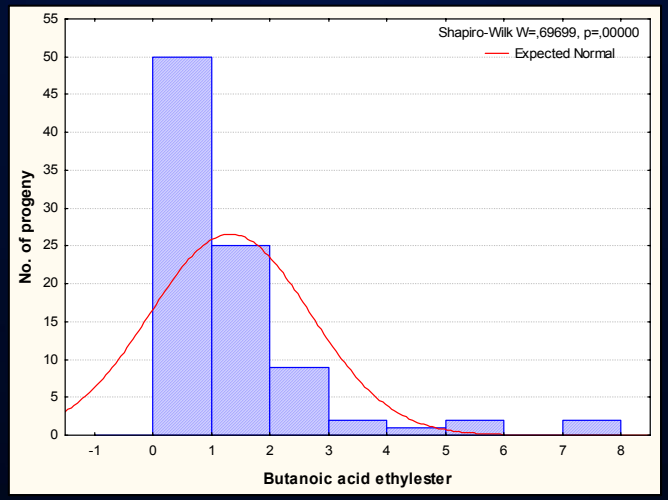
QTLs controlling strawberry aroma

Headspace solid phase micro-extracción + gas chromatography + mass spectrometry (HS-SPME-GC-MS)

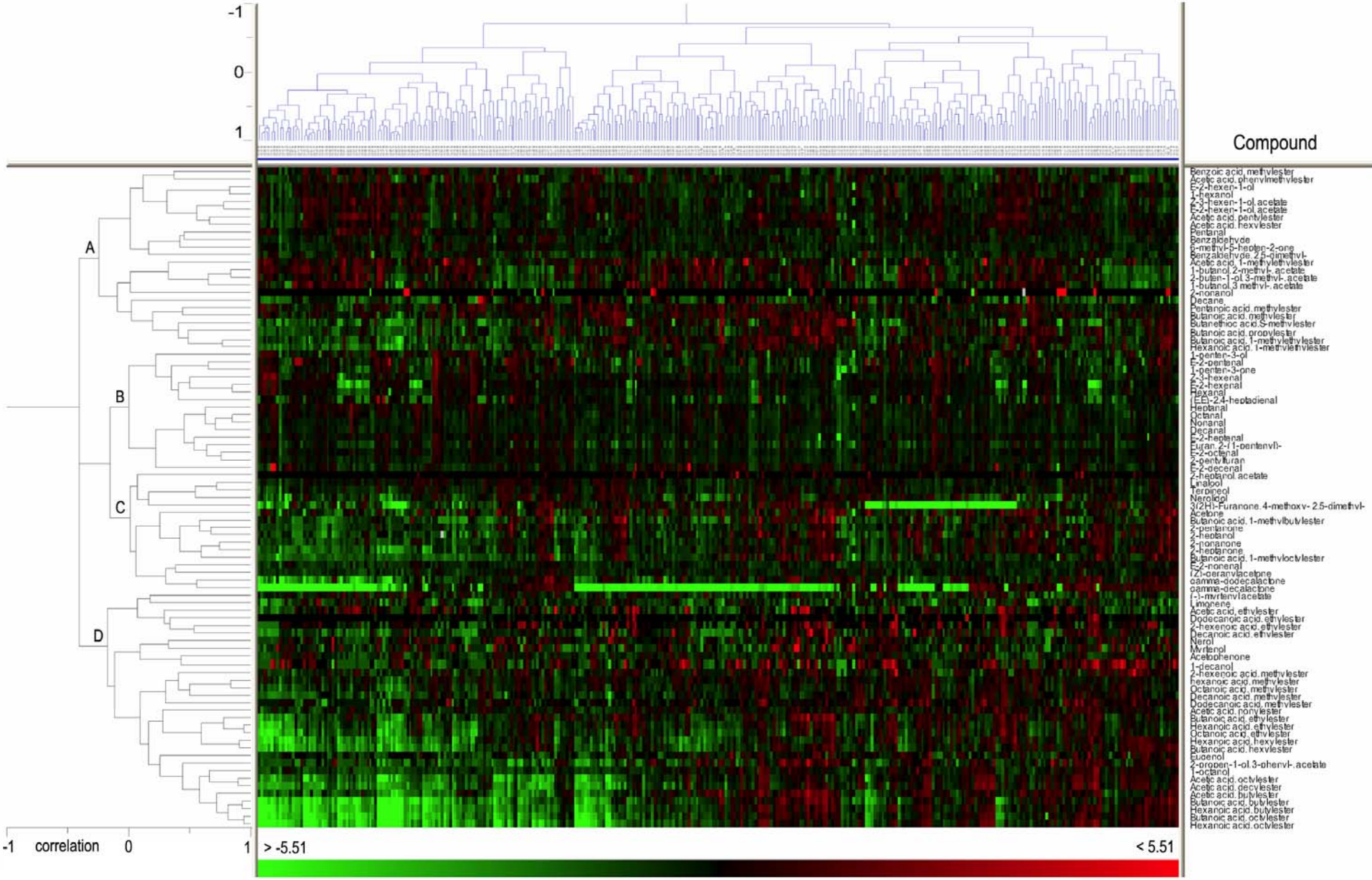
Parental & F_1 lines
3 years

87 volatiles

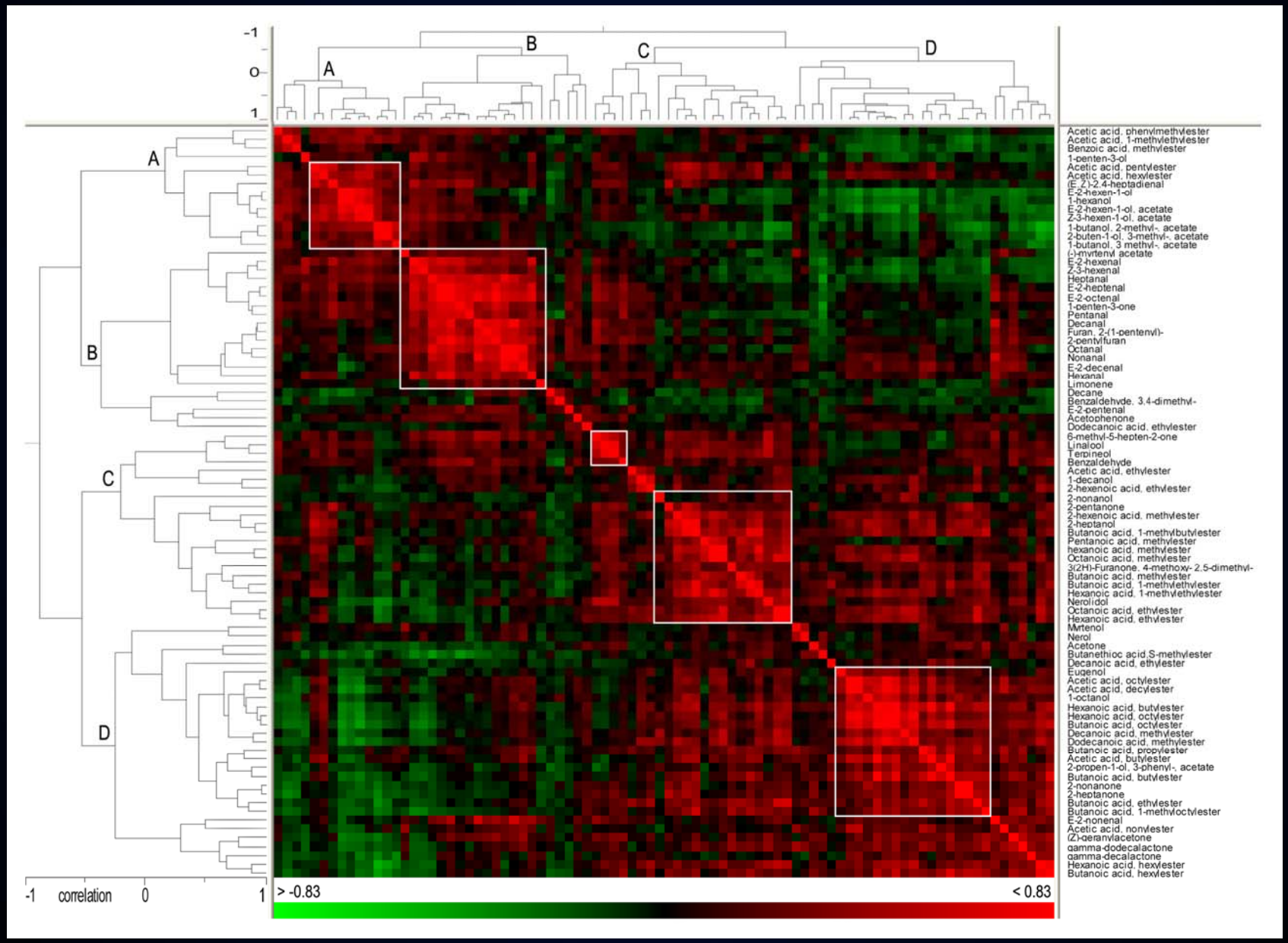
- 43 esters
- 16 aldehydes
- 8 alcohols
- 9 ketones and alkanes
- 6 terpenes
- 5 furans



HCA of volatile profiles in the population



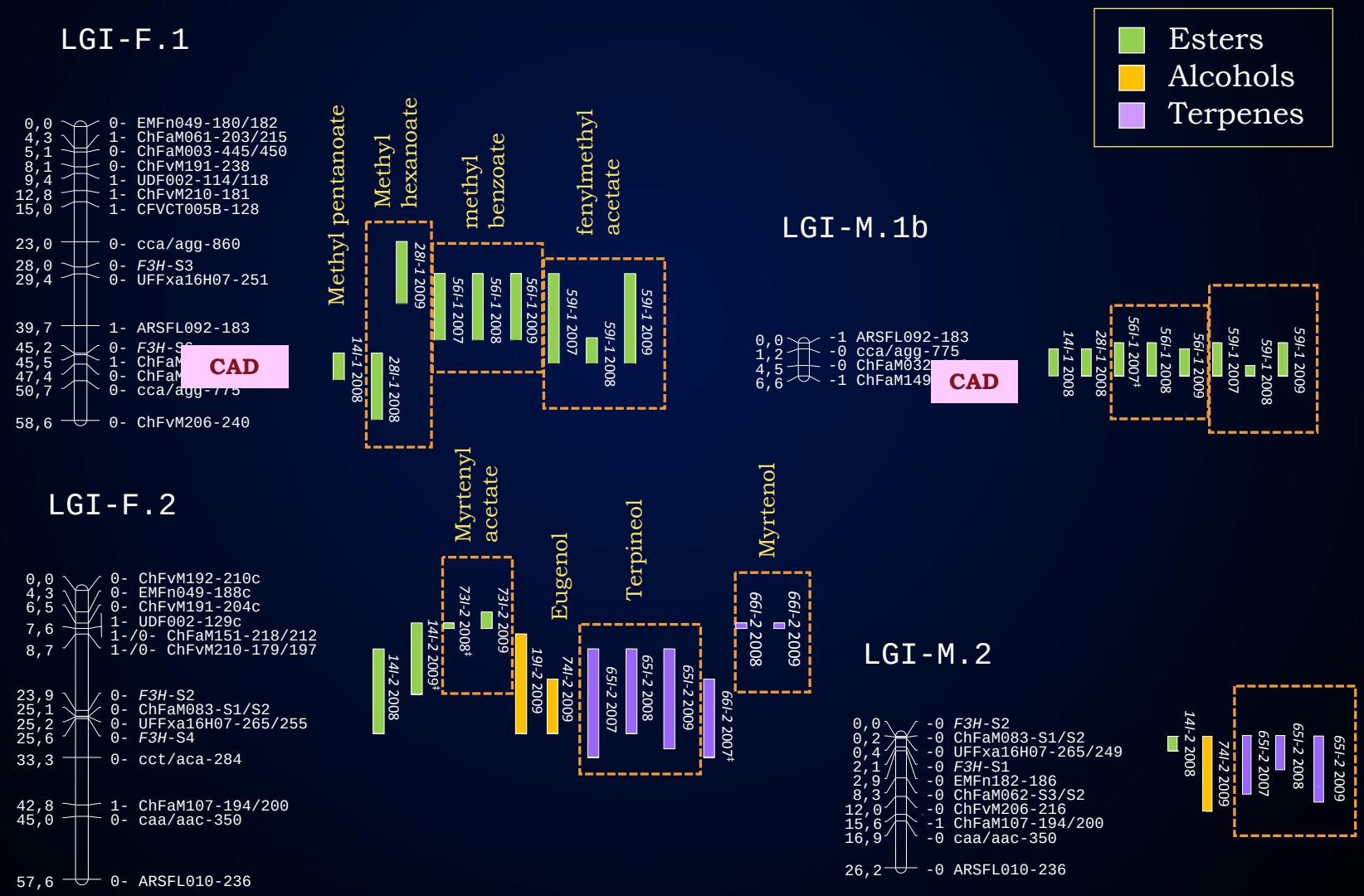
HCA of pair-wise correlations between volatiles



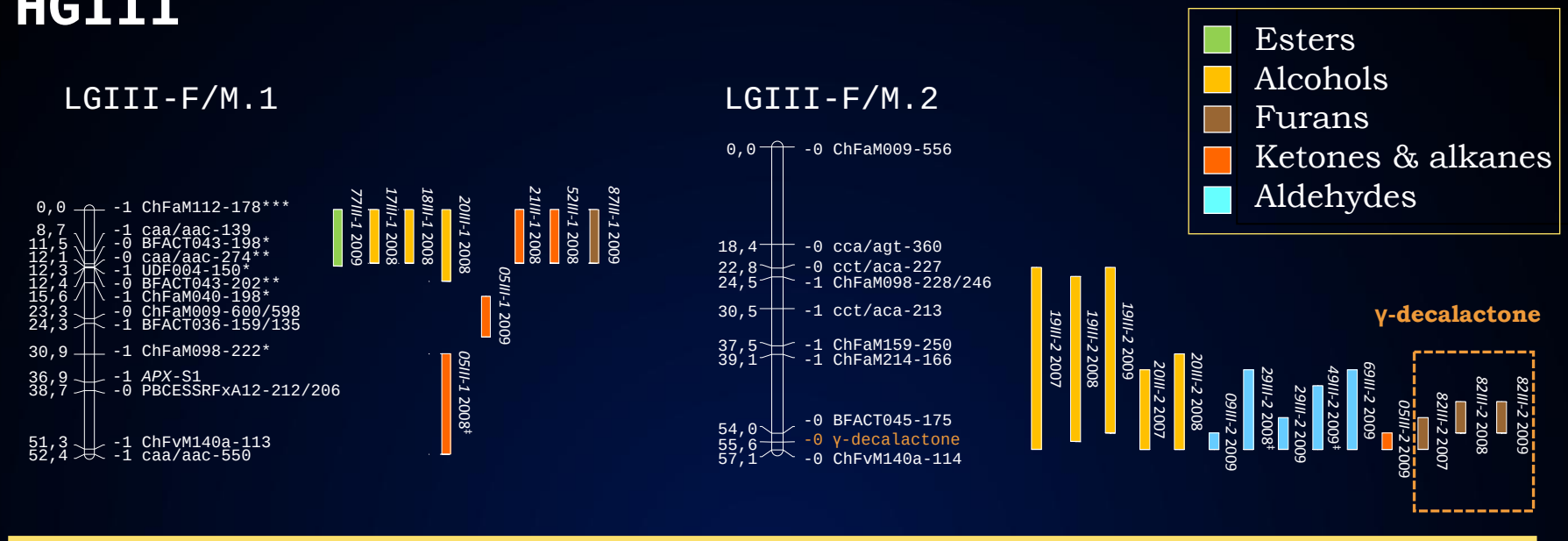
70 QTLs for 48 volatiles

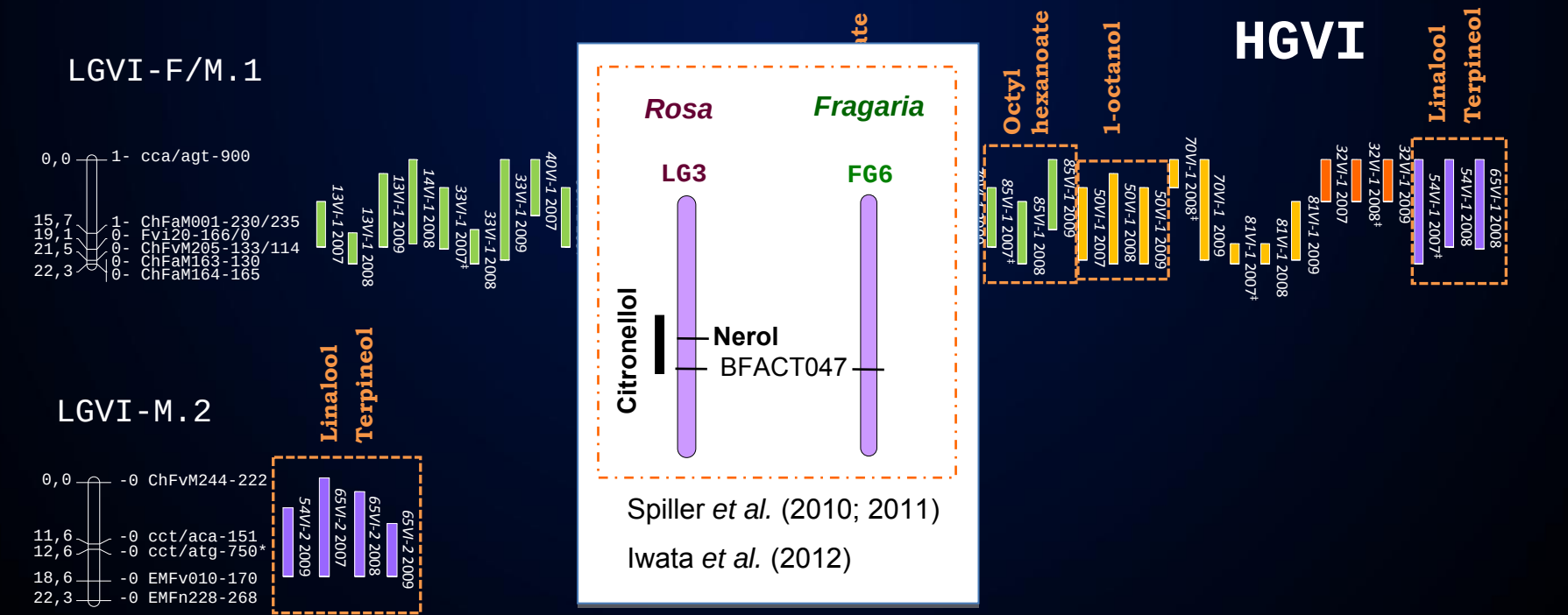
- Stability: 50,0%
- R^2: 14,2% - 92,8%

Homoeology Group I (HGI)



HGIII


Esters
Alcohols
Furans
Ketones & alkanes
Aldehydes
LGIII-F/M.1
0,0 -1 ChFaM112-178***
8,7 -1 caa/aac-139
11,5 -0 BFACT043-198*
12,1 -0 caa/aac-274**
12,3 -1 UDF004-150*
12,4 -0 BFACT043-202**
15,6 -1 ChFaM040-198*
23,3 -0 ChFaM009-600/598
24,3 -1 BFACT036-159/135
30,9 -1 ChFaM098-222*
36,9 -1 APX-S1
38,7 -0 PBCESSRFxA12-212/206
51,3 -1 ChFvM140a-113
52,4 -1 caa/aac-550
77III-1 2009
17III-1 2008
18III-1 2008
20III-1 2008
05III-1 2009
05III-1 2008‡
21III-1 2008
52III-1 2008
87III-1 2009
LGIII-F/M.2
0,0 -0 ChFaM009-556
18,4 -0 cca/agt-360
22,8 -0 cct/aca-227
24,5 -1 ChFaM098-228/246
30,5 -1 cct/aca-213
37,5 -1 ChFaM159-250
39,1 -1 ChFaM214-166
54,0 -0 BFACT045-175
55,6 -0 γ-decalactone
57,1 -0 ChFvM140a-114
19III-2 2007
19III-2 2008
19III-2 2009
20III-2 2007
20III-2 2008
09III-2 2009
29III-2 2008‡
29III-2 2009
49III-2 2009‡
69III-2 2009
05III-2 2009
82III-2 2007
82III-2 2008
82III-2 2009
γ-decalactone


LGVI-F/M.1
0,0 1- cca/agt-900
15,7 1- ChFaM001-230/235
19,1 0- Fvi20-166/0
21,5 0- ChFvM205-133/114
22,3 0- ChFaM163-130
0- ChFaM164-165
13VI-1 2007
13VI-1 2008
13VI-1 2009
14VI-1 2008
33VI-1 2007‡
33VI-1 2008
33VI-1 2009
40VI-1 2007
LGVI-M.2
0,0 -0 ChFvM244-222
11,6 -0 cct/aca-151
12,6 -0 cct/atg-750*
18,6 -0 EMFv010-170
22,3 -0 EMFn228-268
54VI-2 2009
65VI-2 2007
65VI-2 2008
65VI-2 2009
Linalool
Terpineol
Rosa
Fragaria
LG3
FG6
Citronellol
Nerol
BFACT047
Spiller et al. (2010; 2011)
Iwata et al. (2012)
Octyl hexanoate
1-octanol
HGVI
85VI-1 2007‡
85VI-1 2008
85VI-1 2009
50VI-1 2007
50VI-1 2008
50VI-1 2009
70VI-1 2008‡
70VI-1 2009
81VI-1 2007‡
81VI-1 2008
81VI-1 2009
32VI-1 2007
32VI-1 2008‡
32VI-1 2009
54VI-1 2007‡
54VI-1 2008
65VI-1 2008

HGVII

- Esters
- Alcohols
- Furans
- Ketones y alkanes
- Aldehydes

LGVII-F/M.1

0,0 — 0- *SAAT*-S1

11,0 — 0- ChFaM208-229

0,0 — 0- UDF005-234

2,0 — 0- cca/agg-233

0,0 — 0- ChFvM212-122

0- EMFv021-196

31,7 — 0- cca/agg-266**

32,2 — 0- ARSFL099-178

39,8 — 0- ChFaM160-193

41,1 — 0-

43,7 — 0-

FaOMT

59VII-1 2007

59VII-1 2008

59VII-1 2009

87VII-1 2007

87VII-1 2008

87VII-1 2009

LGVII-F/M.2

0,0 — -0 caa/aaa-550

2,2 — -0 ChFaM018-229

14,3 — -1 BFACT029-231

23,6 — -1 cca/agt-125

32,5 — -0 *SAAT*-S2

41,8 — -1 cca/agg-835

46,8 — -0 UDF005-216

47,2 — -1 ChFvM084-153

55,0 — -0 caa/aac-177

55,8 — -0 caa/aaa-222

56,7 — -0 CFVCT023-122

59,9 — -0 caa/aaa-187

60,7 — -1 ChFaM160-187

61,6 — -0 EMFv023-176/199

65,1 — -0 mesifurane

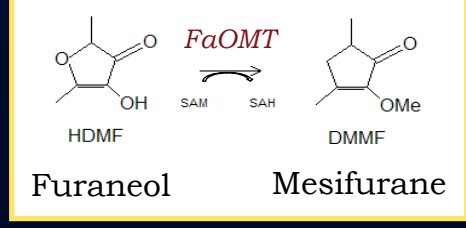
Mesifurane

29VII-2 2009

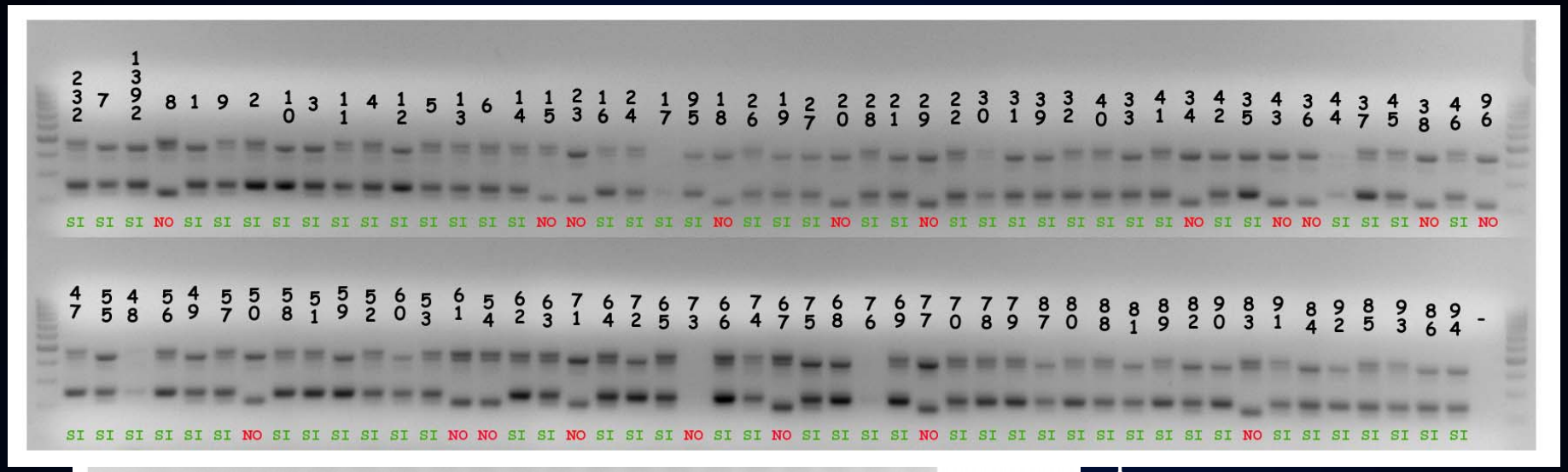
48VII-2 2007

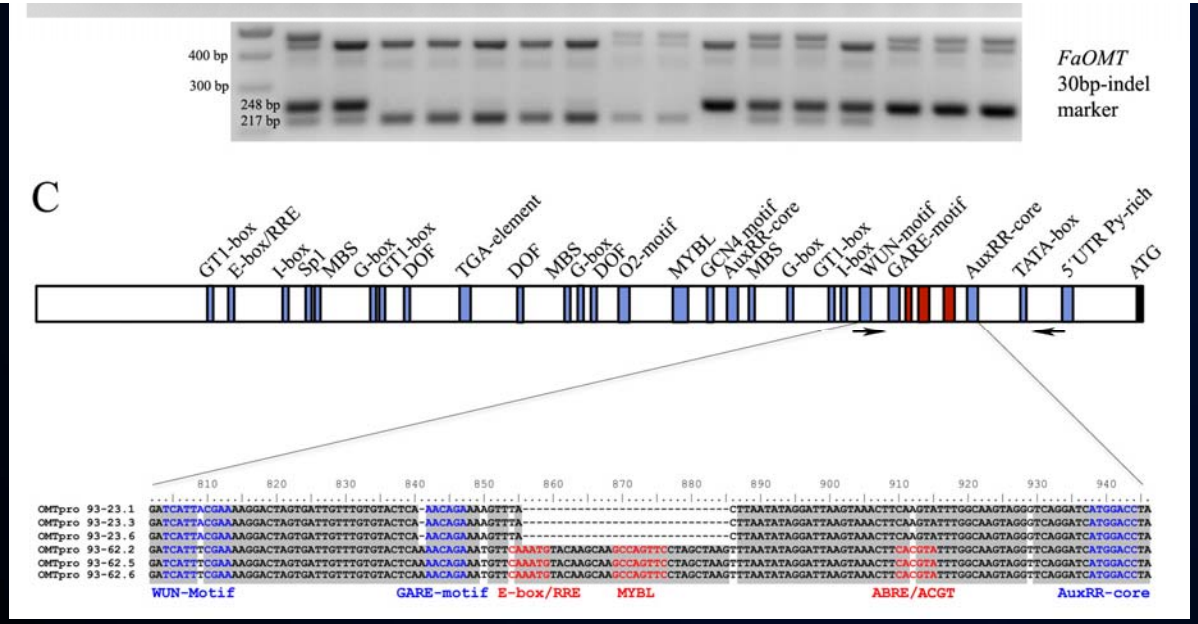
48VII-2 2008

48VII-2 2009



The gene *FaOMT* controls the variation in mesifurane content in strawberry





fully co-segregates with mesifurane content

3 potential cis-regulatory elements are specific of the functional allele

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