



Metabolomics as a tool for plant authentication?

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WAGENINGEN UR

 **BioSystems** | GENOMICS 
The Netherlands Plant Genomics Network 



META-PHOR



METAbolomics for Plants, Health and OutReach



SIXTH FRAMEWORK
PROGRAMME



Food-CT-2006-03622



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 **BioSystems** | GENOMICS 
The Netherlands Plant Genomics Network 

PRI '-omics' technology platforms

TECHNOLOGY

- Genome sequencing
- Transcriptomics (Arrays)
- Proteomics
- Metabolomics

APPLICATION

- All organisms
- Linking gene to function
- Profiling development
- Identifying key enzymes
- Identifying key structural proteins
- Food quality
- Biochemical pathways
- Tracing & tracking
- Biodiversity



Terminology – What is metabolomics?

Metabolomics:

- Technology aimed to give us the broadest possible insight in the biochemical make-up of a biological product
- Differs from traditional analytical biochemistry:
 - Focused on all small molecules (non polymers)
 - High Throughput / large scale
 - Data generation + bioinformatics / biostatistics



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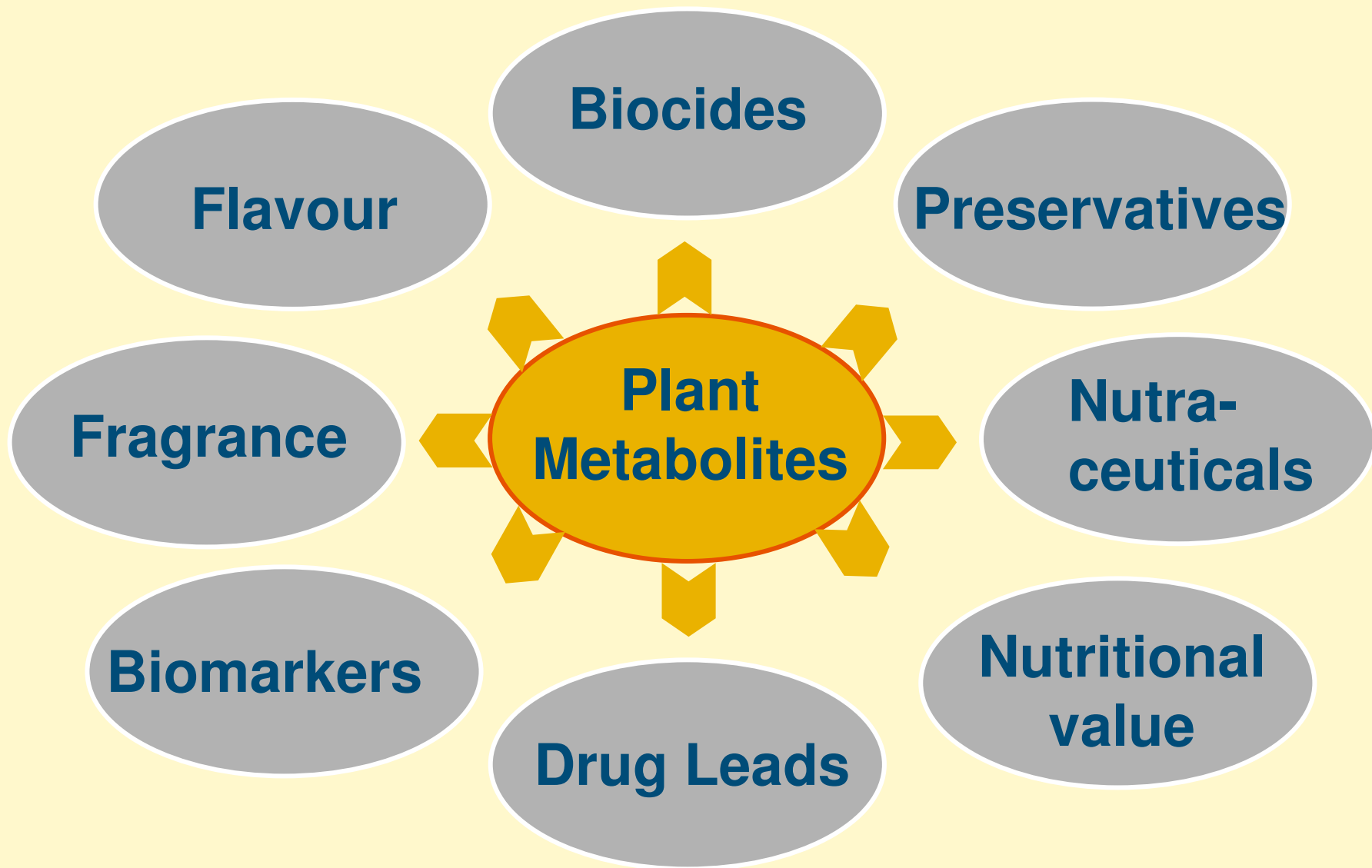
Terminology - Metabolomics

■ Non-targeted analyses

- Fingerprinting – pattern recognition (without identification)
- Profiling – metabolite identification

■ Targeted analyses

- Analyses of selected (groups of) compounds



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Metabolomics to understand biosynthesis pathways

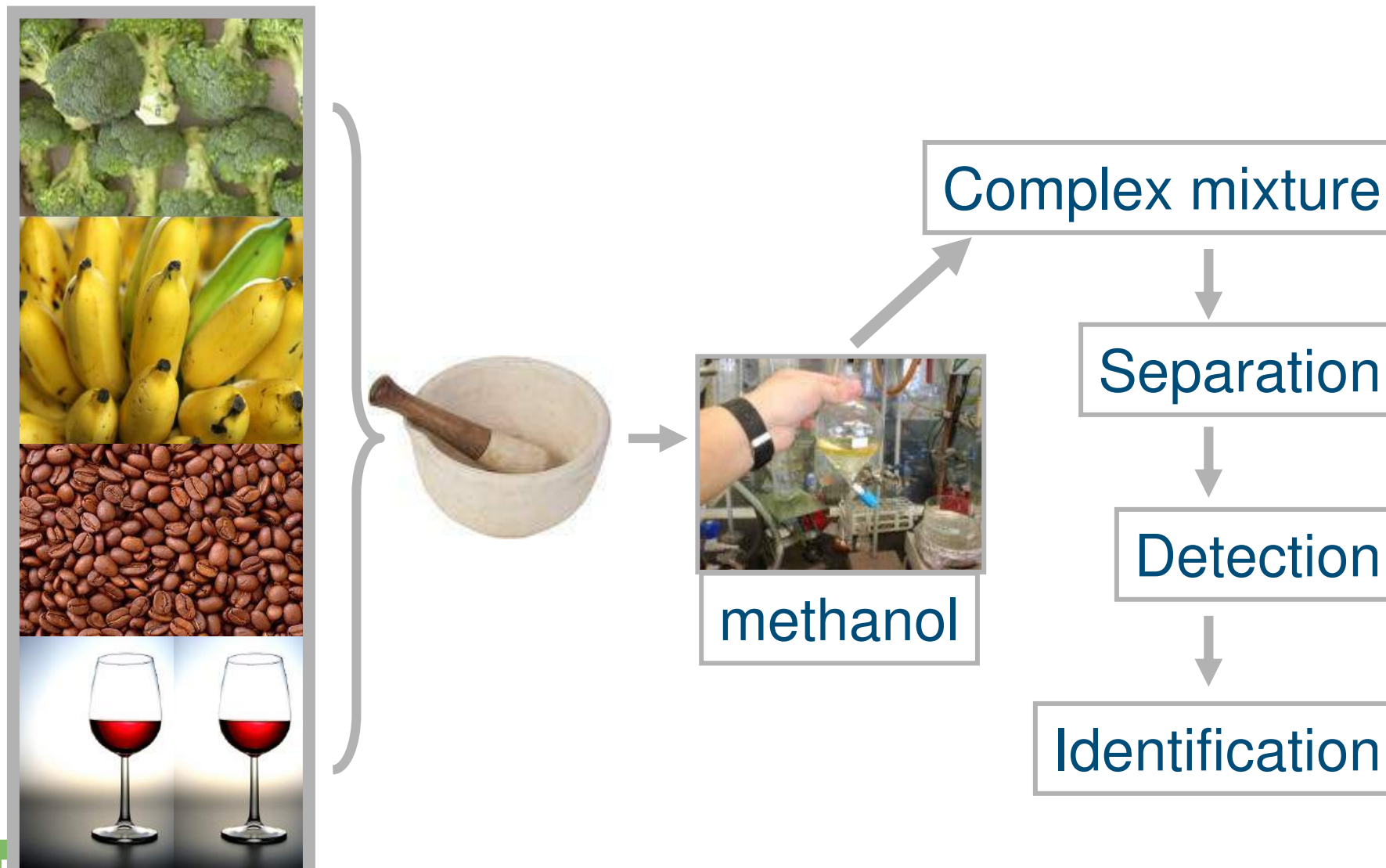


WT



MYB/MYC

Metabolomics – what do you need?



Metabolomics Technology platforms



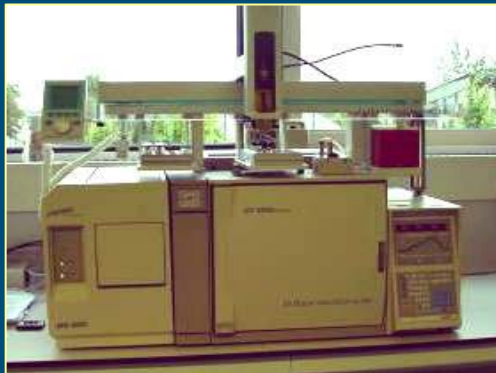
GCMS



Extraction Robot



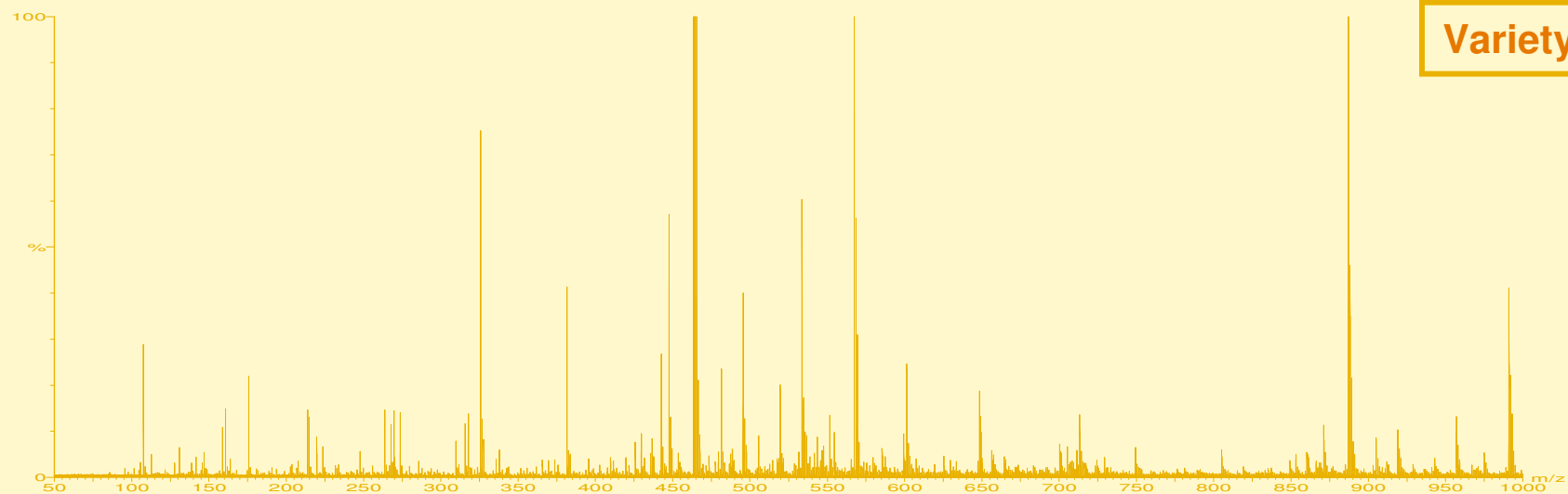
LCMS



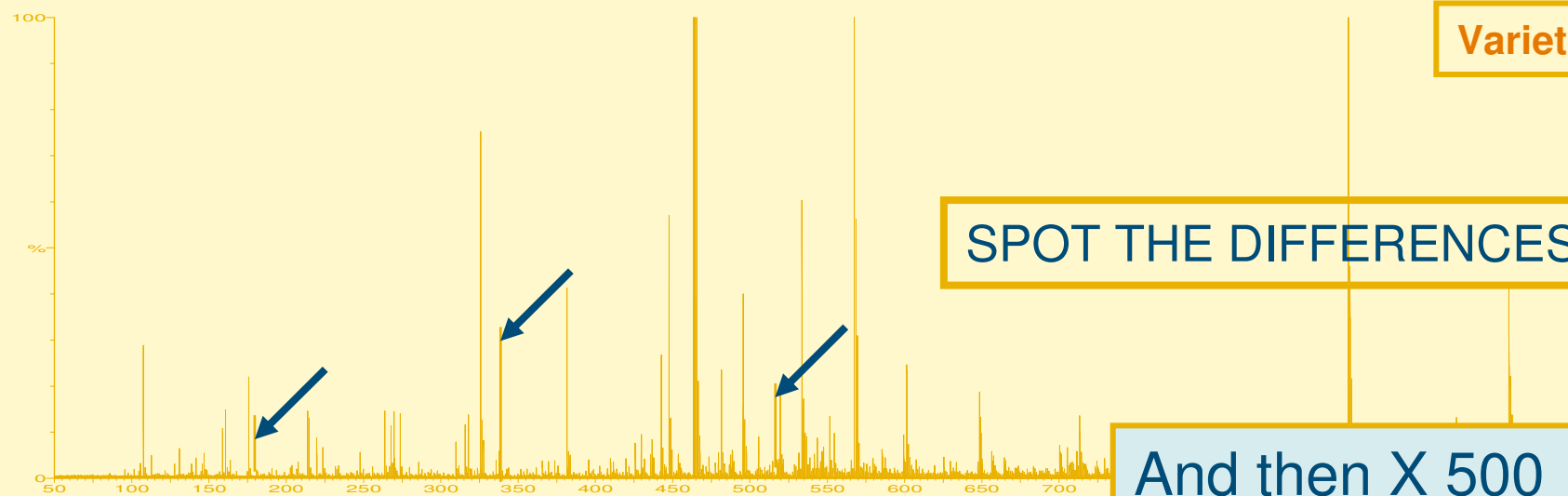
Orbitrap FTMS



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Variety 1



Variety 2

SPOT THE DIFFERENCES!!!!

And then X 500 !!

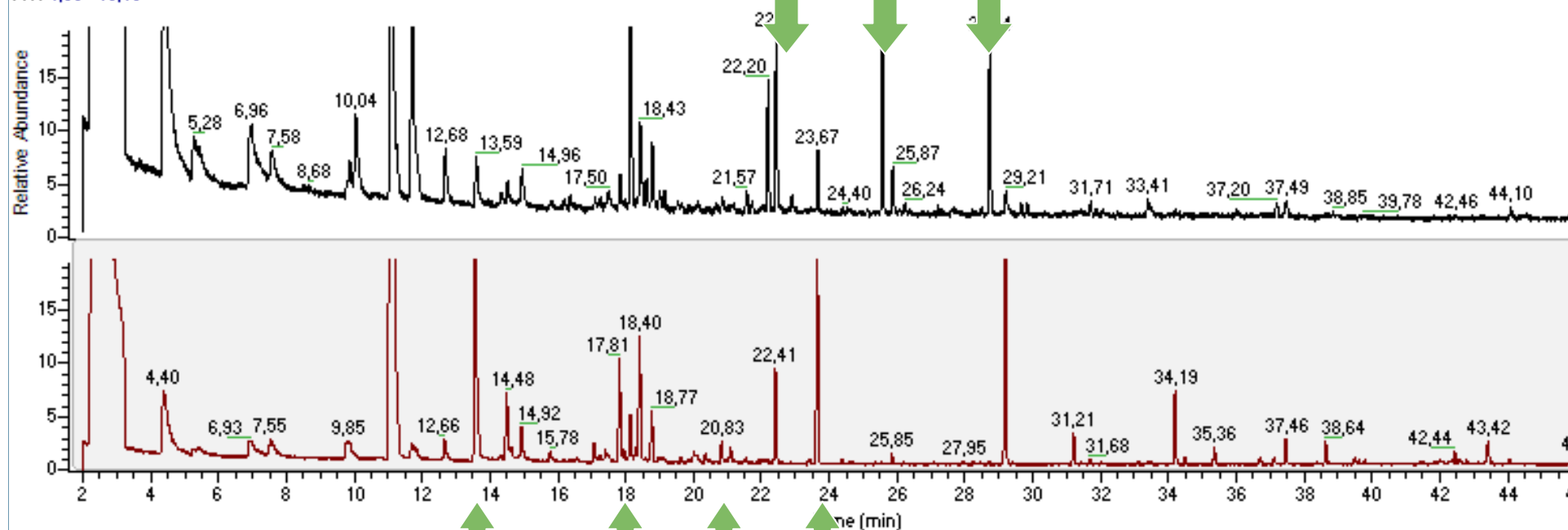


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2 Commercial cooked Basmati's

Aliphatic components

RT: 1,55 - 48,10

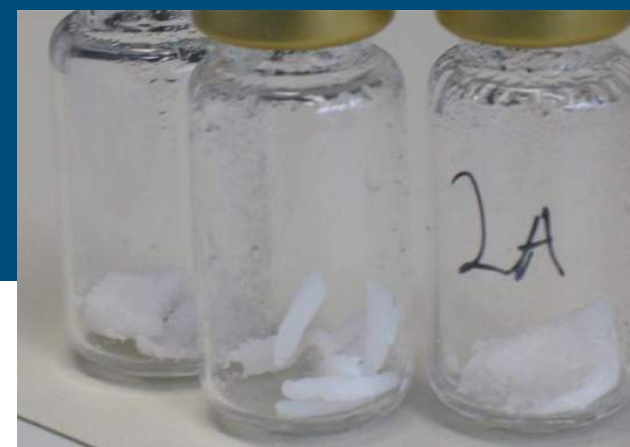


Hexanal

Octanal

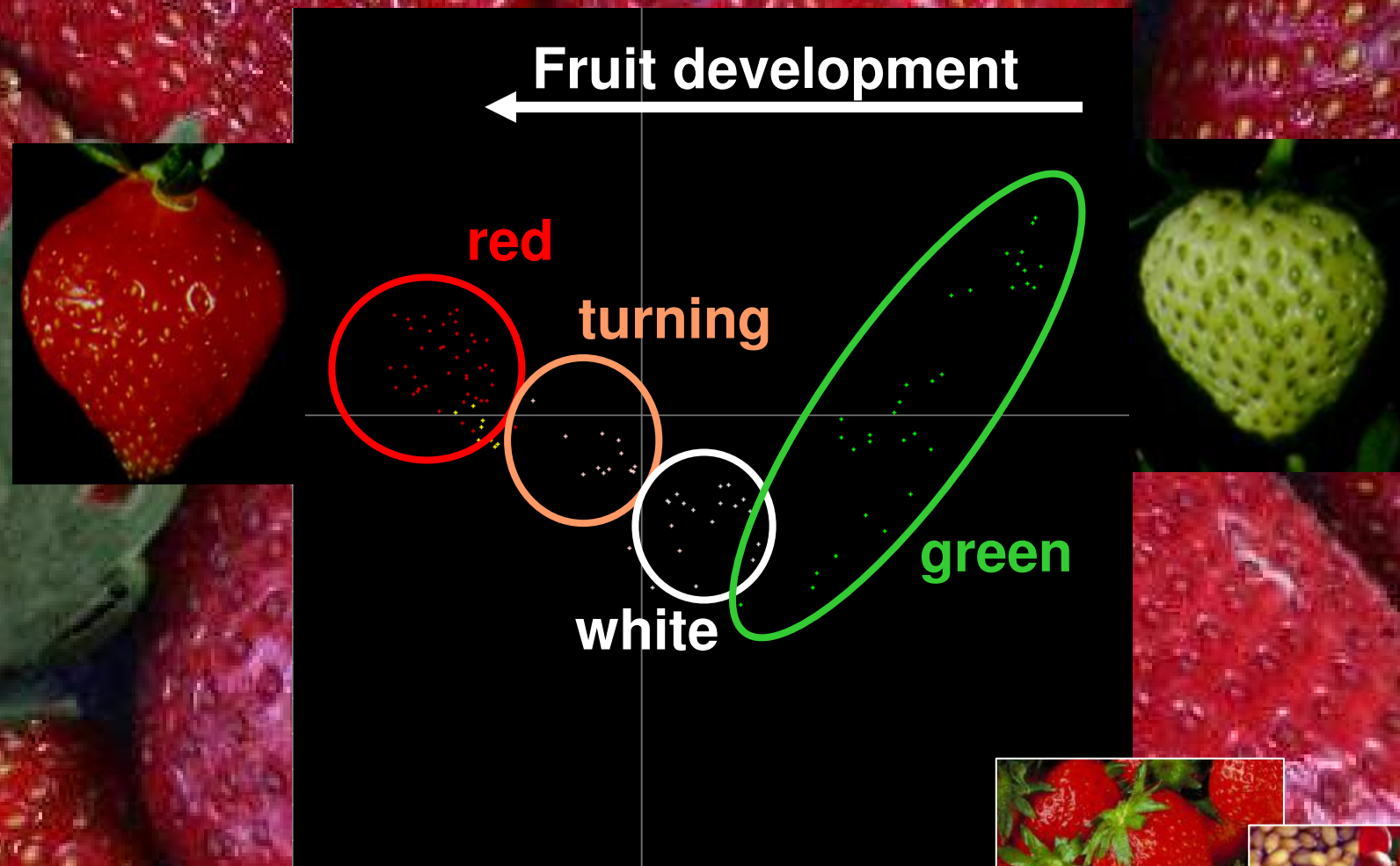
Heptanal

Nonanal



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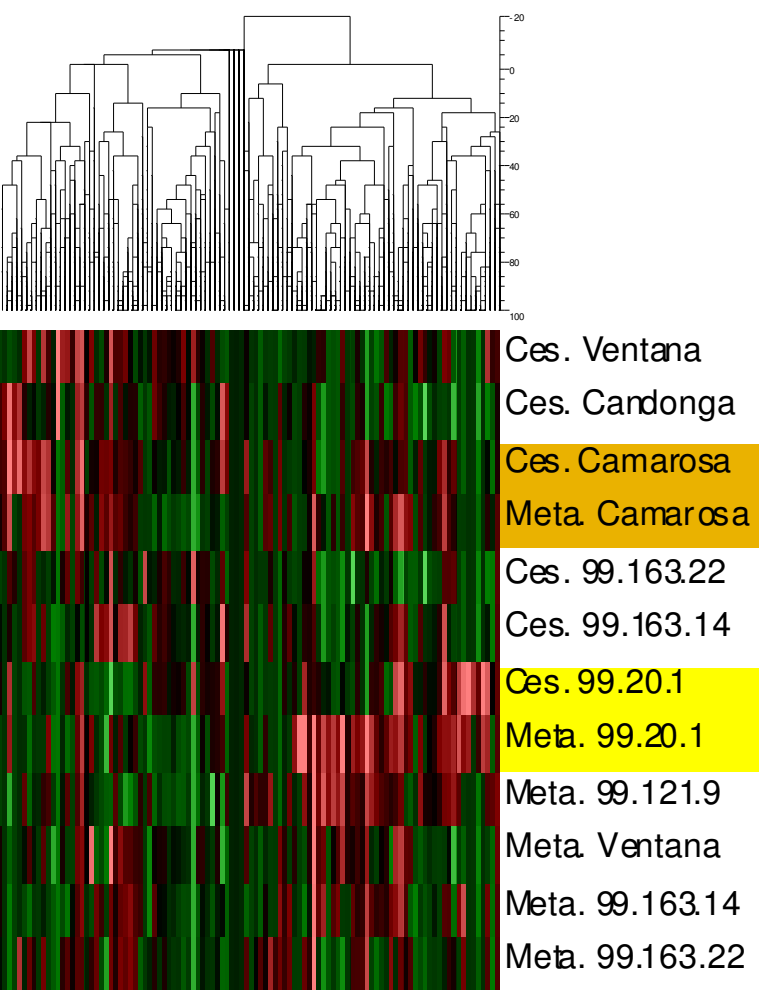
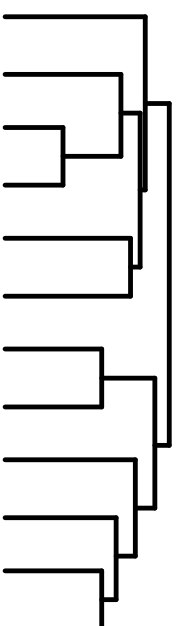
Metabolic profiles are considerably flexible



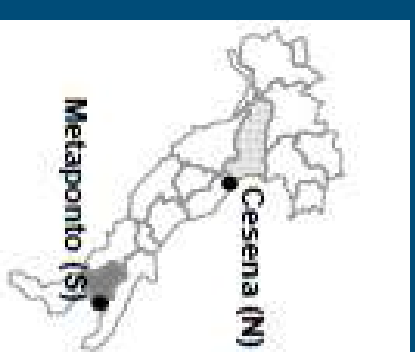
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Effect of growth location on metabolite



Meta = Metaponto
Ces = Cesena



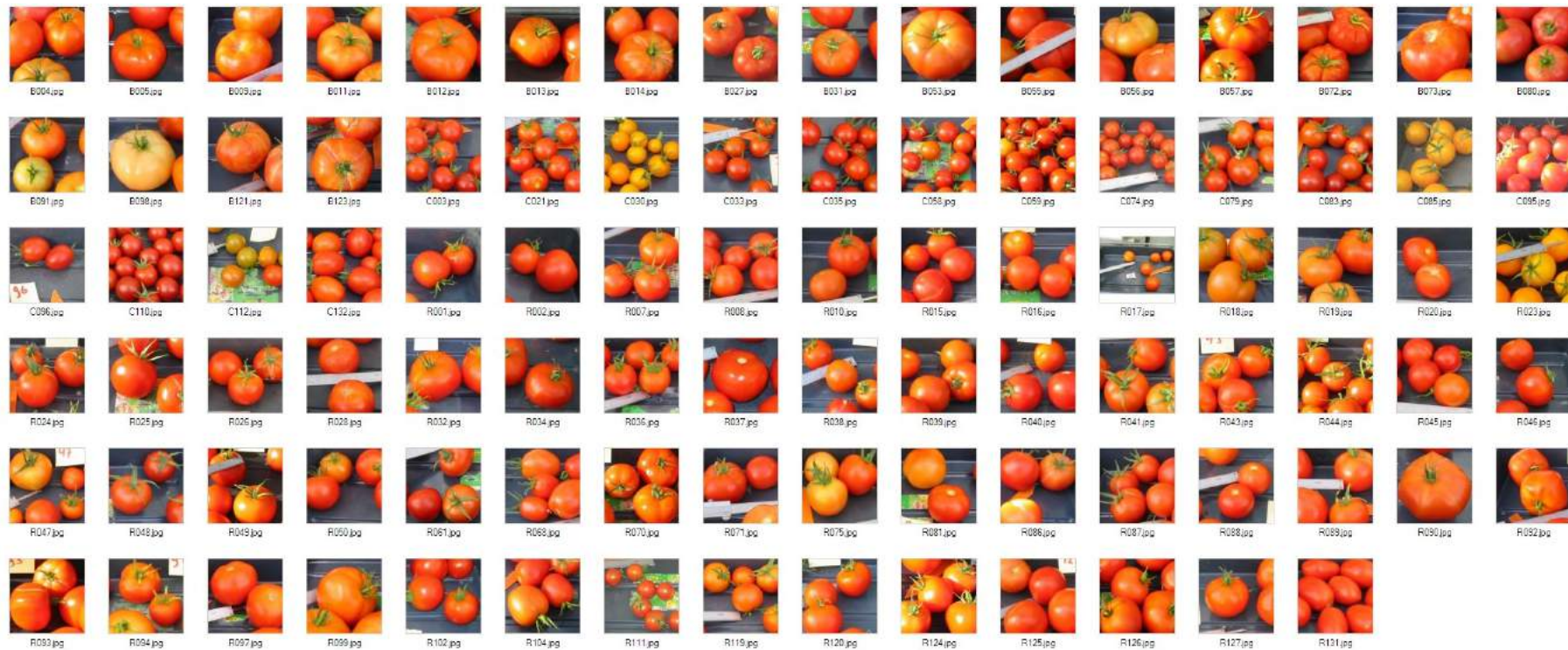


Tomato taste – what is that?

- a multi- omics example

What materials do we have?

► 94 cultivars



Metabolic profiling

► Non-targeted analyses

- | LC-MS (flavonoids, phenolic acids, alkaloids)
- | GC-MS (volatile compounds)

► Targeted analyses

- | Flavour-related tomato volatiles
- | Primary metabolites (sugars, organic acids, amino acids)
- | Carotenoids and tocopherols



Cherry

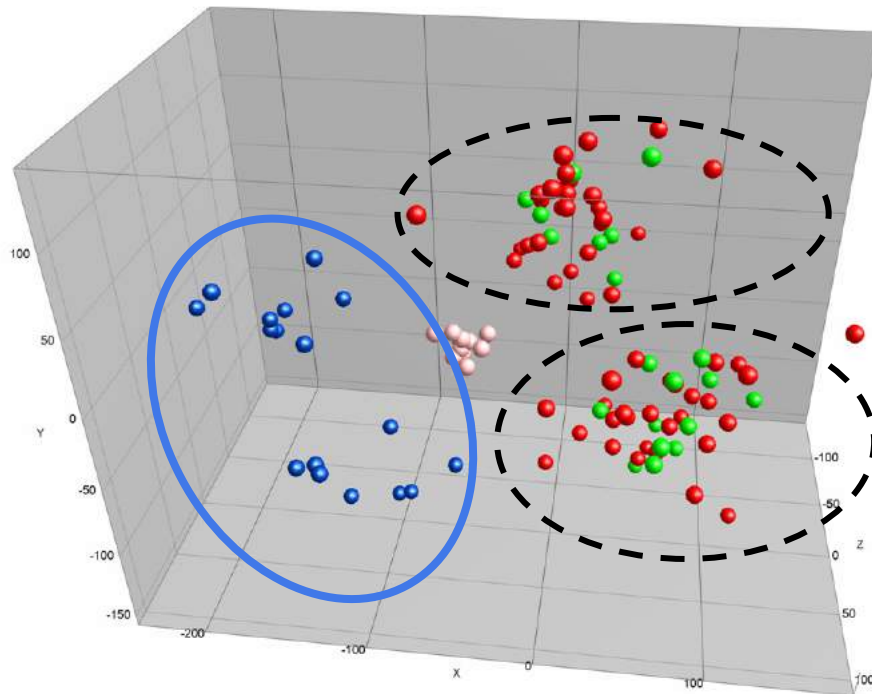


Round

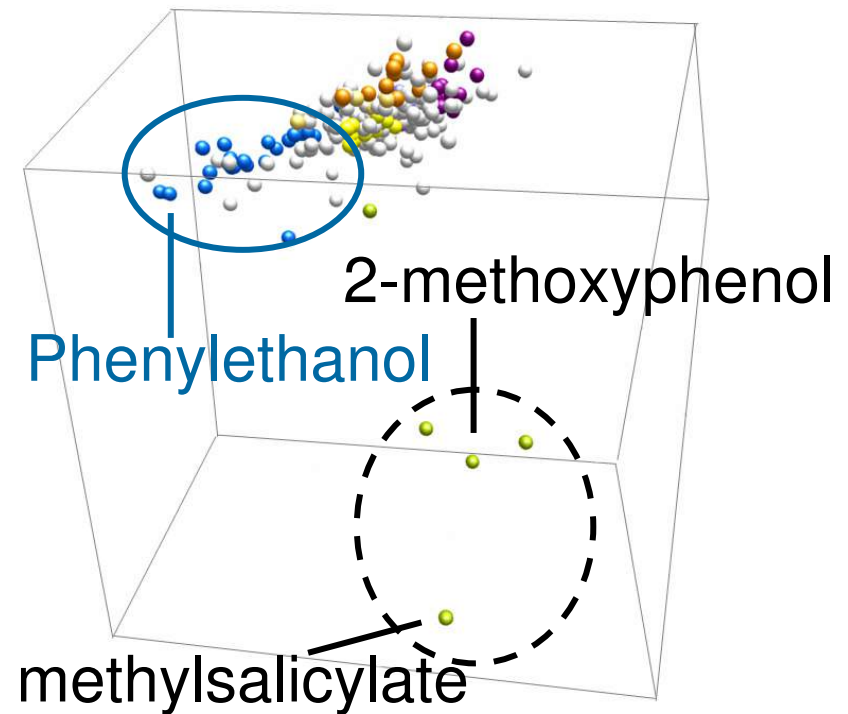


Beef

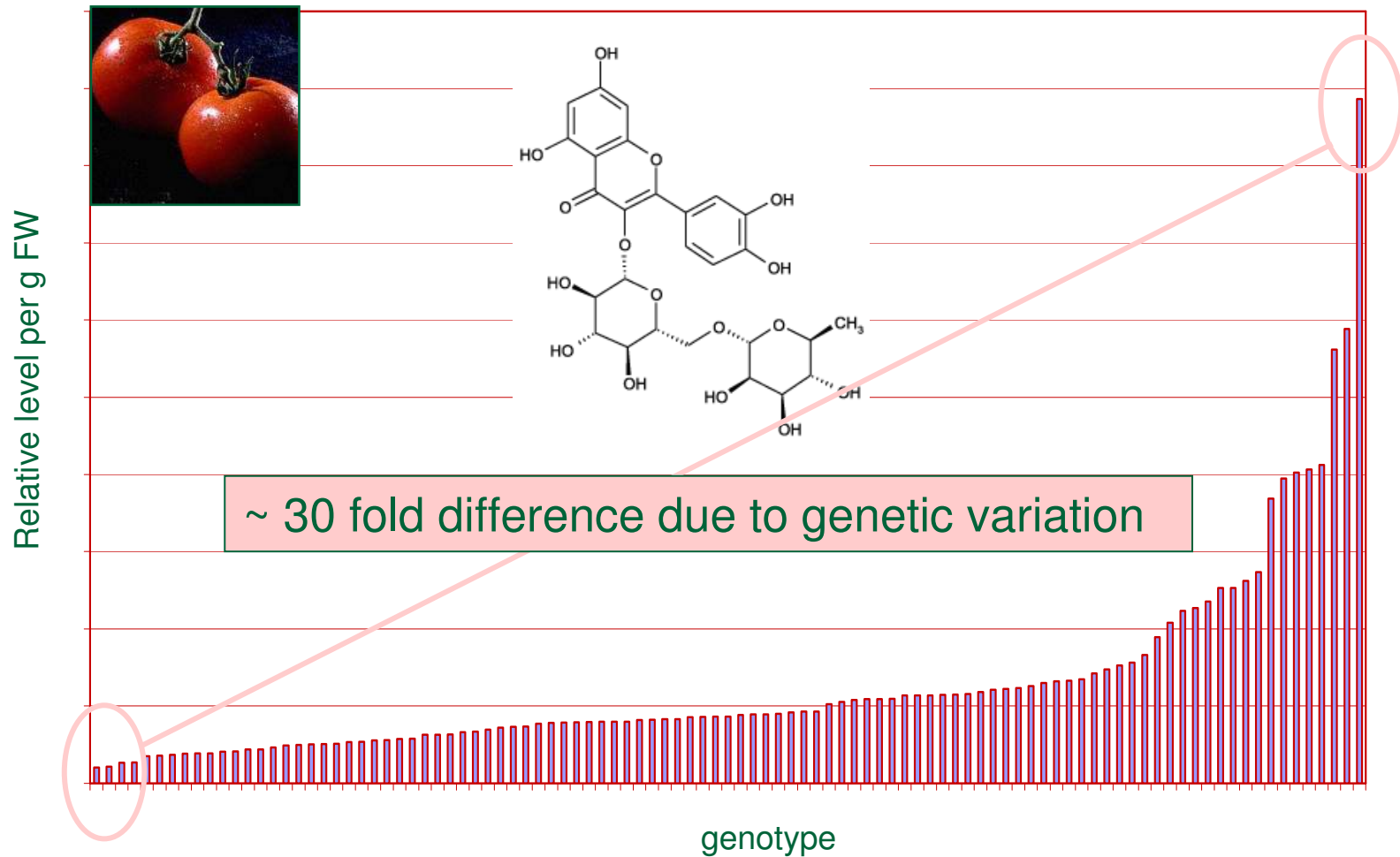
PCA analysis of 94 tomato cultivars based on 322 volatile metabolites

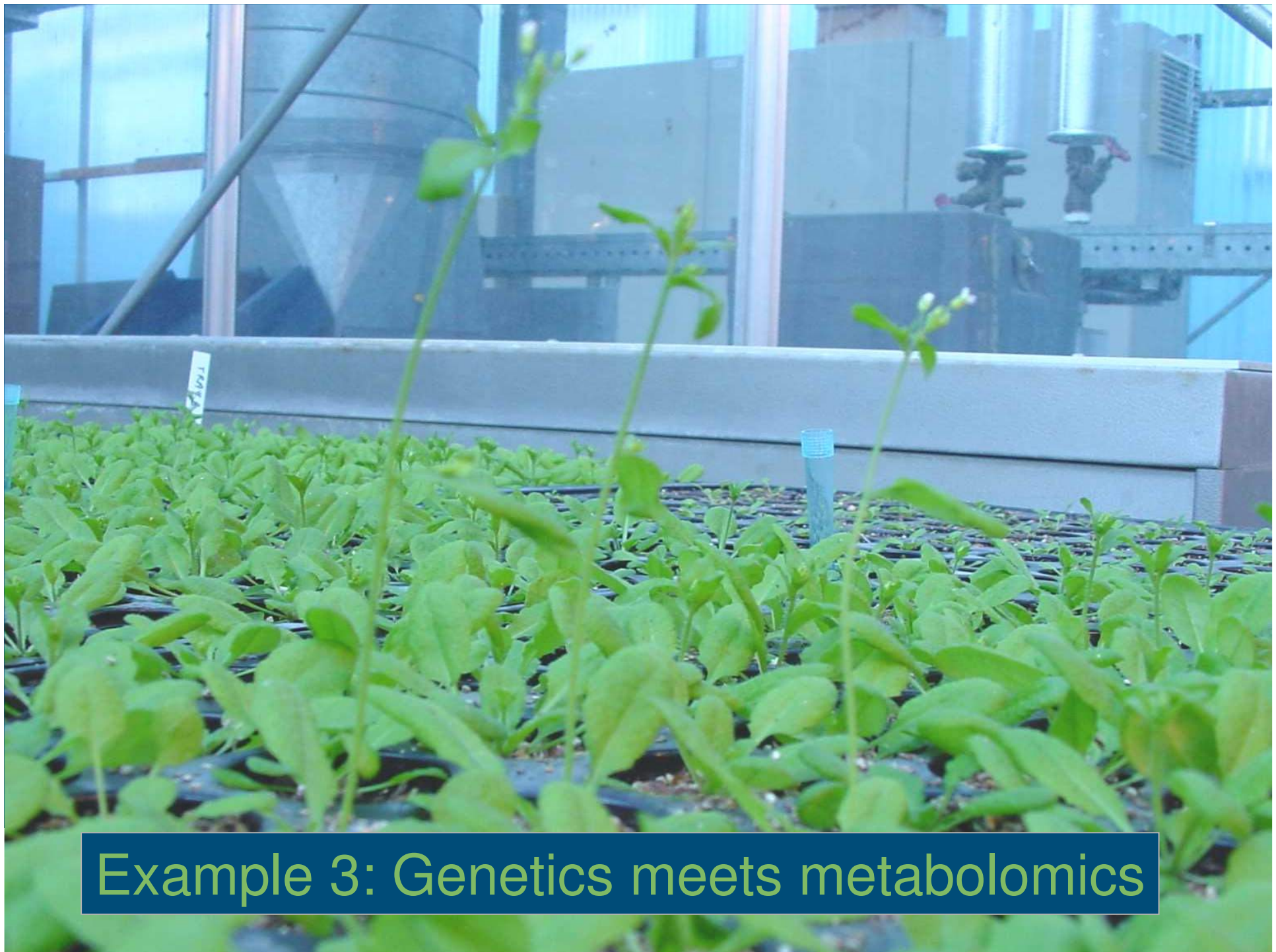


- cherry
- round
- beef
- reference-samples



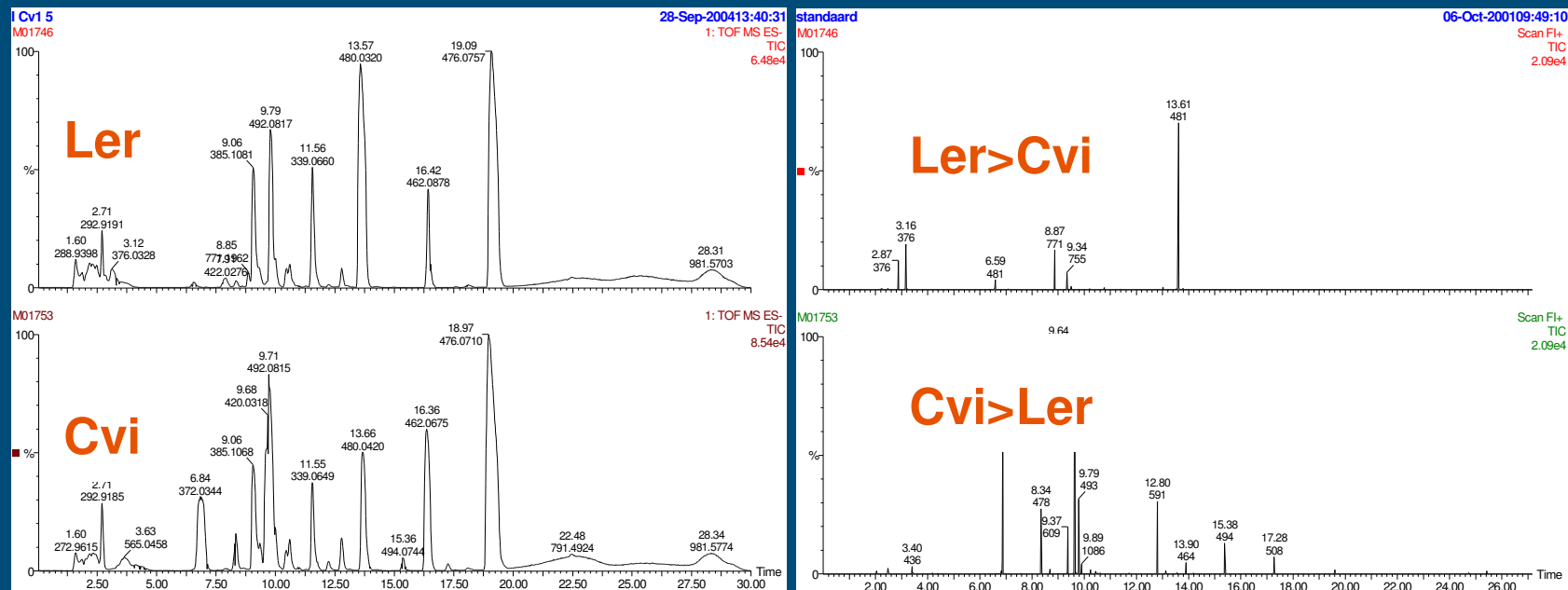
Tomato: quercetin-3-*O*-rutinoside





Example 3: Genetics meets metabolomics

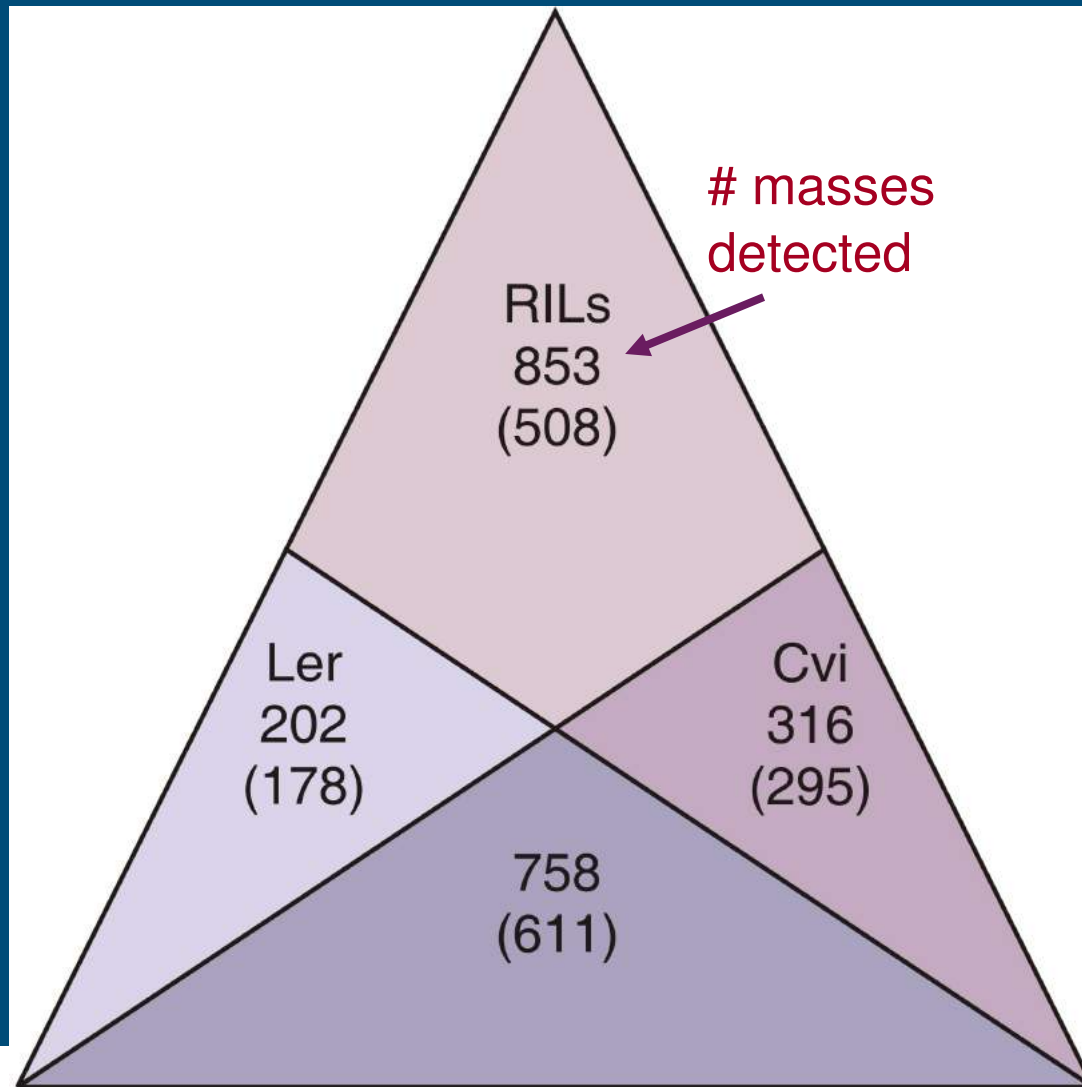
Variation in metabolite composition of parents



original LC-QTOF MS profiles

significantly different compounds
(n=5, factor 2 or more, 99% conf.)

Huge metabolic variation even between ecotypes





Rice Headspace volatile analysis

Key properties of rice fragrance

- Main focus has been on one fragrance compound:
 - 2-acetyl pyrroline (2-AP)
- BUt:
 - There are many breakdown products of lipid peroxidation:
 - Aldehydes and alcohols
 - Aliphatic and branched
 - And many other volatiles of diverse biochemical origins
 - e.g. benzoic acid-derived, terpenoids, amino acid-derived

Dynamic headspace-trapping using Tenax

Air in Air out



Parallel trapping:
series of
flow meters

thermo-
desorption
unit



MS

GC

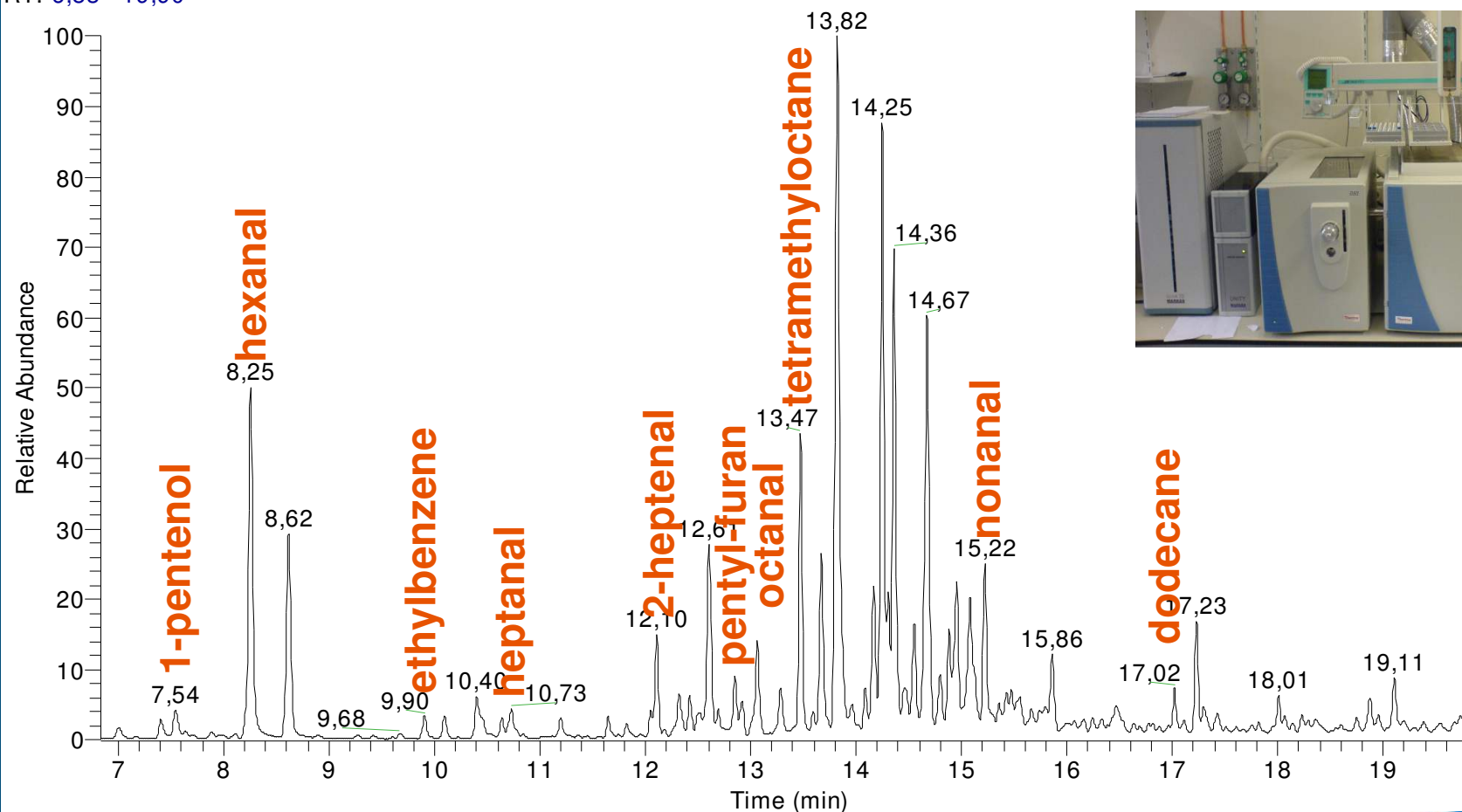


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GCMS rice headspace volatiles

RT: 6,83 - 19,90



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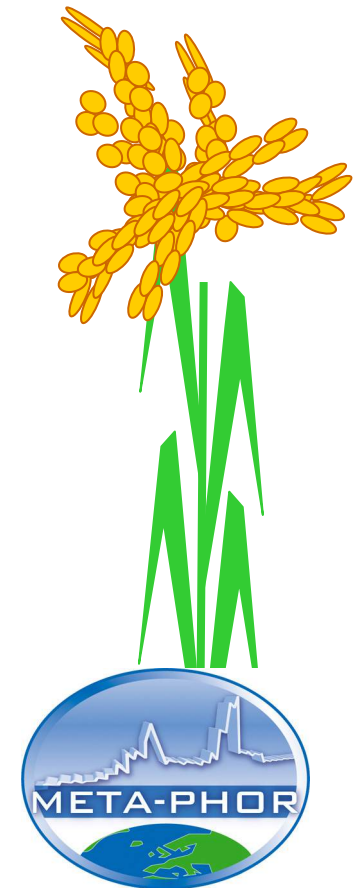
META-PHOR Fragrant rices

SAMPLES/GERMPLASM

- Fresh Jasmines
- Stored Jasmines
- Fresh Basmati's
- Stored Basmati's
- Aromatic / Indica
- Temp. & Trop Japonica

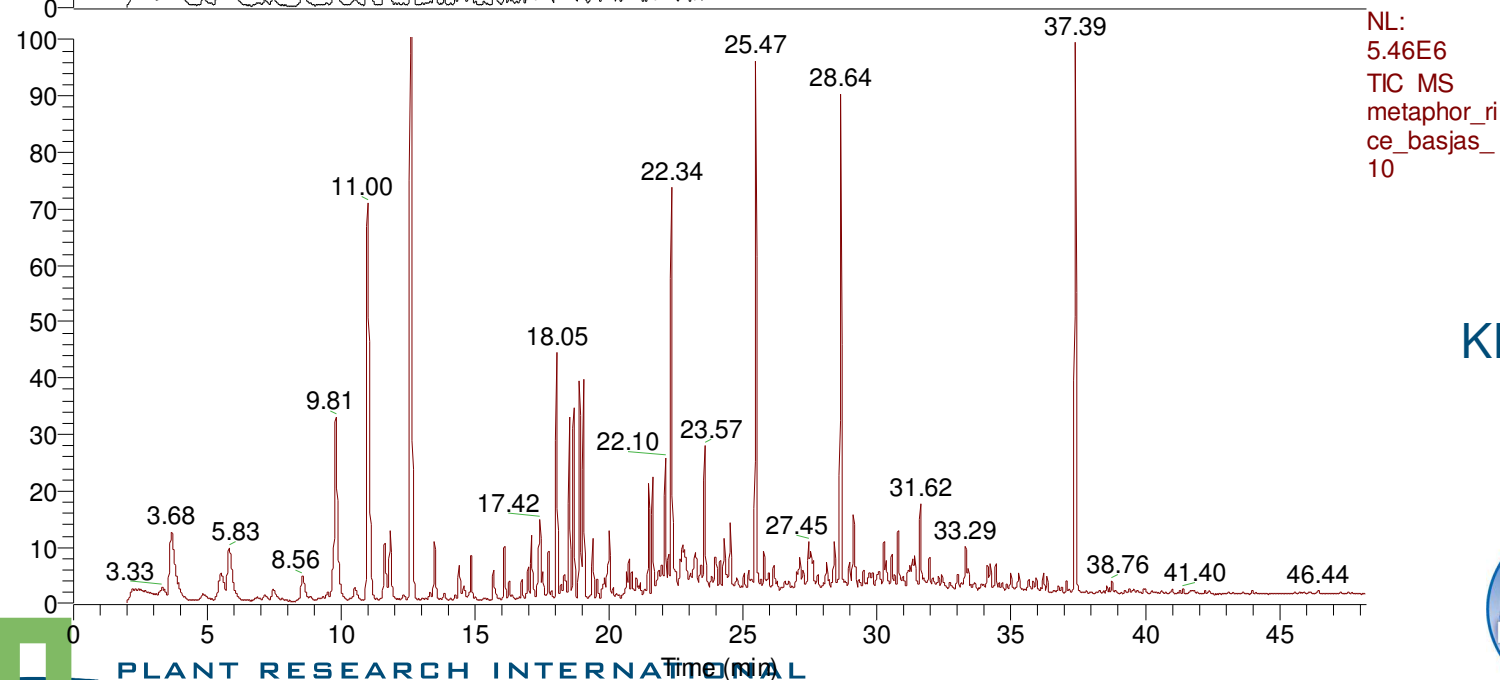
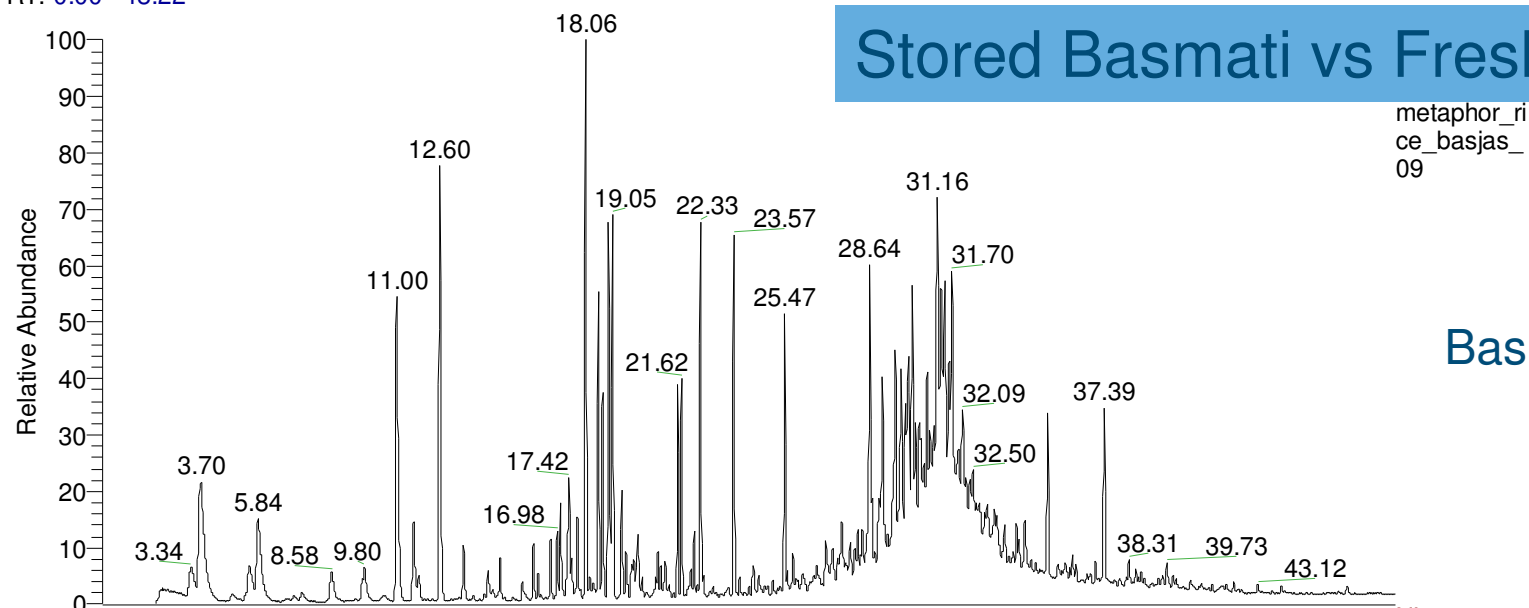
SOURCES

- Thailand
- The Philippines
- Pakistan
- Cambodia
- Australia
- Japan
- Laos
- Iran
- Malaysia
- Indonesia
- Vietnam



RT: 0.00 - 48.22

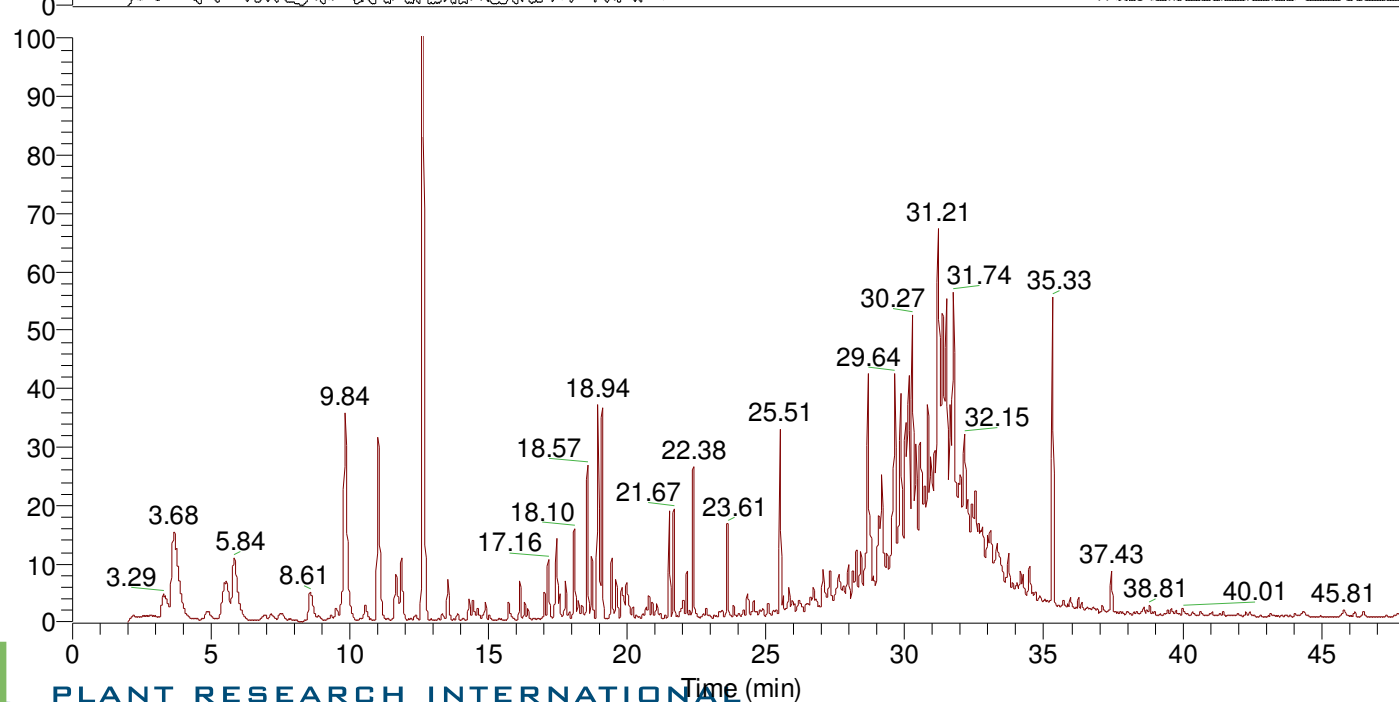
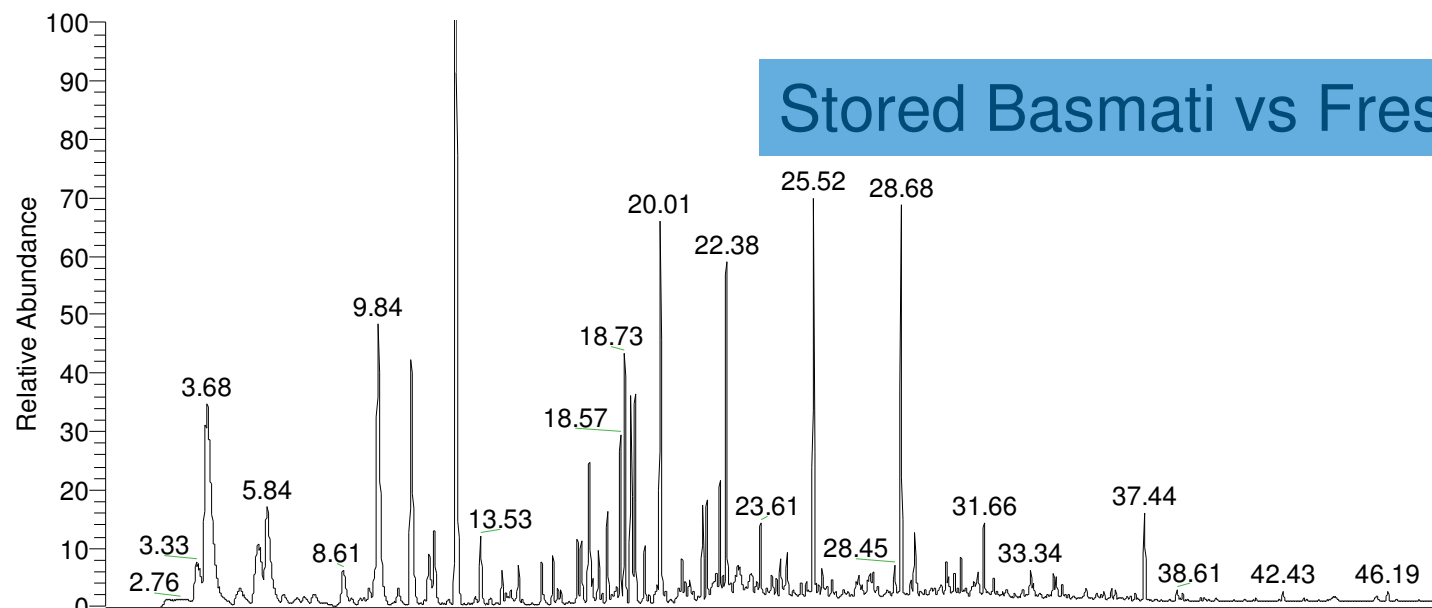
Stored Basmati vs Fresh Jasmine



RT: 0.00 - 48.22

NL:
1.00E7

Stored Basmati vs Fresh Basmati



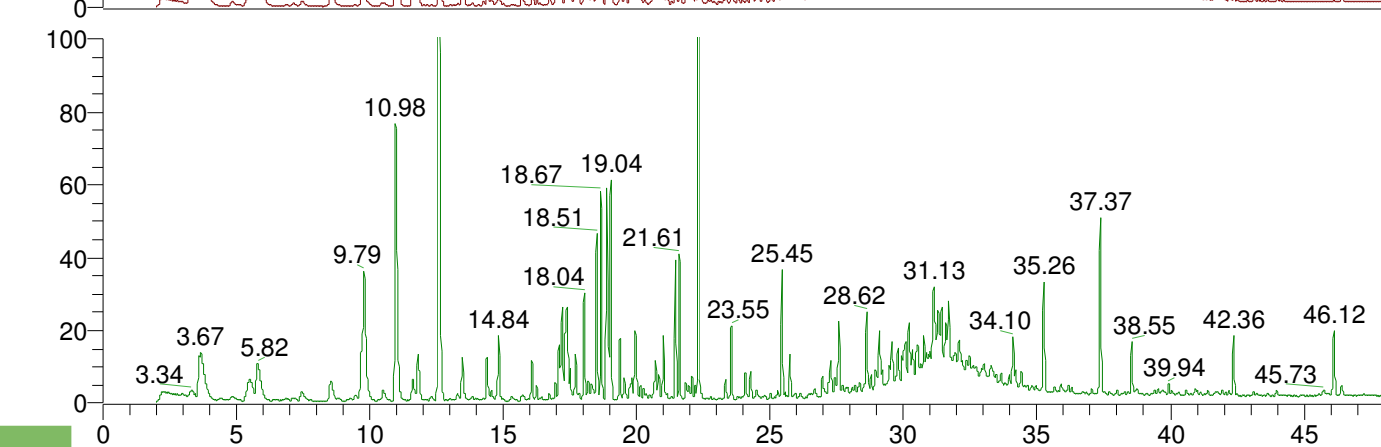
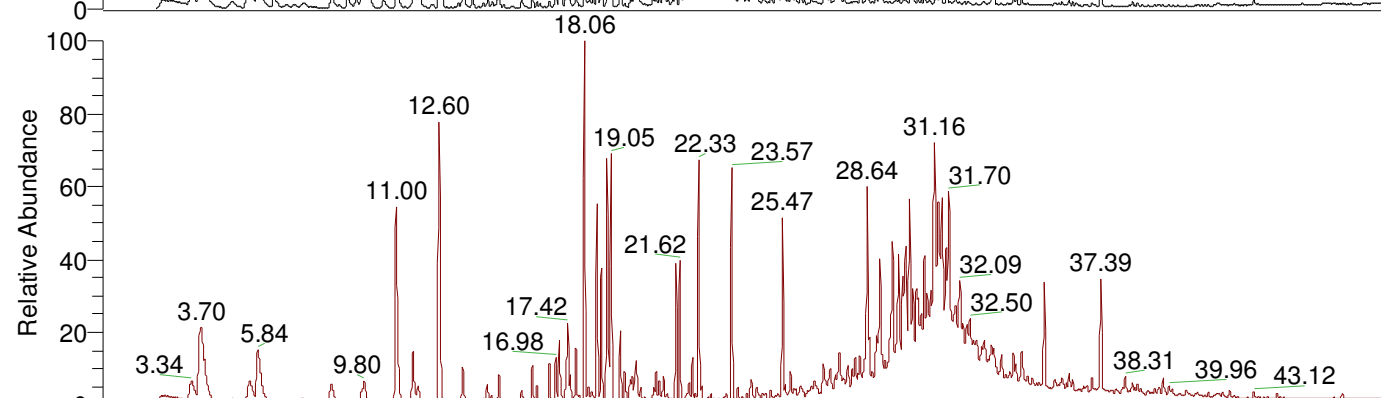
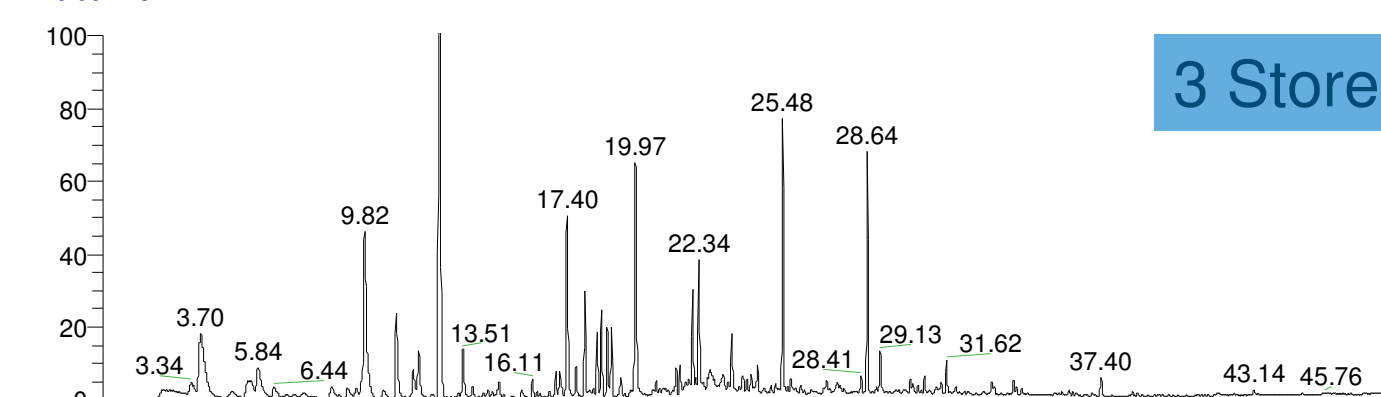
NL:
1.00E7
TIC MS
Metaphor_ri
ce_04



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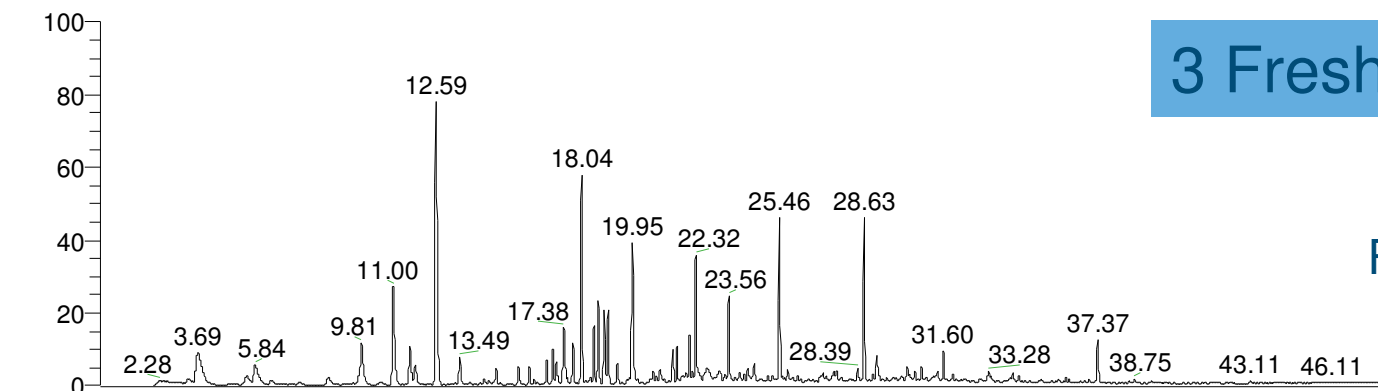
RT: 0.00 - 48.22



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RT: 0.00 - 48.22

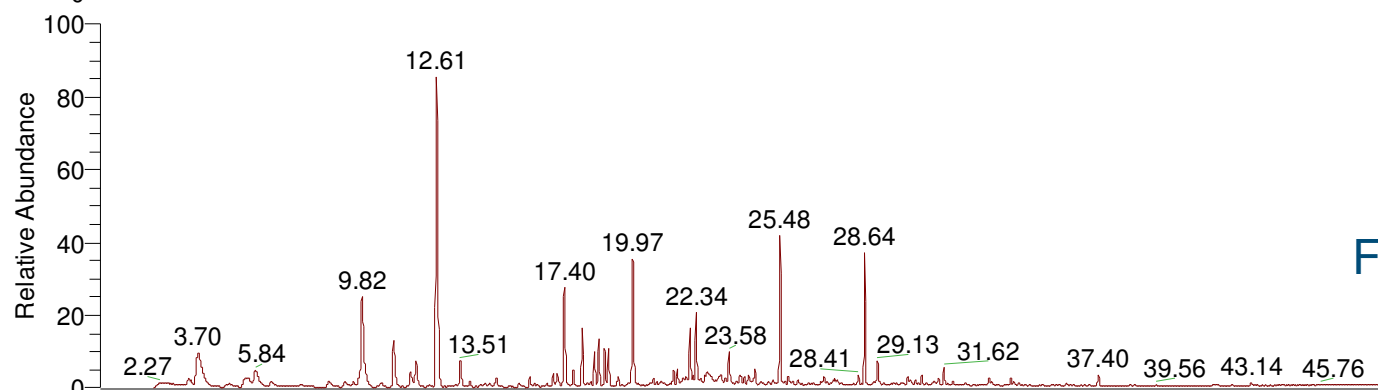


NL:

3 Fresh Basmati's

ce_basjas_
14

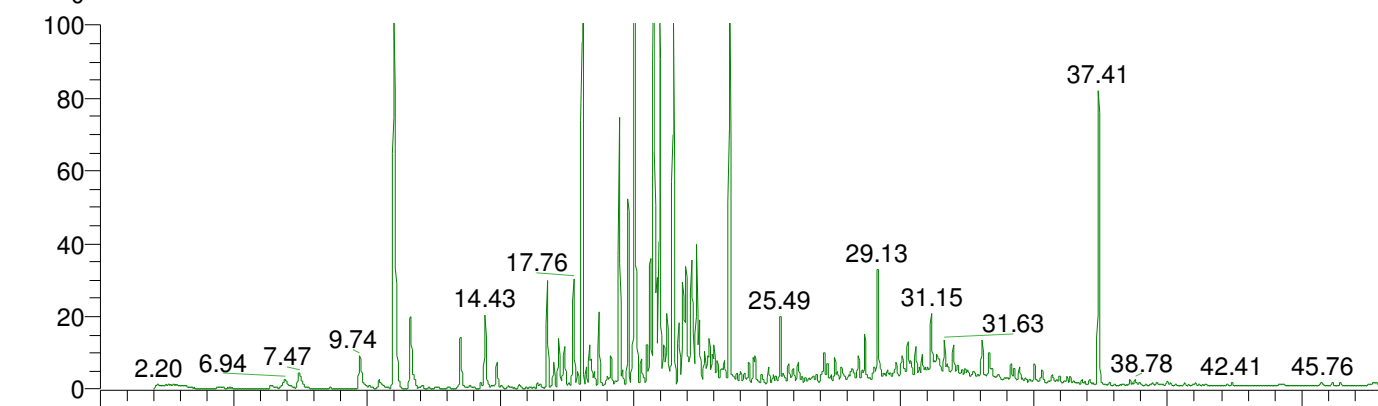
Fresh Basmati 370



NL:

1.00E7
TIC MS
metaphor_ri
ce_basjas_
06

Fresh Basmati 2000



NL:

1.00E7
TIC MS
metaphor_ri
ce_26

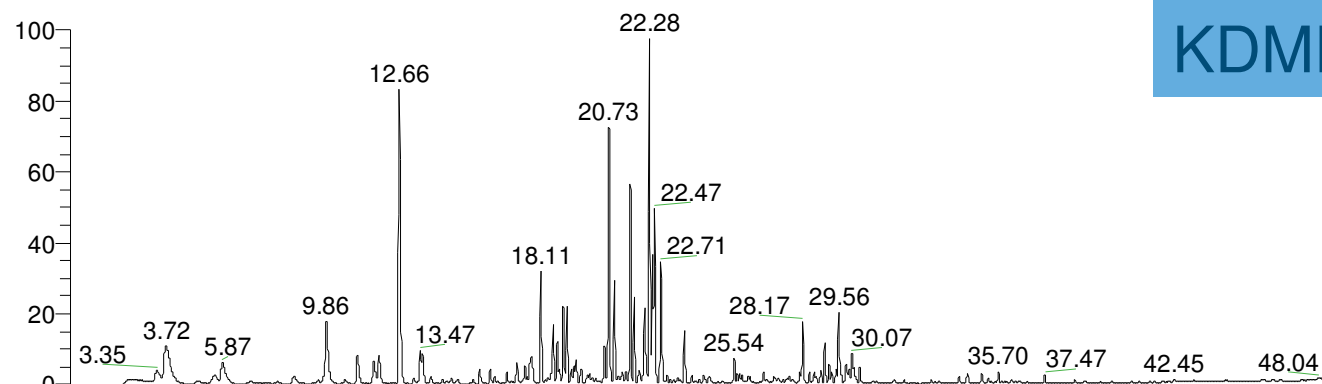
Fresh Shaheen
Basmati



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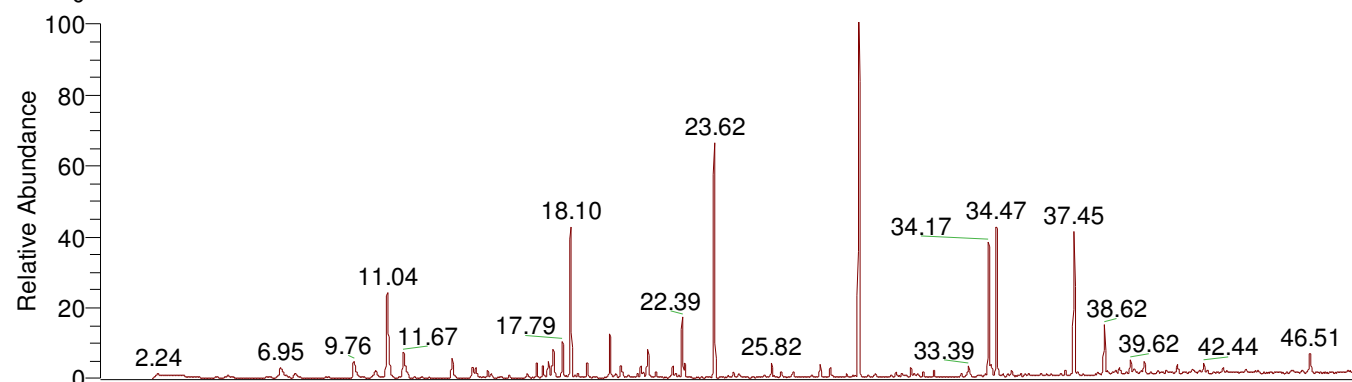
RT: 0.00 - 48.21



KDML Jasmines

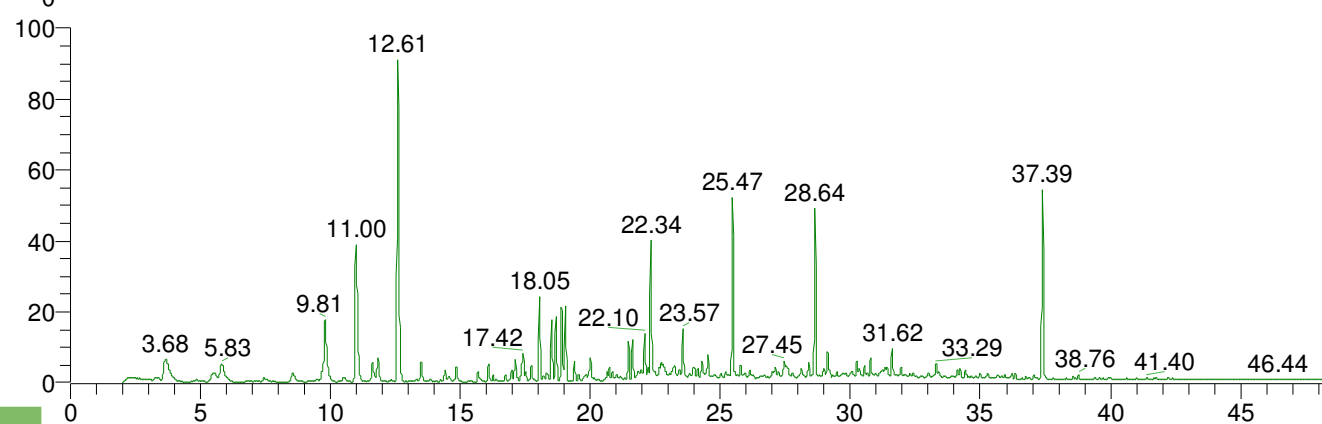
metaphor_ri
ce_basjas_
50

Fresh KDML 105



NL:
1.00E7
TIC MS
metaphor_ri
ce_basjas_
41

Stored KDML 105



NL:
1.00E7
TIC MS
metaphor_ri
ce_basjas_
10

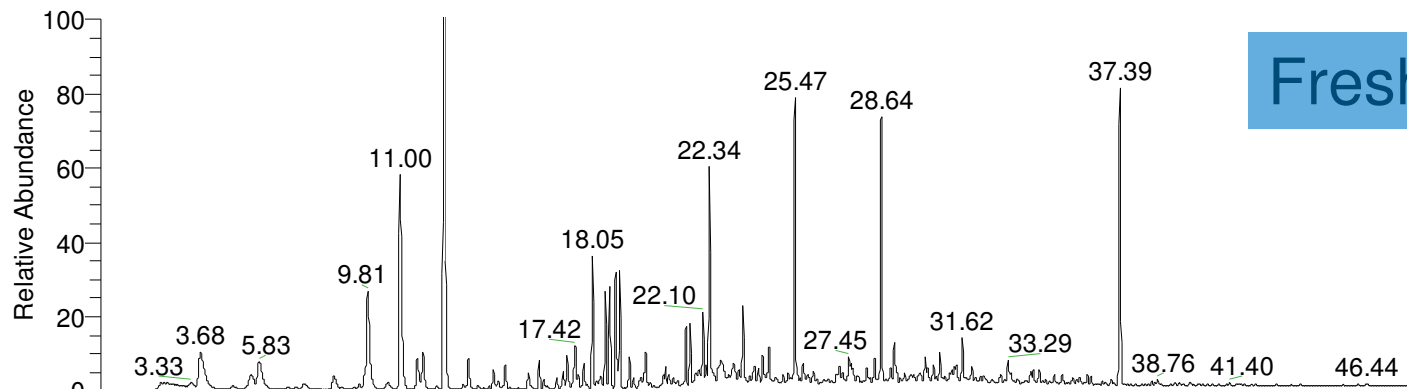
Stored at cold room
KDML 105



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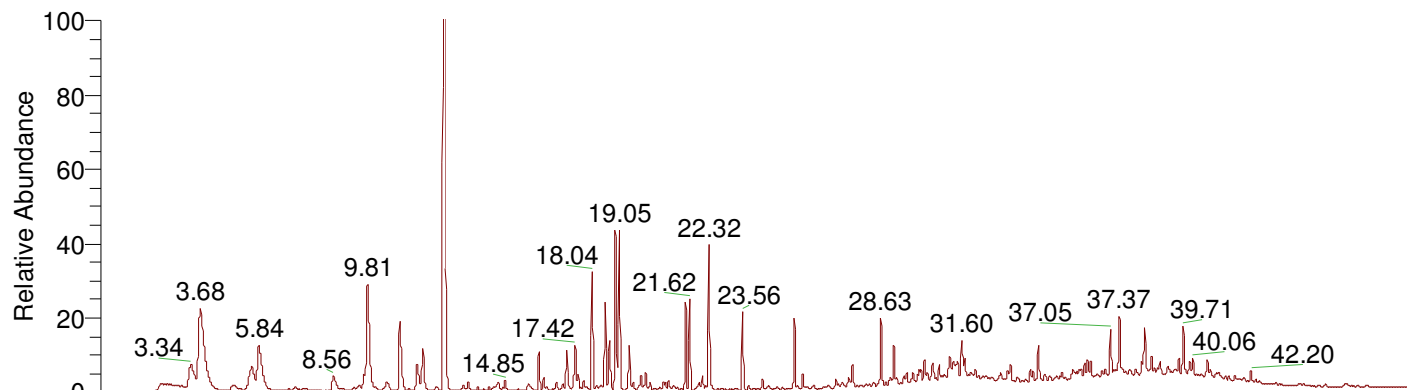


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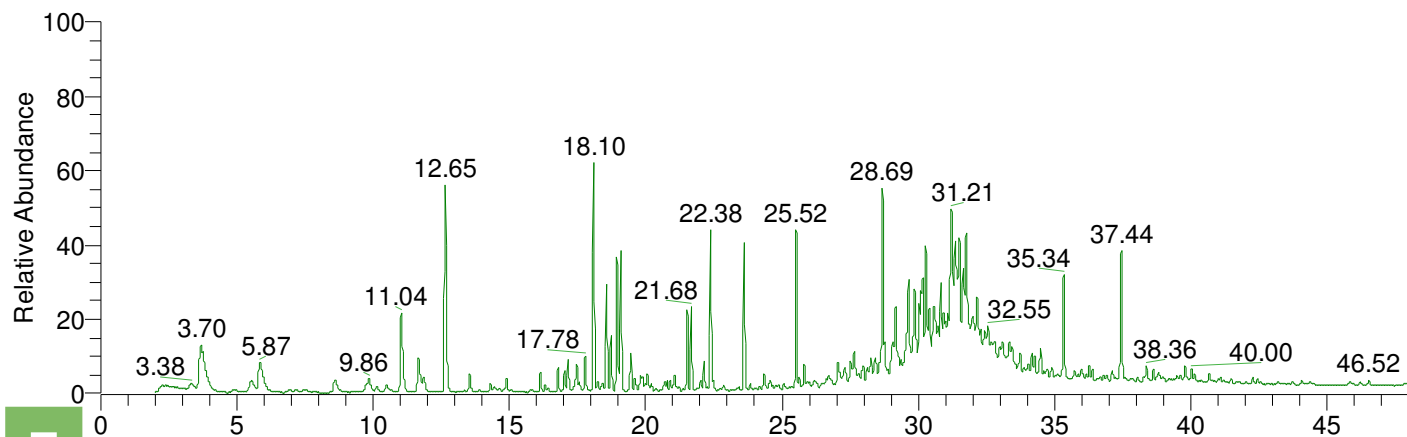


Fresh Jasmines

KDML 105



Q 74

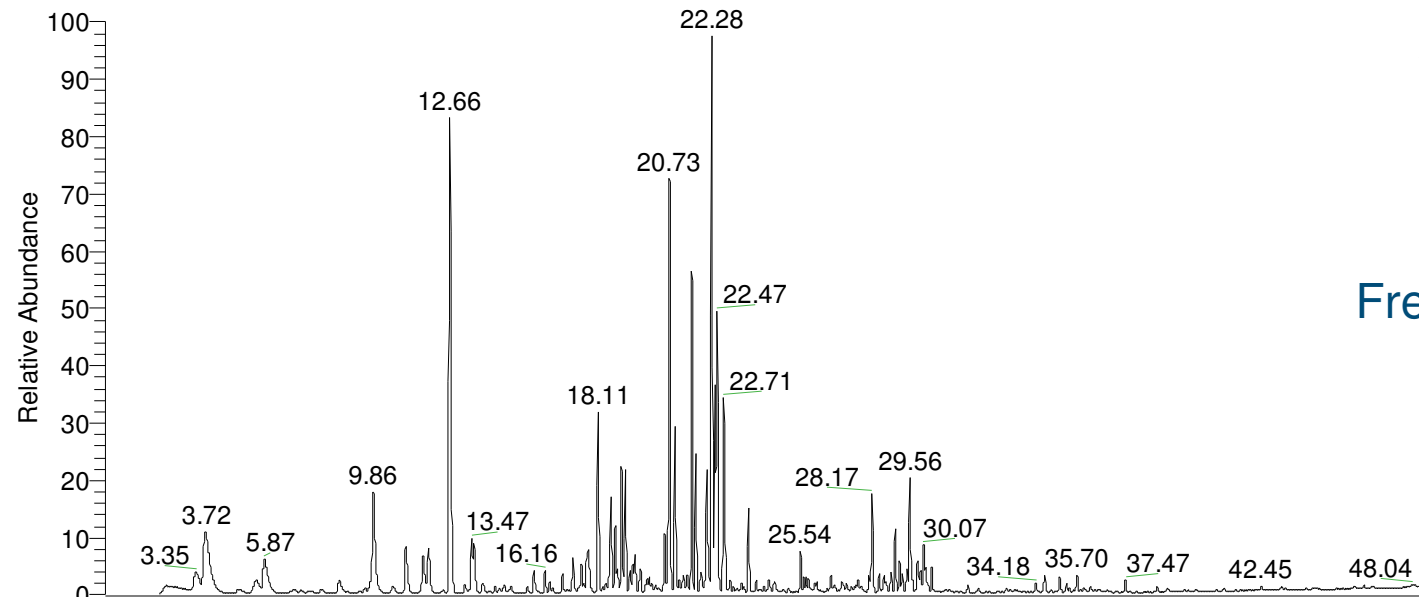


RD 33



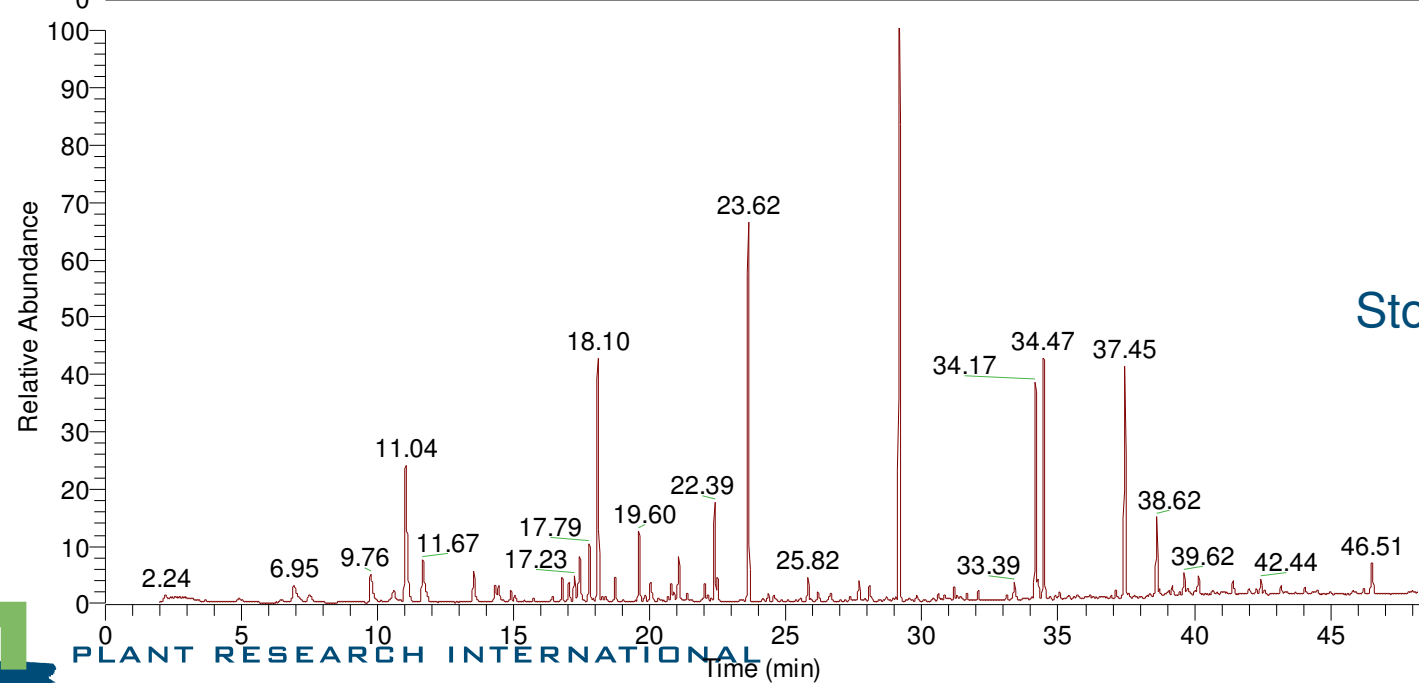
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RT: 0.00 - 48.21



NL:
1.00E7
TIC MS
metaphor_ri
ce_basjas_
50

Fresh KDML 105



NL:
1.00E7
TIC MS
metaphor_ri
ce_basjas_
41

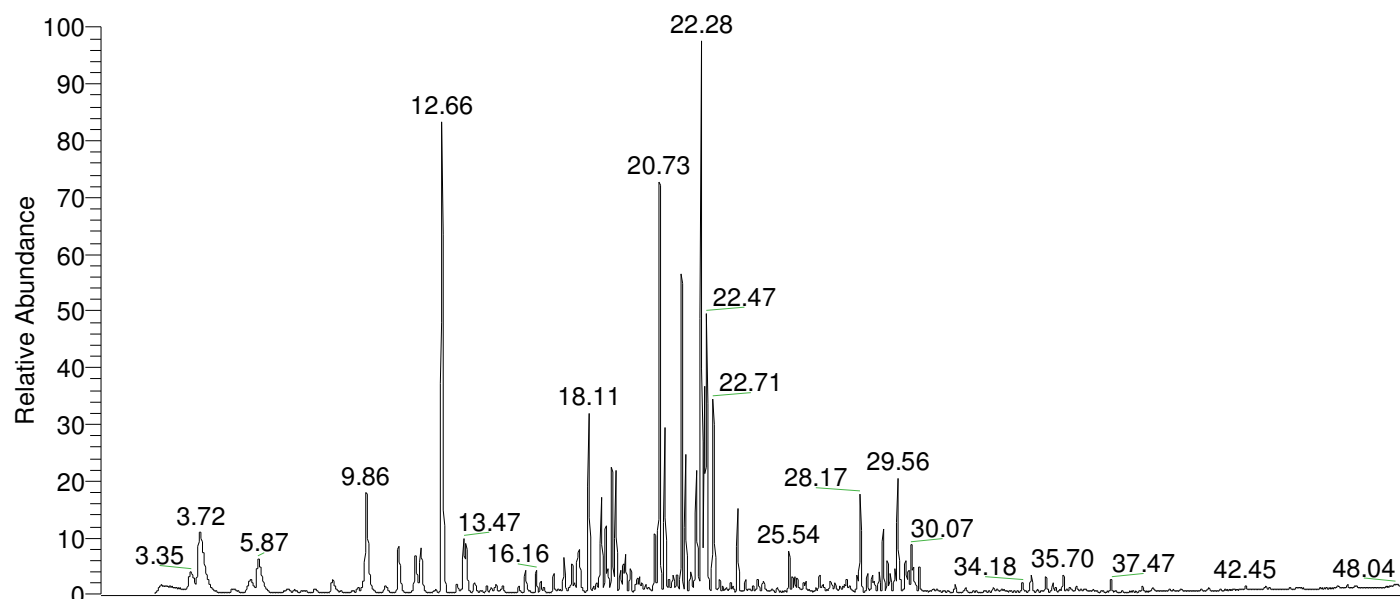
Stored KDML 105



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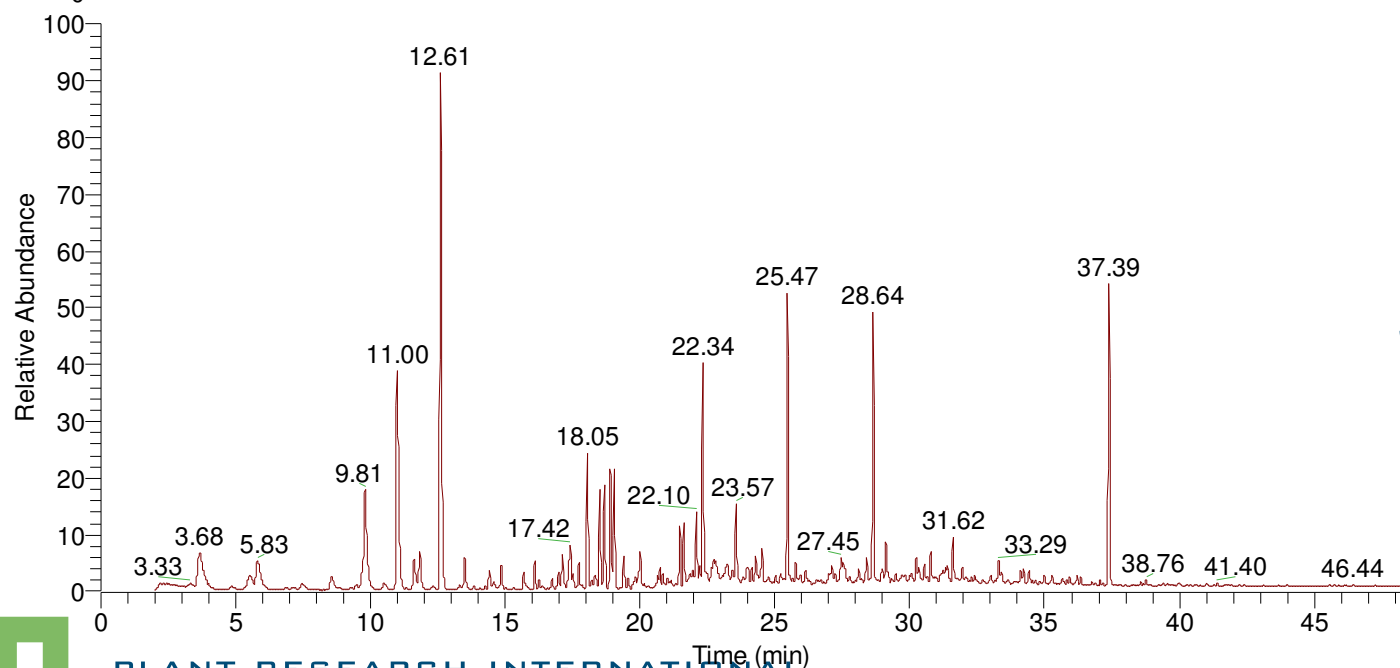


RT: 0.00 - 48.21



NL:
1.00E7
TIC MS
metaphor_ri
ce_basjas_
50

Fresh KDML 105



NL:
1.00E7
TIC MS
metaphor_ri
ce_basjas_
10

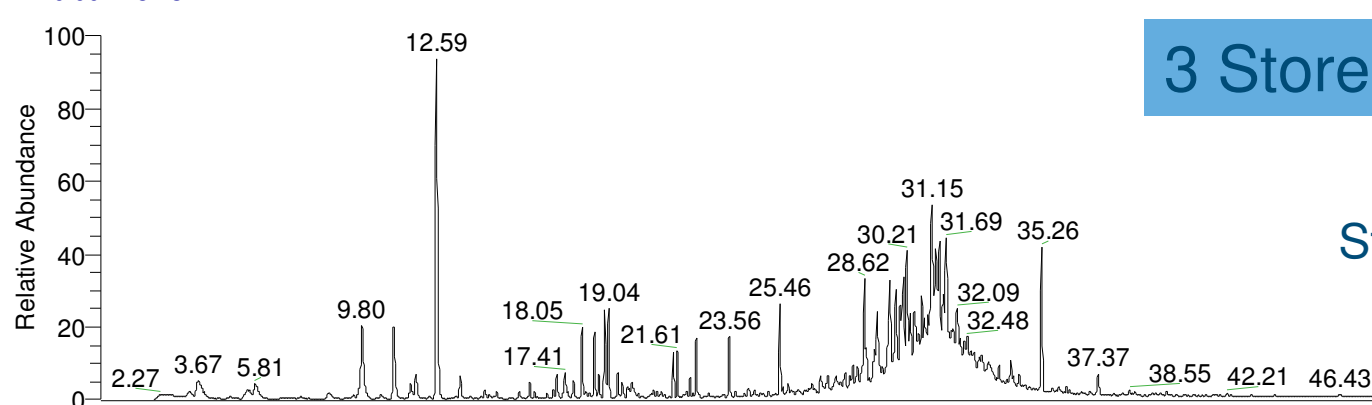
Stored at cold room
KDML 105



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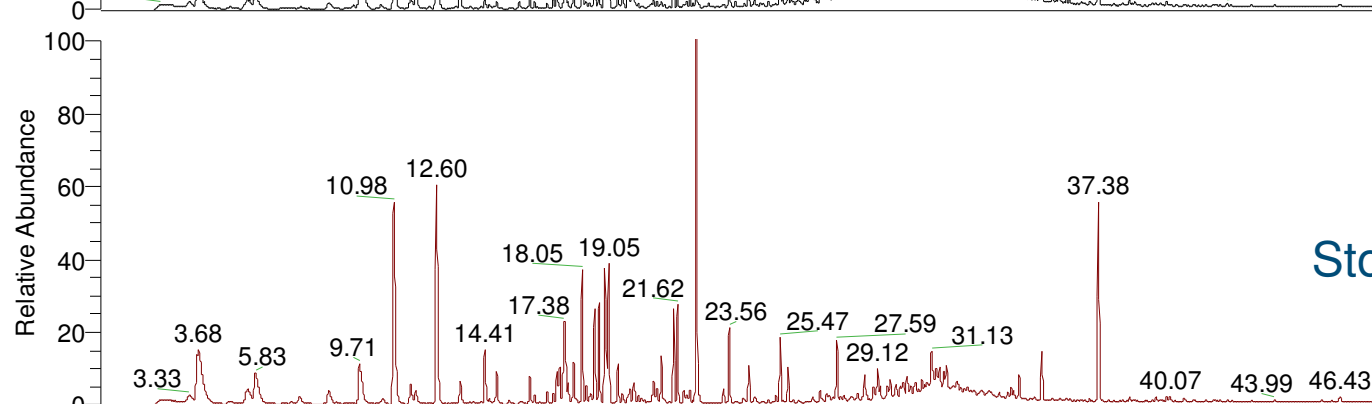
RT: 0.00 - 48.23



3 Stored Basmati's

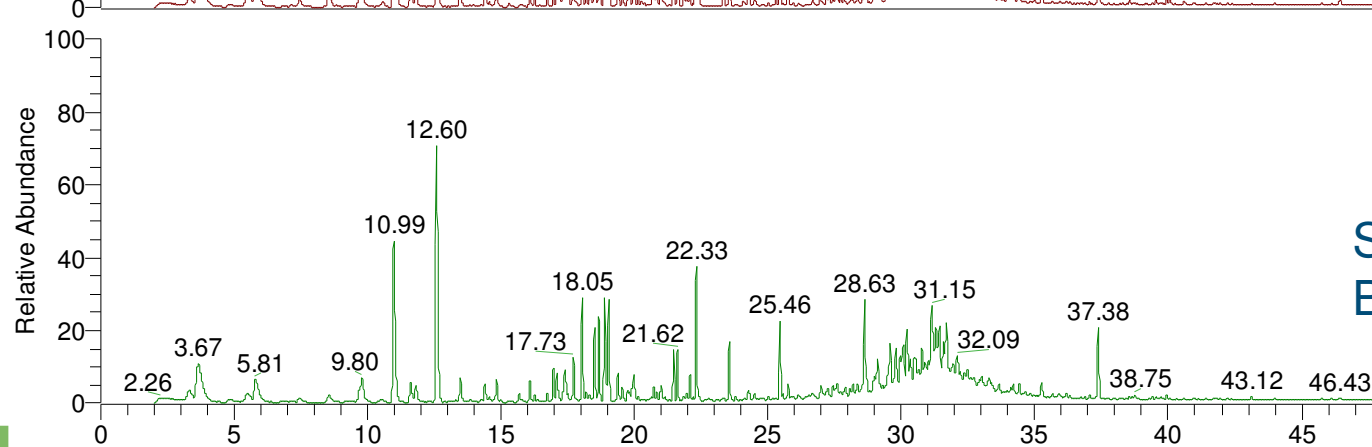
NL:
ce_basjas_
20

Stored Basmati 370



NL:
1.00E7
TIC MS
metaphor_ri
ce_basjas_
25

Stored Basmati 2000



NL:
1.00E7
TIC MS
metaphor_ri
ce_basjas_
23

Stored Shaheen
Basmati



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Metabolomics – tracing and tracking / authentication

- Rice on its way
- Olive oil
- Wine / grape types
- Tea
- Coffee
- Fruit juices
- Apple purees

Conclusions Metabolomics as Authentication tool

- Plant metabolite profiles are a tremendously rich source of biochemical markers
- Metabolomics is already at a stage of applicability for tracing and tracking / adulteration and authentication
- Variety differences are extensive
- Unique markers for each variety are unlikely but qualitative combinations will be identifiable as unique varietal fingerprint

Rice quality Metabolomics Workshop, Laos 2008

