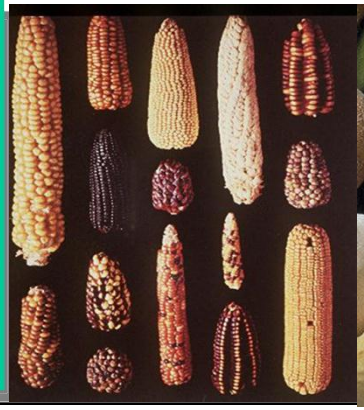
The use of metabolomics approaches to mine the genetic diversity in tomato.

Dr. Arnaud Bovy

Genetic variation in classical Plant Breeding

- **Genetic variation** is the existence of variation in genetic material within a species or between a crossable species
- Basis is **mutation**






Breeding for quality

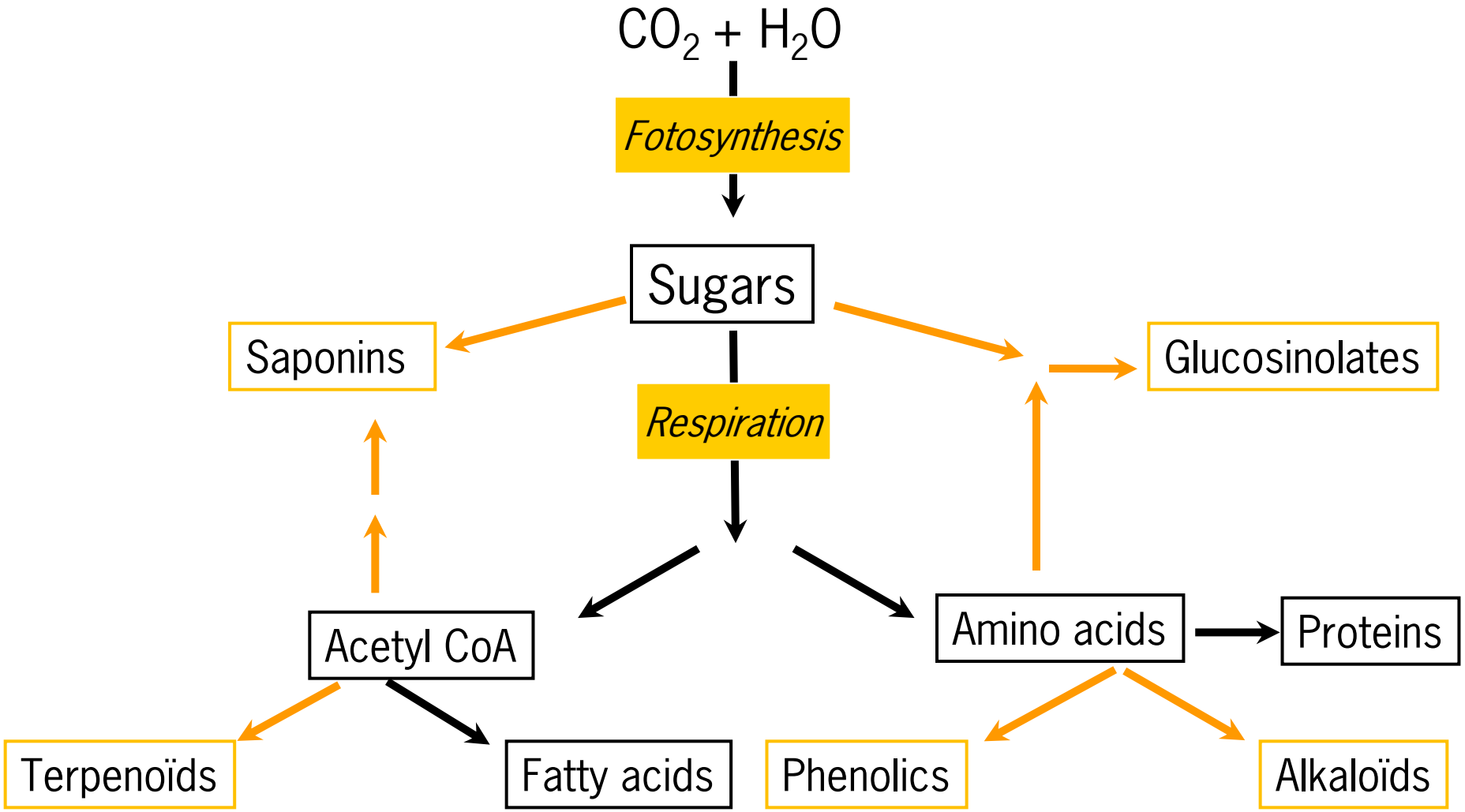
- Quality traits:
  - Flavour
  - Fragrance
  - Nutritional value
  - Colour
  - Shelf-life


Many quality traits are determined by metabolites

‘Rich Food’: rich in secondary metabolites

- Primary metabolites
  - Are present in all plant cells and are essential for growth and development, eg sugars, amino acids,….
- Secundary metabolites
  - (apparently) not essential for growth and development
  - Presence limited to specific compound classes or organs, tissues

How are secondary metabolites produced?



Which secondary metabolites do we know?

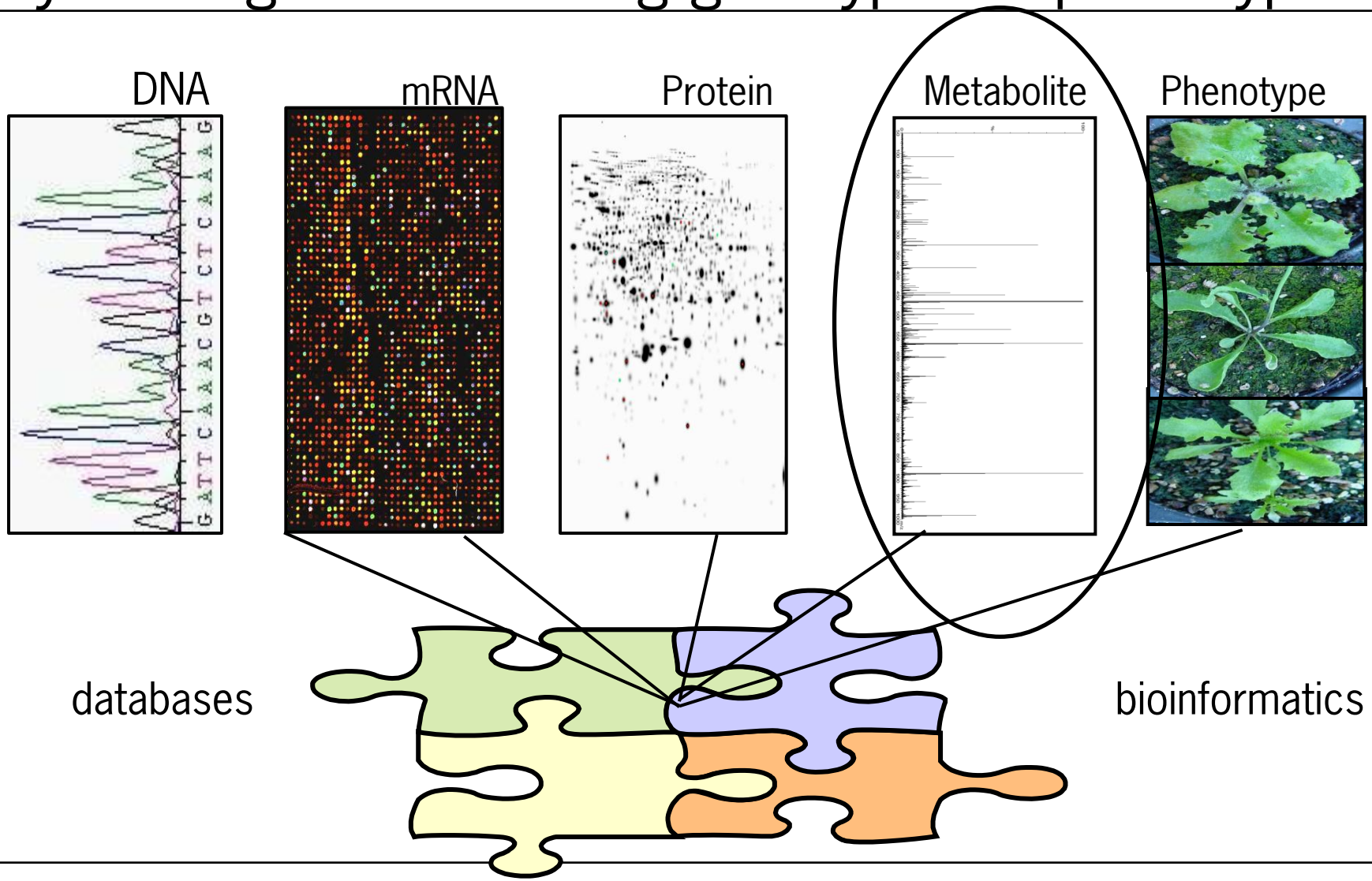
- Flavonoids, terpenoids, carotenoids, alkaloids, glucosinolates……..
- Large groups of compounds:
  - > 5.000 flavonoids
  - > 20.000 terpenoids
  - > 100.000 sec. metabolites in Plant Kingdom

Why does the plant produce sec. metabolites?

- Pathogen resistance
  - Terpenoids, alkaloids, flavonoids
- Cope with abiotic stress (UV, cold, drought, salt)
  - Flavonoids, vitamin C, vitamin E (antioxidanten)
- Attract pollinators and seed dispersers
  - Terpenoids (fragrance)
  - Flavonoids en carotenoids (colour)

- Sec. metabolites are BIOACTIVE compounds

Systems genetics: linking genotypes to phenotypes



Terminology

Metabolomics:

- Technology aimed at obtaining a broad as possible insight in the biochemical make-up of a biological product
- Large scale – datastream requires *in silico* approach
  - Tomato- 94 varieties, 203 analyses, 4.06 million data points, some Gb data – for one type of analysis!

Metabolomics: what can you do with it?

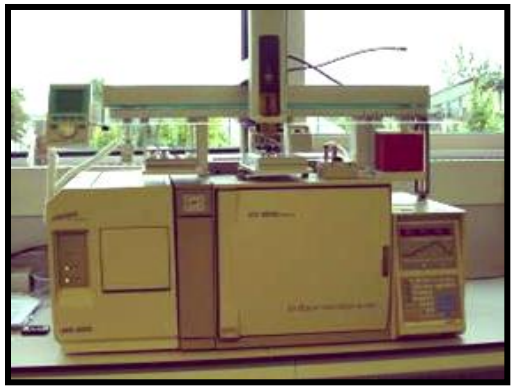
- Get more insight in complex traits
  - Taste
  - Fragrance
  - Nutritional value
- Acquire detailed knowledge on the influence of genetic or environmental factors on the biochemical composition
  - Processing
  - Storage
- Tracking & tracing: markers for authenticity, origin, quality
- Tool to monitor genetic diversity.....

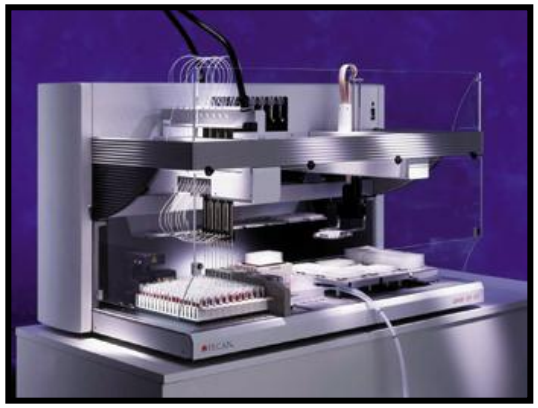
Metabolomics

- Non-targeted analyses
  - Detect as many as possible compounds in a given extract
- Targeted analyses
  - Analyses optimised for selected (groups of) compounds

Technology platform Wageningen

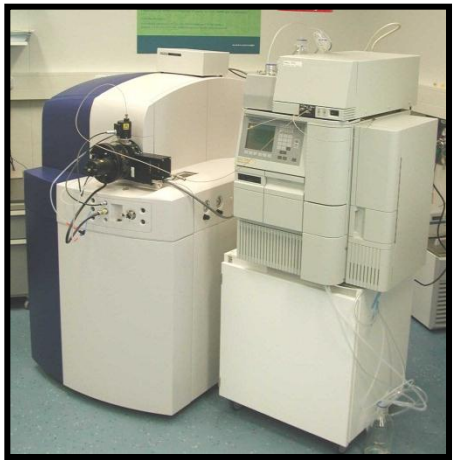
GC-MS





extraction robot

LC-MS



WU-NMR

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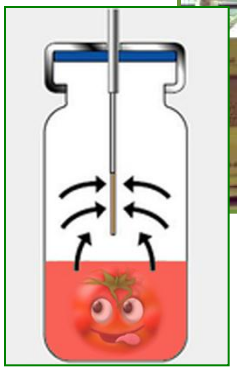
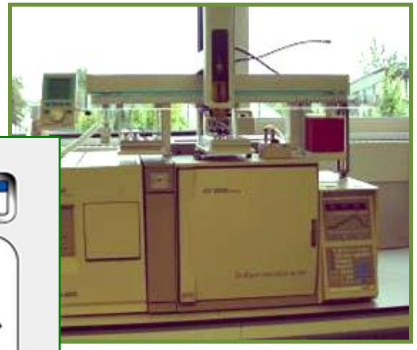
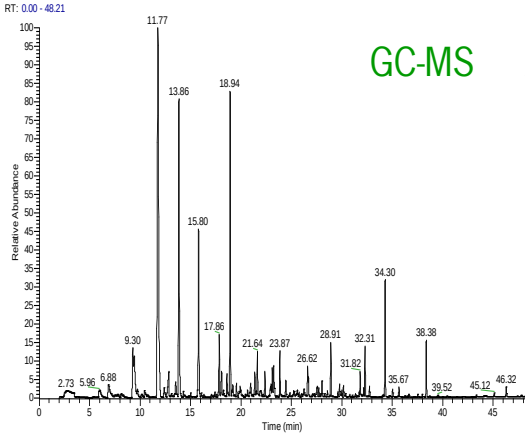
Metabolomics



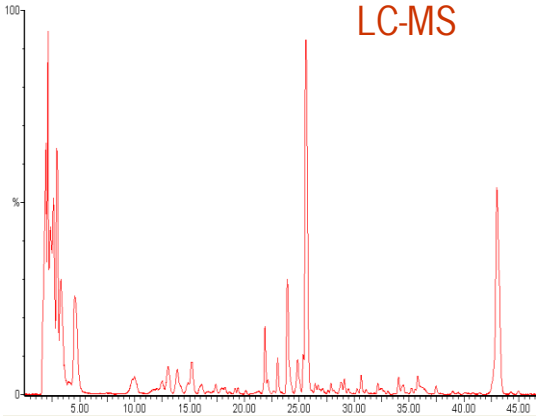


Headspace SPME-GC-MS



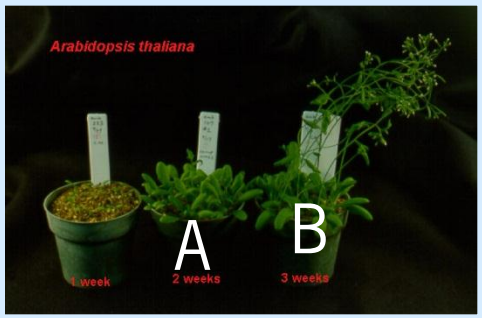
GC-MS

LC-QTOF-MS

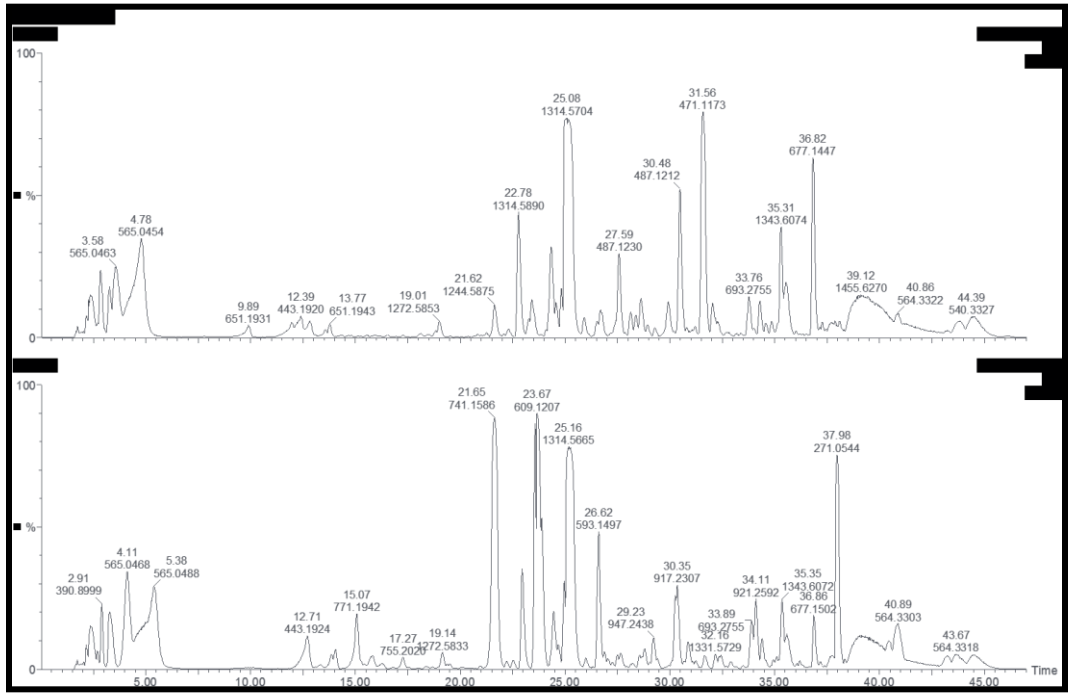


LC-MS

Comparative metabolomics



sample A



sample B

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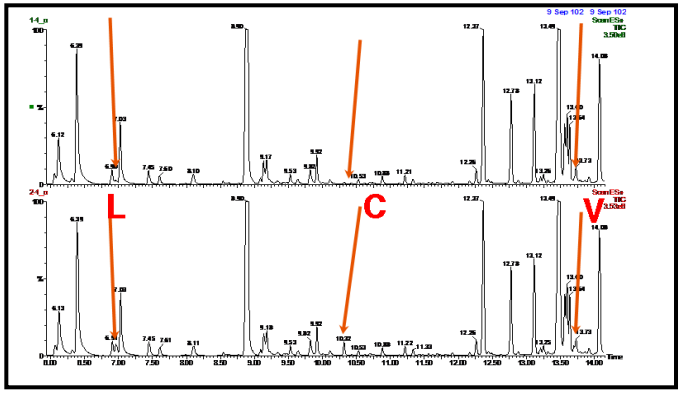
Bioinformatics for metabolomics

2.



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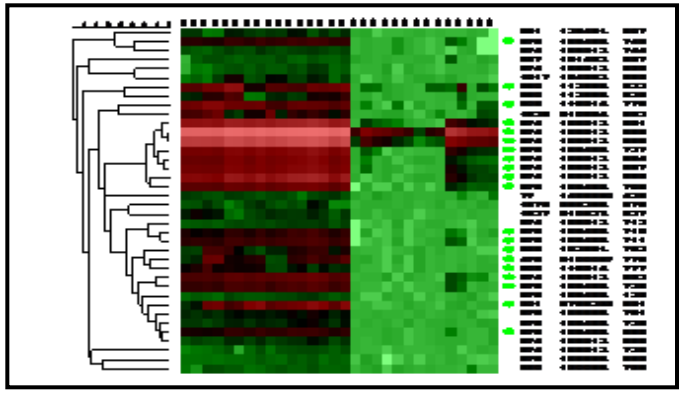

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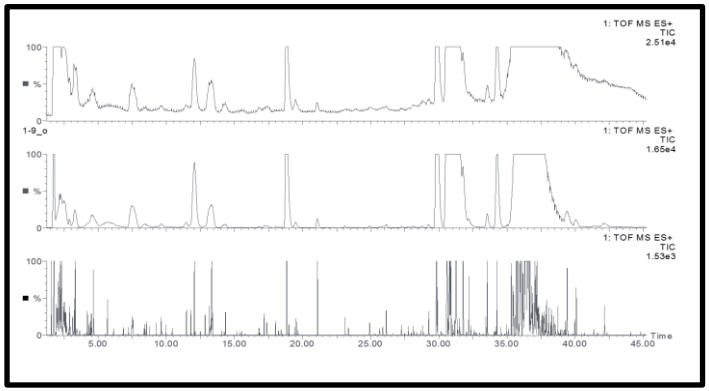

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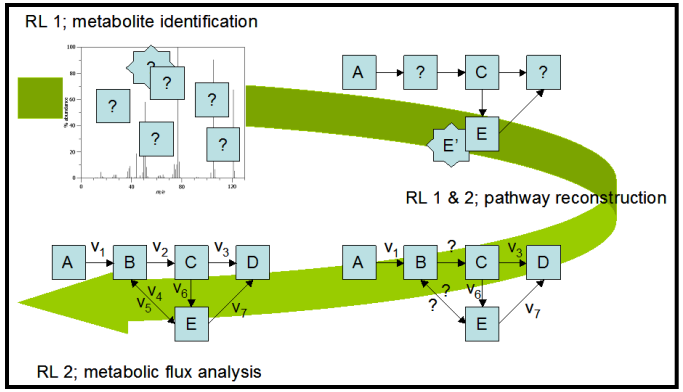
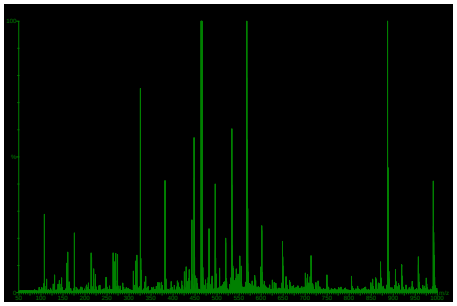


Breeding for quality: general strategy

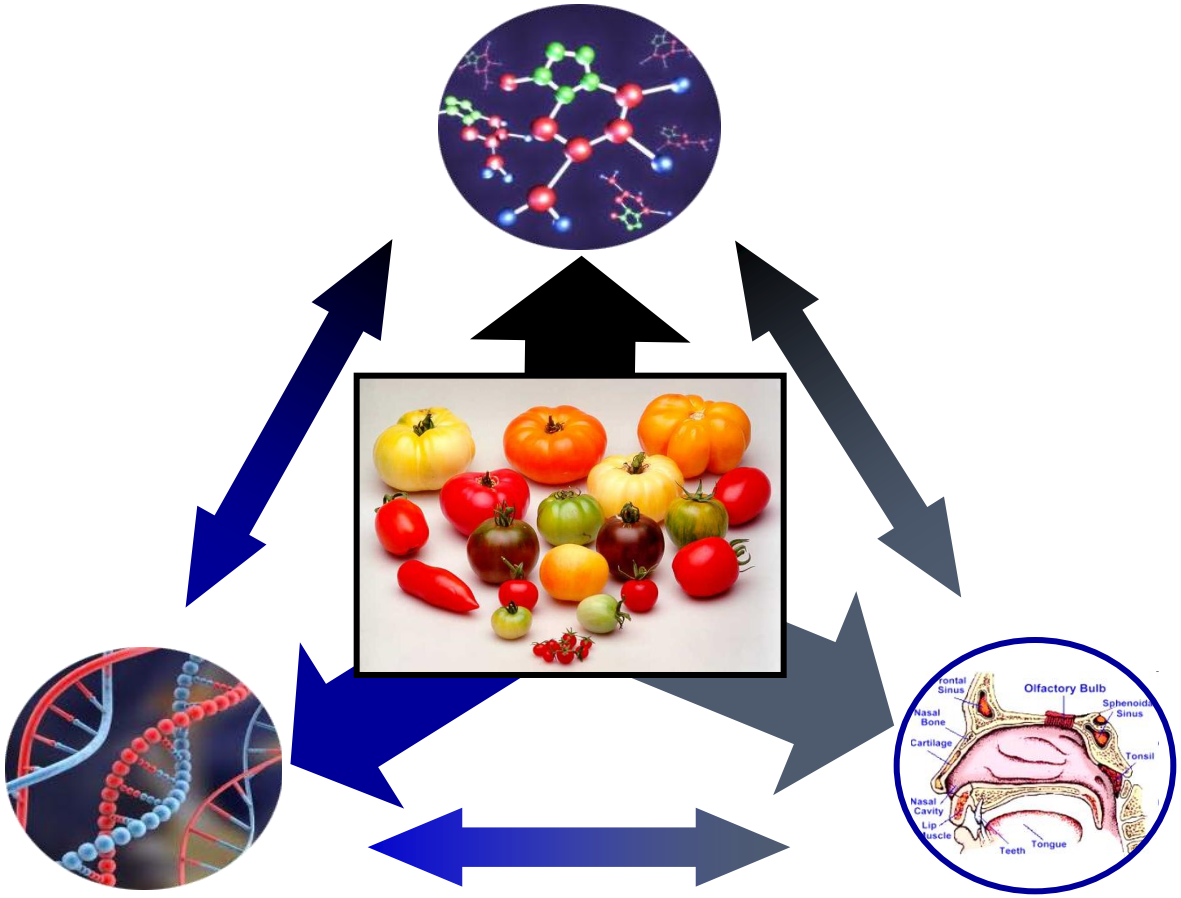
- Explore genetic diversity
  - Collections and breeding populations
  - Link markers and genes to traits
- Unravel metabolic pathways
  - Metabolomics/Biochemistry
  - Gene isolation
- Modify the composition
  - Marker-assisted breeding
  - Genetic engineering
  - Bioconversion



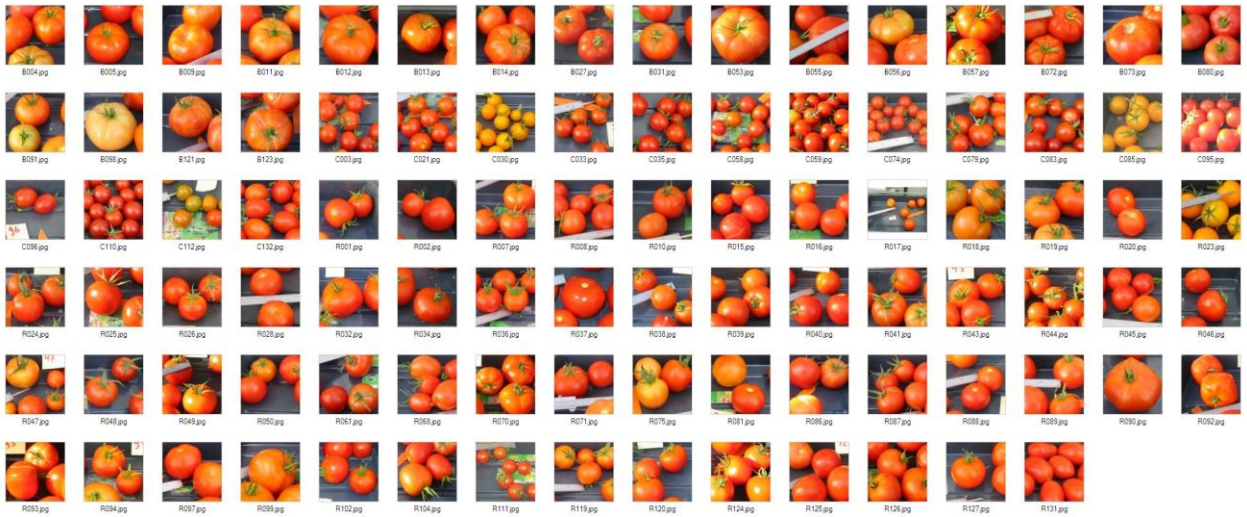
Research examples

- Unravel the taste of tomatoes
- Screen metabolic diversity in wild tomato accessions
  - Collection of wild relatives
  - Introgression line population

Unravel the taste of tomatoes

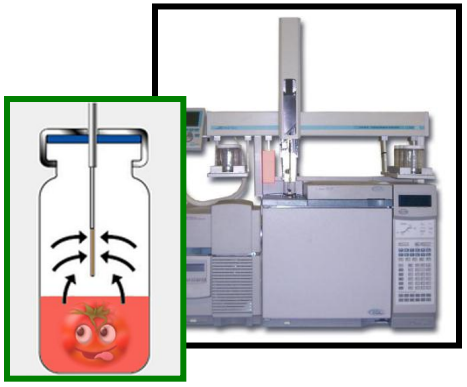


Characterisation of commercial tomato germplasm: 94 cultivars



Volatiles, sugars & acids

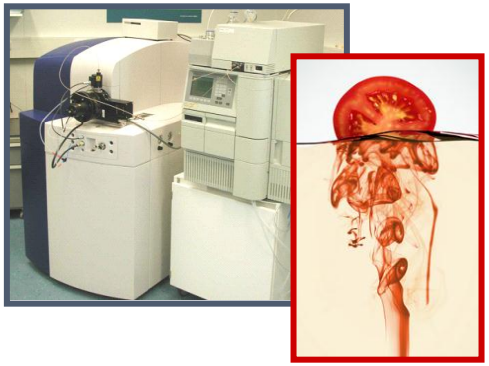
Gas Chromatography-Mass Spectrometry (GC-MS)



Sensory panel

Semi-polar compounds

Liquid Chromatography-Mass Spectrometry (LC-MS)



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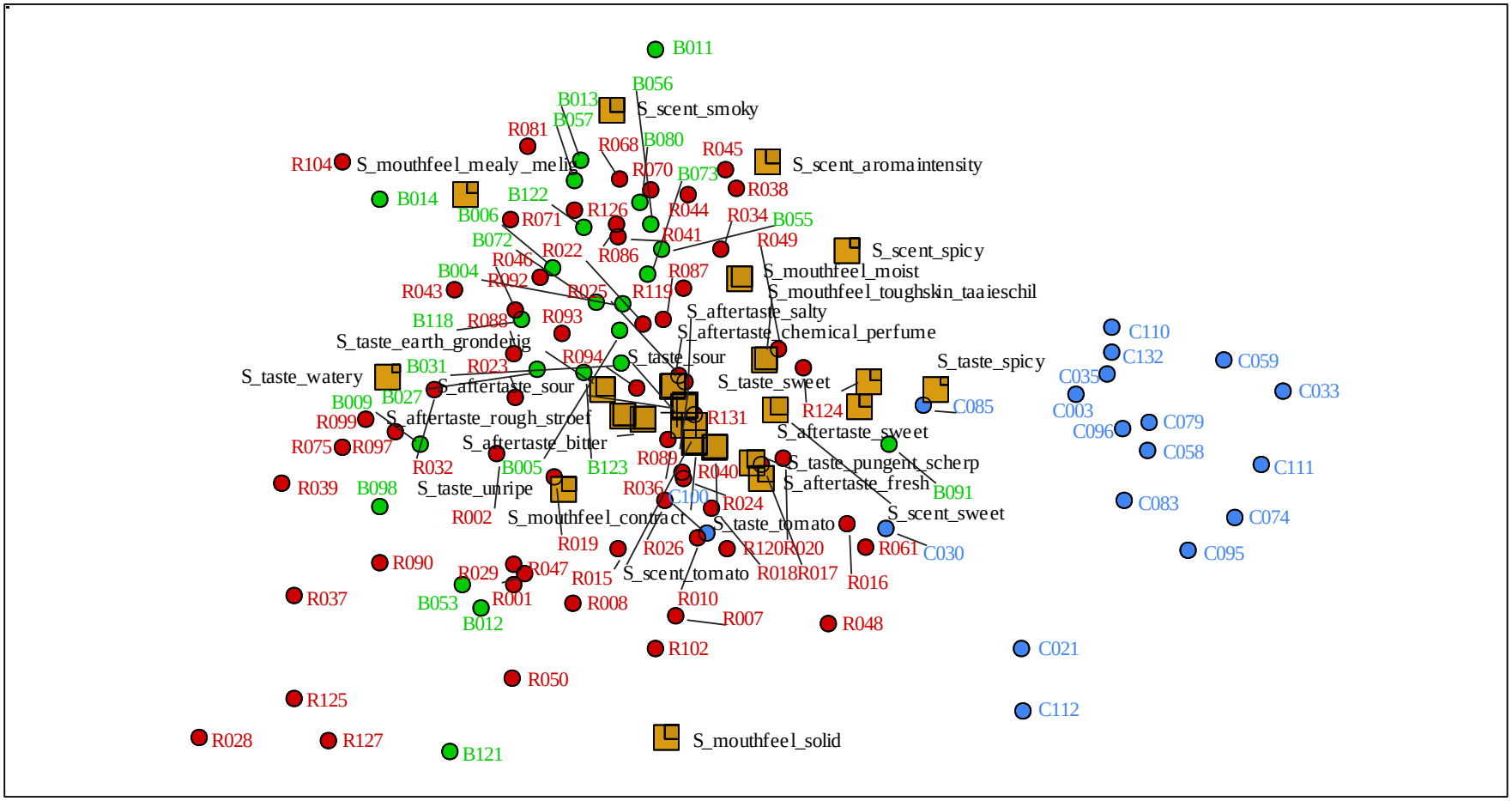
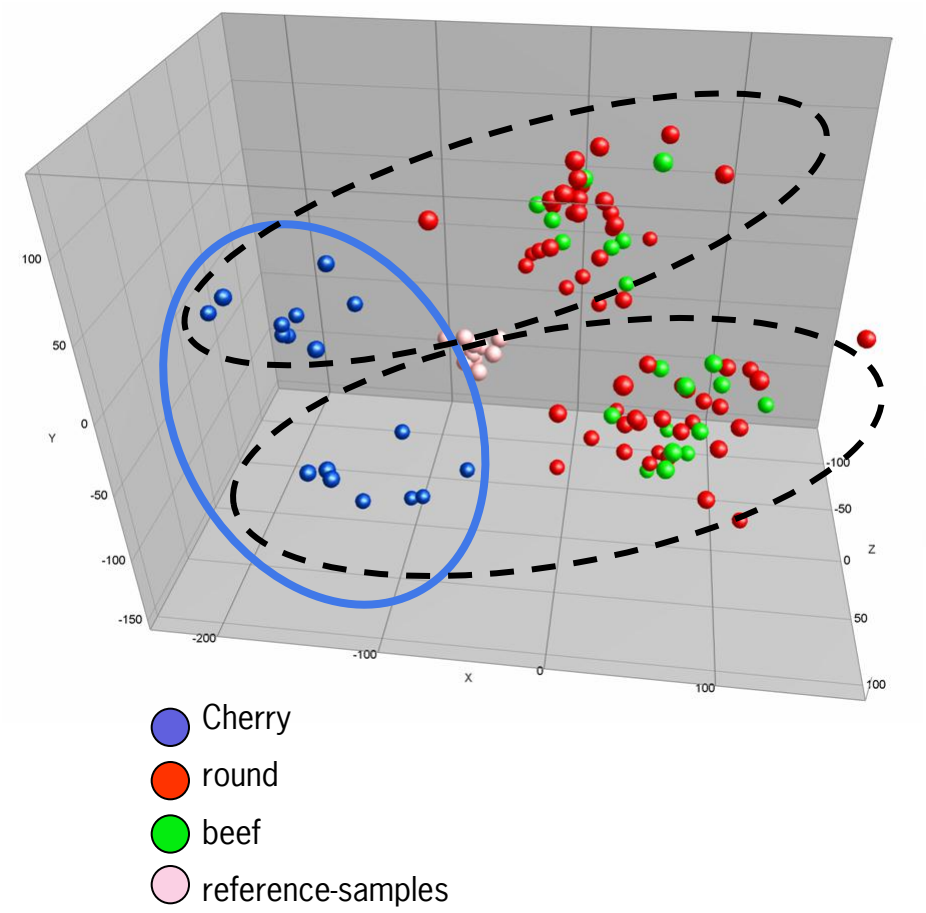
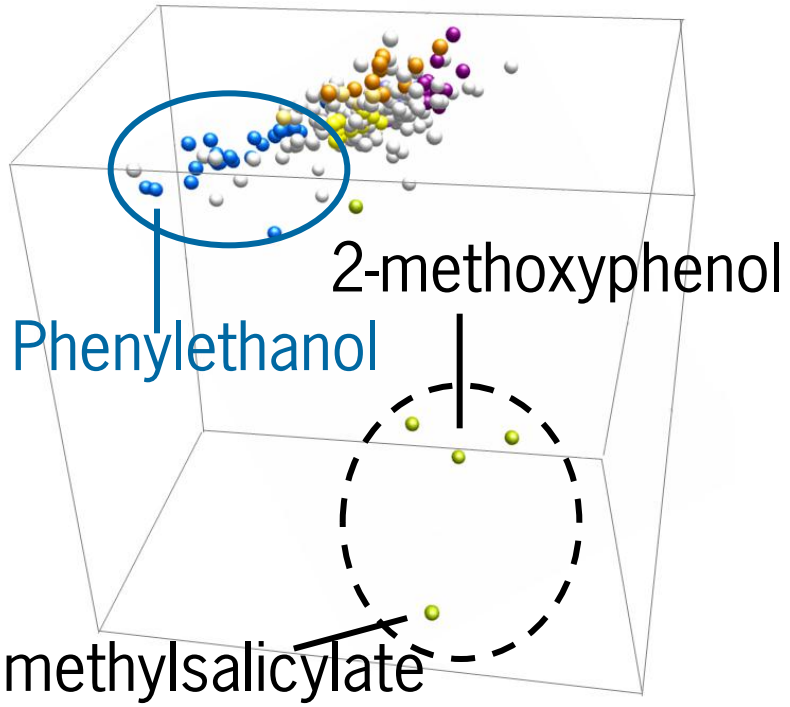
Sensory data



55%

Metabolic contrast based on 300 volatiles





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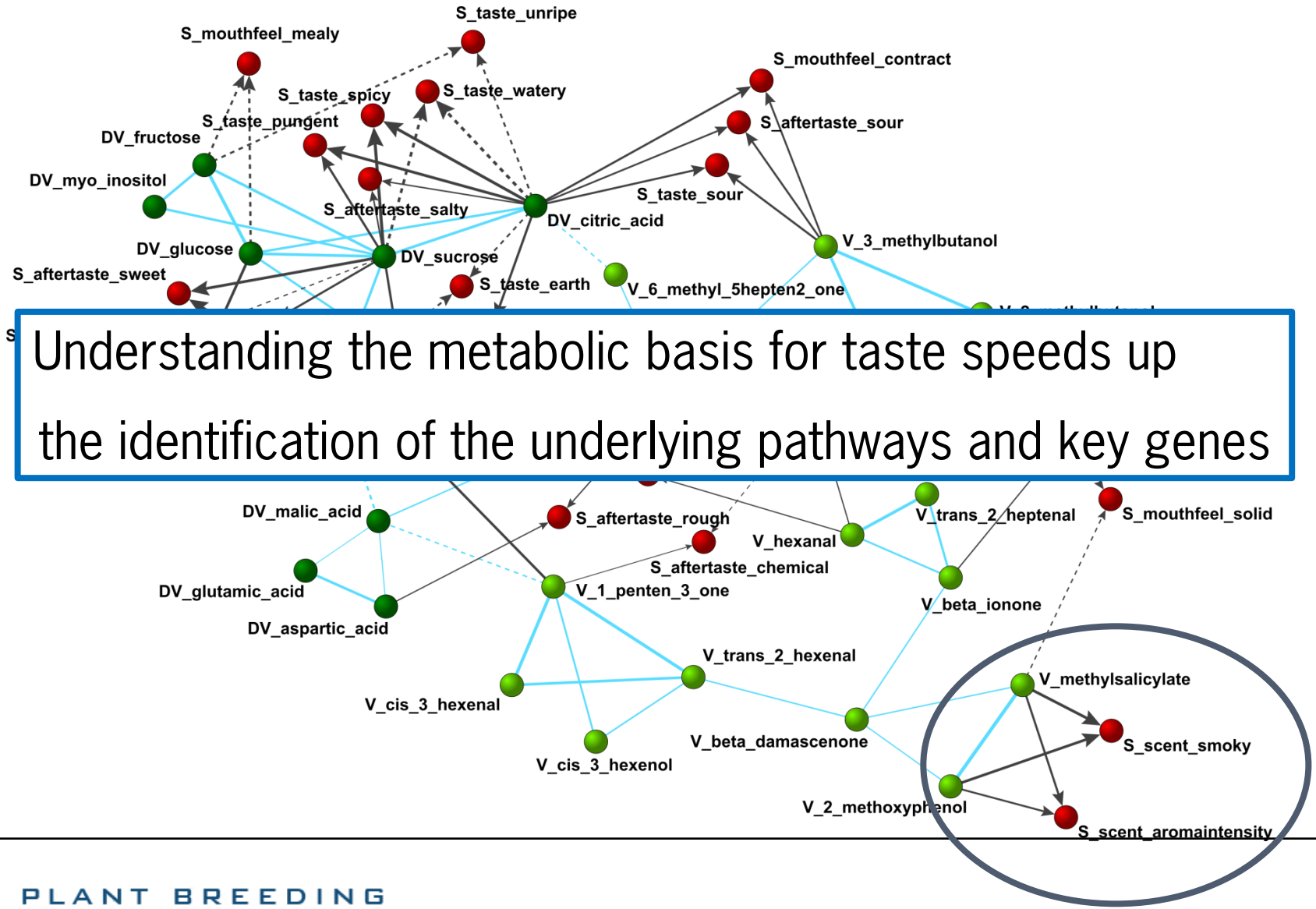
Tikunov et al. (2005) Plant Physiology 139: 1125

Relations between sensory traits and metabolites



Understanding the metabolic basis for taste speeds up the identification of the underlying pathways and key genes

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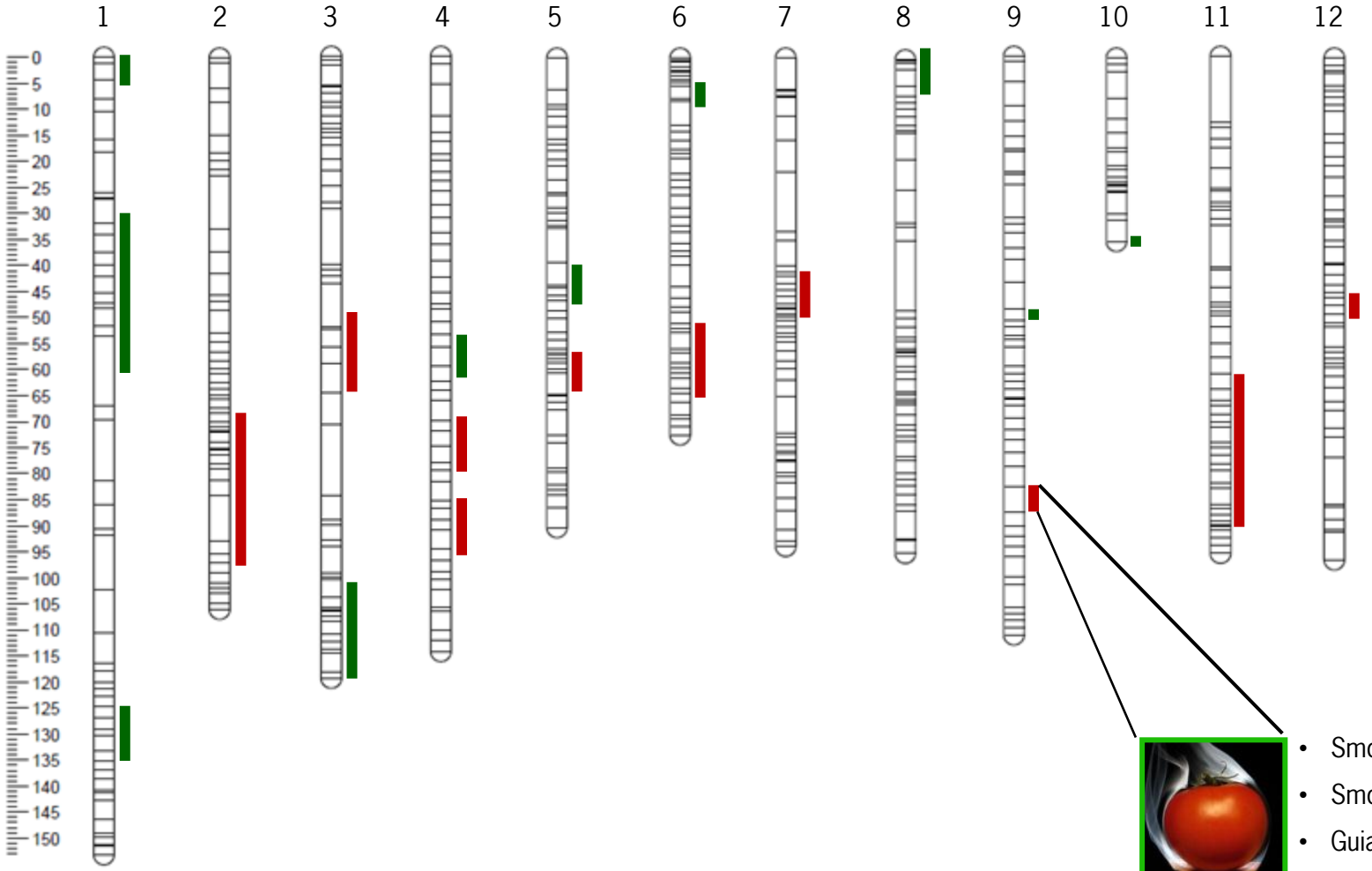
Flavour QTL regions



- Smoky aroma
- Smoky taste
- Guiacol
- Methylsalicylate
- Eugenol

Metabolite QTLs
Sensory & Metabolite QTLs

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Into the wild!

S. cheesmaniae

S. chmielewskii

S. habrochaites

S. huaylasense

S. neorickii

S. pennellii

S. peruvianum

S. pimpinellifolium

C.M. Rick TGRC Tomato Genetics Resource Center

http://tgrc.ucdavis.edu/

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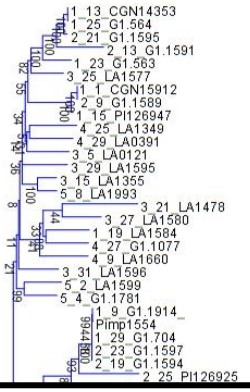
Screening a collection of wild relatives

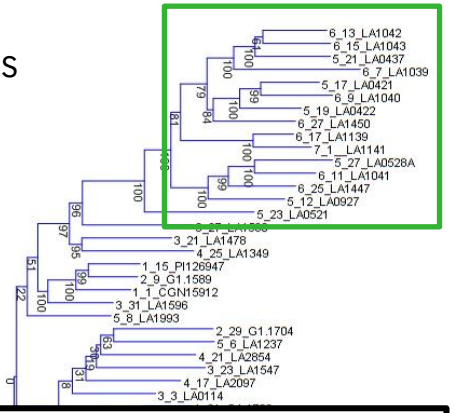
- Materials:
  - 80 *S. pimpinellifolium*
  - 20 *S. galapagensis*
  - 20 *S. cheesmanii*
- Approach:
  - Genotyping (SNP's) and phenotyping (LC & GC-MS)
  - QTL/Association analysis

Bootstrap analysis

5000 SNP markers

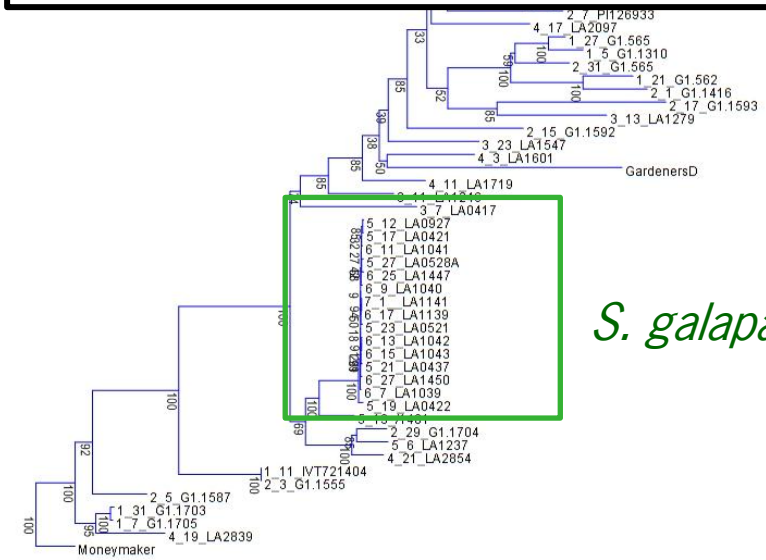


300 metabolites

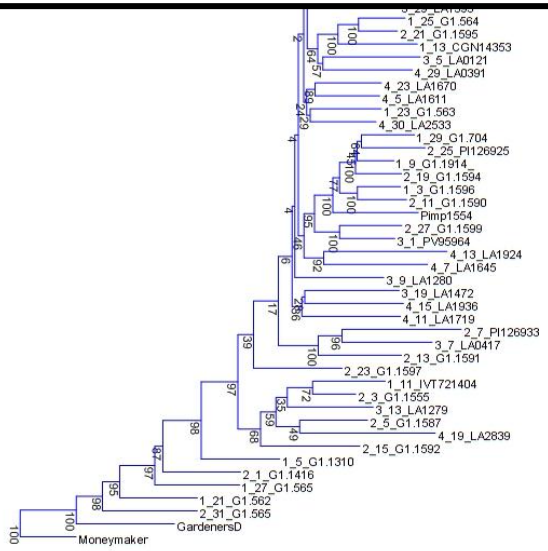


Hypothesis:

When genetic diversity is low, metabolic diversity may be more informative



S. galapagense



S. chmielewskii ILs

S. lycopersicum x *S. chmielewskii*

F_1

BC_n

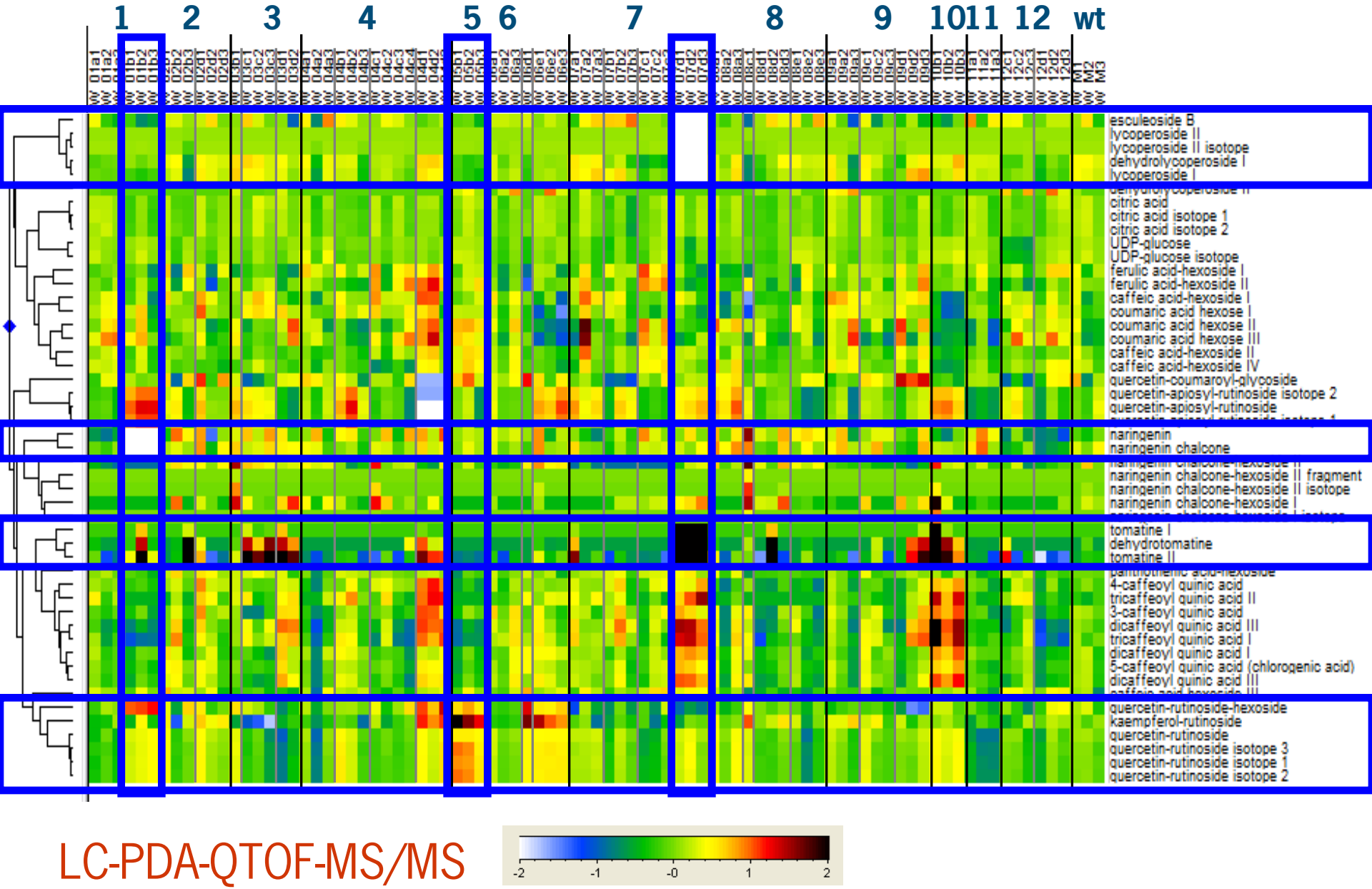
IL01a

IL01b

.....

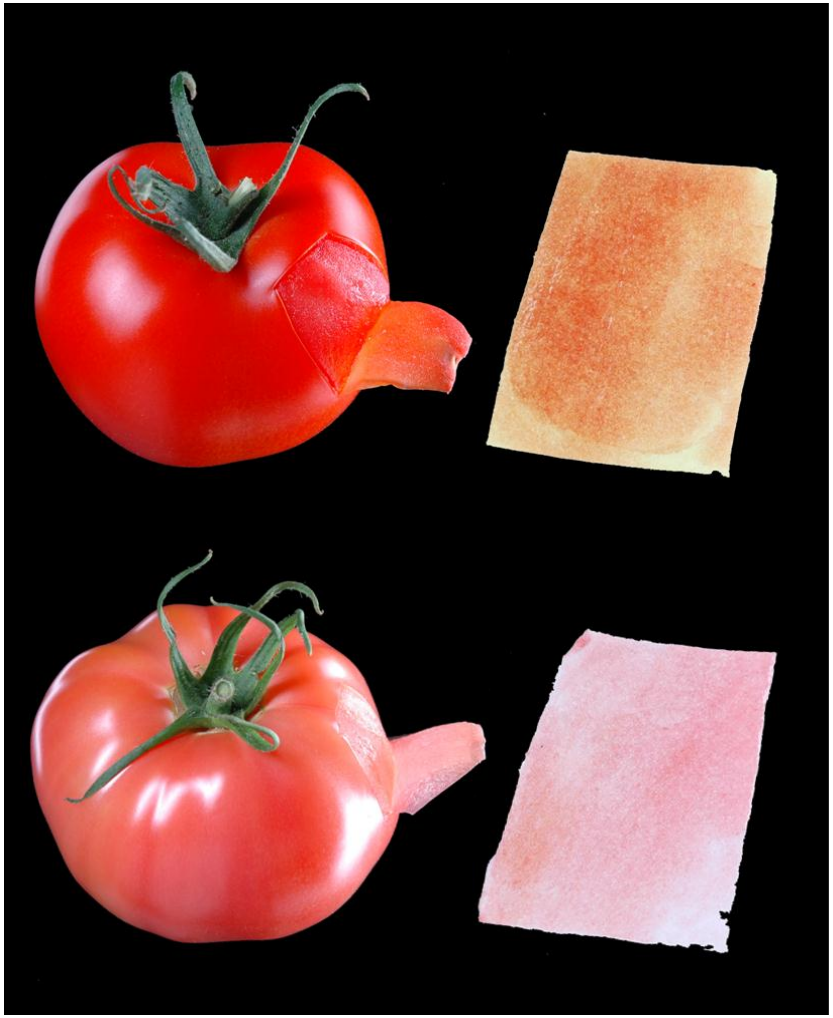
IL12d

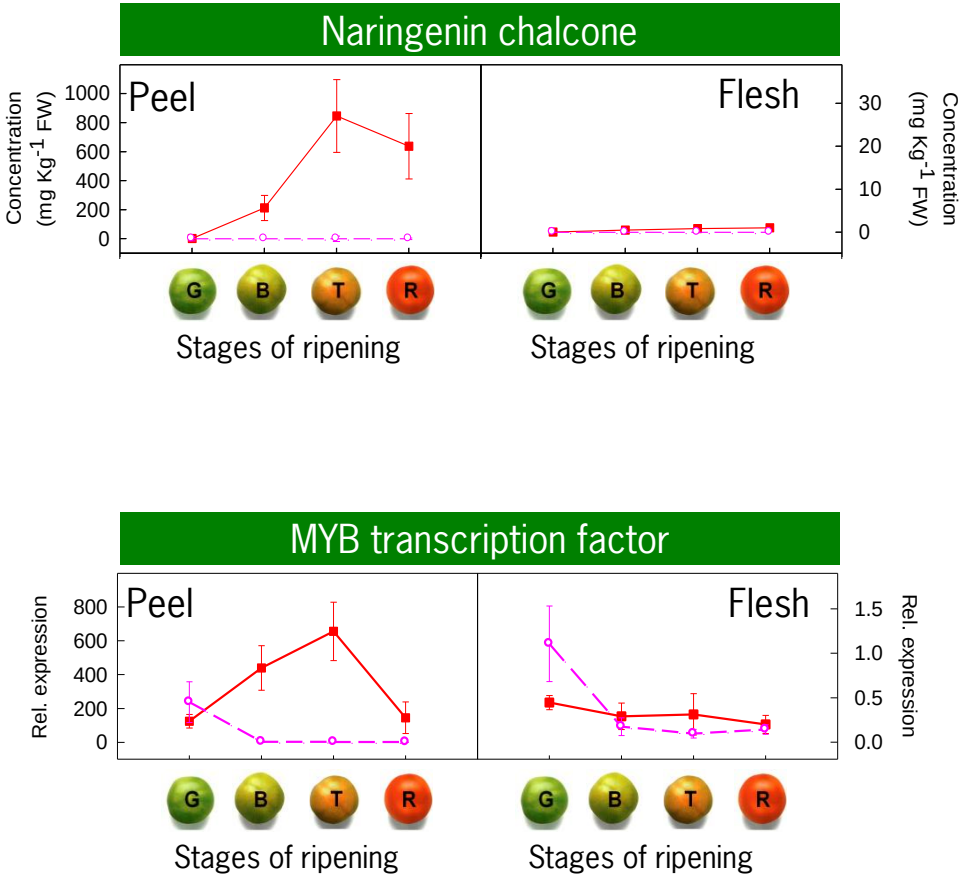
Chromosomal order



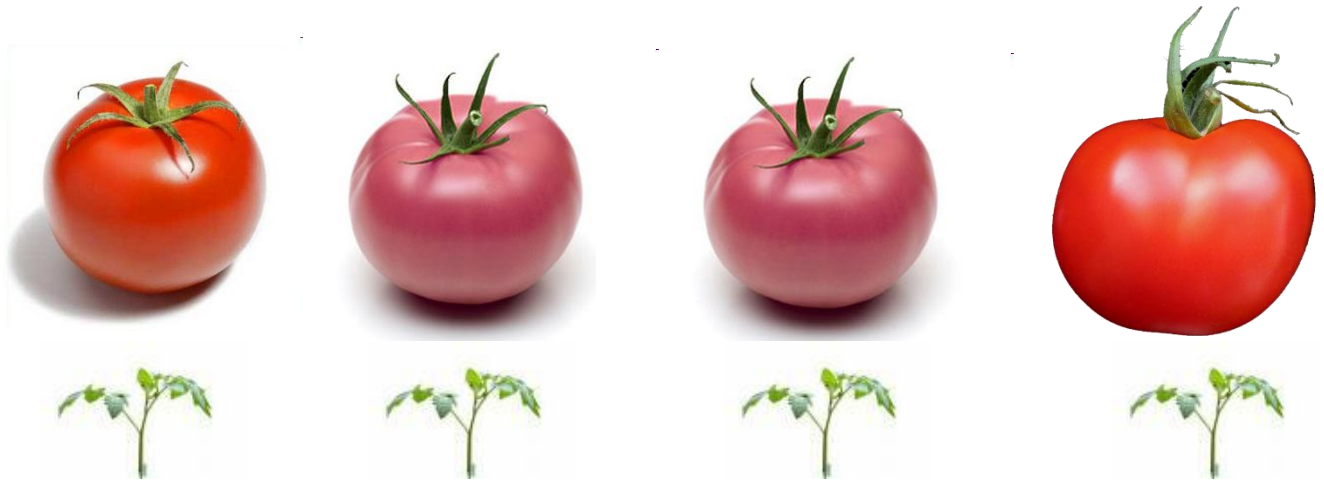
LC-PDA-QTOF-MS/MS

Pink tomatoes: an Asian growth market





What breeders want: molecular markers



Marker - + + -

- The gene is the best possible marker
- First tomato varieties on the Asian market in less than 4 years!

Conclusions

- Huge phenotypic variation in the (commercial) tomato germplasm
- Metabolomics is a useful tool to get insight in complex traits such as flavour, colour and health potential
- Combining metabolomics and genetics leads to the identification of markers for mQTLs
- Metabolomics may be an alternative or complementary tool for biodiversity studies
  - Unbiased
  - May reflect functional variation
  - In case genetic variation is very low
  - BUT metabolites reflect genetics x environment