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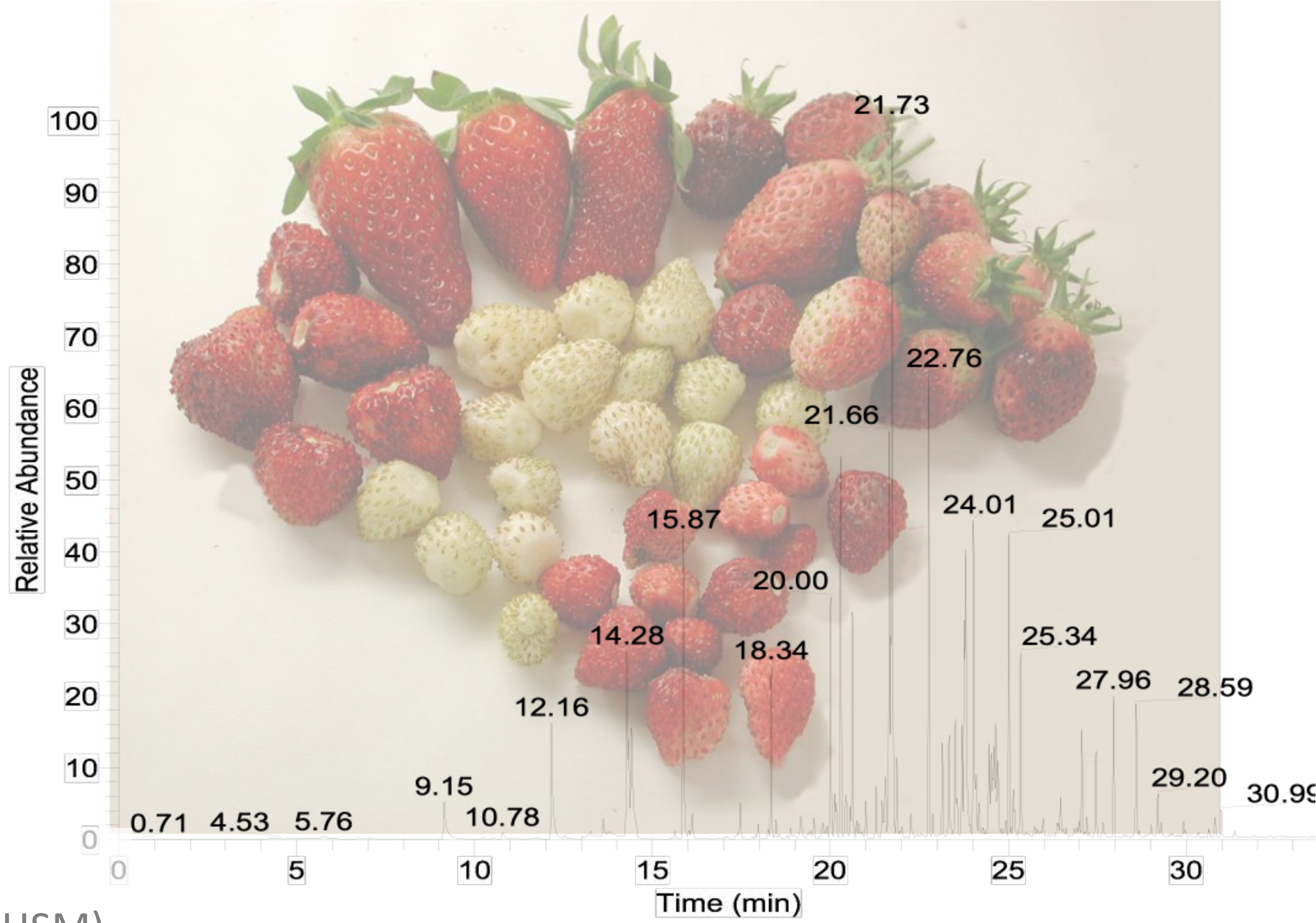
Instituto de Hortofruticultura Subtropical y Mediterránea

How can we translate strawberry flavour into chemical compounds for breeding?



Prof. Dr. Sonia Osorio
Instituto de Hortofruticultura Subtropical y Mediterranea (IHSM)
University of Malaga, Spain

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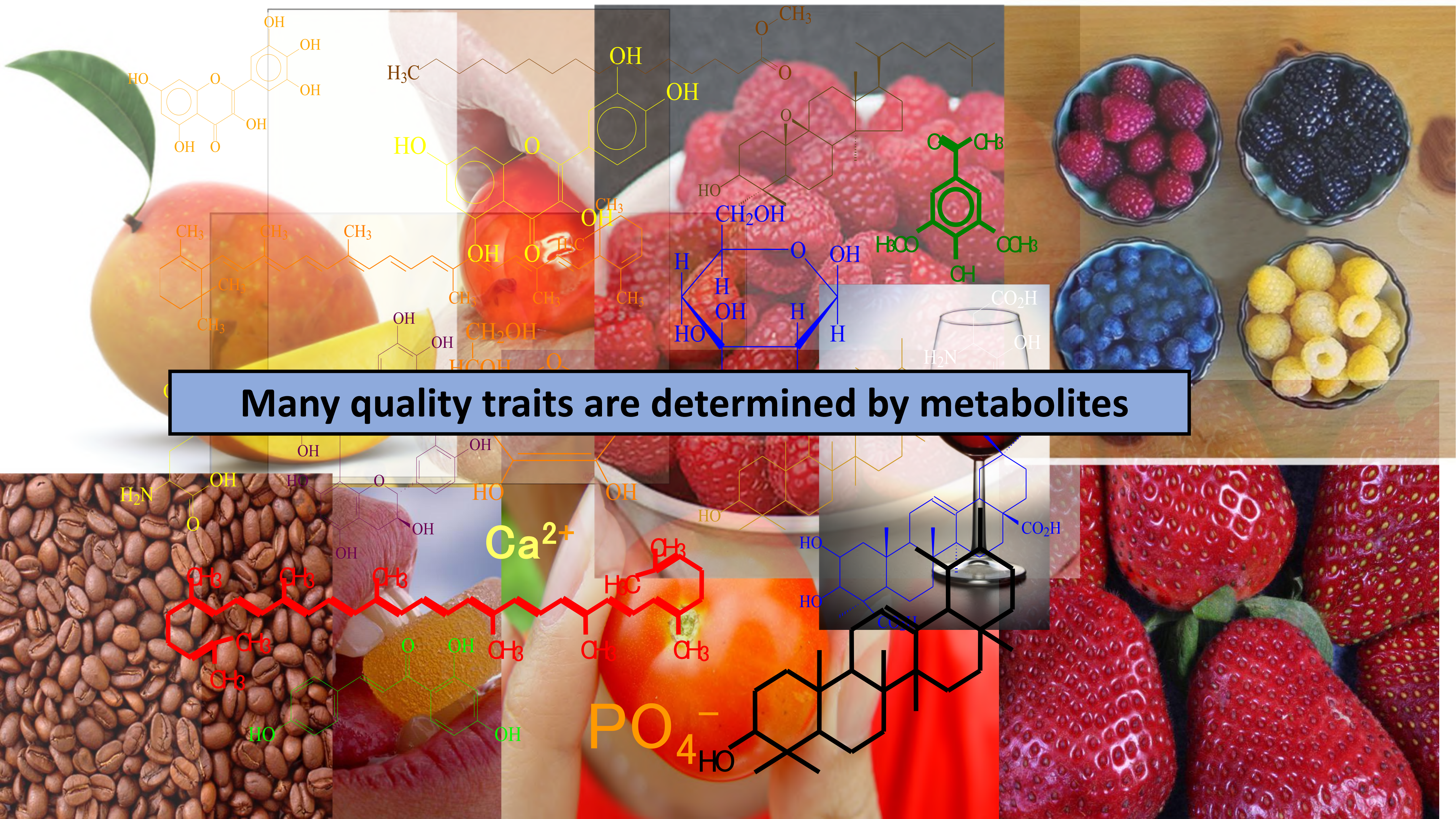
Breeding for fruit quality

- **Quality traits:**
  - Flavour & aroma
  - Nutritional value
  - Colour
  - Shelf-life …
- **Crops**
  - Tomato
  - Pepper
  - Potato
  - Strawberry

Many quality traits are determined by metabolites



What breeders do: exploit genetic variation

- Materials:
  - Cultivated genotypes >> commercial cultivars, landraces
  - Related wild species

F. virginiana

F. mochata

F. ananassa cv Camarosa

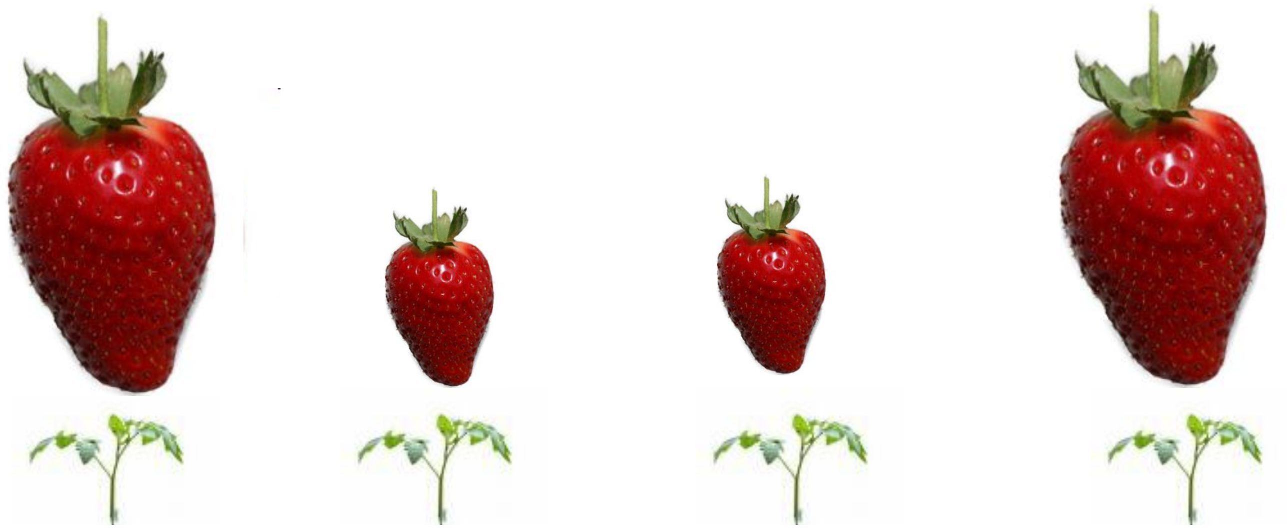
F. chiloensis

F. vesca

F. chiloensis type

F. ananassa cv Senga Sengana

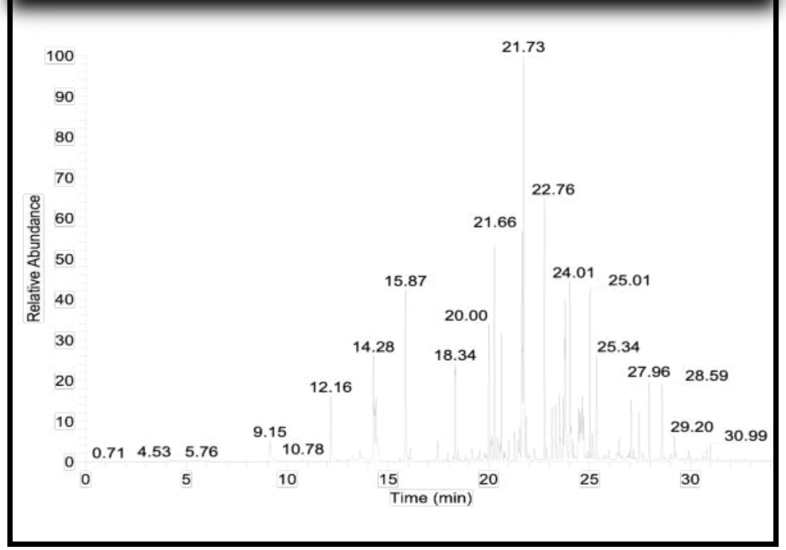
What breeders want: molecular breeding



Marker/gene - + + -

- The marker (gene) predicts the trait
  - Selection in seedlings
  - Gain in breeding efficiency
  - Reduction in time and costs

Breeding for quality: general strategy

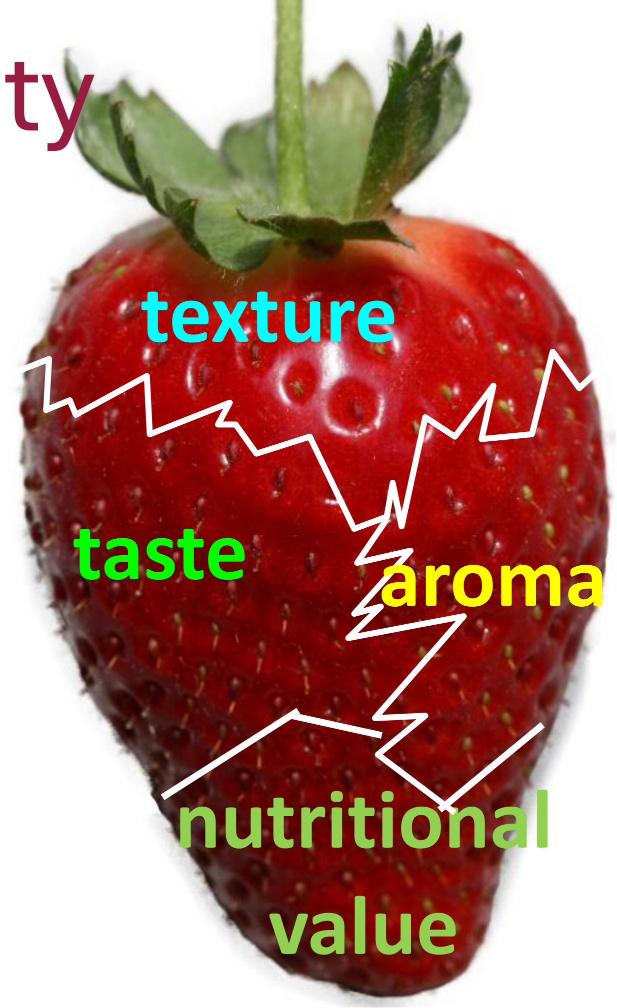


<u>Modify the composition</u>

- Marker-assisted breeding
- Genetic engineering

- Explore genetic diversity
  - Association panels & populations
  - Link genes and markers to traits
- Unravel metabolic pathways
  - X-omics/Biochemistry
  - Gene isolation

Fruit quality - **consumer** desirability



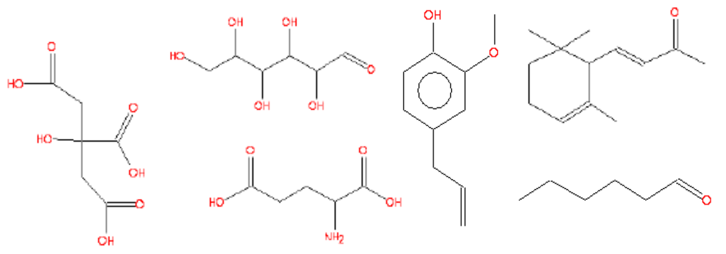
Consumers buy with their eyes:

- Shape
- Color
- Appearance
- Firmness

Consumers buy the same product again when the flavour is good:

- Taste
- Aroma
- Texture
- Nutritional value

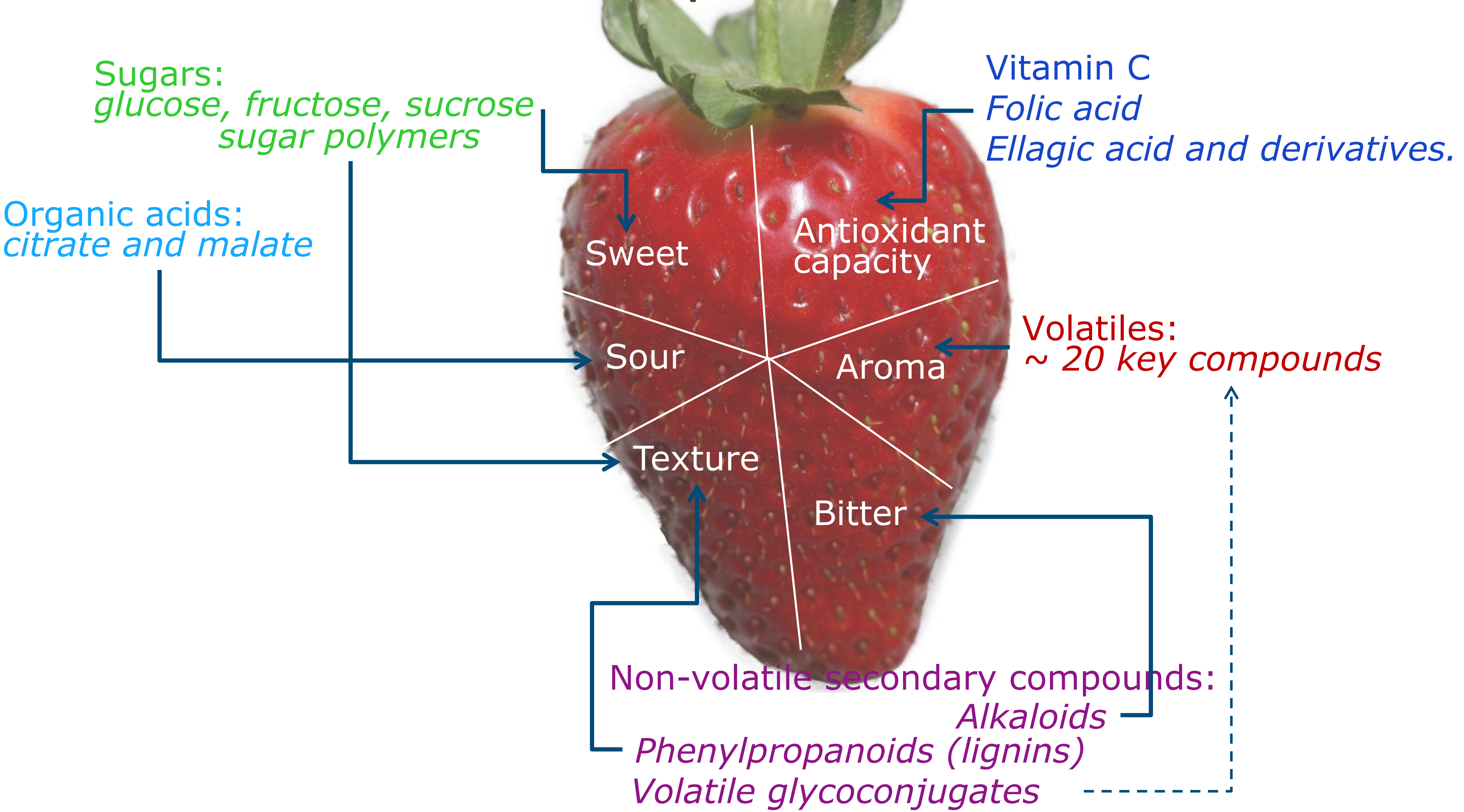
Chemicals (metabolites)



Nutritional value
Vitamins and antioxidants.

Taste, aroma
Sweet, sour, bitter, etc.

Metabolites and fruit quality



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Primary Strawberry Flavour Compounds

Sweetness / Fruity

- Sugars
- Furanones
- Lactones
- Esters

Citrus / Floral

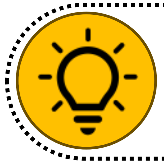
- Organic acids
- Terpenoids

Green / Spicy

- Alcohols
- Aldehydes
- Fatty acid volatiles

Musty

- Sulfur Compounds



Only **20** out of 300 VOCs are important to flavour

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Primary Strawberry Flavour Compounds

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- Lactones
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Green / Spicy

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Citrus / Floral

- **Organic acids**
- Terpenoids

Quality

Yield

Musty

- Sulfur Compounds

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▪ Sugars ▪ Furanones ▪ Lactones ▪ Esters

Citrus / Floral
▪ Organic acids ▪ **Terpenoids**

Green / Spicy
▪ Alcohols ▪ Aldehydes ▪ Fatty acid volatiles

Musty
▪ Sulfur Compounds

Strawberry Fruit Quality
-Scenario-

Decline in quality

- Intense breeding
- Loss of diversity

Domestication

Cultivars (Before 1949)

35% allelic loss

Cultivars (After 2000)

Cultivars (Since 1990)

18% allelic loss

Adapted from Gil-Ariza et al., (2009)

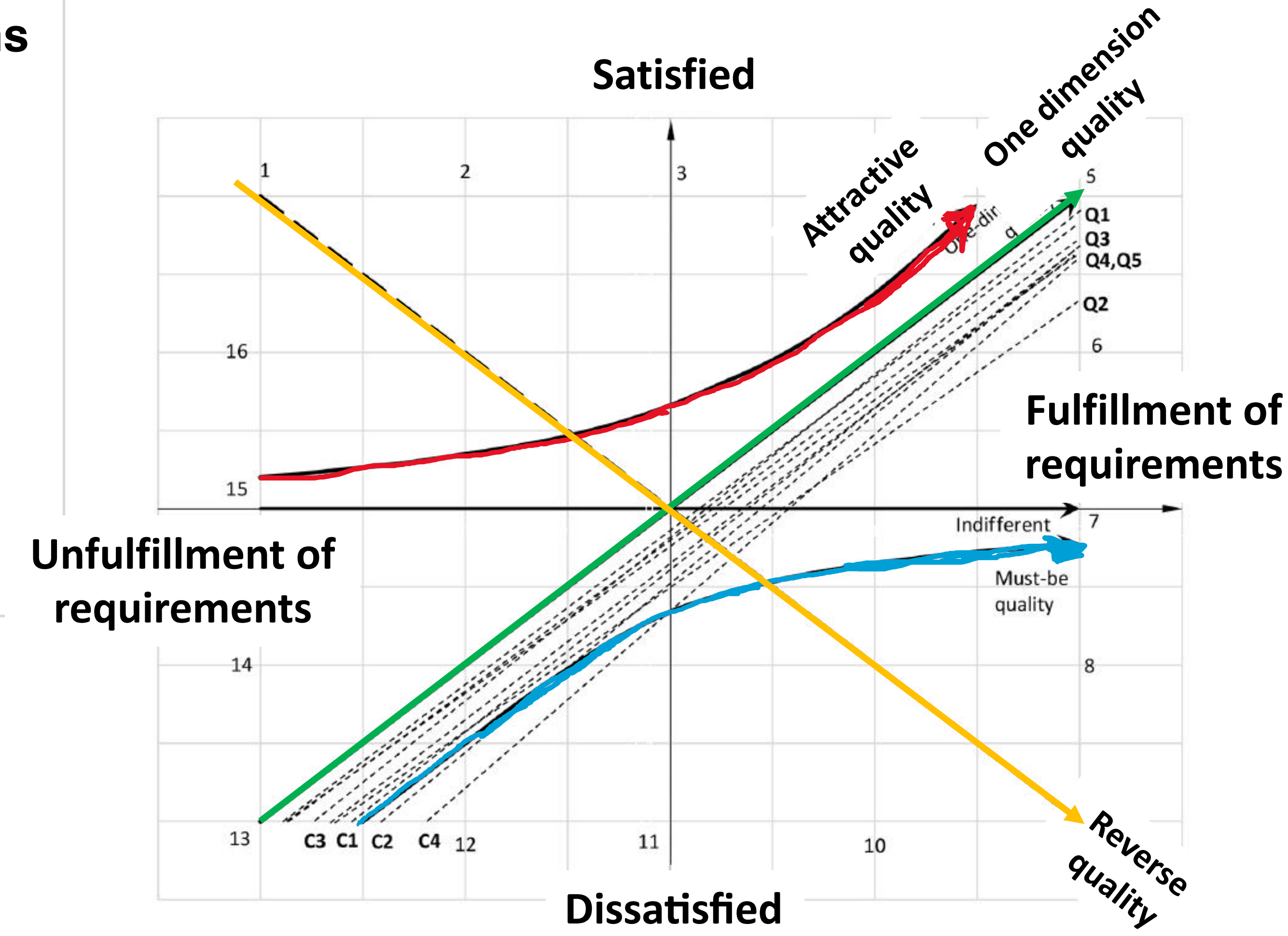
Strawberry Fruit Quality

Decline in quality

- Intense breeding
- Loss of diversity

Consumer complains

- Bad Economic Impact
- Less intake of fruits



Strawberry Fruit Quality

Decline in quality

- Intense past breeding
- Loss of diversity

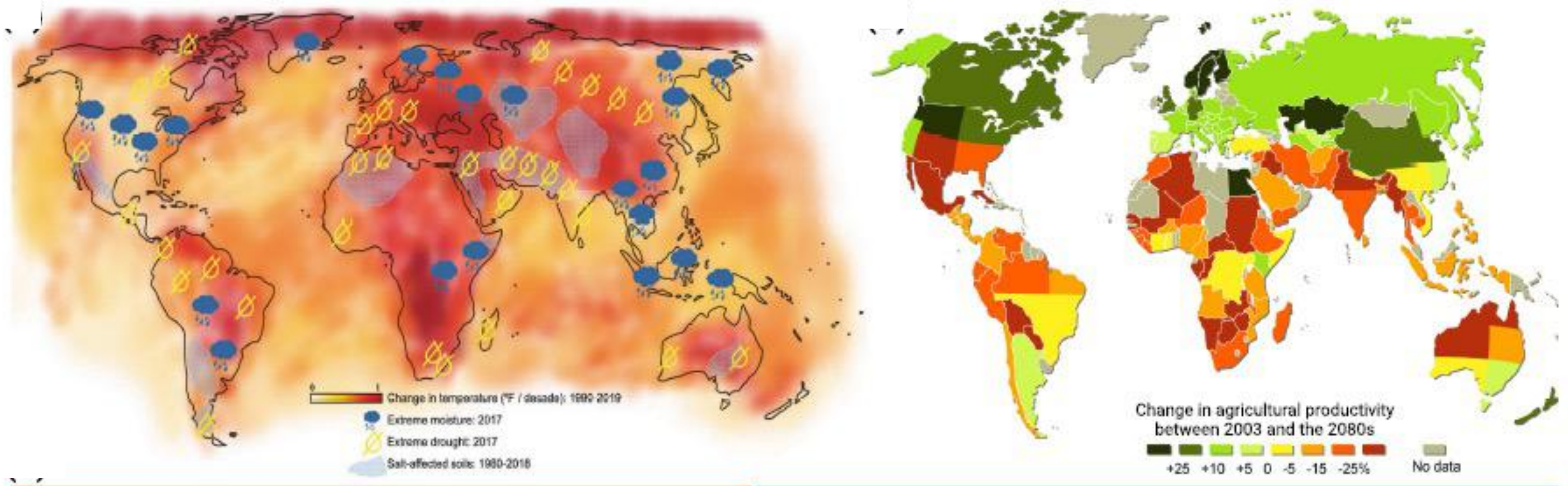
Consumer complains

- Bad economic impact
- Less intake of fruits

Comercial competition

- Less severe legislation
- Lower production prices

Strawberry Fruit Quality



Heat stress

- Change in phenology
- Increase in oxidative stress (ROS production)
- Reduction in antioxidant enzyme activities
- Reduced grain growth and yield
- Decreased photosynthesis
- Reduced stomatal conductance and CO_2 fixation
- Damaged photosynthetic pigments
- Inhibition of seed germination
- Poor cell enlargement
- Loss turgor
- Reduction in biomass
- Reduction in carbohydrate metabolism
- Reduction in production of secondary metabolites
- Dehydration
- Changes in sensing and signaling
- Changes in expression of heat-stress related genes
- Changes in positive/negative regulator gene expression

Cold stress

- Decrease membrane stability
- Metabolism retarded
- Increase in oxidative stress (ROS production)
- Reduction in antioxidant enzyme activities
- Higher electrolyte leakage
- Ion leakage or altered homeostasis
- Protein disintegration
- Chlorophyll degradation
- Protoplast volume shrinkage
- Leaves chlorosis and wilting
- Reduced grain growth and yield
- Inhibition of seed germination
- Physiological and cellular perturbations
- Cellular dehydration and formation of intracellular ice crystals
- Reduced root branching and root surface area
- Reduced water and nutrient uptake
- Changes in sensing and signaling
- Changes in expression of cold-stress related genes

High resilience cultivars

- Climate Change
- Production demand

Strawberry Fruit Quality

Decline in quality

- Intense breeding
- Loss of diversity

Consumer complains

- Bad Economic Impact
- Less intake of fruits

Comercial competition

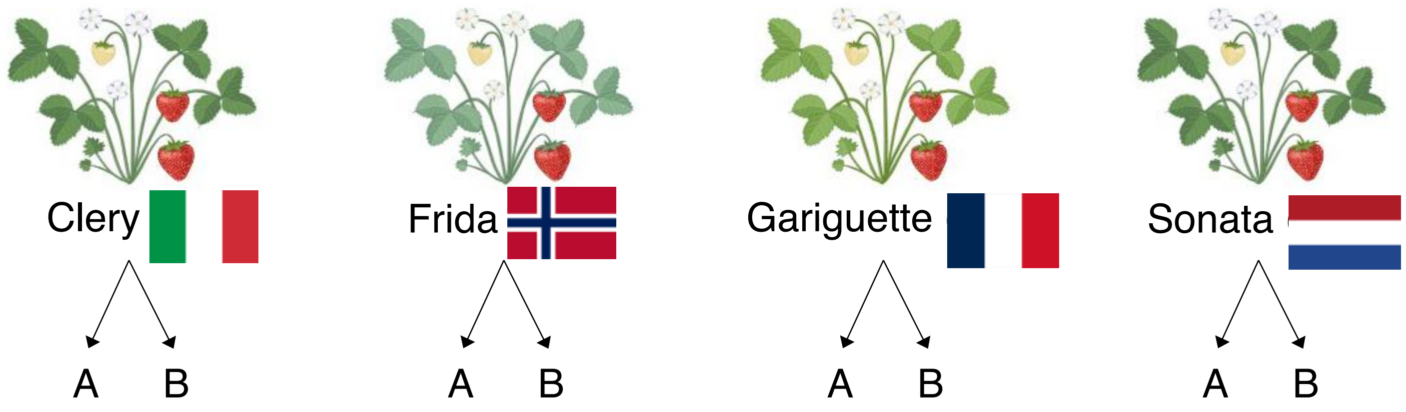
- Less severe legislation
- Lower production prices

High resilience cultivars

- Climate Change
- Production demand

Evaluate the impact of genotype-by-environment (GxE) interactions on Strawberry quality profile

Sample Collection | Multi-Omic Analysis | Bioinformatic Integration



4 Cultivars

2 Picking Dates

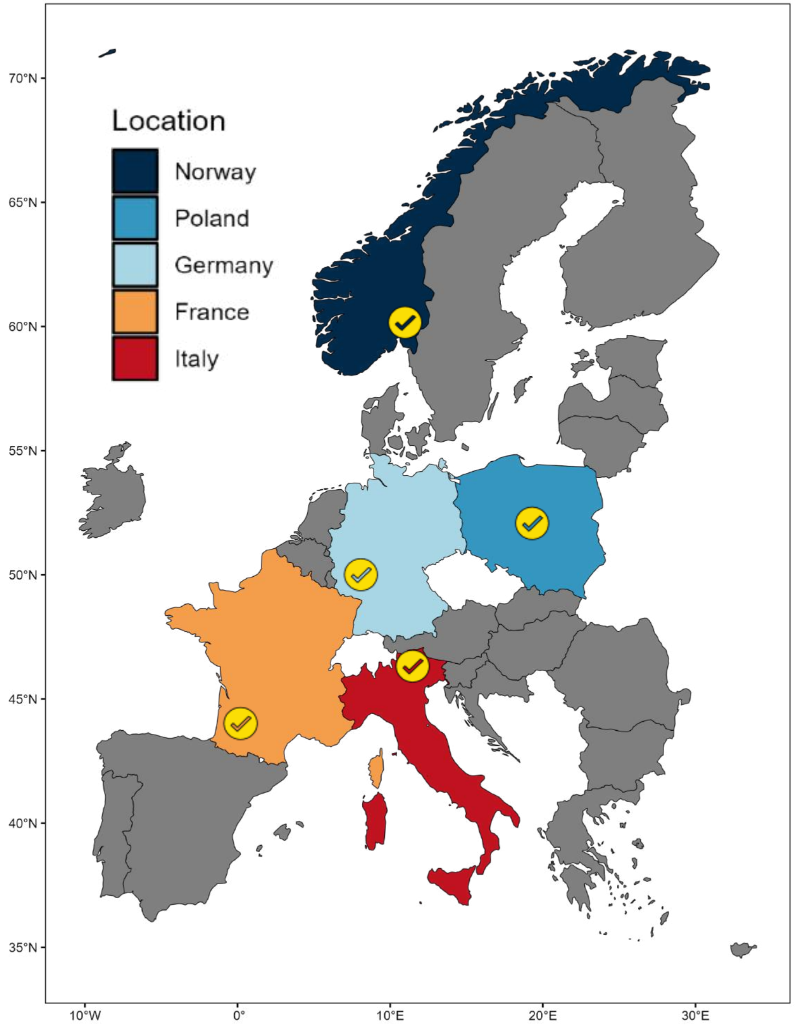
Varieties from different EU breeding programs

Sample Collection | Multi-Omic Analysis | Bioinformatic Integration

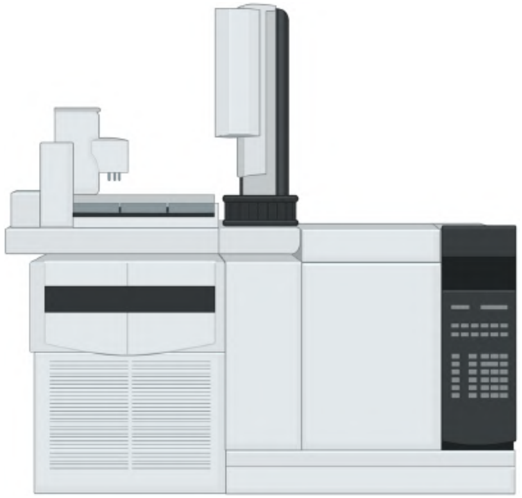
A B A B A B A B

Norway Poland Germany France Italy

Open Field Polytunnel



Metabolomic Analysis



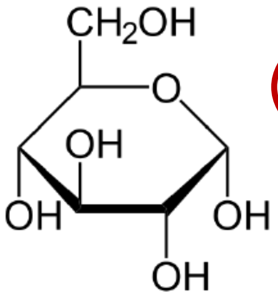
GC-TOF-MS

- **Primary Metabolites**
- **Volatile Organic Compounds**

METABOLOMICS

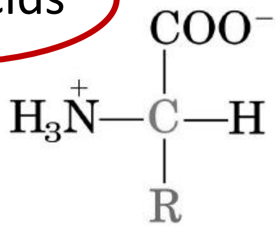
Primary metabolites (aprox. 60-70)

Volatiles (> 100)

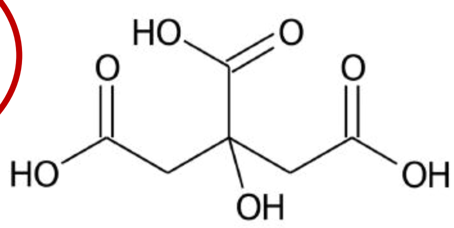


Sugars and sugar derivatives

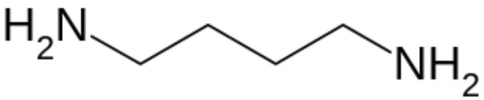
Aminoacids



Organic acids



GC-TOF-MS

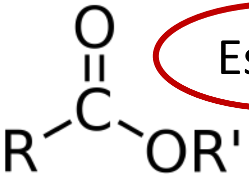


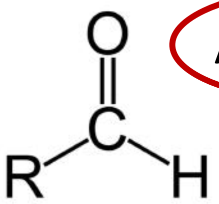
Polyamines and others

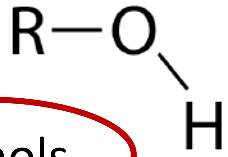
METABOLOMICS

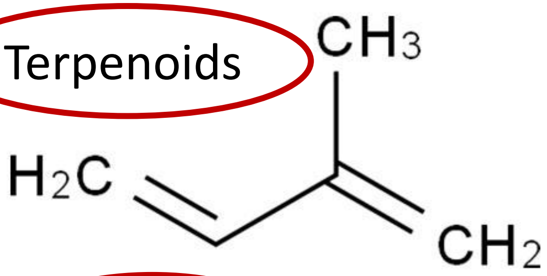
Primary metabolites (aprox. 60-70)
Volatiles (> 100)

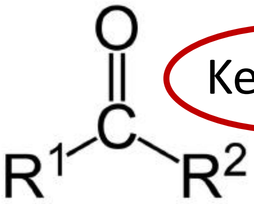
HSPME/GC-MS

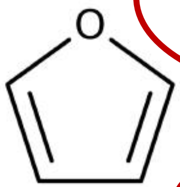

Esters

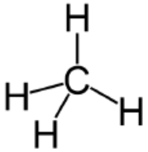

Aldehydes


Alcohols

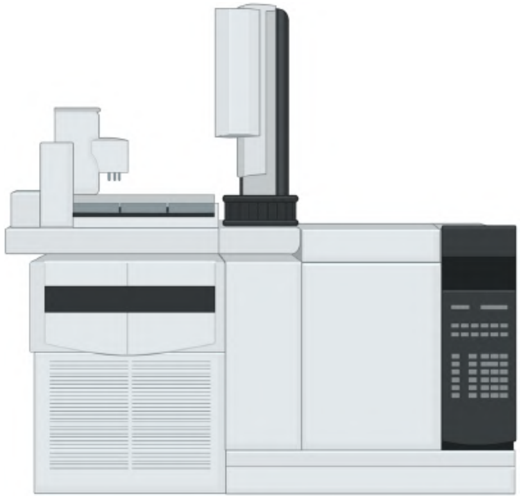

Terpenoids


Ketons


Furanones
Lactones


Fatty acid
volatiles

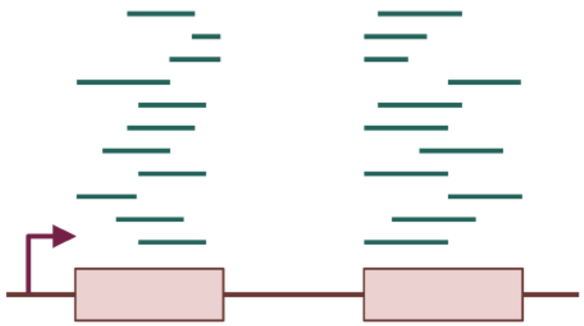
Metabolomic Analysis



GC-TOF-MS

- Primary Metabolites
- Volatile Organic Compounds

Transcriptomic Analysis



RNA-Seq

- Differentially Expressed Genes
- Transcripts were annotated using last *Fragaria x ananassa* vs Camarosa annotation

Chemometric approach

RStudio

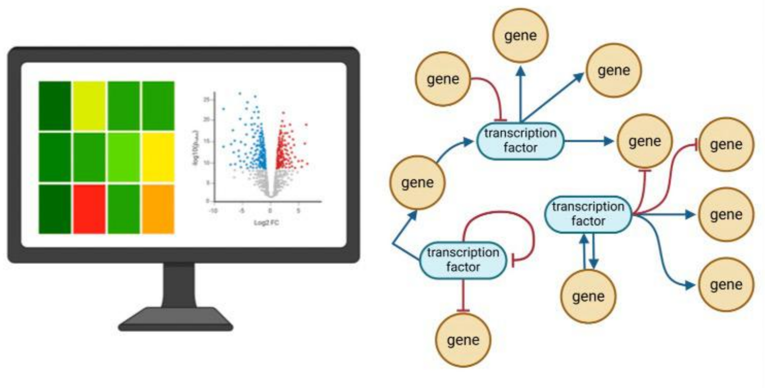
Metabolomic Data → Univariate Analysis → Anova → P value

Metabolomic Data → Multivariate Analisis → PCA

Multivariate Analisis → sPLS-DA → VIP scores

Transcriptomic Data → DEG (DESeq2) → LogFC

Omic Integration → Manual Pathway Analysis



Do genotype-by-environment interactions significantly alter the composition and quality profile of strawberry fruit?

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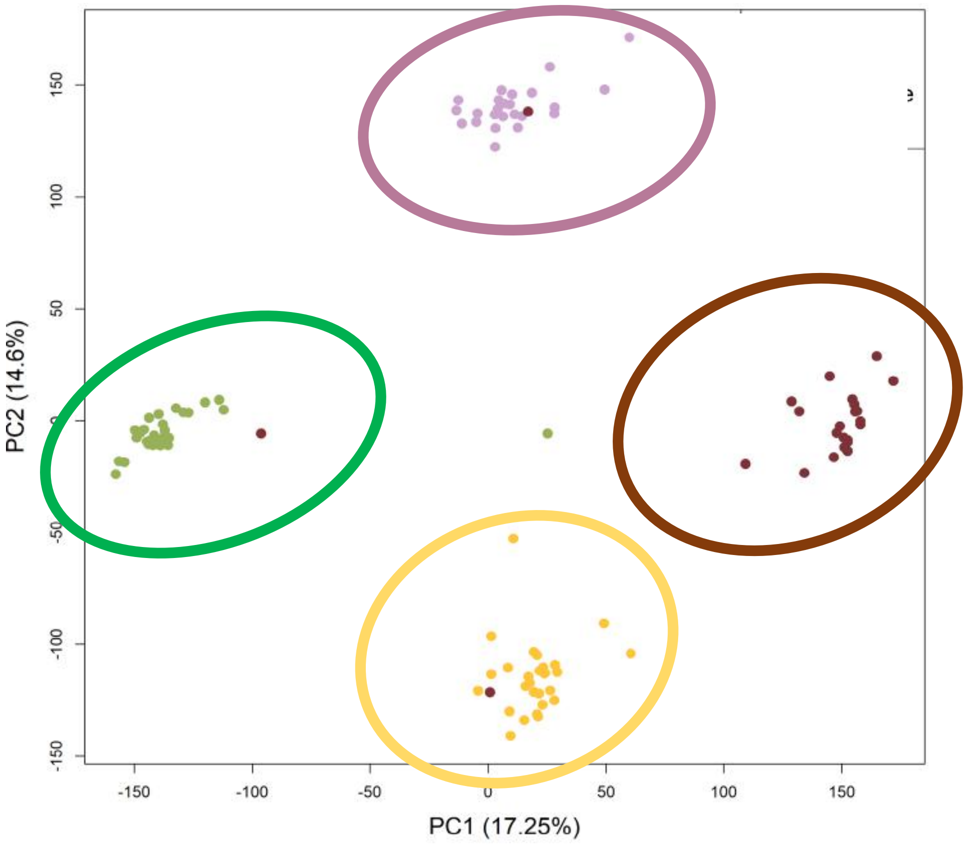
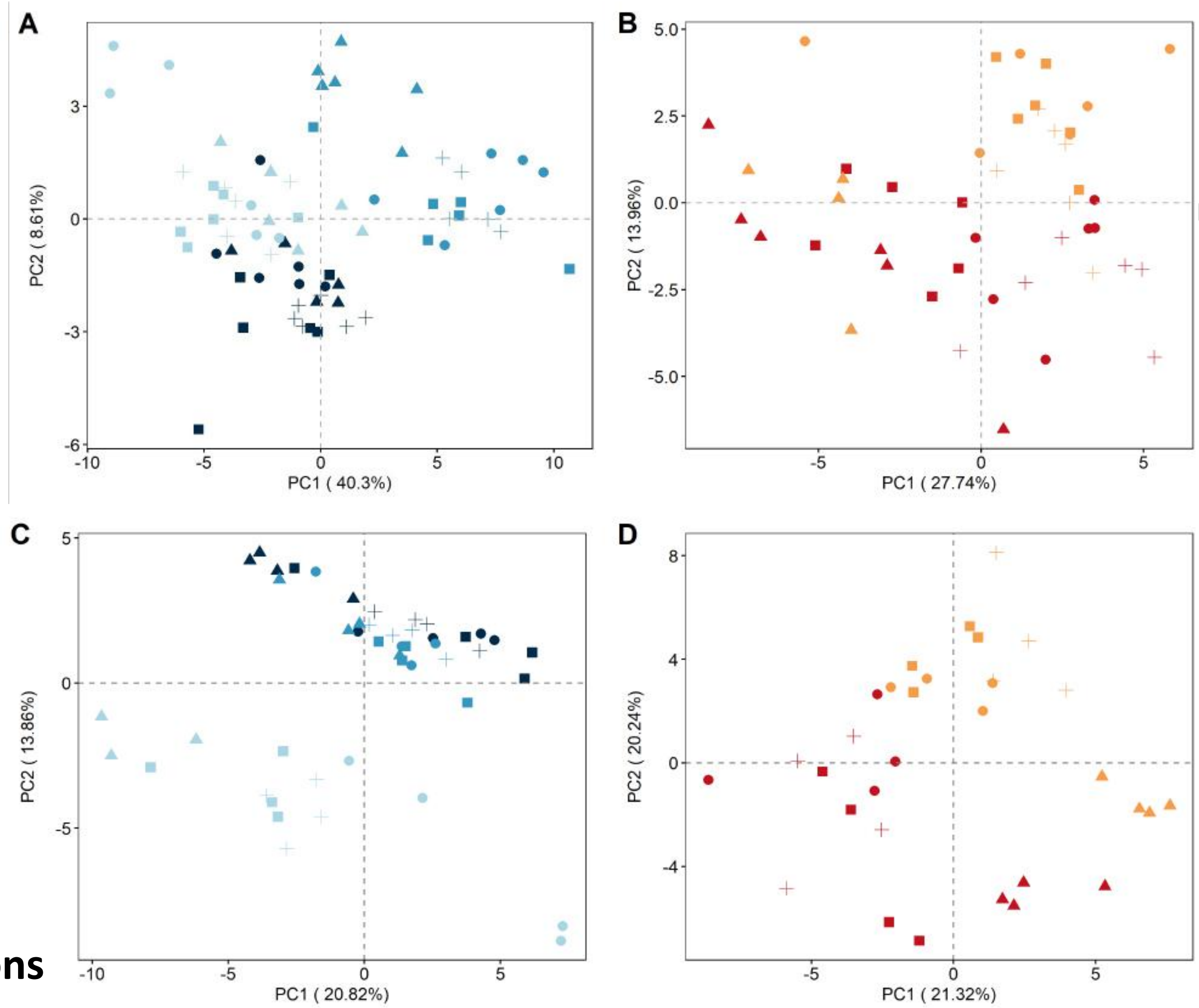
Transcriptomic analysis



Cultivars

Clery Frida Gariguette Sonata

Metabolomic Data



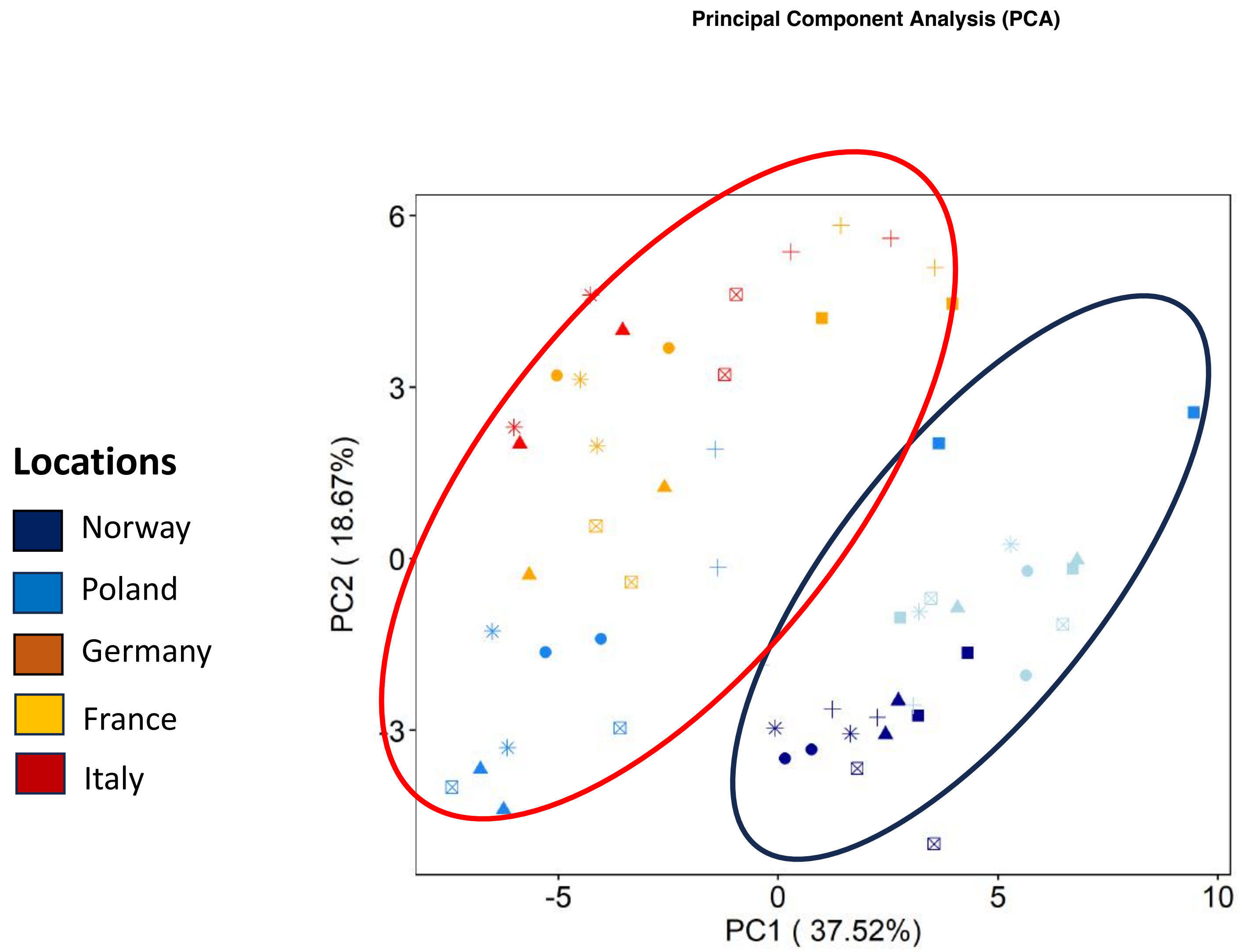
Primary metabolites

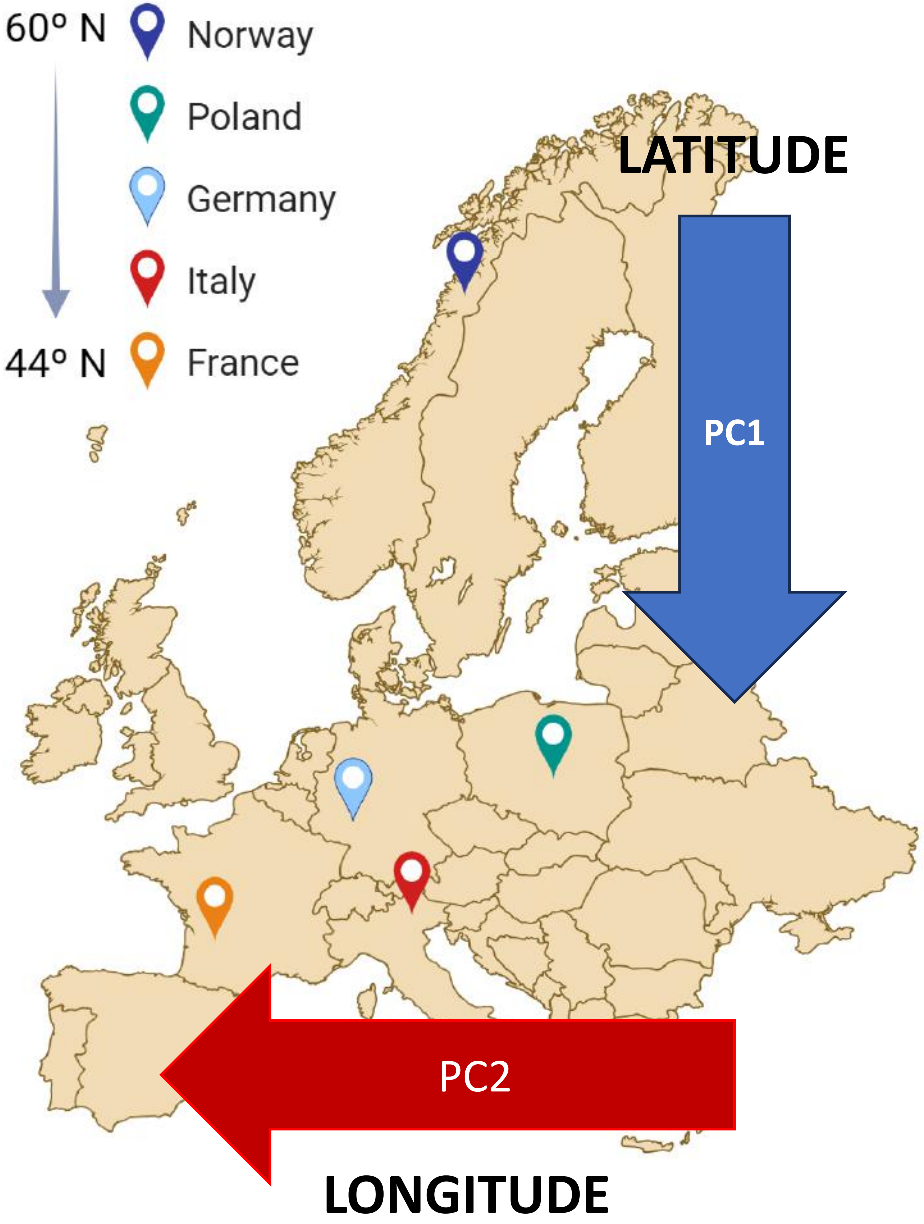
Volatiles

Locations

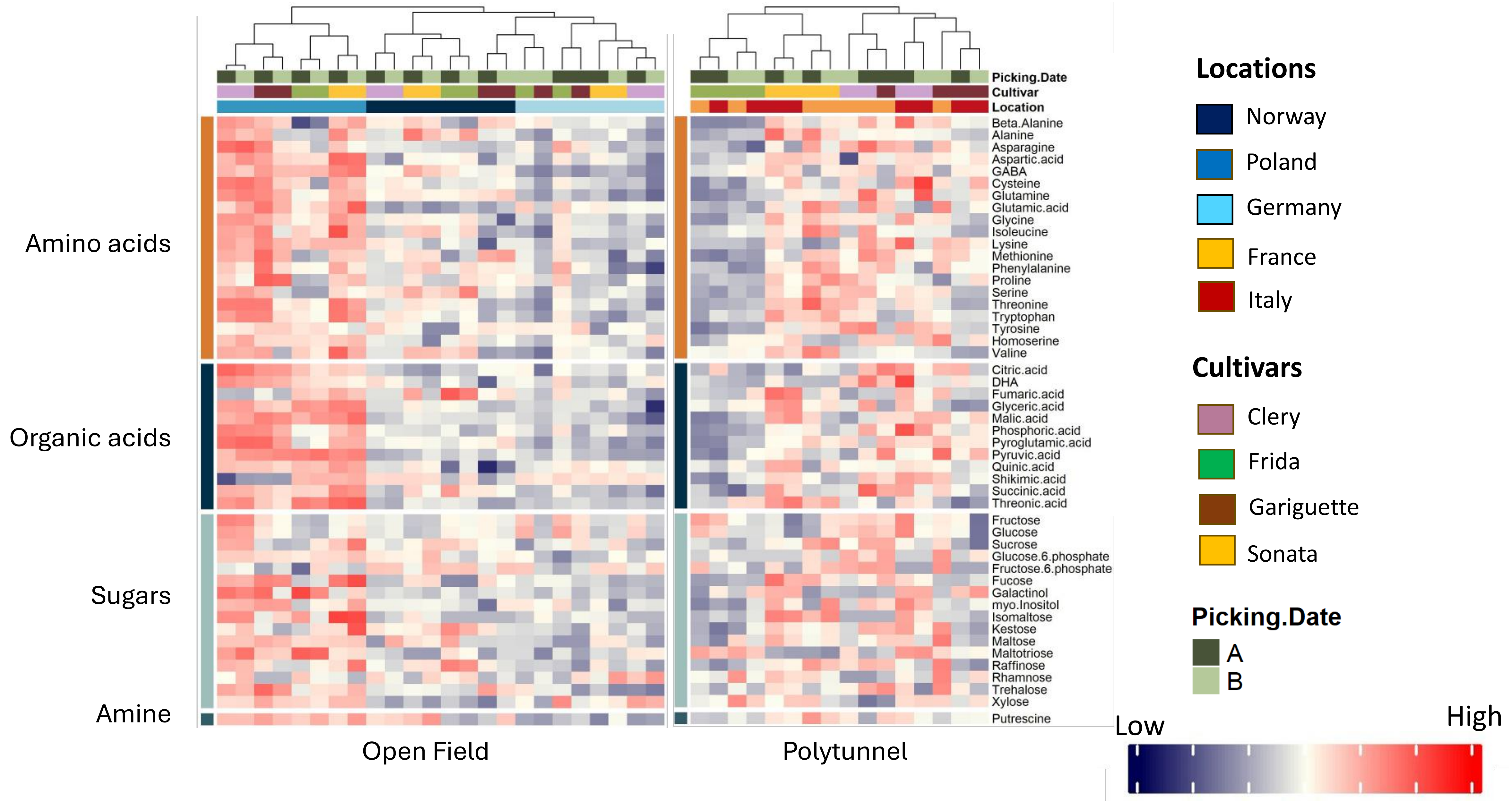
Norway Poland Germany France Italy

Primary Metabolites

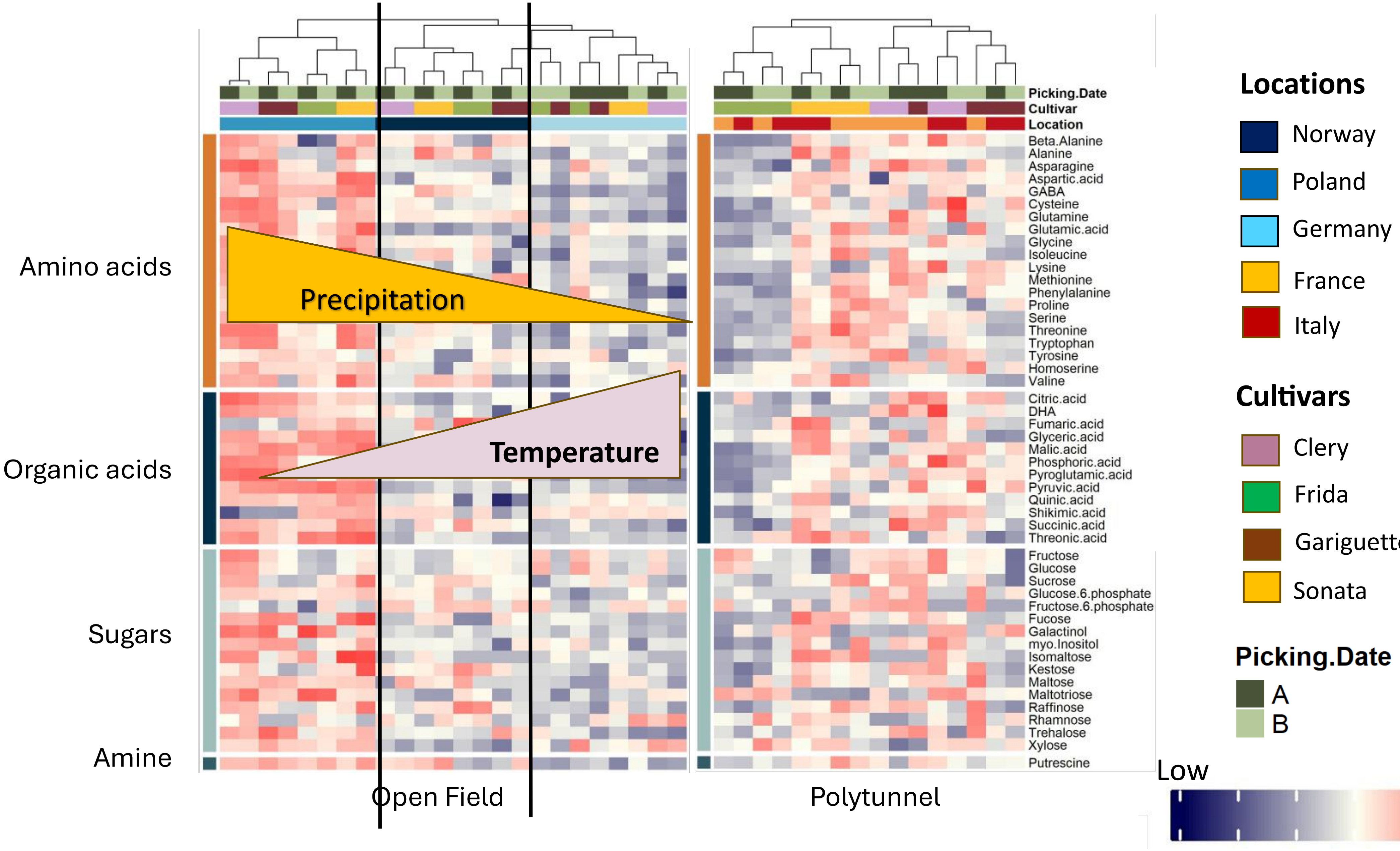


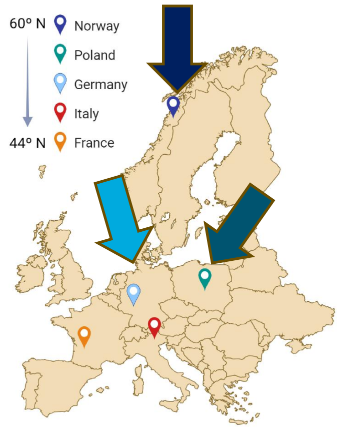


Primary Metabolites

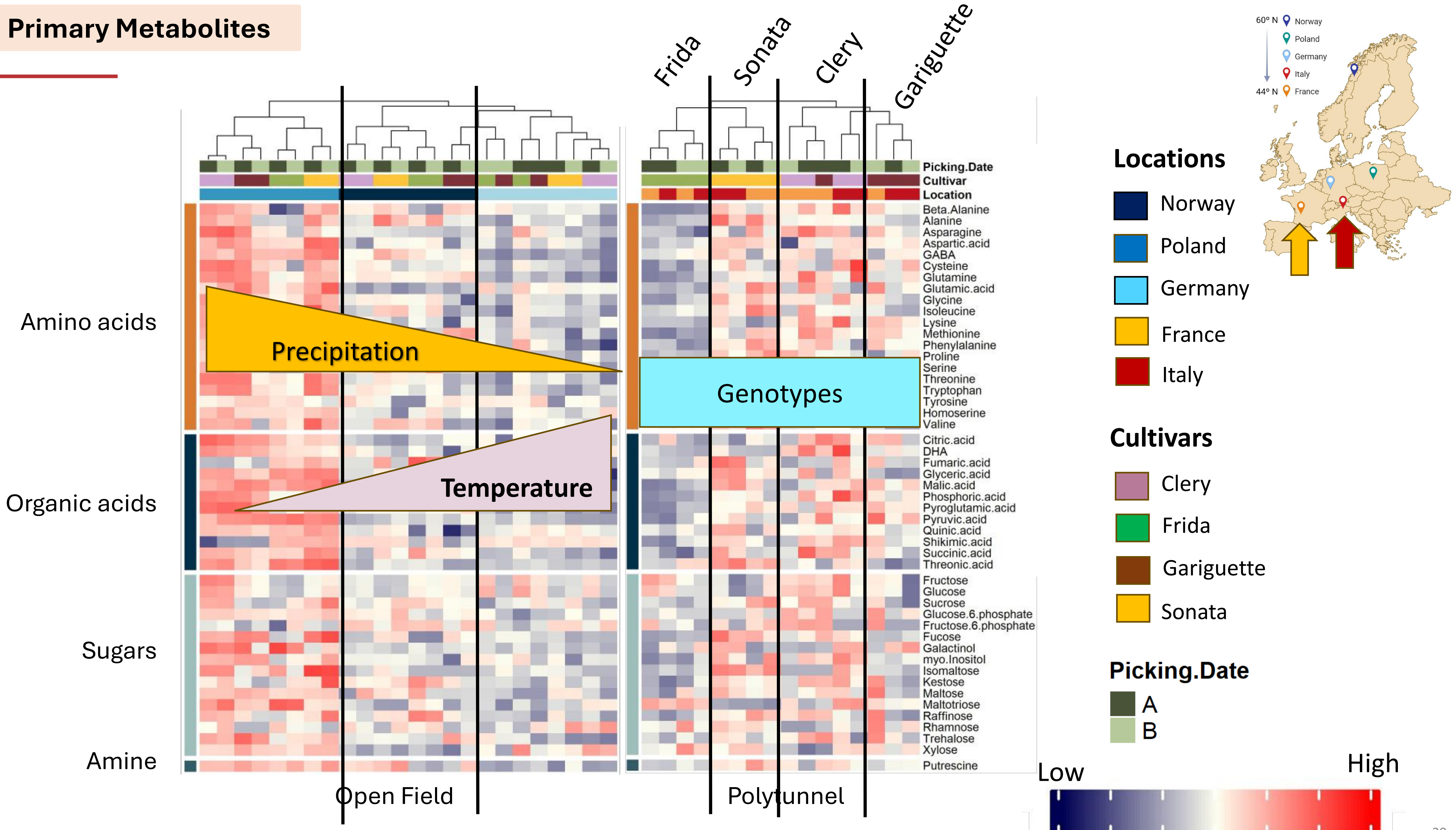


Primary Metabolites





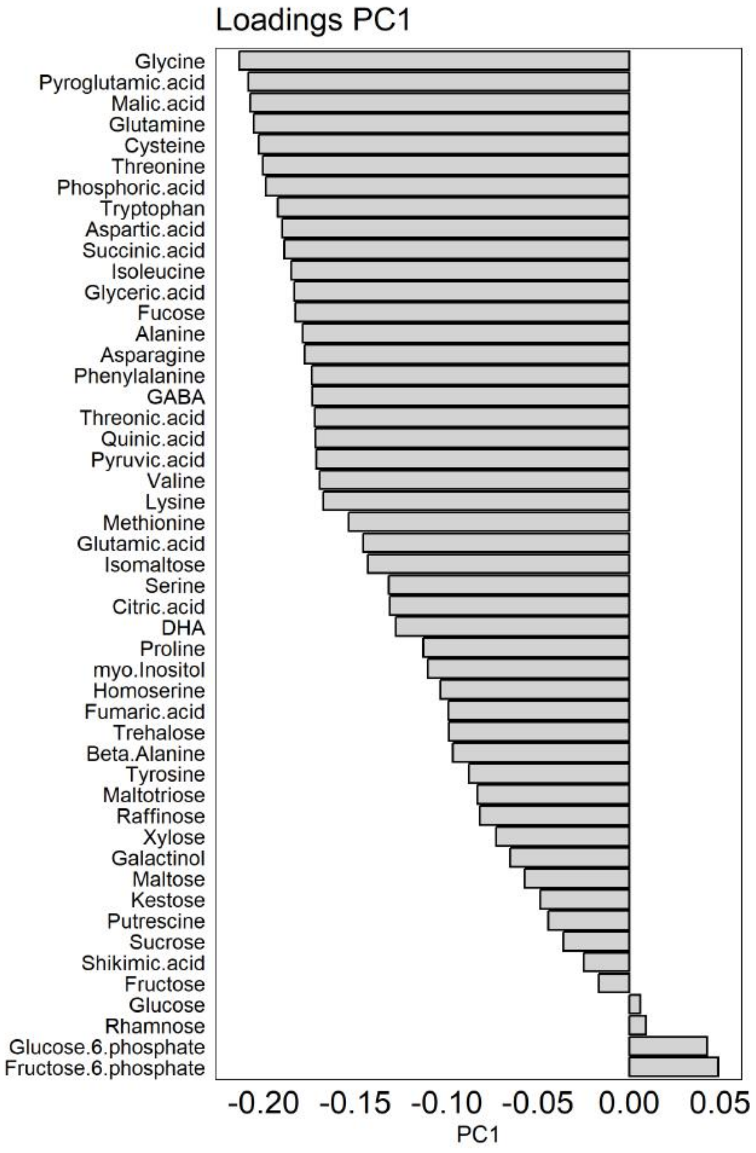
Primary Metabolites

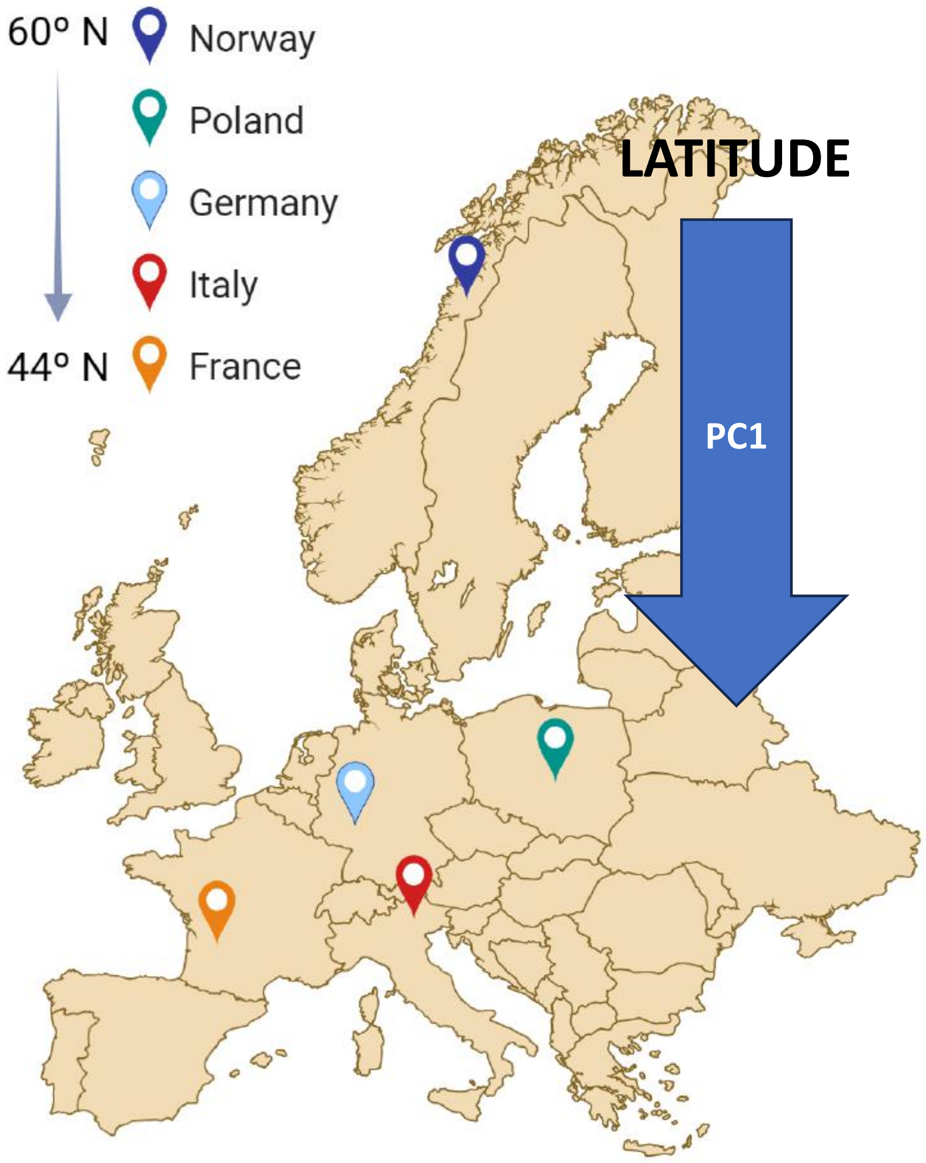


Primary Metabolites

Principal Component Analysis (PCA)

Organic acid
Amino acids



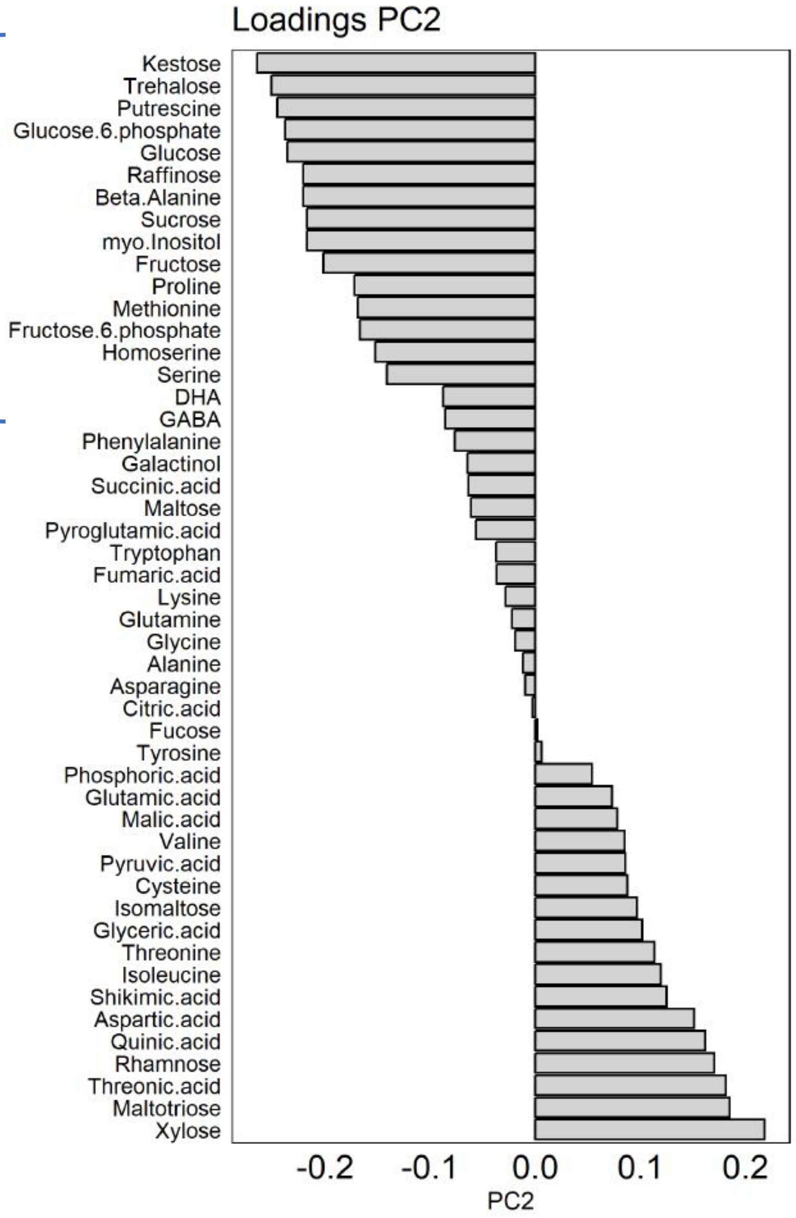


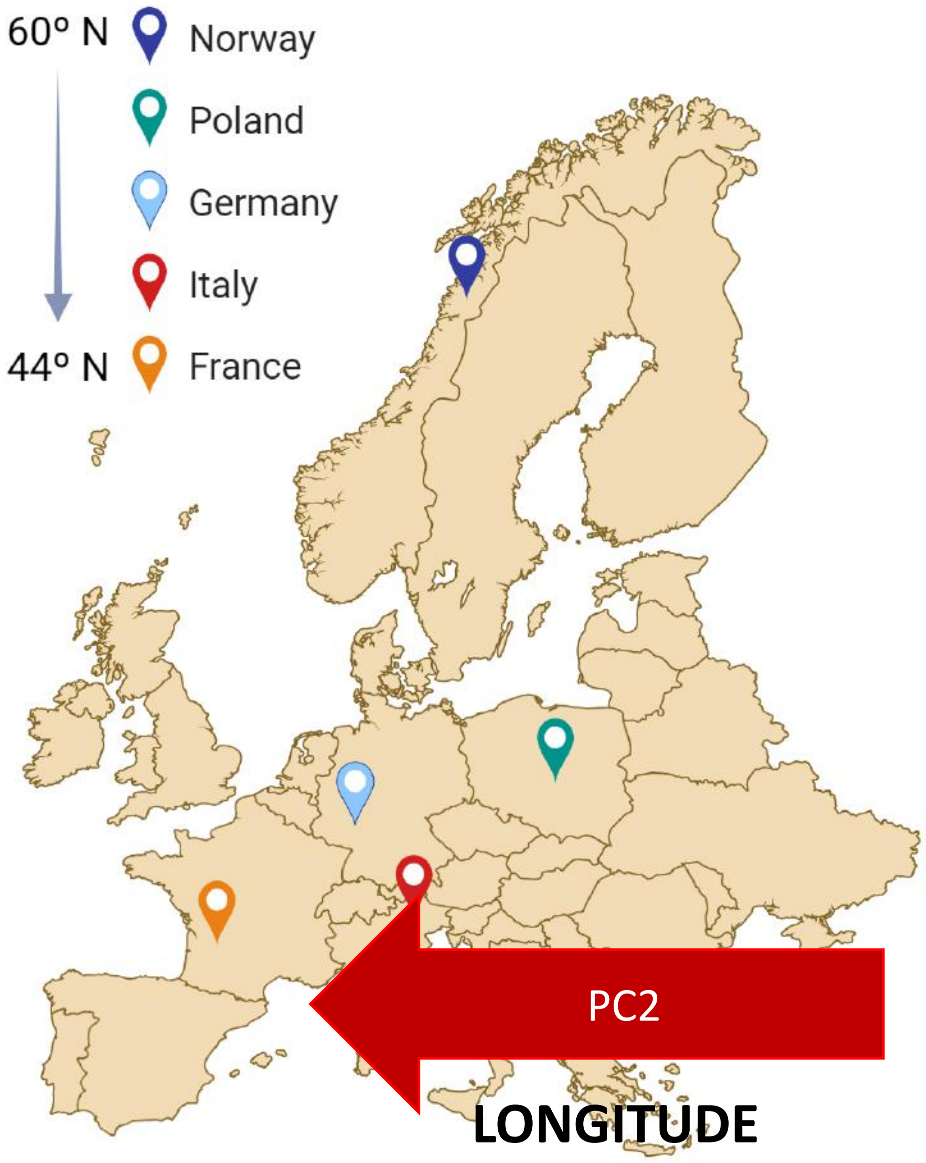
Primary Metabolites

Principal Component Analysis (PCA)

Sugars

Amino acids





Primary Metabolites

Location

Sugars & deriv.

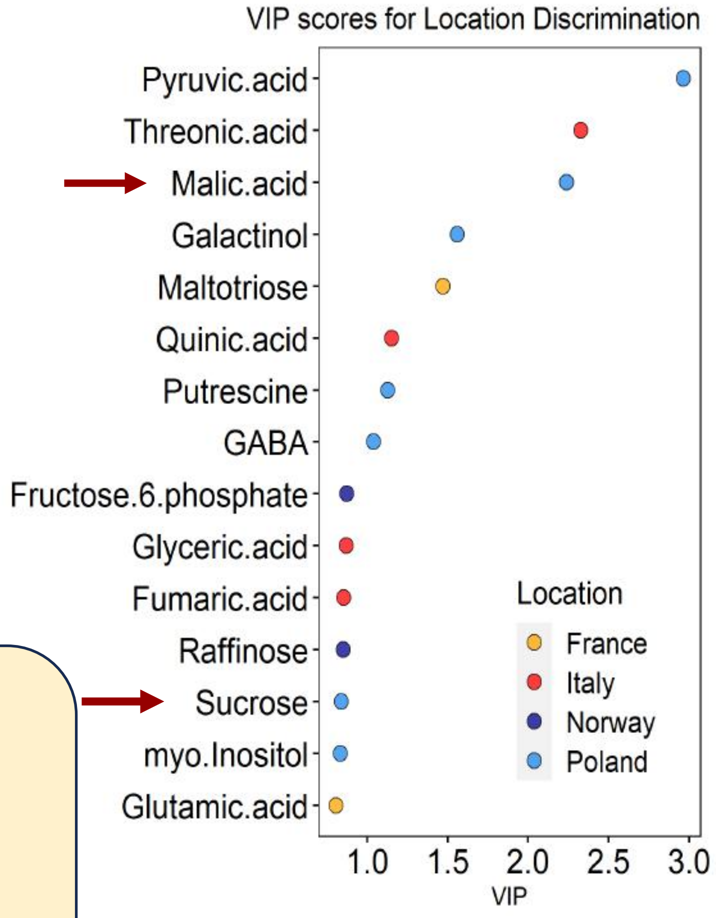
galactinol, maltotriose, fructose-6-P, raffinose, sucrose, and myoinositol

Organic acids

pyruvic acid, threonic acid, malic acid, quinic acid, glyceric acid, fumaric acid, and glutamic acid

GABA

- metabolites related to energy metabolism
- metabolites related to response to abiotic stress
- protective role



*Only metabolites with VIP > 0.8 are shown

Primary Metabolites

Location

Sugars

galactinol, maltotriose, fructose-6-P, raffinose, sucrose, and myoinositol

Organic acids

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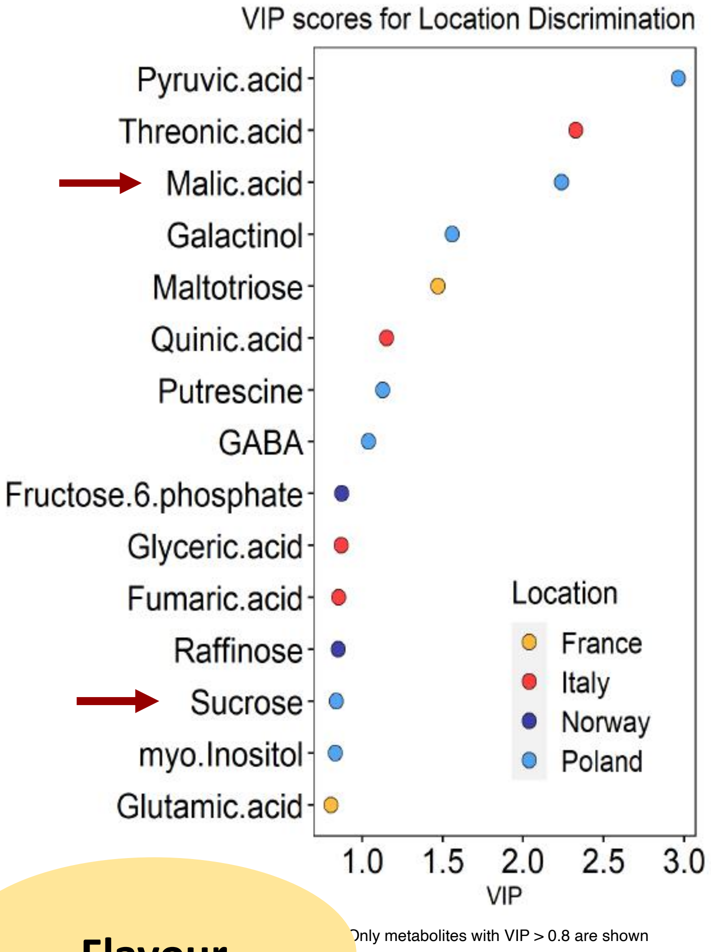
GABA



SOUTHERN LATITUDES GABA ↑ **Protective role**

POLAND Malic acid, Sucrose ↑
- high levels of global radiation
- moderately high temperatures

Flavour

Primary Metabolites

Cultivars

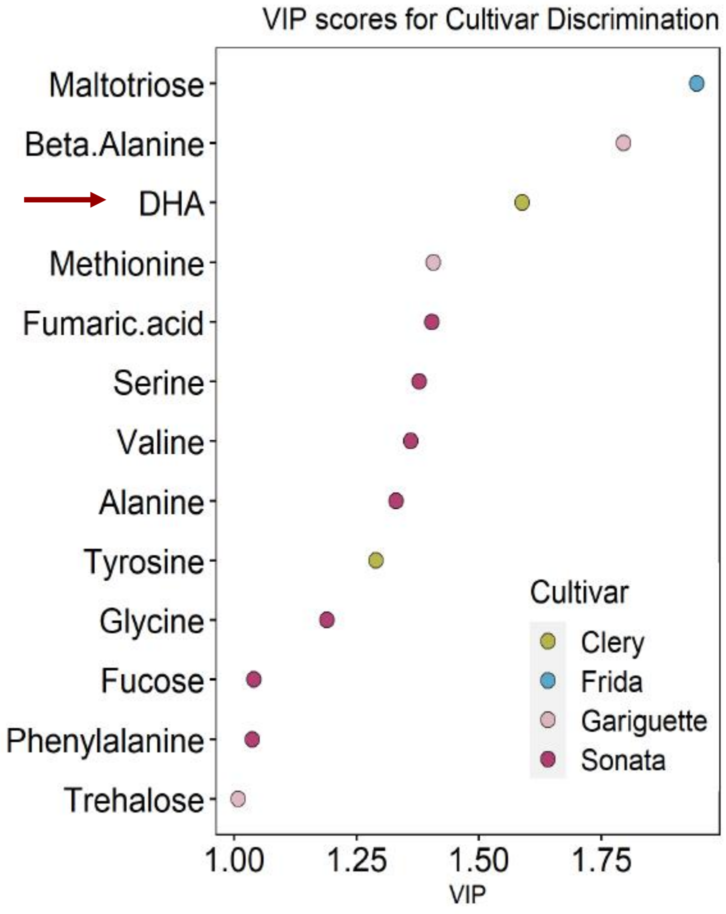
Amino acids

beta-alanine, methionine, serine, valine, alanine, tyrosine, glycine, and phenylalanine

Sugars

maltotriose, fucosa

Amino acid metabolism
Vitamin C metabolism
- Nutritional profile (health benefits)



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Primary Metabolites

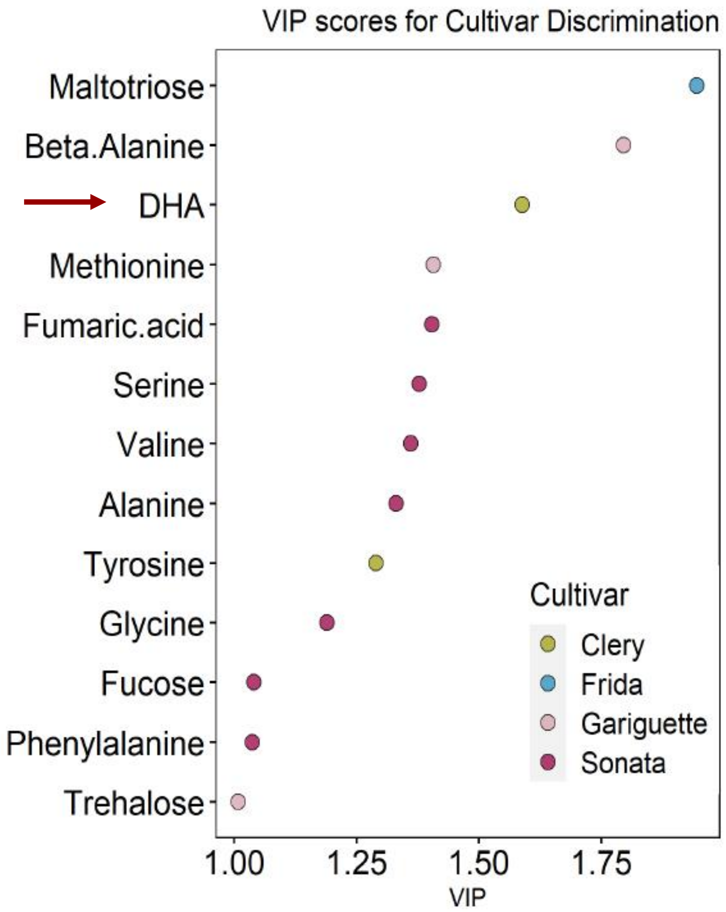
Cultivars

Amino acids

beta-alanine, methionine, serine, valine, alanine, tyrosine, glycine, and phenylalanine

Sugars

maltotriose, fucosa



*Only metabolites with VIP > 1 are shown

SONATA Amino acids ↑ **Nutritional profile**

CLERY DHA ↑ **Vitamin C (health benefits)**

Volatile Compounds

Straight-chain esters

Apocarotenoids

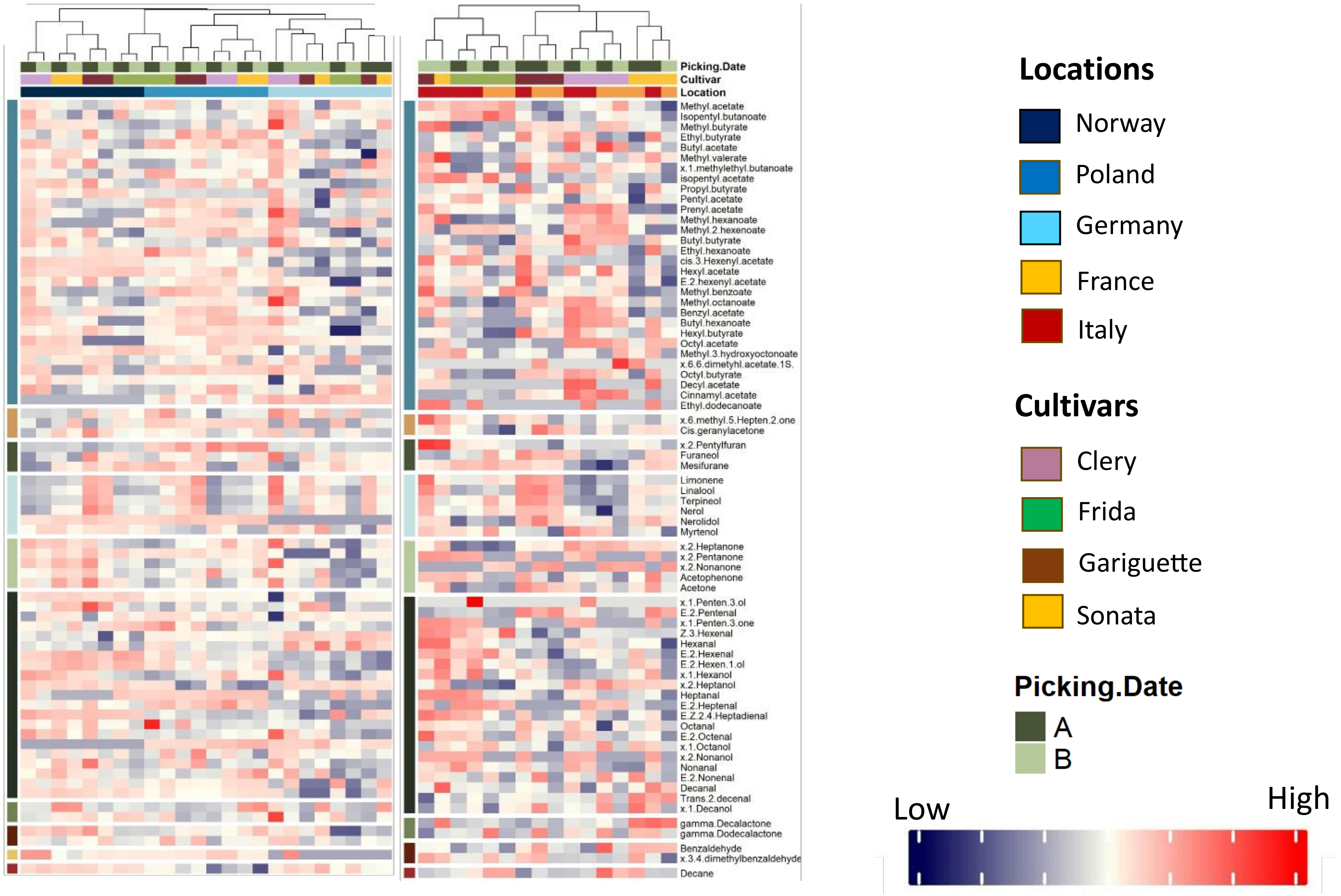
Furans

Terpenoid compounds

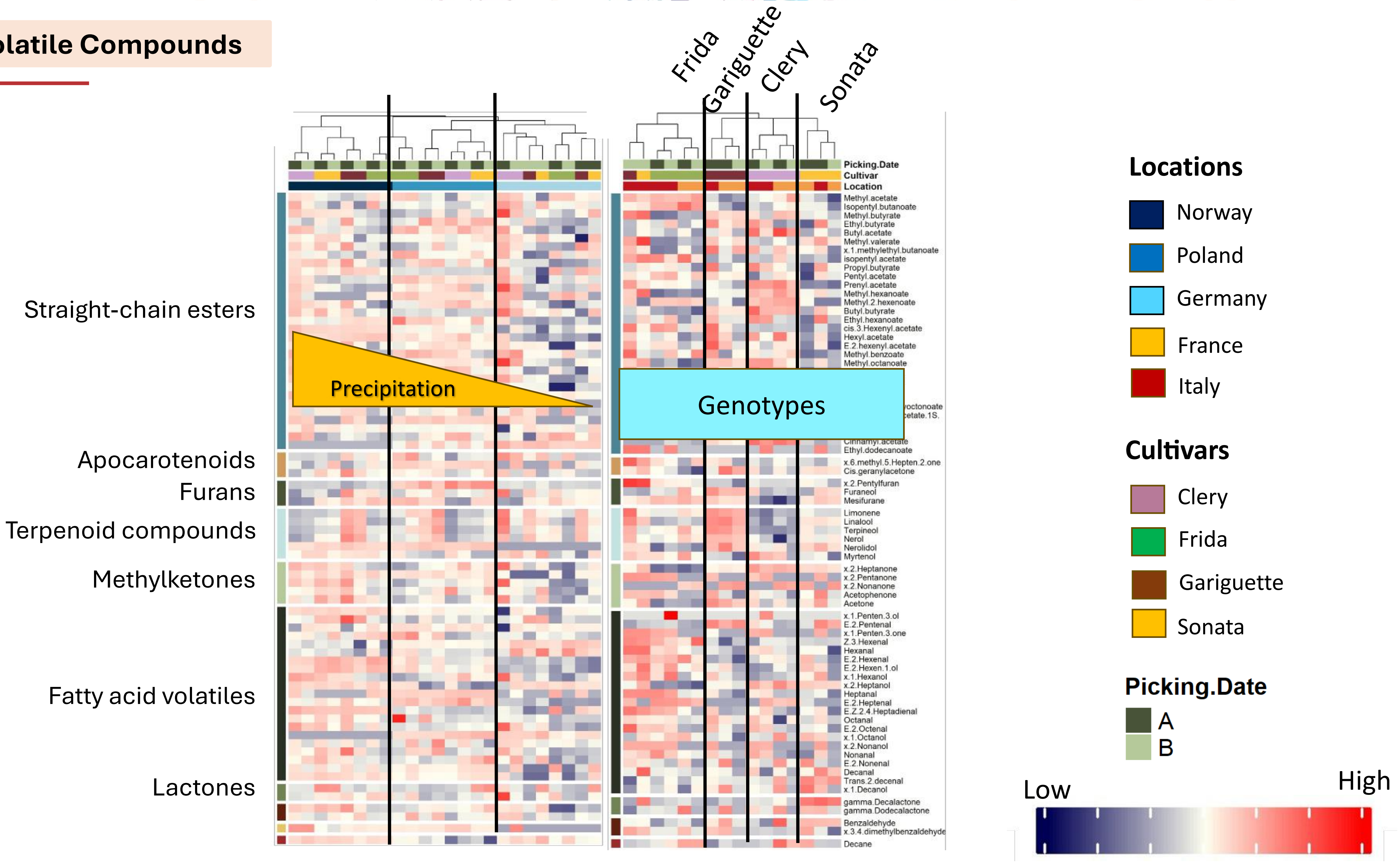
Methylketones

Fatty acid volatiles

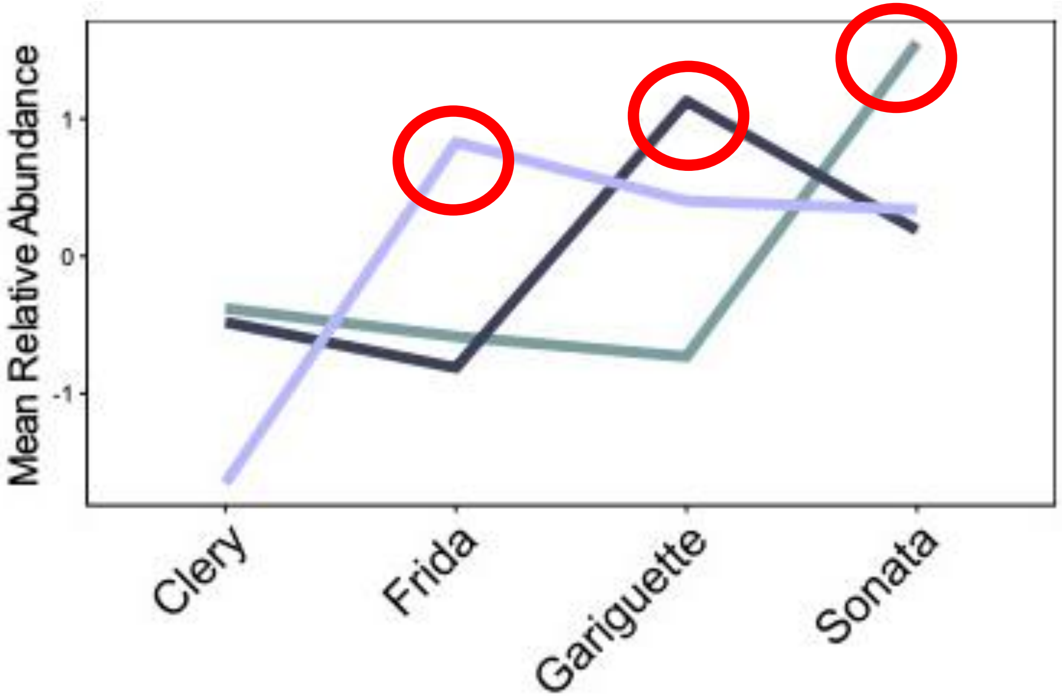
Lactones



Volatile Compounds



How can we search for candidate genes?



gamma-Decalactone → **peach & fruity**

Linalool → **citrus-like**

Mesifurane → **sweet-like**

FLAVOR

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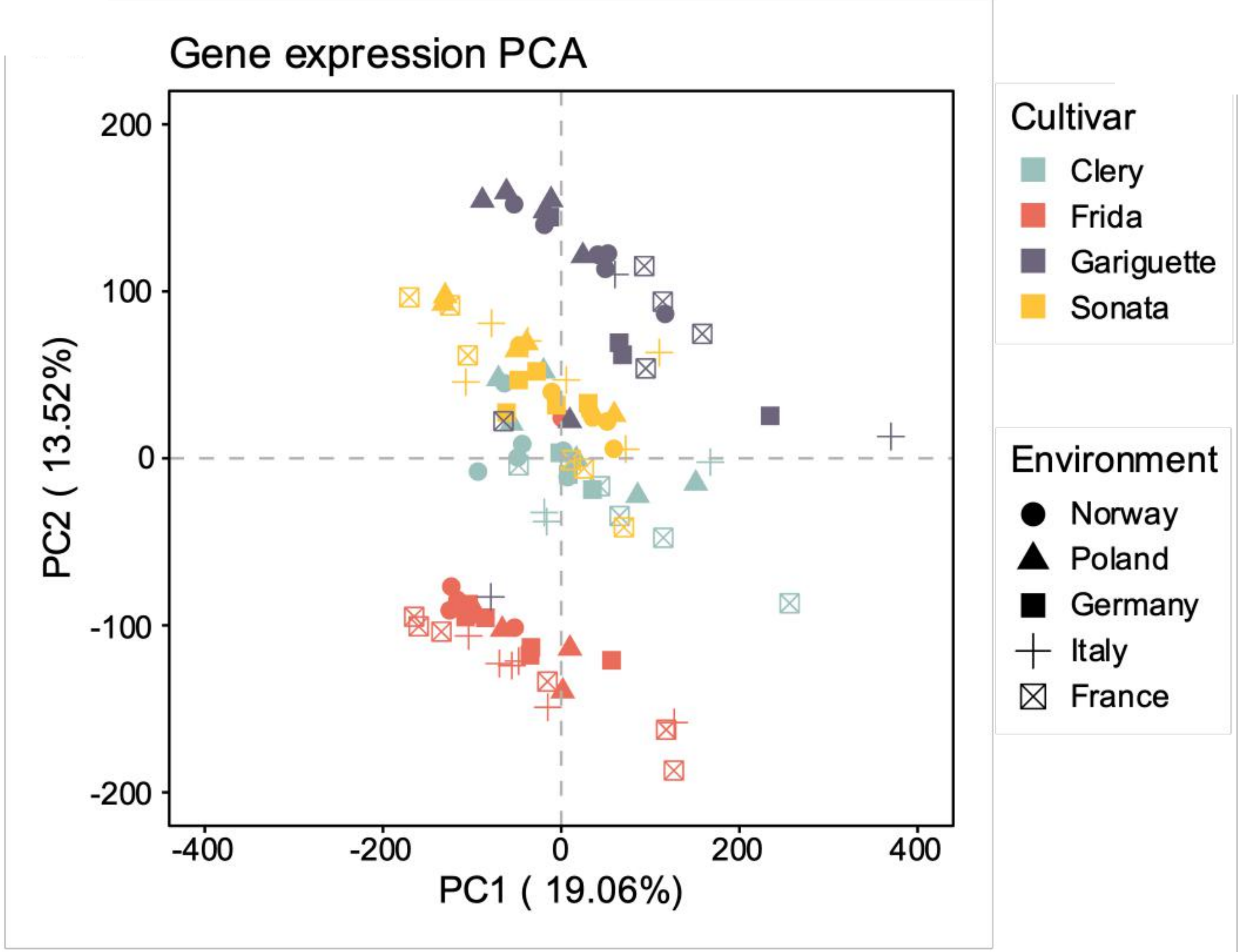
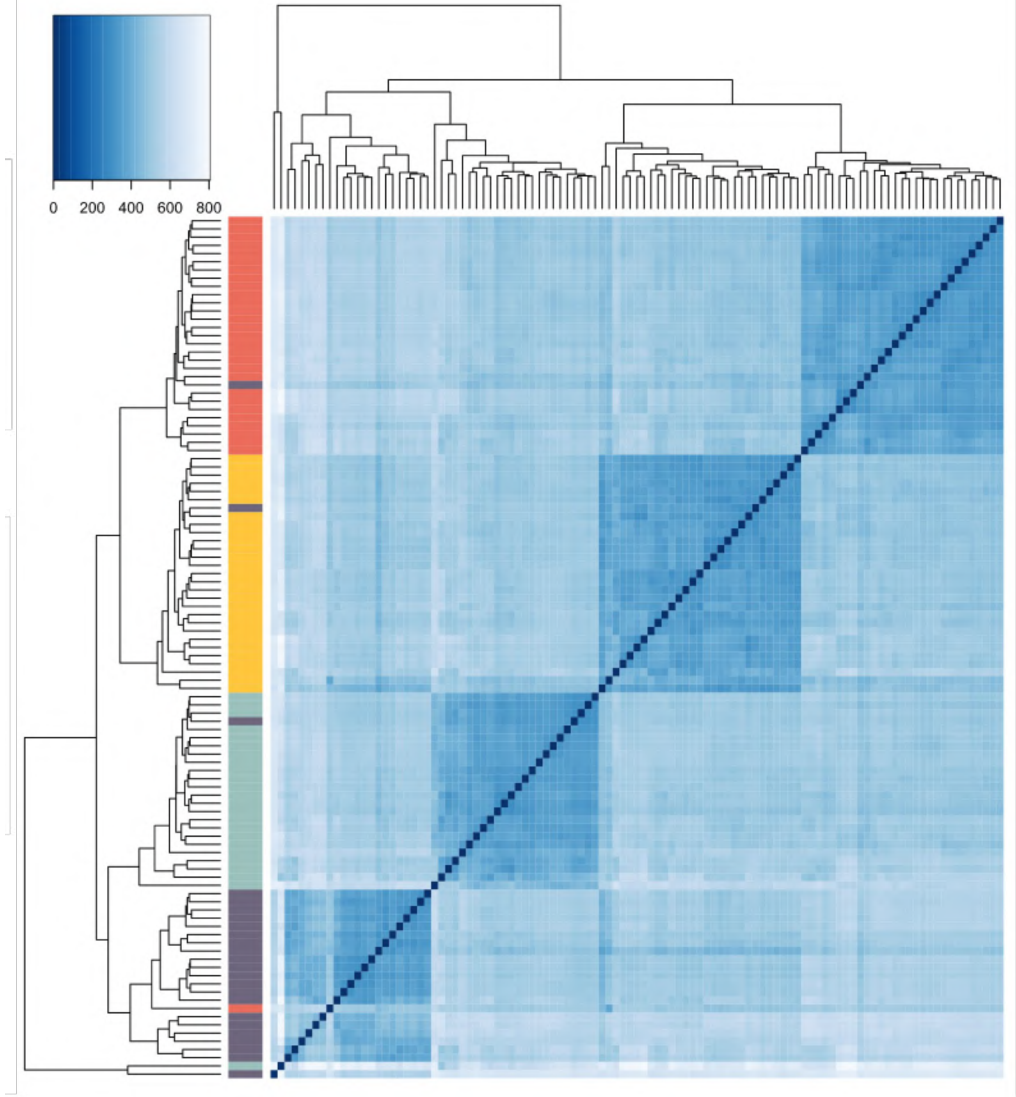
Transcriptome analysis





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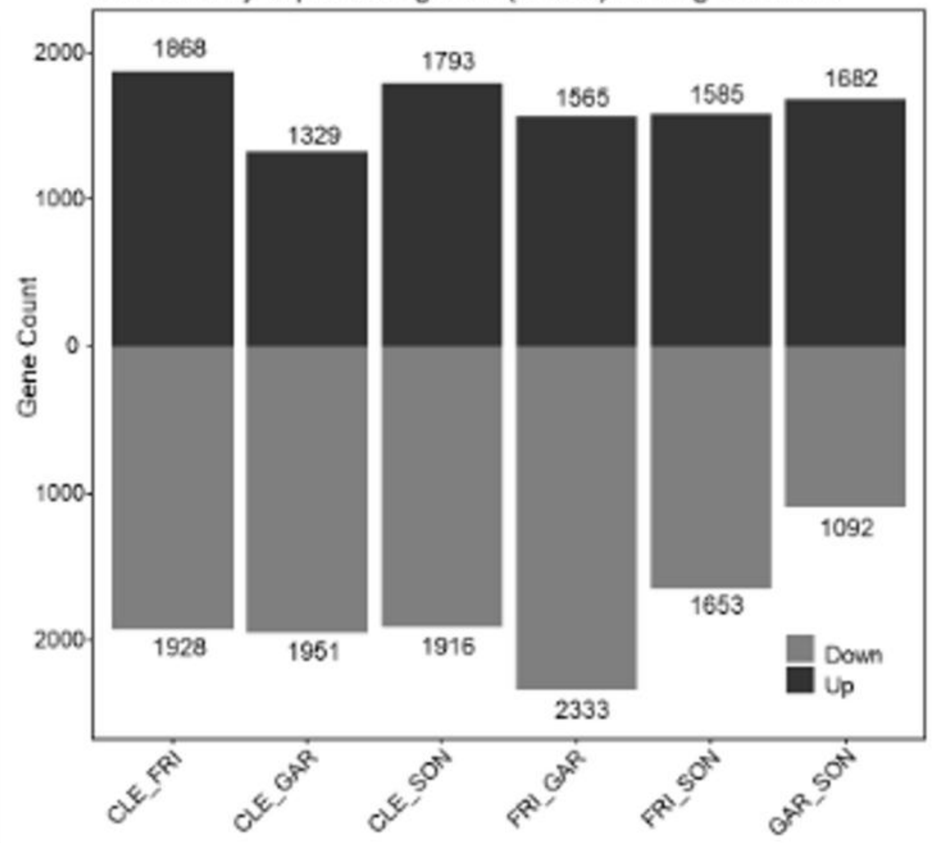
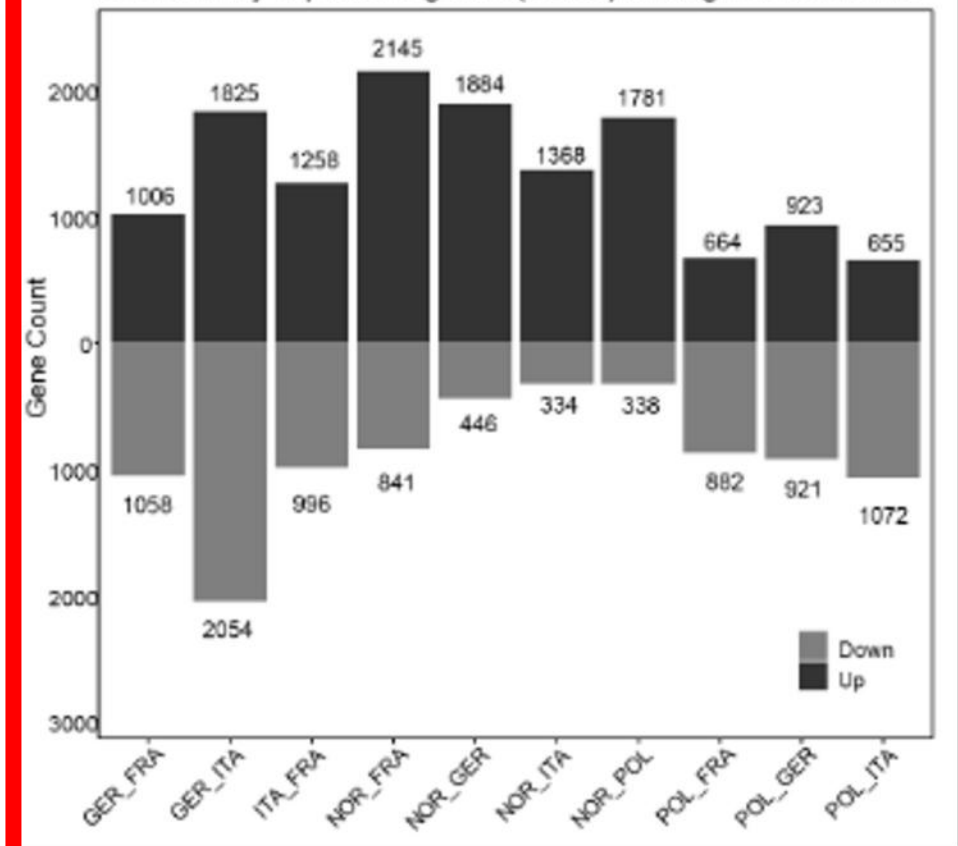
Transcriptome analysis

Differentially expressed genes (DEGs) among **CULTIVARS**

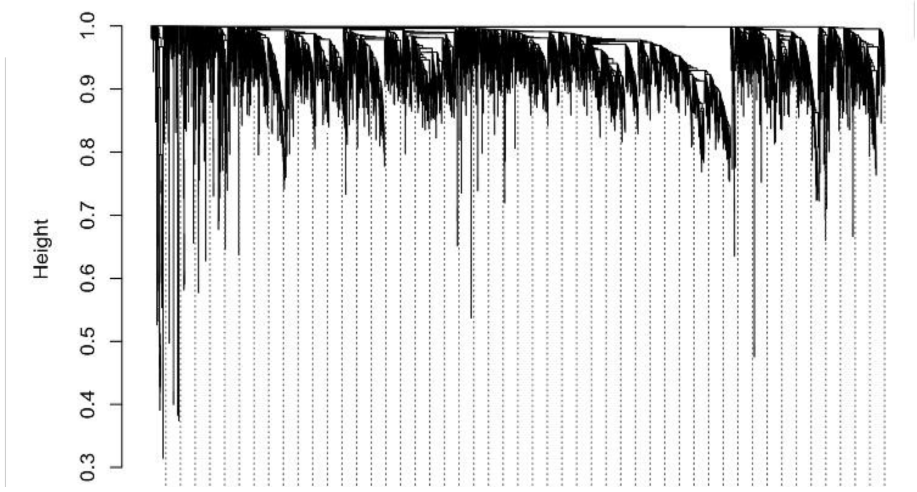


Differentially expressed genes (DEGs) among **ENVIRONMENTS**

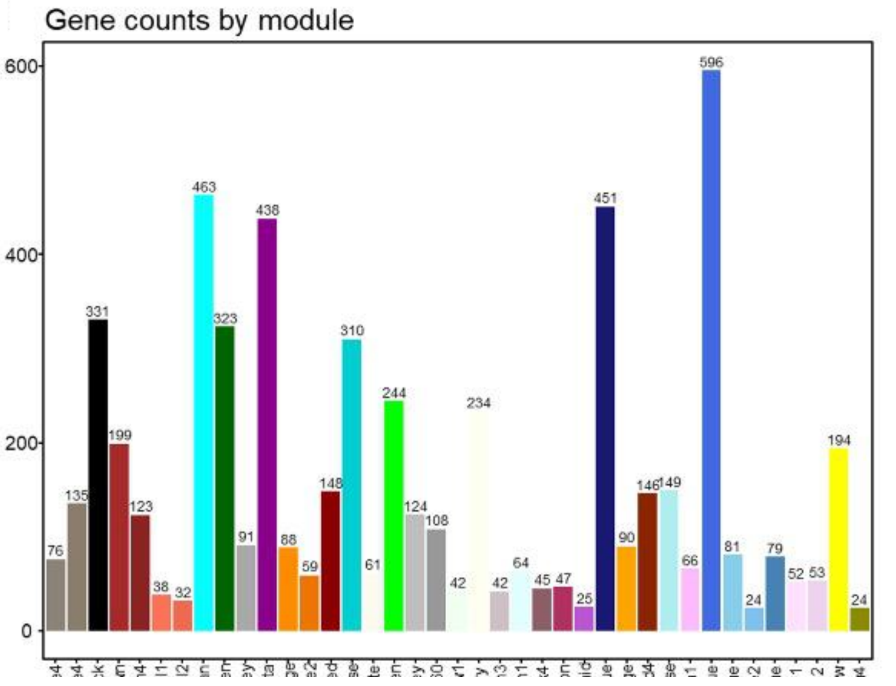


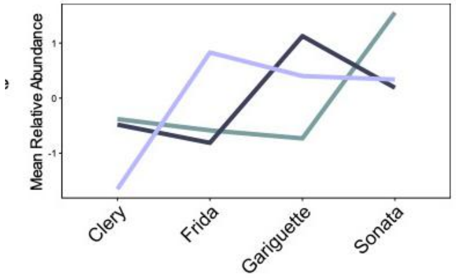
How can we search for candidate genes?

Weighted Gene Co-expression Network Analysis (WGCNA)



39 modulos





Correlation analysis

gamma-Decalactone
Linalool
Mesifurane

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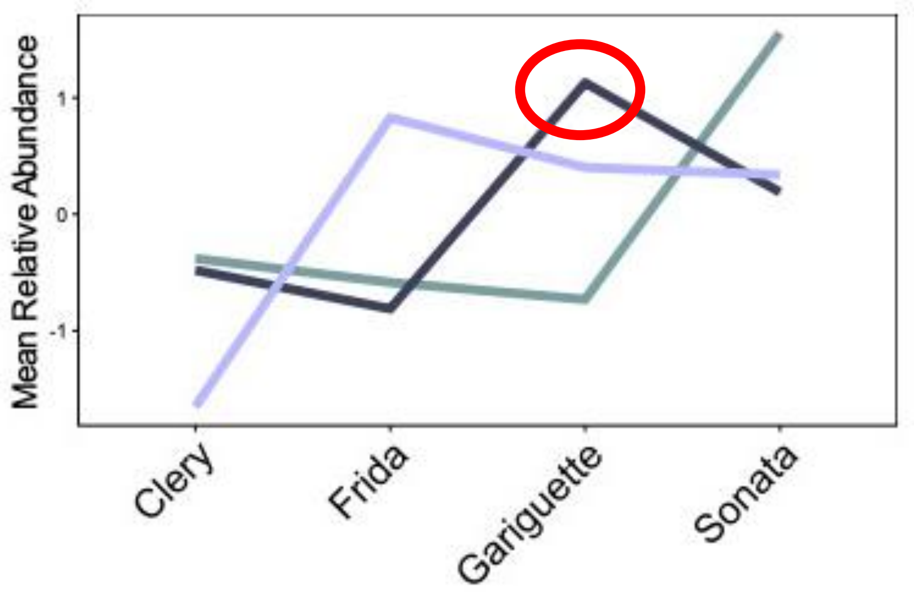
Volatile	Candidate gene
Linalool	5 genes (terpenoid synthases)



High expression in **Gariguette**

IHSM La Mayora
Instituto de Hortofruticultura Subtropical y Mediterránea
UNIVERSIDAD DE MÁLAGA

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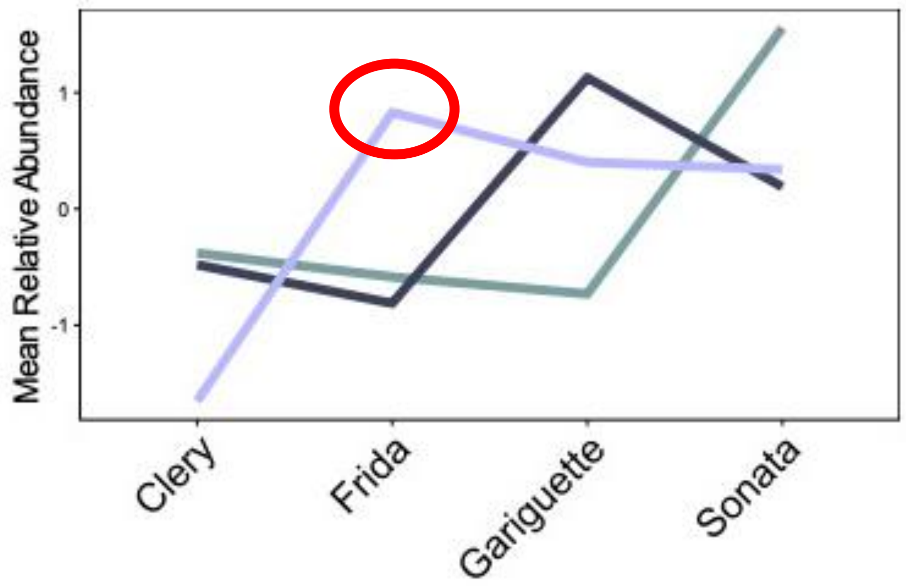
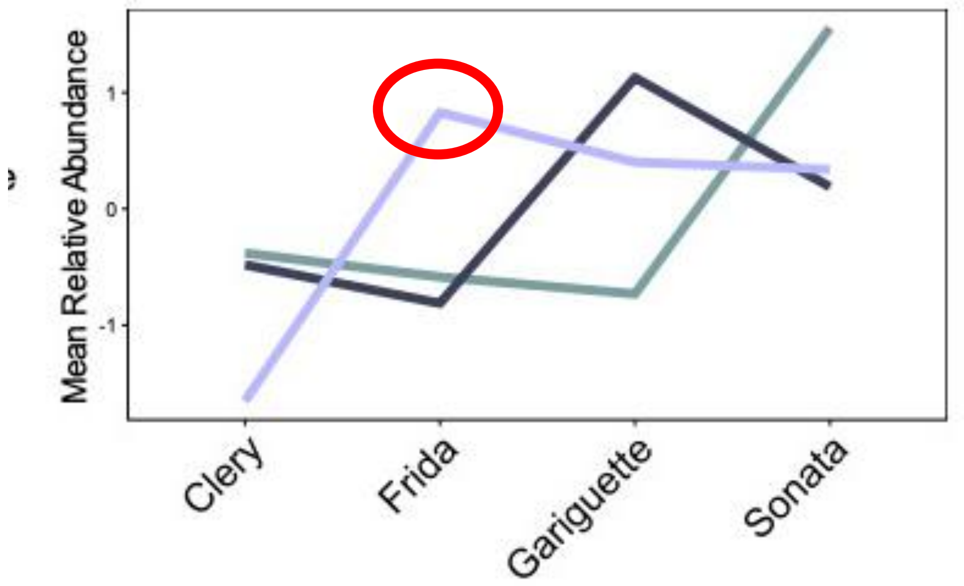


High expression in **Frida**

How can we search for candidate genes?

Volatile	Candidate gene
Linalool	5 genes (terpenoid synthases)
Mesifurane	5 genes (lipid metabolism, phytohormone signaling, solute transport, oxidoreductases (2))
gamma-Decalactone	3 acyltransferases (Urrutia et al., 2017)



High expression in **Sonata**

Road to the ***perfect*** strawberry fruit

- **Genotype-by-environment (G×E) interactions significantly influence** strawberry fruit development, metabolite composition, and transcriptional regulation, with **northern regions showing stronger environmental effects** on volatile organic compounds (VOCs) compared to southern regions.

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- Specific **cultivars demonstrated resilience in different climates**: <u>Clery</u> showed metabolic stability in northern regions; **Frida** highlighted in some compounds in Norway and Italy; **Sonata** was consistent across environments; and **Gariguette** maintained stability in terpenoids across all the environments.

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- **Weighted gene co-expression network analysis** (WGCNA) **identified key transcriptional modules linked to VOC biosynthesis**, suggesting **targeted breeding strategies** to enhance sensory attributes in strawberries across environments.

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Thank you for your attention!