NMR profiling for the characterisation of melon commercial varieties and genetic resources

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This study was carried out within the European META-PHOR Project (FOOD-CT-2006-036220). The objectives of this project are to develop a phenotyping European platform for plants using metabolomics approaches, from biochemical analyses to statistical analyses and databases. This part of the project focused on the major metabolites of melon fruit (*Cucumis melo L.*). We used quantitative ^{1}H-NMR profiling of polar extracts and multivariate analyses to characterize sample clustering or difference and highlight discriminant metabolites between commercial cultivars depending on culture conditions or year and between various genotypes.

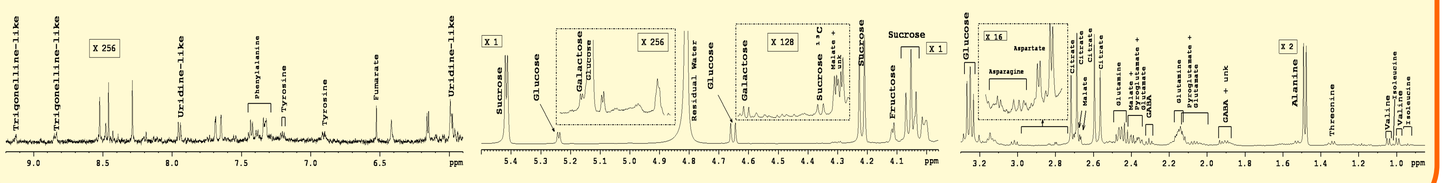
Sample collection and preparation

Commercial varieties were grown in open field in Moissac (South-West France) or open field and greenhouse in Newe Yaar (North Israel). Genetic resources were grown In Newe Yaar (North Israel). Melon fruits were harvested at commercial maturity.

Ethanolic extraction was performed on freeze dried powdered samples of fruit flesh and ^{1}H-NMR data were acquired using an electronic reference allowing absolute quantification of metabolites (Moing *et al*., 2004).

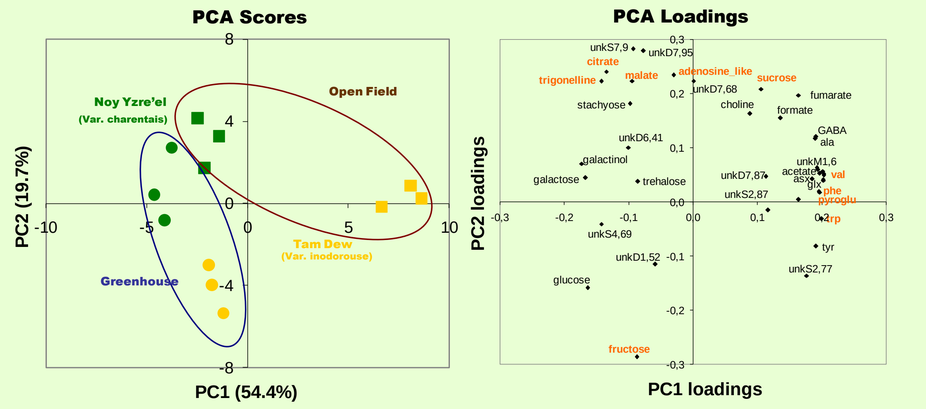
^{1}H-NMR spectra were processed to quantity major metabolites, including sugars, organic acids, amino-acids and some unknown compounds.

Representative ^{1}H-NMR metabolic profile of melon extract



Open Field / Greenhouse effect

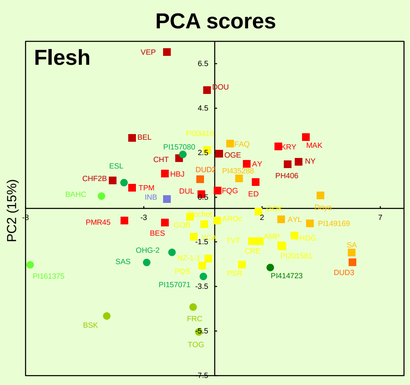
2 cultivars, 41 metabolites

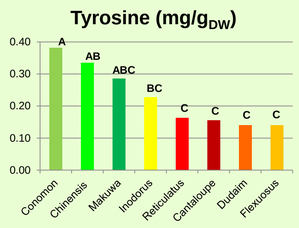


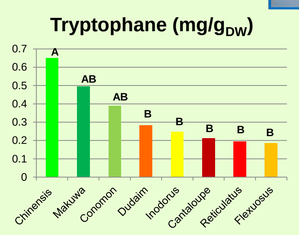
- **PC1 & PC2 separate the cultivars and the growth conditions.**
- **Independently of the growth environment, the two cultivars can be separated, Noy Yzre'el having higher organic acid and sucrose contents.**
- **The growth environment also has an effect on the melon flesh composition, Tam Dew cultivar being more sensitive than Noy Yzre'el (Higher hexose content in Tam Dew grown in greenhouse).**

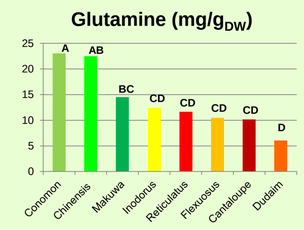
Characterisation of 52 genotypes belonging to *Cucumis melo* species, subspecies *melo* and *agrestis*

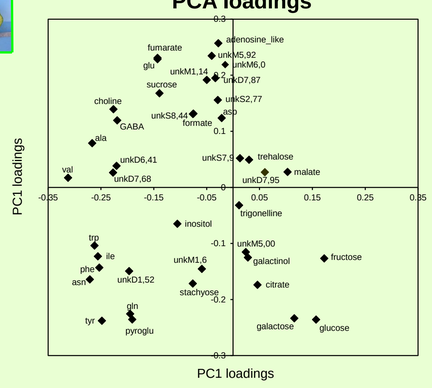
- ***subspecies melo***
  - **Cantaloupe (8 cvs)**
  - **Reticulatus (10 cvs)**
  - **Inodorus (14 cv)**
  - **Dudaïm (2 cvs)**
  - **Flexuosus (6 cvs)**
  - **Chandalak (1 cv)**
- ***subspecies agrestis***
  - **Makuwa (5 cvs)**
  - **Conomon (3 cvs)**
  - **Momordica (1 cv)**
  - **Chinensis (2 cvs)**











➢ ***agrestis/melo* discriminant metabolites**

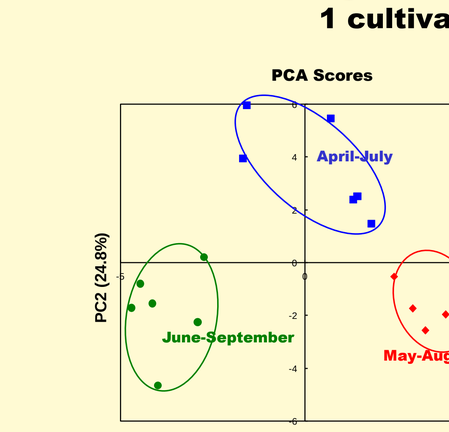
Letters above metabolite concentration are Tukey's test groups. Values with same letter are not statistically significant (P<0.05)

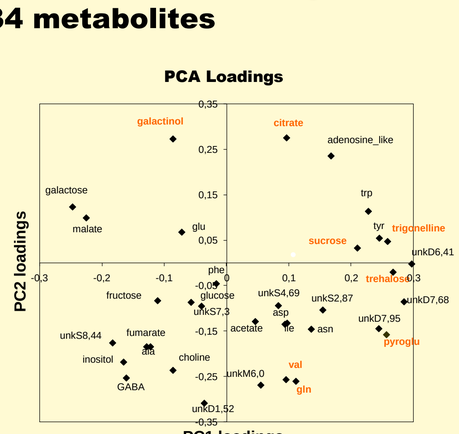
- **A high variability is observed within each sub-species or group.**
- **ANOVA was used to highlight metabolites discriminating subspecies or genotypes.**

- **The PCA scores plot shows that a high variability within each group leads to overlapping of some subspecies (i.e. cantaloupe, flexuosus and makuwa)**
- **The loadings plot shows that the amino acid contents tend to be higher in *Cucumis agrestis* subspecies than in *Cucumis melo* L. This was confirmed by ANOVA and Tukey's test (e.e. for glutamine, tyrosine and tryptophane).**

Year effect

2 cultivars, 3 years, 34 metabolites





- **PC1, PC2 & PC3 separate the cultivars and harvest years.**
- **The differences between years are higher than between cultivars except for 2007. In 2007, Cézanne cv. was much different and accumulated more hexoses and organic acids. The level of sucrose was decreased in comparison with 2006 and 2008.**
- **July 2007, one of the coldest July months over 20 years (Data from http://www.meteo-bordeaux.fr), affected ripening and sucrose accumulation leading to an increased level of organic acids and hexoses.**

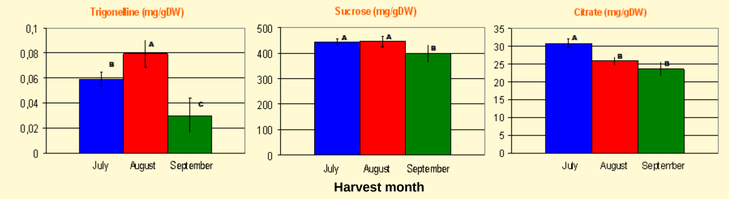
Effect of growth period on one year

1 cultivar, 34 metabolites

- **PC1 separates the three growth periods of Hugo cultivar (Plantation-Harvest).**
- **ANOVA and Tukey's test highlight that major discriminant metabolites between the three growth periods included trigonelline, sucrose and citrate .**
- **Fruits harvested in July and August contained more sucrose while citric acid content decreased in the later growth period.**

➢ **Concentration of some discriminant metabolites**

Letters above metabolite concentration s are Tukey's test groups. Means with same letter are not statistically significant (P<0.05)



Perspectives

➢ The characterisation of the 52 genotypes has also been performed on the fruit peel. Flesh and peel data sets will be submitted to Hierarchical Clustering Analysis in order to compare the obtained "metabolic phylogeny" with the genetic phylogeny.

➢ Within the META-PHOR Project, these data will be combined with metabolome data from other analytical strategies (i.e. GC-MS, LC-MS, …)

References:

[1] Moing. *et al*. (2004). Functional Plant Biology, vol. 31, pp 889-902

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