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Primary metabolism investigation of fleshy fruit species using ¹H-NMR profiling

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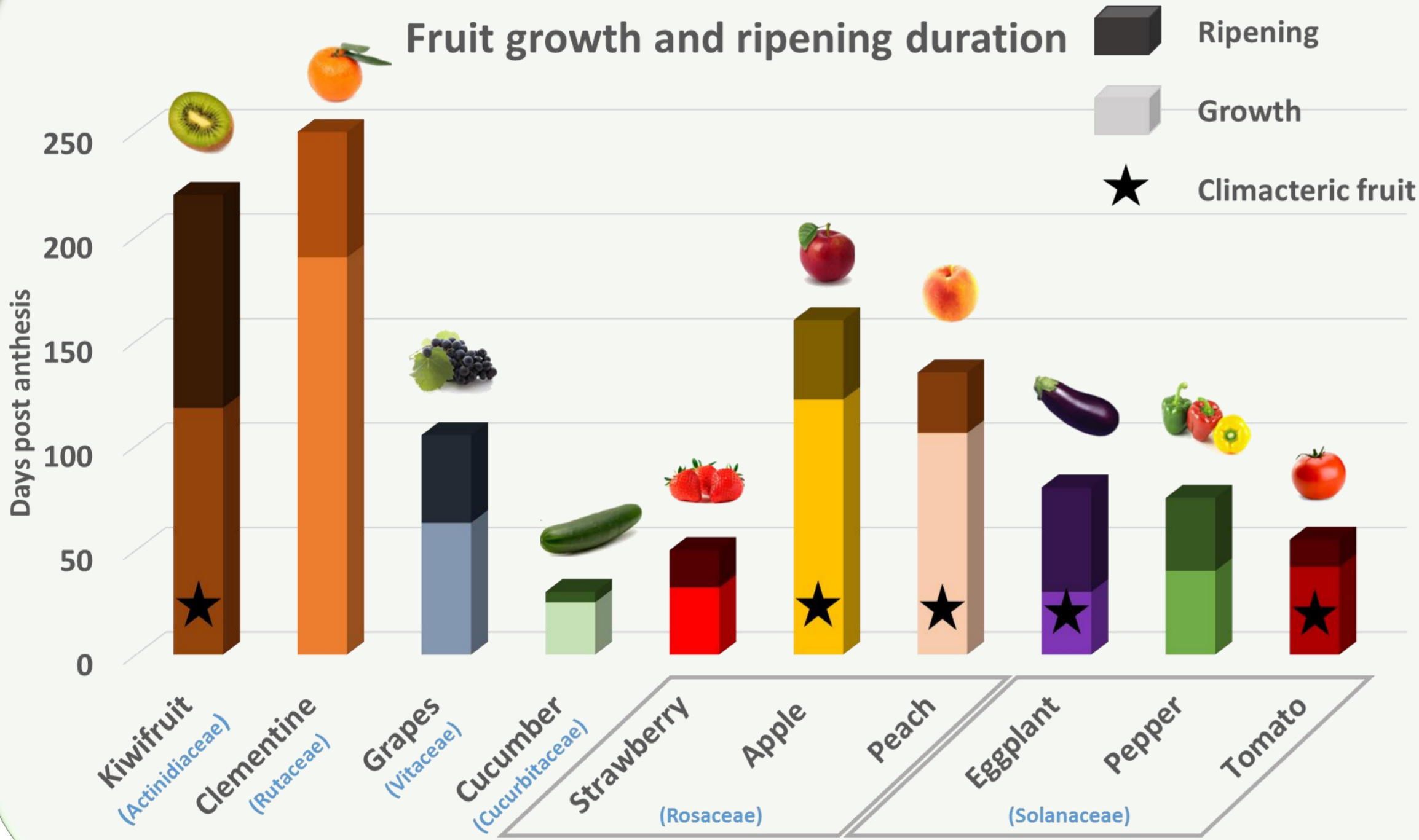
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Context of study

Fruits are crucial for human nutrition and they have a prominent place in the economic market. To continue to improve fruit quality and yield it is important to have a better understanding of the mechanisms involved in its development. In our study, ten fleshy fruit species, differing for fruit growth dynamics, ripening climacteric status, maturation duration, phloem-transported sugars and starch storage level, were investigated during their development. We focused on primary metabolism which provides energy and biosynthetic precursors to support fruit growth and ripening, and is essential for fruit quality.

Experimental design

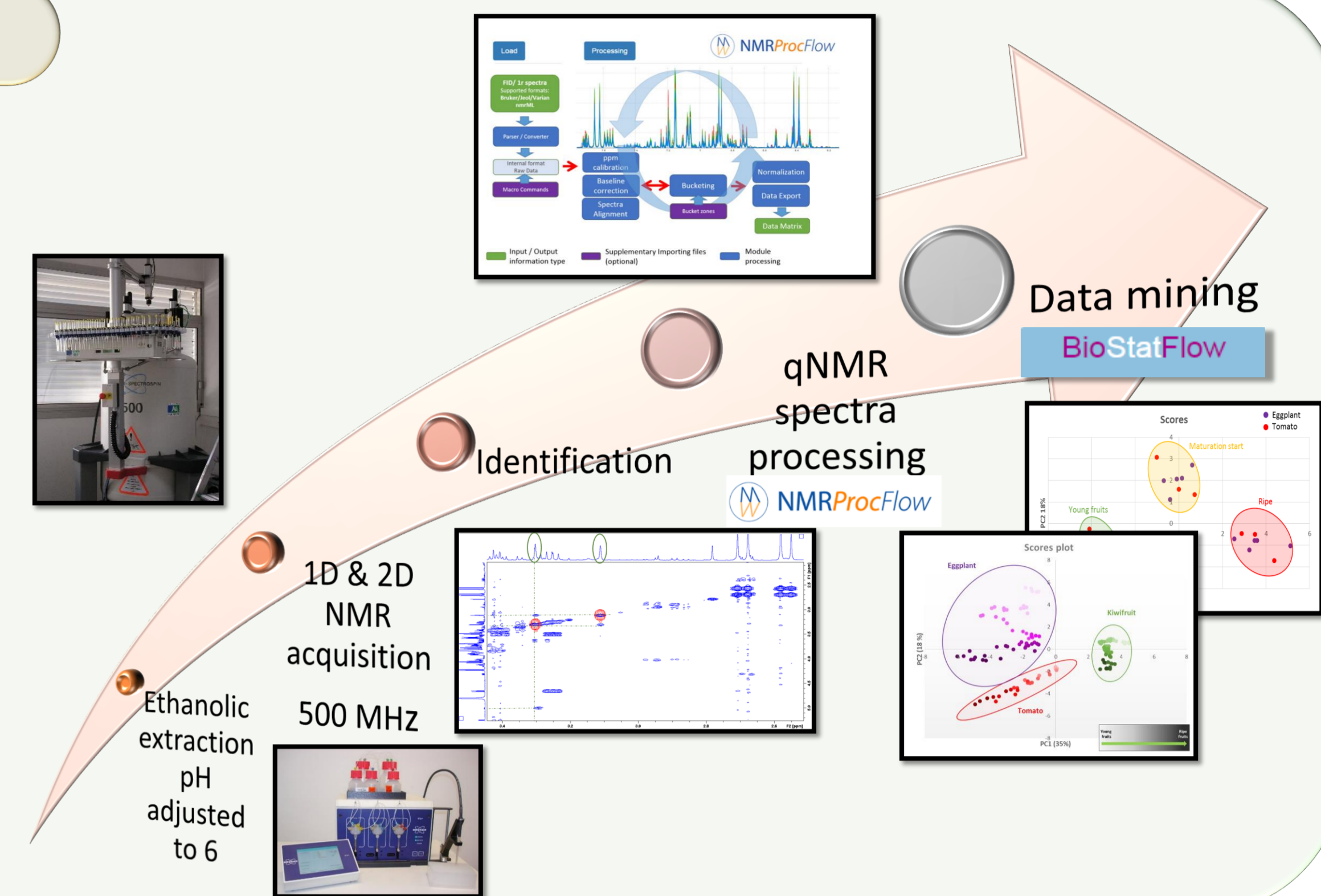


10 fruit species chosen according to different criteria :

- ≠ acidity / sweetness
- ≠ growth duration
- ≠ maturation duration
- climacteric type or not
- ≠ botanical families

9 to 16 developmental stages per species

Identification of major polar compounds
Quantification by ¹H qNMR for 3 species



Identifying common and specific major polar compounds in 10 fruit species

Qualitative comparison

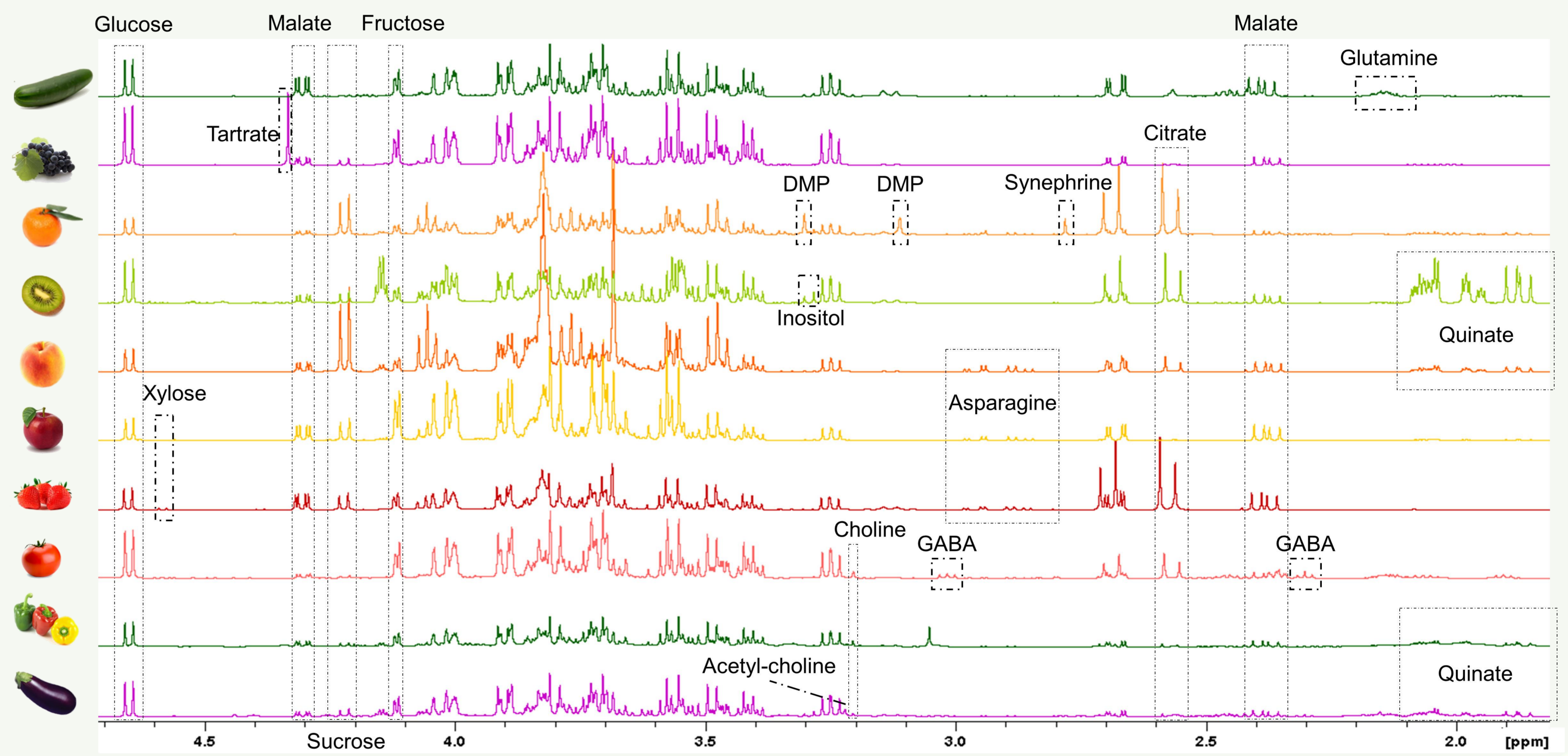
- ☀ Xylose in *Rosaceae*
- ☀ DMP and synephrine in clementine
- ☀ Tartrate in grape berry

NMR-based annotation of a mixture of several stages:
zg (¹H) / zgpg (¹H/¹³C) / CPMGpr(¹H) / Dept 135 (¹³C)
COSY (¹H-¹H) / TOCSY (¹H-¹H) / HSQC (¹H-¹³C)
HMBC (¹H-¹³C) / Jres (¹H-¹H) / TOCSY (¹H-¹H) in selective irradiation

Quantitative comparison

- ☀ More sucrose in peach
- ☀ More glucose & fructose in grape berry
- ☀ More inositol & quinate in kiwifruit

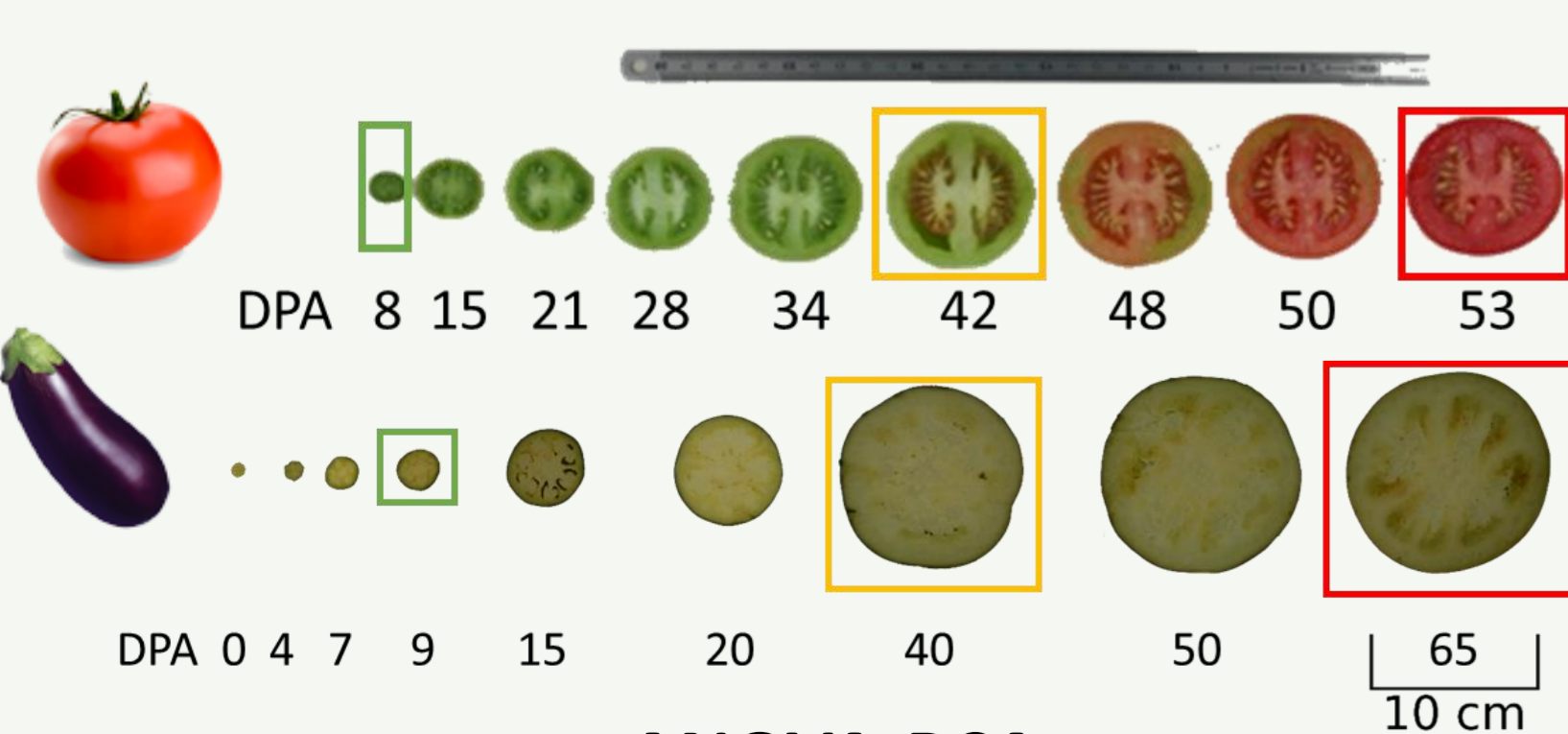
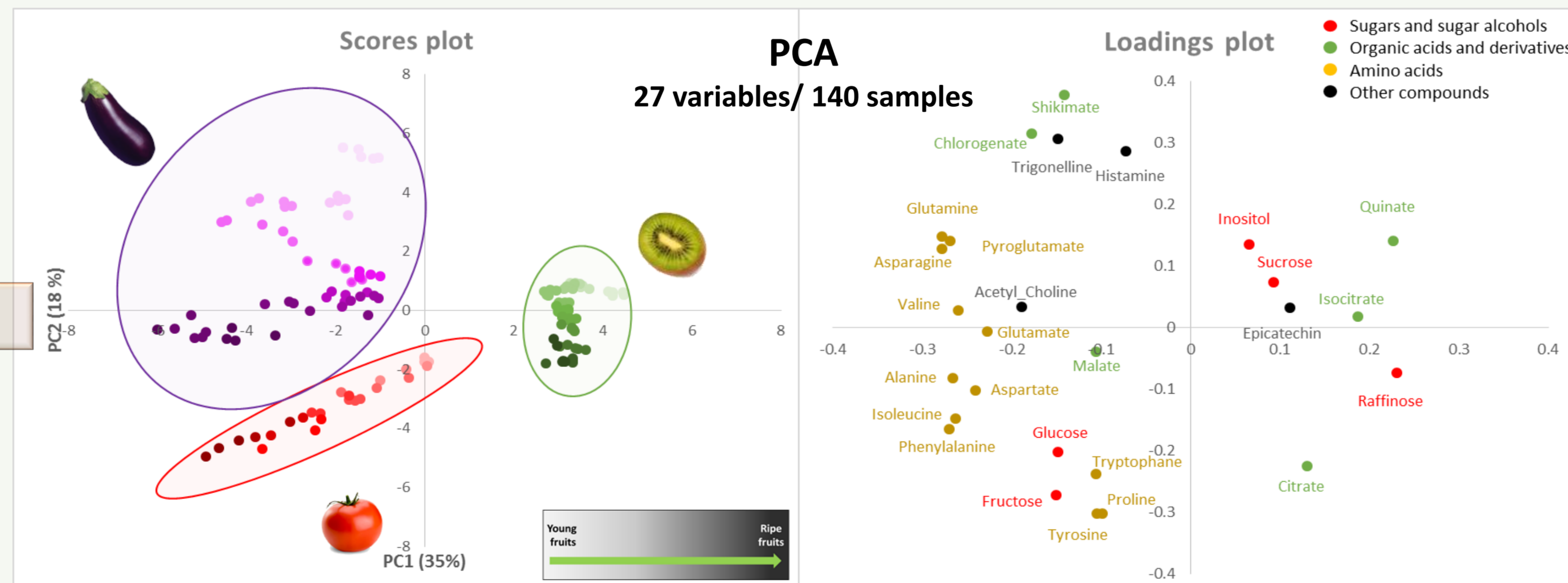
→ Through the developmental quantification



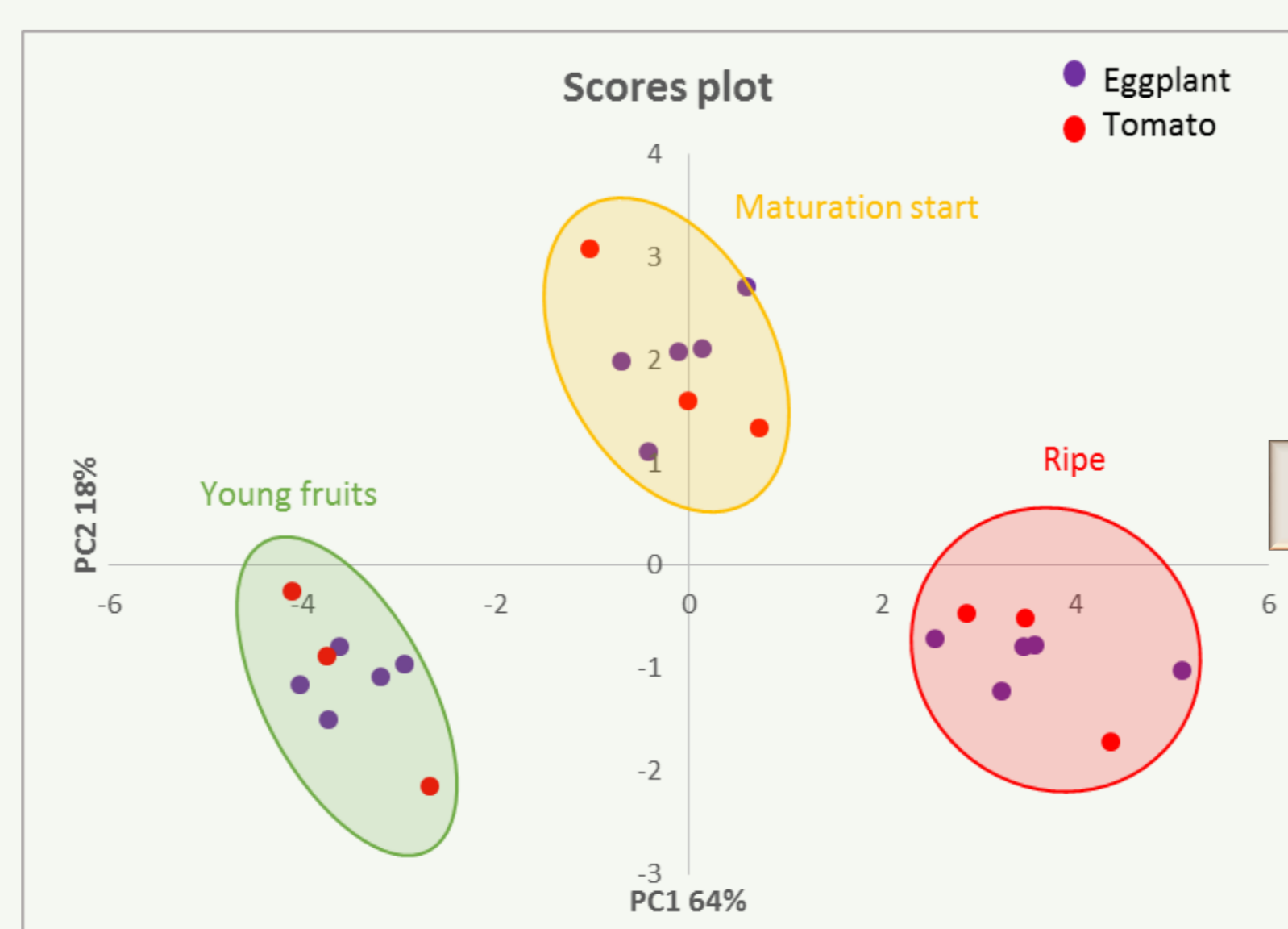
Searching for common and specific changes along fruit development

Clear species and developmental effects

- ☀ **Eggplant** characterised by higher shikimate and chlorogenate contents at young stages
- ☀ **Tomato** characterised by fructose and glucose at late stages
- ☀ **Kiwi** characterised by quinate at early stages



NMR data were complemented with MS-based data of phosphorylated compounds
Hot water extraction, Quantification by IC-QTRAP-MS



Highlighting stage effect through tomato and eggplant development

- ☀ **Young stages** : Highest contents in sucrose, shikimate, quinate, trigonelline and chlorogenate
- ☀ **Start of maturation** : Highest contents in fumarate, pyroglutamate and fructose-1,6-bisP
- ☀ **Ripe** : Highest contents alanine, aspartate and citrate

Conclusion & Perspectives

NMR allowed **identifying** the major polar compounds in fruit species and to **discriminate** them through quantification

Analyses of **cell walls** and **enzymatic capacities** will complement our data set

Metabolic models will be parameterized with absolute quantification data to reveal **common** and **species-specific regulations** of primary metabolism during fruit development

References

Jacob et al., *Metabolomics*, 2017