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> How do genetics, development and drought affect polyphenols in goji?

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> **1. Context**

> 2. Effect of genetic and developmental factors

> 3. Impact of drought

> 1. What are goji berries ?



- Solanaceae family

- Goji/wolfberry -> 3 species:

Lycium barbarum

Lycium chinense

Lycium ruthenicum

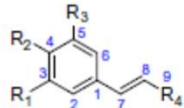
- Traditionally eaten in Asia for their high nutritional value
- Consumption is growing in Europe, only cultivars from *L. barbarum* can be commercialized

> 1. “Superfood”



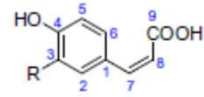
- Fresh weight: 0.5 – 1 g (10-20% of dry matter content)
- Polysaccharides: $1 - 2.5 \times 10^3$ mg 100 g⁻¹ FW
- Carotenoids: 10 – 50 mg 100 g⁻¹ FW
- Vitamin C: 20 – 50 mg 100 g⁻¹ FW
- **Polyphenols: 10 – 50 mg 100 g⁻¹ FW**

> 1. A complex composition in polyphenols at maturity

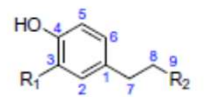


- 1: R₁ - H, R₂ - OH, R₃ - H, R₄ - COO-β-D-Glc¹-β-D-Glc
- 2: R₁ - H, R₂ - OH, R₃ - H, R₄ - COO-β-D-Glc⁴-β-D-Glc
- 3: R₁ - OCH₃, R₂ - OH, R₃ - H, R₄ - COO-β-D-Glc²-β-D-Glc
- 4: R₁ - OCH₃, R₂ - OH, R₃ - H, R₄ - COO-β-D-Glc²-β-D-Glc
- 5: R₁ - OCH₃, R₂ - OH, R₃ - H, R₄ - CH₂O-β-D-Glc²-β-D-Glc
- 6: R₁ - H, R₂ - O-β-D-Glc²-β-D-Glc, R₃ - H, R₄ - COOCH₂CH₃
- 7: R₁ - H, R₂ - O-β-D-Glc²-β-D-Glc, R₃ - H, R₄ - COOCH₂CH₃
- 8: R₁ - OCH₃, R₂ - O-β-D-Glc²-β-D-Glc, R₃ - H, R₄ - COOCH₂CH₃
- 9: R₁ - O-β-D-Glc, R₂ - OH, R₃ - H, R₄ - COOCH₂CH₃
- 10: R₁ - OCH₃, R₂ - OH, R₃ - H, R₄ - COO-β-D-Glc⁴-β-D-Xyl
- 11: R₁ - H, R₂ - OH, R₃ - H, R₄ - COO-β-D-Glc²-β-D-Glc
- 12: R₁ - OCH₃, R₂ - OH, R₃ - H, R₄ - COO-β-D-Glc²-β-D-Glc
- 13: R₁ - OCH₃, R₂ - OH, R₃ - H, R₄ - COO-β-D-Glc
- 14: R₁ - H, R₂ - O-β-D-Glc, R₃ - H, R₄ - COOCH₂CH₃
- 15: R₁ - OCH₃, R₂ - O-β-D-Glc, R₃ - H, R₄ - COOCH₂CH₃
- 16: R₁ - H, R₂ - OH, R₃ - H, R₄ - COOCH₂CH₃
- 17: R₁ - OCH₃, R₂ - OH, R₃ - H, R₄ - COOCH₂CH₃
- 18: R₁ - OCH₃, R₂ - OH, R₃ - OCH₃, R₄ - COOH
- 19: R₁ - OCH₃, R₂ - OH, R₃ - OCH₃, R₄ - CH₂OH
- 20: R₁ - H, R₂ - H, R₃ - H, R₄ - COOH
- 21: R₁ - H, R₂ - OH, R₃ - H, R₄ - COOH
- 22: R₁ - OCH₃, R₂ - OH, R₃ - H, R₄ - COOH
- 23: R₁ - OCH₃, R₂ - OH, R₃ - H, R₄ - CH₂OH

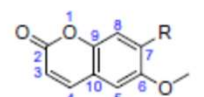
Glc: glucopyranosyl
Xyl: xylopyranosyl
Rha: rhamnopyranosyl



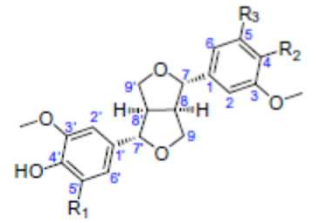
- 24: R - OCH₃
- 25: R - H



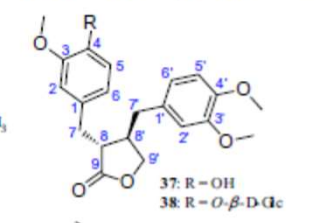
- 26: R₁ - H, R₂ - COOH
- 27: R₁ - OCH₃, R₂ - COOH
- 28: R₁ - OCH₃, R₂ - COOCH₂CH₃



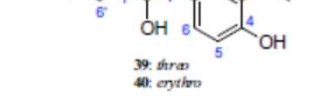
- 29: R - O-β-D-Glc⁴-β-D-Xyl²-β-D-Glc
- 30: R - O-β-D-Glc⁴-β-D-Xyl
- 31: R - O-β-D-Glc
- 32: R - OH



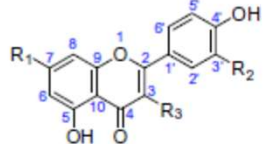
- 33: R₁ - H, R₂ - OH, R₃ - H
- 34: R₁ - H, R₂ - OH, R₃ - OCH₃
- 35: R₁ - OCH₃, R₂ - OH, R₃ - OCH₃
- 36: R₁ - OCH₃, R₂ - O-β-D-Glc, R₃ - OCH₃



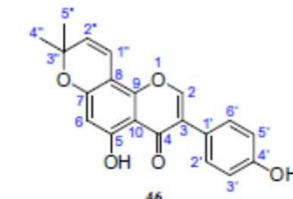
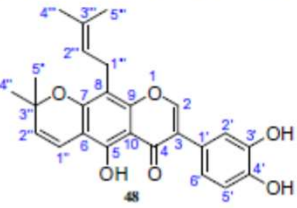
- 37: R - OH
- 38: R - O-β-D-Glc



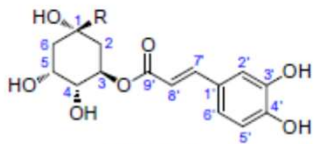
- 39: *thrao*
- 40: *erythro*



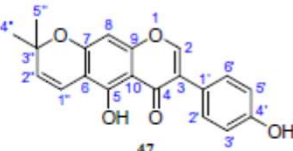
- 41: R₁ - OH, R₂ - OH, R₃ - OH
- 42: R₁ - OH, R₂ - OH, R₃ - O-α-L-Rha
- 43: R₁ - OH, R₂ - H, R₃ - O-β-D-Glc²-α-L-Rha
- 44: R₁ - OH, R₂ - OH, R₃ - O-β-D-Glc²-α-L-Rha
- 45: R₁ - O-β-D-Glc, R₂ - OH, R₃ - O-β-D-Glc²-α-L-Rha



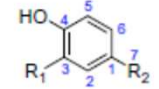
46



- 49: R - COOH
- 50: R - COOCH₃



47



- 51: R₁ - H, R₂ - CHO
- 52: R₁ - H, R₂ - COOH
- 53: R₁ - OCH₃, R₂ - COOH

- 52 compounds (phenolic acids + flavonoids) isolated and 15 identified using HPLC-DAD-ESI-MS (Inbaraj *et al.* 2010)

- 53 polyphenols identified using spectroscopic and chemical analyses and comparison with NMR data (Zhou *et al.* 2017)

> Genetic and developmental controls remain poorly characterized as well as the impact of drought

Inbaraj et al. 2010, *J. Pharm. Biomed.*
Zhang et al. 2016, *Food Chem.*
Zhou et al. 2017, *Food Chem.*

> 1. Context

> **2. Effect of genetic and developmental factors**

> 3. Impact of drought

> 2. Intraspecific comparisons: metabolite contents of ripe fruits



'FPW07'



'FPW08'



'FPW10'



'1708/3'

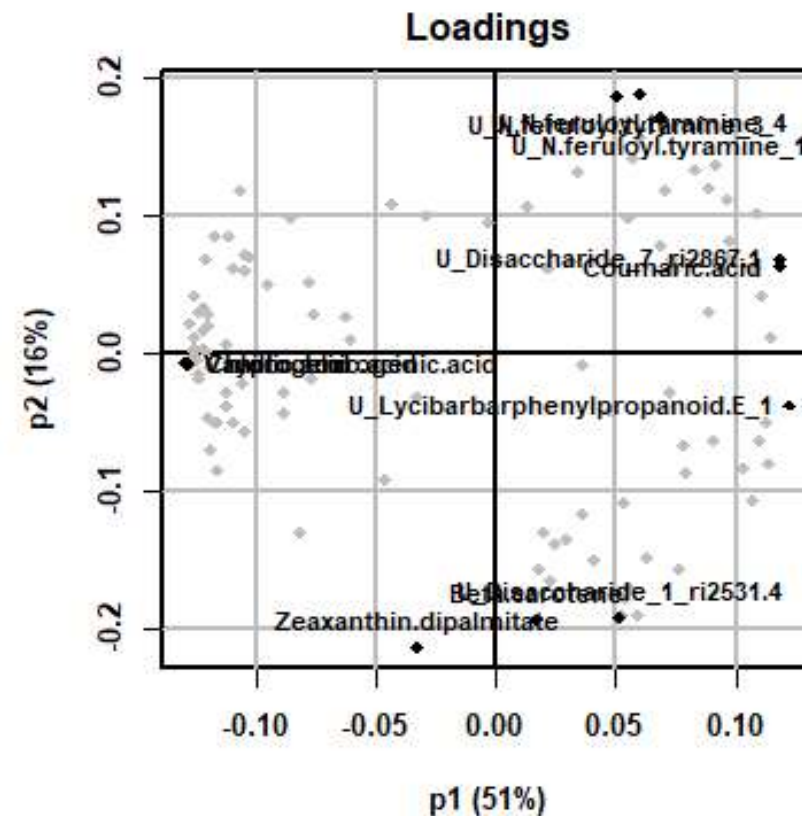
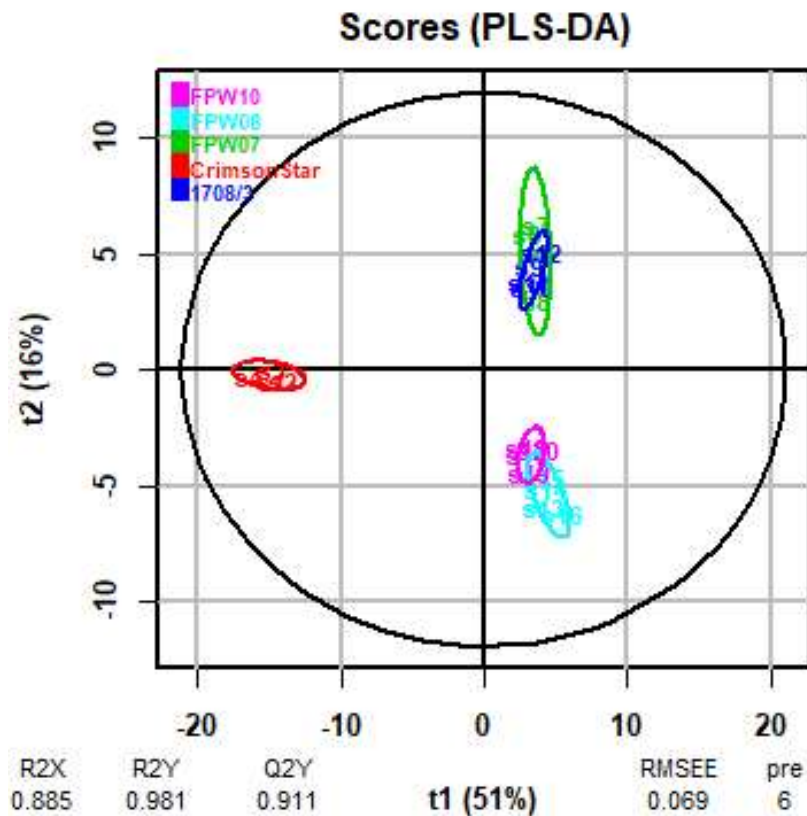


'Crimson star'

- 5 commercial cultivars (*L. barbarum*) grown in the same field in the south of France
- At maturity: 4 samples of at least 10 berries collected from 4 plants
- multi-targeted metabolite analysis:
 - metabolites I > GC-EI-TOF
 - carotenoids } > UPLC-DAD-ESI-TQ
 - polyphenols }

> 2. Metabolite contents of ripe fruits: determination of biomarkers

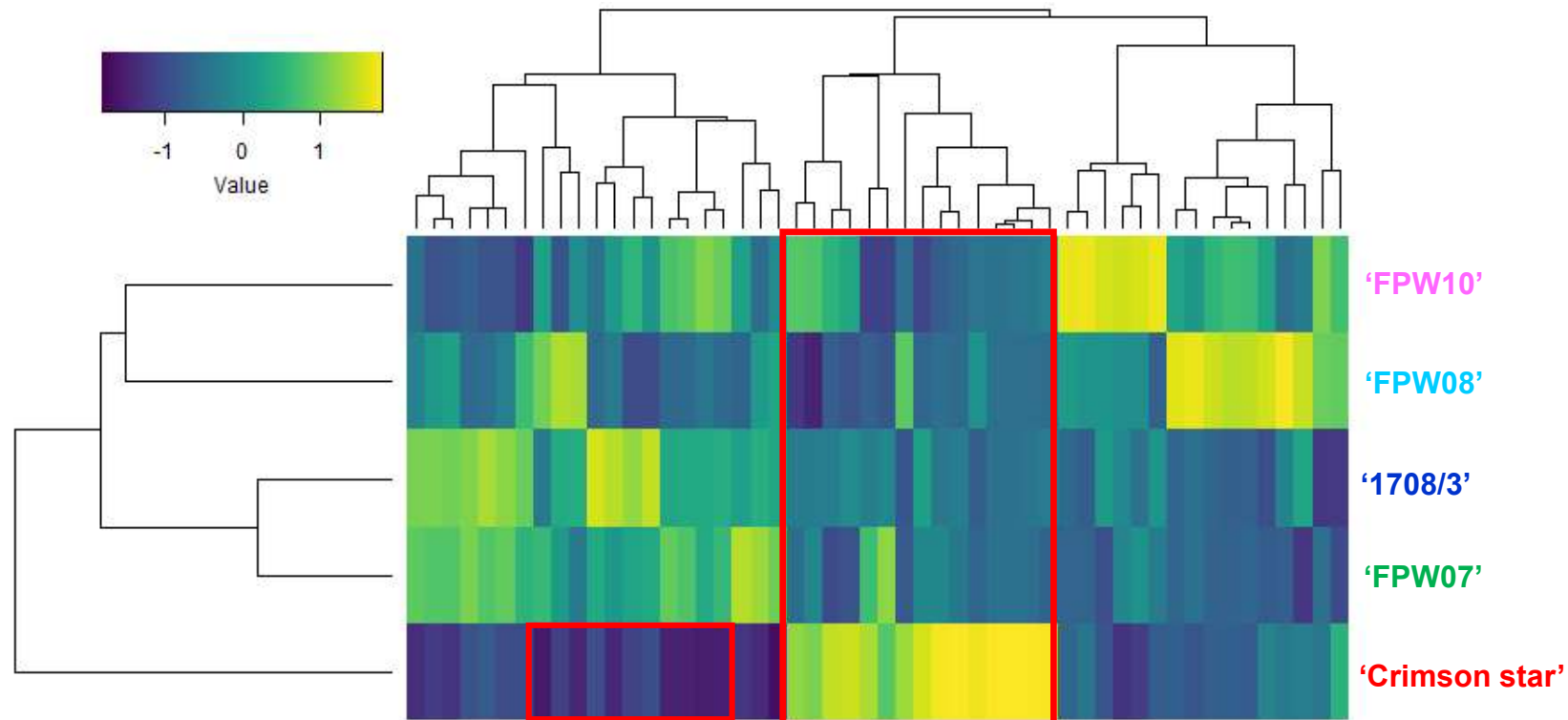
- Comparison of the 5 cultivars on the basis of 117 metabolites (41 polyphenols) -> supervised multivariate analysis (PLS-DA) to find discriminant features/metabolites



‘Crimson star’ differs from the 4 other cultivars

> 2. Metabolite contents of ripe fruits: determination of biomarkers

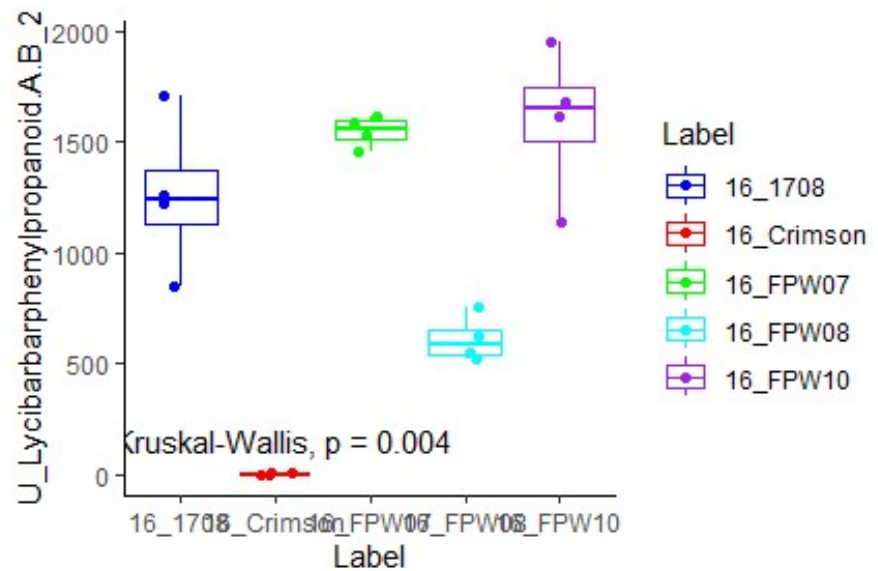
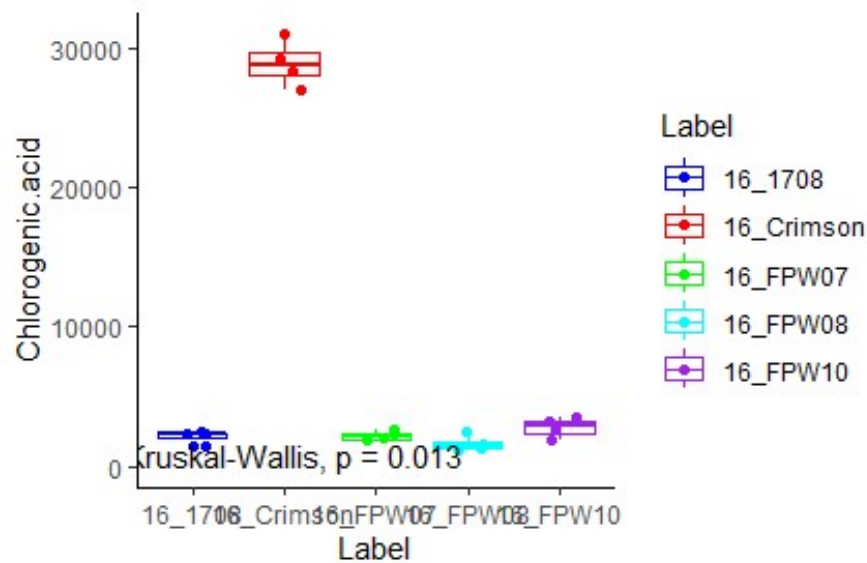
- 53 metabolites with VIP > 1



Asparagine, phenylalanine, quinic acid, glutamic acid, malic acid and chlorogenic acid, cryptochlorogenic acid, and 3,5-dicaffeoylquinic acid, biomarkers of 'Crimson star' and very low amounts of some lycibarbarphenylpropanoids (*L. chinense*)

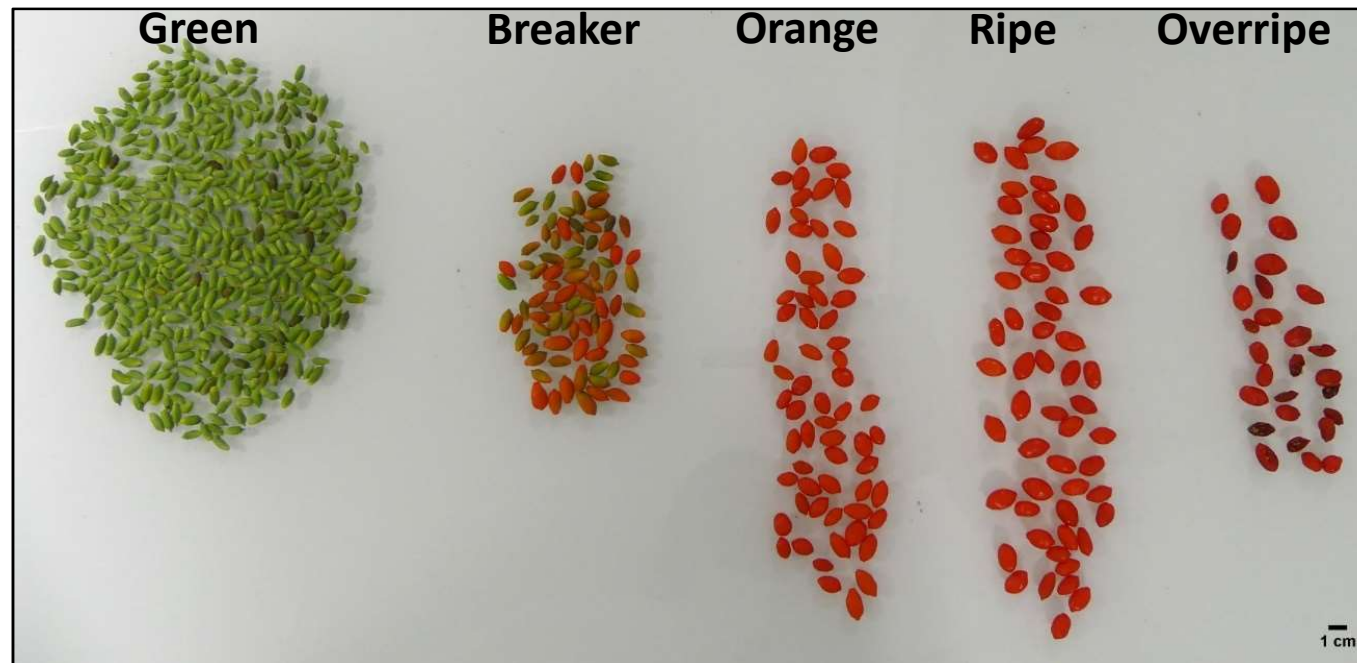
> 2. Metabolite contents of ripe fruits: determination of biomarkers

- Examples: Changes in chlorogenic acid and lycibabarphenylpropanoid A B_2



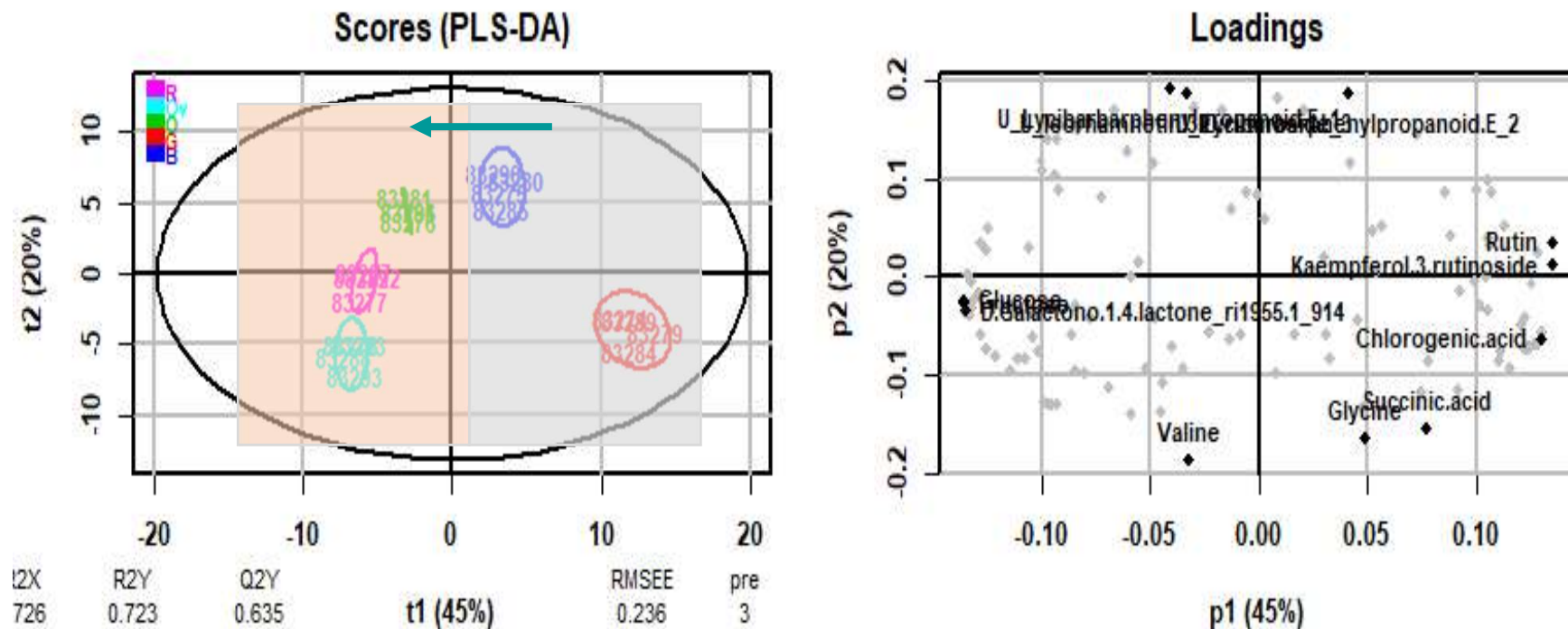
> 2. Variations in metabolite contents during fruit development and ripening

- Analysis of 5 developmental stages



> 2. Variations in metabolite contents during fruit development and ripening

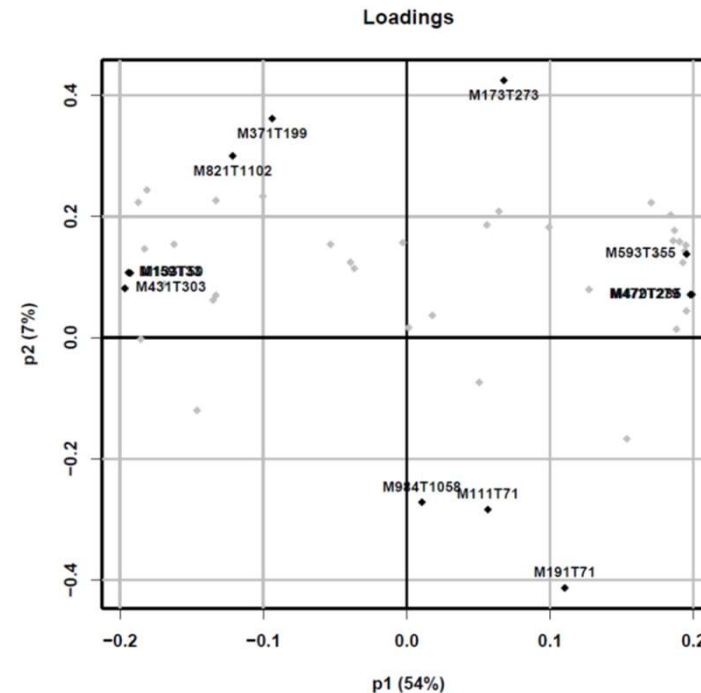
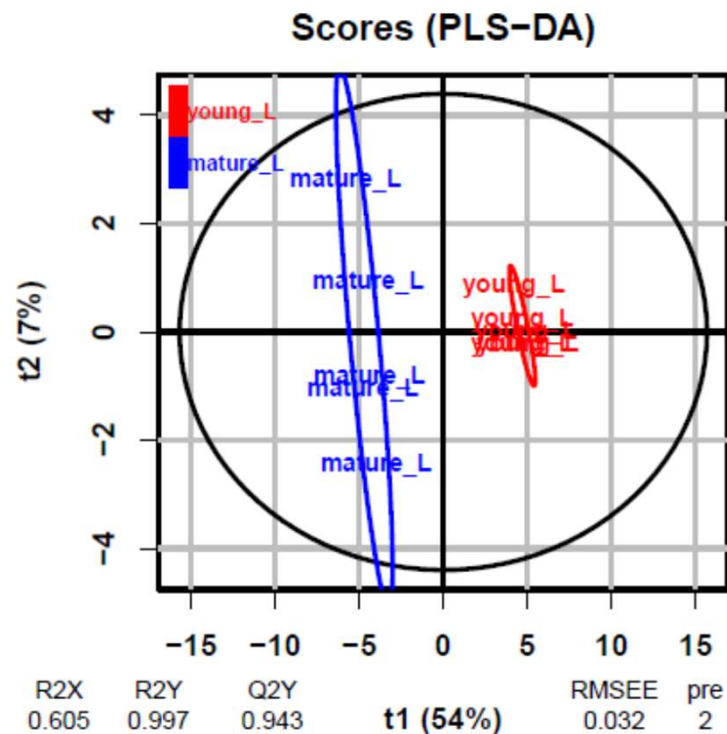
- Determination of discriminant metabolites between the 5 stages



- From green to ripe and overripe: ↗ soluble sugars and carotenoids
- The highest contents in polyphenols were found at green and breaker stages (+ 79% when compared to overripe)

> 2. Comparison of young and mature leaves

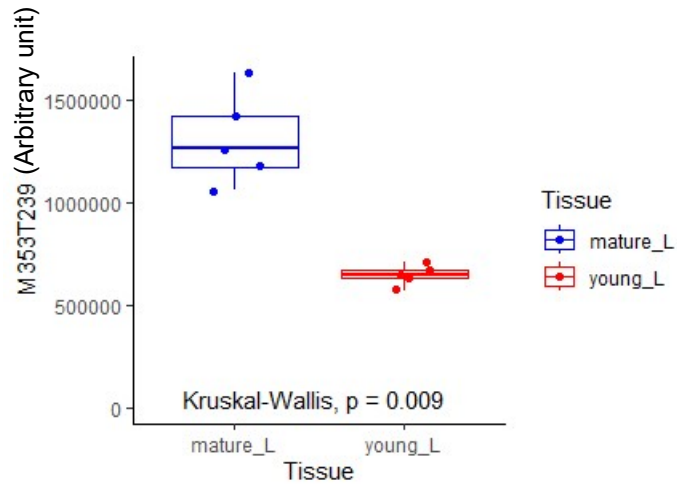
- Analysis of metabolites from young and mature leaves with an untargeted approach -> UPLC-MS/TOF and data processing, statistical analysis, and annotation with W4M



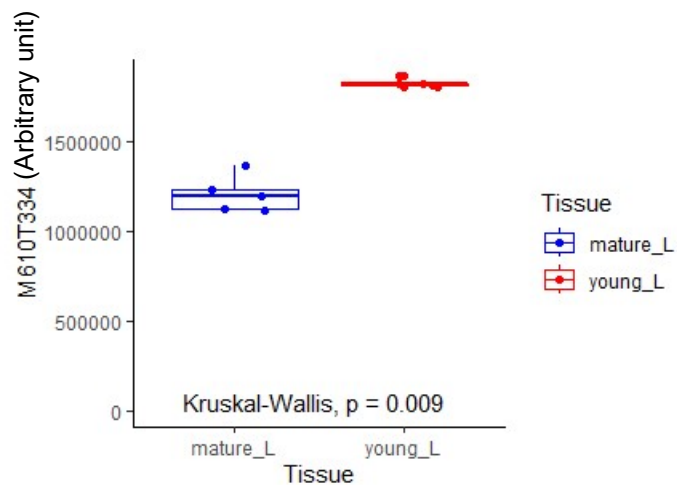
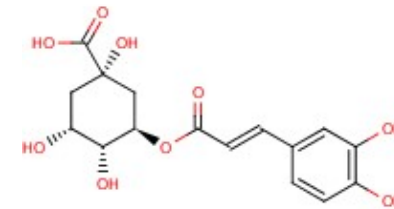
10 features with VIP>1 associated with mature leaves and 15 with young leaves

➤ 2. Comparison of young and mature leaves

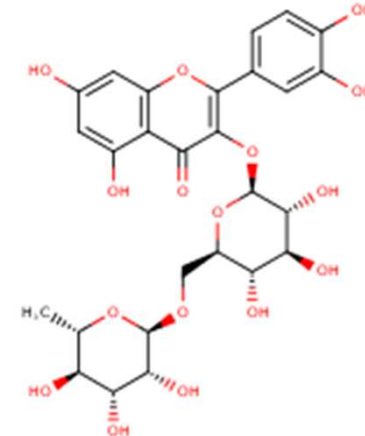
- Examples: variations of 2 features in young and mature leaves



- Monoisotopic mass: 354.095
- Formula: C₁₆H₁₈O₉
- Chlorogenic acid

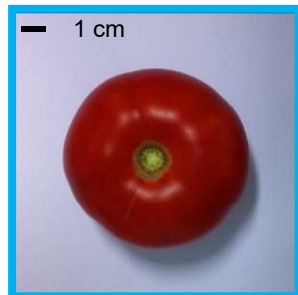


- Monoisotopic mass: 610.153
- Formula: C₂₇H₃₀O₁₆
- Rutin



- > 1. Context
- > 2. Effect of genetic and developmental factors
- > 3. **Impact of drought**

> 2. Goji (*L. barbarum*) response to drought



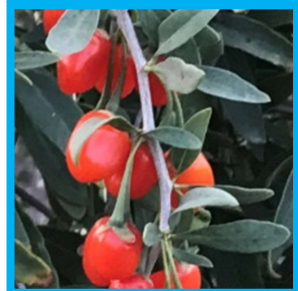
'Levovil'

Tomato

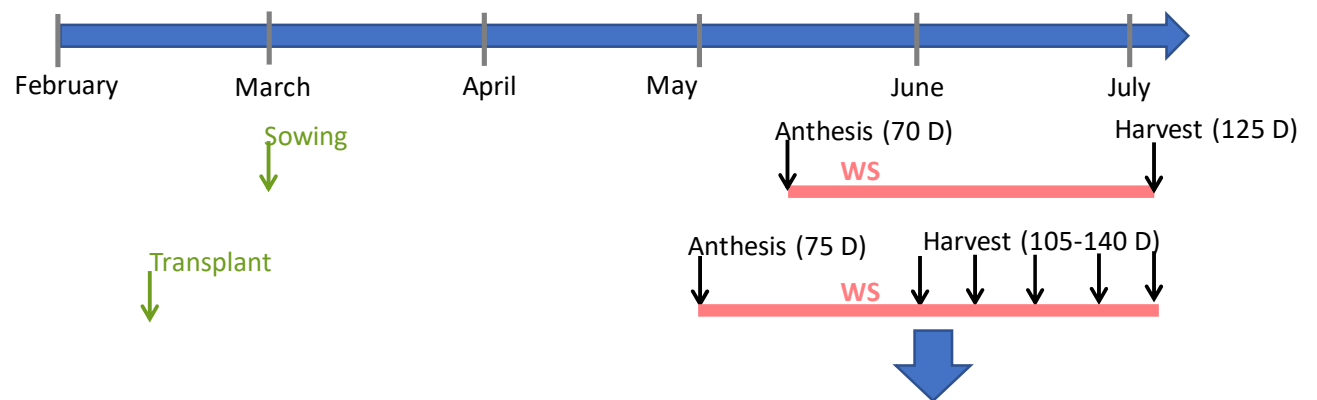


'FPW07'

Goji

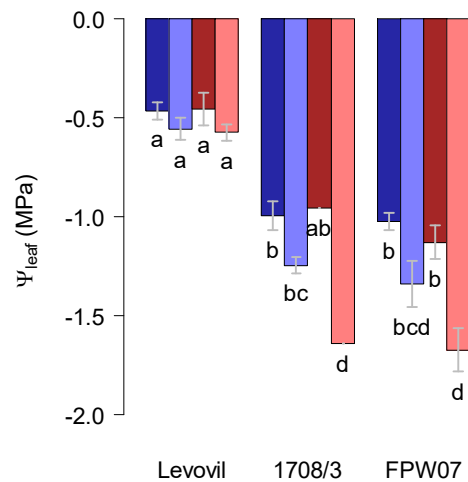


'1708/ 3'

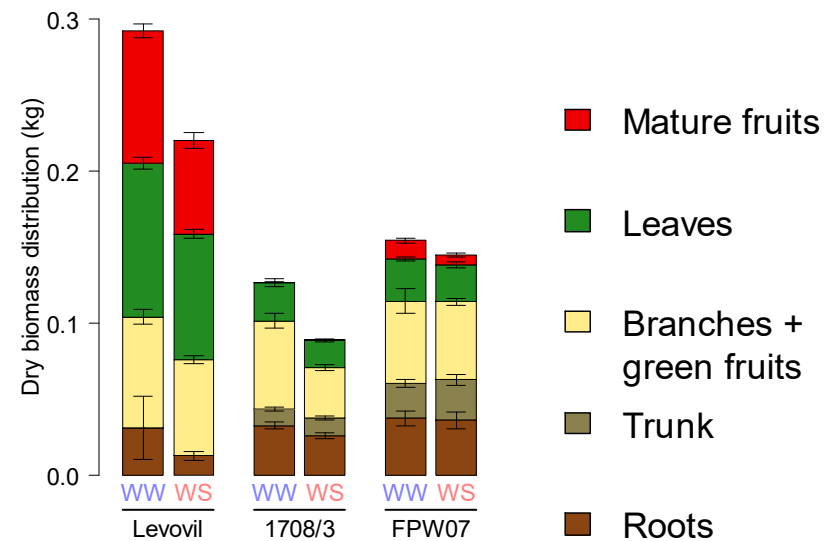


- Plant functioning
- Biomass production
- Leaf and Fruit metabolisms

> 2. WS affected water status, yield and carbon allocation



∟ midday leaf water potential of Goji plants under WS. Goji plants showed an anisohydric behavior



Tomato and Goji plants decreased carbon allocation to fruits

> Polyphenols of goji: take home messages

- > Polyphenols (hydroxycinnamic acids and lycibarbarphenylpropanoids) are markers of inter and intra species differences
- > The highest contents in polyphenols were found in early stages of berry development. The decrease in polyphenols is concomitant with the accumulation of soluble sugars
- > A water stress during the reproductive period negatively impact yield (-50%) and polyphenol contents (-14%) probably through an effect on fruit maturity

> Acknowledgments



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- Laurence Hibrand Saint Oyant
- Annie Chastellier

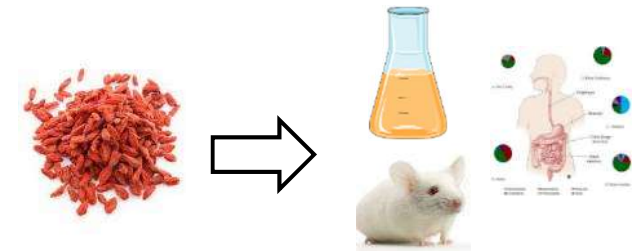
Gojinov project



SYALSA
Gojiquai project



- Nadia Bertin
- Jean-François Landrier
- Thomas Breniere
- Carine Le-Bourvellec
- Claire Cherbuy



> **Thank you for your attention**

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Polyphenols in red flesh
apples

