HIGH-THROUGHPUT PHENOTYPING OF WATER FUNCTIONING AND CARBON METABOLISM IN GRAPEVINE

Laurine Chir, Romain Boulord, Virgilio Freitas, Gaëlle Rolland, Miguel Thomas, Martin Ecarnot, Charlotte Brault, Angélique Christophe, Benoit Pallas, Thierry Simonneau, Vincent Segura*, Aude Coupel-Ledru*

Presenting author: Charlotte Brault ; email: aude.coupel-ledru@inrae.fr ; vincent.segura@inrae.fr

TRANSPIRATION AND PHOTOSYNTHESIS: KEY TRAITS IN THE FACE OF CLIMATE CHANGE

Grapevine production and wine quality are affected by **climate change**[1]. Searching for genotypes that are **more water-efficient** and able to maintain **high photosynthesis** is a key leverage to maintain production and vineyards durability under drought condition[2]. **Phenotyping hundreds of genotypes** in the vineyard is a key requirement to evaluate of the **genetic variability** for these traits.

However, conventional methods for measuring water and carbon related traits are typically expensive, destructive, and not usable at high-throughput.
GOAL: developing and testing new high-throughput phenotyping methods, based on the use of NIRS (Near InfraRed Spectroscopy)[3] and leaf chlorophyll fluorescence[4] to study the genetic variability and determinism of functional traits in grapevine.

PLANT MATERIAL AND TRAITS MEASUREMENTS

Two populations of *Vitis vinifera*
→ Diversity panel[6] → 279 genotypes
→ Semi-diallel cross[5] → 600 genotypes

Measurements

Classical physiological measurements + High-throughput measurements

Net assimilation, stomatal conductance *Licor 6800*

Ψ *Pressure chamber*

Water content, LMA *Weighing*

C, N, metabolites concentrations *Biochemical quantitative analysis*

Fluorescence, stomatal conductance *Licor 600*

NIRS spectra *MicroNIR, NeoSpectra, ASD*

Chlorophyll *SPAD*

CONCLUSIONS AND PERSPECTIVES

Analyses were conducted with measurements taken in 2021 on well-watered plants. The results obtained with NIRS show the **sturdiness** of the calibration phase, which is **promising** for future calibration and analyses. In addition, a calibration phase using fluorescence data is underway.

NIRS high-throughput measurements combined to prediction models reveal a **strong genotypic variability** for the traits related to water use and carbon metabolism. A significant **SNP on chromosome 16 has already been detected for water content and transpiration**. An exploration of the **candidate genes** underlying this association is being conducted.

The experiment is currently being repeated, with a **water deficit** scenario in addition to well-watered conditions. The measurements will be added to the models **to improve** them and assess the genotypic variability for responses to water deficit.

The results are encouraging, especially concerning the **deployment of high-throughput phenotyping in vineyards**.

ANALYSIS OF WATER CONTENT, TRANSPIRATION, LEAF MASS PER AREA, STARCH AND NITROGEN
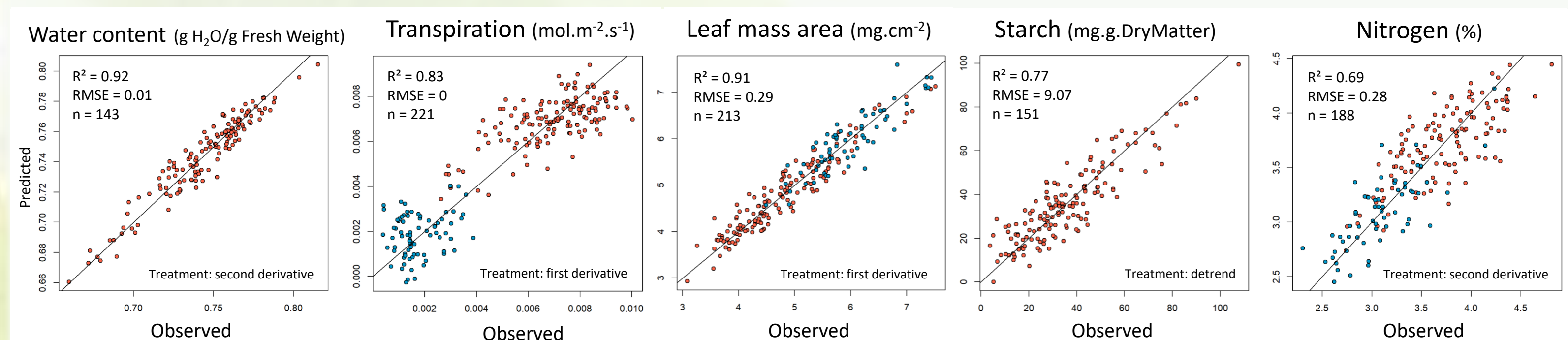
1. SPECTRA PROCESSING

With a focus on **MicroNIR spectra**, several treatments are applied:

Raw spectra → Standard Normal Variate / Detrend / First Derivative / Second Derivative
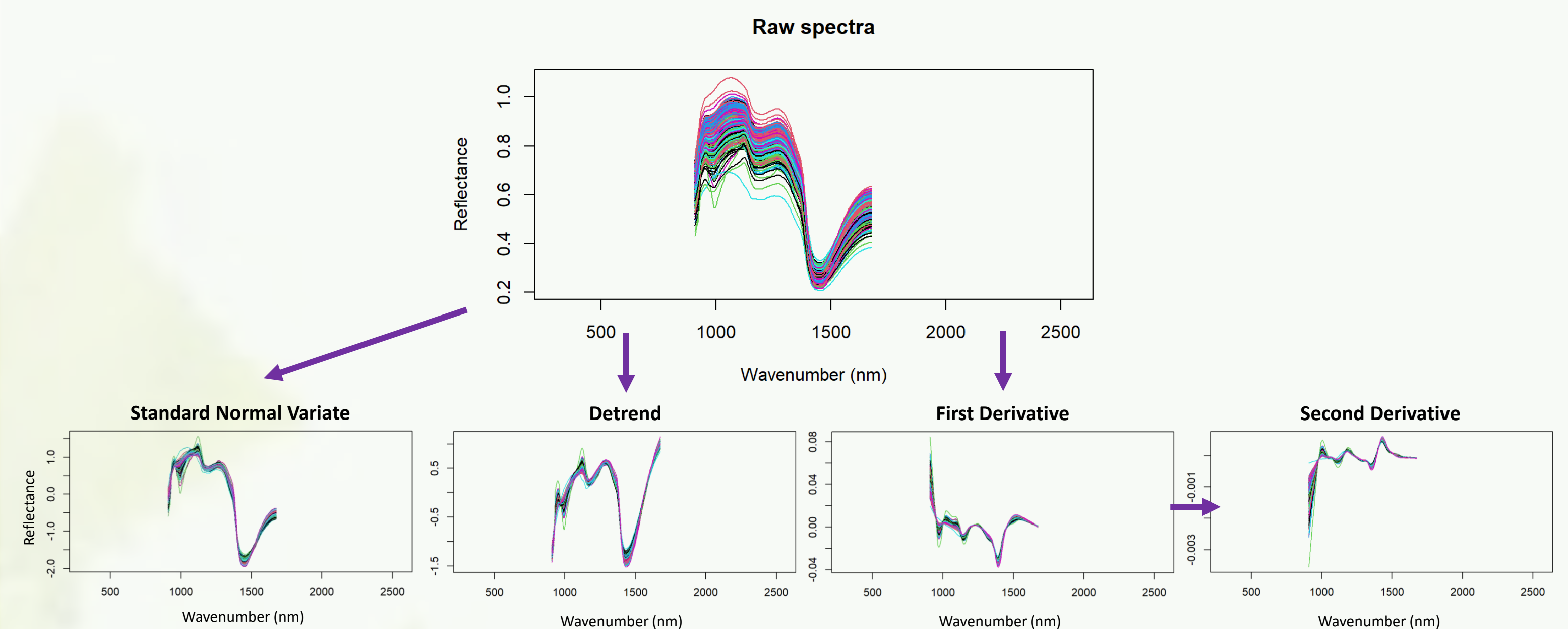
2. CALIBRATION PHASE

In this phase conducted on a subset of genotypes from the two populations (semi-diallel cross and diversity panel), we combined conventional low-throughput measurements of photosynthesis, stomatal conductance, nitrogen and carbohydrates content, with fast leaf measurements using porometry/fluorimetry and NIRS. We use **PLS (Partial Least Squares) regressions** to build statistical models for the prediction of the traits measured at low-throughput from the NIRS spectra measured at high throughput.

Water content (g H$_2$O/g Fresh Weight) R² = 0.92, RMSE = 0.01, n = 143, Treatment: second derivative

Transpiration (mol.m^{-2}.s^{-1}) R² = 0.83, RMSE = 0, n = 221, Treatment: first derivative

Leaf mass area (mg.cm^{-2}) R² = 0.91, RMSE = 0.29, n = 213, Treatment: first derivative

Starch (mg.g.DryMatter) R² = 0.77, RMSE = 9.07, n = 151, Treatment: detrend

**Nitrogen (%)** R² = 0.69, RMSE = 0.28, n = 188, Treatment: second derivative

3. TRAITS PREDICTION

In this phase, high-throughput methods alone are deployed on the whole populations to **predict the traits of interest** using models established in the calibration phase with three repetitions for each genotype.

4. HERITABILITY AND GWAS

Heritabilities and **genotypic values** (**BLUPs)** are calculated on the traits estimated from the high-throughput measurements. Genetic determinants of the traits are analysed with **Genome Wide Association Study** (GWAS), using MM4LMM method[7] and 90K SNPs. The results show that **the traits are heritable**, and that the **genotypic variability is high**. In addition, a **genetic region with genes associated with water content and transpiration was identified with GWAS**.
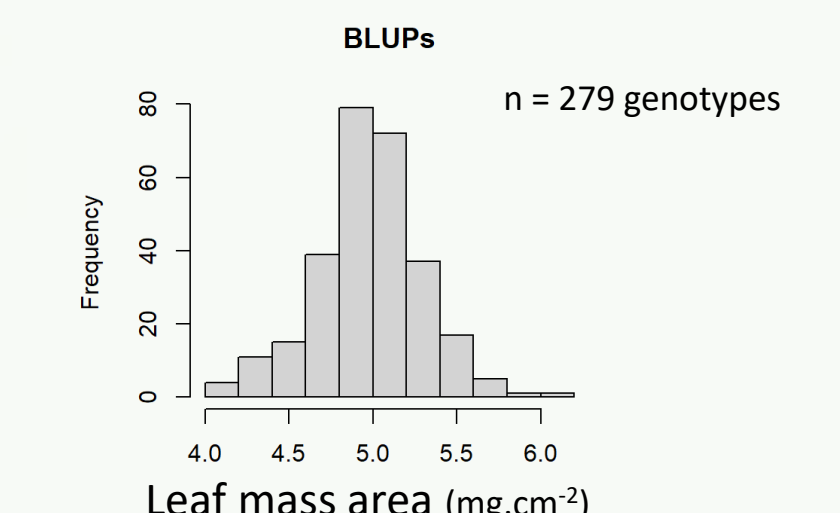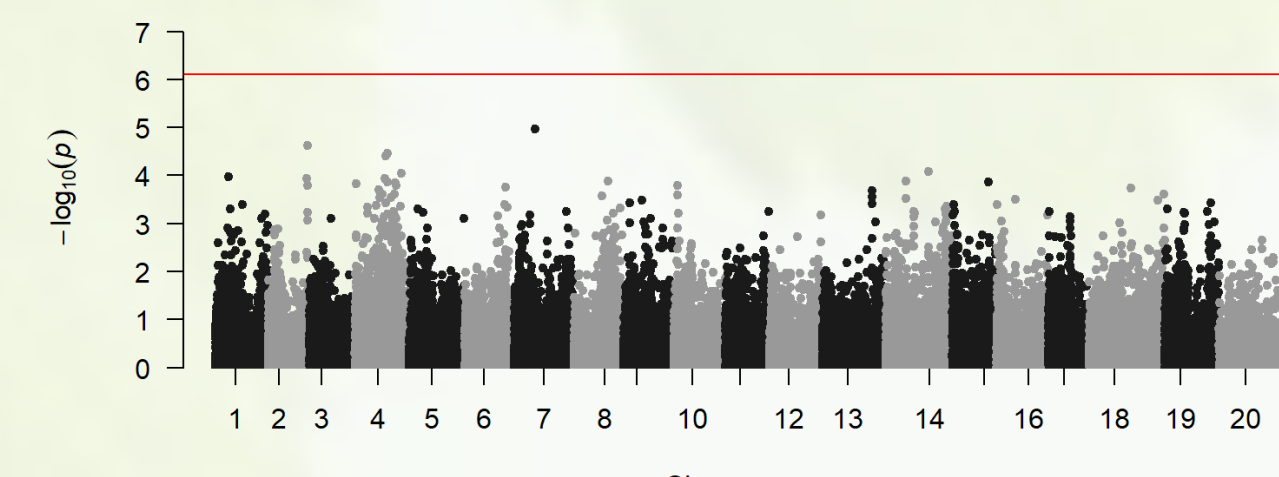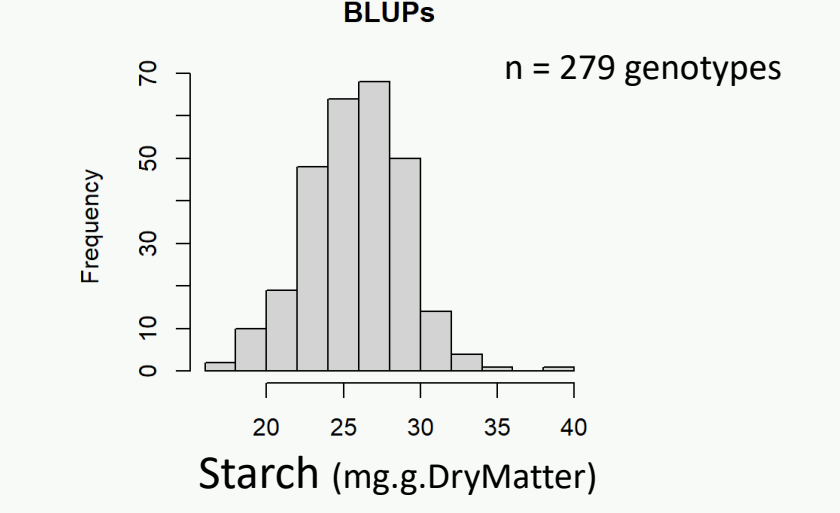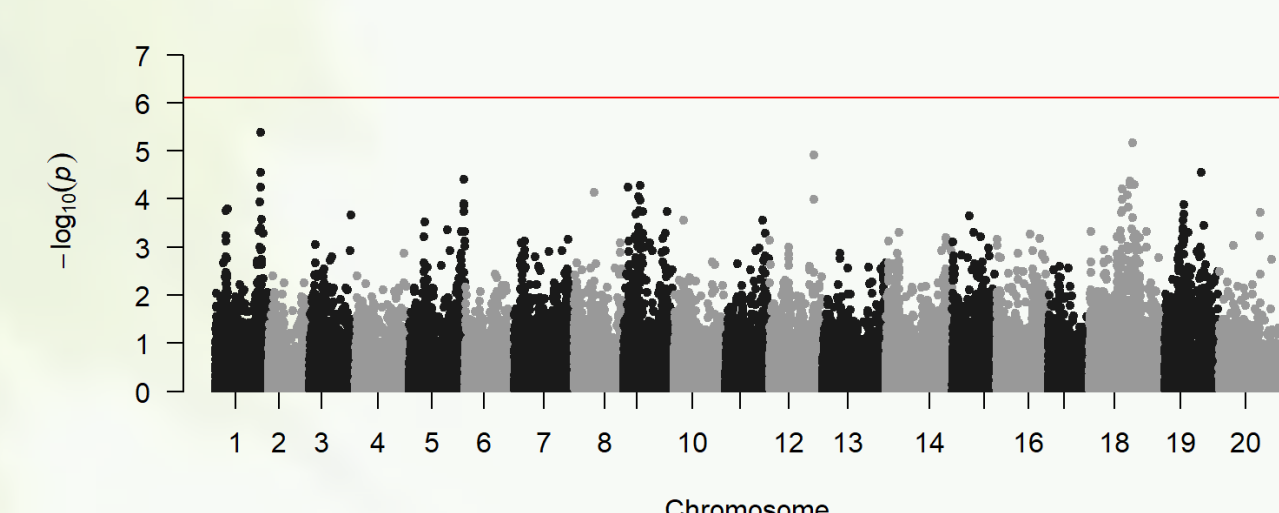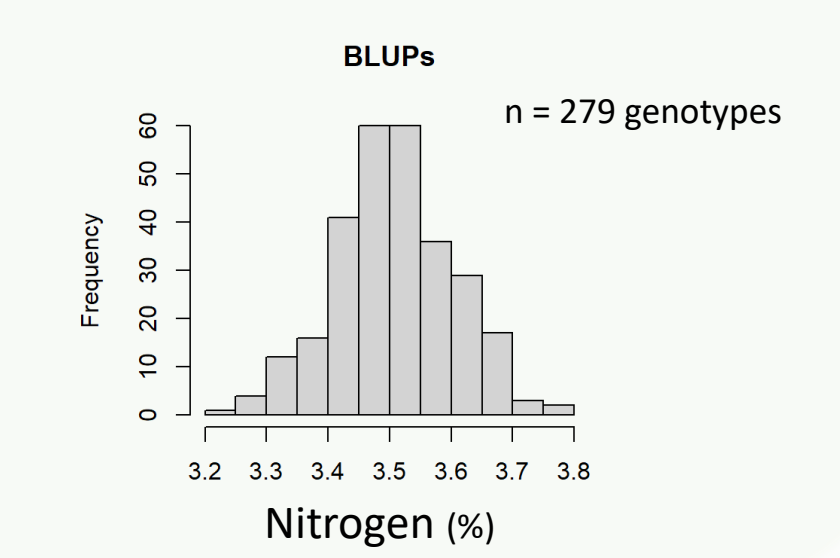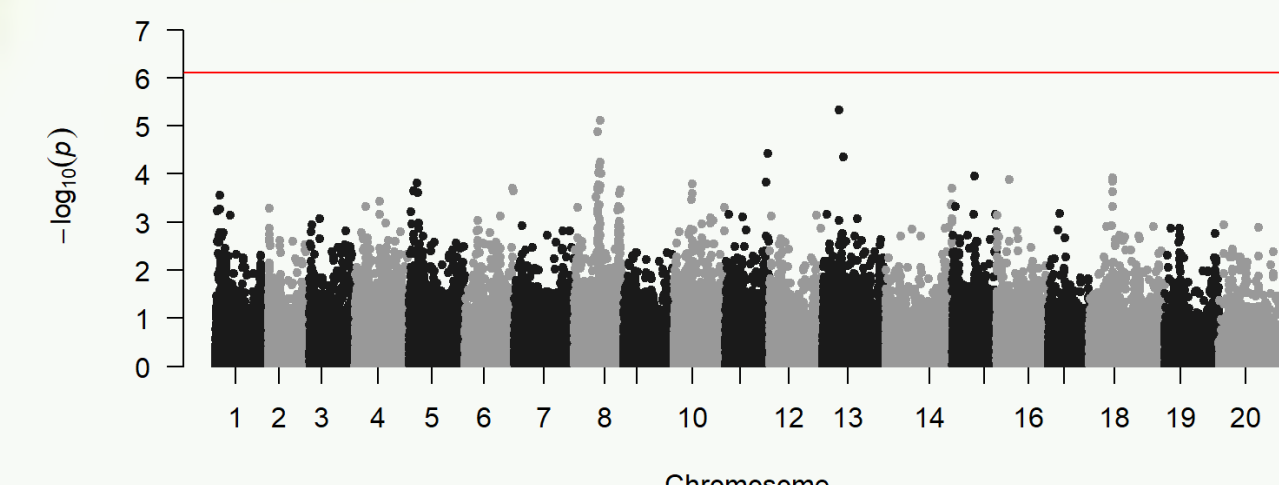
➢ Water content: H² = 0.50
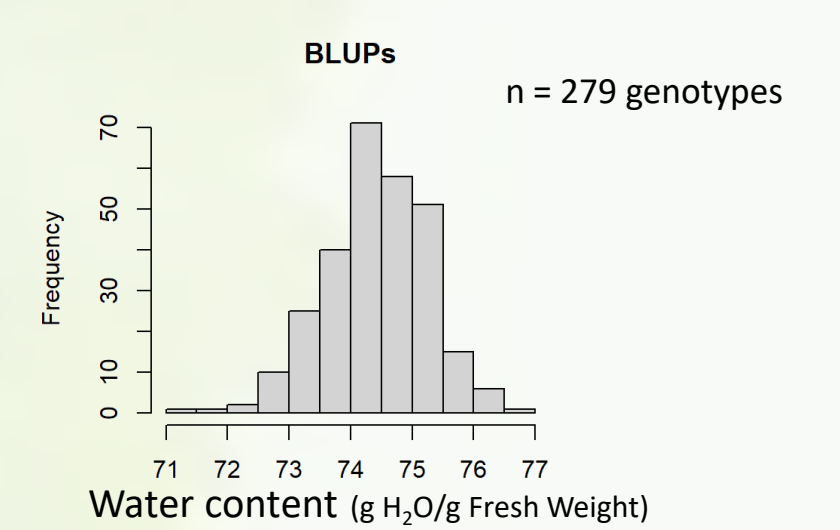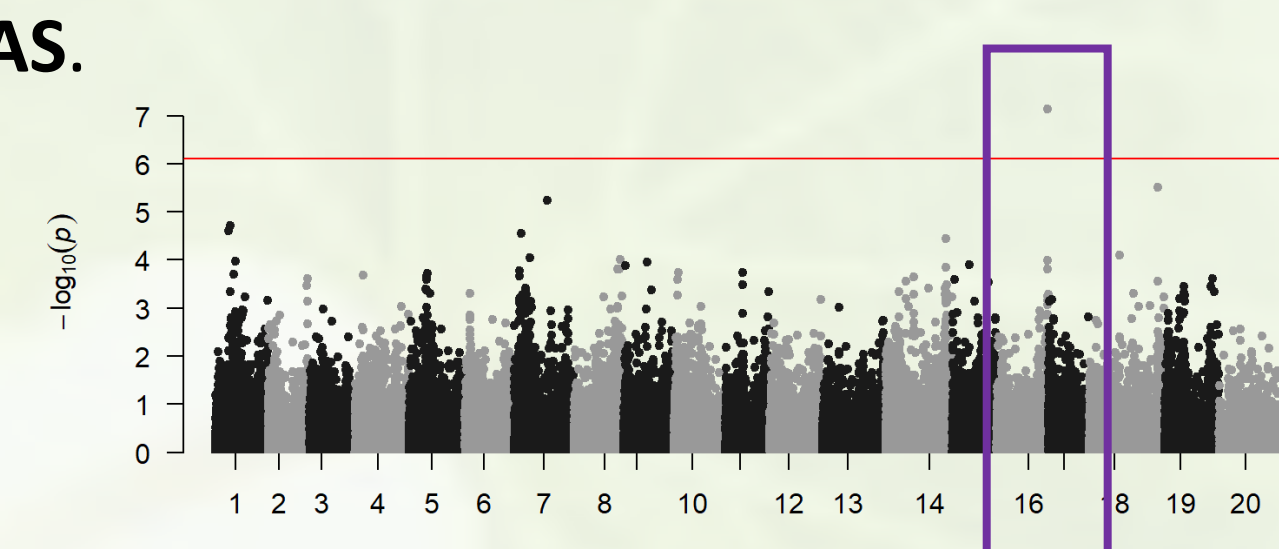
➢ Transpiration: H² = 0.47
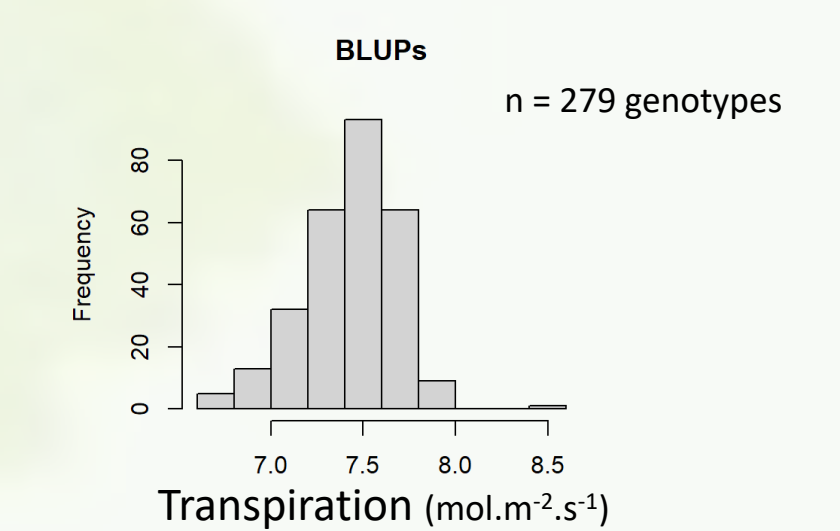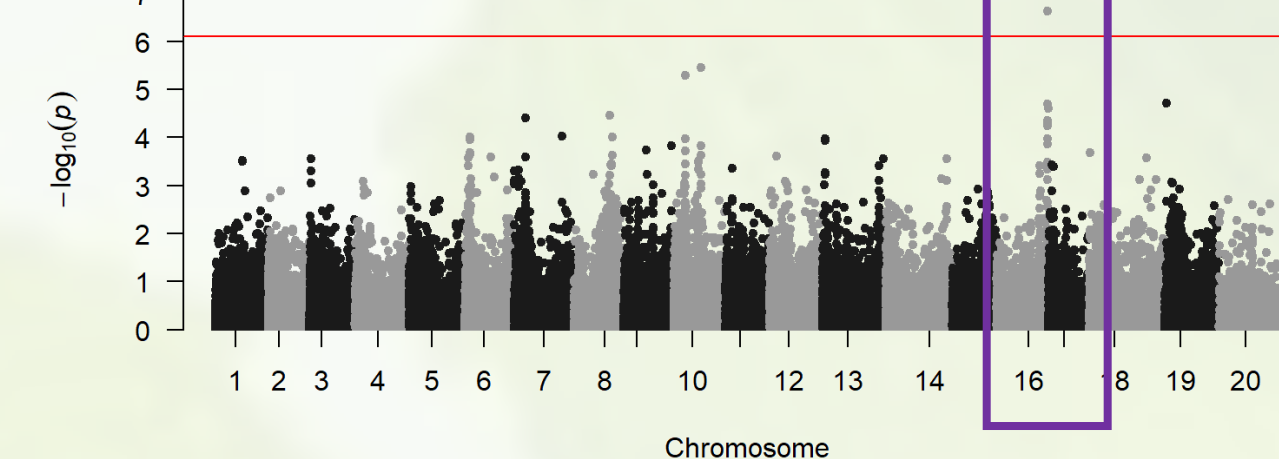
➢ Leaf mass per area: H² = 0.59

➢ Starch: H² = 0.36

➢ Nitrogen: H² = 0.46

BLUPs, n = 279 genotypes

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