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Metabolomic study of tolerance to water limitation for sunflower germination

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SUNRISE objectives: 2012 - 2020

- To improve oil production of sunflower in conditions adapted to climate change
- To understand genetic and molecular mechanisms controlling the development of the plant

- 16 public and private partners

Study context

- Climate change is one of the most serious challenges facing the world, and water limitation is a major abiotic stress which affects plant growth and farming yield
- World demand for oilseed production is increasing

- Necessity to improve tolerance to drought and yield of sunflower crops in such conditions
- For germination and further development stages

Materials and methods

- Genomic pre-selection of 50 genotypes
- Selection of 2 hybrids
 - 1 **tolerant** to water limitation
 - 1 **sensitive** to water limitation
- Based on 2 treatments during germination
 - 15h seed imbibition with **PEG 8000**, **water stress**
 - 15h seed imbibition with **water**, **control**



Analytical strategy

Metabolomics profiling:

Proton NMR
(polar extracts)



LC-LTQ-Orbitrap-HRMS
(semi-polar extracts)

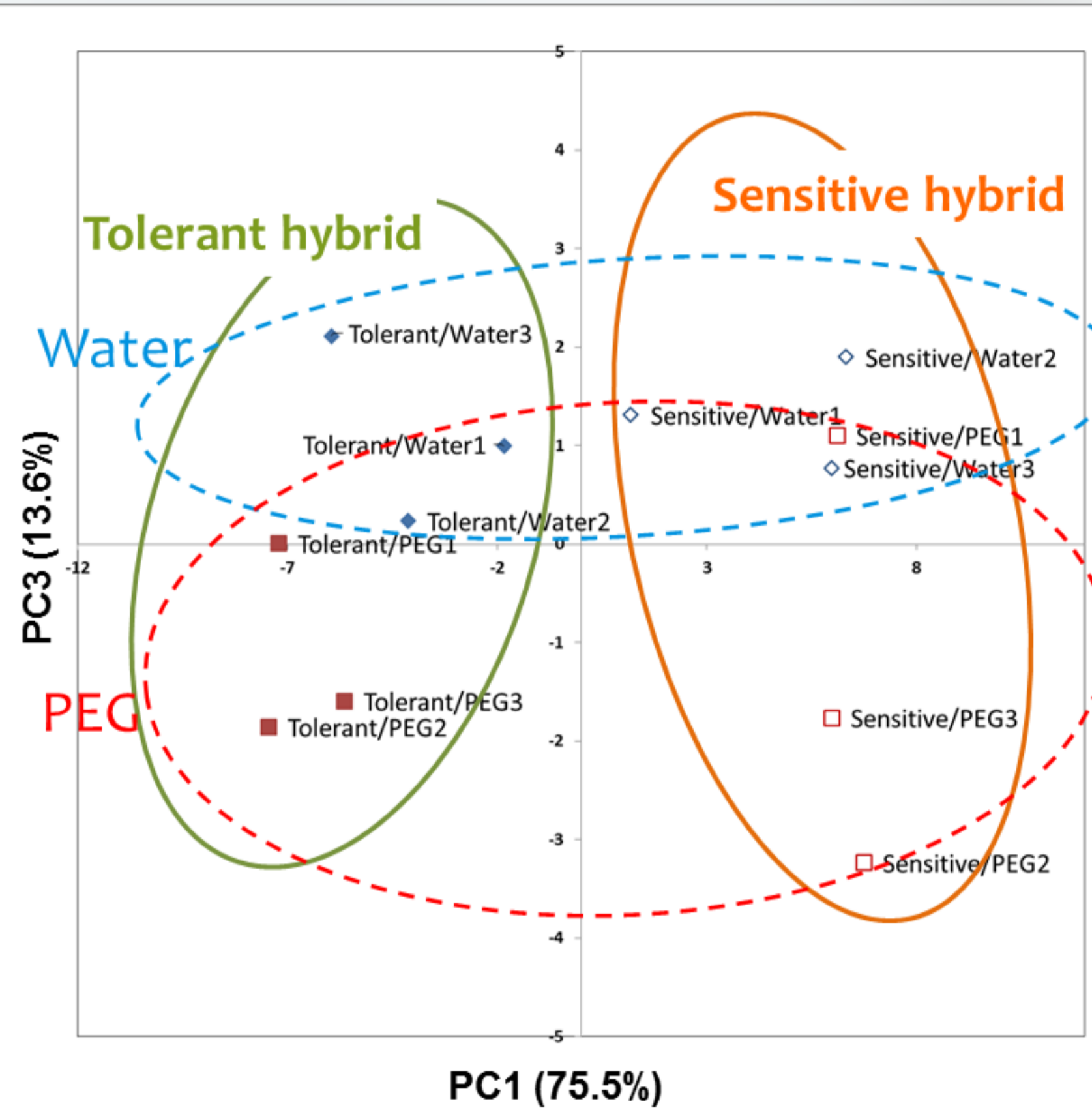


Data processing

- Spectra annotation (NMR and HRMS)
- Extraction of MS data (XCMS)
- Statistical analyses (PCA, ANOVA)
- Biomarker highlighting / identification

Results and discussion

Effect of genotype and treatment on seed composition



Statistical analyses on major compounds

PCA (47 major compounds detected in 1H-NMR and LC-HRMS)

- Good separation between the 2 hybrids
- Treatment effect seems to be observed

ANOVA (two-factor ; $p < 0.05$)

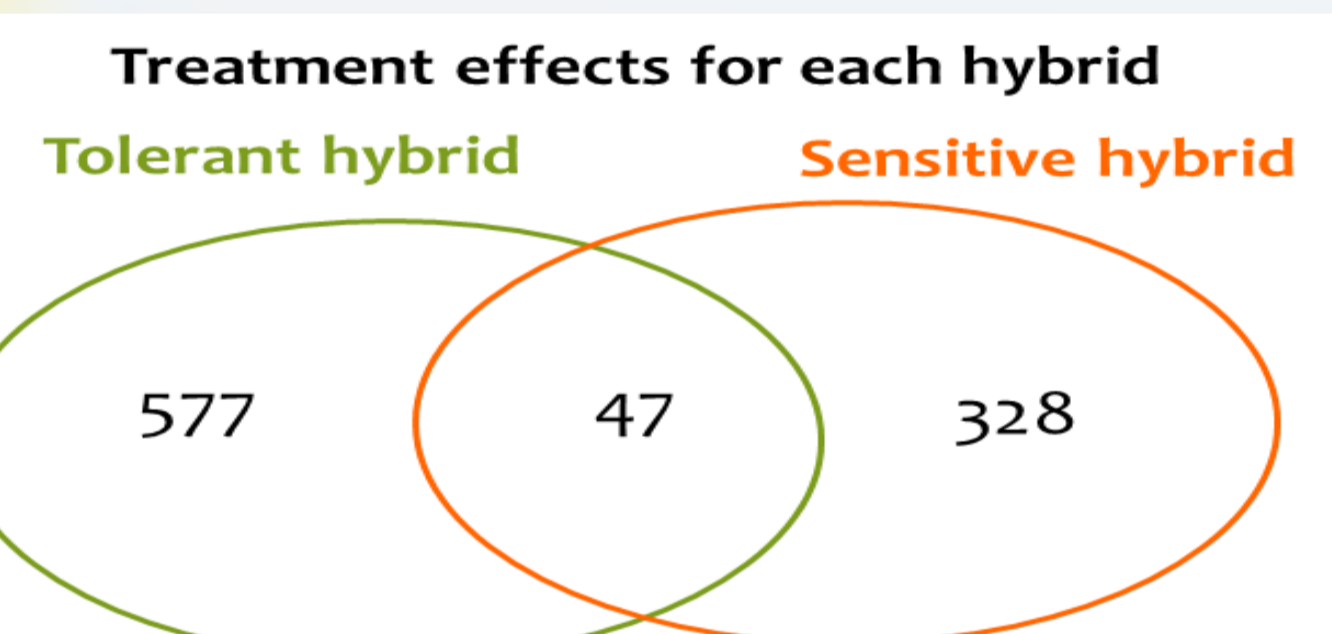
- 42 markers of hybrids among the 47 major compounds
- 0 marker of treatment
- The 2 hybrids differ largely

Study of PEG imbibition for each hybrid

Statistical analysis on entire MS-profiles (4908 variables)

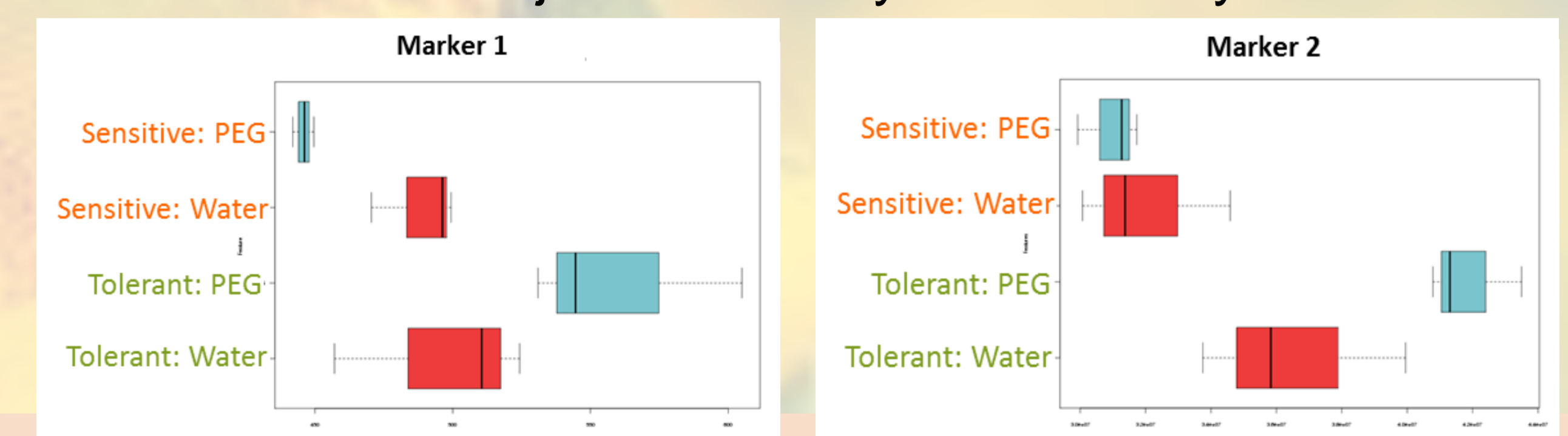
Student's t test per hybrid (4908 variables ; $p < 0.05$)

- More markers are highlighted for the tolerant hybrid



- Different response for the 2 hybrids:

- Opposite treatment response for several markers,
- Treatment effect just for one hybrid for many markers



LC-HRMS annotation		NMR annotation	
Family	Number of compounds	Family	Number of compounds
Hydroxy-cinnamic acid	8	Sugar	2
Flavonoid glycoside	1	Organic acid	4
Phenolic glycoside	2	Amino acid	8
Hydroxystyrene glycoside	2	Quaternary amine	3
Terpene lactone	1	Hydroxy-cinnamate	2
Fatty acid	1		
		72% of major compounds were putatively annotated	

Conclusion & Perspectives

- Larger difference between the hybrids than between the treatments
- Different treatment response for each hybrid
- Combination of metabolomics data with other omics data
- Validation of potential markers needed on a larger set of genotypes

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www.sunrise-project.fr