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# Multi-omics systems biology approach to reveal heterosis and drought response genomic processes in sunflower

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# Multi-omics systems biology approach to reveal heterosis and drought response genomic processes in sunflower

Harold Duruflé



# General context



# Why are we studying sunflower?

## Long-time model in ecology and evolution

Hybrid speciation accompanied by genomic reorganization in wild sunflowers

Loren H. Rieseberg, Chrystal Van Fossen & André M. Desrochers

Nature, 1995

Origin of extant domesticated sunflowers in eastern North America

Abigail V. Harter, Keith A. Gardner, Daniel Falush, David L. Lenz, Robert A. Bye & Loren H. Rieseberg

Nature, 2004

**ndhF sequence evolution and the major clades in the sunflower family**

K J Kim and R K Jansen

PNAS, 1995

Major Ecological Transitions in Wild Sunflowers Facilitated by Hybridization

Loren H. Rieseberg<sup>1\*</sup>, Olivier Raymond<sup>2</sup>, David M. Rosenthal<sup>3</sup>, Zhao Lai<sup>1</sup>, Kevin Livingstone<sup>1</sup>, Takuya Nakazato<sup>1</sup>, Jennifer L...

Science, 2003



Genomic islands of divergence are not affected by geography of speciation in sunflowers

S. Renaut, C. J. Grassa, S. Yeaman, B. T. Moyers, Z. Lai, N. C. Kane, J. E. Bowers, J. M. Burke & L. H. Rieseberg

Nature Communication, 2013

**Genetics of alternative splicing evolution during sunflower domestication**

Chris C. R. Smith, Silas Tittes, J. Paul Mendieta, Erin Collier-zans, Heather C. Rowe, Loren H. Rieseberg, and Nolan C. Kane

PNAS, 2018

The sunflower genome provides insights into oil metabolism, flowering and Asterid evolution

Hélène Badouin, Jérôme Gouzy [...] Nicolas B. Langlade

Nature, 2017

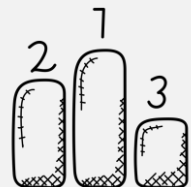
The genomics of linkage drag in inbred lines of sunflower

Kaichi Huang, Mojtaba Jahani, Jérôme Gouzy, and Loren H. Rieseberg

PNAS, 2023

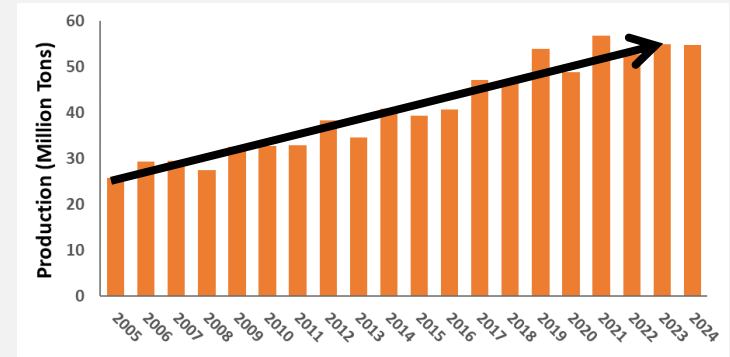
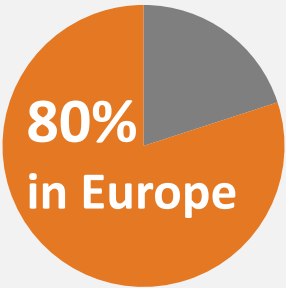


And ... the third largest oilseed crop in the world



# Sunflower, an important crop for Europe

**45** Million tons of seed produced worldwide



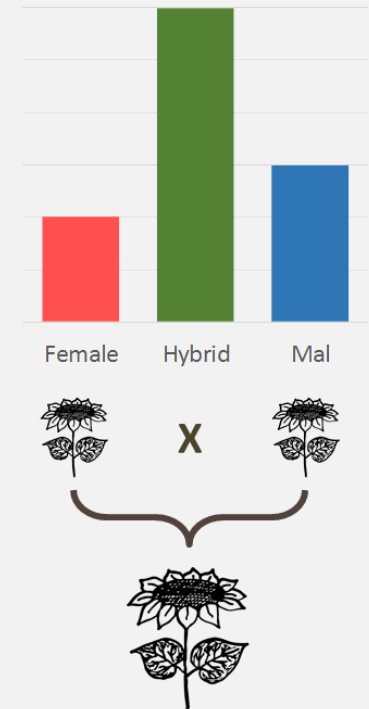
USDA, 2024

**Hybrid production : > 90 % of sunflower production**

Heterosis / Hybrid vigour: phenomenon who given progeny more vigorous than either of the two parents

Washburn et., al. 2017

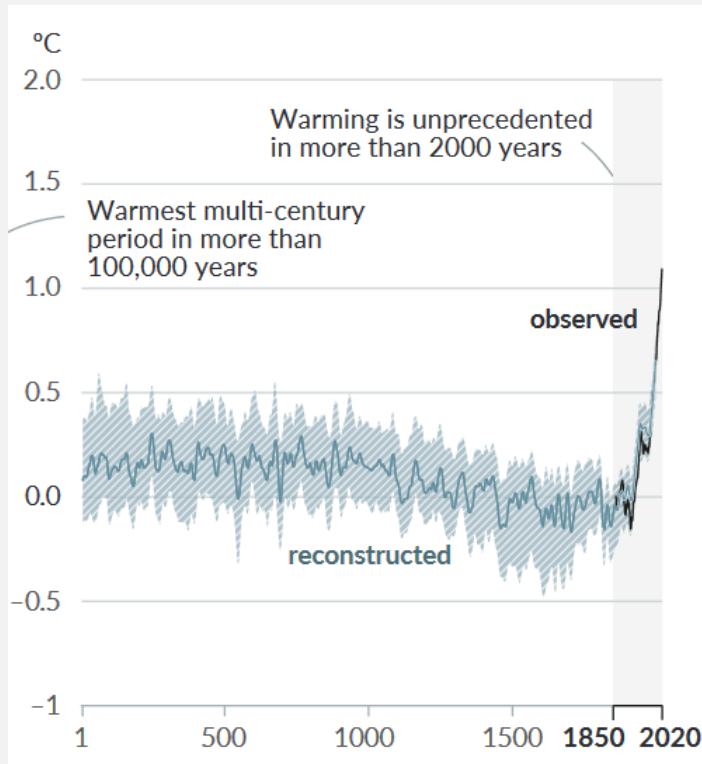
e.g. Higher yields and oil content, Disease resistance, Drought tolerance



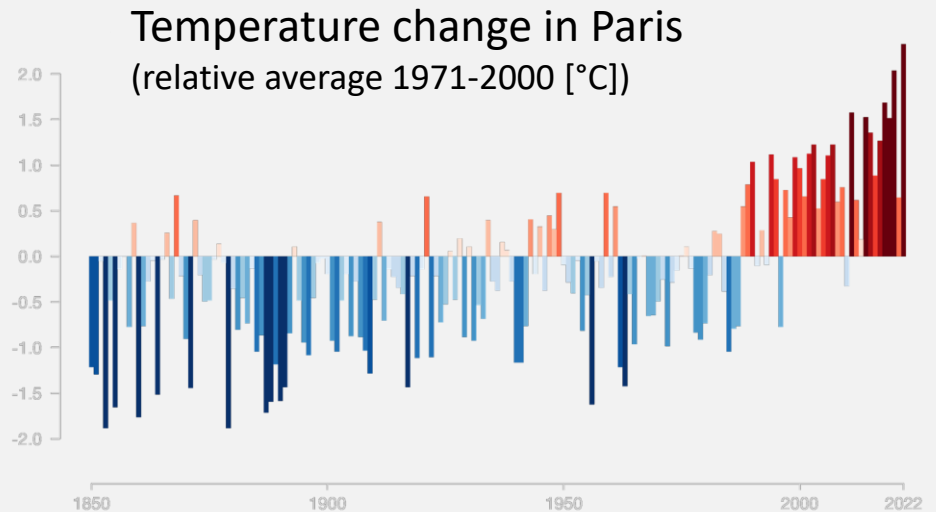


# Environmental context

## Strong impact expected by the climate change



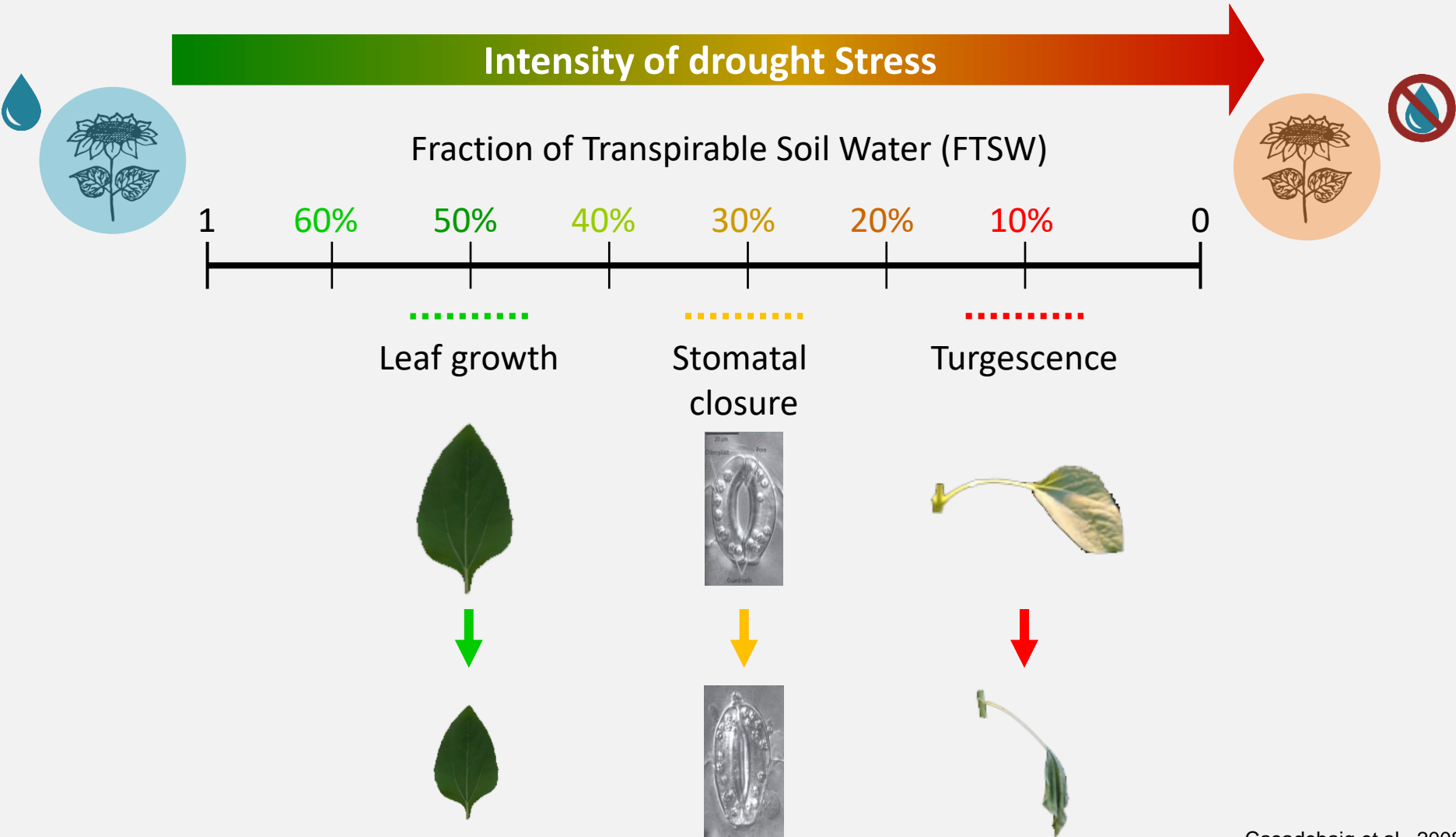
IPCC, 2021



Berkeley Earth, 2022

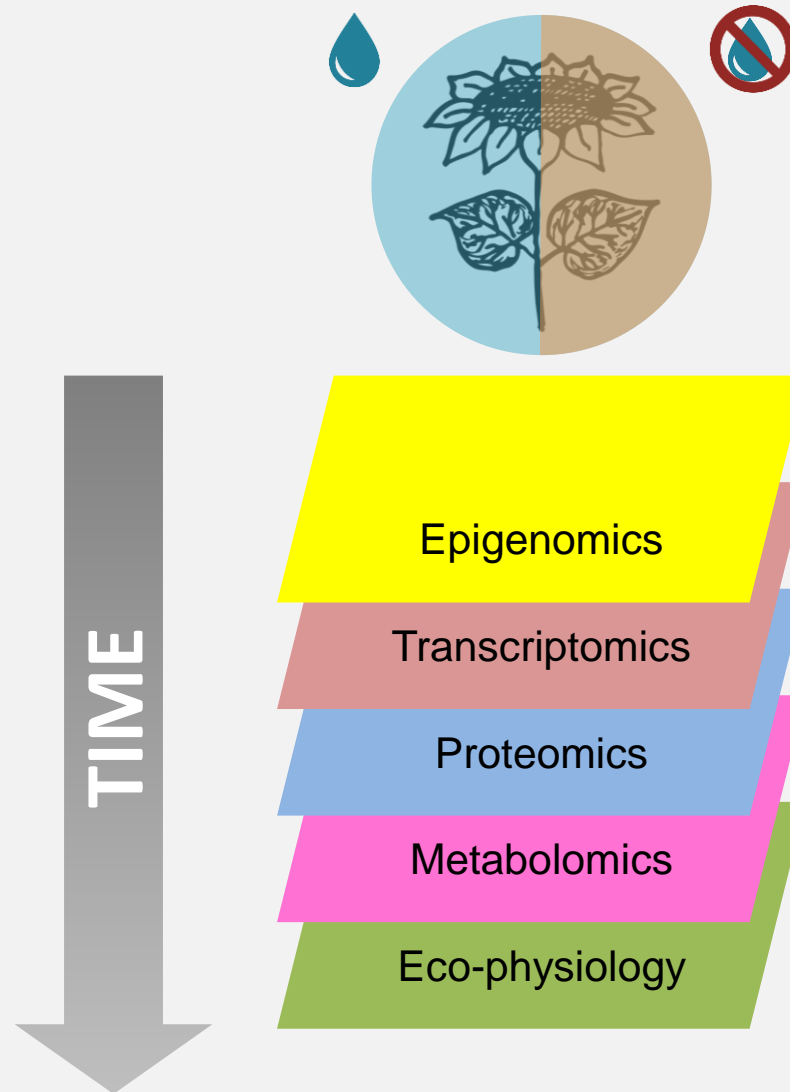
# The drought stress response: complex responses

At the Agronomic, physiology, Molecular levels...



Casadebaig et al., 2008

# The drought stress response: a complex response at multiple scales





# Objectives & Strategies



How to characterize the response to sunflower drought and its genetic variation between parental lines and hybrids?



# Experimental design

24 genotypes (8 parental lines and 16 hybrids)

♀/♂	SF279	SF317	SF326	SF342
SF009	✓	✓	✓	✓
SF092	✓	✓	✓	✓
SF109	✓	✓	✓	✓
SF193	✓	✓	✓	✓

3 biological replicates

2 environmental conditions

Water Deficit  
(WD)



Well Watered  
(WW)



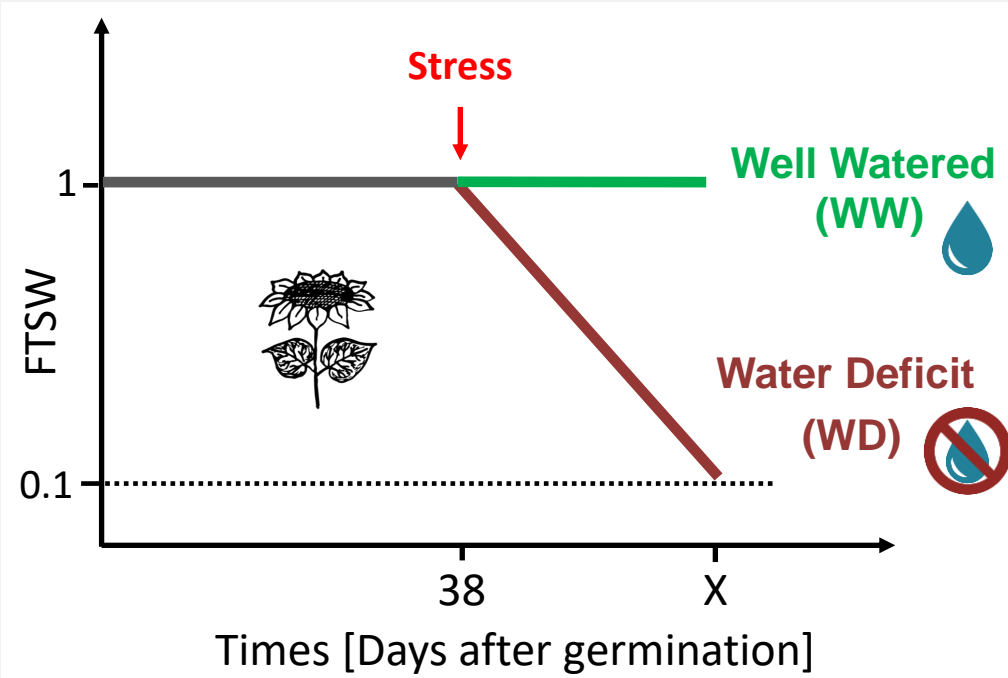
# Objectives and experimental design

Realized with *Heliaphen* robot:

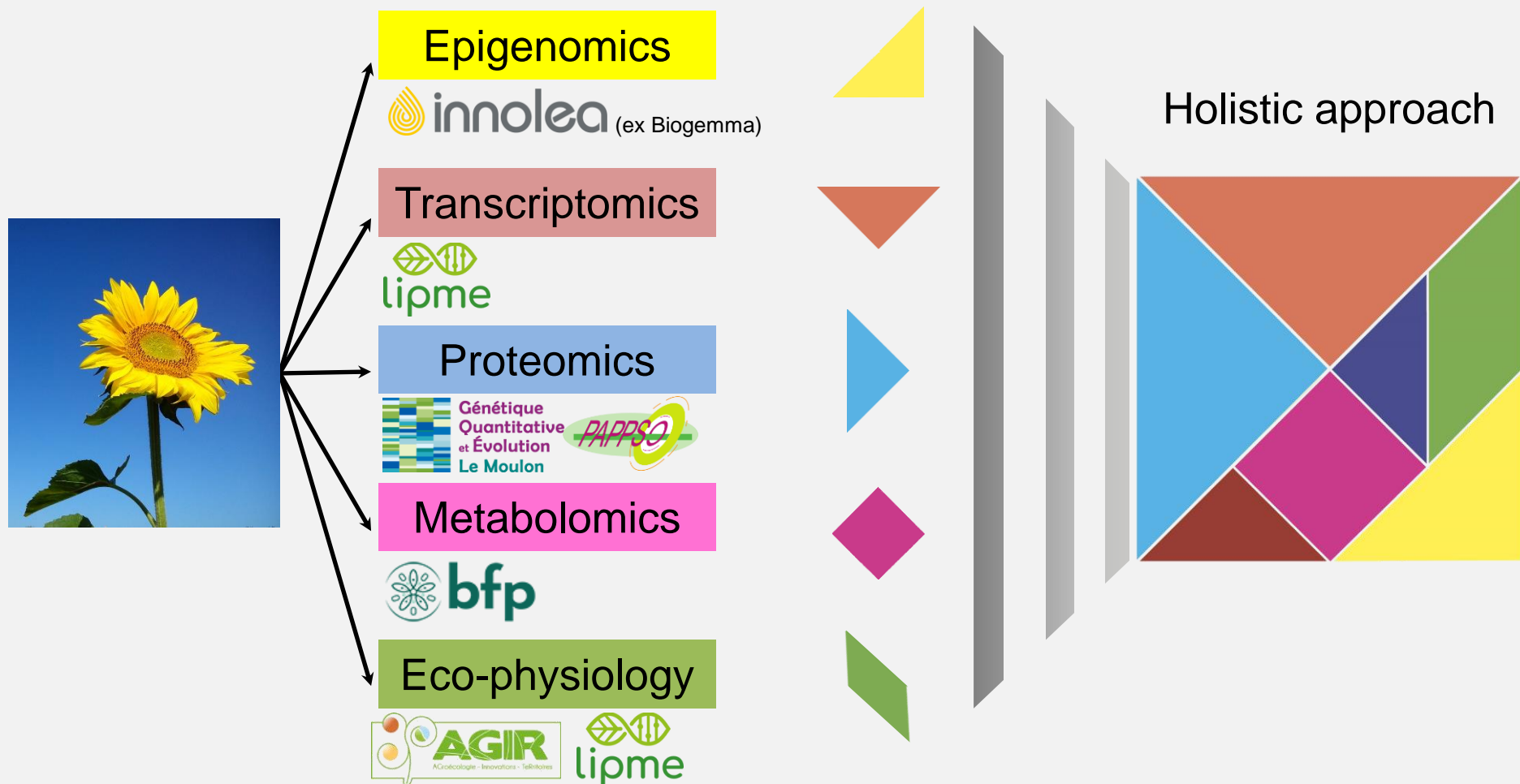
- Controlled stress
- Phenotyping standardization



Heliaphen platform (LIPM, INRA Toulouse)  
(Gosseau et., al. 2019)

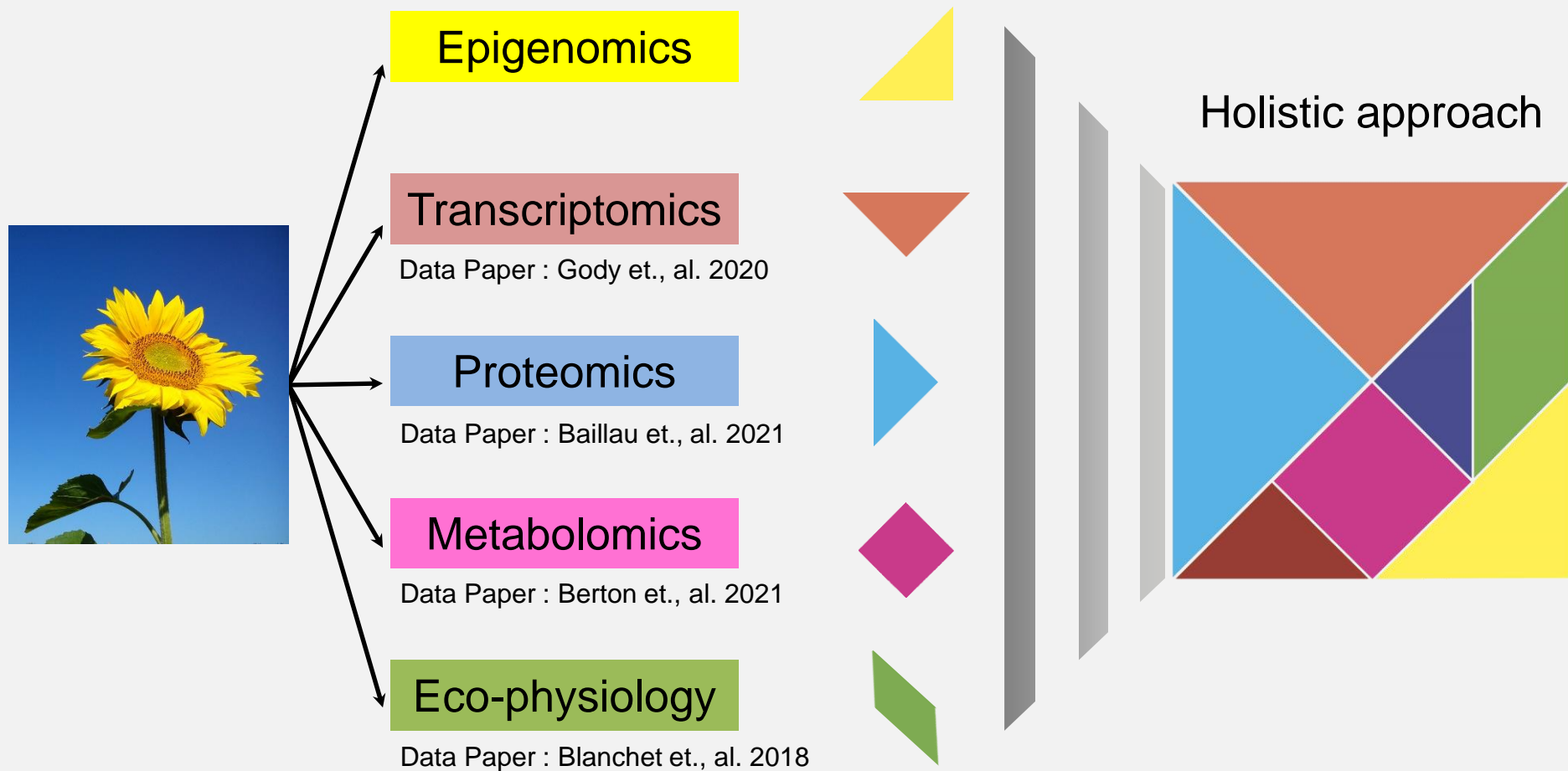


# A transdisciplinary and multi-partner project





# A transdisciplinary and multi-partner project



# Results





# Eco-physiology

Nicolas Blanchet, Pierre Maury



# Eco-physiological data



Eco-physiology

22 Variables

Physiological

Morphological

e.g. Specific Leaf Area,  
Osmotical potential,  
Carbon Isotope Discrimination

e.g. Plant height,  
Number of leaves,  
Leaf expansion

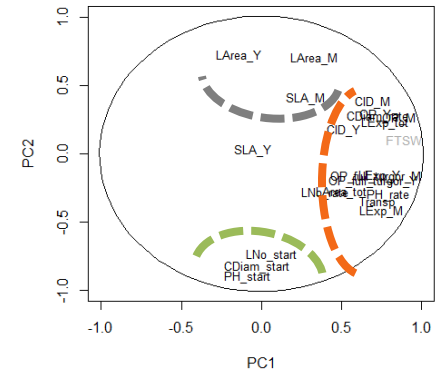
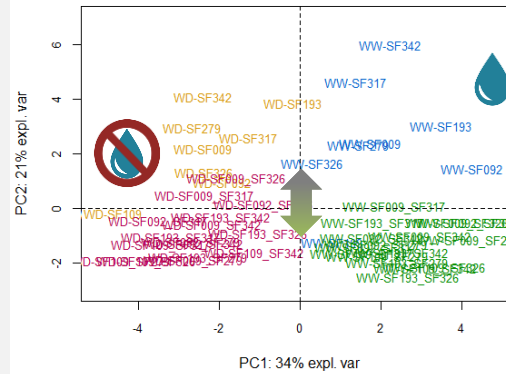
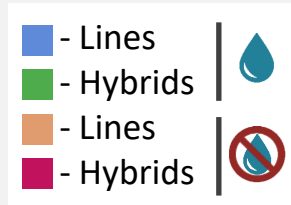
- At the beginning of the stress
- During the stress period
- At the end of the stress



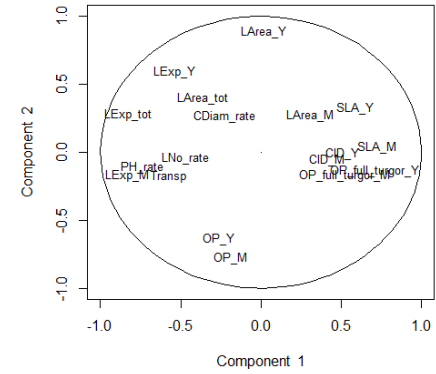
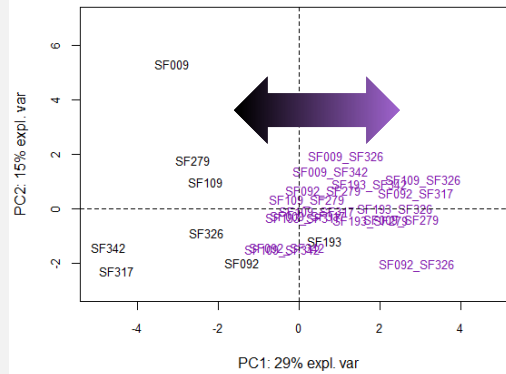
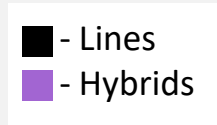
Blanchet et. al., 2018

# Eco-physiological data: multivariate analysis

## PCA of the traits



## PCA of the trait responses to WD (WD-WW)



Durufle et. al., 2023

- Strong impact of the water status: Physiological variables
- Types of genotype show different behaviors: Morphological variables
- Hybrids doesn't have the same behaviour than the parental lines during the stress.

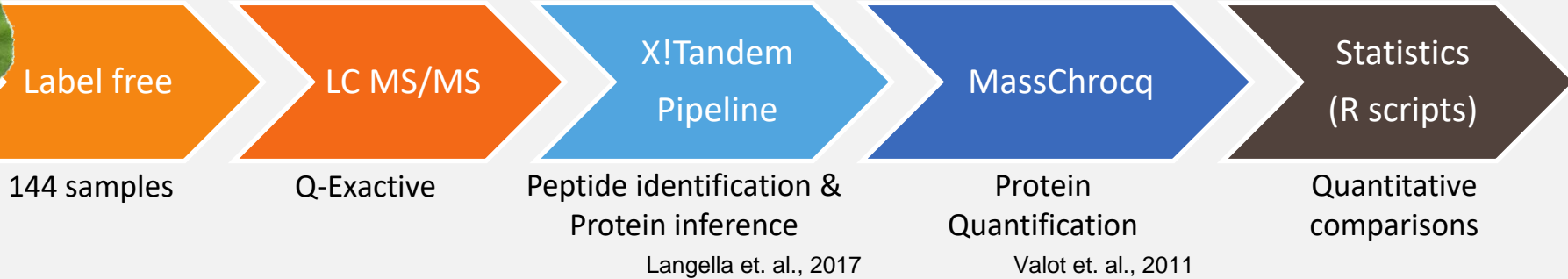


# Proteomics

Thierry Balliau, Mélisande Blein-Nicolas, Michel Zivy



# Proteomics workflow



## Identification

- Database : *Helianthus annuus* Non redundant Peptides XRQ (Heliagene.org)
- **3062 proteins identified**

## Quantification

- Quantitative analysis on **1211 proteins** (after filtering) : Anova



## 471 DAPs identified with statistical tests

- Treatment, Genotypes, Heterosis, Interaction
- Pvalue < 0.05 (adjusted for multiple comparisons)
- Mixed model using kinship matrix

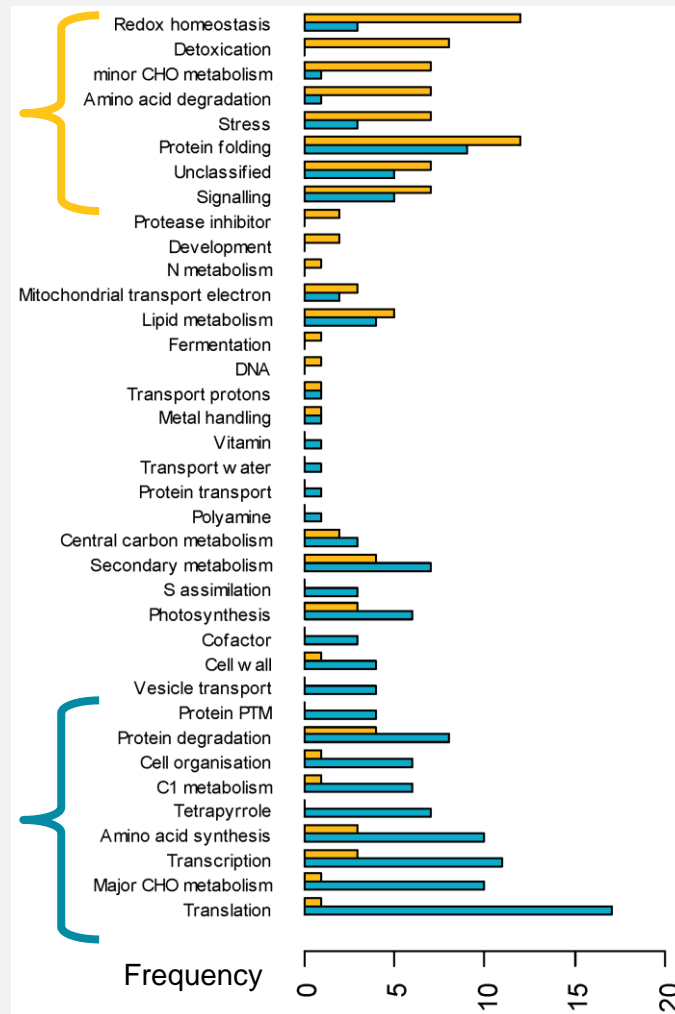
Durufflé et. al., 2023

# Main protein categories involved in the response

Functional category of proteins in response to Water Deficit

 **Upregulated** 

 **Downregulated** 





 Classical global response to the stress

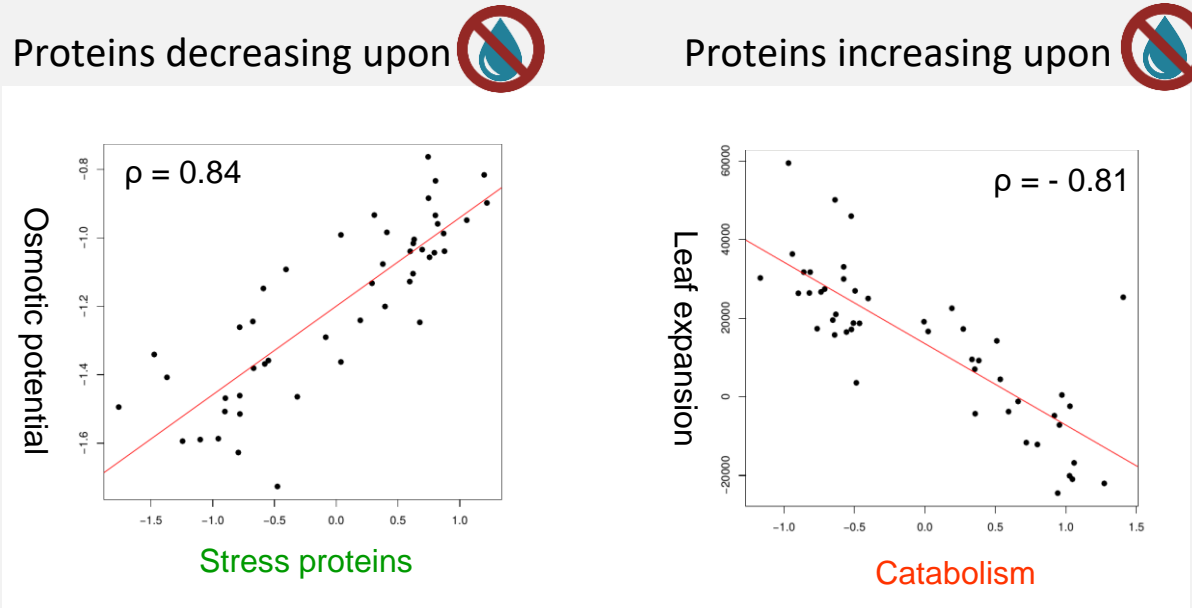
Durufié et. al., 2023





# Basic correlation

Examples of correlation based on the water status (  and  )  
(48 genotype/treatment means)

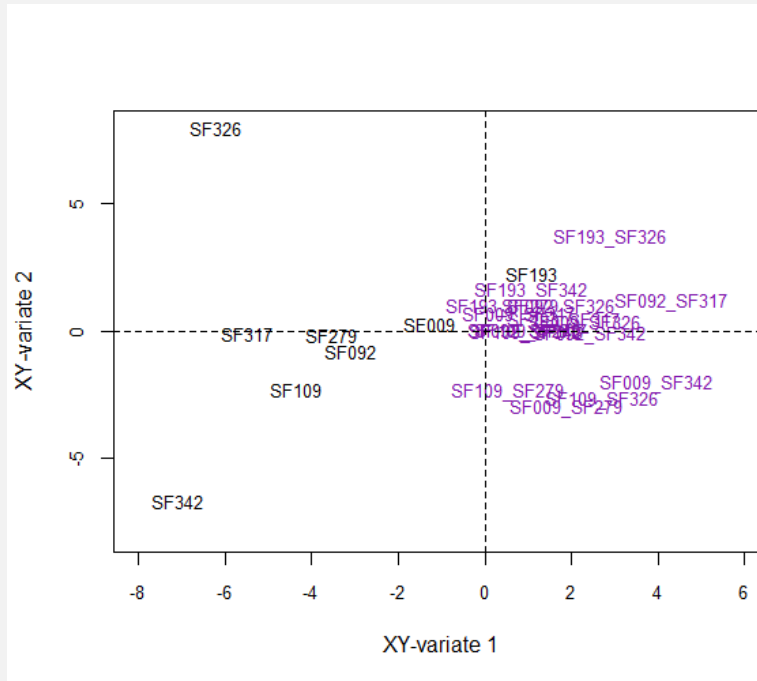


- ☀ Highlighted some correlations between proteins and ecophysiological variables
- ☀ Is it possible to predict WD response with protein levels under WW conditions?

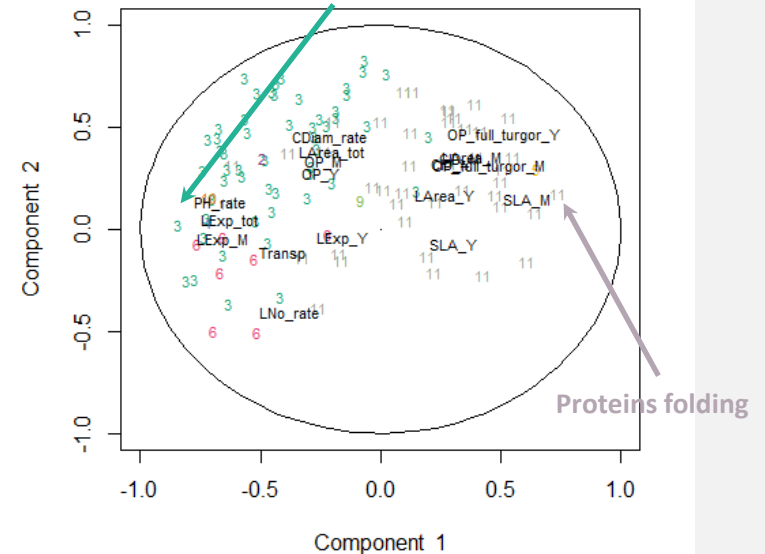
# Proteomics and Predictions

PLS of 3 clusters of **DAPs upregulated under WW** conditions & the response of the **EP traits**

■ - Lines  
■ - Hybrids



Proteins involved in cell wall growth & synthesis of the osmoprotectant glycine-betaine



Durufflé et. al., 2023

☀️ Positive correlation between the abundance of upregulated proteins under the WW condition and the maintenance of growth under WD (e.g. Cluster 3)


Olivier Fernandez, Thierry Berton, Stéphane Bernillon, Yves Gibon, Annick Moing

Metabolomics (2019) 15:56  
<https://doi.org/10.1007/s11306-019-1515-4>

ORIGINAL ARTICLE



## Metabolomic characterization of sunflower leaf allows discriminating genotype groups or stress levels with a minimal set of metabolic markers

Olivier Fernandez<sup>1,5</sup>  · Maria Urrutia<sup>1,2,6</sup> · Thierry Berton<sup>1,7</sup> · Stéphane Bernillon<sup>1,3</sup> · Catherine Deborde<sup>1,3</sup> · Daniel Jacob<sup>1,3</sup> · Mickaël Maucourt<sup>1,3,6</sup> · Pierre Maury<sup>4</sup> · Harold Duruflé<sup>4</sup> · Yves Gibon<sup>1,3</sup> · Nicolas B. Langlade<sup>4</sup> · Annick Moing<sup>1,3</sup>



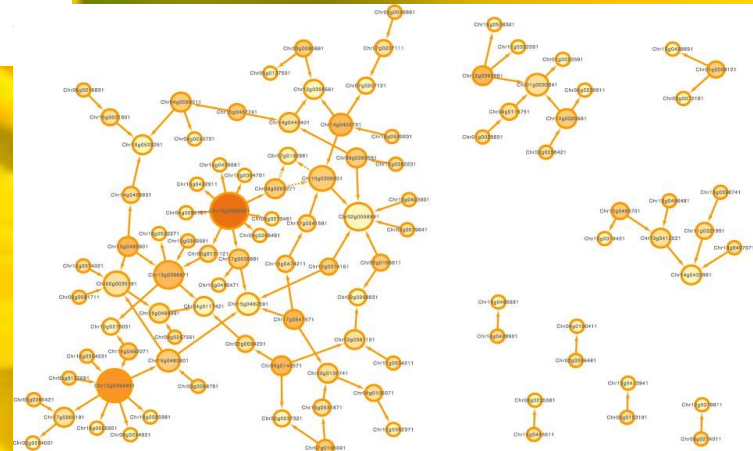
*Bioinformatics*, 38(17), 2022, 4127–4134  
<https://doi.org/10.1093/bioinformatics/btac445>  
Advance Access Publication Date: 6 July 2022  
Original Paper

OXFORD

Gene expression

## Gene regulatory network inference methodology for genomic and transcriptomic data acquired in genetically related heterozygote individuals

Lise Pomiès <sup>1</sup>, Céline Brouard<sup>1</sup>, Harold Duruflé<sup>2</sup>, Élise Maigné<sup>1</sup>, Clément Carré<sup>1</sup>, Louise Gody<sup>2</sup>, Fulya Trösser<sup>1</sup>, George Katsirelos<sup>3</sup>, Brigitte Mangin<sup>2</sup>, Nicolas B. Langlade<sup>2</sup> and Simon de Givry <sup>1,\*</sup>





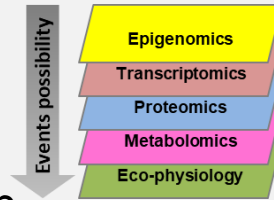
# Conclusions & Perspectives





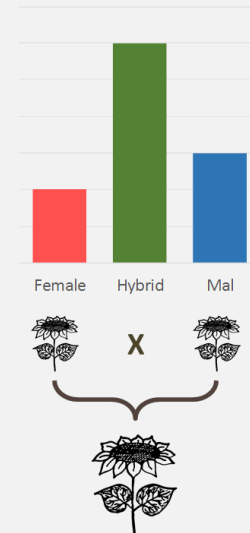
# Conclusions and perspectives

- Large and complex effect of water deficit at multi biological scales
- Hybrids seem to be more reactive to water deficit than parental lines
- Multi-level analysis allows to see impact of the accumulation of regulation
- But, 3 biological replicates is not enough for complex statistical models



## Perspectives:

- Give biological and evolutionary meaning of the integrative studies
  - Functional validation of the most relevant candidates
  - Genetic diversity evolution and breeding
- Improve our knowledge of the regulatory networks underlying heterosis



## Challenges:

- Quantitative genetics approaches to study plasticity
- Increase the amount of protein per variety / genotype

**Nicolas Langlade**

Brigitte Mangin  
Nicolas Blanchet  
Stéphane Muños  
Alexandra Duhnen  
Louise Gody  
Florie Gosseau  
Nicolas Pouilly  
Claire Esperou

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Céline Brouard  
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Thierry Balliau  
Mélisande Blein-Nicolas  
Michel Zivy



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Yves Gibon  
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**VASCO team**

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Philippe Debaeke  
Pierre Maury



Hélène Bergès  
Arnaud Bellec  
Sonia Vautrin



**SUNRISE**  
UNE CULTURE POUR LE FUTUR

**INRAE Toulouse Experimental Unit**

Gilles Tison  
Paul Bataillon  
Philippe Burger  
Rémi Marandel  
Didier Campergue

**Thanks to all people  
involved...**

**...and thank you for your attention**

