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International symposium on adaptation of horticultural plants to abiotic stresses

Apple drought adaptive response: transcriptional and epigenetic approach

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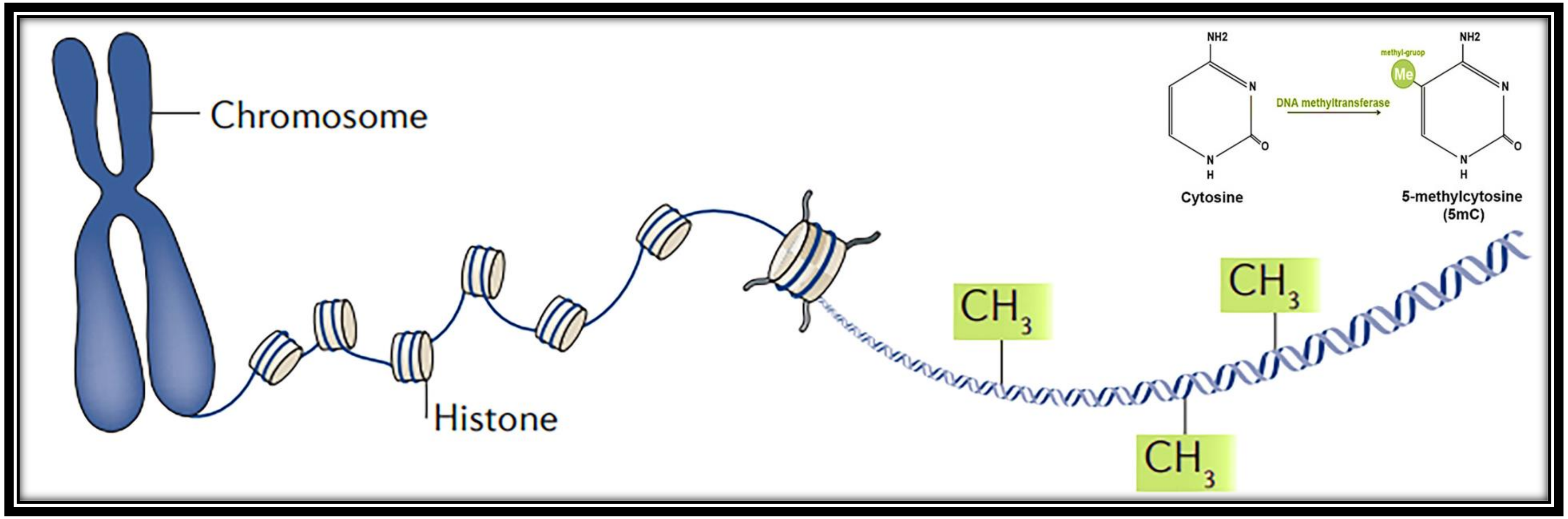
Epigenetics

"[…]epigenetics may be defined as the study of any potentially stable and, heritable change in gene expression or cellular phenotype that occurs without changes in Watson-Crick base-pairing of DNA."

Goldberg et al., 2007

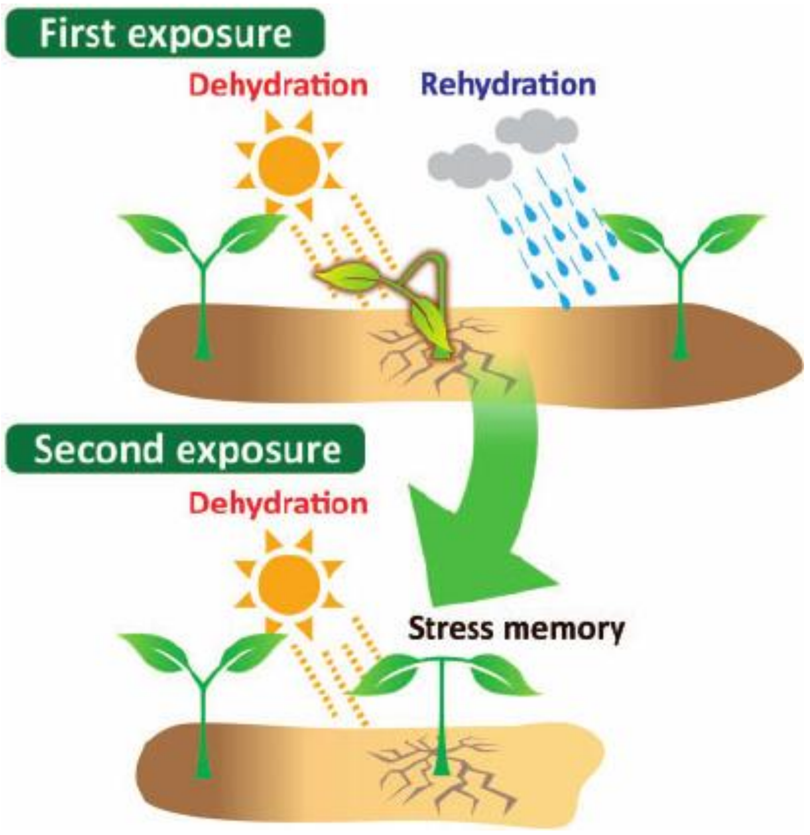


Acetylation

Phosphorylation

DNA methylation

Epigenetics, a tool to improve stress tolerance?



Kinoshita & Seki, 2014

DNA methylation associated to plant adaptive responses

Environmental perturbations may occur repeatedly → use the stored knowledge to adapt to new challenges

<u>Several priming strategies</u>:

- Accumulation of compounds in the cellular compartments;
- Modification of key regulatory proteins, e.g. MAPKs
- Epigenetic mechanisms

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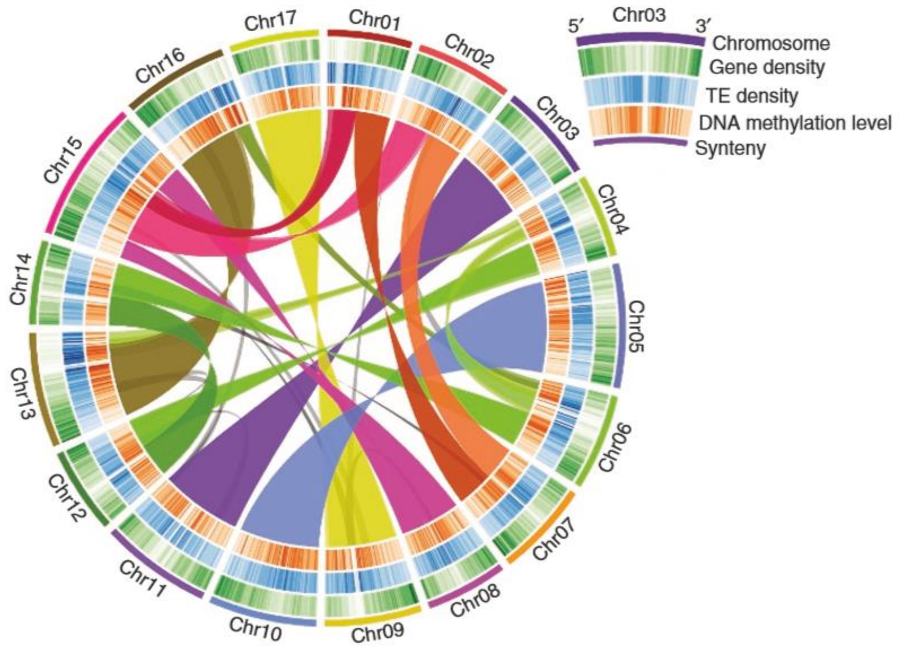
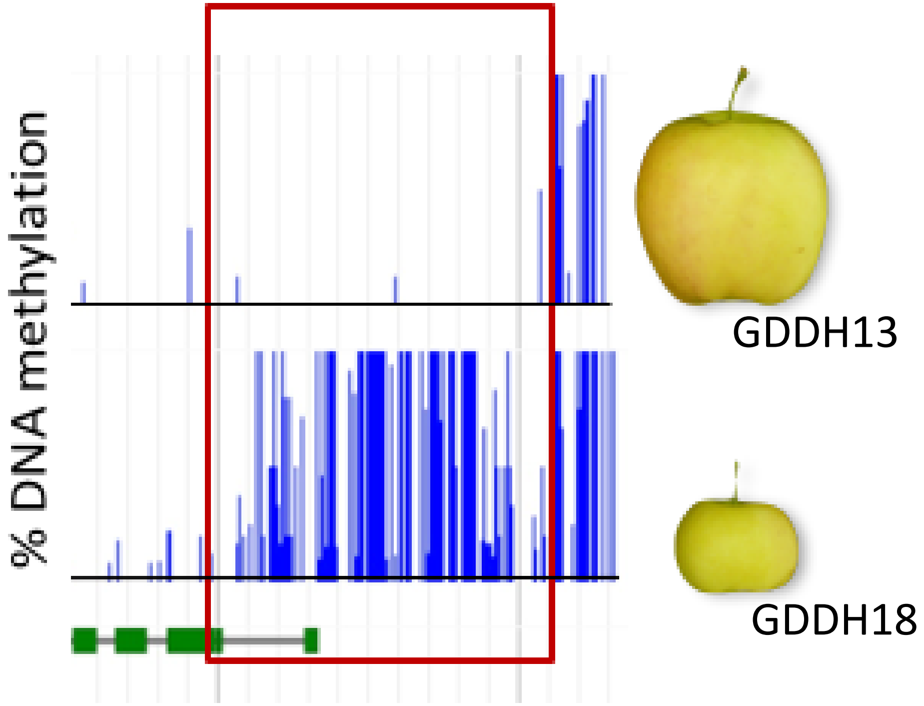
Apple tree, a model for fruit and perennial plants

nature genetics

High-quality *de novo* assembly of the apple genome and methylome dynamics of early fruit development

Nicolas Daccord[1,11], Jean-Marc Celton[1,11], Gareth Linsmith[2], Claude Becker[3,10], Nathalie Choisne[4], Elio Schijlen[5], Henri van de Geest[5], Luca Bianco[2], Diego Micheletti[2], Riccardo Velasco[2], Erica Adele Di Pierro[6], Jérôme Gouzy[7], D Jasper G Rees[8], Philippe Guérif[1], Hélène Muranty[1], Charles-Eric Durel[1], François Laurens[1], Yves Lespinasse[1], Sylvain Gaillard[1], Sébastien Aubourg[1], Hadi Quesneville[4], Detlef Weigel[3], Eric van de Weg[9], Michela Troggio[2] & Etienne Bucher[1]





1-AMINO-CYCLOPROPANE-1-CARBOXYLATE SYNTHASE 8 gene (ACS8, MD15G0127800)

Ethylene synthesis

Daccord *et al.* 2017

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Drought, a threat to crop production

FACTS:

- New environmental constrains - cycles of low water availability is challenging fruit and crop production.

- Necessity of increase the efficiency via breeding and/or improving agricultural behavior

QUESTIONS :

- How plants behave after DS ?
- How long lasts the epigenetic memory in plants?
- Is this memory transmissible by grafting?

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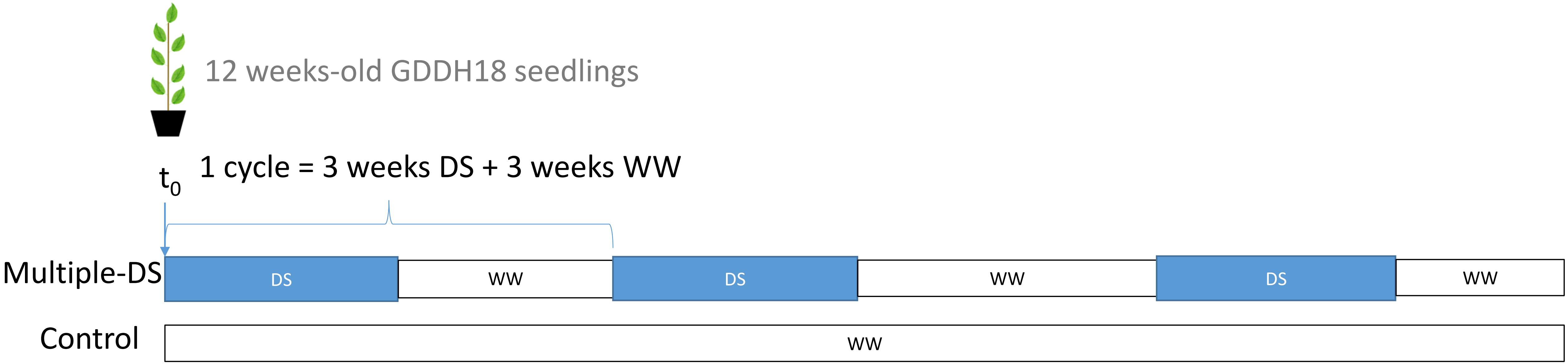
Can we develop an epigenetic tool to improve plants?

Experimental design



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WW: Well Watered
WD: Water Deficit

Morphological response

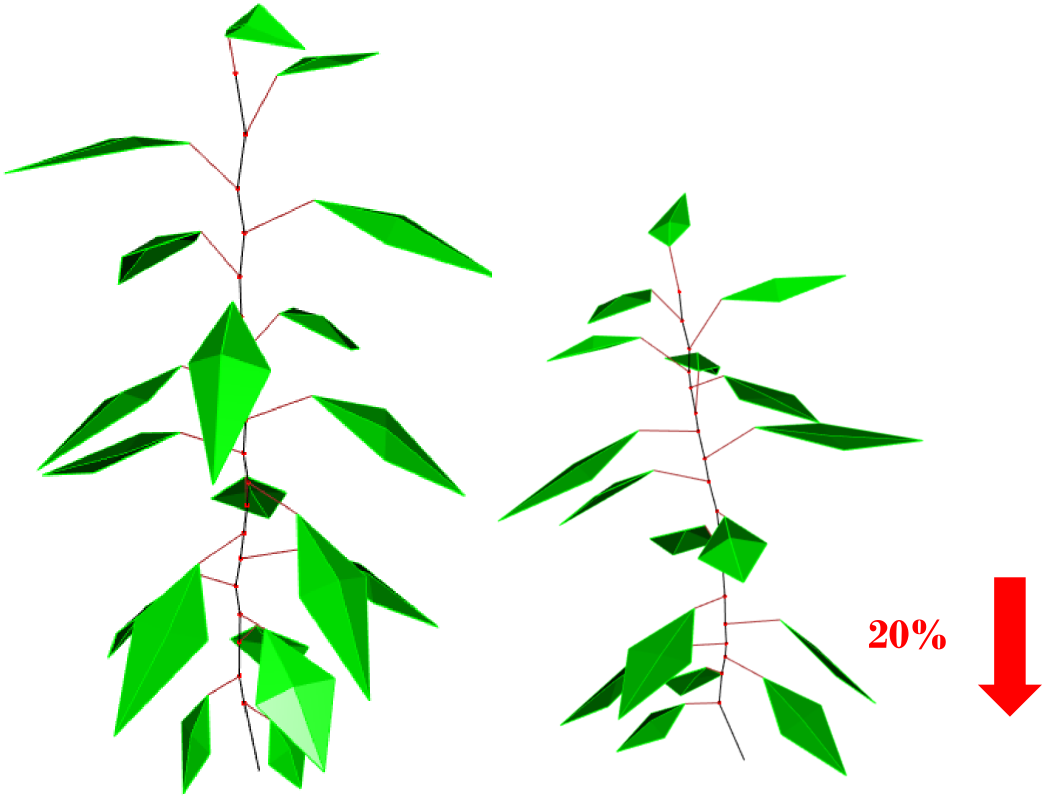
Do plants growth in a different way depending on the submitted water regime?

3D imaging of plants after 2nd DS

Monitoring (every 7 days):
- height
- number of nodes
- internode length

R script

MicroScribe, portable coordinate measurement machine (CMM) – collection of 3D data



Control plants

411,5 mm ± 24,7 mm

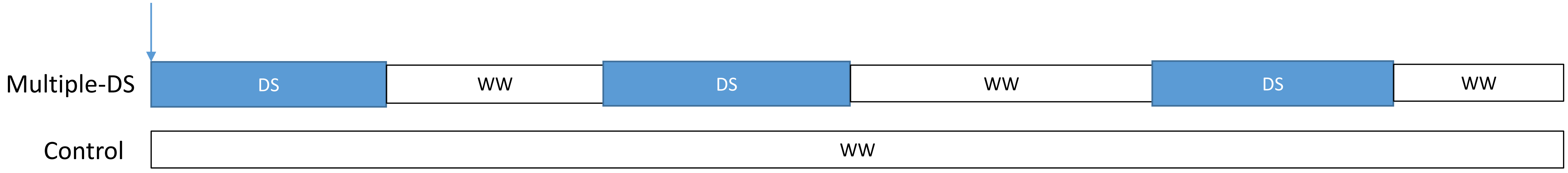
DS plants

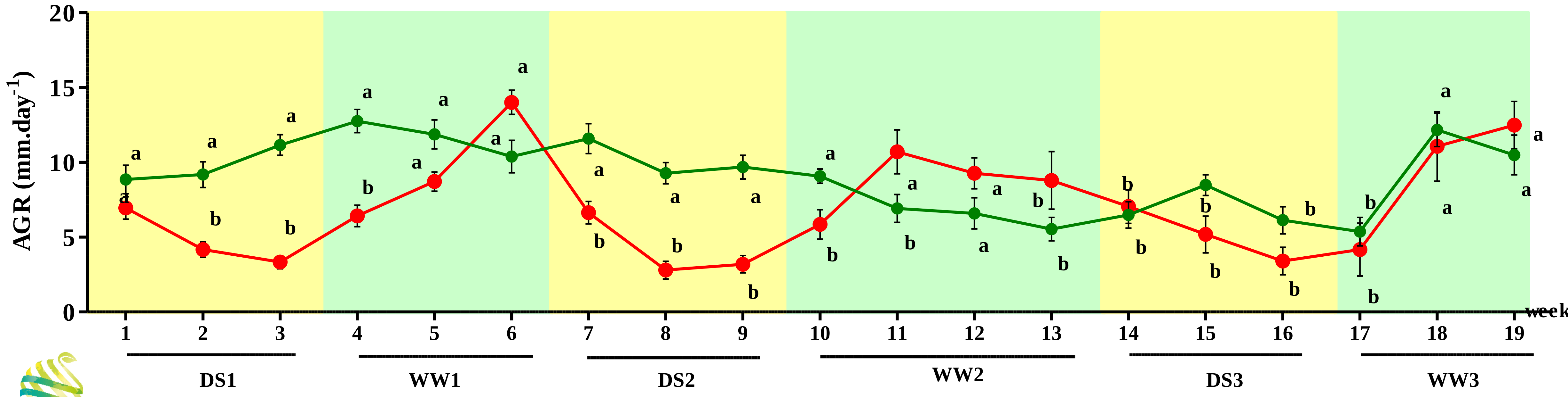
326,4 mm ± 18,5 mm

12 weeks-old GDDH18 seedlings

Morphological response

Do plants growth in a different way depending on the submitted water regime?





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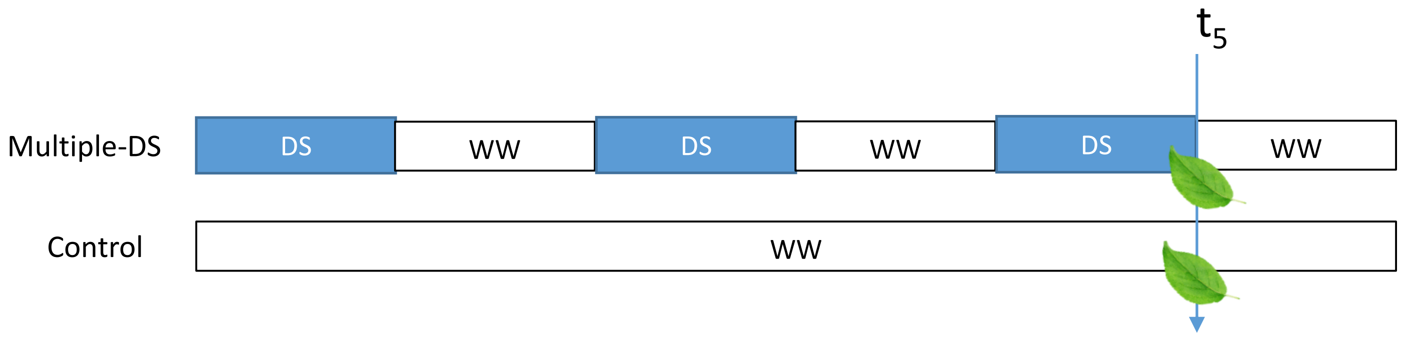
WW:	Well Watered
WD:	Water Deficit
AGR:	Arbitrary Growth Rate

Control group

Multiple-Drought Stress group

Morphological response

Is leaf morphogenesis affected by drought stress?

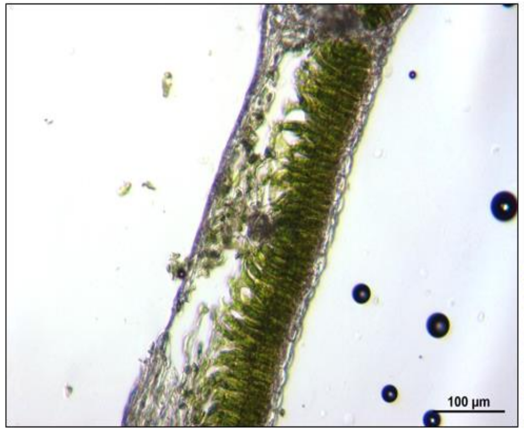


Collect a well expanded leaves developed

1 leaf from 3 different plants

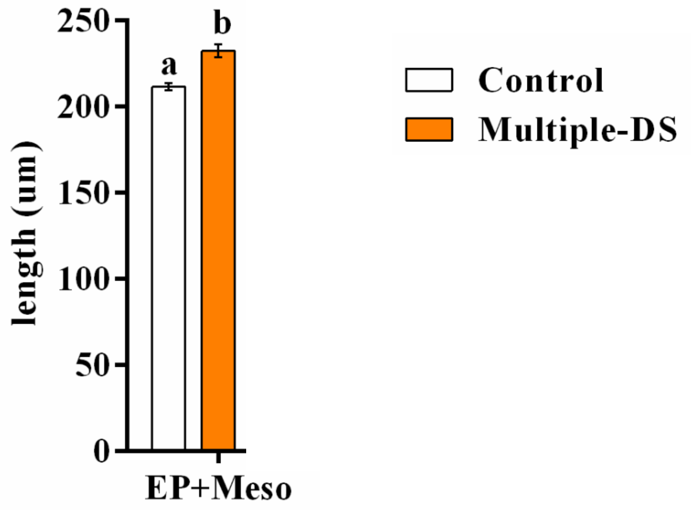
Each leaf three cuts (60µm), each cut two pictures.

54 pictures each condition (3 Reps)



Control leaf

Multiple-DS leaf



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Morphological response

SUM UP preliminary results

Phenotyping traits after multiple drought stress *vs* control group

- Number nodes decreased
- Stem diameter decreased
- The anatomy of leaves showed differences
- AGR in 3rd DS : is there memory?

What about molecular aspect?

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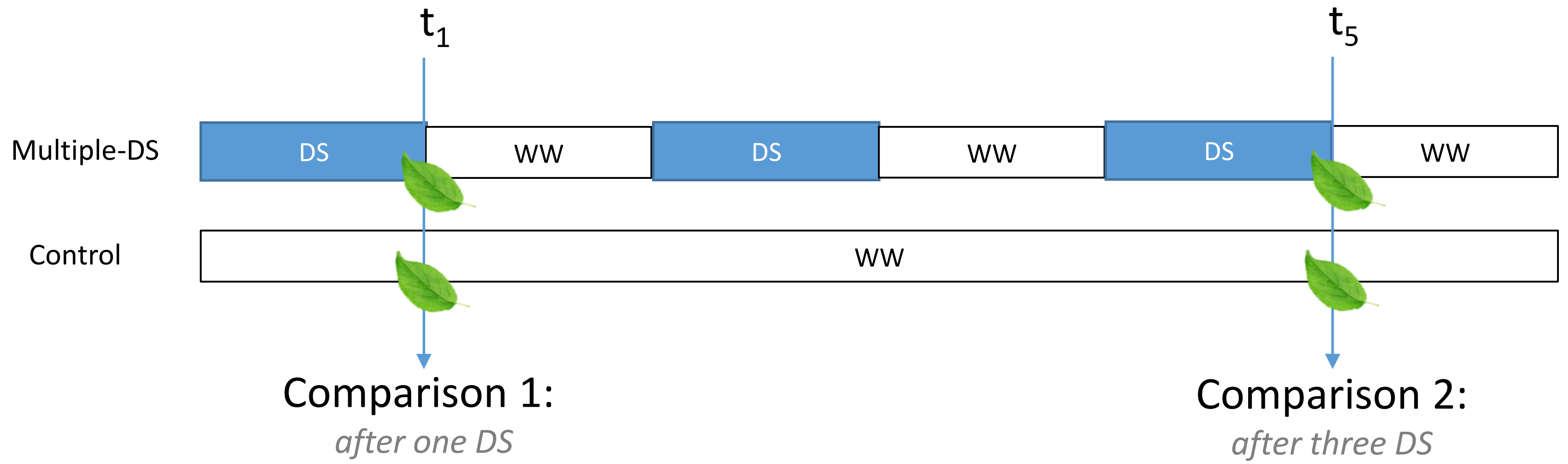
Transcriptional reprogramming

How different water regimes affect plant transcriptome?



RNA extraction from leaves

Fastq files input

FastQC *Quality Control*

Salmon *Mapping/quantification GDDH13 transcriptome as reference*

DEseq2 *Pairwise comparison; DEGs extraction*

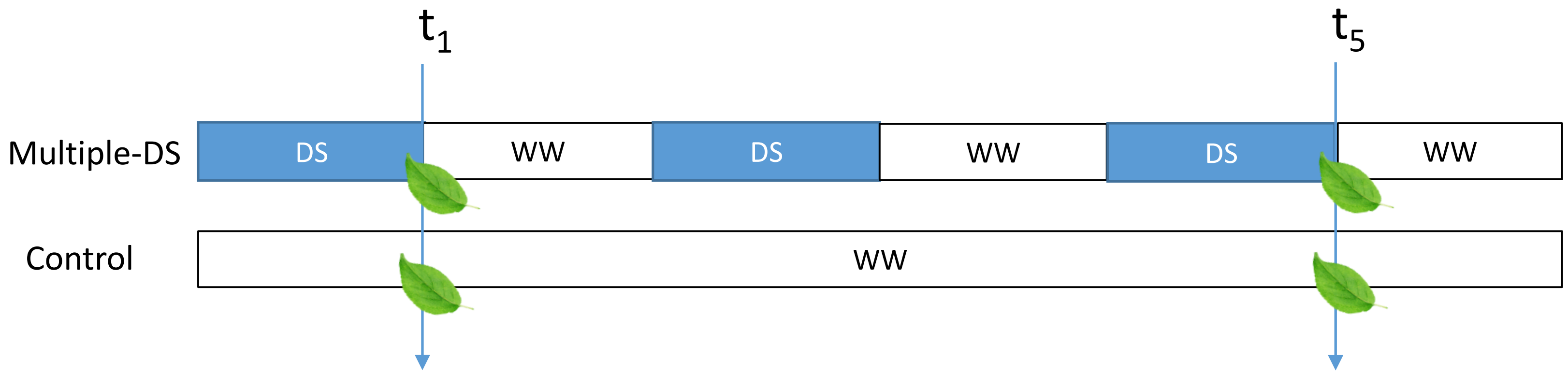
AnaDiff tool

AnaDiff- Sandra Pelletier

DOI: 10.5281/zenodo.6477918

Transcriptional reprogramming

RNA-seq results in leaves : differentially expressed genes



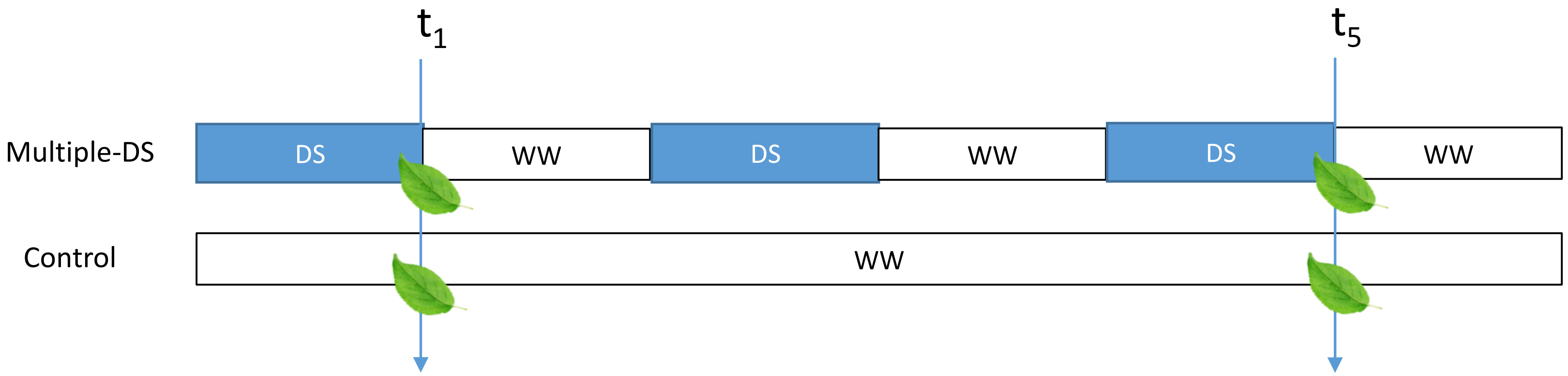
	Comparison 1: *after one DS*	Comparison 2: *after three DS*
Log2 fold change >1	2040 membrane component oxydative stress	44 Ion transport metabolic process
Log2 fold change <1	1995 reproduction/dvp process/ anatomical structure dvp/ cell cycle	238 ATP metabolism

13x less DEGs

After multiple drought cycles plants showed a less complex reprogramming dynamic to the same environment stress driver.

DNA methylation

Does the level of DNA methylation vary over drought stress cycles?



Comparison 1:
after one DS

Comparison 2:
after three DS

DNA extraction from leaves

Whole genome bisulfite sequencing (WGBS)

In-house pipeline to DMRs identification: **BiSePS**

Identification of DMRs – sliding window approach;
windows set to 100 bp and step length 100 bp

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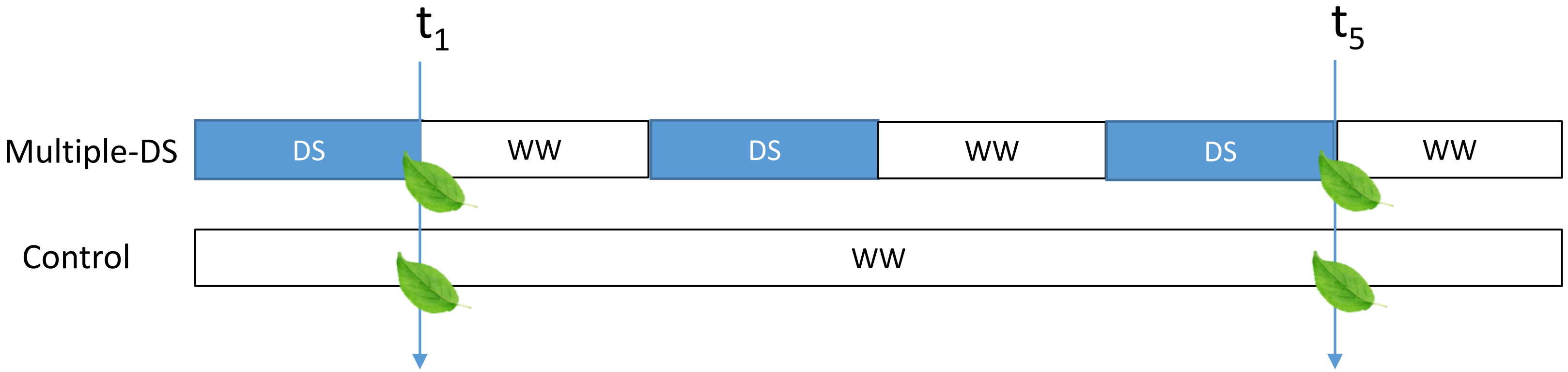
BiSePS- Skander Hatira
DOI: 10.5281/zenodo.6841705

DNA methylation

Does the level of DNA methylation vary over drought stress cycles?



	Comparison 1: *after one DS*	Comparison 2: *after three DS*
CHH	393	215
CpG	58	2939
CHG	12	992

H : is any base except G

9x more DMRs

Multiple-DS plants accumulate more DMRs in the context of CpG and CHG allover the DS cycles

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Take-home message

- Multiple cycles of drought affect morphological structure of apple trees.

- Memory observed on leaf anatomy

- Multiple cycles of drought stress result in a high modification in DNA methylation level and a low transcriptional changes

Back to our biological questions

- **How plants behave after DS ?**
  Phenotyping differences maintained after multiple DS.

- **How long lasts the epigenetic memory in plants ?**
  Ongoing project to study long-term memory of DS.

- **Is this memory transmissible by grafting?**
  Ongoing project to understand epigenetics marks after grafting.

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- BIDEFI – Sylvain Gaillard, Eric Montaudon, Sandra Pelletier
- AFEF (Montpellier) - Evelyne Costes

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