Sepal morphology and gene expression in *Arabidopsis thaliana*

NETBIO Meeting
08/12/2020

Diego Hartasánchez
Laboratoire Reproduction et Développement des Plantes

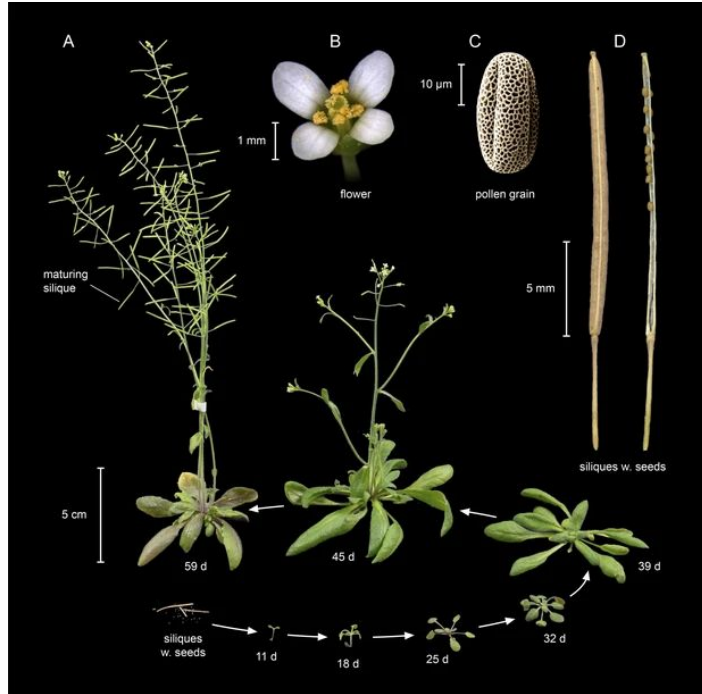# Sepal morphology and gene expression in *Arabidopsis thaliana*

Organ size and shape is amazingly reproducible.

Gene expression is a fundamentally stochastic process.

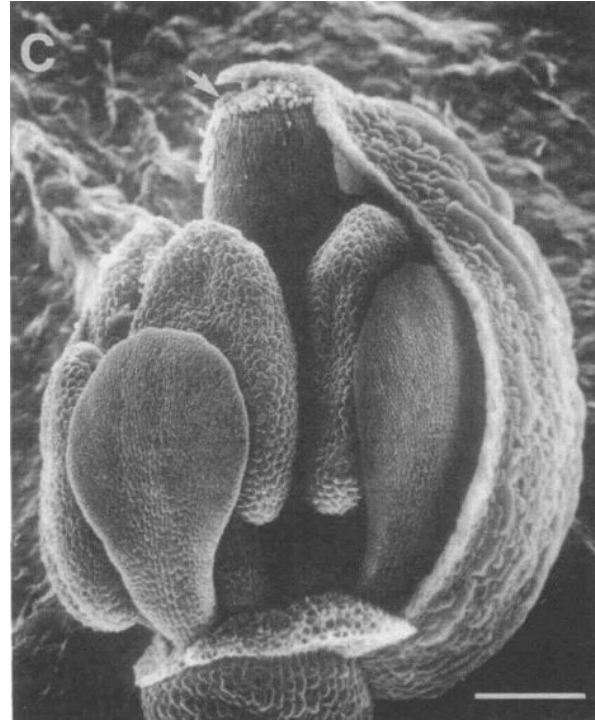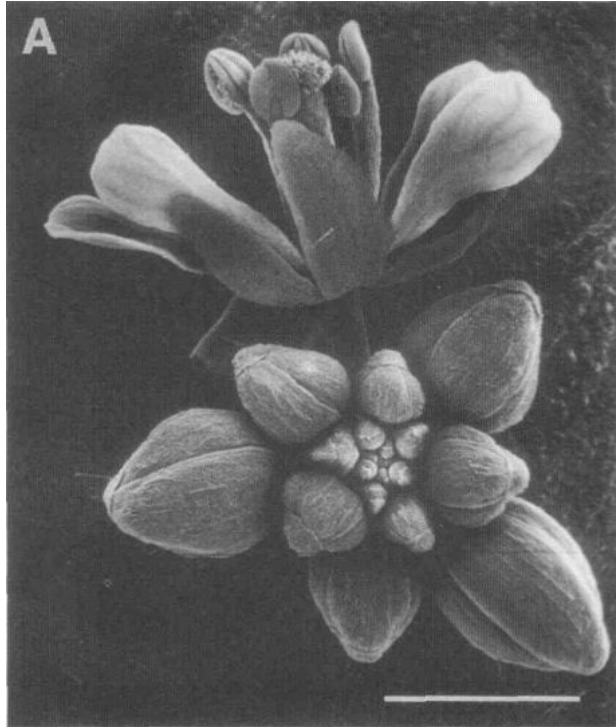# Sepal morphology and gene expression in *Arabidopsis thaliana*

Arabidopsis thaliana

Krämer, eLife 2015;4:e06100

Sepal morphology and gene expression in *Arabidopsis thaliana*
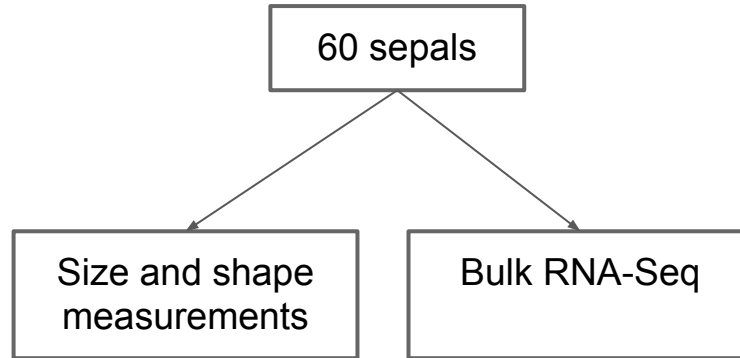
Abaxial sepal from stage 11 flowers

Dataset

6 *Arabidopsis thaliana* wild type (Col-0) plants:

- **DEF** plants: Standard conditions (20 days in short days and then long days at **22**ºC)
- **JKL** plants: Slight stress conditions (20 days in short days and then long days at **27**ºC)

10 abaxial sepals from stage 11 flowers from each plant

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                         ┌──────────────┐
                         │  60 sepals   │
                         └──────────────┘
                          /            \
              ┌────────────────┐   ┌──────────────┐
              │ Size and shape │   │ Bulk RNA-Seq │
              │ measurements   │   │              │
              └────────────────┘   └──────────────┘
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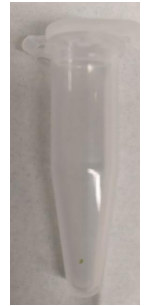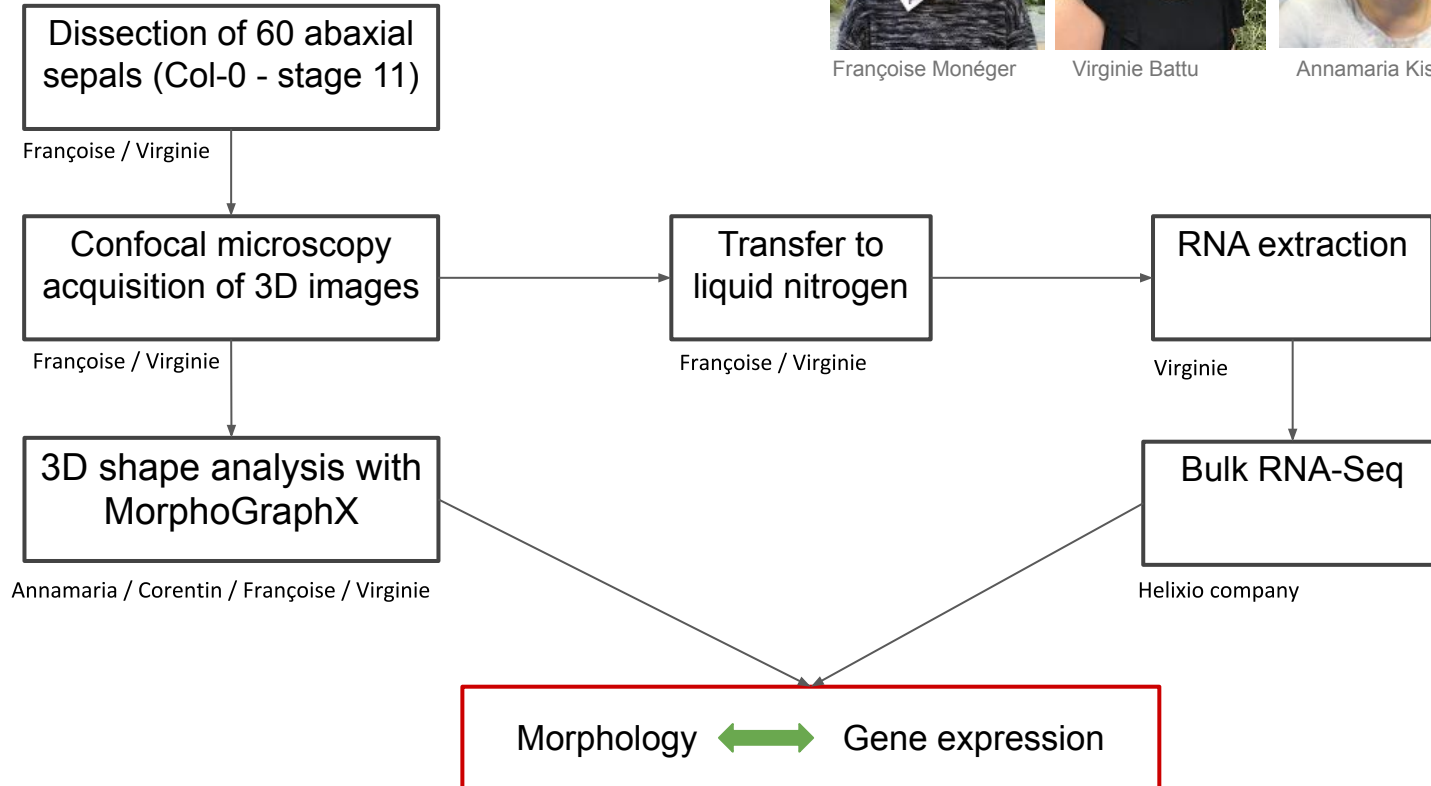
Experimental protocol

Françoise Monéger   Virginie Battu   Annamaria Kiss   Corentin Mollier

Dissection of 60 abaxial sepals (Col-0 - stage 11)

Françoise / Virginie

Confocal microscopy acquisition of 3D images

Françoise / Virginie

Transfer to liquid nitrogen

Françoise / Virginie

RNA extraction

Virginie

3D shape analysis with MorphoGraphX

Annamaria / Corentin / Françoise / Virginie

Bulk RNA-Seq

Helixio company

Morphology ⬌ Gene expression

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3D shape analysis pipeline

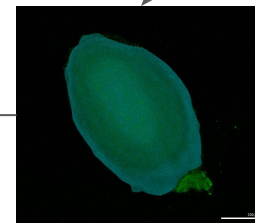Françoise Monéger   Virginie Battu   Annamaria Kiss   Corentin Mollier
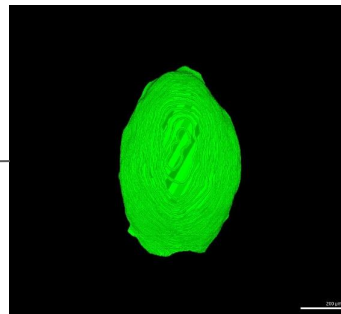
Confocal image
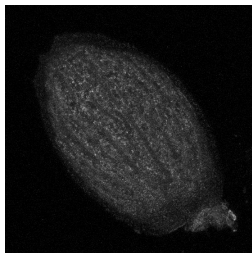
Otsu normalization & xy smoothing
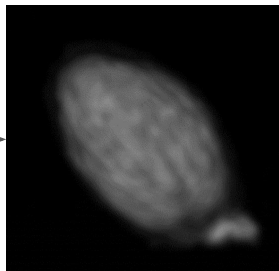
Pre-treatment & EdgeDetect in MGX
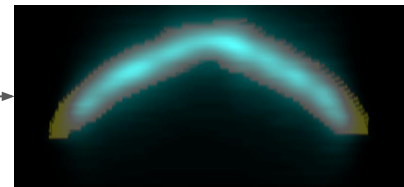
Manual cropping

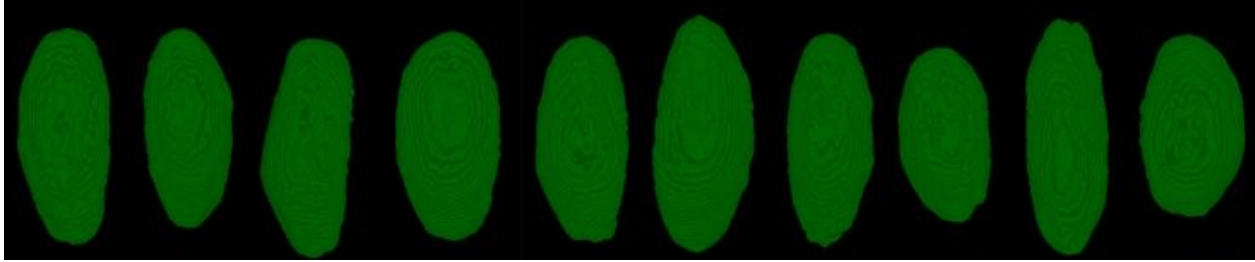Extract size and shape measurements

MorphoGraphX object
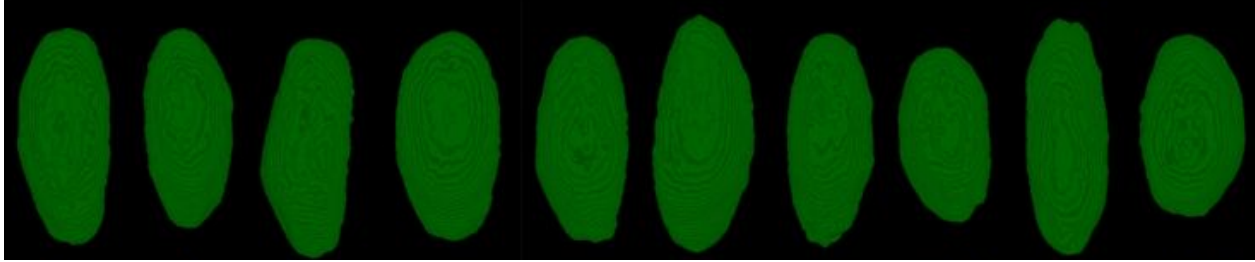
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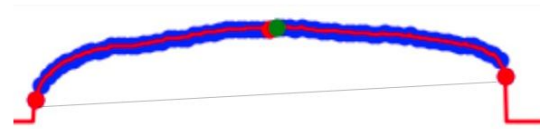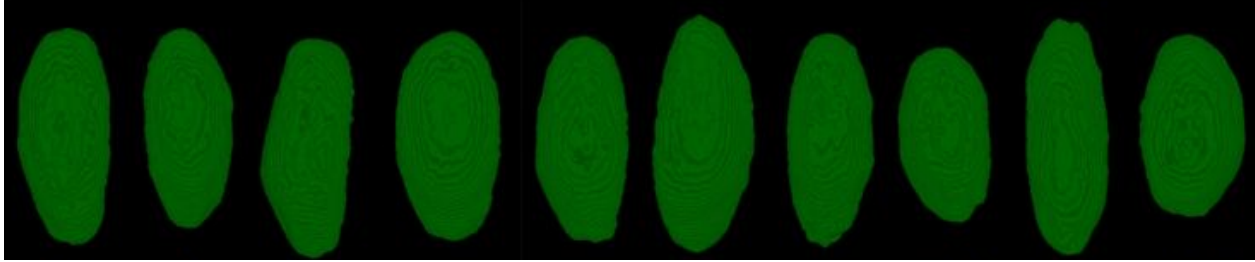

Annamaria Kiss

- Longitudinal
  - Flat length
  - Curved length
  - Longitudinal height
  - Longitudinal radius (LR)

- Transversal
  - Flat width
  - Curved width
  - Transversal height
  - Transversal radius (TR)

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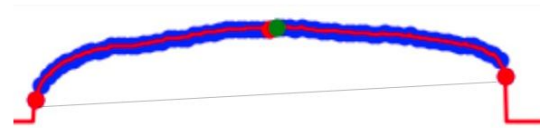 - Transversal radius (TR)
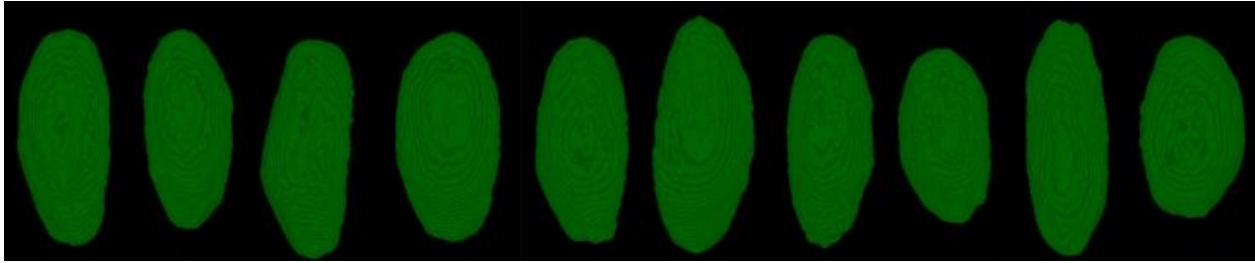
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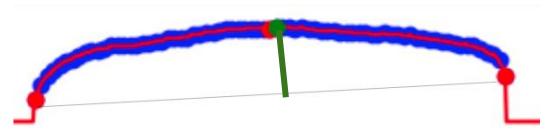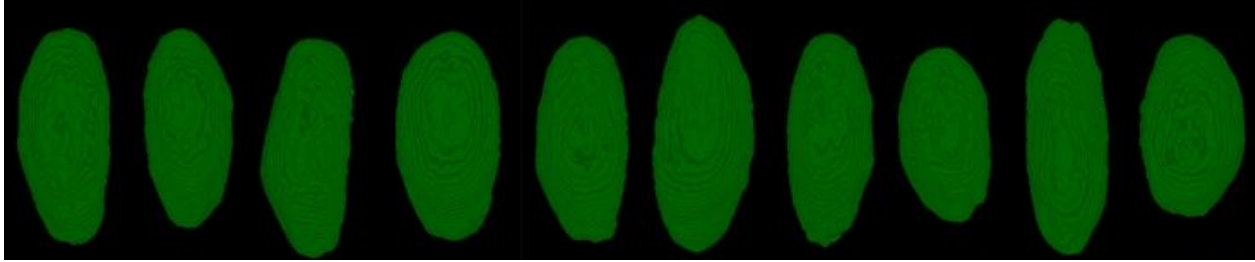 - Transversal height
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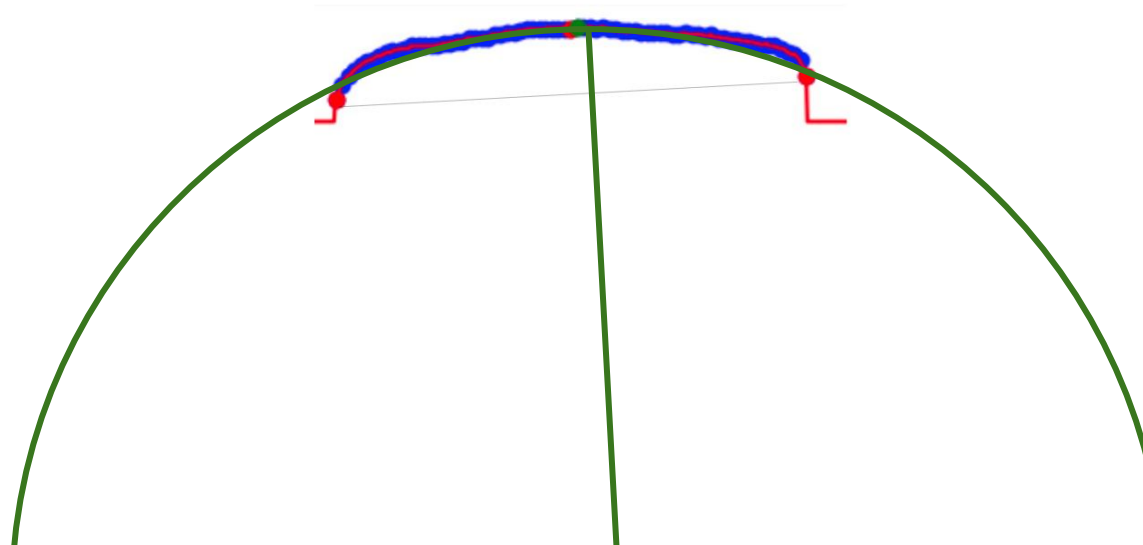 - Transversal radius (TR)
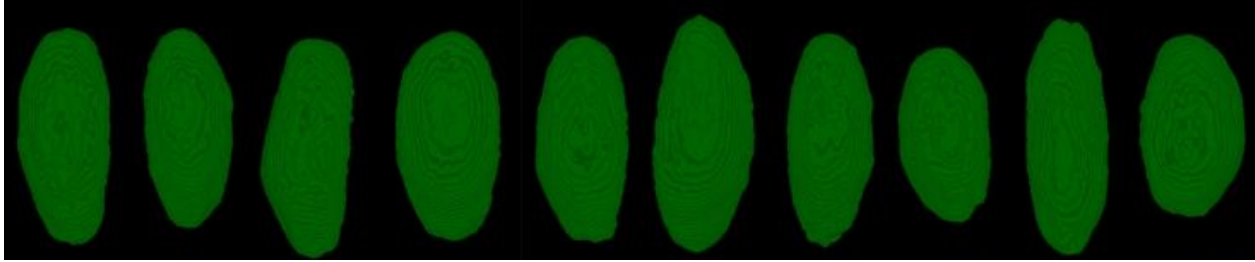
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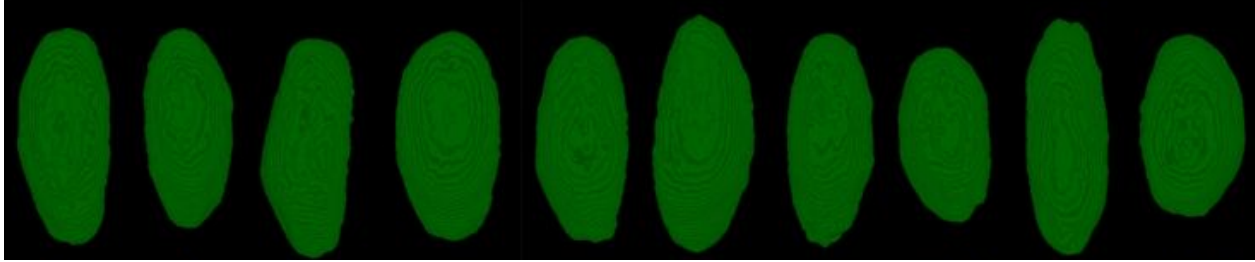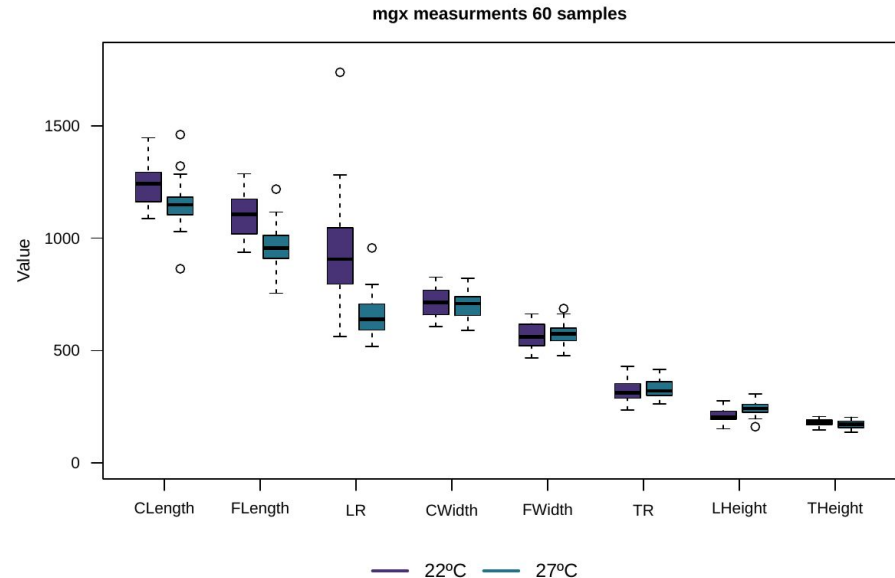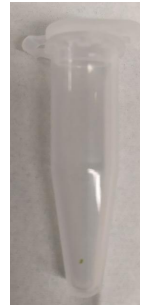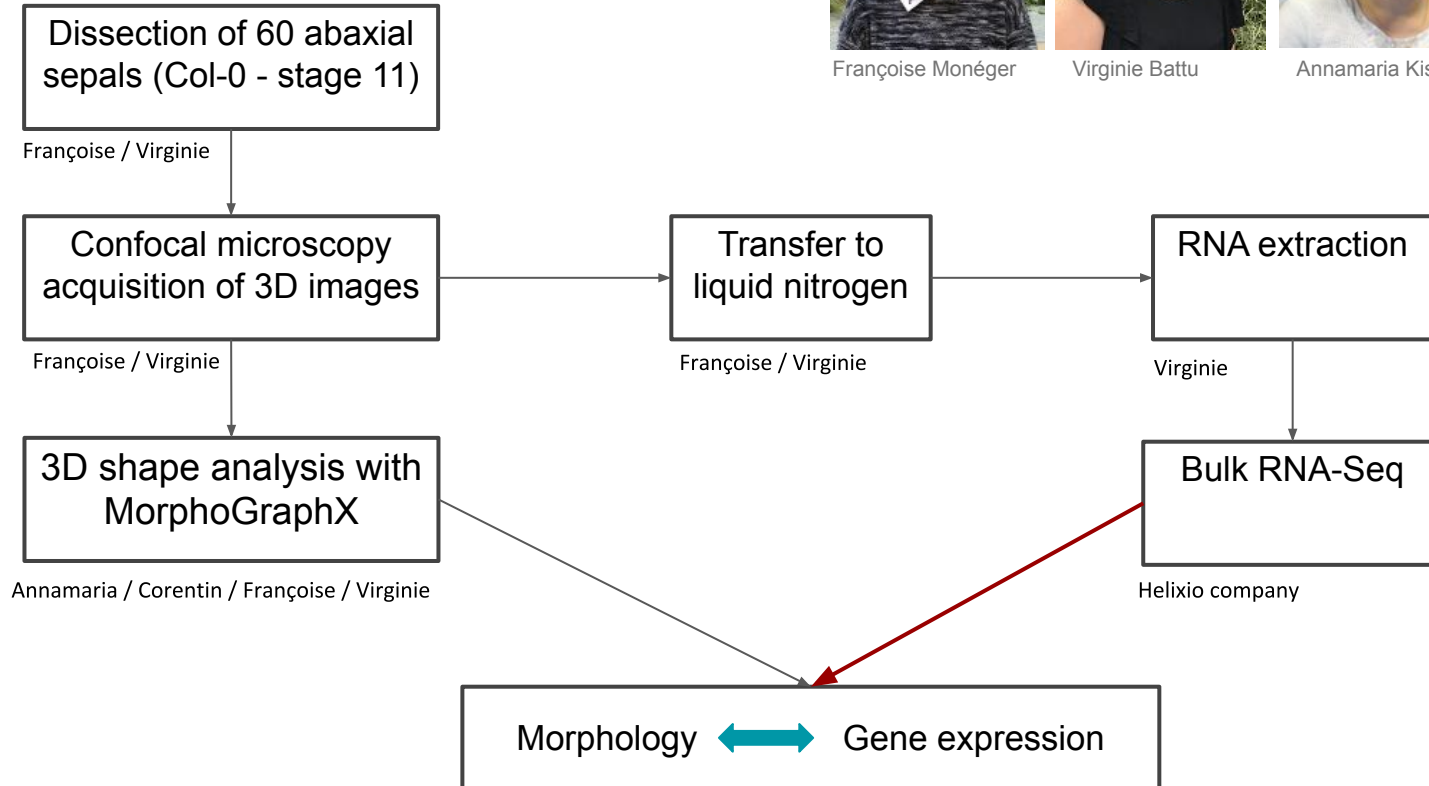 - Transversal height
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mgx measurments 60 samples

22ºC   27ºC

Experimental protocol

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Morphology ⟷ Gene expression

Sepal RNA-Seq analysis

- FASTQ files from Helixio (30 million reads per sample)
- Pseudoalignment with Kallisto
- Transcripts to genes with tximport
- Gene count normalization and filtering with DiCoExpress (Lambert et al., 2020)
- Exclusion of 4 outliers (56 samples)

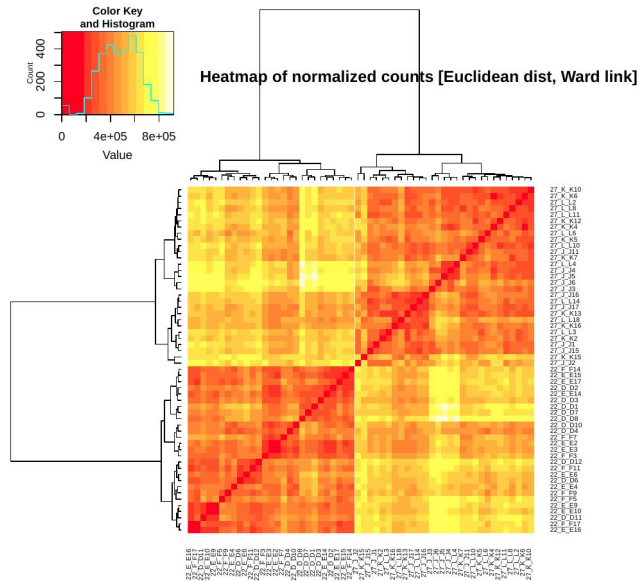➡️ 15858 genes expressed in sepals

Sepal RNA-Seq analysis

Work in progress

- Principal component analysis - DiCoExpress (Lambert et al., 2020)
- Weighted gene co-expression network analysis - WGCNA (Langfelder & Horvath, 2008)
- Sparse partial least squares regression - sPLS (Lê Cao et al., 2008)
- Highly variable gene detection
- Candidate gene co-expression network reconstruction
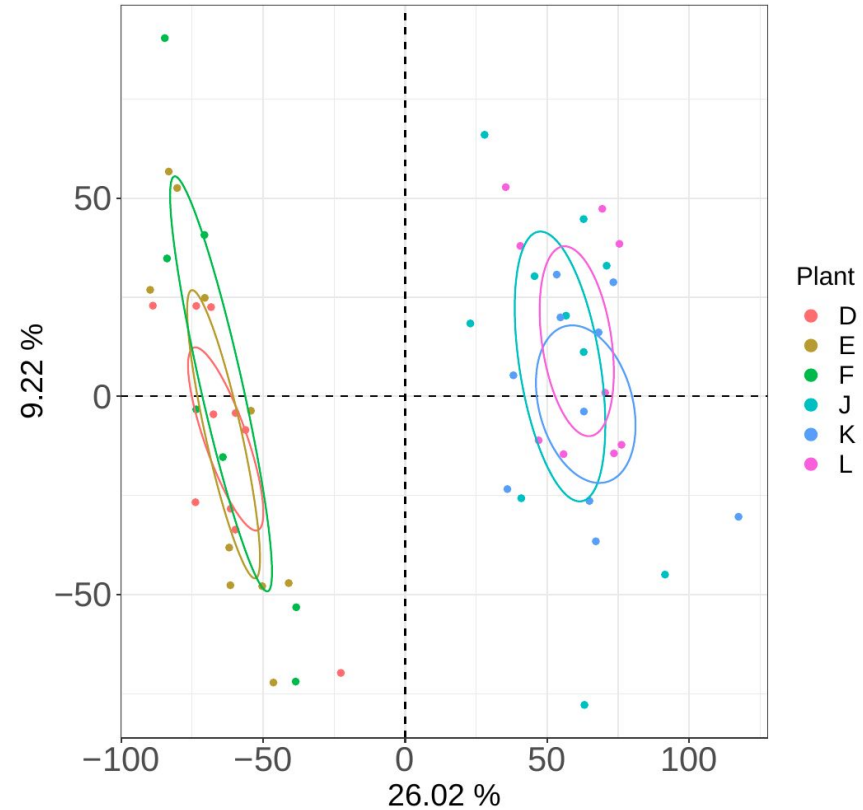- Differential gene expression analysis - DiCoExpress (Lambert et al., 2020)

Sepal RNA-Seq analysis

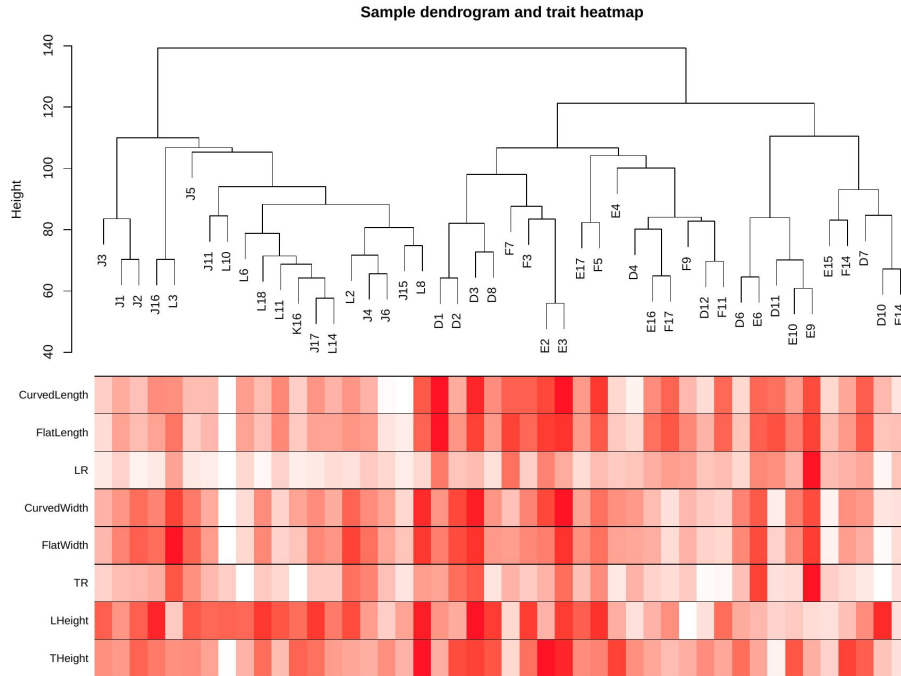DiCoExpress (Lambert et al., 2020)

- PCA separates 22º and 27º plants
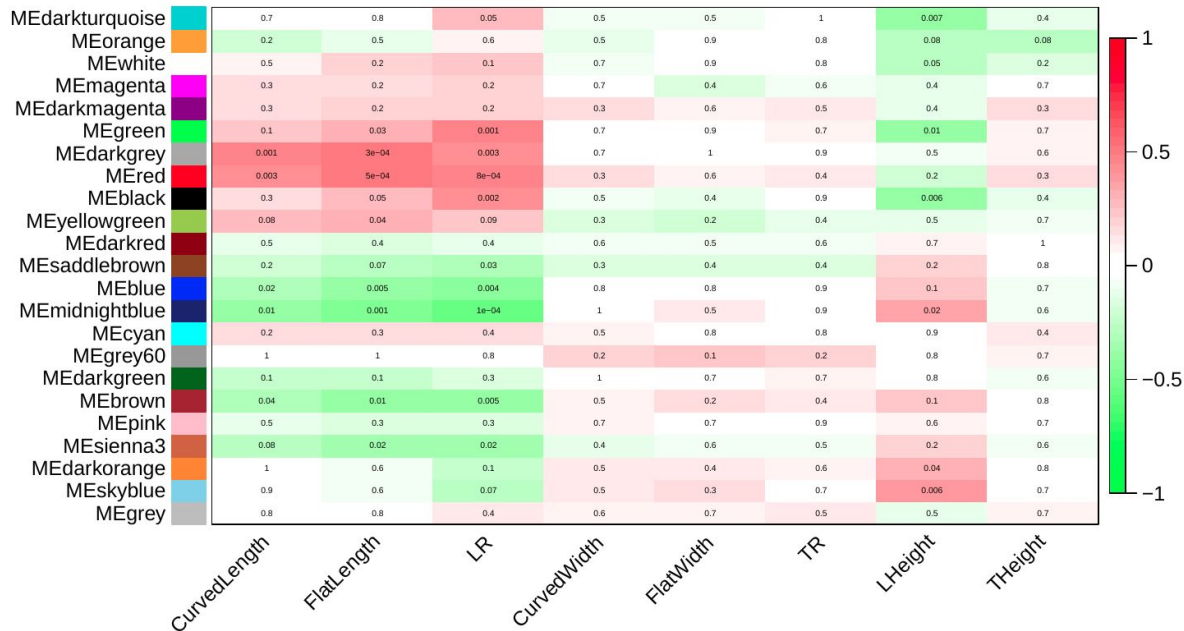

Heatmap of normalized counts [Euclidean dist, Ward link]

PCA on normalized counts

Sepal RNA-Seq analysis

WGCNA for 46 samples (Langfelder & Horvath, 2008)

Sample dendrogram and trait heatmap

Sepal RNA-Seq analysis
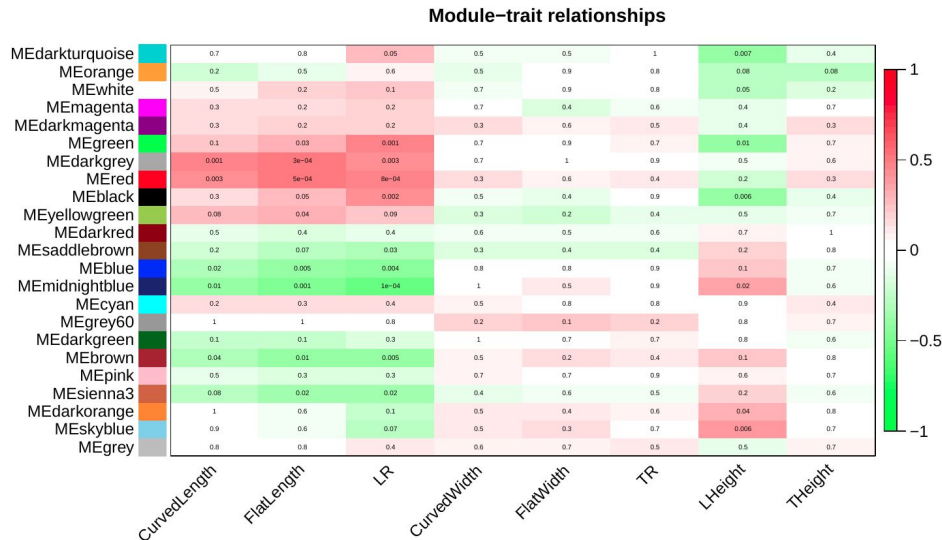
WGCNA for 46 samples (Langfelder & Horvath, 2008)

Module−trait relationships

Sepal RNA-Seq analysis

WGCNA for 46 samples (Langfelder & Horvath, 2008)

- Midnightblue module enriched in cell wall related genes
- P-value of t.test between 22º and 27º expression correlates with trait significance

Module−trait relationships

|	CurvedLength	FlatLength	LR	CurvedWidth	FlatWidth	TR	LHeight	THeight
MEdarkturquoise	0.7	0.8	0.05	0.5	0.5	1	0.007	0.4
MEorange	0.2	0.5	0.6	0.5	0.9	0.8	0.08	0.08
MEwhite	0.5	0.2	0.1	0.7	0.9	0.8	0.05	0.2
MEmagenta	0.3	0.2	0.2	0.7	0.4	0.6	0.4	0.7
MEdarkmagenta	0.3	0.2	0.2	0.3	0.6	0.5	0.4	0.3
MEgreen	0.1	0.03	0.001	0.7	0.9	0.7	0.01	0.7
MEdarkgrey	0.001	3e−04	0.003	0.7	1	0.9	0.5	0.6
MEred	0.003	5e−04	8e−04	0.3	0.6	0.4	0.2	0.3
MEblack	0.3	0.05	0.002	0.5	0.4	0.9	0.006	0.4
MEyellowgreen	0.08	0.04	0.09	0.3	0.2	0.4	0.5	0.7
MEdarkred	0.5	0.4	0.4	0.6	0.5	0.6	0.7	1
MEsaddlebrown	0.2	0.07	0.03	0.3	0.4	0.4	0.2	0.8
MEblue	0.02	0.005	0.004	0.8	0.8	0.9	0.1	0.7
MEmidnightblue	0.01	0.001	1e−04	1	0.5	0.9	0.02	0.6
MEcyan	0.2	0.3	0.4	0.5	0.8	0.8	0.9	0.4
MEgrey60	1	1	0.8	0.2	0.1	0.2	0.8	0.7
MEdarkgreen	0.1	0.1	0.3	1	0.7	0.7	0.8	0.9
MEbrown	0.04	0.01	0.005	0.5	0.2	0.4	0.1	0.8
MEpink	0.5	0.3	0.3	0.7	0.7	0.9	0.6	0.7
MEsienna3	0.08	0.02	0.02	0.4	0.6	0.5	0.2	0.6
MEdarkorange	1	0.6	0.1	0.5	0.4	0.6	0.04	0.8
MEskyblue	0.9	0.6	0.07	0.5	0.3	0.7	0.006	0.7
MEgrey	0.8	0.8	0.4	0.6	0.7	0.5	0.5	0.7

Sepal RNA-Seq analysis

- sPLS on DEFJKL plants
- Parameters: 4 components, 20 genes and 2 measurements per component.
- First component: LR and FlatLength

Sepal RNA-Seq analysis

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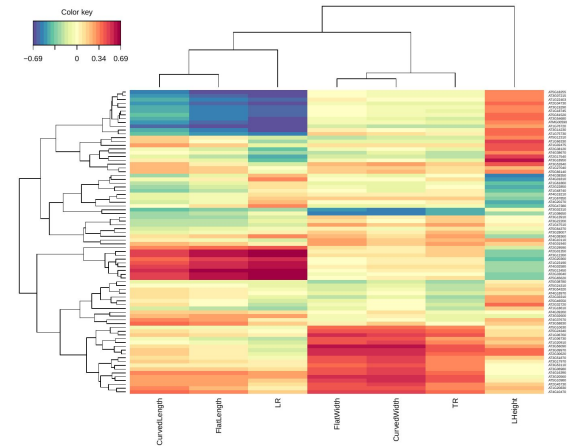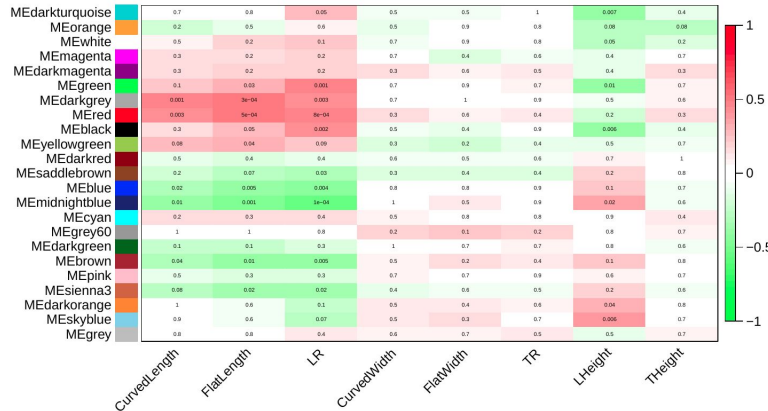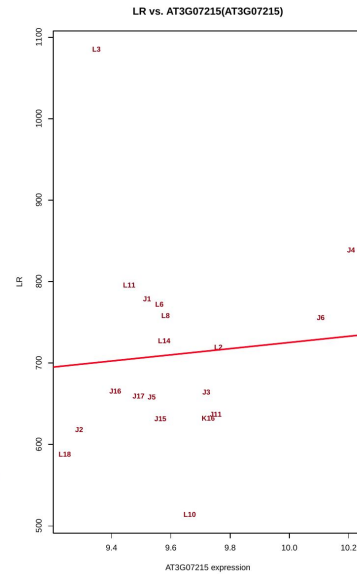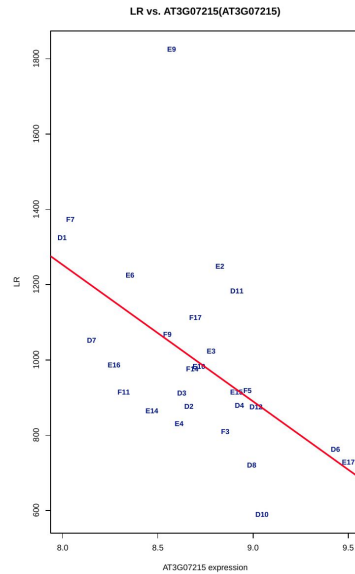- Top genes in first component are exactly the same the top genes in the midnightblue module!
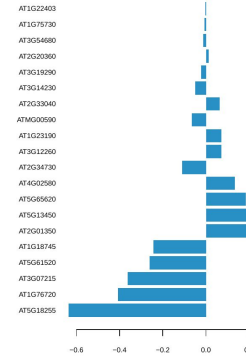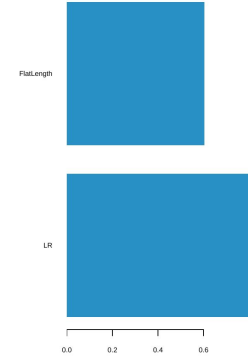
Sepal RNA-Seq analysis

- sPLS on DEFJKL plants
- The problem with genes correlated with LR
- The correlation is mainly due to differential expression and different LR between 22º and 27º

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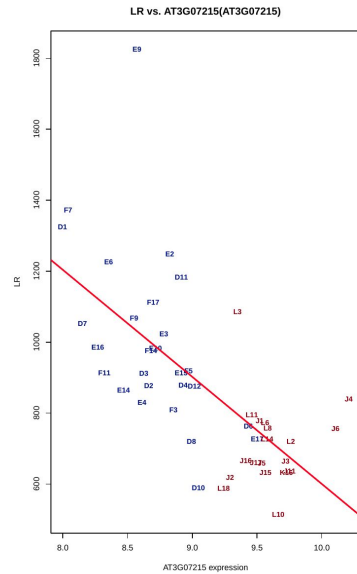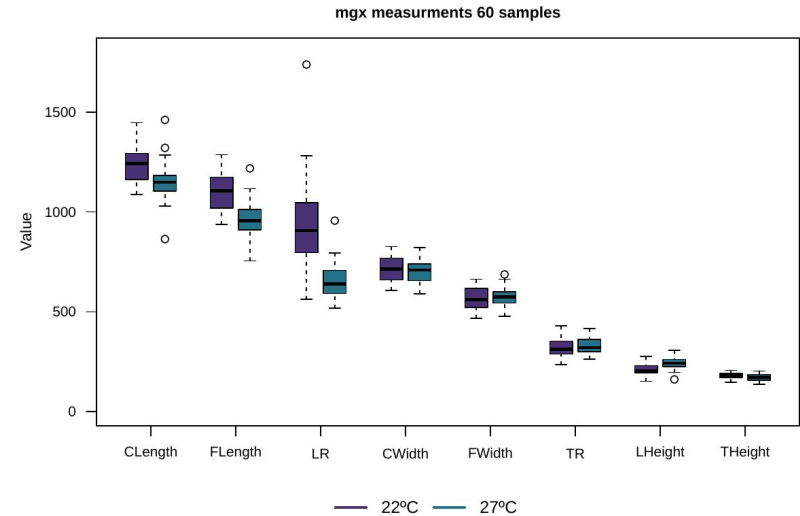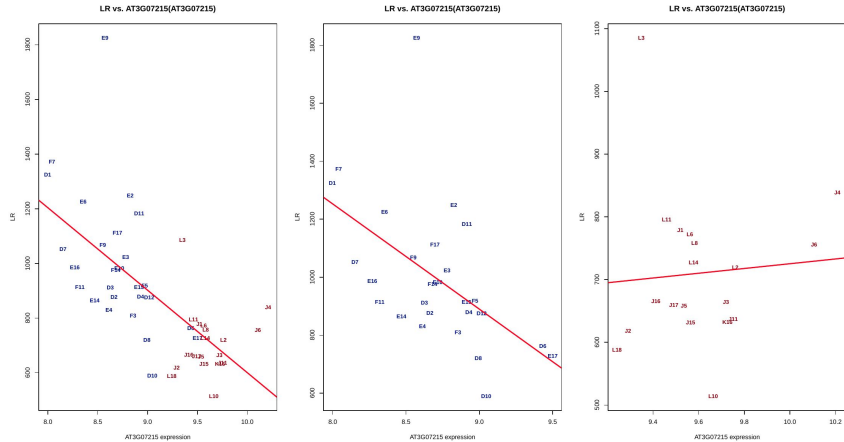
Sepal RNA-Seq analysis

- WGCNA on 22ºC and 27ºC plants separately
- For 22ºC plants:

Module−trait relationships
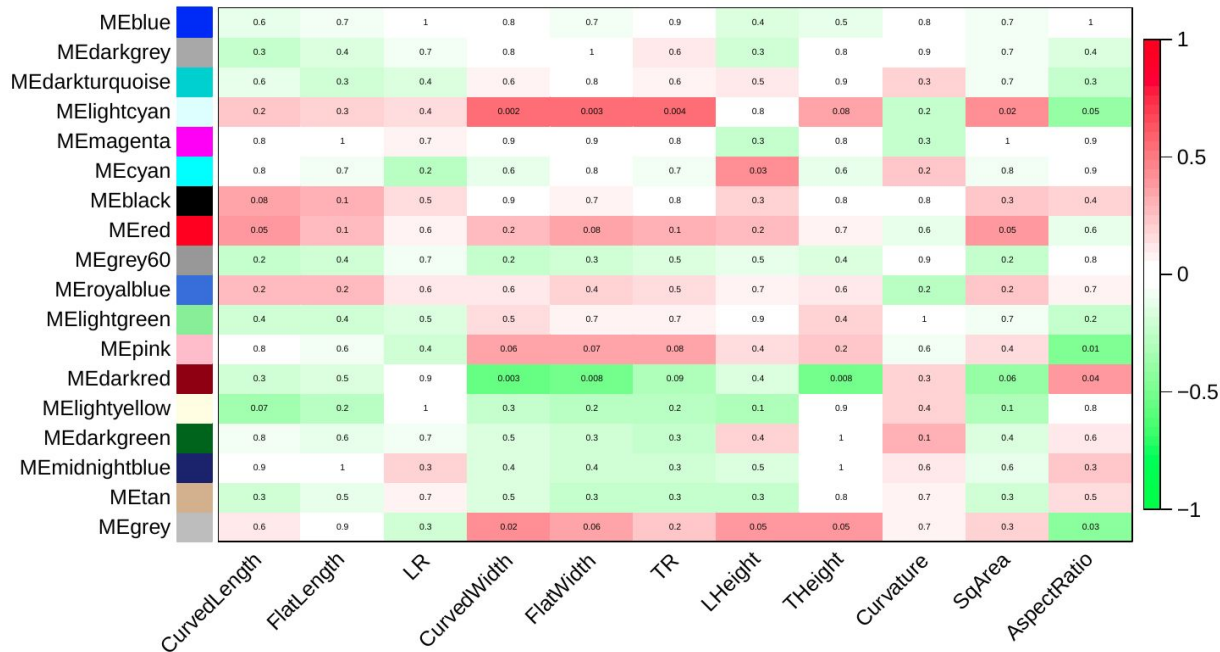
Sepal RNA-Seq analysis

- sPLS on 22ºC plants
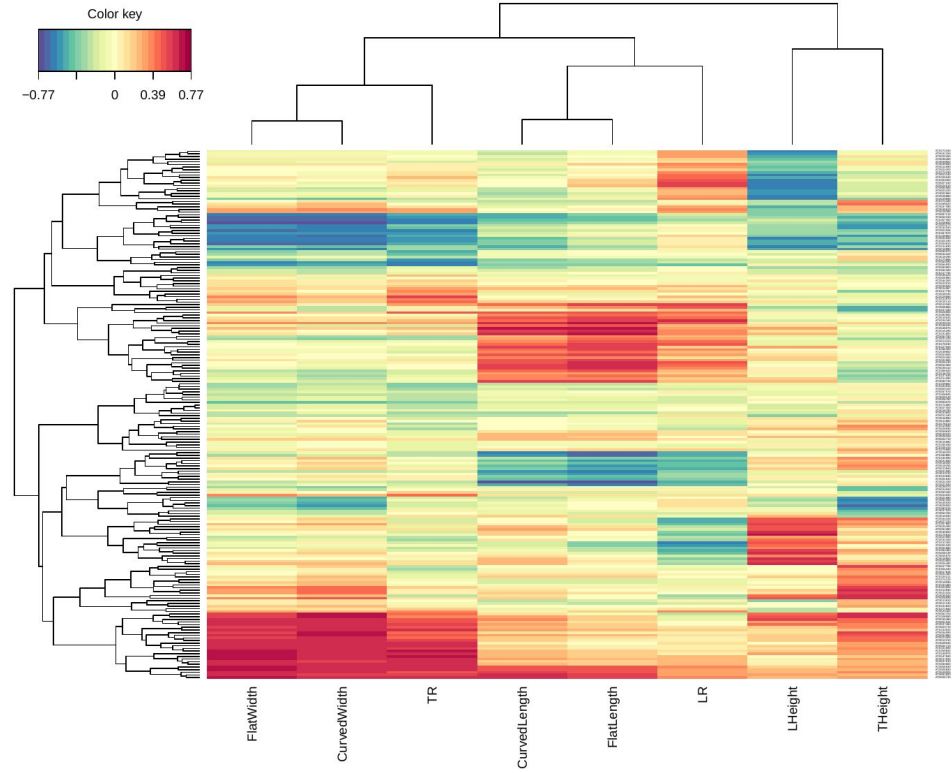
Sepal RNA-Seq analysis

- sPLS on 22ºC and 27ºC plants independently
- Find intersection
- Find candidate genes
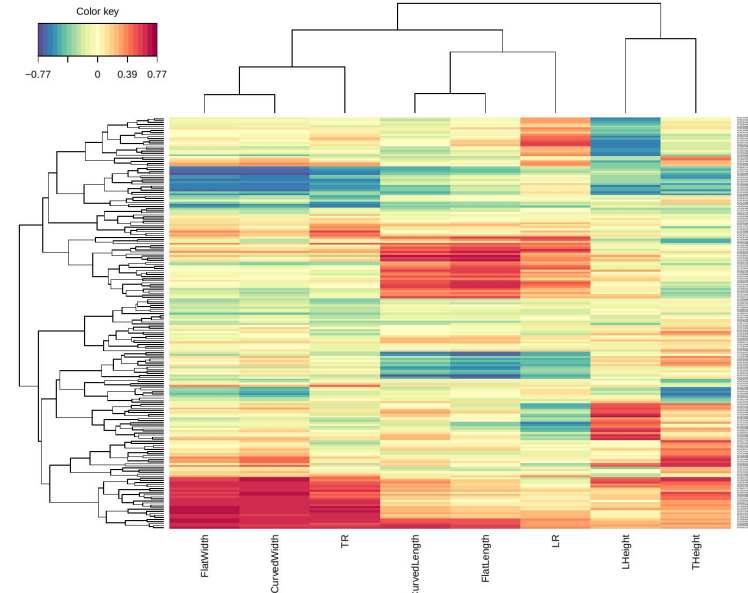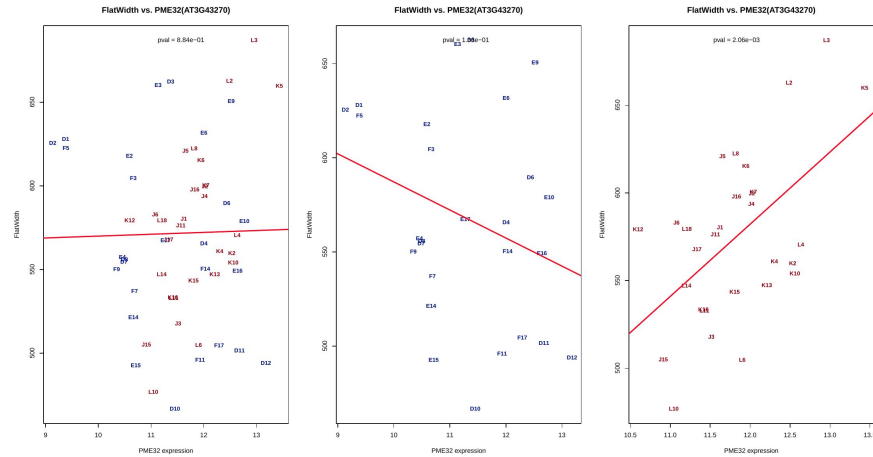
Sepal RNA-Seq analysis

- 2-degree small correlation network for candidate genes
  - Find top 5 genes that correlate with our candidate genes
  - And top 10 genes that correlate with them
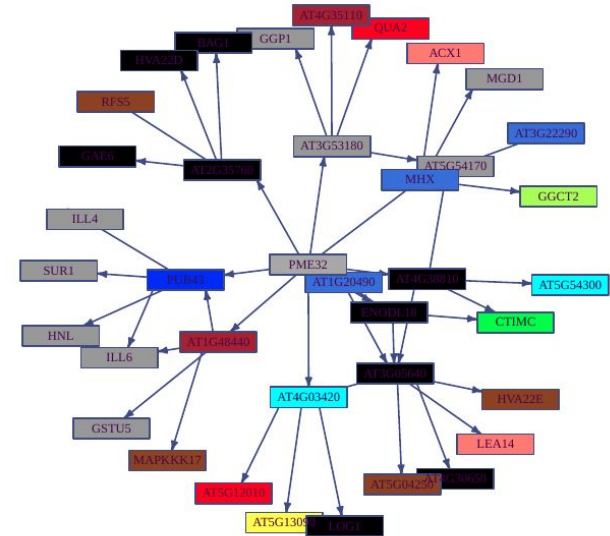
Sepal RNA-Seq analysis

Highly variable gene detection as in Cortijo et al. 2019
- Squared coefficient of variation corrected by trend
- Take top 5% genes
- 407 HVG common to both conditions
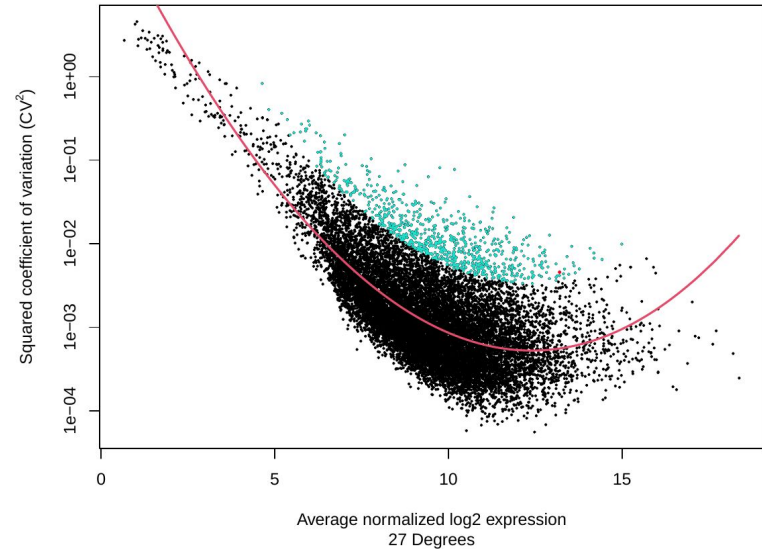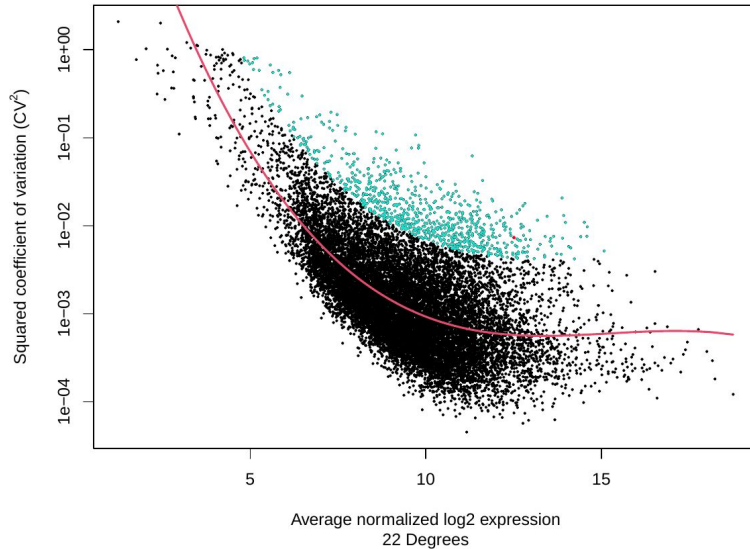
Sepal RNA-Seq analysis

DiCoExpress (with 56 plants plants)

- Differential expression (6 groups)
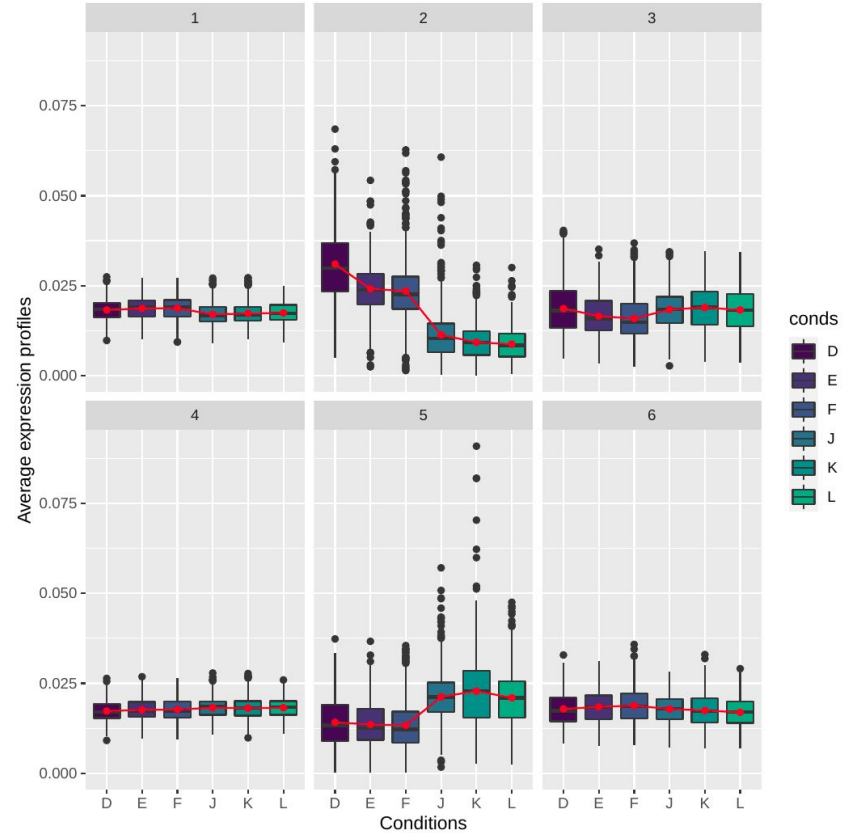
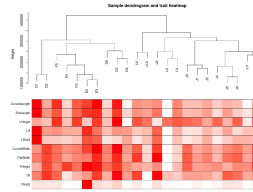# Sepal morphology and gene expression in *Arabidopsis thaliana*

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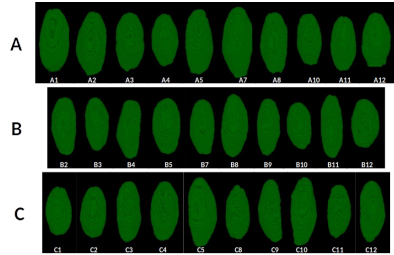
22° vs 27°
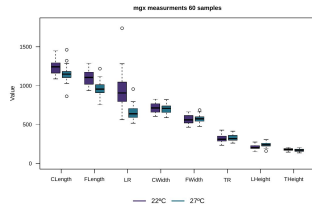
GO enrichment
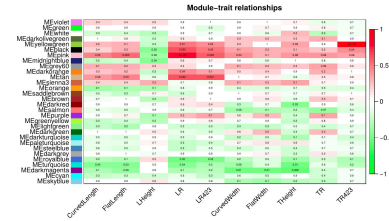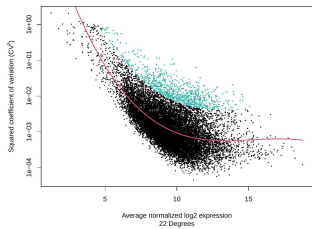
WGCNA

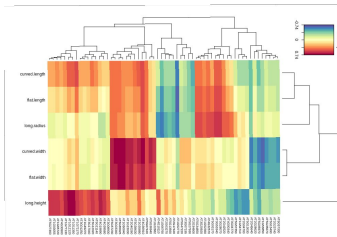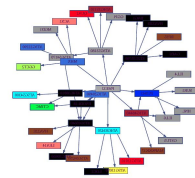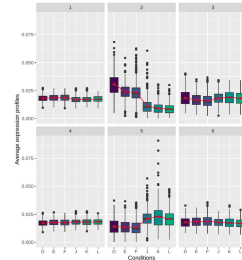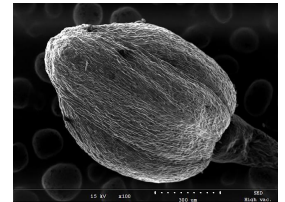Module-trait relationships

Variation in gene expression

sPLS

Correlation networks

Differential expression

Candidate genes

Acknowledgements

Françoise Monéger
Arezki Boudaoud
Virginie Battu
Annamaria Kiss
Corentin Mollier
Abigail Delgado-Vaquera

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Laboratoire Reproduction et Développement des Plantes

Marie-Laure Martin-Magniette
Marina Brasó-Vives