Sepal morphology and gene expression in Arabidopsis thaliana

NETBIO Meeting
08/12/2020

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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*
Sepal morphology and gene expression in *Arabidopsis thaliana*

Abaxial sepal from stage 11 flowers

Smyth et al., 1990 The Plant Cell, Vol. 2, 755-767
6 Arabidopsis thaliana wild type (Col-0) plants:

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

10 abaxial sepals from stage 11 flowers from each plant
Experimental protocol

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

Confocal microscopy acquisition of 3D images

Transfer to liquid nitrogen

RNA extraction

3D shape analysis with MorphoGraphX

Bulk RNA-Seq

Morphology ↔ Gene expression

Françoise Monéger  Virginie Battu  Annamaria Kiss  Corentin Mollier
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Annamaria / Corentin / Françoise / Virginie

Helixio company
3D shape analysis pipeline

- Confocal image
- Otsu normalization & xy smoothing
- Pre-treatment & EdgeDetect in MGX
- Extract size and shape measurements
- MorphoGraphX object
- Manual cropping

Françoise Monéger
Virginie Battu
Annamaria Kiss
Corentin Mollier
Size and shape measurements

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

- **Transversal**
  - Flat width
  - Curved width
  - Transversal height
  - Transversal radius (TR)
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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
Sepal RNA-Seq analysis

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

WGCNA for 46 samples (Langfelder & Horvath, 2008)
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 22° and 27° expression correlates with trait significance
- sPLS on DEFJKL plants
- Parameters: 4 components, 20 genes and 2 measurements per component.
- First component: LR and FlatLength

Sepal RNA-Seq analysis
Sepal RNA-Seq analysis

- sPLS on DEFJKL plants
- Parameters: 4 components, 20 genes and 2 measurements per component.
- First component: LR and FlatLength
- Top genes in first component are exactly the same the top genes in the midnightblue module!

![Module–trait relationships](image)

![Loadings on comp 1](image)
- sPLS on DEFJKL plants
- The problem with genes correlated with LR
- The correlation is mainly due to differential expression and different LR between 22° and 27°

Sepal RNA-Seq analysis
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 22° and 27°
**Sepal RNA-Seq analysis**

- **WGCNA on 22°C and 27°C plants separately**
- **For 22°C plants:**

### Module–trait relationships

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<th>MEPink</th>
<th>MEblack</th>
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<th>MElightblue</th>
<th>MElightgreen</th>
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<th>MEmagenta</th>
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The diagram shows the module–trait relationships with colors representing different modules and the intensity of the color indicating the strength of the relationship. The traits include CurvedLength, FlatLength, CurvedWidth, FlatWidth, TR, LHeight, Threight, Curvature, SArea, and AspectRatio.
Sepal RNA-Seq analysis

- sPLS on 22°C plants
Sepal RNA-Seq analysis

- sPLS on 22°C and 27°C 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
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
DiCoExpress (with 56 plants plants)

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

**Sepal morphology**

22° vs 27°

**GO enrichment**

**WGCNA**

**Module-trait relationships**

**Variation in gene expression**

**sPLS**

**Correlation networks**

**Differential expression**

**Candidate genes**
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