DMP du projet "FLOWER-LAYER"

Plan de gestion de données créé à l'aide de DMP OPIDoR, basé sur le modèle "INRAE - Trame générique projet v1" fourni par INRAE - Institut national de recherche pour l'agriculture l'alimentation et l'environnement.

Renseignements sur le plan

Titre du plan	DMP du projet "FLOWER-LAYER"
Domaines de recherche (selon classification de l'OCDE)	
Langue	fra
Date de création	2020-10-22
Date de dernière modification	2020-10-28

Renseignements sur le projet

Titre du projet

FLOWER-LAYER

Résumé

How cells coordinate their growth and division to generate organs with defined shapes is a long-standing question in biology. In plants, organs are formed by clonally-distinct cellular layers that remain independent throughout organ development. In the mean time, organ identity, shape and size are specified by master regulators. The main objective of the project is to understand how a master regulator can trigger organ development in all cellular layers in a coordinated manner, ensuring the robust acquisition of a proper identity, size and shape. We will tackle this question using *Petunia hybrida* flowers, whose petals are organized in a tube ending with colourful limbs. We obtained chimeric flowers in *Petunia hybrida*, originating from the layer-specific excision of a transposon inserted in the *PhDEF* gene, a MADS-box gene controlling petal development. These chimeras revealed that expression of *PhDEF* in the epidermis of the petal directs growth of the limbs, while its expression in the internal layers directs growth of the tube. This suggests that the tube and the limbs constitute two independent developmental modules, whose growth is controlled in a layer-specific fashion. Moreover, we obtained evidence for non-cell-autonomous effects between layers since *PhDEF* expression in the internal layers of the petal restores some petal epidermal features.

The objective of this project is to characterize the layer-specific PhDEF regulatory netwok in the *Petunia hybrida* petal, in order to understand how PhDEF can direct tube or limb growth independently from specific cellular layers. For this, after a detailed characterization of the chimeric flowers we obtained, we will recreate these chimeras as transgenic plants. This will allow us to identify PhDEF target genes and interactors specifically for each cellular-layer, by a combination of cell sorting, RNA-Seq, ChIP-Seq and co-IP. We will also identify the non-autonomous targets of PhDEF induced in one layer by expression of PhDEF in another layer. Finally we will functionally characterize some key target genes, aiming to understand how they can direct tube or limb growth in a layer-specific fashion. Altogether this project should advance the field of plant developmental biology by addressing how a master regulator directs organ growth and identity in all cellular layers in a coordinated manner.

Sources de financement

- Agence nationale de la recherche (ANR) : ANR-19-CE13-0019

Date de début 2020-04-01

Date de fin 2024-03-31

Produits de recherche :

1. scRNA-Seq pilot experiment (mixture of wt, def, star and wico mature petals) (Jeu de données)
2. Phenotypic data from star and wico flowers (Jeu de données)
3. Transcriptomic data from star and wico petals at 3 developmental stages (Jeu de données)
4. Transgenic Petunia hybrida lines to control cell-layer specific gene expression (Collection)

Contributeurs

Nom	Affiliation	Rôles
Marie Monniaux		• Coordinateur du projet • Personne contact pour les données (scRNA-Seq pilot, Phenotypic data, Transcriptomic data, Transgenic lines) • Responsable du plan de gestion de données

DMP du projet "FLOWER-LAYER"

Informations sur le plan de gestion

Question sans réponse.

Laboratoire de Reproduction et Développement des Plantes (RDP)
ENS de Lyon
46 allée d'Italie 69364 Lyon Cedex 07

22/10/2020

version 2 -24 mois

22/03/2022

Informations sur le projet

AAPG 2019

Agence Nationale de la Recherche (ANR)

ANR Jeune Chercheur/Jeune Chercheuse (JCJC)

ANR-19-CE13-0019

FLOWER-LAYER

Investigating the contribution of cell layers to petal development in Petunia flowers

Centre National de la Recherche Scientifique (CNRS), France

Question sans réponse.

Laboratoire de Reproduction et Développement des Plantes (RDP), ENS de Lyon

01/04/2020 - 31/03/2024 (42 months)

Présentation générale des données du projet

Phenotypic data from star and wico flowers

Star and wico flowers, originating from the cell-layer-specific excision of a transposon inserted into the petal identity gene PhDEF, were measured for tube length and limb area. Pictures were taken from the side and from the top (compressing the flower against a glass slide to flatten the limbs) and measurements were done with ImageJ on at least 2 flowers from 4 independent revertant lines.

Scanning electron microscopy pictures of petal epidermis were taken for wild-type, phdef, star and wico mature flowers, in the tube and in the limbs

Petal cross-sections stained with toluidine blue were imaged for wild-type, phdef, star and wico mature flowers, in the tube and in the limbs.

These data are integrated in a preprint deposited on bioRxiv: doi.org/10.1101/2021.04.03.438311

Transcriptomic data from star and wico petals at 3 developmental stages

Petals from wild-type, star and wico flowers (1 line) were collected at three developmental stages, and sepals from the phdef mutant were collected at the late stage only. For each stage, the petals from 2 flowers were collected, and 3 biological replicates were performed. Tissue was grinded and RNA was extracted on a fraction of the tissue. The total RNA, after rRNA depletion, was sequenced with Illumina Next Seq 500. Reads were mapped on the P. hybrida genome and differential expression was analyzed with DESeq2.

These data are integrated in a preprint deposited on bioRxiv: doi.org/10.1101/2021.04.03.438311

Transgenic Petunia hybrida lines to control cell-layer specific gene expression

At 6 months: transgenic Petunia hybrida lines were generated as an attempt to express PhDEF in one cell layer of the petal only in an inducible manner. Three constructs were generated and transformed in planta. Plants are now at the third generation after transformation.

At 24 months: problems of weak promoters, weak fluorescent proteins, suboptimal inducible system and transgene silencing have made us reevaluate the transgenic strategy initially planned. We are testing new layer-specific promoters now, we have switched to strong fluorescent proteins validated in the petunia petal, and switched to a different inducible system that works in petunia. It is too early to know if the constructs will be working or not.

scRNA-Seq pilot experiment (mixture of wt, def, star and wico mature petals)

Protoplasts from mature petals or sepals from wild-type, phdef-151, star and wico flowers were obtained with a 5-hour long digestion in an enzymatic mix. Protoplasts were purified and concentrated, and protoplasts from the 4 different genotypes were pooled together at equal concentrations. Single protoplast RNA was sequenced on the 10X Genomics Chromium platform of the Center for Cancer Research in Lyon (CRCL), aiming for 10 k cells and 80 k reads per cell.

Droits de propriété intellectuelle

Data will remain the property of the RDP laboratory.

Not applicable.

Confidentialité

Not applicable.

Not applicable.

If necessary, a confidentiality agreement will be established.

Partage des données à l'issue du projet

Phenotypic data from star and wico flowers

The ANR encourages open science and open data access.

The phenotypic data have been integrated into a preprint deposited on bioRxiv (doi.org/10.1101/2021.04.03.438311), that will be submitted to a peer-reviewed journal in the near future.

No

The phenotypic data have been integrated into a preprint deposited on bioRxiv (doi.org/10.1101/2021.04.03.438311), that will be submitted to a peer-reviewed journal in the near future. After acceptance by a journal the publication will be loaded on the open archive HAL depository.

No licence.

Already available on the preprint deposited on BioRxiv.

The publication will always be accessible on BioRxiv or HAL.

No.

Transcriptomic data from star and wico petals at 3 developmental stages

The ANR encourages open science and open data access.

The transcriptomic data have been integrated into a preprint deposited on bioRxiv (doi.org/10.1101/2021.04.03.438311), that will be submitted to a peer-reviewed journal in the near future.

Collaborators working on petal development might be interested in looking at deregulation of their genes of interest in our transcriptomic dataset.

No.

Before publication of the transcriptome, it will be shared to collaborators by transfering an excel file with normalized read counts through a secure file transfer system (system for big file transfer from the ENS de Lyon). Just before publication, the transcriptome will be deposited on the Transcriptome Shotgun Assembly (TSA) from NCBI and will be accessible for any user.

No licence.

The transcriptome data is already available next to the bioRxiv preprint.

Data will always be accessible through the TSA NCBI database.

Data will have a TSA master record.

Not applicable.

Transgenic Petunia hybrida lines to control cell-layer specific gene expression

The ANR encourages open science and open data access.

Seeds from validated transgenic lines will be sent to collaborators upon request, only after publication of the data generated with them. A MTA will specify the obligation of the recipient.

Not applicable.

Question sans réponse.

Question sans réponse.

Question sans réponse.

Question sans réponse.

scRNA-Seq pilot experiment (mixture of wt, def, star and wico mature petals)

The ANR encourages open science and open data access.

Reads and cell clusters from the scRNA-Seq pilot experiment will be shared freely with the community upon request, after it has been properly analyzed. Shortly before publication, raw and processed data will be deposited on NCBI's Gene Expression Omnibus (GEO).

Collaborators working on petal development might be interested in looking at gene expression in cell clusters of the petunia petal.

Question sans réponse.

Shortly before publication, raw and processed data will be deposited on NCBI's Gene Expression Omnibus (GEO).

Question sans réponse.

Shortly before publication of the results.

Data will always be available on NCBI's Gene Expression Omnibus (GEO).

GEO number

Question sans réponse.

Description et organisation des données

Phenotypic data from star and wico flowers

Excel file with phenotypic measurements.

None.

None.

Not applicable.

Date of modification always added to the name of the file. Raw data on the first sheet of the excel file, never modified.

Question sans réponse.

Transcriptomic data from star and wico petals at 3 developmental stages

Raw read counts are normalized with DESeq2. This generates a large txt or csv file with gene identifiers and normalized read counts, which is further used for analysis.

Excel file with description of samples and methodology for tissue collection.

None.

Not applicable.

The raw read counts data are never modified. Normalized read counts have been generated with DESeq2 and transferred into an excel file used for analysis. The name of this excel file contains the date of modification and old versions are stored in the same folder.

Question sans réponse.

Transgenic Petunia hybrida lines to control cell-layer specific gene expression

All transgenic lines are registered into a local excel file, then after validation they are registered into our data management sofwtare Labcollector, accessible for all members of the laboratory.

The plasmids used to generate the transgenic lines are registered into Labcollector. Each transgenic plant will get a unique identifier and details about the ascendency are registered into an excel file stored on our local server Biodata. This file is regularly saved.

None.

Not applicable.

Question sans réponse.

scRNA-Seq pilot experiment (mixture of wt, def, star and wico mature petals)

Raw read counts are treated with CellRanger to map reads to gene identifiers. Cell clusters are computed with Seurat (R package). Calculations are performed on the computing cluster of the ENS de Lyon (PSMN). Outputs from CellRanger and Seurat are stored in folders on our local server Biodata.

Excel or word files describe the samples and scripts applied to generate data. These files are stored in the same folder as the data, on our local server Biodata.

Question sans réponse.

Question sans réponse.

Question sans réponse.

Question sans réponse.

Stockage et sécurité des données

All data is stored on the servers of the laboratory (Biodata servers from ENS de Lyon) which are saved daily. An automatic daily back-up of the datafolder is made on the server: "http://sauvegardes.biologie.ens-lyon.fr" (which is provided by IT support services of the home institution, ENS de Lyon).

The pieces of data to be shared will be occasionnally shared with collaborators via the secure large file transfer system for ENS de Lyon. Occasional transfer to USB sticks for personal use on different computers.

Transcriptomic data: around 20 Gb.
scRNA-Seq data: several hundreds Gb.
Other pieces of data: negligible.

All data is stored on the servers of the laboratory (Biodata servers from ENS de Lyon) which are saved daily. For scRNA-Seq, data is stored on the local computing clusters (Calculus and PSMN).

The servers of the laboratory are located in the ENS de Lyon.

Yes.

Data will be shared to collaborators upon request.

All data will be shared freely upon request (open science access encouraged by the ANR).

Free access after discussion about the scientific use for the data.

Physical laboratory notebooks register the generation of biological data (sample collection for transcriptome, generation of transgenic lines). Informatic protocols, as well as all generated and analyzed data, are regularly updated and stored on local servers, and are accessible to all group members.

Archivage et conservation des données après la fin du projet

All raw data and the final analyses files will be kept for long term. Intermediate analysis files will be trashed.

Phenotypic data will be stored on local servers. Transcriptomic and scRNA-Seq data will be accessible for long term through the NCBI TSA or GEO database. Information about transgenic lines will be accessible by Labcollector.

The director of the laboratory will be responsible for the long term data conservation.

Question sans réponse.

The RDP laboratory pays for space on the local servers.