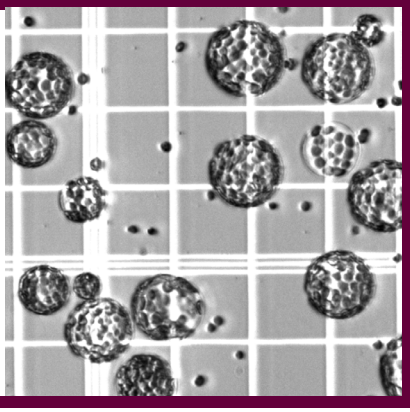
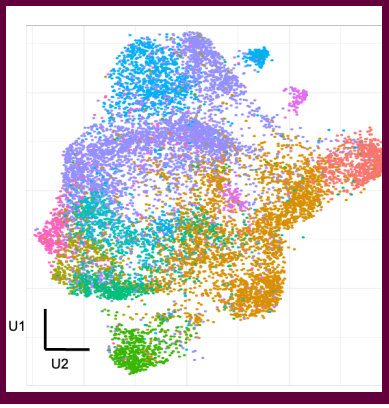
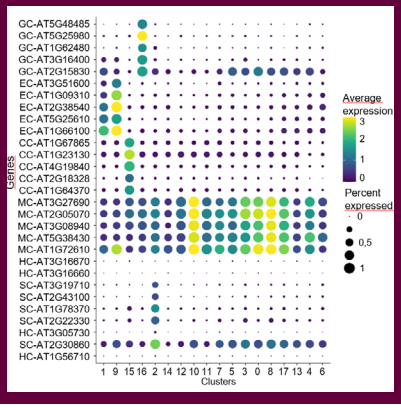
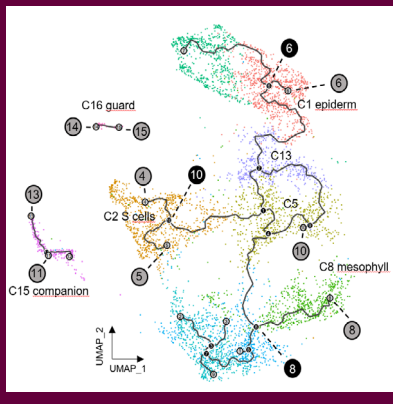
SPS Saclay Plant Sciences | université PARIS-SACLAY

SPS Summer School 2026









« Plant single cell RNAseq workshop: From current technologies to data analyses »

June 28th - July 3th, 2025

Versailles, France

Participant's guide

Contents

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INRAE Département BAP

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GDR Groupement de recherche BIMMM Bio-Informatique Moléculaire : Modélisation et Méthodologie

Waters™ Biosciences
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Singleron

Venue

Institute Jean-Pierre Bourgin for Plant Sciences
INRAE Centre Île-de-France Versailles-Saclay
Route de St-Cyr (RD 10)
78000 Versailles

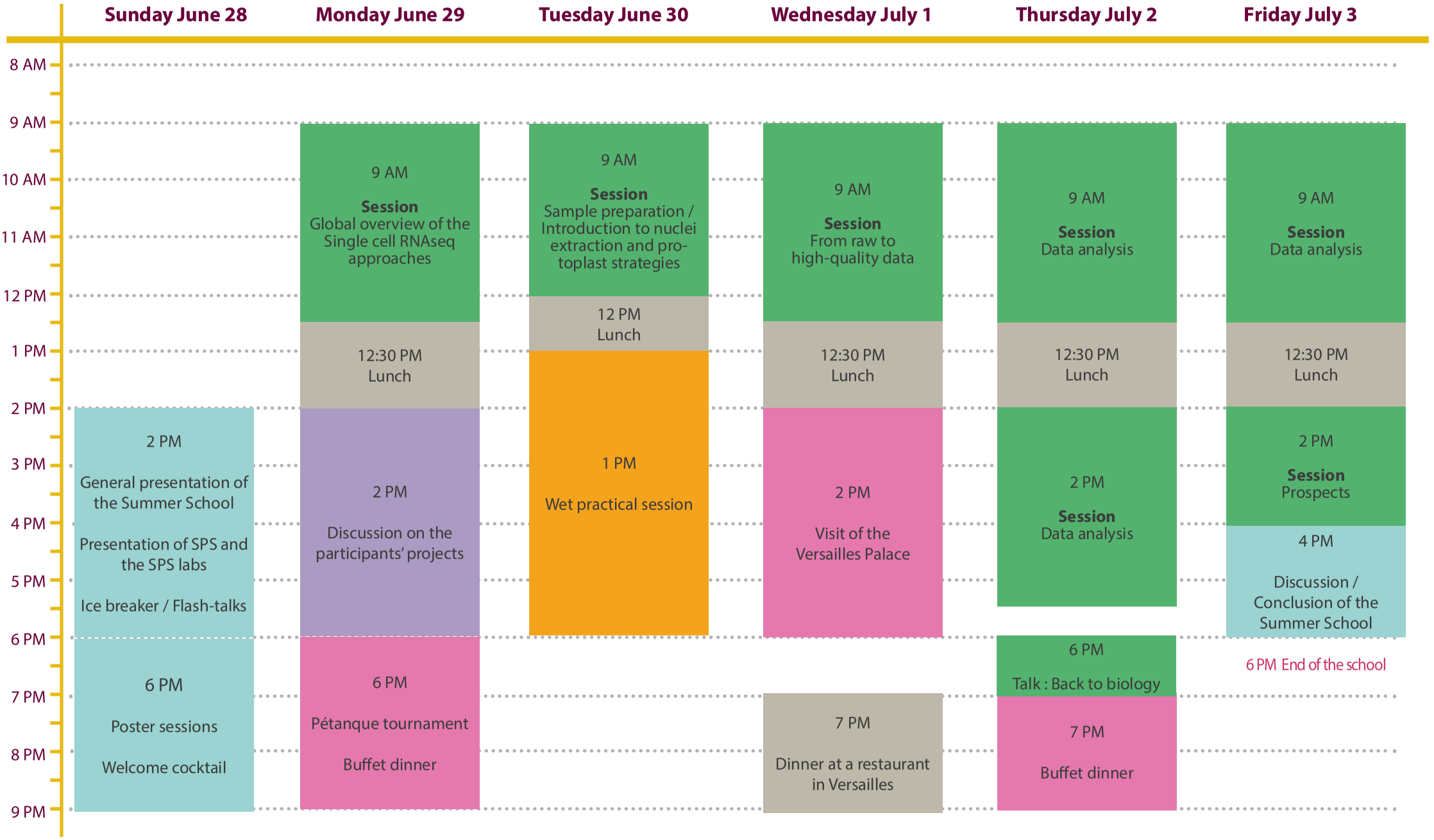
Hotel

Hôtel Le Bout du Parc
92-94 allée du Cordon Boisé
78000 Versailles
https://www.hotel-boutduparc.com/

Summer School e-mail address

SPS-Summer-School@inrae.fr

Planning at a glance



Program

Sunday June 28

2 PM – 6 PM: Welcome introduction
General presentation of the Summer School
Presentation of SPS and the SPS labs
Flash-talks of participants' research (2 to 3 Powerpoint slides, 5 min max.)

6 PM: Welcome cocktail and poster session

Monday June 29

9 AM – 10:30 AM: Session « Global overview of the Single cell RNAseq approaches » (Building 19)
"Well defining your biological question and what type of data can you expect from Single cell RNAseq" - *In this first part we will discuss on how to properly design your experiment depending on your biological question, and the type of data and analyses you can expect from scOmics.*

10:30 AM – 11 AM: Coffee break (Building 19)

11 AM – 12:30 PM: Session « Global overview of the Single cell RNAseq approaches » (Building 19)
"Overview of the main Single cell technologies" - *We will present the pros and cons of each main sc technologies, as well as the pros and cons to do single cell vs single nuclei. This will help you identify the best option to answer your biological question.*

12:30 PM – 2 PM: Lunch at the INRAE cafeteria

2 PM – 6 PM: Discussion with the participants (Building 19)
Using the morning session, we will discuss what would be the best technologie and strategy to answer your biological question. Particiants will be in group of 2-4 to discuss and then present their conclusions.

6 PM – 9 PM: Buffet dinner and pétanque tournament (Building 43 and/or outside of the INRAE cafeteria)

Tuesday June 30

9 AM – 10:30 AM: Session « Sample preparation » (Building 19)
Freya Persyn - "An introduction to flow cytometry for plant samples"

10:30 AM – 11 AM: Coffee break (Building 19)

11 AM – 12 PM: Introduction to nuclei extraction and protoplast strategies (Building 14)
- *Introduction to nuclei extraction and protoplast strategies.*
- *Tricks and Tips*

12 PM – 1 PM: Lunch at the INRAE cafeteria

1 PM – 6 PM: Wet practical session (Building 14)
- *Nuclei extraction from different plant tissues*
- *Measurement of nuclei amount and quality assessment using microscopy*
- *Loading and retrieval of nuclei using a BD Rhapsody and Singleron flow cell*

Dinner not included in the Summer School

Wednesday July 1

9 AM – 10:30 AM: Session « From raw to high-quality data » - Part 1 (Building 19)
"From reads to filtered count matrix"
Cellranger websummary report
Introduction to RStudio and Seurat object
Practical session with R: from cellranger output to a filtered matrix

10:30 AM – 11 AM: Coffee break (Building 19)

11 AM – 12:30 PM: Session « From raw to high-quality data » - Part 2 (Building 19)

12:30 PM – 2 PM: Lunch-box / Trip to the Versailles Palace

2 PM – 6 PM: Tour of the Versailles Palace (BE ON TIME, see next page)

7 PM – 9 PM: Dinner at the restaurant « Au chien qui fume », 72 rue de la Paroisse, 78000 Versailles

Thursday July 2

9 AM – 10:30 AM: Session « Data analysis » - Part 1 (Building 19)
"From filtered matrix to cells clusters"
Normalisation
Dimension reduction and clustering Selection of highly variable genes
PCA
Visualisation
Clustering

10:30 AM – 11 AM: Coffee break (Building 19)

11 AM – 12:30 PM: Session « Data analysis » - Part 2 (Building 19)
"From filtered matrix to cells clusters"
Practical session

12:30 PM – 2 PM: Lunch at the INRAE cafeteria

2 PM – 5:30 PM: Session « Data analysis » - Part 3 (Building 19)
"From cells clusters to annotated cells"
Data integration
Annotation of cell clusters
Differential analysis
Practical session

5:30 PM – 6 PM: Break (Building 19)

6 PM – 7 PM: Session « Back to biology » (Building 19)
"Exploring cell specialization and cell coordination in plant leaf exposed to pathogens by using scRNAseq" - *The immune response of a cell depends both on its identity and on the kinds of immunogenic signals it perceives. Unravelling the intricacy of these responses within a multicellular organ is essential to better understand how plants defend themselves against invaders and to unveil new ways of making plants more resilient.*

7 PM – 9 PM: Buffet dinner (Building 43 and/or outside of the INRAE cafeteria)

We are grateful to:
- the INRAE MIGALE bioinformatics facility (MIGALE, INRAE, 2020. Migale bioinformatics Facility, doi: 10.15454/1.5572390655343293E12) for providing help and/or computing and/or storage resources,
- Lorette Noiret (Sorbonne Université, Paris) for providing ressources for these courses and practical sessions.

Friday July 3

Attention:
The rooms have to be vacated after breakfast (check-out). Make sure your things are packed and ready on time.

9 AM – 10:30 AM: Session « Data analysis » - Part 4 (Building 19)
Trajectory analyses
Practical session

10:30 AM – 11 AM: Coffee break (Building 19)

11 AM – 12:30 PM: Session « Data analysis » - Part 5 (Building 19)

12:30 PM – 2 PM: Lunch at the INRAE cafeteria

2 PM – 4 PM: Session « Prospects » (Building 19)
Additional experiment and validation methods to annotate your cell cluster:
- Spatial transcriptomic overview and the different current technologies,
- Multiplexed in situ hibridization using HRC-RNA Fish,
- Tissue embeding methods,
- scATACseq and Multiomic.

4 PM – 6 PM: Discussion / conclusion of the Summer School (Building 19)

6 PM: End of the Summer School

Abstracts of the participants

Blue light-dependent development in the model organism Ectocarpus: new insights into photo-development in brown seaweed

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Light quality is a major determinant of developmental patterning in photosynthetic organisms, but the photoreceptor-mediated mechanisms underlying this process in brown algae—a lineage with independently evolved multicellularity—remain poorly understood. Here, we investigated the model brown alga *Ectocarpus*, exposing it to red (656 nm) and blue (445 nm) light to dissect photoreceptor-mediated developmental responses by a series of developmental, physiological and transcriptomic analyses. Blue light enabled normal development, with partheno-sporophytes forming basal and upright sporangia-bearing filaments. In contrast, red light impaired normal development, inducing mono-polar germination with aberrant cell morphology. Remarkably, supplementing red light with low-intensity blue light (2 µmol photons m^{-2} s^{-1}) restored normal growth and development, revealing a highly sensitive blue light photoreceptor. Dose-response experiments indicated that sub-threshold blue light intensities (<1 µmol photons m^{-2} s^{-1}) rescue growth but not upright filament development, suggesting at least two distinct blue light signaling pathways. Similar light-dependent developmental defects and rescue patterns were observed in the haploid gametophyte stage. Photosynthetic performance increased within 6 h of blue light exposure, preceding visible cell differentiation and growth resumption (detectable after ~72 h). Transcriptomic analysis of red-grown filaments exposed to blue light revealed 1553 differentially abundant transcripts, including rapid regulation of photosynthesis-related genes (LHCs), sigma factors, and TIC20-like genes. Ongoing CRISPR-Cas12a-mediated deletions of photoreceptor genes aim to elucidate the molecular basis of light signaling in brown algae. These findings uncover blue light as a key regulator of development and physiology in *Ectocarpus* and provide new insights into photoreceptor-mediated signaling in brown algae.

Engineering integrase-based genetic recorders to link cell lineage and gene expression history during lateral root development

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Understanding how individual cells transition toward new developmental fates is a central challenge in plant developmental biology. During lateral root formation in *Arabidopsis thaliana*, only a subset of pericycle cells becomes competent to initiate new organs. These founder cell transitions involve dynamic changes in gene expression and cell identity that are difficult to capture using conventional approaches. While single-cell transcriptomics has greatly improved our ability to characterize transcriptional heterogeneity within plant tissues, linking cellular states to lineage relationships and the history of gene expression events remains a major challenge.
In this project, we are developing synthetic genetic tools based on serine integrases to record cellular events directly in plant genomes. Integrase-mediated recombination systems can introduce stable DNA barcodes or record gene expression activity within individual cells, creating genomic records that can later be recovered together with transcriptomic information.
Using *Arabidopsis thaliana* as a model system, we are engineering integrase-based recording circuits designed to operate during lateral root initiation. To accelerate construct development, we are also establishing a rapid prototyping pipeline based on transient expression in plant protoplasts combined with flow cytometry to quantify recombination efficiency and circuit activity.
In the longer term, we aim to integrate these lineage-recording systems with single-cell and spatial transcriptomic approaches to link transcriptional states, lineage relationships, and spatial organization during root organogenesis. This work aims to develop new tools that complement single-cell transcriptomics and enable deeper reconstruction of plant developmental trajectories.

Cellular and Physiological Reponses of Arctic Diatoms to Sea Ice Conditions

Rory Burke [1], Arthur Plassart[2,3], Chris Bowler [3], Marcel Babin [2], Eric Maréchal [1]

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Diatoms are a group of unicellular photosynthetic eukaryotes that have successfully colonized virtually every marine and freshwater habitat on the planet. They are thought to be responsible for up to 40% of all marine primary production, and thus contribute substantially to global biogeochemical cycles of carbon and other macronutrients. In the arctic ocean, diatoms are the most important primary producers, and are capable of growing underneath and even within the sea ice despite the incredibly harsh abiotic environment this habitat represents. Despite their vital importance to this vulnerable ecosystem, relatively little is known about how diatoms cope with sea ice conditions at a molecular level. Here, we describe the survival, growth and physiology of three different arctic diatom species under high salinity and low temperature stress representative of the sea ice environment. These species display differing responses to such conditions, indicative of their specific adaptations to varying extents of sea ice cover throughout the seasons. These results serve as the basis for ongoing transcriptomics and lipidomics analysis of the diatom species undergoing sea ice associated abiotic stress, aimed at describing the cellular and molecular adaptations necessary for survival in this harsh environment.

Spatiotemporal characterization of cellular changes at the grapevine graft junction

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Grafting is a fundamental technique in viticulture, yet the cellular mechanisms underlying graft success remain poorly understood. Currently, no study has investigated the grapevine graft junction at singlecell resolution. This project aims to characterize the spatiotemporal cellular dynamics at the graft interface using single-cell RNA sequencing (scRNA-seq) on protoplasts, combined with spatial transcriptomics (Stereo-seq). By identifying distinct cellular clusters and reconstructing cell trajectories, we aim to map the populations present at the interface of grafted partners, which are critical for adhesion and successful union. Preliminary experiments have highlighted technical challenges in protoplast isolation, including low yield and limited reproducibility, emphasizing the need for specialized training. The combination of single-cell and spatial transcriptomics will allow unprecedented insight into the molecular and cellular mechanisms driving graft compatibility and success in grapevine. This study will provide key information on cell identity, spatial organization, and temporal changes during the grafting process, potentially informing future breeding and propagation strategies. The results will also contribute to a broader understanding of plant grafting mechanisms and provide a framework for applying single-cell approaches in woody species.

Epiplasticity: uncovering the mechanism and ecological significance of heritable induced resistance in *Arabidopsis thaliana*

Mia Cohen[1,2], Chris Cazzoneli[2], Luke Dunning[1], Saima Shahid[1], Jurriaan Ton[1]

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Plants experience threats from pests and diseases, which they must resist to survive. After exposure to a stress, plants develop immunological memory, which improves their ability to resist recurrent attacks. In some cases, this memory can be transmitted to progeny, as heritable induced resistance (HIR). Previous work has shown that HIR depends on active DNA de-methylation. However, the exact epigenetic mechanisms driving trans generational memory, including the epigenetic loci responsible, are unknown. Identifying specific epialleles controlling HIR has been confounded by the intrinsic variability of the HIR response. To address the ecological function of this variability, I will explore the costs and benefits of HIR under ecologically relevant conditions, by tracking the reproductive fitness of different *Arabidopsis* populations grown in competition with each other. To enable identification of specific epi-alleles, despite variation in response, I will expose multiple generations of *Arabidopsis* in high-competition microcosms to disease pressure. I expect this to drive positive selection for HIR-conferring epi-alleles, and therefore reduce the variability of the HIR phenotype. This will facilitate the discovery of epi-alleles by global DNA-methylome analysis. In parallel, I will use single-cell RNA-seq to track gene expression changes in the reproductive tissue of HIR-conferring *Arabidopsis*. I will identify which cell-type specific transcriptional changes enable the transmission of stress memory to the next generation. Thus, this project will further our understanding of HIR, from mechanistic underpinnings to ecological significance.

Dissecting the Molecular Basis of Root Cortical Parenchyma Developmental Trajectories Using Single-Cell Transcriptomics

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Roots are the primary interface between plants and the soil environment, regulating water and nutrient uptake, perceiving mechanical and chemical signals, and interacting dynamically with the rhizosphere. Within the root, cortical parenchyma represents a key tissue contributing to stress adaptation, as their developmental plasticity enables structural and functional adjustments to local soil constraints. However, the molecular and cell-type specific mechanisms governing developmental trajectories of these cells remain poorly understood. Our aim is to investigate tissue-specific downstream responses within cortical parenchyma cells to environmental stresses (e.g. development of sclerenchyma, aerenchyma) using single-cell transcriptomic technologies. By leveraging maize genotypes that exhibit natural variation in cortical layer development, we seek to identify novel molecular pathways regulating cell plasticity and evaluate expression trajectories across distinct tissues in the cortex. This high-resolution dataset will be used to validate known candidate genes and discover new genetic regulators of root cortical plasticity.

Understanding the Formation of Root Barriers: Periderm Development Using Single-Cell Approaches

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During radial growth of plant organs, the vascular cambium provides new vasculatures while the cork cambium forms a protective barrier, shielding the vasculature from the environment. These cambia form concentric rings and divide bifacially. The vascular cambium produces wood inward and bast outward, while the cork cambium forms phelloderm inward and cork outward, generating the periderm. Our previous studies using the *Arabidopsis* root as a model revealed that auxin is essential for the initiation and maintenance of the cork cambium. Auxin is also required for vascular cambium formation, yet the molecular mechanisms determining auxin these two cambia specific responses in both cambia remain largely unknown. Cytokinin is known to interact with auxin in meristem regulation and is required for vascular cambium initiation, but its role in periderm formation is still poorly understood. To address cambia specificity, we performed transcriptome profiling in plants with specifically impaired auxin signaling in either the cork or vascular cambium. Several genes were downregulated in both cambia, suggesting the existence of shared regulatory components. In parallel, we employed single-cell RNA sequencing of cambia-enriched tissues to understand vascular and cork cambium identities and identify novel regulators of cambia stem cells. We are currently deciphering the auxin-cytokinin crosstalk in periderm development through spatiotemporal perturbation approaches and we could show that both auxin and CK act synergistically to promote periderm development. In summary, our research sheds light on regulatory networks governing radial growth, with the ultimate goal of providing novel solutions for increasing wood and cork production.

Gene Regulation in Plant Meristematic Stem Cells upon Viral Infections

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Plant viral infections can lead to complete crop failure and to both significant yield and economic losses. However, the meristem is often the only tissue that remains virus-free due to the host's antiviral defenses. Meristem culture is used to obtain virus-free plants from infected material, yet the molecular mechanisms underlying viral exclusion from meristematic stem cells remain poorly understood. Although many plant viruses are prevented from entering the meristem through host pathways such as RNA interference, some viruses can still invade stem cells. Thus, viral germline infection may depend on the virus ability to infect stem cells within the shoot apical meristem (SAM). While exclusion is the most common outcome, invasion can enable vertical transmission or trigger long-lasting antiviral responses, including recovery, defined by the absence of symptoms and viral accumulation in newly developing tissues following an acute infection. Gene expression regulation behind these mechanisms remains largely unexplored. Using *Arabidopsis thaliana* as a model, infections with meristem-excluded TuMV and TCV, together with the invasive, recovery-triggering TRV, will be analyzed to obtain and compare RNAseq data from rare meristematic nuclei upon challenge by these viruses. This dataset will be used to identify candidate genes involved in viral exclusion, invasion, recovery, and, potentially, vertical transmission. Understanding the interaction between viruses and meristematic stem cells can be the basis for developing targeted antiviral strategies for crop protection.

Decoupling the distinct cell communication mechanisms that regulate protein mobility under stress

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Intercellular communication through plasmodesmata is key for plant development by enabling mobility of signaling molecules, proteins, and sugars. To restrict molecules from entering the channel, callose is synthesized at the plasmodesmata aperture. Callose-independently, MCTP (Multiple C2 Transmembrane Protein) physically impedes molecular movement through the channel. SHORTROOT (SHR) moves through plasmodesmata to maintain the endodermis, but phosphate starvation initiates callose deposition to block SHR movement to the quiescent center to facilitate meristematic exhaustion. This suggests that both callose-dependent and callose-independent mechanisms may be initiated in parallel by different tissues. To decouple the roles of callose-dependent from callose-independent mechanisms, we quantified SHR movement through root meristematic tissues in response to: phosphate starvation, salicylhydroxamic acid (SHAM, a callose-independent promoter of plasmodesmata permeability) treatment, and a combinatorial condition. We found that some cell types likely initiate callose-dependent mechanisms while others likely initiate callose-independent mechanisms to control SHR movement under phosphate starvation. To isolate the roles of each mechanism, we evaluated movement in cells that were isolated from protoplasts and had reformed cell walls then divided into multicellular complexes. These multicellular complexes were then treated with SHAM and paraquat, an inhibitor of both mechanisms, and then movement of a plasmodesmata permeable probe both into cells from the cellular environment and intercellularly was evaluated. We found that callose played roles in inhibiting entry of the probe into cellular complexes while SHAM promoted intercellular movement. Overall, this work provides insight towards decoupling the functional roles of mechanisms controlling cell communication as tissues respond to environmental cues.

Single-Cell RNA sequencing reveals differences in male meiotic programmes in different reproductive modes and mutants

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Meiosis is a special cell division process that genetically diversifies offspring. Most model plant species have canonical meiosis, but some plants have non-canonical meiotic activities, such as male-asymmetric reproduction and inverted meiosis. We already have extensive knowledge of the process in multiple species using various approaches. However, we still lack single-cell data in plant meiosis that can offer a transcriptomic profile of the plant meiosis. Although single-cell technologies have been applied to various plant tissues, meiocytes pose unique challenges for single-cell platforms, for which no successful research has yet been conducted.
In this project, we aimed to develop and identify a methodology and a single-cell platform that would be suitable for the male meiocytes. We would also like to establish the meiosis transcriptomic profile and identify clusters associated with specific timepoints during meiosis. This data will shed more light on the genetic regulation influencing each stage and potentially reveal more key regulated genes related to meiosis. Another main objective was to use this developed method to compare differences between genotypes and species that inherit different meiotic events to understand the gene expression dynamics.
From the preliminary data, we have generated the Transcriptomic Profile of the meiocytes across meiosis in Tomatoes and compared it with rec8 mutant. Known meiotic genes have been screened and used to validate our data. We have also identified Tapetum cell development progression in our data.

Analysis of Molecular Regulatory Mechanisms for Secondary Cell Wall Patterning of Metaxylem and Protoxylem Vessels

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Xylem vessels enable long-distance water transport because their secondary cell wall (SCW) architecture affects hydraulic efficiency and vulnerability to cavitation. In *Arabidopsis*, the NAC transcription factors VND6 and VND7 are master regulators that specify metaxylem and protoxylem vessel identity, respectively. These vessel types develop distinct SCW patterns-pitted/reticulate in metaxylem versus spiral/annular in protoxylem-but the downstream regulatory logic that distinguishes VND6- and VND7-dependent developmental programs remains unclear. Here we hypothesize that the characteristic SCW patterning in metaxylem and protoxylem vessels is regulated by distinct downstream target genes of VND6 and VND7. To dissect these programs at cellular resolution, we use dexamethasone-inducible VND6 and VND7 systems that trigger ectopic differentiation of metaxylem- or protoxylem-like vessel cells. We performed single-nuclei RNA sequencing (snRNA-seq) to capture the transcriptomic landscape of the induced cells. Candidate genes identified based on their expression profiles will undergo functional validation and phenotypic analysis to confirm their roles in SCW patterning. Overall, this project provides a framework to connect transcriptional programs to wall architecture and yields targets for functional tests using genetic perturbation and phenotypic assays of SCW deposition. Defining the molecular determinants of xylem SCW patterning will advance our understanding of vessel specialization.

Deciphering *NF-YA1* transcription factor targets in nodule and gall formation

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Legumes can establish beneficial endosymbiosis with nitrogen-fixing rhizobia, forming nodules that support plant growth. However, legume plants are also susceptible to pathogens such as root-knot nematodes (RKN), which induce galls and cause significant yield losses. Despite their contrasting outcomes, both processes depend on extensive changes in gene expression and chromatin organization within root cells. Our work focuses on how the transcription factor NF-YA1, a master regulator of nodule development also contributes to regulating gall formation. To understand NF-YA1-dependent networks, we are conducting multi-organ, single-nucleus RNA sequencing (snRNA-seq) on wild-type and nf-ya1 mutant nodules and galls. This strategy will identify cell type-specific NF-YA1 targets, as well as developmental communalities and differences between nodule and gall organogenesis. Among the candidate targets of *NF-YA1*, we also identified *NANO1* (**N**F-Y**A**1-dependent long non-coding RNA involved in **no**dulation 1), a long non-coding RNA. Reporter analyses shows that *NF-YA1* and *NANO1* exhibit overlapping spatiotemporal expression patterns in nodules and galls. Functional characterization using RNA interference (RNAi) knock-down and overexpression lines reveals that *NANO1* acts as a negative regulator of nodule formation without disrupting nodule differentiation. These preliminary results suggest that *NANO1* may fine-tune organ initiation. Together, this study provides a cell-type-resolved view of how transcription factors and long non-coding RNAs coordinate root developmental reprogramming during beneficial symbiosis and pathogenic infection.

Study and characterization of the MADS-box gene *FRUITFUL* (*FUL*) during Global Proliferative Arrest (GPA) in *Arabidopsis thaliana*

José Rafael Pérez-Moraga, Vicente Balanzá, Cristina Ferrándiz

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Monocarpic plants, many of them major crops, undergo only one reproductive cycle. In these plants, when fruit production exceeds a certain threshold, the meristems switch from active growth to a controlled shutdown, ending flowering and ultimately leading to plant death. This process is known as Global Proliferative Arrest (GPA). GPA depends strongly on fruit set and is genetically controlled by age and other internal variables. However, unknowns remain regarding how this genetic control system is regulated. The aim of this thesis is to provide new insights into the genetic regulation of GPA, focusing specifically on *FRUITFULL* (*FUL*), a MADS-box transcription factor that controls flowering time and plays a fundamental role in GPA, as the *ful* loss-of-function mutant does not undergo GPA and flowers indefinitely. To achieve this goal, we have identified conserved domains in the promoter of *FUL* dicot orthologs. These motifs are being functionally evaluated to assess their importance throughout inflorescence development and GPA by genome editing with CRISPR/Cas9. We also aim to identify the transcription factors that bind the most relevant motifs and are responsible for controlling *FUL* expression during plant reproductive development. All this information will be integrated into models of genetic control of GPA.

A Comparative Single-Cell Framework for Investigating Rare Epidermal Cell Differentiation in Grasses

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Silica and cork cells are paired specialized epidermal cell types in the leaves of Poaceae grasses that contribute to structural reinforcement through coordinated silica and suberin deposition, particularly over vascular tissues. This structural specialization has been associated with enhanced herbivore deterrence, pathogen resistance, and improved water regulation. Due to their relative rarity, transient developmental states, and the recalcitrant nature of many grasses, traditional bulk transcriptomic approaches lack the resolution necessary to resolve their lineage specification, leaving the molecular mechanisms underlying their differentiation poorly characterized. To address this limitation, this project employs a comparative single-cell transcriptomic framework using *Brachypodium distachyon* and *Sorghum bicolor* to investigate conserved regulatory processes governing silica and cork cell differentiation. Reanalysis of a published *Brachypodium* leaf developmental atlas incorporating trajectory inference and gene regulatory network approaches has revealed transcriptionally distinct epidermal subpopulations and candidate regulatory programs associated with lineage progression. As a comparable leaf developmental atlas is not yet available for sorghum, ongoing work focuses on optimizing single-cell and single-nuclei isolation strategies to generate a high-quality reference dataset. Establishing this comparative framework will enable the identification of conserved mechanisms underlying rare epidermal cell differentiation in grasses and contribute to a broader understanding of cell-type diversification within Poaceae.

Elucidating the cell type-specific permissivity to retrotransposition

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Transposable elements (TEs) are mobile genetic elements with the ability to continuously alter the genetic landscape by creating new genes and regulatory sequences. Plant genomes are particularly rich in TEs and many agricultural traits have been linked to transposon activity. However, insights into the dynamic and real-time mobilization of TEs remain scarce. To close this gap, a new method called RUM (Real-time detection of transposon jUMping) is developed to monitor the dynamics of transposon mobilization in real time and at single-cell resolution. By utilizing a synthetic, estradiol-inducible long terminal repeat (LTR) retrotransposon construct containing GREEN FLUORESCENT PROTEIN (GFP) sequences, the retrotransposition process of LTR retrotransposons is emulated and visualized. By coupling of RUM to Fluorescence-Activated Cell Sorting (FACS), cells associated with transposition events are successfully isolated in *Arabidopsis*. To uncover the dynamicity and permissivity of different cell types to transposition events, we aim to conduct single-cell RNA sequencing (scRNA-seq) on nuclei collected via FACS following RUM activation. RUM-positive and pre-FACS nuclei samples will be subjected to scRNA-seq to identify cell types enriched or depleted in transposition events and scRNA-seq results will be validated experimentally using marker genes specifically transcribed in the identified cell types. By combining RUM, FACS and scRNA-seq we hope to elucidate the underlying mechanisms of retrotransposition and ultimately work towards controllable transposition.

Barrier breakthrough: Molecular determinants of exodermal divergence for root resilience

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As climate change intensifies, drought has emerged as one of the most severe abiotic stresses, reducing crop yield. To ensure food security, we need to tap into all avenues of protecting our crops against climatic stresses. Chickpea (Cicer arietinum), a major legume with high nutritional and several health benefits suffers annual yield loss (~50%) due to drought. The root exodermis, a specialized cell layer, plays a crucial role in water retention and protects against pathogens through deposition of suberin and lignin as apoplastic barriers. Despite its significance, the mechanisms governing exodermal barrier formation remain poorly understood, due to its absence in the model plant *Arabidopsis*. We recently discovered that chickpea varieties from the main types desi and kabuli exhibit dynamic inducible and constitutive exodermal barrier deposition, respectively. My objective is to investigate the underlying mechanism governing these exodermal responses and differentiation. First, I aim to elucidate the gene regulatory networks controlling this exodermal barrier formation through single cell transcriptomics of chickpea cultivars with contrasting barrier dynamics. These will reveal the developmental and stress-responsive pathways that orchestrate barrier formation. Second, I will test the role of key transcription factors (TFs)/genes through genetic modifications via hairy root transformation, molecular interaction, and promoter activity assays. This will provide insights into how these regulators regulate this barrier differentiation, and stress response that impact barrier traits. The insights gained from this proposal will not only advance our understanding of cell-type-specific stress responses, but facilitate its translation for targeted breeding strategies aim at enhancing stress resilience and improving crop yield.

Hormonal Crosstalk Regulating Cell Cycle Progression in the *Arabidopsis* Root Meristem

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In the root meristem, cell populations balance proliferation and differentiation through a tightly orchestrated cell cycle. Phytohormones such as auxin, cytokinin, and brassinosteroids regulate this process by modulating core cell cycle genes, thereby controlling the rate and timing of cell division and maintaining meristem activity. Despite these insights, how multiple hormonal activities are coordinated within individual cells and across cell cycle phases remains poorly understood. Advances in single-cell RNA sequencing (scRNA-seq) now enable hormone-responsive transcriptional programs to be examined at cellular resolution and have begun to reveal dynamic modules linked to specific cell cycle phases: cytokinin activity peaks at the G1-S and G2-M transitions, and brassinosteroid signaling rises at the onset of G1 but declines before mitosis. While single-cell datasets describing cytokinin and brassinosteroid signaling have emerged, auxin responses during the meristematic cell cycle remain unresolved to date.
To address this gap, single-cell transcriptomes from roots treated with auxin, cytokinin, and their combination will be integrated into an established cell cycle reference framework for the *Arabidopsis* root, enabling hormone responses to be mapped across cell cycle phases. Combining scRNA-seq with live-cell imaging of hormone reporters will reveal phase-specific regulatory modules and candidate mediators of hormonal crosstalk. Phase-specific perturbations of hormonal pathways through misexpression of candidate genes will validate key cell cycle regulators. Together, these approaches will establish a mechanistic framework explaining how interacting hormonal networks coordinate cell cycle progression and shape plant growth at cellular resolution.

Exploring the cellular dynamics of clubroot galls through the transcriptomic lens

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Clubroot is a disease caused by the soil-borne pathogen *Plasmodiophora brassicae*. It primarily affects plants in the Brassicaceae family including *Arabidopsis thaliana*. Infection induces pronounced cellular reprogramming in underground host organs, leading to the formation of characteristic galls and stunted plant growth. Although disease progression and gall development are well described, the molecular and transcriptional mechanisms underlying cell type–specific responses to infection remains poorly understood. Here, we employed a single-nucleus RNA sequencing (snRNA-seq) to mock and *Plasmodiophora* infected *Arabidopsis*. We were able to capture approximately 300,000 nuclei and identified almost all major cell types in root undergoing secondary growth. In addition, unique cell states associated with infection and specific maker genes for these cell clusters were identified. Furthermore, several genes showing dynamic expression in response to pathogen infection and during vascular reprogramming were identified across distinct cell types. Overall, this study will provide a high-resolution view of cellular heterogeneity and dynamic transcriptional reprogramming during gall development.

Uncovering the biosynthesis of a low-abundance triterpenoid and its role in shaping plant–rhizosphere interactions

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Low-abundance triterpenoid specialized metabolites produced by plant roots can act as potent signaling molecules in rhizosphere interactions. One such metabolite produced by solanaceous plants induces the hatching of potato cyst nematodes and plays a key role in plant–nematode interactions. Despite its ecological importance, the biosynthetic pathway of this metabolite remains largely unknown due to its extremely low abundance and structural complexity. Interestingly, this metabolite has only been detected in plants grown in microbe-containing soil and is absent under sterile conditions, suggesting that its production may be influenced by rhizosphere microorganisms.
Here, we aim to elucidate the biosynthetic pathway of this metabolite and investigate its ecological role. Using tomato and potato as model systems, candidate biosynthetic genes were identified through bulk RNA sequencing and co-expression analyses, and CRISPR-based genome editing confirmed that several of these genes are required for the pathway. To uncover the spatial regulation of this pathway, we analyzed single-cell RNA sequencing datasets from tomato roots. This analysis revealed strong cell-type specificity of pathway genes and co-expression of multiple components within the same root cell type, indicating that the pathway is spatially organized at the cellular level.
To explore the physiological relevance of this pathway, we examined mutants impaired in its function. These mutants exhibit reduced nitrogen accumulation under nitrogen-deficient conditions, suggesting a potential link between this metabolite and plant nutrient acquisition. Ongoing metabolomics and rhizosphere microbiome analyses aim to determine how disruption of this pathway affects root exudate composition and plant–microbe interactions.

Single-Cell Investigation of Epigenetic and Transcriptional Variability in Salt Stress Responses in *Arabidopsis thaliana*

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Soil salinity is a major abiotic stress threatening agricultural productivity, exacerbated by climate change as well as irrigation-driven salt accumulation. In *Arabidopsis thaliana*, salt stress responses have been widely characterised using bulk transcriptomic approaches, however, these analyses mask cell-type-specific regulatory dynamics and may therefore obscure potential sources of variability in stress response. Emerging evidence suggests that epigenetic regulation and cellular state, including cell-cycle phase, may contribute to heterogeneous stress responses across distinct cell populations.
This project aims to explore the epigenetic and transcriptional basis of salt stress variability at a single-cell resolution. A multiomic strategy combining single-cell RNA sequencing and single-cell ATAC sequencing will be implemented to characterise gene expression profiles and chromatin accessibility landscapes in *Arabidopsis* roots under control and saline conditions. Integrative analysis will enable the identification of stress-responsive cell populations and the regulatory elements underlying variable gene expression. High-resolution confocal microscopy will complement these approaches by facilitating the assessment of cell-cycle dynamics and chromatin organisation in live specimens.
In addition, the influence of salt stress on cell-cycle progression will be investigated to examine whether proliferative state controls transcriptional response, exploring how epigenetic configuration and cellular state may shape stress-induced transcriptional plasticity.
This approach will provide insight into how chromatin organisation contributes to cell-type-specific environmental responses, therefore advancing our understanding of stress adaptation in plants.

SAPTICoN : a robust no-code pipeline to analyze [plant] single cell transcriptomics data sets

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Preprint

GitLab

1. CONTEXT AND AIMS

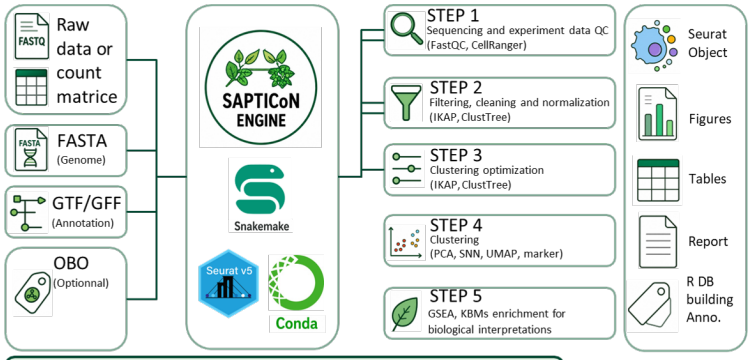
Few end-to-end tools for plant single cell data

Non-model species and limited annotations

Reproducible workflow, accessible for non coding skill

Applicable for all genome with fasta and GFF/GTF files

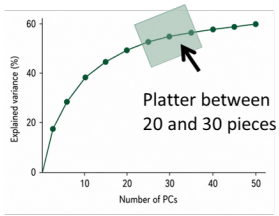
2. PIPELINE OVERVIEW



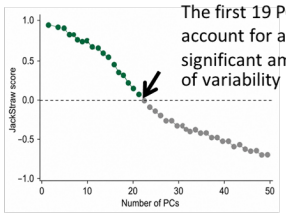
Compatible with raw data or count matrices : Reproducibility using Snakemake framework + Conda.

3. CLUSTERING OPTIMIZATION

A. Elbow plot



B. JackStraw (Significance threshold)

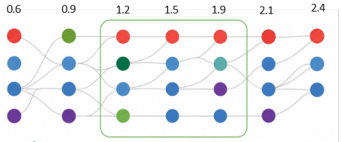


C. IKAP (summary)

Configuration (PCs number)	R optimal (rapport R3, SNN)	IKAP score
21 PCs	R = 1.9	0.87
26 PCs	R = 2.4	0.86
28 PCs	R = 2.5	0.85

IKAP: 21 PCs, r= 1.9

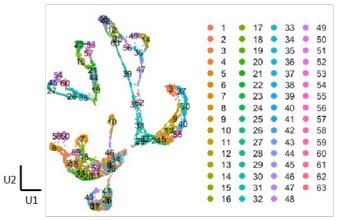
D. Clustree (cluster stability resolution (r))

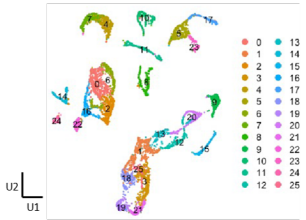


Clustree: 25 PCs, r= 0.9-1.4

Agreed compromise for clustering: 21 PCs, resolution (k)= 1.9

4. BENCHMARK : ARABIDOPSIS ROOT CASE





Cluster reciprocity

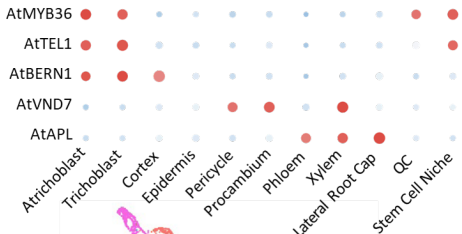
2,5x fewer clusters whilst retaining the biological structure

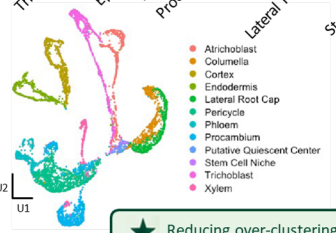
94 % cells retained compared to the reference dataset

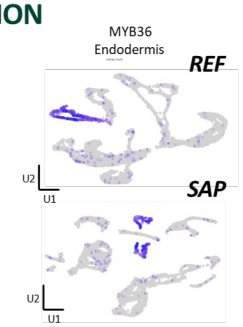
High confidence kept for biological relevancy

Read Organellar (Low) : Mito <5% chloroplast < 0.05%

5. BIOLOGICAL VALIDATION



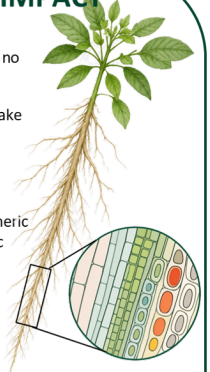




- Cluster GSEA analysis
- Known Biological Marker (KBMs) expression profile assessment
- KBMs cellular marker enrichment (Fisher test)

Reducing over-clustering without losing biological signal

6. CONCLUSION AND IMPACT



- A pipeline accessible to biologists with no coding experience
- High reproducibility thanks to Snakemake and Conda frameworks
- Integrated Clustering optimization
- Support for non-model species, via generic annotation based on common genomic files
- Data ready for exploration, annotation and further analysis

SAPTICoN: simplifies the analysis of single-cell organisms whilst ensuring robustness and biological interpretability

Open source | No code

References : *Sahan et al., 2022. Cell plant.*

Glossary : *QC: Quality Control*
KBMs: Known Biological Marker
PCs: Principal Component

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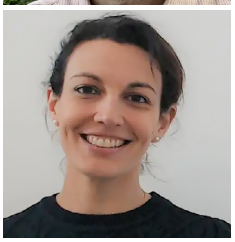

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