Offre de stage de Master / Master Internship offer

Internship supervisor and Host laboratory:

- Lab: 'Reproduction et développement des plantes' at the ENS de Lyon
- Group: 'Biophysique et développement'
- Supervision: Pradeep Das, Assistant professor at the ENS de Lyon, pradeep.das@ens-lyon.fr,04.72.72.89.37
- Cosupervision: Sam Collaudin, PhD student, samuel.collaudin@ens-lyon.fr, 06.50.80.47.22

Research project title:

Understanding the cellular-level dynamics of homeotic gene expression during flower development via an interdisciplinary approach

Project description:

The ABC model of floral organ identity determination is now a classical example of how combinatorial gene activities effect patterning during development. In the 25 years since the model was first proposed, the field has come to understand a lot about how the so-called A-, B- and C-class homeotic genes interact with each other, both genetically and molecularly, and how they in turn regulate the expression of specific target genes. However, one aspect that is still poorly understood is exactly how the ABC genes themselves become expressed in very specific spatial domains and at very specific times during flower development.

To address this question, we chose to focus on the expression dynamics of the C class gene, AGAMOUS (AG). It has been shown that AG expression first begins to appear in the centre, but not in the periphery, of 3 day-old flowers. However LEAFY and WUSCHEL, which are the main activators of AG, are present in the flower from day 1, well before the onset of AG expression. Similarly. APETALA2, which plays a key role in repressing AG expression, is also expressed at day 1. To understand how these regulators bring about the precise spatio-temporal regulation of AG, we developed a simple dynamic model based on reaction-diffusion equations that reflected the principal known interactions of these regulators. We also incorporated floral growth during the relevant stages in the model. A mathematical analysis of the model suggested that the auto-activation of AG coupled with the repression by APETALA2 defines a threshold in AG expression. It also suggested that AG diffusion plays an important role in its capacity to be highly expressed in the central dome just after activation.

To validate the different hypotheses output by the model, we generated AG translational reporter lines (figure below) and developed tools to quantify expression at the cell level and describe AG expression in different genotypes. The goal of the internship will be to use these lines and tools to test the model. The student will use available Python scripts but no knowledge in computer sciences is required. According to their progress, the student will feed back on the hypotheses of the model.

Work to perform:

- 3D live-imaging of flower development with fluorescent markers and confocal microscopy
- Image analysis to quantify gene expression in each cells, using available tools
- Statistical analysis to be able to compare gene expression in different genotypes, using available methodology
- Plant culture
- Optional molecular biology experiments to generate new lines
- Comparison between data and model predictions; model improvement could be proposed

Lab publications:

- Fernandez R, Das P, Mirabet V, Moscardi E, Traas J, Verdeil JL, Malandain G, Godin C. 2010. Imaging plant growth in 4D: robust tissue reconstruction and lineaging at cell resolution. Nat Methods. 7(7):547-53.
- Rozier F, Mirabet V, Vernoux T, Das P. 2014. Analysis of 3D gene expression patterns in plants using whole-mount RNA in situ hybridization. Nat Protoc. 9(10):2464-75.
- Milani P, Mirabet V, Cellier C, Rozier F, Hamant O, Das P, Boudaoud A. 20140. Matching patterns of gene expression to mechanical stiffness at cell resolution through quantitative tandem epifluorescence and nano-indentation. Plant Physiol. 165, 1399–1408.

- Das P. 2011. Imaging and modeling growth and morphogenesis in plants. Curr Opin Genet Dev. 21(5):606-611.
- Das P, Ito T, Wellmer F, et al. 2009. Floral stem cell termination involves the direct regulation of AGAMOUS by PERIANTHIA. Development. 136(10):1605-11.

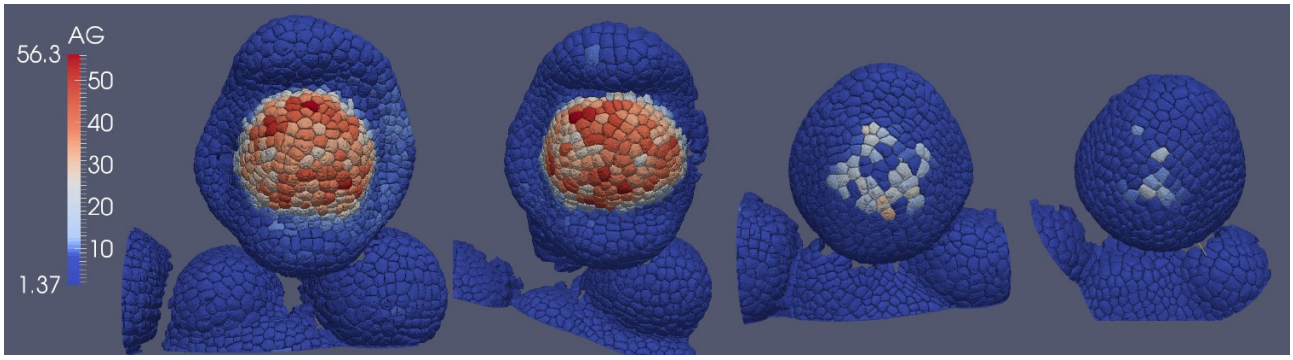

Figure: AG expression quantified at a cellular level in stage 2 and 3 flowers of an AG translational reporter (AG-2xVenus)