

Year of CfP: 2007

Project No: 07047 Completed

Project title: Leaf growth and soil water deficit in <i>A. thaliana</i> and apple tree: The 3 dimensions

Unit managing the project: LEPSE (Ecophysiology of Plants under Environmental Stresses) (Inra, SupAgro)

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Country involved in the project: UK

Research units from the Foundation's scientific network involved: AGAP

Subthematic axes: IPB-1 (Integrative Plant Biology 1: *Genetics and genomics, plant breeding, ecophysiology*)

Objectives:

Total leaf biomass in a plant is an integration of variables depending on elementary processes such as leaf production, duration and rate of expansion of each individual leaf, leaf thickness, cell production and cell expansion. As a first step to a modelling approach of whole plant leaf growth, it is necessary to elucidate how the different leaf growth variables are related to one another. Until now, the causal or functional links between underlying leaf growth variables have not been clearly identified. In addition, the scale and the tissue at which the control of growth occurs, is not clearly established.

The contribution of the different processes potentially involved in the responses of *A. thaliana* leaf growth to environmental stresses (focusing mainly on drought) is currently being evaluated in the LEPSE research unit. An automated phenotyping platform (PHENOPSIS) is set up to grow plants in reproducible environmental conditions, including optimal conditions and well-characterized drought stresses.

Until now, growth was only considered as expansion in area and was broken down further into a set of underlying variables such as leaf expansion rate and duration of leaf expansion, epidermal cell number and size. Reproducible relationships between these variables and micro-meteorological conditions could be established. However, leaves also grow in the third dimension.

The objectives of this project are

- to analyse the relationships between cellular leaf growth variables
- to identify functional links between them
- to evaluate their contribution to the plasticity of leaf growth in response to drought. For this purpose, an ecophysiological approach will be combined with statistical modelling both in apple tree, one of the most economically important tree crops grown worldwide and *A. thaliana*, as a plant model system.

Protocols will be established to reproducibly analyse cell area, cell number and cell volume in the different leaf tissue layers in both species. Automatic procedures for image analysis will be developed to increase the throughput of the experiments. Then, leaf growth will be altered both via genetic and environmental perturbation, taking advantage of the large collection of genetic backgrounds available in the two groups (*A. thaliana* and apple tree) and the capacity to impose reproducible drought treatments in LEPSE. Throughout the project, a statistical modelling approach to analyse and interpret the mass of data collected at different scales will be developed.

Analysis of interactions between leaf area plasticity and leaf thickness is lacking in both studies (*A. thaliana* and apple tree). Combining the expertise of different partners in dicotyledonous leaf growth and in statistical and functional modelling, will allow the development of a model leaf growth in 3-D.

Action carried-out and results obtained:

Leaf growth processes can be evaluated at different organisational levels, such as cells, the individual leaf and the whole plant. On the cell and leaf level, most studies have been limited to the leaf surface with data collected on leaf surface area and the number and surface area of epidermal cells. However, leaf sub-epidermal tissues, the palisade and spongy mesophyll, contain the main actors in photosynthesis and their cellular organisation may affect leaf transpiration and the accumulation of photosynthates, which further influences whole plant growth. As studies into the three dimensional development of leaves are rare, the interaction between the different cell layers (epidermal and sub-epidermal) during growth is largely unknown and the effects of particular environmental stresses, such as soil water deficit, on internal leaf tissues have not been thoroughly investigated.

The aim of the present project was to develop a procedure for the three dimensional imaging and analysis of leaves to obtain leaf growth variables specific to internal leaf tissues. The further aim was to characterize leaf growth in three dimensions, under well-watered conditions and soil water deficit, for *Arabidopsis thaliana* and apple, two dicots with a largely differing leaf cellular organisation.

The development of the procedure for three dimensional imaging and analysis of leaves was successful and gave consistently high-quality images for both *A. thaliana* and apple [Plant Methods, 2010, 6: 17]. The procedure consisted of a leaf sample preparation phase, an imaging phase and finally an image analysis protocol. Leaf sample preparation included leaf fixation and conservation to allow for the typically large quantities of samples issued from phenotyping platforms (Phenopsis, Lepse, INRA Montpellier) [<http://bioweb.supagro.inra.fr/phenopsis/>], and efficient sample clearing and coloration for successful tissue penetration and image acquisition. Three-dimensional imaging was performed on the multiphoton microscope (Zeiss LSM 510 Meta NLO equipped with a Chameleon femtosecond laser) of the Montpellier RIO Imaging platform and provided high resolution images of the entire thickness of leaves, enabling the three dimensional visualisation and analysis of internal leaf tissues. Finally, image analysis macros were developed in the open source software ImageJ [<http://rsb.info.nih.gov/ij/>] which allowed annotated and structured storage of image data and variables measured on images, and standardized, semi-automated and assisted measurement of leaf growth variables, including leaf surface area and thickness, tissue proportions, cell densities and cell volumes in the leaf epidermis and mesophyll. Three dimensional visualisations of leaf images and leaf structural models was performed in the MedINRIA ImageViewer software (Asclepios Research project, INRIA Sophia Antipolis) [<http://www-sop.inria.fr/asclepios/>].

The procedure was applied in a study of leaf growth, from emergence until maturity, in *A. thaliana* and apple and provided data on the longitudinal (tip-to-base) gradient in leaf development and the transversal gradient between leaf tissues, including data on epidermal and mesophyll proportions, cell densities and cell volumes over time. For *A. thaliana* the study was extended to the characterization of the effects of soil water deficit on cell proliferation and differentiation in the epidermis and mesophyll of a range of genotypes, including mutants affected in leaf transpiration.

Publications:

Wuyts N, Palauqui JC, Conejero G, Verdeil JL, Granier C and Massonnet C (2010) High-contrast three-dimensional imaging of the *Arabidopsis* leaf enables the analysis of cell dimensions in the epidermis and mesophyll. Plant Methods, 6-17

Fabre J, Dauzat M, Nègre V, Wuyts N, Tireau A, Gennari E, Neveu P, Tisné S, Massonnet C, Hummel I & Granier C (2011) PHENOPSIS DB: an Information System for *Arabidopsis thaliana* phenotypic data in an environmental context. BMC Plant Biology 11:77.

Prospects for the future:

An efficient protocol for three dimensional imaging was developed and published and allows for in depth studies on cellular organisation in developing leaves from initiation until senescence of

Arabidopsis thaliana, apple and other species. It offers the potential to extend our current knowledge of leaf growth processes, under influence of both genotype and environment, to the inner cell layers of leaves and provide the necessary data for the establishment of a complete cellular model of leaf growth which can be integrated into a whole plant growth model.

Groups involved in this project are looking for further financial supports to analyse further data and images produced during the project with still the objective to establish a 3D model of leaf development at the cellular level.

Total Agropolis Fondation funding: € 117,943 (salary for a post-doctoral fellow, travel expenses, running costs)

Funding categorie(s): Agropolis Fondation post-doctoral fellowship

Project duration: 15 March 2008 – 1 December 2010

Keywords: *Arabidopsis* – apple tree – leaf growth – modelling – drought – stress