Leaf growth and soilwater deficit

Leaf growth and water deficit in Arabidopsis thaliana and apple: the three dimensions.

ABSTRACT

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 thoruoghly 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 procdure for three dimensional imaging and analysis of leaves was succesful and gave consistenlty 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 succesful 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 visualisation of leaf images and leaf structural models was performed in the MedINRIA ImageViewer software (Asclepios Resaerch 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.

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PERSPECTIVES

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 extent 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.