PHENOPSIS, a plant phenotyping automaton : 10 years later

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Our group aims at analysing and modeling the responses of plant growth and transpiration to environmental conditions, with a particular focus on soil water deficit. The PHENOPSIS 1 platform was set-up a decade ago [Granier et al., 2006] with the purpose of phenotyping large sets (> 500) of *Arabidopsis thaliana* plants under highly reproducible soil humidity conditions. Since the release of the first version of PHENOPSIS 1, our group has performed extensive developments and PHENOPSIS has been triplicated to reach a capacity of more than 1500 plants.

Before PHENOPSIS…

Soil water content was maintained at a given value by weighing and watering pots on a balance to reach a target weight manually during experiments.

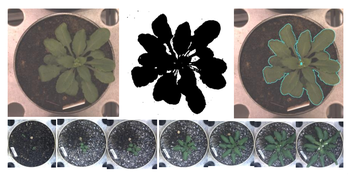
Plant images were taken manually and analysed to extract phenotypic shoot growth traits.

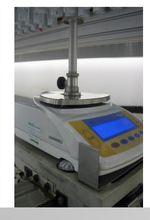
Throughput of the experiments : only 3 to 6 A. thaliana genotypes could be grown together in a same experiment with enough replicates [Cookson et al., 2005 & 2006].

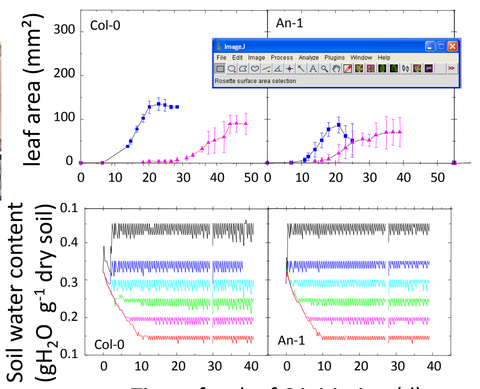
PHENOPSIS 1 was built in 2002

A camera fixed on a mobile arm is moving automatically from pot to pot to take plant images once or several times a day.

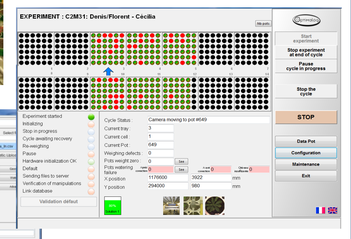
An irrigation station and a balance fixed on the mobile arm are moving from pot to pot to weight and adjust soil water content automatically once or several times a day, following instructions.







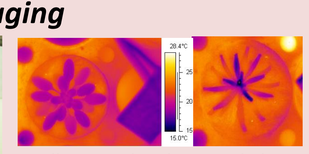
An intuitive software drives the automaton with a user-friendly interface and several applets for data security.



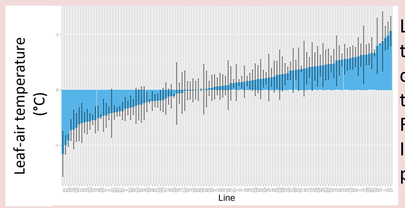
Throughput of the experiments : 12 to 120 *A. thaliana* genotypes could be grown together in a same experiment with different soil water contents and enough replicates for genetic analyses [Aguirrezabal et al., 2006; Tisné et al., 2008]

Improvement of the imaging station capacities since 2002 to study …

Infra-red imaging



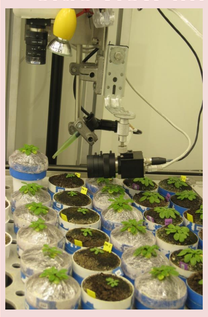
Well-watered and stressed plant Leaf temperature

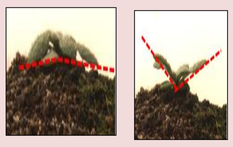


Leaf-air temperature difference in the *Ler* X Cvi Recombinant Inbred Lines population

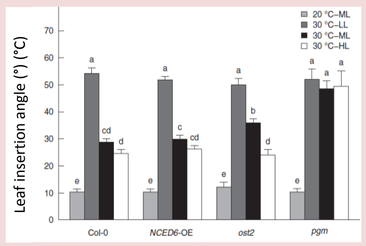
…the genetic control of leaf temperature and transpiration [Vasseur et al., 2014]

Side view imaging



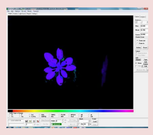


High temperature causes an increase in leaf angle when plants are grown under moderate light (ML).

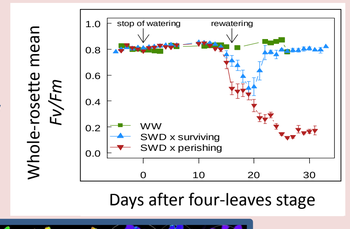


…the role of carbon balance on leaf hyponasty [Vasseur et al., 2011; Pantin et al. (under review)]

Chlorophyll fluorescence imaging

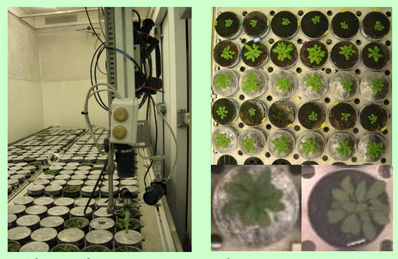


- CSV Files
- Jpeg files
- Pim files
- Tiff files



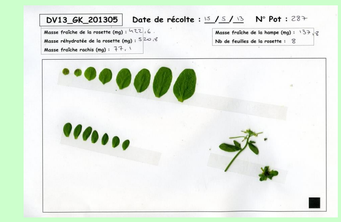
…response to drought stress and recovery after re-irrigation [Bresson et al., in prep]

New phenotypic traits measured manually or semi-automatically at high-throughput since 2002 to study…

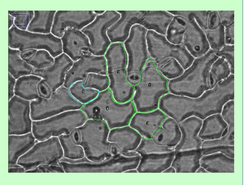


Plant biomass and transpiration

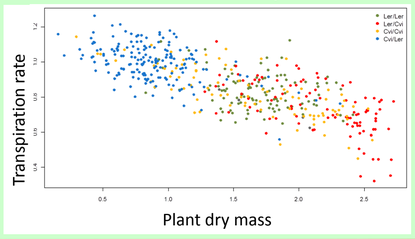
Plant or leaf photosynthesis

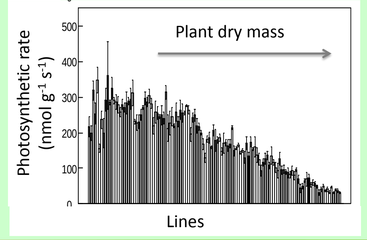


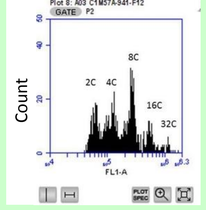
Total leaf area



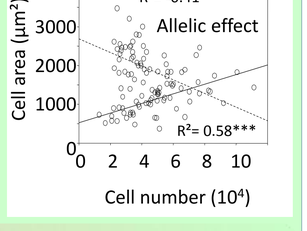
Cell number & area



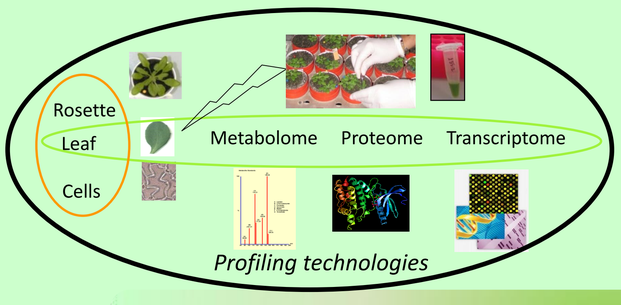


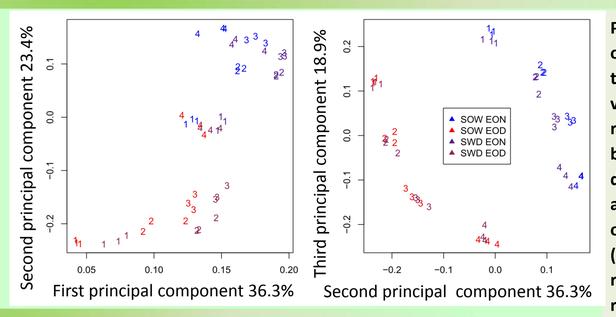


Endoreduplication



… the control of water use efficiency [Vasseur et al., 2012] / **cellular control of growth** [Massonnet et al., 2011]





Principal Component Analysis of transcripts, ~25000 genes, **to estimate the source of variation between samples revealed a strong effect of both the stage of leaf development (from 1 to 4) and the time of the day : end of day (EOD) & end of night (EON) with a high reproducibility of biological replicates; 3 experiments in optimal watering (OW) and 3 in water deficit (WD) conditions.**

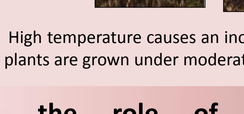
…the biochemical and molecular controls of plant responses to drought [Hummel et al., 2010; Pantin et al., 2012; Baerenfaller et al., 2012]

Not only drought stresses but also other abiotic or biotic stresses can now be assessed isolated or in combinations

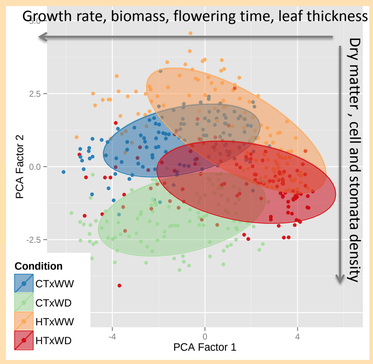
Three automatons are set-up in 3 growth chambers to impose different air temperatures and /or light intensities.

Comparison of plants grown in Control Temperature (CT), High Temperature (HT), Water Deficit (WD) and Well Water (WW) in natural accessions and *Ler* x Cvi RIL [Vile et al. 2012; Bresson et al., 2013 & 2014; Vasseur et al., 2014]

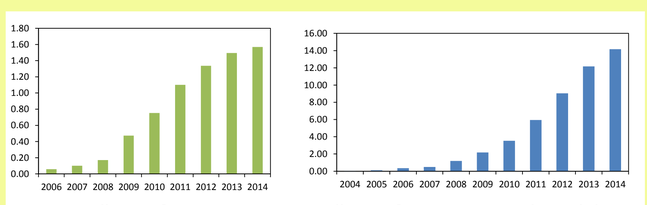
The watering station has been improved with the use of 2 peristaltic pumps for automatic watering with 2 different nutrient solutions.



Control and NaCl traited plant



PHENOPSIS DB : a database and a web interface for automatic or semi-automatic image and data storage, reuse and analysis [Fabre et al., 2011]



In Phenopsis Database
http://biowebsupagro.inra.fr/phenopsis

Micrometeorological files
Irrigation files

'Genotype' section (1500 genotypes)
Hyperlinks to stock center websites

'Variable' section
Hyperlinks to Plant and Trait Ontology websites
Protocols

Quantitative phenotypic data

Images and Image J macros for analyses

R scripts for data analyses and visualisation

Some technical informations…

Duration of an automaton cycle for 504 plants depends on the set of required data

	cycle duration
Infra Red imaging	1h
Watering	2h30
Weighting	1h15
Chlorophyl Fluorescence imaging (Fv/Fm)	3h30
Top view Imaging	30 min
Side view imaging	1h15

Securities and controls

Phenopsis has evolved and offers now a lot of securities and controls. But the discerning eye of the user is always the best controller!

- Alarms (sms, emails) for defaults (mechanical, images, irrigation, pot weight …)
- Watering limits and controls
- Automatic peristaltic pump control during automaton cycles
- Pause and restart cycle in progress
- Distance control of the machine
- Doors of growth-chambers security when the automaton is running for human safety
- Many experiments can be run at the same time In the same robot (different programs)
- Automatic control of the % use of nutrient solution (% available)

Conclusion - Future developments

Today, 3 PHENOPSIS automatons set-up in 3 growth chambers allow to grow 1500 *A. thaliana* plants in reproducible environmental conditions. A high number of phenotypic traits can be measured routinely using non-invasive methods. Destructive measurements can complete these datasets when necessary.

Tomorrow, Phenopsis will be adapted for flexibility in pot sizes with 3 options : small pots (250ml as actually, 504 pots per robot), median (pots 1L, 224 pots per robot), and big pots (5L) (70 pots per robot). Tests have been performed on lettuce and canola.

See references online:
https://www6.montpellier.inra.fr/lepse_eng/M3P/Infrastructure/PHENOPSIS-platform/Comunication