IJPB Phenoscope Platform Agreement (March 2023)

1- Presentation of the Platform

Within the INRAE research center in Versailles, the Phenoscope platform is one of the platforms of the Plant Observatory ('OV'), the technological component of IJPB (Jean-Pierre Bourgin Institute) dedicated to multi-level phenotyping of plants.
The Phenoscope platform is under the scientific responsibility of Olivier Loudet (Research Director) and is managed by two permanent staff (a computer engineer –Olivier Zurfluh– and an assistant engineer –Elodie Gilbault–). The Phenoscope is a high-throughput phenotyping robot dedicated to cultivate and observe large numbers of plants grown in individual pots. It is typically exploited to monitor and compare the vegetative growth of many genotypes under different watering regimes and strictly-controlled environmental conditions. The platform is composed of 2 independent robots located in the same growth room. Each robot handles 735 individual pots.

More information and citations:
https://phenoscope.versailles.inrae.fr/

Tisné *et al.*, The Plant Journal, 2013

2- Organization of the Platform

The scientific head of the platform is Olivier Loudet. He takes decision on the relevance of the experiment and validate the proposed plan. Olivier Zurfluh manages the mechanical, electronics and informatics points. Elodie Gilbault manages the experiments themselves, plant culture and extraction/analysis of phenotypes and datasets. The **service proposed on this platform is necessarily all-inclusive** (we can't allow you to use the robots freely): the platform staff takes care of all experiment's steps. When possible, the external user can be asked to help for specific actions required to prepare the experiment on the Phenoscope, like sowing and thinning.

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We are here

3- Rules of access and services of the Platform

How do I apply?

Any request starts with you contacting us with some basic informations (number of genotypes, specific conditions, deadline).

We reserve the right to eventually decide not to make a proposition of experiment for some requests.

Our requests

For all experiments, users must:

- Provide **recent seeds that have been produced simultaneously for all genotypes to compare** (if possible under controlled conditions, especially for genotypes differing in flowering time)
- Check the **germination quality** (uniformity and rate) of the seeds supplied prior to the experiment (germination rate 48h after sowing following 3 days of seed stratification in the dark)
- In some cases, we will have to check the uniformity of the seed size (with sieves)

For experiments with more than ten genotypes, the **seeds must be provided already aliquoted** for each experiment with the requested amount of seeds (not less and not much more).

Users must **inform us of any phenotypes already known** for the studied genotypes. The Phenoscope is not set up (nor useful) to characterise and study the growth response of genotypes with obvious phenotypes such as dwarfness, delayed development or poor/slow germination.

Proposed service

Growth conditions:

- Short days: 8h photoperiod (strongly favored) or Long days: 16h photoperiod
- Light: white LED light intensity is around 250µmol.m-².sec-1
- Temperature: 21°C during day / 18°C during the night
- Relative Humidity: 65%

Different conditions from those indicated here will not be our priority and will cost more, because they will constrain the whole growth room (i.e. 2 Phenoscope robots) for a long time (the experiments themselves but also often a +/- long period to preliminary test our treatment scenarios under these conditions).

The pots follow each other along the path of the Phenoscope according to a sequence initially defined and unchanged throughout the experiment. Their continuous rotation makes it possible to homogenize the environmental conditions perceived by individual plants and to treat them individually and successively (watering and phenotypic acquisition).

Roughly, here are our standard treatments (on a specific peatmoss soil substrate):

- **Control Treatment** = 60% Soil Water Content (SWC), non-limiting nutrition
- **Mild Water (W) Stress Treatment** = between 40 and 25% SWC (30% SWC is our typical mild stress)
- **Severe Water (W) Stress Treatment** = 20% SWC
- **Nitrogen (N) Stress Treatment** = watering with a nutrient solution with lower nitrate (N-limiting)
- **'Combined' WxN Treatment** = mild W and N combined stress
- Salt Stress Treatment = watering with a nutrient solution with 50mM NaCl (requires prior testing)

Different treatment scenarii (or soil) from those listed here are possible, but require to be adjusted in advance on one (and often several) preliminary experiments under our specific conditions. **This can take up to several monthes.**

Phenotypic **parameters essentially quantifying plant growth** are acquired daily at the imaging stations (visible and infrared) in an automated and non-destructive way (Figure 1a).

Here are the different traits that are measured directly from the images (Figure 1a):

- Projected Rosette Area (PRA): a proxy for leaf area derived from vertical images (Figure 1c)
- Circle Radius: Radius of the circle encompassing the rosette
- Leaf colour is converted from RGB (Red Green Blue) to an HSV scale (Hue, Saturation, Value)
- ConvexHullArea: Surface of the convex polygon encompassing the rosette (Figure 1b)
- Average leaf temperature: from the IR camera, normalised for room temperature variations

Then some derived traits are calculated:

- Compactness = PRA / ConvexHullArea
- Relative Expansion Rate (RER): relative growth rate (Figure 1d)
- RERi: Instantaneous growth rates (Figure 1e)

The watering data is recorded from the weighing station and makes it possible (in theory) to evaluate water consumption. Still, this data is made very noisy by the variations in evaporation (which largely exceed plant transpiration) so we usually don't provide this data.
In other words, **the Phenoscope is not designed to record water use efficiency** or other parameters that require to control soil evaporation.

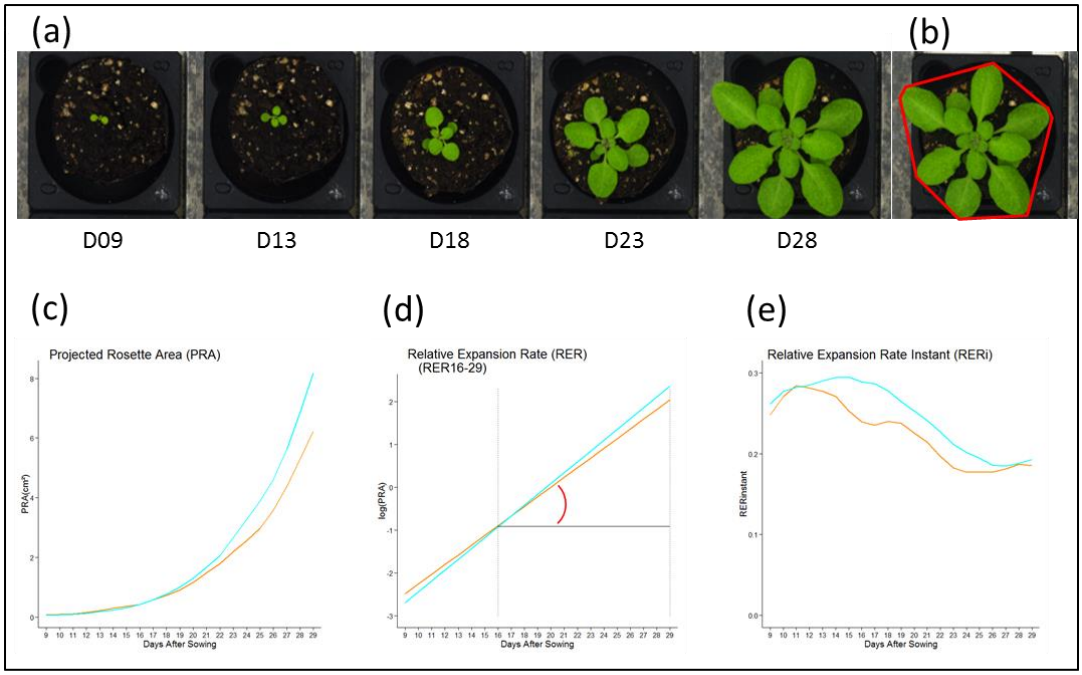

Figure 1 : Experimental design and traits
(a) Pictures of plants at different days on the Phenoscope
(b) ConvexHull to calculate the compactness
(c, d, e) In blue : Well Watered (Soil Water Content = 60%), In orange : Water Deficit (Soil Water Content = 30%)
(c) Growth kinetics on Phenoscope, obtained by extracting the projected rosette area (cm2) from the daily photographs
(d) Relative Expansion Rate : example day 16 to day 29
(e) Relative Expansion Rate Instant : for each day, RERi is calculated with a window of 6 days (example : RERi day 16 = RER13-19)

Validation of experiment and tolerances

In case of absence of light for more than 4 hours continuously (short days) or 6 hours continuously (long days), or in case of a temperature peak above 27°C, we will rerun the experiment.

Imaging: pictures allowing to estimate PRA are typically taken each day for each plant from day 9 after sowing (installation on Phenoscope) until the end of the experiment usually ~30 days after sowing (+ an InfraRed image per day and per plant).

> It may happen that some days the image is not available. We consider that the experiment is successful when no more than 2 images are missing during the ~exponential phase of growth (between day 19 after sowing and the end of the experiment).

Watering: Soil water content is typically adjusted twice a day (once during the day and once during the night) for each plant, maintaining SWC within the target SWC +/- 5 points after watering.

> It can happen that some waterings are not carried out correctly for a specific pot. We consider that the treatment is successful if no more than 2 successive waterings were missed.

> In complex/sensitive watering cases we have our own tolerances to validate the success of an experiment, based on our experience of the effect of these watering variations around the target SWC.

For a given experiment, if we can't validate the phenotypic data for more than 20% of the replicates (individuals) by modality (genotype x treatment), we rerun the experiment, unless this is due to poor germination of the provided seeds. This doesn't include obvious outliers (plants whose development is stopped -or growth strongly retarded- at an early stage, for whatever reason) that may appear stochastically in a given population regardless of experimental conditions.

Provided results and initial analyses

The file with all the phenotypes extracted from the experiment will be provided once the platform staff has completed validation of the experimentation, image analysis and verification of the data. **This usually takes about 2 weeks minimum**.
A pdf with a first quick analysis will also be provided, as well as the complete explanations specific to the experiment.

- Experiment data (plug lot, nutrient solution, watering and nutrition)
- Climate data (synthetic graphs over the duration of the experiment, temperature, RH)
- Phenotypic raw data : csv file (a line of this file corresponds to a plant for a given day with : descriptive columns - Genotype and Treatment - then a column per trait measured for this plant for the given day - see phenotypic parameters described above)
- WebPheno access to the specific experiment database, including RGB pictures (raw and segmented). Images are saved on our server on the long term.
- First quick analysis of growth phenotypes, including growth curves, boxplots of all traits on the last day of the experiment, treatment response curves, growth rate dynamics, organized by genotype x treatment modality, and basic statistics.

Anything not listed here will require specific developments or analyses, with associated delays and costs.

Destructive sampling of the aerial part of the plants is possible at anytime and is taken care of by you, with possible support from Elodie Gilbault. **Roots are not accessible** (peatmoss soils).

4- Price scale fixing and invoicing

Please contact us for this as there are too many different cases and situations.

5- Use of the Phenoscope data

Apart from the rare specific case of subcontracting services, any use of the data resulting from an experiment on the platform using the work of staff dedicated to the Phenoscope will require:

- **sharing the results and conclusions** drawn from this experimentation after your full analysis

- **including at least one staff member of the platform among the authors of a possible publication resulting from these analyses**. For basic experiments, one staff member of the platform who has contributed technically to the experimentation is enough (Elodie Gilbault). In the case when a scientific contribution was also needed to design the experiment, adjust the conditions or analyse/interpret the results, or in case of a collaboration, Olivier Loudet should also be listed as author. In the case when technical and computer developments were needed specifically for this experiment, Olivier Zurfluh should also be listed as author

Obviously, co-authorship requires that we have been able to **validate a complete manuscript** at least **10 days prior to the intended initial submission date**. Even when not a co-author himself (cases when Elodie Gilbault is the only name included), Olivier Loudet must see and validate the complete manuscript. **Manuscripts should never be submitted without our prior approval**. Our IJPB policy doesn't allow us to be co-authors on manuscripts submitted to 'predatory journals' (those without a proper peer-review process and editorial board).
We shall work together to agree on the use of the provided data, but we reserve the right to withdraw from the authors list at any time.