

PILOT LEAF GROWTH EXPERIMENT
(WORKPACKAGE 1 - DELIVERABLE 1.2)

OBJECTIVES :

The purpose of this experiment is the comparison of leaf growth of 3 genotypes under the same target environmental conditions in the labs of all/many participating partners of AGRON-OMICS. This will allow assessment of biological and inter-laboratory variability and is crucial for the profiling and modelling approaches. In parallel, we need to learn about the differences when the same genotypes are grown under the conditions normally used at the respective location.

PERSONS IN CHARGE :

Christine Granier (granier@supagro.inra.fr) - Partner 2b
Lothar Willmitzer (Willmitzer@mpimp-golm.mpg.de) – Partner 3a

REFERENCE IN AGRON-OMICS ANNEX 1 :

Deliverable D1.2, described page 90

DEGREE OF CONFIDENTIALITY : Not confidential – Available on Website

STARTING DATE AND ENDING DATE :

Starting date: Beginning of March or later but it has to be finished end of May!

LIST OF PARTICIPANTS :

Partner 1 (Gerrit Beemster, gebee@psb.ugent.be)
Partner 2a (Christian Meyer, meyer@versailles.inra.fr)
Partner 2b (Catherine Massonnet, massonne@supagro.inra.fr)
Partner 3a (Rhonda Meyer, meyer@mpimp-golm.mpg.de)
Partner 3b (josip.perkovic, josip.perkovic@tuebingen.mpg.de)
Partner 4 (Gaelle Messerli, mgaelle@ethz.ch)
Partner 5 (Mike Bevan, michael.bevan@bbsrc.ac.uk)
Partner 6 (Vicky B-Wollaston, Vicky.b-wollaston@warwick.ac.uk)
Partner 7 (Emma Wigmore, emma@arabidopsis.info)
Partner 9 (Silvia Rubio-Diaz, silvia.rubio@umh.es)

DETAILED DESCRIPTION OF THE EXPERIMENT

3 genotypes : Col-4, Ws and *Ler*

Repetitions : 10 repetitions in ‘shared’ conditions described below by partner 2b (INRA-LEPSE). The 10 repetitions in shared conditions are plants numbered from 1 to 10
As a consequence, plants are named with the partner number-the genotype-the number of the repetition as follow :

-P3a-Ws-10 is the plant with a Ws repetition, repetition 10, grown with common protocol in partner 3a lab.

-P1-Ler-7 is the plant with a *Ler* repetition, repetition 7, grown with common protocol in partner 1 lab

The protocol shared by all partners is described below.

Please, if possible, also grow the 3 genotypes with 10 repetitions in conditions that you normally use in your lab. The 10 repetitions in your 'specific' conditions are plants numbered from 11 to 20 :

-P1-Ler-11 is the plant with a *Ler* repetition, repetition 11, grown with specific conditions in partner 1 lab

At the end of this experiment we will measure on the 60 plants (30 with shared protocol and 30 with specific protocol) grown in the lab of each participant :

From scans :

total rosette leaf area, number of leaves on the rosette, final individual leaf area, position of the leaf with maximal area, length and width of leaf with maximal area, length and width of leaf 6

From epidermal imprints :

epidermal cell size in leaf 6, epidermal cell number in leaf 6

From frozen leaf 5 :

A few molecular and biochemical characteristics in leaf 5.

Keep in mind that it is also **mandatory** to fill annex 1a and 1b carefully to describe your experiment as precisely as possible...

PROTOCOL SHARED BY ALL PARTICIPANTS

Before sowing :

30 labelled pots from partner 2b (INRA LEPSE) are sent to each partner.

7kg of soil from partner 2b (INRA LEPSE) is sent to each partner. The soil is a mixture of agricultural soil and organic compost (V/V, 50/50) made by partner 2b (INRA LEPSE). It is distributed at 32% of humidity ($0.33 \text{ g H}_2\text{O g}^{-1}$ dry soil) in a closed bag. Each participant has to keep the soil in the closed bag until the day of sowing. (To avoid fungi development, do not wait too long before starting the experiment.)

Seeds have to be ordered on the NASC website (partner 7) : N933 is Col-4, NW20 is *Ler* and N2360 is *Ws*. Put the phrase AGRONOMICS in the notes so you do not get charged and you will receive the necessary amount of seeds. When you receive the seeds, store the seeds at 4°C until the sowing date. 24h before sowing just put the necessary quantity of seeds (= 60 minimum) in a micro-centrifuge tube and cover them with water. Keep the seeds covered by water in the tubes at 4°C during for 24h.

The growth conditions in the chamber have to be fixed at:

- day-length: 16h (example : light on at 6am and off at 10pm).
- light intensity: $150 \mu\text{mol m}^{-2} \text{ s}^{-1}$ at the rosettes level (new lamps, washed glasses, washed walls, reduced plant distance from light source can help to reach this value).

Note: Ideally, light intensity measurement should be performed when lamps are burning at their set output, i.e., not soon after they come on during the light cycle. Measuring after at

least 2 hours from light onset is strongly recommended. In addition, the value should be an average taken at representative points in the growth chamber (i.e., 'edges' vs center).

- light quality: ideally a mix of cool-white fluorescent tubes and sodium or HQi lamps (if not possible, just write-down in annex 1 the characteristics of your lamps).
- air humidity: 70 to 75 %.
- ideally leaf temperature should be 21°C (for Col-0 plant grown in the PHENOPSIS platform, this corresponds to air temperature of 20 °C during the day and 21.5 °C at night).

Note: if you have the possibility or by default you use data loggers, air temperature and air humidity can be recorded for the full duration of the experiment or for selected days (i.e., around harvest time).

The day of sowing :

Fill each pot with 196 g of soil. As the pots are approximately 12g, each individual pot has to weigh 208 g. Add 10 ml of nutrient solution (see composition in annex 2) at the pot surface just before sowing. Aspirate seeds and water with a suitable pipette and sow 3 soaked seeds in the centre of the pot (approximately 0.5cm between each of them). After sowing, arrange your pots randomly in trays and spray some water on the pot surface. Then cover the pots in the trays with aluminium foil and put them in the growth chamber. Leave them covered during 48h at 21°C.

From germination to harvest :

After 48h, remove the aluminium foil to ensure that plants do not etiolate. Ideally, fill the pots and sow the seeds on a Friday in the afternoon, and remove the aluminium foil on Monday morning. From this moment (removal of the aluminium foil) until the end of the experiment, weigh and water your plants daily with nutrient solution ideally on a pot to pot basis. The watering will have to be done in the morning 3 hours after the light is on (depending on settings, for example, around 9-10am). For each pot the **target weigh is 218 g**. The following week is crucial : the germination stage is very sensitive to drought and, as the root is very small, do not hesitate to spray water on the pot surface once or twice a day depending on the air circulation in your growth chamber: do not allow the soil surface to dry out.

Notations :

When more than 2 over 3 plants for each pot have reached the stage 1.0 (cotyledons open fully) as described in Boyes et al., 2001¹, discard your plants to leave just one healthy plant with the 2 cotyledons fully opened per pot. **Note the date of this stage.**

Later, also **note the date when the 6th leaf is visible** (stage 1.06 according to Boyes et al., 2001). When the stem starts to elongate try to identify which leaf is leaf number 5 and mark it by planting in the pot a small object (for example, a small plastic tag).

¹ Boyes D.C., Zayed A.M., Ascenzi R., McCaskill A.J., Hoffman N.E., Davis K.R. & Gortlach J. (2001) Growth stage-based phenotypic analysis of arabidopsis: A model for high throughput functional genomics in plants. *Plant Cell*, **13**, 1499-1510.

The day of sampling and leaf growth measurements :

Note the date of the stage when the first flower is open (Stage 6.00 according to Boyes et al., 2001). At this stage, 8h after the light is on (ie 2pm if the light is on at 6am), cut leaf 5 without its petiole (lamina + midvein), and put it as fast as possible (less than 20seconds) in pre-cooled micro-centrifuge tubes in liquid nitrogen (that will be distributed pre-labelled by partner 3a). Leave the samples individually at -80°C . Don't forget to put a small hole in the tube cap to avoid over-pressure building in liquid nitrogen.

Then cut the remaining rosette. Clean it with a paintbrush rapidly to be sure there is no soil on it anymore and then weigh the whole rosette rapidly on a balance (10^{-3}g precision). Note the weigh on the sheet of paper distributed by Partner 2b (template INRA-LEPSE shown below).

Detach individual leaves formed after the two cotyledons (**only lamina, without the petiole**) in their order of emergence and stick the leaves on the sheet prepared with double-sided adhesive. As leaf 5 is absent because it has been harvested for profiling, just mark 'L5' between leaf 6 and leaf 4. Also note on the A4 sheet the number of leaves (total number of leaves of the rosette without the two cotyledons and including leaf 5).

Don't forget to fill this sheet of paper with the accession name and the number of the repetition!

Spread a thin layer of nail varnish on the upper surface of leaf number 6. Let it dry and then make a scan of the A4 sheet with your scanner. Name the file accordingly :

-P3a-Ws-11 is the scan with a Ws repetition grown with partner 3a specific protocol, repetition 11

-P1-Ler-7 is the scan with a Ler repetition grown with common protocol in partner 1 lab, repetition 7

Carefully, peel out the thin layer of dry varnish with transparent adhesive tape and stick it on the microscope slide (the microscope slides are labelled and distributed by Partner 2b, INRA-LEPSE, the varnish and the adhesive tape are also distributed by partner 2b).

Slides have to be sent to Partner 2b (INRA-LEPSE) for image analyses.

**Catherine Massonnet UMR LEPSE Bat 7 INRA-SUPAGRO
Place Viala 34060 Montpellier France**

Scans have to be sent to Partner 2b (INRA-LEPSE) for image analyses.

massonne@supagro.inra.fr

Samples of leaf 5 for profiling have to be sent to partner 3a (MPI) on dry ice.

**Lothar Willmitzer Max-Planck-Institute of Molecular Plant Physiology
Wissenschaftspark Golm, Am Mühlenberg 1 D-14476 Potsdam**

ADDITIONAL NOTES: [anything including accidents, remarks, problems, any other change to the plan]

Light quantity was estimated at..... $\mu\text{mol m}^{-2} \text{s}^{-1}$

TEMPERATURE CONDITIONS :

Air temperature during the night has been measured : Yes No

If yes, which sensor :

If yes, when :

If yes, where in the chamber :

Air temperature during the day has been measured : Yes No

If yes, which sensor :

If yes, when :

If yes, where in the chamber :

Leaf temperature during the night has been measured : Yes No

If yes, which sensor :

If yes, when :

If yes, where in the chamber :

Leaf temperature during the day has been measured : Yes No

If yes, which sensor :

If yes, when :

If yes, where in the chamber :

HUMIDITY CONDITION :

Air humidity during the night has been measured : Yes No

If yes, which sensor :

If yes, when :

If yes, where in the chamber :

Air humidity during the day has been measured : Yes No

If yes, which sensor :

If yes, when :

If yes, where in the chamber :

ADDITIONAL NOTES: [anything including accidents, remarks, problems, any other change to the plan]

Annex 2. Composition of the nutrient solution shared by all partners

The Pilot leaf grow experiment will last approx, 45 days
Each partner will add approx. 5ml per pot daily (30 pots)
Each partner will need 6750 ml of nutrient solution for the experiment

**Oligoelements have been prepared and distributed
by partner 2b (each partner will receive 5ml but will need 4ml)**

OLIGO-ELEMENTS	For 20L
H ₃ BO ₃	7.42g
MnSO ₄ , H ₂ O	7.6g
CuSO ₄ , 5H ₂ O	0.126mg
ZnSO ₄ , 7H ₂ O	5.75g
(NH ₄) ₆ Mo ₇ O ₂₄ , 4H ₂ O	2.48g
H ₂ O	Adjusted to 20L

10L of nutrient solution		
	Mother? solution for 100ml	For 10L
HNO ₃ (purity 52%)	-	4,93ml
H ₂ PO ₄ NH ₄	2.876g/100ml	4ml
KNO ₃	8.845g/100ml	8ml
OLIGO-ELEMENTS	-	4ml
Fe (E.D.D.H.A)	-	0,48g
H ₂ O		Adjust to 10L

pH has to be around 5.2-5.8. Adjust it if it is higher or lower.

Annex 3. Template of A4 sheet distributed to all partners by Partner 2b for notations (the corresponding 'empty' pdf file is available on the Agron-Omics WEBSITE).

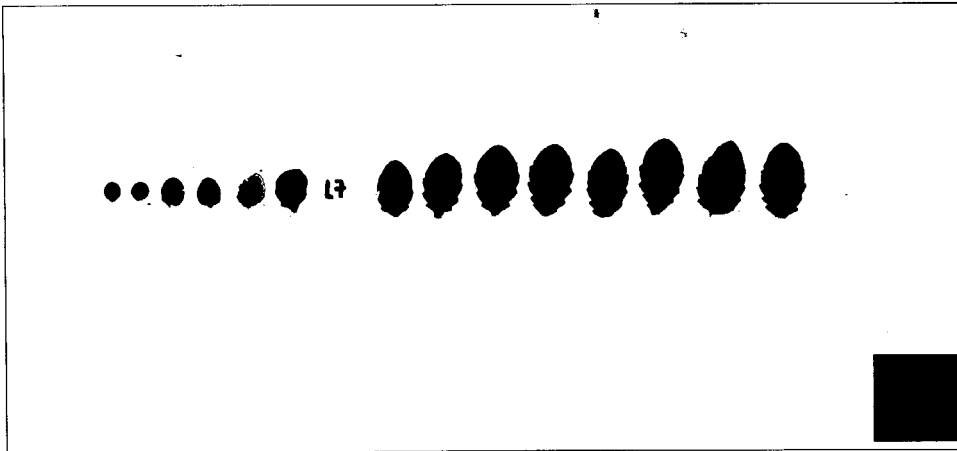


Partner n° : **2b** Lab : **LEPSE**

Fresh weight of rosette (g) : **0.447 g**
Number of rosette leaves : **15**

Accession : **TEST**
Repetition : **XX**
Sowing date : **ww/yy/zz**

2 flat cotyledons (date) : **AA/BB/ZZ**
6th leaf visible (date) : **cc/dd/zz**
first flower open (date) : **EE/FF/ZZ**



Please, stick all the leaves from the rosette without their petiole, Do not stick the two cotyledons, Do not stick leaf 7 (harvested for profiling). Stick the leaves in order of their emergence on the rosette and write down L7 at leaf 7 position.

Pilot Leaf Growth Experiment- AGRON-OMICS consortium