Supplemental data: Detailed description of the protocol distributed to all 10 laboratories

for the comparative experiment.

3 genotypes: Col-4, Ws and Ler

Repetitions: 10 repetitions in 'shared' conditions described below by laboratory L3.

Before sowing:

30 labeled pots from laboratory L3 are sent to each laboratory.

7 kg of soil from laboratory L3 is sent to each laboratory. The soil is a mixture of agricultural soil and organic compost (V/V, 50/50) made by laboratory L3. It is distributed at 30 % of humidity (0.30 g H_2O 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: N933 is Col-4, NW20 is L*er* and N2360 is Ws. Each laboratory will receive approximately 30 seeds per genotype except L3 who will receive 90 seeds per genotype for the measurement of a larger set of leaf growth variables. 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 (= 30 minimum per genotype) 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 µmol m⁻² 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 lamps (if not possible, just write-down 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) 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 <u>target weigh is 223 g</u> corresponding to a soil water content of 0.40 g H_2O g⁻¹ dry soil. 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., 2001, 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).

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, cut the 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 (10⁻³g precision). Note the weigh on the sheet of paper distributed by L3.

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. Also note on the A4 sheet the number of leaves (total number of leaves of the rosette without the two cotyledons).

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.

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 L3, the varnish and the adhesive tape are also distributed by L3).

Scans and slides have to be sent to L3 for image analyses.

Measured variables:

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

Annex: composition of the nutrient solution shared by all laboratories

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 P3 (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
ZnSO₄, 7H₂O	5.75g
(NH ₄) ₆ M0 ₇ O ₂₄ , 4H ₂ O	2.48g
H2O	Adjusted to 20L

10L of nutrient solutio	n	
	Mother solution for 100ml	For 10L
HNO3		4,93ml
H ₂ PO ₄ NH ₄	2.876g/100ml	4ml
KNO₃	8.845g/100ml	8ml
OLIGO-ELEMENTS	-	4ml
Fe (E.D.D.H.A)	-	0,48g
H2O		Adjust to 10L