unPAK <u>CURE phenotype measurement protocols</u> last updated October, 2018

Guidance for trait measurement: Two traits are required for contribution to the unPAK database, rosette diameter and total fruit production. Rosette diameter is measured with a caliper (in mm) or ruler at or after bolting. Fruits are counted as 'good fruit' with seeds present and 5mm in length.

Additional traits can be measured or counted easily in a classroom setting following specific unPAK protocols (see separate PDF with research apprentice protocols titled "unPAK experimental protocols"); then these traits can be included in the database: inflorescence height at maturity, fruit length, leaf number, branching, number of aborted fruits.

Measuring additional traits could also be used as a means for students to learn to creatively develop replicated measurement protocols of their own. However, if they do not follow unPAK protocols, they cannot be included in the data base.

To request additional formal protocol development for the entire unPAK network and inclusion in database please contact (rutterm@cofc.edu).

CURE unPAK phenotype protocol

Note: unPAK specific protocols for additional traits can be found at arabidopsisunpak.org.

Rosette diameter:

diameter.at.bolt: During early growth in Arabidopsis, leaves are arranged in a basal rosette. This circular rosette of leaves is typically flush to the potting mix surface. At onset of bolting, we measure rosette diameter as the maximum diameter. Using a digital caliper set to millimeters, we measure across the widest point in the rosette from the edge of the tip of one leaf to the opposite edge of the rosette. To confirm that the widest point is selected, we rotate the calipers until no additional diameter is a longer distance and retain the longest measurement.

To measure rosette diameter at a consistent point during development of the plant, measurement occurs at onset of bolting, i.e. when the inflorescence has begun to elongate. We consider date of onset of bolting as when the main inflorescence has reached a height of 5 mm.

Photo credit: A. Bisner

rdiam.snapshot: If rosette diameter is not measured at the onset of bolting, but the widest point measured consistent with the protocol above, the trait is considered a "snapshot" of plant development. Date of measurement (time since germination) and whether or not the plant has bolted must also be recorded in this case.

Fruit (fitness) measurements:

fruitnum: Total number of fruit (siliques) with seeds are counted when the main stem has stopped growing. This developmental point is when the terminal bud has senesced or developed into a fruit. When the plant has stopped growing on the main stem, fruit are counted on the whole plant including all branches (basal branches and branches on the main stem). To be counted as a fruit, the length must be at least 5 mm, with evidence of seeds within the fruit. If only the replum (the separation between the two valves of the silique) remains (as the valves have dehisced), the replum must be at least 5mm and there must be evidence of multiple seeds produced (indentations on the replum; or seeds as in picture below).

Open fruits without remaining seeds still count as good fruits.

Replum

Photo credit: A. Bisner

fruitnum.snapshot: If fruit are counted at a different developmental point than described for fruitnum above, then the date of measurement must be included and the traits is considered a "snapshot".

aborted.fruits: This trait is not typically measured during unPAK CUREs. It is included here for clarity and completeness. When "fruitnum" are counted not all reproductive output results in a fully developed silique. This may include flowers, buds or fruit that are <5mm and are likely to contain only one or no seeds. Not all flowers will develop into fruit. The total remaining flowers, buds, underdeveloped pistil, and fruit <5 mm on all branches and main stem are considered aborted or unfilled fruits. As above, if aborted fruits are counted at a different developmental point, then the date of measurement must be included and the traits is considered a "snapshot" "aborted.fruits.snapshot".