

Supplemental Figure S1. Analysis of Phe accumulation and export in wild type, *gdu1-1D* and *lht1-1*.

Plants were grown and assayed for Phe uptake as described in Material and Methods.

(A) Qualitative analysis by thin layer chromatography of the composition of the medium after efflux analysis.

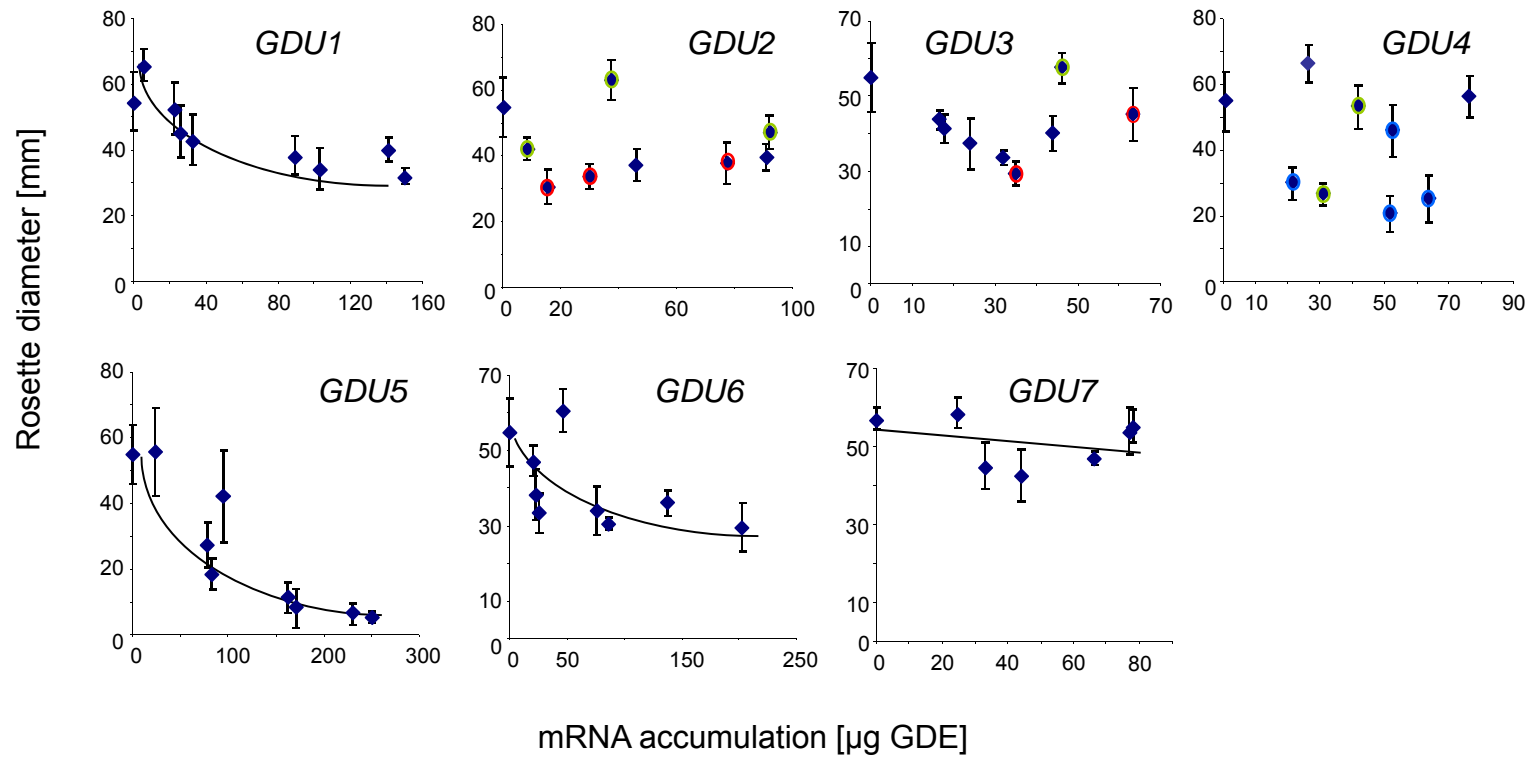
After taking up Phe for 10 min from a solution containing 10 μM or 1 mM Phe and 5.6 kBq [¹⁴C]Phe, wild type plants (WT), *lht1-1*, *gdu1-1D* and *gdu1-1D lht1-1* mutants were transferred into a fresh medium lacking Phe. The compounds released into the medium after 1 h were separated by thin layer chromatography and the radioactivity was localized on the plate with a storage phosphor screen. Loading of various amounts of [¹⁴C]Phe proved that the radiolabeled compound present in the medium is Phe. The positions of the loading and the migration front of the solvent are indicated.

(B) Export of Glu, Phe and His is increased in *gdu1-1D*.

Plants absorbed compounds supplied at 1 mmol L⁻¹ for 10 min and were transferred into a fresh medium for 20 min. Radioactivity present in the medium and the plant was determined by scintillation counting. Percentages correspond to the ratio radioactivity in the medium divided by the total absorbed radioactivity (i.e. present in the medium and the plant after the efflux experiment). Mean ± SE of three biological replicates. Significantly different from the wild type (t-test): * P<0.05; ** P<0.01.

(C) The reduction of Phe accumulation in *gdu1-1D* and *lht1-1* are additive.

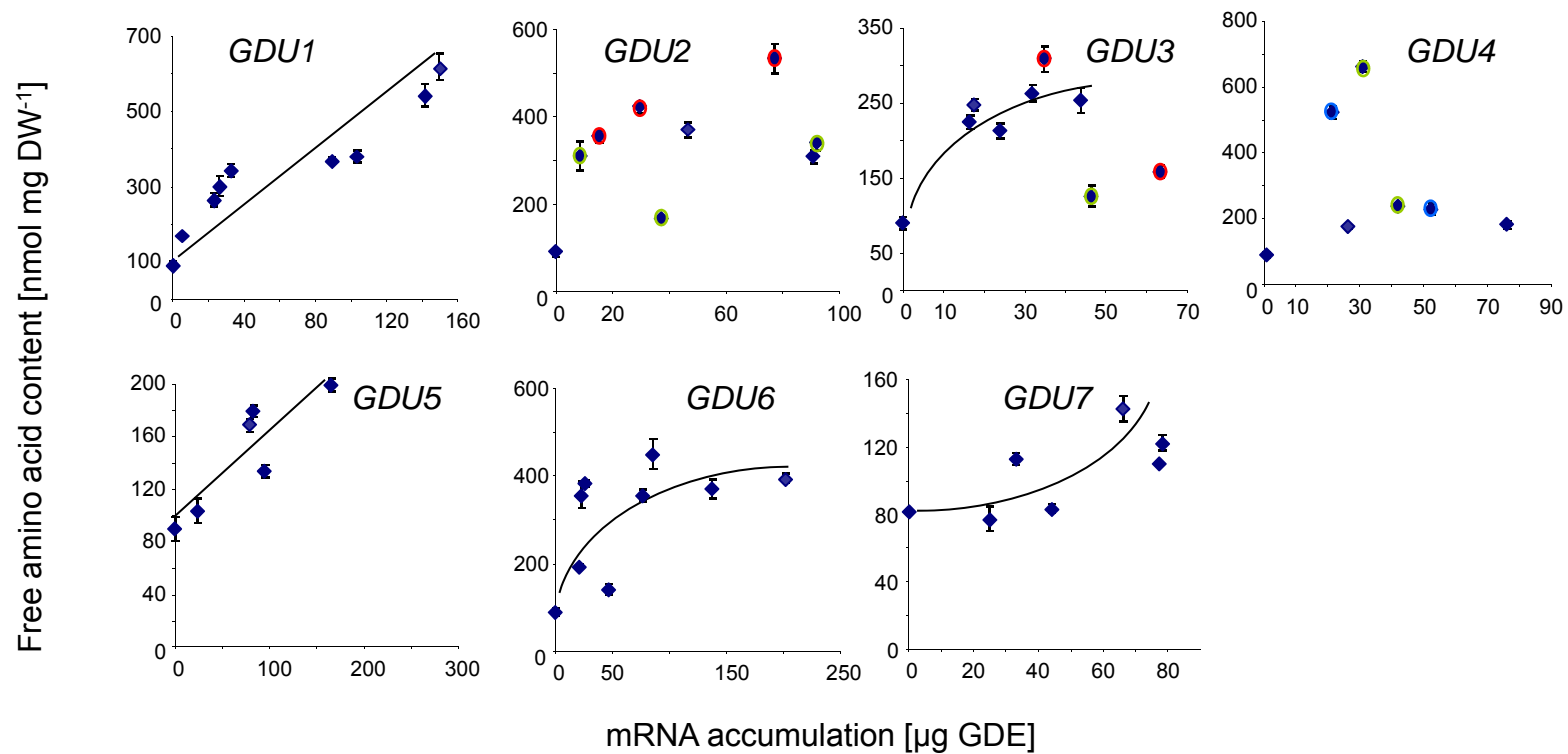
Time course analyses of Phe accumulation in WT, *lht1-1*, *gdu1-1D* and the double mutant *gdu1-1D lht1-1* were performed using a solution of 0.1 mM Phe. Phe uptake by *lht1-1* was linear and lower than that of the WT, as previously described (Hirner et al, 2004). Phe uptake in the double mutant *gdu1-1D lht1-1* is non-linear, as observed for *gdu1-1D*, and lower than that of *gdu1-1D*. Mean ± SE of three biological replicates.



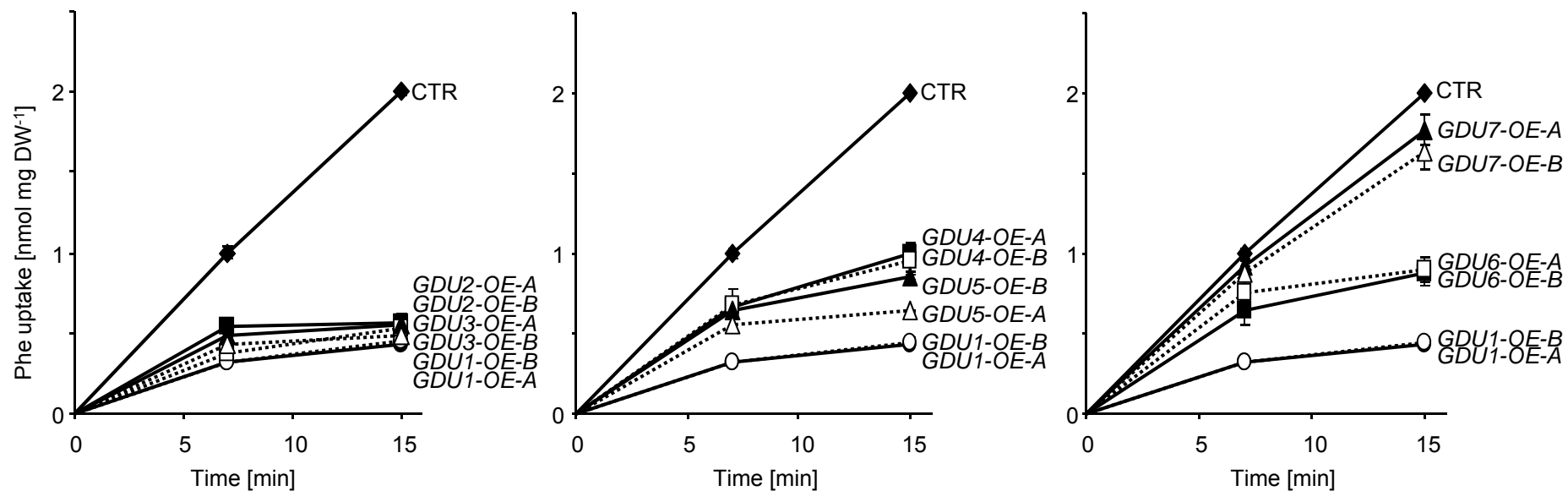
Supplemental Figure S2. Correlation between the mRNA levels and the size of plants over-expressing the *GDU* genes.

The size of about 10 progenies for each of 6 to 8 lines per construct was measured. Total RNAs were extracted from ground leaves. The accumulation of the *GDU* transcripts was determined by quantitative RT-PCR and Northern blotting analyses, with similar results. Rosette diameter was reported to the mRNA accumulation of the overexpressed gene for each line. Errors bars represent the standard deviations.

Circled data points represent lines that over-express another *GDU* gene in addition to the one placed under the control of the 35S promoter. Red circle: *GDU6*; green circle: *GDU1*; blue circle: *GDU3*.

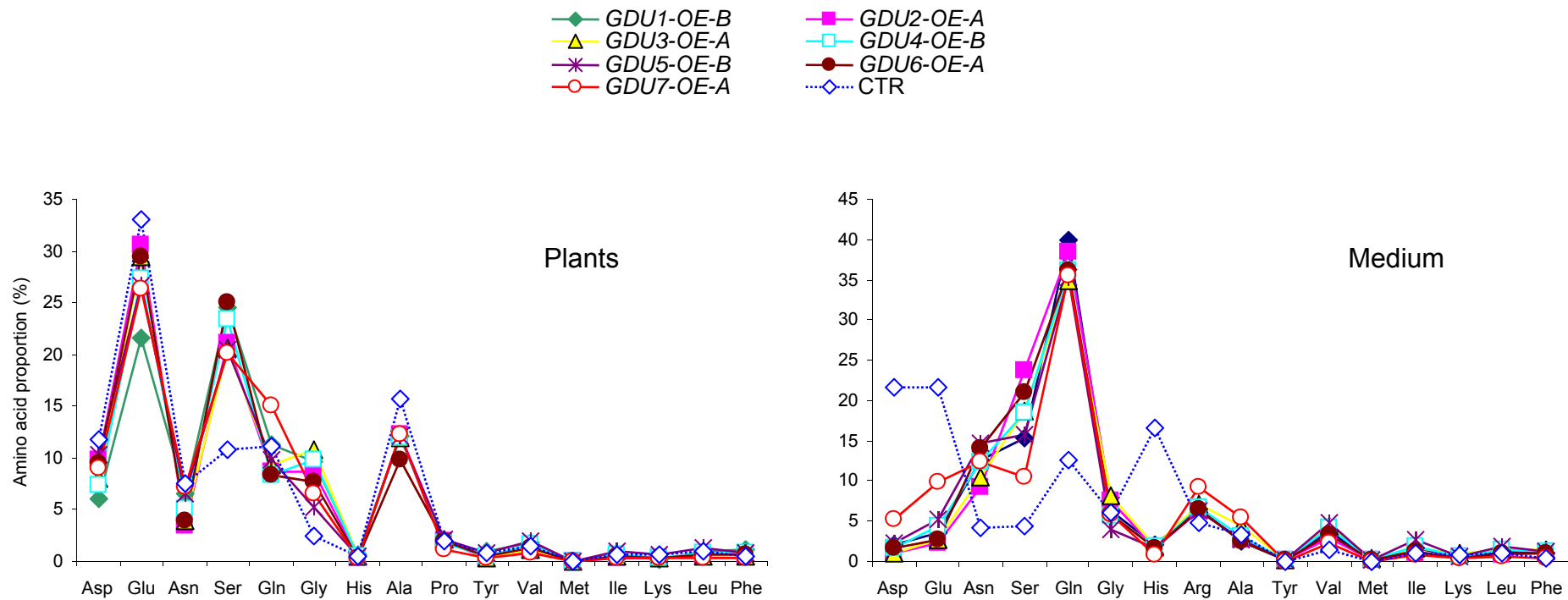


Supplemental Figure S3. Correlation between the mRNA levels and the free amino acid content of plants over-expressing the *GDU* genes. Leaves from about 10 plants for each 6 to 8 lines per construct were pooled and ground. Aliquots of the ground material used for mRNA quantitation (see legend from Supplemental Figure S2) were freeze-dried. Free amino acids were extracted from dried material and quantitated in duplicate using the ninhydrine assay on two separated aliquots of the ground material. Errors bars represent the standard deviation of the four values. The amino acid content was reported to the mRNA accumulation of the overexpressed gene for each line. Circled data points represent lines that over-express another *GDU* gene in addition to the one placed under the control of the 35S promoter. Red circle: *GDU6*; green circle: *GDU1*; blue circle: *GDU3*. No correlation between the mRNA levels and the rosette diameter for *GDU2*, *GDU3* and *GDU4* over-expressors was found when all the data points were used. Weak correlation appeared only when the single over-expressors (see text) were used. Curves were drawn using only the non-circled data points. Two and three lines (for *GDU4* and *GDU5* respectively) were not assayed for amino acid content, because the plants of these lines were too small to collect enough material for both RNA and amino acid analyses.

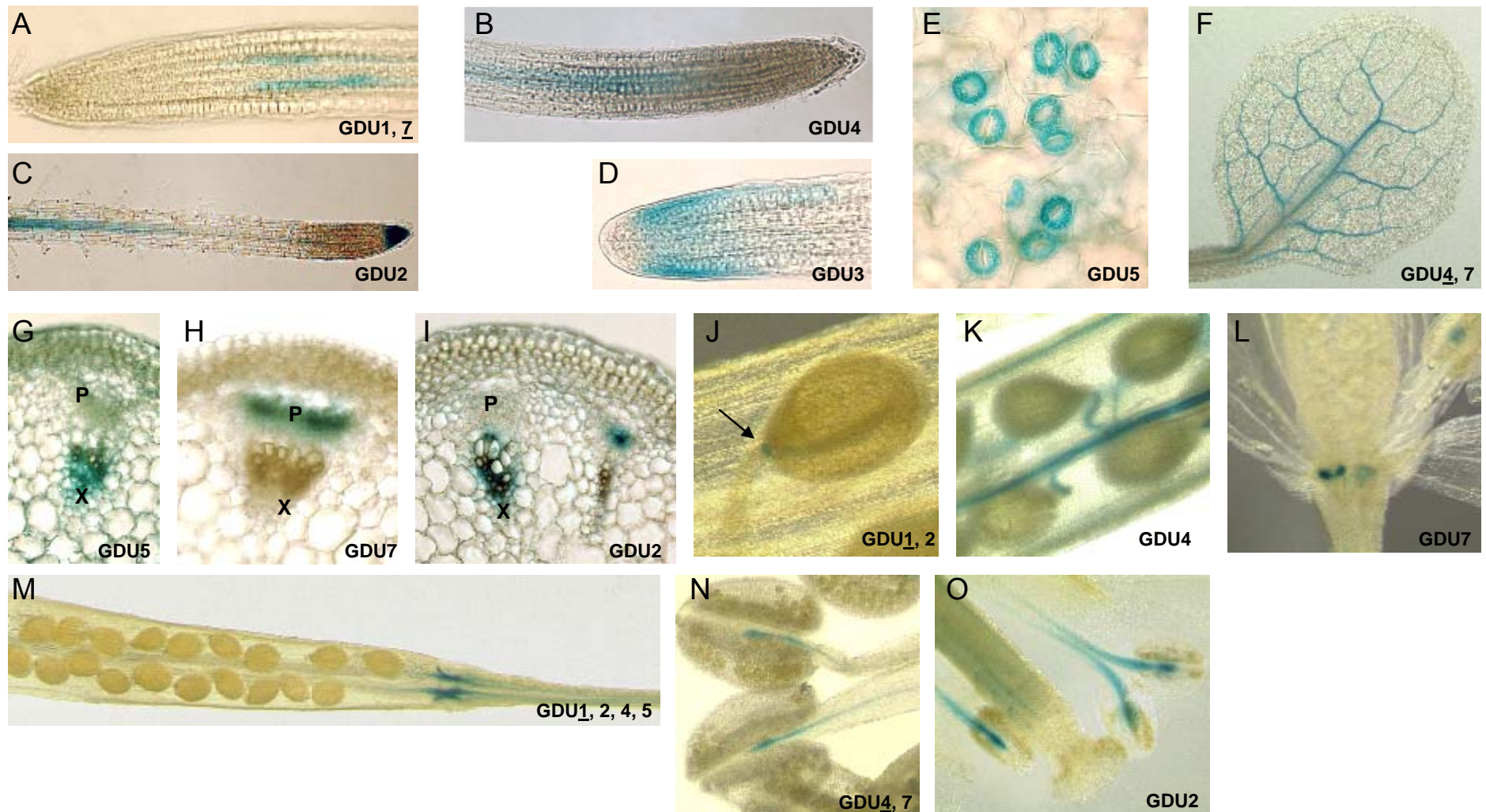


Supplemental Figure S4. Time course analysis of Phe accumulation in wild type and *GDU* over-expressing plants.

Phenotype characteristics of the over-expressors are given in Supplemental Table S1. Plants were grown and assayed for Phe uptake as described in Material and Methods in a solution of 0.3 mM Phe. Phe uptake by *GDU1*-, *GDU2*-, *GDU3*-, *GDU4*-, *GDU5*- and *GDU6*-OEs are non-linear and reduced compared to the control (CTR). Phe uptake in *GDU7*-OEs appears linear but lower than the wild type. Errors bars, when visible, represent SE of three biological replicates.



Supplemental Figure S5. Amino acid profiles of plants grown in liquid culture. Proportion of amino acids present in the plants (left) and medium (right) after a 2-day growth in liquid culture (data from Supplemental Table S3).



Supplemental Figure S6. Localization of the activity of the *GDU* promoters in the organs of Arabidopsis.

GUS activity is revealed by histochemical staining in plants expressing GUS under the control of the *GDU* promoters.

(A) to (D). GUS expression in roots from plantlets grown in vitro for 10 days. Plants contain the promoter-GUS construct corresponding to *GDU1* (A), *GDU4* (B), *GDU2* (C) and *GDU3* (D).

(E) to (I). GUS expression in vegetative aerial organs of Arabidopsis plants. Plants contain the promoter-GUS construct corresponding to *GDU5* (E), *GDU4* (F), *GDU5* (G), *GDU7* (H) and *GDU2* (I), in guard cells (E), leaf (F) and stem cross section (G, H and I).

(J) To (O). GUS expression in reproductive organs of Arabidopsis plants. *GDU1* promoter activity localizes in the chalaza of seeds (arrow) (J). *GDU4* in siliques (K). *GDU7* in nectaries (L). *GDU1* in siliques (M). *GDU4* (N) and *GDU2* (O) in stamens.

The name of the genes that display the same expression pattern are given in the lower right corner (the name of the gene corresponding to the construct used for the picture is underlined). X: xylem; P: phloem.