Figures and figure supplements

The role of photorespiration during the evolution of C₄ photosynthesis in the genus Flaveria

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Figure 1. The genus *Flaveria* as a model organism to study C₄ evolution. Schematic view of the photorespiratory pathway (A), the NADP-ME type C₄ pathway as it can be found in C₃ Flaveria species (B) and the C₂ photosynthesis pathway (C). (D) Phylogeny and physiological properties of selected Flaveria species. The phylogeny was redrawn according to McKown et al. (2005), CO₂ compensation points are taken from Ku et al. (1991), incorporation of ¹⁴CO₂ is from Moore et al. (1987) and the ratios of GLDP B (expressed in all chlorenchyma cells) and GLDP A (expressed in bundle sheath cells only) are from Schulze et al. (2013). (Abbreviations: AGT: serine glyoxylate aminotransferase; AlaAT: alanine aminotransferase; AspAT: aspartate aminotransferase; GDC: glycine decarboxylase complex; GGT: glutamate, glyoxylate-aminotransferase; GLYK: D-glycerate 3-kinase; GOX: glycolate oxidase; HPR: hydroxy pyruvate reductase; MDH: malate dehydrogenase; NADP-ME: NADP dependent malic enzyme; PEPC: phosphoenolpyruvate carboxylyase; PGLP: 2-phosphoglycerate phosphatase; PPDK: pyruvate, phosphate-dikinase; RUBISCO: Ribulose-1,5-bisphosphat-carboxylase/-oxygenase; SHM: serine hydroxymethyltransferase; 2-OG: oxoglutarate; 2-PG 2-phosphoglycolate; 3-PGA: 3-phosphoglycerate; Gln: glutamine; Glu: glutamate; OAA: oxaloacetate; PEP: phosphoenolpyruvate; TP: triosephosphate).

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Figure 2. Variation of transcript profiles of the individual Flaveria species between the four experiments. (A) Hierarchical sample clustering of all expressed transcripts. The tree was calculated with the MEV program using the HCL module with Pearson correlation and the average linkage method. (B) Principal component analysis of transcript levels. The first three components explain 27% of the total variance.

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Figure 3. Abundance of photorespiratory transcripts and proteins in leaves of individual Flaveria species. Normalized transcript (A) and protein (B) amounts are plotted as heat maps. Transcript amounts were determined by Illumina sequencing of the leaf transcriptomes and read mapping on selected F. robusta full length transcript sequences. Protein amounts were determined by protein gel blots. See Figure 3—source data 1 for absolute transcript levels, Figure 3—source data 2 for protein quantification and Figure 3—figure supplements 1 and 2 for immunoblots. Fp: F. pringlei (C3); Fro: F. robusta (C3); Fc: F. chloraefolia (C3–C4); Fpu: F. pubescens (C3–C4); Fa: F. anomala (C3–C4); Fra: F. ramosissima (C3–C4); Fbr: F. brownii (C4-like); Fb: F. bidentis (C4); Ft: F. trinervia (C4).
Figure 3—figure supplement 1. Results of the protein analyses. DOI: 10.7554/eLife.02478.010

Figure 3—figure supplement 2. Results of the protein analyses. DOI: 10.7554/eLife.02478.011
Figure 4. Flux Balance Analysis of the C₂ photosynthetic pathway. Predicted fluxes if (A) major amino acids and the corresponding oxoacids and dicarboxylic acids are allowed to freely diffuse between cells, (B) the α-ketoglutarate and glutamate transfer between mesophyll and bundle sheath was constrained (C) additionally the transfer of alanine and pyruvate between mesophyll and bundle sheath was constrained (D) transfer of all nitrogen containing compounds except for glycine and serine, which are used by the C₂ cycle were constrained. Fluxes are given in µmol s⁻¹ m⁻². Values in brackets show minimum and maximum of flux resulting from flux variability analysis. Flux of dissolved gases, sucrose, inorganic compounds and processes that carry flux below 1 µmol s⁻¹ m⁻² are not shown. The sums of absolute fluxes over the plasmodesmata for the different variants were (A): 17.8 µmol s⁻¹ m⁻²; (B): 18.4 µmol s⁻¹ m⁻²; (C): 19.0 µmol s⁻¹ m⁻²; (D): 22.1 µmol s⁻¹ m⁻². See Figure 4—source data 1 for plasmodesmatal fluxes.

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Figure 5. Mechanistic interaction between $C_2$ and $C_4$ cycle. (A) Predicted fluxes when the model is parameterized to include activity of the $C_4$ cycle enzymes. Fluxes are given in $\mu$mol s$^{-1}$ m$^{-2}$. Values in brackets show minimum and maximum of flux resulting from flux variability analysis. The sum of absolute flux over plasmodesmata was 21.9 $\mu$mol s$^{-1}$ m$^{-2}$. Flux of dissolved gasses, sucrose, inorganic compounds and processes that carry flux below 1 $\mu$mol s$^{-1}$ m$^{-2}$ are not shown. See Figure 5—source data 1 for plasmodesmatal fluxes. (B) Predicted activities of Ala-AT in mesophyll (black line) and bundle sheath (gray line) cells and predicted transfer of $\alpha$-ketoglutarate from mesophyll to bundle sheath cells (black dashed line) and glutamate from bundle sheath to mesophyll cells (gray dashed line) at low $C_4$ cycle activities. (C) Changes in biomass production with varying (low) activity of the $C_4$ cycle in a $C_2$ plant.

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Figure 6. Abundance of C₄ related transcripts and proteins in leaves of individual Flaveria species. Normalized transcript (A) and protein (B) levels are plotted as heat maps. Transcript amounts were determined by Illumina sequencing of the leaf transcriptomes and read mapping on selected F. robusta full length transcript sequences. Protein amounts were determined by protein gel blots. See Figure 6—source data 2 for absolute transcript level, Figure 6. Continued on next page.
Figure 6—source data 2 for protein quantification and Figure 3—figure supplement 1 for immunoblots. (C) Mean values of transcript levels from all four experiments were clustered by hierarchical using the HCL module of MEV program with Pearson correlation and the average linkage method. The relative transcript abundance for PEPC, PPDK, NADP-ME and Ala-AT (mean values from all four experiments) are plotted for all nine species. Fp: F. pringlei (C₃); Fro: F. robusta (C₃); Fc: F. chloraeafolia (C₃–C₄); Fpu: F. pubescens (C₃–C₄); Fa: F. anomala (C₄); Fra: F. ramosissima (C₄); Fbr: F. brownii (C₄–like); Fb: F. bidentis (C₄); Ft: F. trinervia (C₄).
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Figure 6—figure supplement 1. Results of the protein analyses.
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