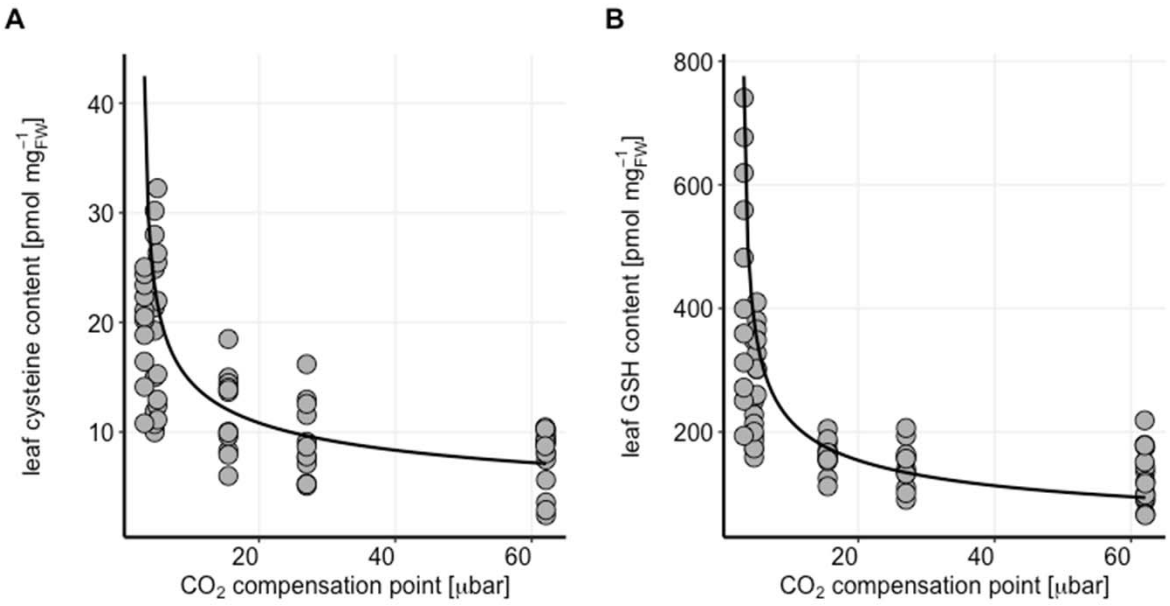
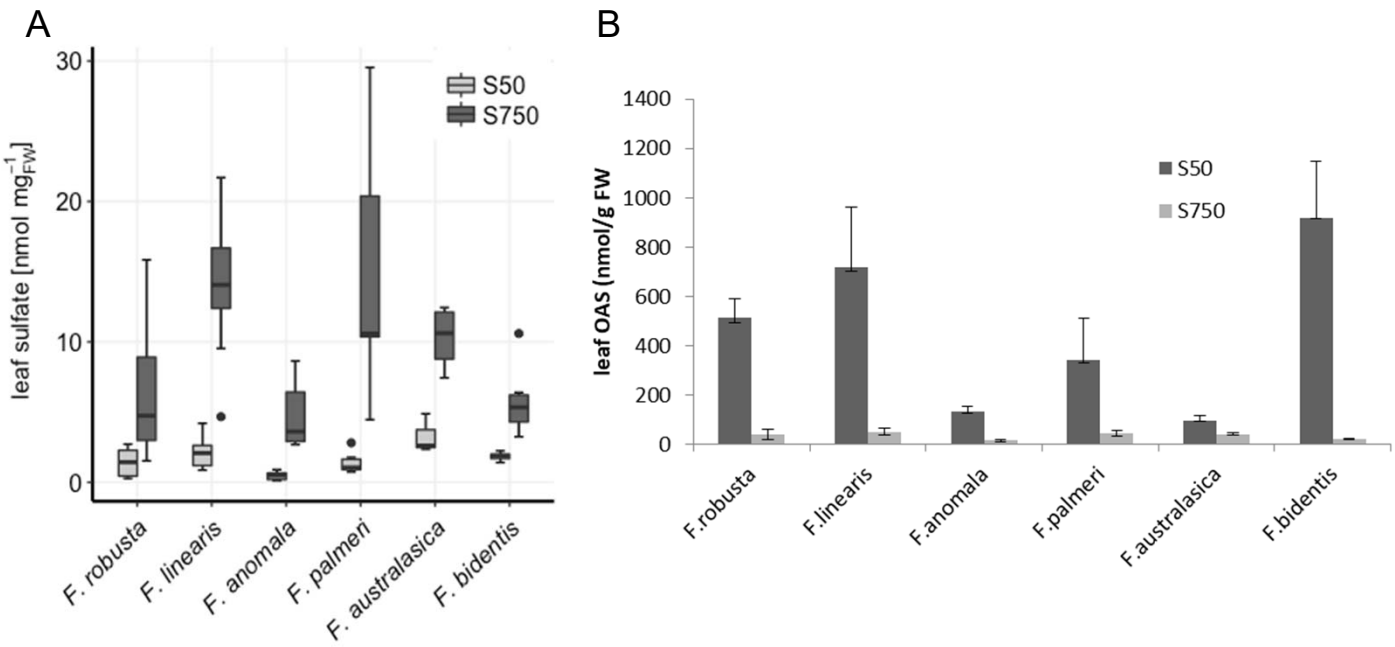


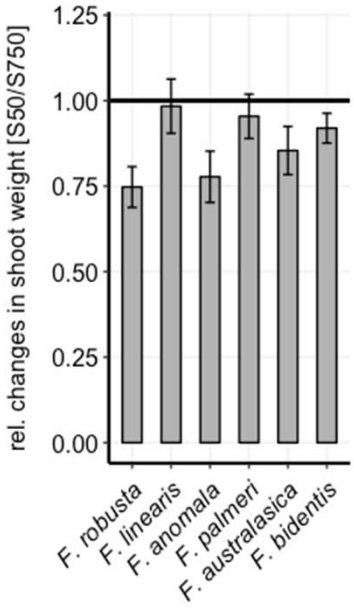
Supplemental Figure S1. Leaf cysteine and GSH content in *Flaveria* spp. in dependence on their CO_2 compensation point.
Leaf cysteine (A) and GSH (B) concentrations were analyzed in three-week-old seedlings of six species of the genus *Flaveria*. The seedlings were grown under controlled nutrient conditions. CO_2 compensation points were adopted from (Ku et al., 1991).



Supplemental Figure S2. Influence of S deficiency on leaf sulfate and OAS concentration in *Flaveria* species.

Leaf sulfate (A) and OAS (B) concentration was analyzed in 23-day-old seedlings of six Flaveria species. The plants were exposed to low sulfate (50 µM sulfate; S50) or adequate sulfate (750 µM sulfate; S750) conditions for 16 days. Data are shown as box plot (25%-75%), the line represents median, the whiskers represent 1.5 IQR, n=8 (A) or as means and SD, n=4 (B). *F. robusta* – C_3; *F. linearis* and *F. anomala* – C_3-C_4; *F. palmeri* – C_4-like; *F. australasica* and *F. bidentis* – C_4.

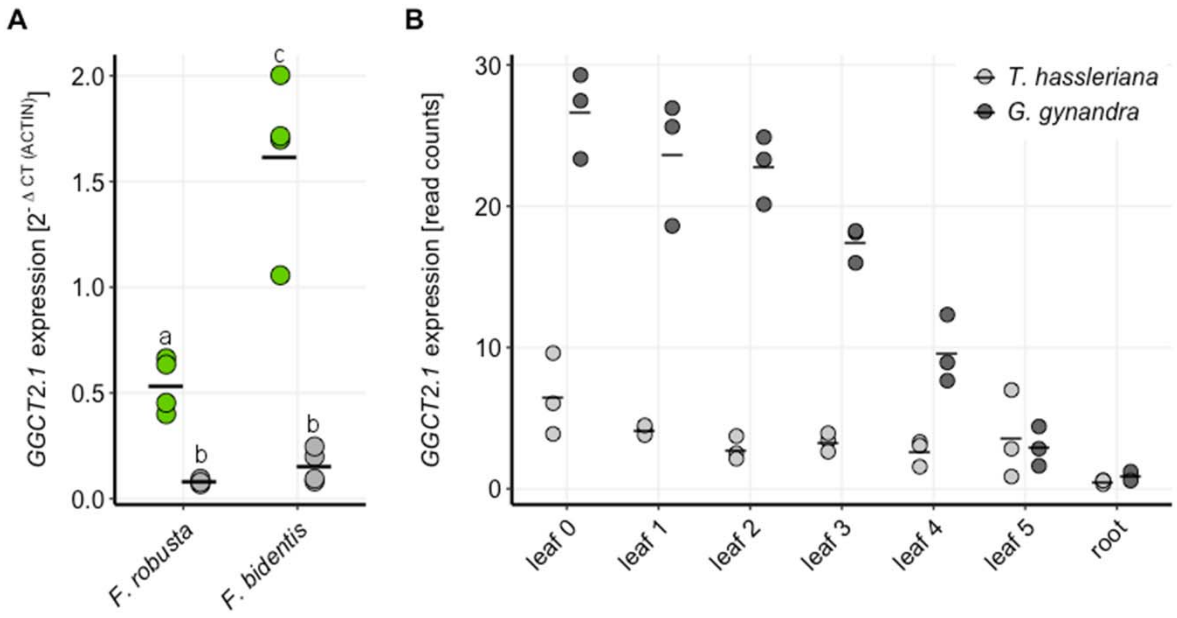
A



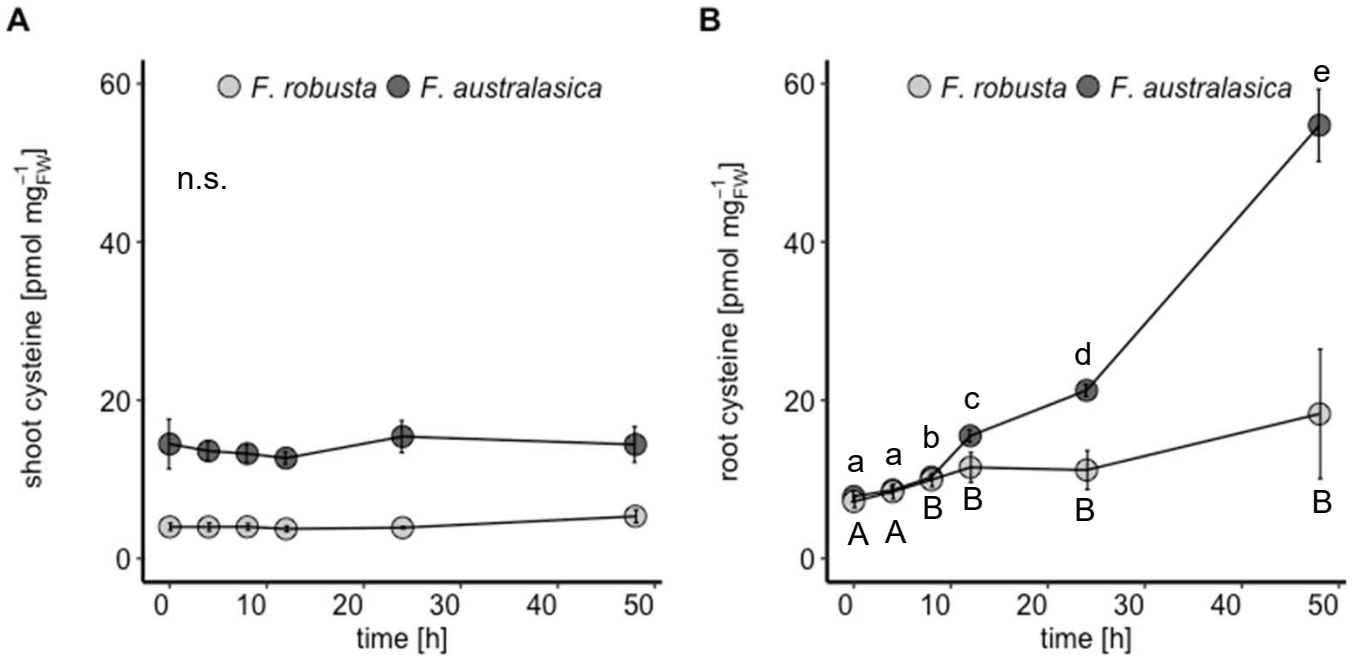
B

species	nutr.	CO_2 compensation point [µbar]	CO_2 assimilation [µmol m^{-2} s^{-1}]	photosynthetic efficiency [µmol m^{-2} s^{-1} Pa^{-1}]
F. robusta	S50	88.6 ± 18.5	10.03 ± 1.39	0.53 ± 0.21
	S750	64.8 ± 8.9	13.08 ± 2.19	0.61 ± 0.15
F. australasica	S50	5.9 ± 1.7	20.09 ± 2.94	2.27 ± 0.62
	S750	6.1 ± 1.5	22.12 ± 3.50	2.28 ± 0.36
F. bidentis	S50	6.8 ± 1.6	14.73 ± 2.15	1.97 ± 0.39
	S750	4.0 ± 1.0	16.44 ± 1.29	2.16 ± 0.19

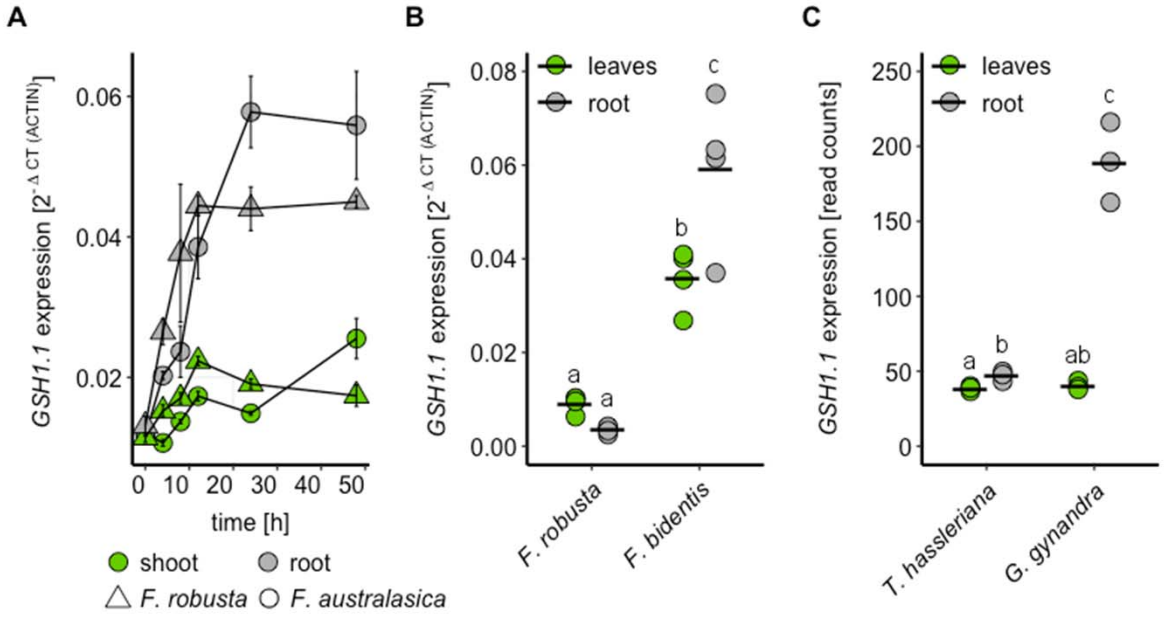
Supplemental Figure S3. Influence of S deficiency on shoot biomass and photosynthetic performance in *Flaveria* species.
A Shoot biomass was analyzed in 23-day-old seedlings of six Flaveria species. The plants were exposed to low sulfate (50 µM S; S50) or adequate sulfate (750 µMS; S750) conditions for 16 days. **B** The photosynthetic performance of *F. robusta*, *F. australasica*, and *F. bidentis*, was analyzed in 5-week old plants, after 14 days exposure to low sulfate (50 µM sulfate; S50) or adequate sulfate (750 µM sulfate; S750) conditions by gas exchange analysis. The data are shown as means± SEM, n=4. *F. robusta* – C_3; *F. linearis* and *F. anomala* – C_3-C_4; *F. palmeri* – C_4-like; *F. australasica* and *F. bidentis* – C_4.



Supplemental Figure S4. *GGCT2.1* expression in C_3 and C_4 species.
A *GGCT2.1* transcript levels relative to *ACTIN* in shoots and roots of 20-day-old seedlings of *F. robusta* (C_3) and *F. bidentis* (C_4), green are data from shoots, grey data from roots, n=4. Letters mark significant differences ($p \leq 0.01$, Student´s T-test). **B** Transcript abundance of *GGCT2.1* in leaves of different developmental stages and roots of *T. hassleriana* (C_3) and *G. gynandra* (C_4) analyzed by RNA sequencing (Külahoglu et al. 2014).

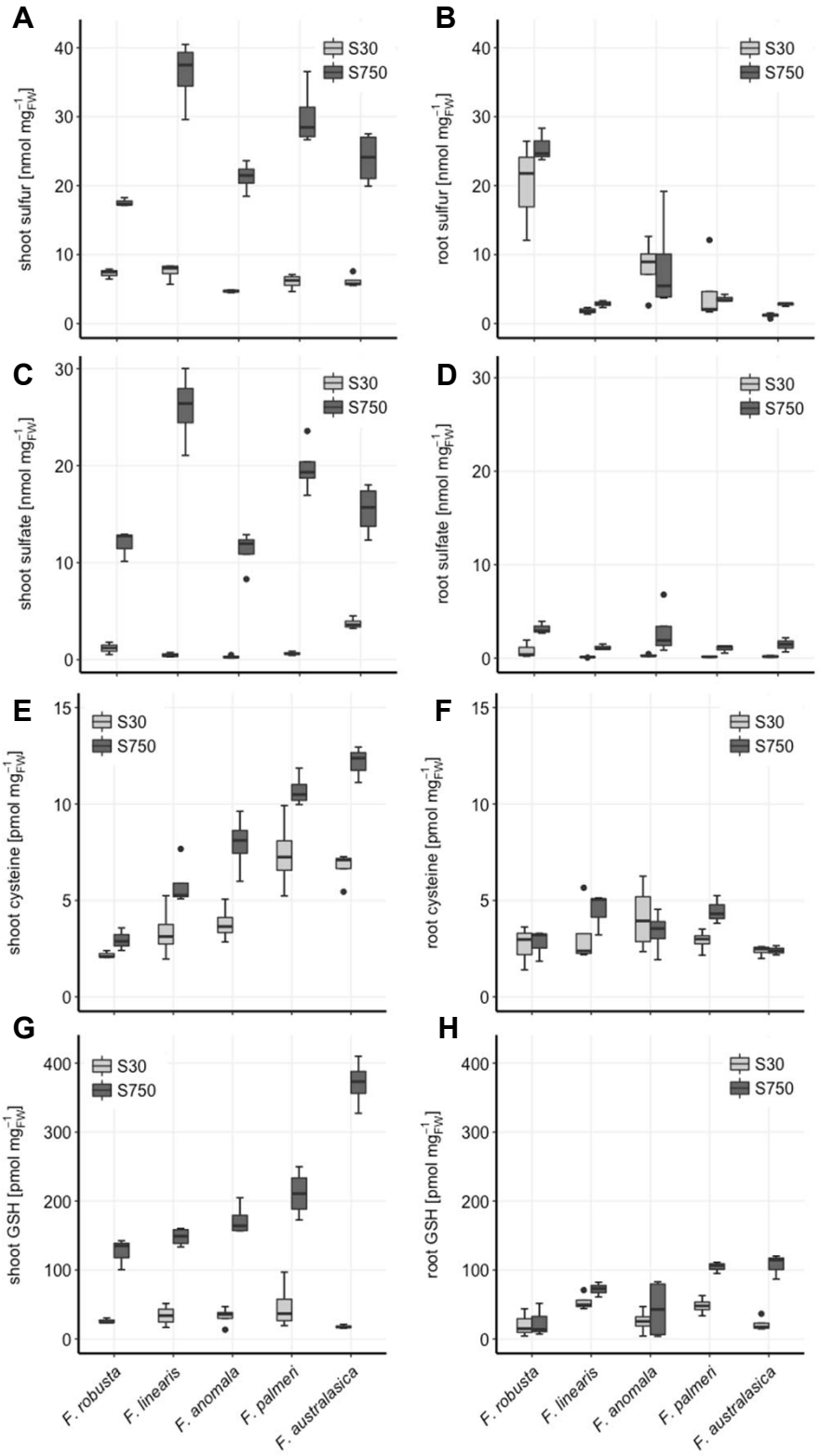


Supplemental Figure S5. Effect of BSO treatment on shoot and root cysteine concentrations in *F. robusta* and *F. australasica*.
Cysteine concentrations in shoots (**A**) and roots (**B**) of 20-day-old seedlings of *F. robusta* (C_3) and *F. australasica* (C_4) in a time course of 48 h after transfer to medium supplemented with 2 mM BSO. Data are presented as means and SEM, n=4. Different letters mark significant differences ($p \leq 0.01$, Student´s T-test).

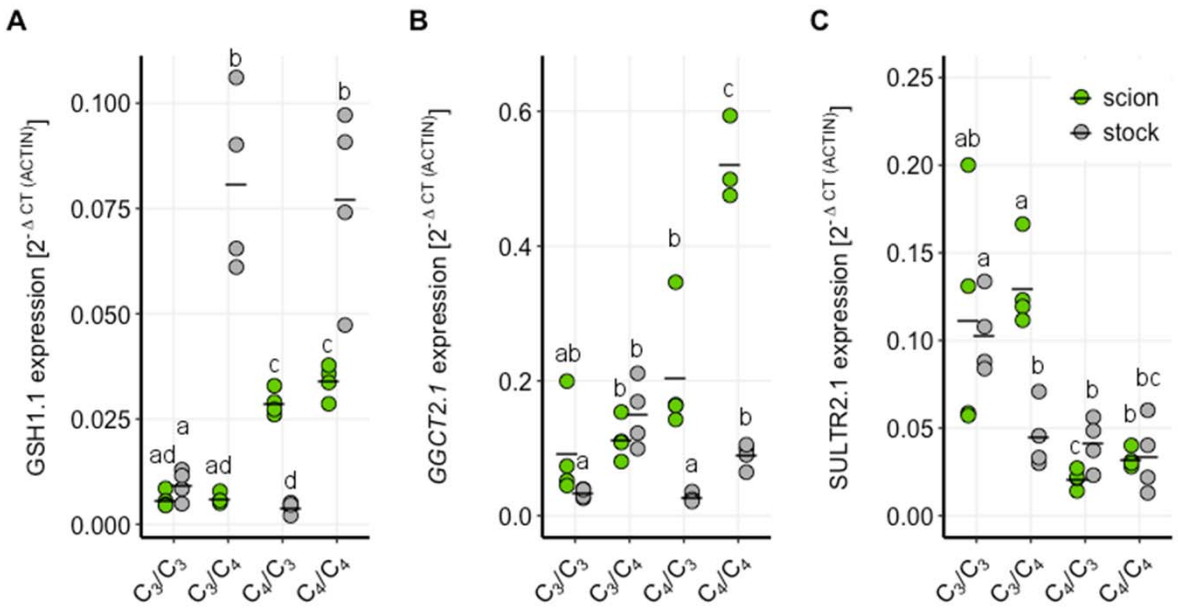


Supplemental Figure S6. Expression patterns of *γECS* in roots and shoots of C_3 and C_4 species.

A: Transcript levels of *γECS* (*GSH1.1*) relative to *ACTIN* in shoots and roots of 20-day-*F. robusta* (C_3) and *F. australasica* (C_4) seedlings in a time course of 48 h after transfer to medium supplemented with 2 mM BSO. Data are presented as means and SEM, n=4. **B** *γECS* (*GSH1.1*) transcript levels relative to *ACTIN* in shoots and roots of 20-day-old seedlings of *F. robusta* and *F. bidentis* (C_4), n=4. **C** Transcript abundance of *γECS* (*GSH1.1*) in leaves of different developmental stages and roots of *T. hassleriana* (C_3) and *G. gynandra* (C_4) analyzed by RNA sequencing (Külahoglu et al. 2014). Different letters mark significant differences ($p \leq 0.05$, ANOVA).



Supplemental Figure S7. Concentration of total sulfur and other sulfur pools in shoots and roots of *Flaveria* species.
Shoots and roots of 23-day-old seedlings of five *Flaveria* species exposed to low sulfate (30 µM sulfate; S30) or adequate sulfate (750 µM sulfate; S750) for 16 days were analysed. **A** Shoot sulfur concentration. **B** Root sulfur concentration. **C** Shoot sulfate concentration. **D** Root sulfate concentration. **E** Shoot cysteine concentration. **F** Root cysteine concentration. **G** Shoot GSH concentration. **H** Root GSH concentration. Data are shown as box plot (25%-75%), the line represents median, the whiskers represent 1.5 IQR, n=4.



Supplemental Figure S8. Expression of *γECS*, *GGCT2.1*, and *SULTR2.1* in scions and stocks of interspecific grafts of *F. robusta* and *F. bidentis*. Seedlings of *F. robusta* (C_3) and *F. bidentis* (C_4) were grafted 5 days after germination and analyzed 35 days after grafting. **A** Transcript levels of *γECS* (*GSH1.1*) relative to *ACTIN*. **B** Transcript levels of *GGCT2.1* relative to *ACTIN*. **C** Transcript levels of *SULTR2.1* relative to *ACTIN*. Crossbar = mean; n=4. Different letters mark significant differences ($p \leq 0.05$, ANOVA).

Supplemental Table S1. Species of the genus *Flaveria* used in this study. Photosynthetic classification and CO_2 compensation points are listed according to Ku *et al.* (1991).

species	photosynthesis type	CO_2 compensation point [μbar]
F. robusta	C_3	62.1 ± 1.0
F. linearis	$C_3 - C_4$	27.0 ± 1.7
F. anomala	$C_3 - C_4$	15.5 ± 0.7
F. palmeri	C_4 - like	4.7 ± 0.3
F. australasica	C_4	5.1 ± 0.4
F. bidentis	C_4	3.2 ± 0.4

Supplemental Table S2. Oligonucleotide primers used in this study.

gene	*A. thaliana* orthologue	direction	sequence	melting temperature	amplicon length (bp)	primer efficiency
ACTIN	At5g09810	fwd	AATGGAAGCTGCTGGTATTCA	63.8	187	*F.r.* 97.2%
		rev	CAACCACCTTGATCTTCATGC	64.8		*F.b.*96.8%
GGCT2.1	At5g26220	fwd	ATCAAATAAGTACTACCTCGGGCC	60°C	97	*F.r.* 105.5%
		rev	TACATAGTCTCGGTTGTTTCCACA	59.7°C		*F.b.*102.1%
GSH1.1	At4g23100	fwd	GATATTTGGAAATGAGGGGTGCTG	68.3	85	*F.r.* 105.7%
		rev	TCATACAATAAGCCCACCCAAAGA	67.3		*F.b.*105.2%
PSP	Atlg18640	fwd	AGTAATGATAGGTGATGGTGCCAC	65.1	78	*F.r.* 93.8%
		rev	CCACCATAGCAGATAAACAGGTCA	66.1		*F.b.*93.4%
SULTR2.1	At5g10180	fwd	TGCTACAATTTCTAACGGGGCTAT	60.1°C	94	*F.r.* 95.9%
		rev	TCATAGTTGATTAATCCGGGGAGC	60.3°C		*F.b.*101.2%