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The long-term adaptive response of air-grown *Arabidopsis ggt1*

photorespiratory mutants involves a drastic reduction in leaf RuBisCO content

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1. The photorespiratory pathway in plants

- In the light, RuBisCO oxygenase activity produces 3-phosphoglycerate (3-PGA) and 2-phosphoglycolate (2-P-glycolate) in leaf chloroplasts. The photorespiratory pathway recycles the 2-P-glycolate to produce 3-PGA, CO₂ and ammonia (Fig. 1).
- Because it is a wasteful process, photorespiration has become a target for plant improvement. However, this cycle is connected to important primary metabolic pathways, and there is still debate about how a reduced photorespiratory cycle would affect them.
- We have investigated primary plant metabolism in *Arabidopsis* mutants with reduced glutamate-glyoxylate aminotransferase (GGT) activity: a key photorespiratory enzyme with a direct link to both amino and organic acid metabolisms.

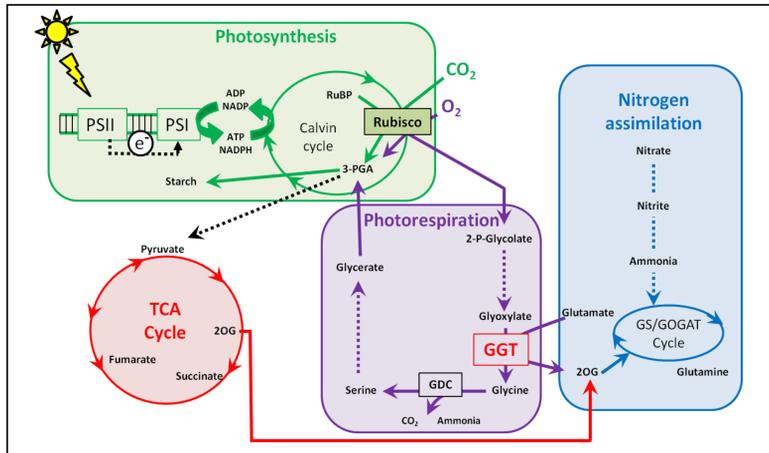


Figure 1. The photorespiratory cycle within a simplified primary metabolism network

2. *ggt1* T-DNA insertion lines

Two genes encode GGT in *A. thaliana*, but *GGT1* is the predominantly expressed *GGT* gene in leaves (where photorespiration occurs) (Fig. 2C). We have identified two *GGT1* knock-out lines in the *A. thaliana* Col-0 ecotype (Fig. 2A) that contain a low, residual leaf GGT activity (Fig. 2D). Both *GGT* KO lines exhibit a photorespiratory phenotype (dwarfism, paler green leaves) when grown in air that is not observed in high CO₂ (3000 μL.L⁻¹, low RuBisCO oxygenase activity) (Fig. 2B).

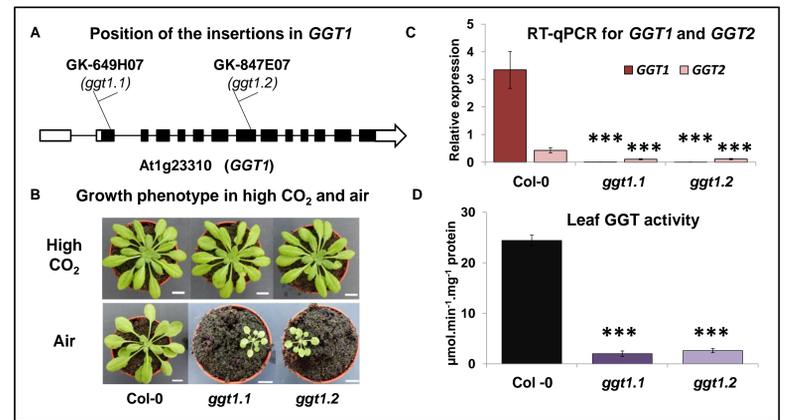


Figure 2. Characterization of two *Arabidopsis thaliana ggt1* mutants

3. Limitation of photorespiration in air-grown *ggt1* mutants affects primary metabolism and reduces photosynthesis independently of RuBisCO activation state

Metabolites analyzed by GC-MS from 5-week old air-grown plants showed that photorespiration was reduced in *ggt1* mutants (accumulation of glyoxylate, diminution of serine and glycerate levels) (Fig. 3A). The levels of three TCA cycle organic acids (Fig. 3B) were decreased also, suggesting that low photorespiration might affect TCA cycle activity. Nevertheless, C:N balance was not affected (Fig. 3C).

Leaf gas exchange was performed on *ggt1* mutants with a homemade chamber to investigate the effect of low GGT activities on leaf photosynthesis. Net CO₂ uptake was nearly 2-fold lower in both mutants (Fig. 4A). Since glyoxylate has been reported to decrease RuBisCO activation state thus inhibiting initial RuBisCO activity *in vitro*, RuBisCO was investigated. Total and initial RuBisCO activities per leaf area were both 3-fold lower in air-grown *ggt1* mutants (Fig. 4B), but surprisingly, the activation state remained unchanged (Fig. 4C). SDS-PAGE analyses of leaf total soluble protein content per surface revealed a drastic reduction of RuBisCO large subunit content (Fig. 4D), which could explain the low RuBisCO activities and the reduced CO₂ assimilation rates.

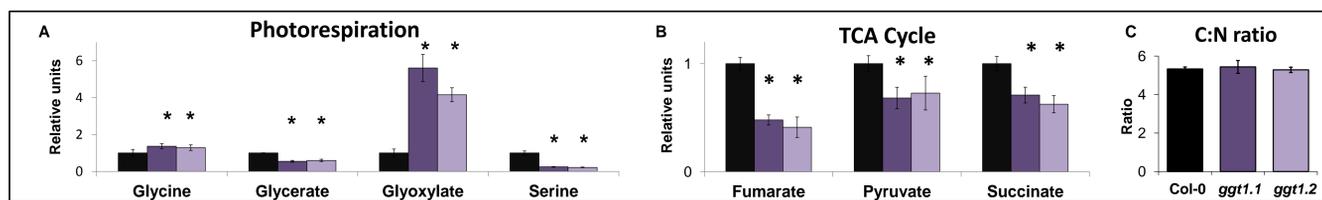


Figure 3. Photorespiratory and TCA cycle metabolites in air-grown *ggt1* leaves with corresponding C:N ratio

4. Altered photorespiratory C-recycling inhibits photosynthesis possibly through Calvin cycle RuBP regeneration

Leaf gas exchange and chlorophyll fluorescence were used to investigate the initial processes leading to the low leaf RuBisCO content in air-grown *ggt1* mutants. When high CO₂ grown plants were transferred to air, a rapid decrease of net CO₂ assimilation (Fig. 5A) associated with an increased non-photochemical fluorescence quenching (NPQ; indicating a high proton gradient across the thylakoid membrane) (Fig. 5B) were observed only in *ggt1* leaves. A high NPQ suggests that photosynthetic ATP production was reduced. Indeed, total ATP/ADP ratios were seen to be lower in mutant leaves compared to the control after transfer, while RuBisCO activation state was not altered (Table 1). Such observations could reflect a lower Calvin cycle activity (due to a reduction in photorespiratory C-recycling) in *ggt1* plants.

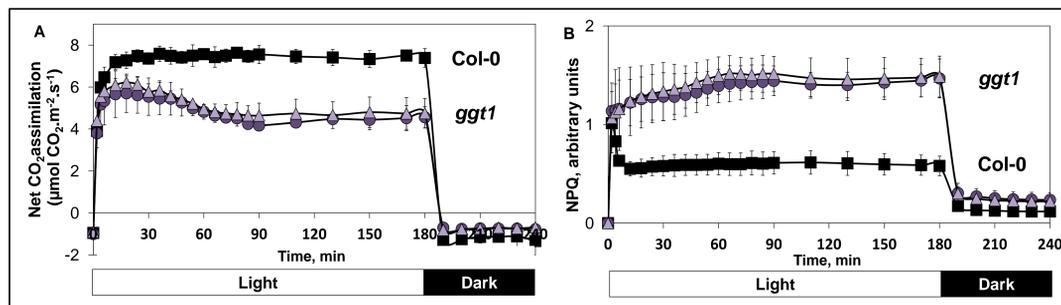


Figure 5. Net CO₂ assimilation and NPQ of *ggt1* mutants after transfer from high CO₂ to air

	Before transfer			After transfer		
	Col-0	<i>ggt1.1</i>	<i>ggt1.2</i>	Col-0	<i>ggt1.1</i>	<i>ggt1.2</i>
Initial RuBisCO activity (nmoles 3-PGA.min ⁻¹ .cm ⁻²)	21.9 ± 4.0	18.2 ± 1.9	15.7 ± 1.1	32.2 ± 7.8	25.5 ± 3.7	21.9 ± 3.0
Total RuBisCO activity (nmoles 3-PGA.min ⁻¹ .cm ⁻²)	25.9 ± 3.6	23.3 ± 1.9	19.0 ± 2.0	38.7 ± 6.1	32.1 ± 4.4	27.5 ± 2.0
RuBisCO activation state (%)	84.3 ± 7.2	78.0 ± 5.6	82.9 ± 6.1	82.48 ± 9.26	79.52 ± 5.52	79.4 ± 7.4
ATP/ADP ratio	1.6 ± 0.9	1.3 ± 0.7	1.5 ± 0.8	3.9 ± 1.3	1.4 ± 1.0	1.9 ± 0.5

Table 1. RuBisCO properties and ATP/ADP ratio of *ggt1* mutants after transfer from high CO₂ to air

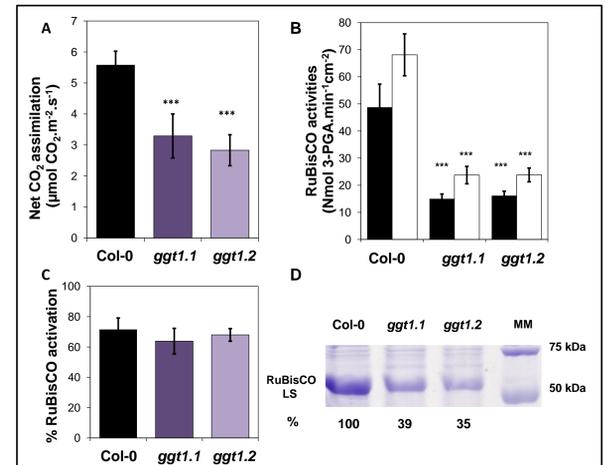


Figure 4. Net CO₂ assimilation rates, RuBisCO activities and protein content of air-grown *ggt1* mutants

5. Conclusions and working model

- ggt1* photorespiratory mutants show a dwarf phenotype in air, coupled to a 60% decrease in leaf RuBisCO content.
- This phenotype is probably the result of a long-term adaptive response to a low photosynthetic activity due to a reduced Calvin cycle activity arising from the disruption in photorespiratory C-recycling.
- Reduced C-fixation limits C-skeletons for N-assimilation that limits the synthesis of RuBisCO, thus leading to small slow-growing plants that maintain a constant C:N balance.

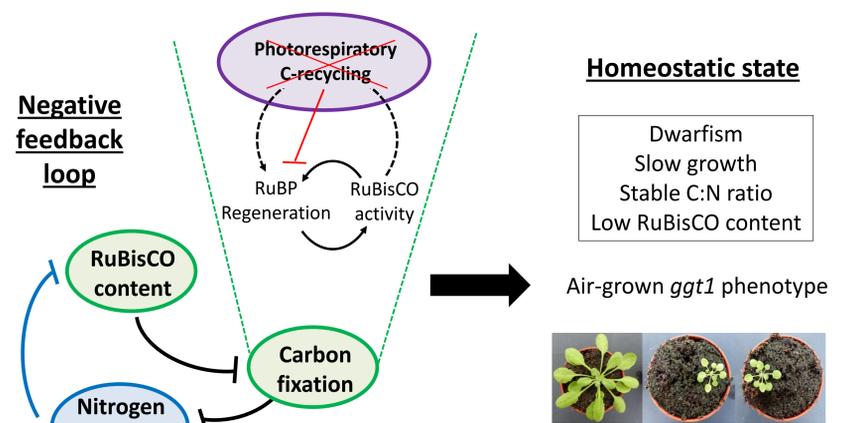


Figure 6. Working model for the air-grown *ggt1* phenotype