

Measuring whole plant CO₂ exchange with the environment reveals opposing effects of the *gin2-1* mutation in shoots and roots of *Arabidopsis thaliana*

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Abbreviations: FW, fresh weight; *gin2-1*, knockout mutant of hexokinase1; NPS, net photosynthesis; SEM, standard error of mean; WT, wild type.

Using a cuvette for simultaneous measurement of net photosynthesis in above ground plant organs and root respiration we investigated the effect of reduced leaf glucokinase activity on plant carbon balance. The *gin2-1* mutant of *Arabidopsis thaliana* is characterized by a 50% reduction of glucokinase activity in the shoot, while activity in roots is about fivefold higher and similar to wild type plants. High levels of sucrose accumulating in leaves during the light period correlated with elevated root respiration in *gin2-1*. Despite substantial respiratory losses in roots, growth retardation was moderate, probably because photosynthetic carbon fixation was simultaneously elevated in *gin2-1*. Our data indicate that futile cycling of sucrose in shoots exerts a reduction on net CO₂ gain, but this is over-compensated by the prevention of exaggerated root respiration resulting from high sucrose concentration in leaf tissue.

Assessment of plant-environment carbon dioxide relations is hampered by the difficulties to discriminate photosynthetic CO₂ fixation from respiratory release in the above-ground organs¹ on the one hand and methodological restrictions to measure root respiration in soil grown plants on the other hand. The contribution of root-derived CO₂ efflux from soils is frequently assessed by root exclusion techniques, shading or other methods that reduce the plant-associated component. However, all these methods have the disadvantage of severely interfering with the system under investigation. Alternative methods based on stable or radioactive carbon isotopes are less invasive, but require complex and costly equipment and, because they are mostly based on pulse labeling strategies, are not suitable for long term observations.²

For the quantification of root respiration over full diurnal cycles, hydroponic systems have been employed that use aerated nutrient solutions and infrared analysis of CO₂ in the off-gas^{3,4} or enclosed systems, in which oxygen consumption is measured using Clark type electrodes or similar techniques.^{5,6} While the latter bears the risk of exerting a substrate limitation on respiration, the former has a rather low time resolution because of the

slow equilibration of CO₂ concentration in aqueous and gas phase.

We analyzed whole plant CO₂ exchange with the environment using a recently developed device that allows simultaneous measurement of net photosynthesis (NPS) in green organs and respiration of roots kept in an aeroponic environment.⁷ Aeroponic measurement of root respiration combines the advantages of non-invasiveness with fast time response and high sensitivity of gas phase measurements of CO₂ based on infrared absorption.

Following an analysis of plant-environment CO₂ exchange in the starchless *pgm* mutant of *Arabidopsis*⁷ we extended our investigation to the *gin2-1* knockout mutant of hexokinase 1 (At4g29130). This mutant has a strongly reduced glucokinase activity that results in sensitivity to high light and altered hormone responses.⁸ Besides its function as a glucose sensor, glucokinase is a key enzyme in re-synthesis of sucrose hydrolyzed by invertase,⁹ which has been termed “futile sucrose cycling” and is believed to be important in stabilizing the carbohydrate status of leaf tissues.^{10,11}

For soil-grown plants a 50% reduction in glucokinase activity in leaf tissues has been reported for the *gin2-1* mutant

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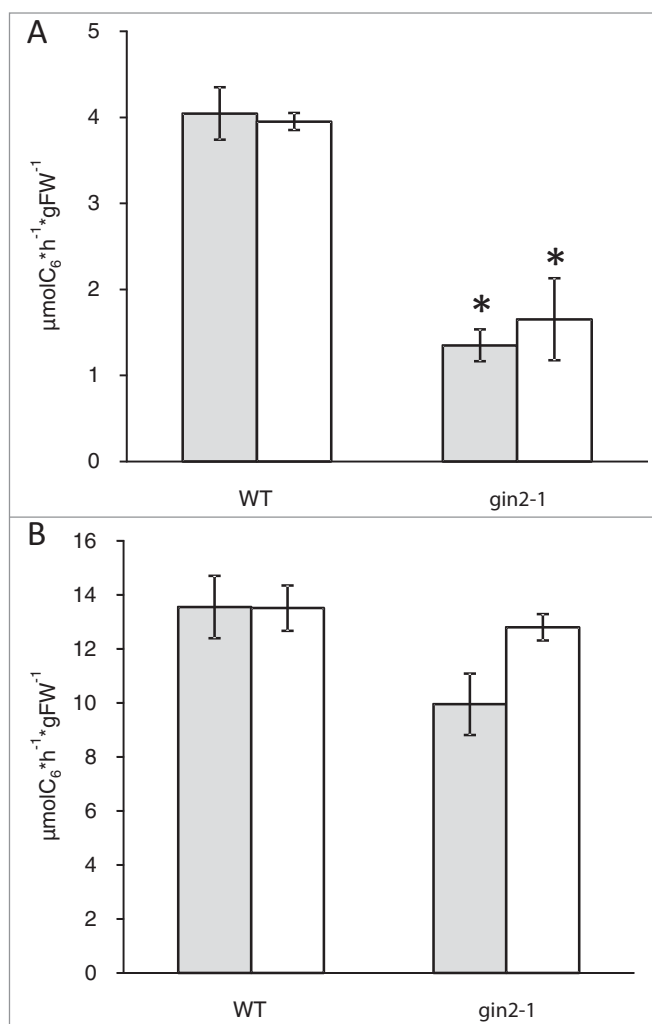


Figure 1. Maximum enzymatic glucose phosphorylation in $\mu\text{mol C}_6$ per hour and gFW of shoot (A) and root (B) immediately before the end of the night (gray) and the end of the day (white). Results represent means with SEMs ($n = 5$). Group means varying significantly at the 5% level were marked with asterisk. Plants were cultivated hydroponically under long day conditions (16 h light, intensity: $150 \mu\text{mol m}^{-2} \text{s}^{-1}$, 22°C ; 8 h dark, 16°C) for 7 days, following cultivation under short day conditions (8 h light, intensity: $150 \mu\text{mol m}^{-2} \text{s}^{-1}$, 22°C ; 16 h dark, 16°C) for 3 weeks. Seedlings were brought into hydroponic culture 21 d after sowing in soil (GS20) / vermiculite (1:1) applying short day conditions.

grown under various light conditions.⁸ We observed the same reduction in leaves of hydroponically cultivated plants using the method of Wiese *et al.*,¹² which is based on the oxidation of glucose-6-phosphate to 6-phosphogluconolactone (Fig. 1A). In contrast to leaves, roots of wild type plants had a threefold higher glucokinase activity as compared to leaves, and there was no reduction in the *gin2-1* mutant (Fig. 1B), indicating that an enzyme not encoded by the HXK1 gene is dominating in root tissue. It can therefore be concluded that at least the metabolic consequences of the *gin2-1* mutation affect only shoot but not root tissues.

Concentrations of soluble sugars were measured at the end of the light and the end of the dark period following the method described in Brauner *et al.*⁷ Glucose was the most abundant soluble sugar in wild type (WT) and *gin2-1* at both time points and accumulated during the day in leaves of *gin2-1* but not in WT (Fig. 2A). Higher glucose than sucrose levels are typical of *Arabidopsis* plants grown under high light intensities and long day conditions. The accumulation of hexoses points to a high rate of operation for sucrose cycling, which is limited by hexokinase activity.⁹ At the end of the day, glucose levels in *gin2-1* were twice as high as in WT, clearly pointing to a limitation in re-entering hexoses, produced by hydrolysis of sucrose, into the carbohydrate metabolic network

of the mutant. Although at much lower levels, fructose followed the same pattern as glucose with an increase during the light phase in *gin2-1* (Fig. 2B). Sucrose accumulated during the light phase in WT as well as in the *gin2-1* mutant, but the increase was stronger in *gin2-1*, thus leading to significantly higher sucrose levels in the mutant at the end of the day (Fig. 2C).

Because we have previously found stimulation of root respiration in the starchless *pgm* mutant that has elevated sugar concentrations in leaves,⁷ we measured NPS of shoots and respiration of roots of *gin2-1* and

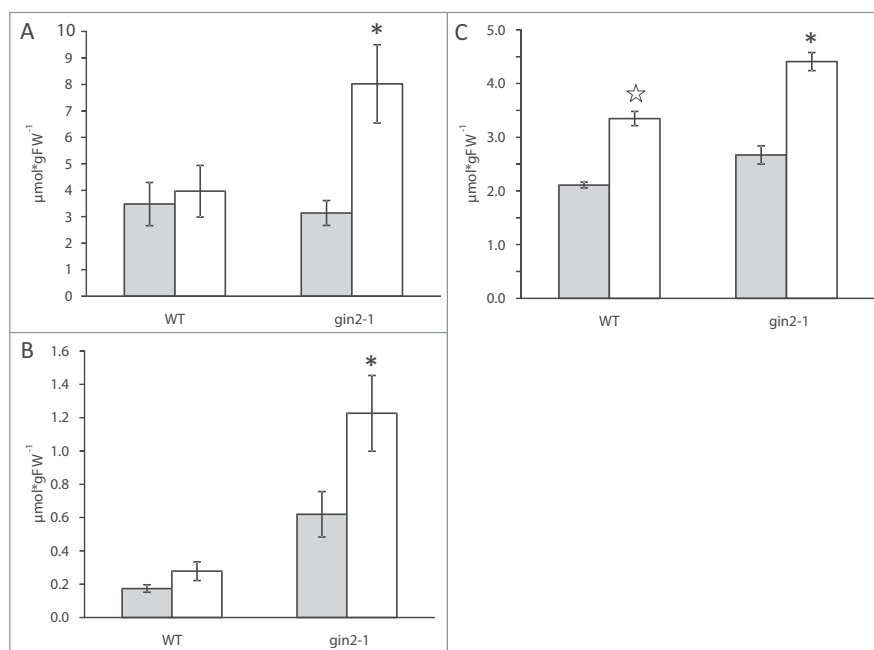


Figure 2. Concentrations of glucose (A), fructose (B) and sucrose (C) in leaf tissues of *Arabidopsis* at the end of the night (gray) and the end of the day (white). Results represent means with SEMs ($n = 5$). Group means varying significantly at the 5% level were marked with different asterisks.

WT. Profiles of these measurements are shown in Fig. 3. It can be seen that, on a fresh weight basis, *gin2-1* displayed a 20% increase in NPS of shoots during the light phase, and nearly a doubling of root respiration over the entire diurnal cycle. Respiration of the shoot during the night was unaltered. At the whole plant level WT lost 28% of NPS by respiration while *gin2-1* lost 37%. This raised carbon deficit was reflected in a 23% reduced total biomass formation after the 50 day growth phase (WT: 1.22 ± 0.11 gFW; *gin2-1*: 0.94 ± 0.08 gFW; $n = 10$). Both genotypes displayed a root-to-shoot ratio of 0.33. Considering the strong stimulation of root respiration, the reduction in biomass was much lower than in the starchless *pgm* mutant, which had a similarly increased root respiration under the experimental conditions described in Brauner *et al.*⁷ The most obvious explanation for this finding is a compensation of respiratory losses of the root by increased daily fixation in the shoot, and this could very likely be caused by a reduction of futile cycling of sucrose. Hydrolysis of sucrose by vacuolar and / or neutral invertases and the consecutive phosphorylation of hexoses by hexokinases that precedes re-synthesis of sucrose amounts to about 25% of sucrose turnover in *Arabidopsis* leaves¹¹ and creates a significant energy sink, thus reducing photosynthetic efficiency. However, sucrose cycling serves to stabilize the sucrose concentration in leaf mesophyll cells,¹⁰ and the biomass reduction in the *gin2-1* mutant demonstrates that the costs for this stabilizing effect are over-compensated by the prevention of increased root respiration that would otherwise result from sucrose accumulation in leaf cells.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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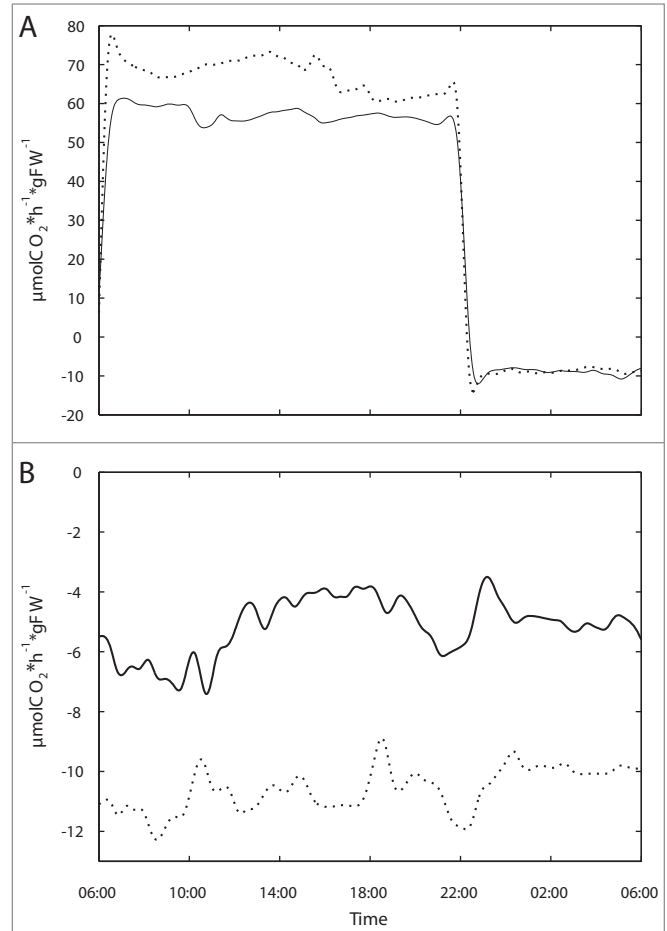


Figure 3. CO₂ uptake rate per hour. Profiles of shoot NPS and respiration (A) and root respiration (B) over full diurnal cycles. Shoot and root gas exchange are based on plant fresh weight; positive values denote CO₂ uptake: WT, solid lines; *gin2-1*, dotted lines. Results represent means ($n = 5$).