Leaf day respiration involves multiple carbon sources and depends on previous dark metabolism

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Abstract

Day respiration (R_d) is the metabolic, non-photorespiratory process by which illuminated leaves liberate CO_2 during photosynthesis. R_d is used routinely in photosynthetic models and is thus critical for calculations. However, metabolic details associated with R_d are poorly known, and this can be problematic to predict how R_d changes with environmental conditions and relates to night respiration. It is often assumed that day respiratory CO_2 release just reflects 'ordinary' catabolism (glycolysis and Krebs 'cycle'). Here, we carried out a pulse-chase experiment, whereby a $^{13}CO_2$ pulse in the light was followed by a chase period in darkness and then in the light. We took advantage of non-targeted, isotope-assisted metabolomics to determine non-'ordinary' metabolism, detect carbon remobilisation, and compare light and dark ^{13}C utilisation. We found that several concurrent metabolic pathways ('ordinary' catabolism, oxidative pentose phosphates pathway, amino acid production, nucleotide biosynthesis, and secondary metabolism) took place in the light and participate in net CO_2 efflux associated with day respiration. Flux reconstruction from metabolomics leads to an underestimation of R_d, further suggesting the contribution of a variety of CO_2-evolving processes. Also, the cornerstone of the Krebs 'cycle', citrate, is synthetised de novo from photosynthates mostly in darkness, and remobilised or synthesised from stored material in the light. Collectively, our data provides direct evidence that leaf day respiration (*i*) involves several CO_2-producing reactions and (*ii*) is fed by different carbon sources, including stored carbon disconnected from current photosynthates.

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