Poor Evidence for C_4 Photosynthesis in the Wheat Grain

Research on C_4 photosynthesis has seen a revival in recent years, leading to a *Nature Scientific Reports* article announcing the discovery of C_4 photosynthesis in grains of the C_3 plant wheat (*Triticum aestivum*; Rangan et al., 2016). The authors indicate the presence of C_4 cycle enzymes, which they interpret as evidence for the existence of a C_4-type carbon-concentrating mechanism in the wheat pericarp. "Extraordinary claims require extraordinary evidence," however, and the evidence presented in this case is based entirely on transcriptome data of the entire wheat grain, with a notable absence of biochemical or physiological support, or the exact location of expression. One needs to consider the wider context of C_4 photosynthesis, and this challenges the interpretation provided by Rangan et al. (2016).

It is a common misconception that in C_4 photosynthesis C_3's primary CO_2-fixing enzyme Rubisco is simply replaced by phospho*enol*pyruvate carboxylase (PEPC). PEPC indeed initially incorporates CO_2 as HCO_3^- into the C_4 acid oxaloacetate, but this molecule is difficult to chemically reduce beyond the level of malate to that required for sugar, cellulose, and other carbohydrates, while still recycling the substrate PEP. So the CO_2 is later released to increase the CO_2 concentration in a specialized compartment, such as the bundle sheath (Leegood, 2002). There, the photosynthetic assimilation of CO_2 into carbohydrates occurs, via the same pathway as in C_3 plants, by Rubisco, ATP, and NADPH. This C_4 carbon-concentrating mechanism thus requires a PEPC:Rubisco activity ratio of roughly 1:1; a larger ratio means that ATP is spent on running a futile C_4 cycle. Without Rubisco, PEPC is only able to fix CO_2 via anaplerotic reactions. Two observations contradict the conclusions of Rangan et al. (2016) in this regard.

The authors show that the expression level of rbcS, the small subunit of Rubisco, is decreased by 99% relative to the expression in the leaf, and conclude that, together with an increase in PEPC expression and other C_4 cycle enzymes, this indicates a shift from C_3 to C_4 photosynthesis. Without providing any measurements of the activity of Rubisco or PEPC to substantiate their claim, they refer to previous work showing that PEPC is 100 times as active in incorporating CO_2 into organic compounds as Rubisco (Duffus and Rosie, 1973). Assuming this to be the case here, the low concentration of Rubisco would leave insufficient capacity for photosynthetic fixation of the vast majority of CO_2 supplied by PEPC via a C_4 pump. It implies that the uptake of CO_2 by PEPC is to supply the large demand in amino and fatty acids. Support for the idea that PEPC facilitates the production of these compounds, and not carbohydrates, was earlier provided by pulse-chase experiments (Bort et al., 1995; Rolletschek et al., 2004).

The C_4 cycle constitutes an energy-driven CO_2 pump from a compartment of relatively low CO_2 concentration to a largely gas-tight compartment in which the CO_2 concentration is increased to reduce photorespiration (Leegood, 2002). Immunolabeling studies in wheat grains have shown that PEPC is not localized in the cross-cells of the pericarp, as suggested by Rangan et al. (2016), but in the aleurone layer and endosperm (Araus et al., 1993; González et al., 1998), the sites where one would expect the highest concentration of respiratory CO_2. With Rubisco located in the chloroplasts of the pericarp (Tambussi et al., 2005), this would render a C_4-like CO_2 pump ineffective.

In our view, the claim of a C_4 carbon-concentrating mechanism in wheat grains therefore cannot be upheld with the supplied data. In any such analysis it is important to distinguish production of C_4 acids from the C_4 photosynthetic pathway.

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