

Poor evidence for C₄ photosynthesis in the wheat grain

Research on C₄ photosynthesis has seen a revival in recent years, leading to a *Nature Scientific Reports* article announcing the discovery of C₄ photosynthesis in grains of the C₃ plant wheat (*Triticum aestivum*; Rangan et al., 2016). The authors indicate the presence of C₄ cycle enzymes, which they interpret as evidence for the existence of a C₄-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 C₄ photosynthesis, and this challenges the interpretation provided by Rangan et al. (2016).

It is a common misconception that in C₄ photosynthesis C₃'s primary CO₂ fixing enzyme Rubisco is simply replaced by phosphoenolpyruvate carboxylase (PEPC). PEPC indeed initially incorporates CO₂ as HCO₃⁻ into the C₄ 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 CO₂ is later released to increase the CO₂ concentration in a specialized compartment, such as the bundle sheath (Leegood, 2002). There, the photosynthetic assimilation of CO₂ into carbohydrates occurs, via the same pathway as in C₃ plants, by Rubisco, ATP and NADPH. This C₄ 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 C₄ cycle. Without Rubisco, PEPC is only able to fix CO₂ 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 C₄-cycle enzymes – this indicates a shift from C₃ to C₄ 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 CO₂ 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 CO₂ supplied by PEPC via a C₄ pump. It implies that the uptake of CO₂ 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 C₄ cycle constitutes an energy-driven CO₂ pump from a compartment of relatively low CO₂ concentration to a largely gas tight compartment in which the CO₂ concentration is increased to reduce photorespiration (Leegood, 2002). Immunolabelling 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 site where one would expect the highest concentration of respiratory CO₂. With Rubisco located in the chloroplasts of the pericarp (Tambussi et al., 2005) this would render a C₄-like CO₂ pump ineffective.

In our view, the claim of a C₄ 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 C₄ acids from the C₄ photosynthetic pathway.

Florian A. Busch & Graham D. Farquhar

Research School of Biology
The Australian National University
Acton, ACT 2601, Australia

References

- Araus JL, Bort J, Brown RH, Bassett CL, Cortadellas N** (1993) Immunocytochemical localization of phosphoenolpyruvate carboxylase and photosynthetic gas-exchange characteristics in ears of *Triticum durum* Desf. *Planta* **191**: 507-514
- Bort J, Brown RH, Araus JL** (1995) Lack of C₄ photosynthetic metabolism in ears of C₃ cereals. *Plant, Cell & Environment* **18**: 697-702
- Duffus CM, Rosie R** (1973) Some enzyme activities associated with the chlorophyll containing layers of the immature barley pericarp. *Planta* **114**: 219-226
- González Ma-C, Osuna L, Echevarría C, Vidal J, Cejudo FJ** (1998) Expression and localization of phosphoenolpyruvate carboxylase in developing and germinating wheat grains. *Plant Physiology* **116**: 1249-1258
- Leegood RC** (2002) C₄ photosynthesis: principles of CO₂ concentration and prospects for its introduction into C₃ plants. *Journal of Experimental Botany* **53**: 581-590
- Rangan P, Furtado A, Henry RJ** (2016) New evidence for grain specific C₄ photosynthesis in wheat. *Scientific Reports* **6**: 31721
- Rolletschek H, Borisjuk L, Radchuk R, Miranda M, Heim U, Wobus U, Weber H** (2004) Seed-specific expression of a bacterial phosphoenolpyruvate carboxylase in *Vicia narbonensis* increases protein content and improves carbon economy. *Plant Biotechnology Journal* **2**: 211-219
- Tambussi EA, Nogués S, Araus JL** (2005) Ear of durum wheat under water stress: water relations and photosynthetic metabolism. *Planta* **221**: 446-458