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Turning sugar into oil: making photosynthesis blind to feedback inhibition

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Since the advent of metabolic engineering 30 years ago it has been a goal to modify crops to accumulate alternative higher value products and/or to increase yield. Direct targeting of metabolic enzymes has been difficult because of strong endogenous regulatory mechanisms that can confound such changes, either impeding the metabolic alteration or producing a growth and yield penalty. Beechey-Gradwell *et al.* (2020) show that in *Lolium perenne*, an important temperate pasture and forage grass, introduction of two genes involved in lipid synthesis and storage results in significant accumulation of oil and increases photosynthesis and growth, elevating calorific value and overall yield.

Genetic modification of crop plants began ~30 years ago when it became possible through newly emerging plant transformation and recombinant DNA technology to alter the magnitude of endogenous gene expression and introduce new genetic information. The benefits of this technology in the form of insect pest resistance and herbicide resistance have been revolutionary for agriculture (Paul *et al.*, 2018). Metabolic engineering has been harder, however, because metabolism is a mainstream process that is highly regulated. For example, any modification of metabolism will potentially alter carbon and energy sensing and signalling, a strongly regulated process analogous to the regulation of blood sugar in humans. It can be argued too that any attempt to engineer overall productivity through photosynthesis will also need to engage with this regulatory system because fixation of carbon is regulated by carbon and energy homeostatic regulatory systems.

Metabolic engineering of lipid metabolism

Forage grasses such as *Lolium perenne* are crucial for livestock agriculture. Their nutritional quality, as well as yield, has a significant impact on the production of meat and milk for human consumption (Hegarty *et al.*, 2013). Several studies have reported that the digestible energy content of ruminant livestock diets can be increased by supplementing with lipid (usually in

the form of vegetable oil) up to an optimum of ~5–8% of dry mass (DM) and that this not only boosts productivity but can also reduce enteric methane emissions (Hegarty *et al.*, 2013). Lipids are the most energy-dense dietary nutrient (38 kJ g⁻¹). Increasing the lipid content of forage by just 3% of DM provides >1 MJ of gross energy kg⁻¹. Lipids normally only make up ~3% of the DM of *L. perenne*, with the majority present in chloroplast thylakoid membranes in the leaves (Hegarty *et al.*, 2013). However, several research groups have reported strategies to increase the lipid content of plant vegetative tissues by engineering them to accumulate triacylglycerol (reviewed by Vanhercke *et al.*, 2019). Beechey-Gradwell *et al.* (2020) have overexpressed the enzyme diacylglycerol acyltransferase (DGAT), to drive triacylglycerol biosynthesis, together with a stabilized version of the oil body surface protein oleosin (cys-OLE), to protect the triacylglycerol from being turned over (Winichayakul *et al.*, 2013); the combined effect is an ~3% increase in leaf lipid content. Beechey-Gradwell *et al.* (2020) show that high lipid (HL) *L. perenne* plants expressing cys-OLE/DGAT not only accumulate lipid but also have enhanced net carbon capture. This finding supports their previous work in *Arabidopsis thaliana* (Winichayakul *et al.*, 2013) and is not intuitive given that even 3% lipid represents a significant additional investment of carbon. The energy density of lipid is more than twice that of carbohydrate, the energy requirements of lipid synthesis are much higher than those for complex carbohydrates, and the carbon conversion efficiency (without CO₂ recycling) is also much lower (Schwender *et al.*, 2004). It is also noteworthy that none of the other metabolic engineering approaches that has been used to enhance triacylglycerol content in vegetative tissues has so far been reported to confer a photosynthesis or growth advantage (Vanhercke *et al.*, 2019).

Metabolic engineering of photosynthesis and yield

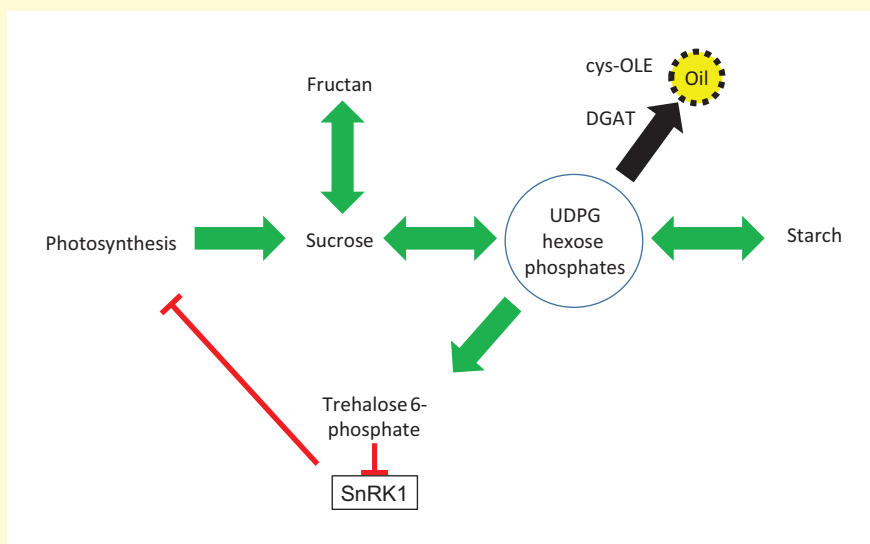
There are very few examples where modification of metabolism has improved growth, photosynthesis, and yield in a

crop (Paul *et al.*, 2018). Targeting of individual metabolic enzymes has so far been unsuccessful in the development of new higher yielding or abiotic stress-resilient genetically modified (GM) crops. There are reports of modifying photorespiration producing large increases in biomass of tobacco (South *et al.*, 2019). However, in this study, the carbon gain is difficult to explain through the changes in glycolate metabolism targeted (Evans, 2019). Instead more biomass in transgenics could have come about through the introduction of shading of the control plants by the transgenics in field tests (Fischer *et al.*, 2019). Amongst other claims of improved photosynthesis, one of the most convincing is the engineering of tobacco for accelerated recovery from photoprotection which gives large biomass improvements (15%) in the field (Kromdijck *et al.*, 2016). Increasing the activity of the Calvin cycle enzyme, sedoheptulose-1,7-bisphosphatase, also shows encouraging results, increasing photosynthesis and biomass in Arabidopsis and tomato. In wheat, yield was increased in glasshouse conditions and, in tobacco, biomass in the field under elevated CO₂ was increased (Simkin *et al.*, 2019). However, increasing photosynthesis through direct targeting of the photosynthetic process resulting in a consistent yield increase in the field has yet to be successfully demonstrated in a major food crop. *Lolium perenne*, in contrast, is an important feed for livestock. The current study has been conducted in convincing detail. Photosynthesis and growth have been characterized under contrasting nitrogen

sources and at ambient and elevated CO₂. Rather than simply providing final biomass harvest data, relative growth rate has been determined together with specific leaf area (SLA) and leaf mass fraction. Both SLA and photosynthesis per unit leaf area are increased. SLA is the factor most positively associated with growth rate (Poorter and Pothmann, 1992) and often there is a negative association between SLA and photosynthetic rate per unit leaf area (Pellny *et al.* 2004). Provision of substrate for fatty acid synthesis within the chloroplast results in one-third of the carbon being lost as CO₂. In *A. thaliana* plants expressing cys-OLE/DGAT, it was previously proposed that this CO₂ was being recycled by Rubisco (Winichayakul *et al.*, 2013). However, gas exchange experiments ruled out this explanation in HL *L. perenne*. As an alternative, the diversion of carbohydrate into a lipid carbon sink sequesters carbon away from carbon-sensing mechanisms. This could mitigate the signals that would normally down-regulate photosynthesis as part of carbon and energy metabolic homeostasis, meaning that photosynthesis is 'blind' to carbon accumulation and can carry on unimpeded whilst carbon accumulates (Box 1). It would be interesting to see if the sugar signal trehalose 6-phosphate (T6P) or elements of the T6P/SnRK1 signalling pathway were altered in HL *L. perenne*. Similar indirect sink effects on photosynthesis have been achieved through modification of the T6P signalling system in maize transgenics (Oszvald *et al.*, 2018); potentially the effects on photosynthesis and growth could be

Box 1. A possible model to explain de-repression of photosynthesis (or 'blind photosynthesis') by creation of a new carbon sink which bypasses the T6P/SnRK1 signalling system

Sucrose, fructan, and starch metabolic pathways engage with central metabolic pools in a two-way process. Synthesis of these end-products draws from UDP glucose (UDPG) and/or hexose phosphate substrate; whilst their breakdown resupplies this pool. Trehalose 6-phosphate (T6P) is also made from this pool as a signal of carbon availability. T6P inhibits the feast-famine protein kinase, SnRK1. Inhibition of SnRK1 by T6P is associated with down-regulation of photosynthesis (Zhang *et al.*, 2009) and reduced levels of T6P with up-regulation of photosynthesis (Oszvald *et al.*, 2018). Synthesis and storage of a new end-product in the form of oil (triacylglycerol) by DGAT/cys-OLE expression (Beechey-Gradwell *et al.*, 2020) draws carbon out of the UDPG/hexose phosphate pool in a one-way process. This reduces metabolically available carbon and the amount of T6P, which enables the activation of photosynthesis for longer.



explained in this way. Clearly further work is needed to understand the impact of sink manipulation on carbon capture and partitioning at the level of whole-plant physiology.

Future perspectives

The findings of Beechey-Gradwell *et al.* (2020) suggest that engineering carbon sequestration in sink tissues in the form of triacylglycerol presents an interesting new tool to address this question as well as providing a useful technology for enhancing both biomass and energy densification of crops.

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Keywords: Assimilate partitioning, growth and development, lipid, *Lolium perenne*, metabolic engineering, photosynthesis, triacylglycerol.

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