A preponderance of scientific evidence makes clear that humanity must transition to carbon neutrality by 2050, if not sooner, to avoid the worst effects of climate change on civilization and ecosystems (Masson-Delmotte et al., 2018). To do so, not only must the use of fossil fuels be curtailed and replaced, for example by using biofuels, but carbon capture and storage (CCS) will be necessary to return atmospheric CO$_2$ to near current levels (Edenhofer et al., 2011, 2014).

An attractive route to increasing atmospheric carbon capture and biofuel production, without displacing food crops or disrupting ecosystems, is to grow cover crops in the off season on farmland otherwise sitting barren and prone to soil erosion and nutrient runoff (Phippen and Phippen, 2012; Dunn et al., 2016). To that end, researchers are domesticating pennycress (Thlaspi arvense L.; field pennycress) (Box 1) into an oilseed cash cover crop grown during the autumn through spring months throughout temperate regions of the world (Sedbrook et al., 2014; Chopra et al., 2020). At a seed yield of 1700 kg ha$^{-1}$ (projected to be economically profitable and what breeding programs are now attaining) and a harvest index of 0.3, pennycress plants would fix ~0.4 t of carbon per hectare annually (McDermitt and Loomis, 1981) while producing 600 liters of oil ha$^{-1}$. If planted on half of the 32.4 Mha US Midwest Corn Belt (Fan et al., 2013), pennycress could annually fix 40 Mt of carbon (the emissions of ~31 million automobiles) and produce 9.8 billion liters of oil and 17.5 Mkg of seed meal.

While native pennycress oil is suitable for conversion to biodiesel and biojet fuel (Moser, 2012; Fan et al., 2013), the oil composition is being improved upon for various applications. For example, mutations in FATTY ACID ELONGATION1 (FAE1) abolish seed oil erucic acid (22:1) content (Box 1), thereby improving the cold flow properties for biodiesel. Mutations that increase oleic acid content improve oil stability and shelf life in that this monounsaturated fatty acid is more oxidatively stable than the abundant polyunsaturated fatty acids linoleic (18:2) and linolenic (18:3) acids in native pennycress oil (Durrett et al., 2008).

Wild pennycress seeds contain ~30–35% oil on a dry weight basis. Given the value of higher oil content and the fact that the indigestible seed coat fiber (composed of structural polysaccharides, condensed tannins, and lignin) must be reduced to improve the nutritional value of the seed, breeding and metabolic engineering efforts are underway to redirect carbon in developing seeds (Chopra et al., 2018). To help guide these efforts, Tsogtbaatar et al. (2020) have provided the first quantitative assessment of carbon uptake and utilization by developing pennycress embryos. By measuring the composition of the liquid endosperm that sustains embryo development, they established in vivo embryo culture conditions that enabled similar physiological development and biomass accumulation rates to embryos in planta. With this culture system, Tsogtbaatar and co-workers measured the uptake and incorporation of carbon...
by pennycress embryos and demonstrated a carbon conversion efficiency (CCE) of 93.4%, which is considerably higher than that observed for other oilseeds. For example, other Brassicaceae such as *Brassica napus* (oilseed rape) and *Camelina sativa* (camelina) possess a CCE of 86% and just 32%, respectively (Goffman et al., 2005; Carey et al., 2020).

**Distinct pathways enable efficient CO₂ capture**

How is pennycress able to achieve such a high CCE compared with related species? Follow-up experiments by Tsogtbaatar et al. (2020) suggest at least two mechanisms, in two different organelles, by which pennycress embryos are able to efficiently re-incorporate the CO₂ generated when pyruvate is decarboxylated to generate acetyl-CoA in the mitochondria and plastids (Box 2).

One mechanism, first demonstrated through pioneering stable isotope labeling studies in *Brassica napus* (Schwender et al., 2004), involves the refixation of CO₂ by Rubisco to form 3-phosphoglycerate (PGA) (Box 2). In the absence of the remainder of the Calvin cycle, this PGA is ultimately converted to acetyl-CoA and used to synthesize fatty acids. In *B. napus*, this so-called ‘Rubisco shunt’ is estimated to generate 37–51% of the PGA in the embryo, thus reducing carbon loss and contributing to the high CCE of this species. Other seeds with relatively high CCE, such as soybean, also appear to utilize the Rubisco shunt (Allen et al., 2009). In contrast, recent work has shown that developing camelina embryos lack this particular capability and thus possess a much lower CCE of 32% (Carey et al., 2020). With a similar stable isotope labeling strategy, Tsogtbaatar and co-workers were able to show that like *B. napus*, pennycress embryos also use Rubisco to recapture CO₂, forming ~25% of PGA in this manner.

In addition to the Rubisco shunt, pennycress embryos also appear able to recapture CO₂ through the reverse action of isocitrate dehydrogenase in the tricarboxylic acid (TCA) cycle (Box 2). Typically, this enzyme functions to decarboxylate isocitrate (formed from citrate) to α-ketoglutarate. However, Tsogtbaatar and co-workers demonstrate that when they label pennycress embryos with [U-13C₅]glutamine, a significant proportion of the citrate and isocitrate formed is labeled on five carbon atoms. As the carbon backbone of the labeled glutamine enters the citric acid cycle as α-ketoglutarate, the forward action of the citric cycle would result in molecules labeled on four carbons due to the decarboxylation of α-ketoglutarate. Consequently, the only way to facilitate the labeling of five carbon atoms of citrate is to directly convert the labeled α-ketoglutarate to isocitrate with the concomitant
incorporation of CO$_2$, through the reverse action of isocitrate dehydrogenase (Box 2). The reverse action of this enzyme in plants is not without precedent, as it has also been demonstrated to occur in developing *B. napus* and soybean seeds (Schwender et al., 2006; Allen et al., 2009), where it presumably contributes to the higher CCE of these species.

**Provisions of substrates for fatty acid elongation**

The labeling experiments of Tsogtbaatar et al. (2020) also reveal how different pathways might function to provide carbon and reductant for the synthesis of erucic acid. This very long chain monounsaturated fatty acid is synthesized in the cytosol by the fatty acid elongation complex which uses acetyl-CoA and NADPH to elongate oleoyl-CoA. Here, the reverse activity of isocitrate dehydrogenase is significant as it allows the increased production of citrate which can be exported from the mitochondria. In the cytosol, acetyl-CoA can be derived from this citrate through the action of citrate lyase, suggesting how additional 2C units can be provided for the synthesis of the large quantities of erucic acid produced in pennycress (Box 2).

Elongation of fatty acids also requires reductant. Here, labeling experiments using different $[^{13}C]$glucose substrates...
demonstrated that the oxidative pentose phosphate pathway (OPPP) was active in developing pennycress seeds. The oxidative portion of this pathway is important for the generation of NADPH and can occur in both the cytosol and plastids. Careful analysis of hexose phosphate labeling patterns by Tsogtbaatar and co-workers suggested that in developing pennycress embryos the OPPP produces more NADPH in the cytosol, which they suggested is used to generate the abundant erucic acid found in wild-type pennycress oil.

**Implications for crop improvement**

The insights generated by this work provide directions to improve the yield and composition of pennycress. One obvious implication is that given an already high CCE, increasing oil content will probably need to occur at the expense of either the protein, carbohydrate, and/or phenolic polymer components of the seed. As protein meal represents a valuable co-product, the extra carbon for increased oil content should preferably be derivable from carbohydrate fractions such as cellulose or starch and phenolic polymer structural components including condensed tannins and lignin, which together comprise 36.3% of the carbon in the seed. Evidence for the viability of this approach already comes from pennycress mutants possessing reduced seed coat fiber content and a relative increase in seed oil content without affecting plant growth and seed yield (Box 1).

Pennycress crop improvement efforts have included the elimination of erucic acid from the seed oil through the targeted mutagenesis of the FAE1 gene using genome editing and ethyl methanesulfonate (EMS) mutagenesis approaches (McGinn et al., 2019; Chopra et al., 2020). The work of Tsogtbaatar et al. (2020) suggests that pennycress embryos possess pathways that devote carbon and reductant for the synthesis of erucic acid. It will therefore be interesting to determine at the gene and metabolite level how pennycress embryos respond to the disruption of erucic acid synthesis in the fae1 mutant. What happens to the now unneeded acetyl-CoA and NADPH generated in the cytosol ostensibly for elongation? Our unpublished results indicate that fae1 mutant seeds possess lower seed oil content compared with the wild type (Box 1), suggesting the introduction of metabolic inefficiencies into fatty acid synthesis. These could result from poor utilization of substrates, as has been suggested to occur in camelina where high flux through the OPPP lowers the CCE (Carey et al., 2020). Alternatively, the aberrant accumulation of biosynthetic precursors could negatively regulate fatty acid production, as has been shown to occur during the synthesis of unusual fatty acids in transgenic plants (Bates et al., 2014). Future work that builds on the approaches and results developed by Tsogtbaatar and co-workers will therefore provide new insights into how oil seeds regulate the flow of carbon into oil and other seed components.

**Keywords:** 13C-labeling, carbon conversion efficiency, isocitrate dehydrogenase, jet fuel, oilseed, pennycress, plant metabolism, Rubisco.

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