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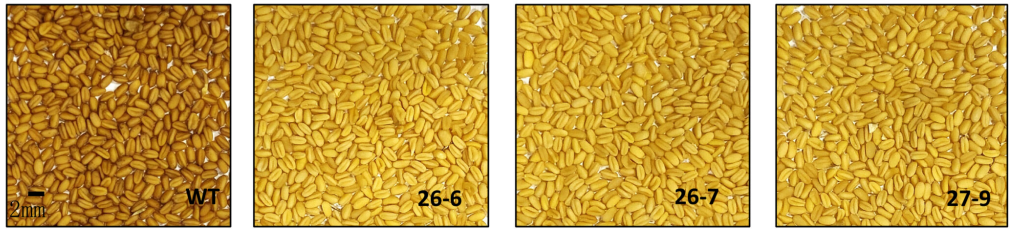
Creating Yellow Seed Camelina sativa with Enhanced Oil Accumulation by CRISPR-Mediated Disruption of Transparent Testa 8

Background/Objective

- Plant oils (triacylglycerol, TAG) are increasingly used for biofuel feedstocks because they have a high energy density and are compatible with the current energy infrastructure. Camelina (*Camelina sativa L.*), a hexaploid member of the Brassicaceae family, is an emerging oilseed crop being developed to meet the increasing demand for plant oils as biofuel feedstocks. In other Brassicas, high oil content can be associated with a yellow seed phenotype, which is unknown for camelina.
- To explore this knowledge gap, we identified three *CsTT8* isoforms in *C. sativa* and used CRISPR/Cas9 technology to create null mutants.

Approach

We sought to create yellow seed camelina using CRISPR/Cas9 technology to disrupt its *Transparent Testa 8 (TT8)* transcription factor genes and to evaluate the resulting seed phenotype. We identified three *TT8* genes, one in each of the three camelina subgenomes, and obtained independent *CsTT8* lines containing frameshift edits.



Editing of the *CsTT8* changed seed coat color to light yellow. Seed coat color of WT Suneson and three null *TT8* modification lines, 26–6, 26–7 and 27–9 respectively.

Results

In the *Cstt8* mutants, flavonoid accumulation was reduced by 44%. Carbon allocation was redirected toward enhanced synthesis of fatty acids which accumulated to as high as 38% of dry weight (DW) without changes in starch and protein contents. Disrupting all three TT8 alleles successfully created yellow seed camelina with increased TAG yield of more than 21%.

Significance/Impacts

Our results demonstrate the potential of creating new germplasm in camelina by manipulating *TT8* to enhance lipid biosynthesis. Understanding the regulation of lipid metabolism by *TT8* and other lipogenic factors may provide additional gene targets that can be manipulated to increase oil yields. The use of materials described herein with increased FA and TAG content, and others derived from them, have the potential to increase the yield of feedstocks for biofuels and bioproducts that can contribute toward a net-zero carbon bioeconomy.

Cai, et al. 2024. “Creating Yellow Seed *Camelina sativa* with Enhanced Oil Accumulation by CRISPR-Mediated Disruption of Transparent Testa 8.” *Plant Biotechnology Journal*. DOI: 10.1111/pbi.14403.