



Modifying Camelina Seed Oil Production Using CRISPR-Cas9 Gene Editing

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Introduction

- **Camelina sativa** is a mustard family seed oil crop being studied by biochemists as a future source of biofuel.
- **Triacylglycerol (TAG)**, the main component of oil, is primarily synthesized by two major enzymes:
 - **Diacylglycerol acyltransferase (DGAT1)**
 - **phospholipid: diacylglycerol acyltransferase (PDAT1)**
- **Acetyl-triacylglycerols (acetyl-TAG)**, an oil produced in **transgenic Camelina** from prior lab studies, has **desirable biofuel properties** including reduced viscosity and a low freezing point. Production of acetyl-TAG in transgenic Camelina is competed by production of TAG as both utilize Diacylglycerol (DAG) as a common substrate. (Figure 1).

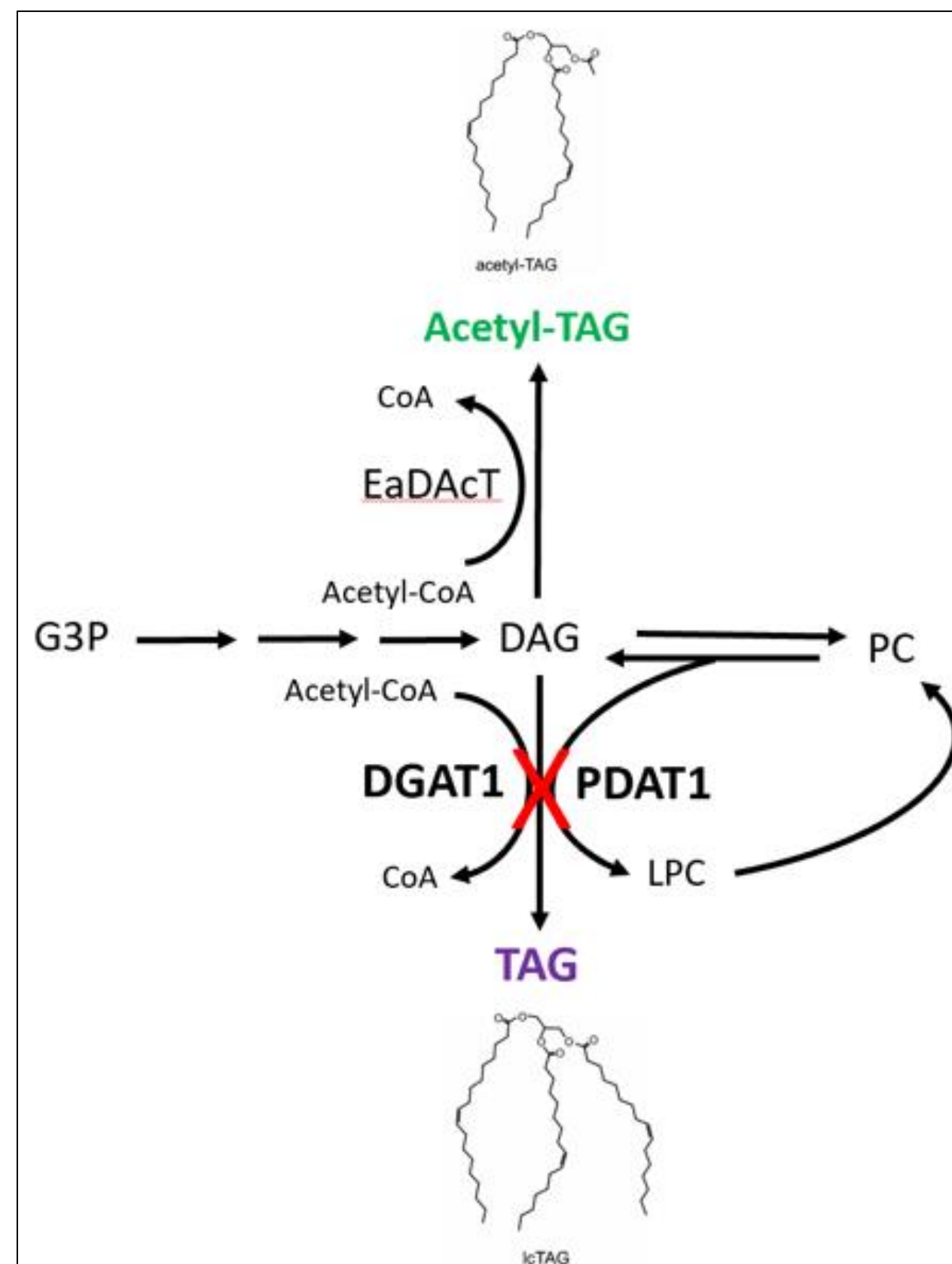
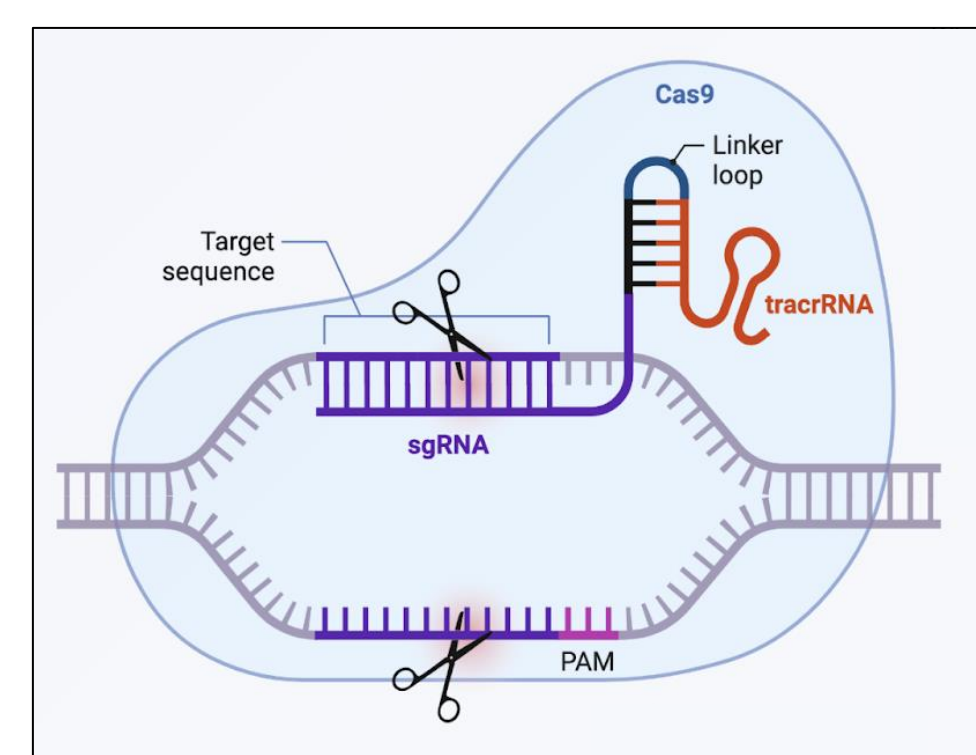


Fig. 1: Competing synthesis pathway of Acetyl-TAG and TAG in Camelina



<https://brianmcauley.net/bio-6b/6b-lecture/crispr>

Fig. 2: CRISPR-Cas9 making targeted cuts across dsDNA using guide RNA (gRNA) with PAM site. PAM site allows for Cas9 nuclease to cut.

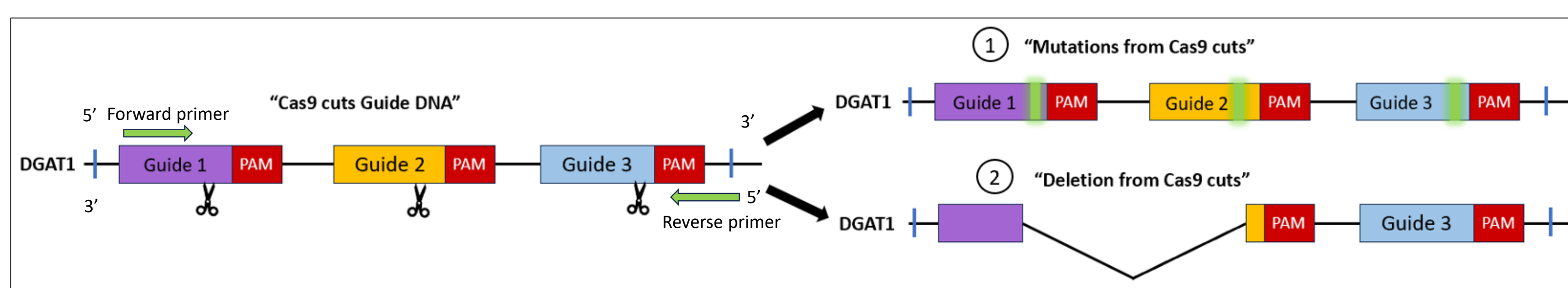
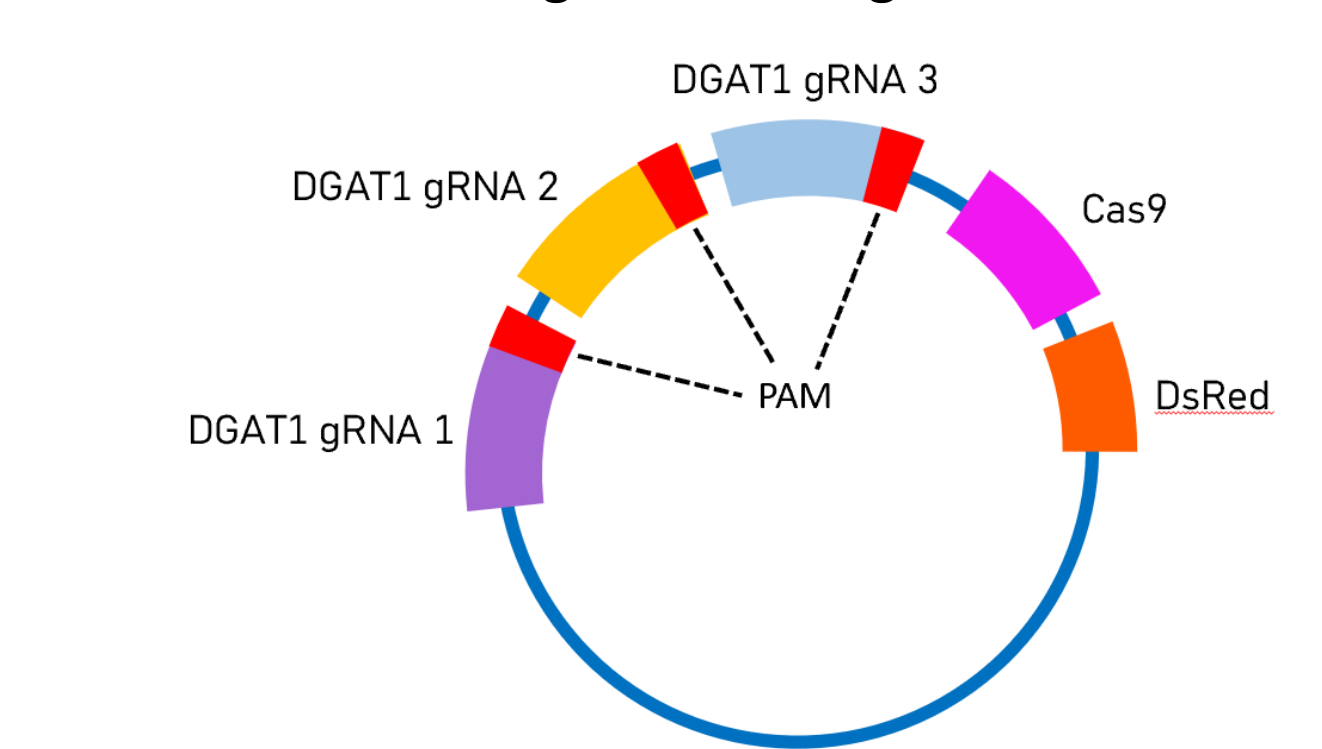


Fig. 3: Possible outcomes from Cas9 cutting mechanism. In this study we are looking for either point mutations, indels, or large deletion events in DNA homologs along the three gRNAs that would prevent the production of DGAT1 enzyme that synthesizes TAG.

Methods using CRISPR-Cas9

- 1 A plasmid containing three gRNAs, Cas9, and DsRed genes is designed

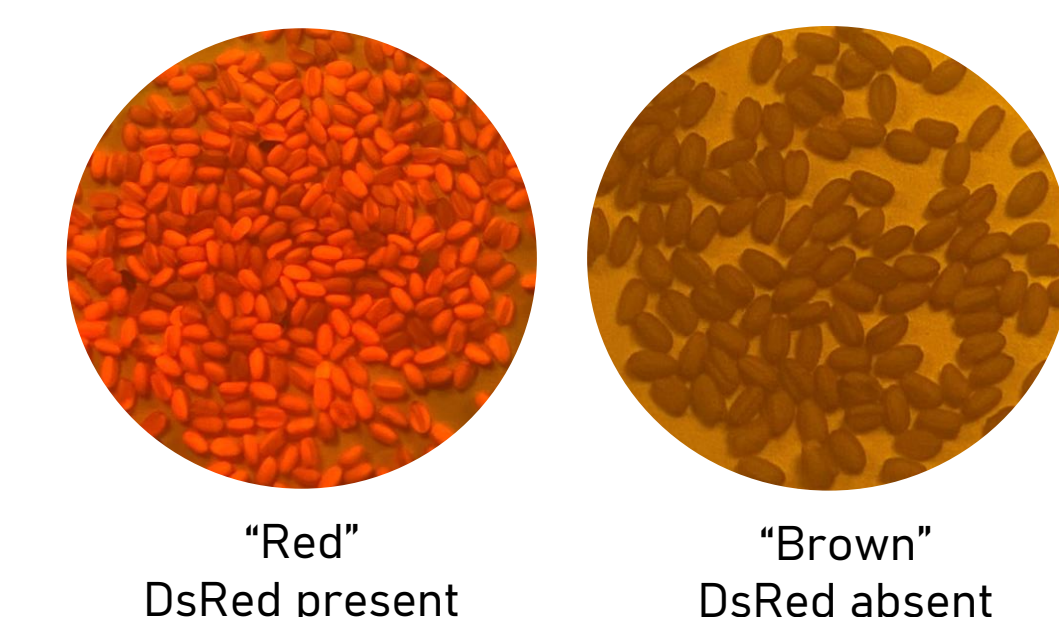


- 2 Constructed plasmid is inserted into *Agrobacterium* vector

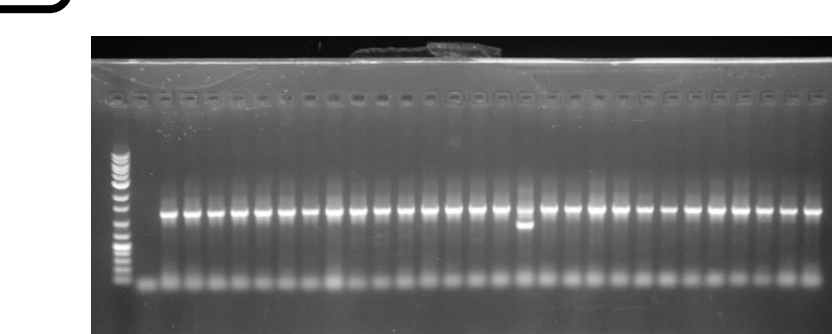
- 3 Transforming Camelina using *Agrobacterium* to insert T-DNA with gRNAs, Cas9, and DsRed from plasmid via floral dip technique.

- 4 Expression of Cas9 with gRNA specific to Camelina DGAT1 copies creates mutations. Crossover events during cell division distribute copies of DsRed, forming transgenic "red" seeds and non-transgenic "brown" seeds.

- 5 Genetic selection using DsRed fluorescent protein.



- 6 PCR screening for deletions



- 7 Sequencing DGAT1 deletions

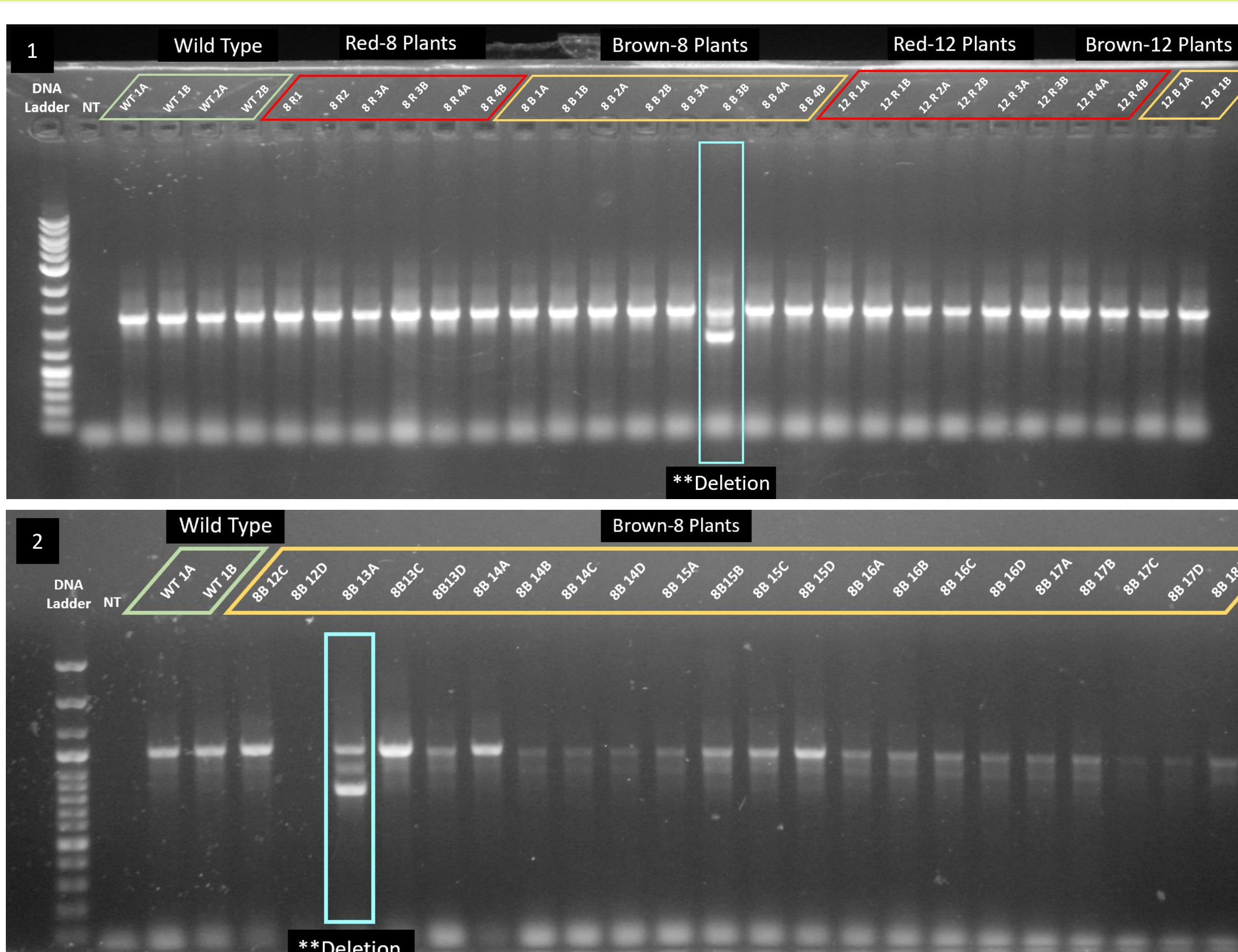
Parent generation (T₀)

1st generation (T₁)

2nd generation (T₂)

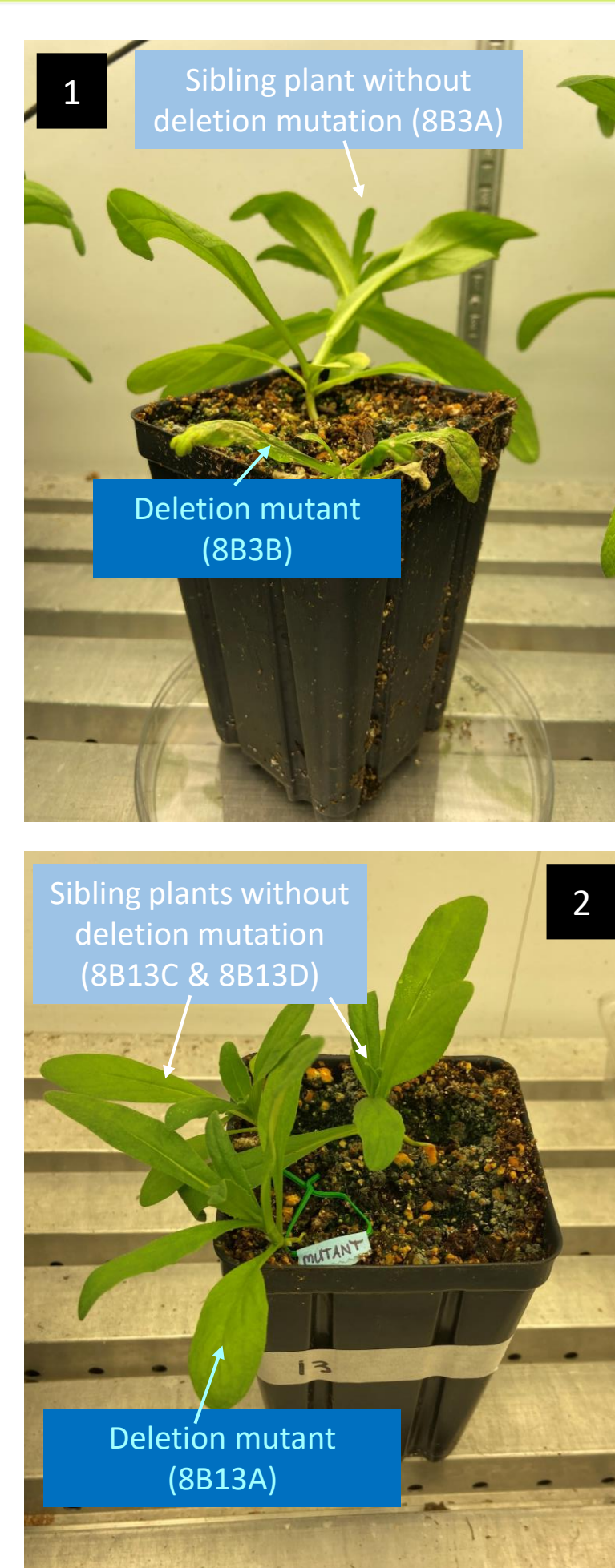
Objective: Observe desired deletions of DGAT1 and maintaining mutated DGAT1 allele into future generations of Cas9 free Camelina.

Results



Conclusion & Future Work

- The results of the genetic screening and sequence data show that deletion of the DGAT1 gene in Camelina is possible.
- Continuation of this study includes following the seed of Camelina deletion-mutant 8B 13A to analyze oil composition, progeny (T₃), and qPCR.
- Future work should aim to screen for deletions in additional Camelina plants and in plants of diploid genomes like Pennycress.



- **Part 1:** Ninety-nine second (T₂) Red and Brown Camelina plants were screened for DGAT1 deletion.
- Only **one observable deletion mutation occurred** (Gel 1) in a plant from line 8. However, this plant possessed a **defective phenotype**, having stunted growth with sickly, wrinkled and spotted leaves (Photo1).
- Under sequence analysis, the deletion of DGAT1 occurred on **chromosome 15 between guide RNA binding sites 1 & 3**.
- One possibility is that off-target effects of Cas9 may have caused unwanted mutations leading to the defective phenotype.
- **Part 2:** Using results from Part 1, we continued planting line T₂ - 8 DGAT1-CRISPR sibling plants. From 72 plants, **one additional deletion mutation was observed**. The three bands in the gel indicate two of the homologs for DGAT1 have been deleted; one wild-type homolog or point mutation is maintained (Gel 2). This plant has a normal phenotype (Photo 2).

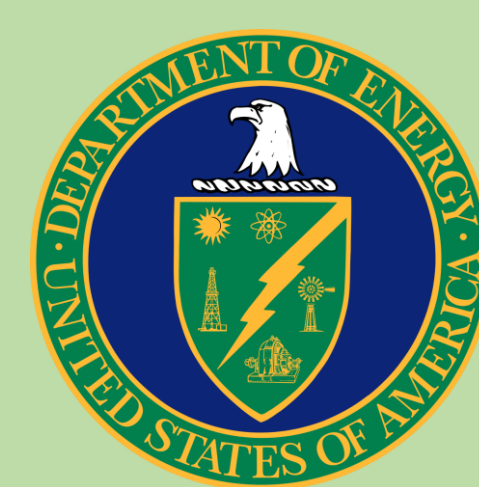
DGAT1 Deletion		[Deleted Region]	
8B3B	GGCGGAGAGTTTGTGGATCTTCGTCGA	GGGACAAACG	CCGTGTTAACGATGACGCTCAGGGAC
Cs15	GGCGGAGAGTTTGTGGATCTTCGTCGA	GGGACAAACG	CCGTGTTAACGATGACGCTCAGGGAC
Cs01	GGCGGAGAGTTTGTGGATCTTCGTCGA	GGGACAAACG	CCGTGTTAACGATGACGCTCAGGGAC
Cs19	GGCGGAGAGTTTGTGGATCTTCGTCGA	GGGACAAACG	CCGTGTTAACGATGACGCTCAGGGAC
Guide RNA 1		PAM	Guide RNA 2
		PAM	Guide RNA 3

Fig.4: Sequence Data of DGAT1 Deletion in Camelina 8B3B in comparison to Chromosomes 15, 1, and 19.

Fig.5: SNPs of 8B3B sequencing around the deletion area shows that the deletion occurred on Chromosome 15

8B3B	GATTATTCAACTTGTGTAGTAGTTCCTTATTGCTGTAAACAGTAGACTCATCATCGAAAACTGATGA
Cs15	GATTATTCAACTTGTGTAGTAGTTCCTTATTGCTGTAAACAGTAGACTCATCATCGAAAACTGATGA
Cs01	GATTATTCAACTTGTGTAGTAGTTCCTTATTGCTGTAAACAGTAGACTCATCATCGAAAACTGATGA
Cs19	GATTATTCAACTTGTGTAGTAGTTCCTTATTGCTGTAAACAGTAGACTCATCATCGAAAACTGATGA

Acknowledgements



Department of Energy: DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0023142

Durrett Lab: Kansas State University – Department of Biochemistry & Molecular Biophysics

United States Department of Agriculture: National Institute of Food and Agriculture

Award no. 2019-67032-29071

Award no. 2020-67013-30897