Stacking and evaluation of key compositional traits in pennycress

Terryn Hutchings, 2021 Integrated Plant Systems – Undergraduate Research Experience
Mentors: Catherine Espinoza Patharkar, Rahul Patharkar

Herbicide tolerance in pennycress can be conferred by a single amino acid change in the acetolactate synthase (*ALS*) gene, serine 643 to isoleucine, that is introduced through homology-directed repair (HDR). Stacking herbicide tolerance with other key compositional traits in the species *Thlaspi arvense* (pennycress), has the potential to bring pennycress closer to domestication as a winter cover crop. Key compositional traits have been identified and produced by knockout mutations for transparent testa 8 (*tt8)*, fatty acid elongation 1 (*fae1)*, and alkenyl hydroxalkyl producing 2 (*aop2)* genes through classical mutagenesis along with CRISPR-Cas9-mediated gene editing. Our goal was to stack herbicide tolerance with these traits to produce quadruple and triple mutants and then evaluate these traits. A plant found to be heterozygous for both TDNA and the *ALS*^{S643I} mutation was crossed to a plant homozygous for *tt8*, *fae1,* and/or *aop2*. F_1 progeny was screened for TDNA and heterozygous *ALS*^{S643I} mutations then self-fertilized to produce F_2 progeny with a small population of TDNA free plants having homozygous mutations of the aforementioned traits. These F_2 seeds were screened using Near-infrared Spectroscopy (NIR) to identify seeds lacking erucic acid, which is indicative of mutations in *fae1*, then the seeds were planted out and genotyped through Sigma Extract-N-Amp DNA Extraction, PCR Allele Competitive Extension (PACE), and Sanger Sequencing. Of 156 F_2 seeds, 3 plants were found to be quadruple mutants, having homozygous *ALS*^{S643I}, *tt8*, *fae1*, and *aop2* mutations. Of 252 F_2 seeds, 1 plant was found to have homozygous *ALS*^{S643I}, *tt8*, and *fae1* mutations. Through these results, it was verified that a linkage exists between *tt8* and *aop2* genes. To evaluate the *ALS*^{S643I} phenotype, we used a random block design and applied Pursuit herbicide at 0.5X, 1X, and 1.5X concentrations at 1.5- and 3-weeks growth to herbicide tolerant and WT pennycress. Plants were largely unaffected by herbicide at all tested concentrations and growth stages with the exception of stunting in plants treated at 1.5 weeks receiving 1X and 1.5X concentrations. The identified quadruple and triple mutants have stacked traits including herbicide tolerance, a thinner yellow seed coat, reduced glucosinolates including sinigrin (quadruple mutants only), reduced erucic acid, reduced fiber, faster germination and increased oil. These economically and environmentally adventitious traits stacked into a single plant will serve as future breeding material bringing pennycress closer to a commercial cover crop.

IPREFER is supported by Agriculture and Food Research Initiative Competitive Grant No. 2019-69012-29851 from the National Institute of Food and Agriculture.