The Impact of Waterlogging on Pennycress Morphology and Yield and Modeling Effective Water Availability of Pennycress Natural Populations

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https://www.pennycressresilience.org/

Project Goals: This project employs evolutionary and computational genomic approaches to identify key genetic variants that have enabled *Thlaspi arvense* L. (Field Pennycress; pennycress) to locally adapt and colonize all temperate regions of the world. This, in combination with knowledge of metabolic and cellular networks derived from first principles, is guiding precise laboratory efforts to create and select high-resilience lines, both from arrays of random mutagenesis and by employing cutting-edge CRISPR genome editing techniques. This project will deliver speed-breeding methods and high-resilience mutants inspired by natural adaptations and newly formulated biological principles, to be introduced into a wide range of commercial pennycress varieties to precisely adapt them to the desired local environments.

Field pennycress (*Thlaspi arvense* L.; pennycress) is a winter annual oilseed cover crop with extreme cold hardiness and a short life cycle enabling off-season integration into corn and soybean rotations across the Midwest. While undergoing rapid domestication over the past several years, pennycress has become a promising bioenergy crop due to high seed oil content with a fatty acid composition suitable for biofuel. Pennycress fields are susceptible to winter snow melt and spring rainfall, leading to waterlogged soils where the plants' roots are submerged under water. Waterlogging has been reported to cause yield loss and negative impacts on oil quality in *Brassica napus*, a close-relative of pennycress. The objective of this research was to determine if waterlogging had a significant effect on morphology and yield of two pennycress reference lines (Spring 32-10 and MN106) and to develop a model to predict the effective water availability for natural pennycress populations. One week of waterlogging during the reproductive stage of development caused several negative phenotypes, including early senescence, aborted silicles, and a reduction in root tissue compared to controls. Additionally, seed count and seed weight in Spring 32-10 waterlogged plants were significantly decreased compared to the controls, implying yield loss in pennycress fields that experience waterlogging from heavy spring rainfall events. The Spring 32-10 and MN106 accessions showed differences in morphology and seed yield in response to waterlogging, demonstrating natural variation in pennycress accessions can contribute to waterlogging resilience. To identify the climate-divergent natural pennycress accessions for waterlogging resilience, we also developed a mathematical model that leverages ORNL's climatype modeling framework to predict effective water availability at high geospatial resolution. The model integrates multiple layers of pre-processed climatic information (e.g., soil water concentration, precipitation, evapotranspiration, temperature, solar radiation, aridity, and slope) in a weighted linear combination to produce a distribution of effective water availability scores. A score is computed for each of the 500 natural accession locations in our current collection (https://www.pennycressresilience.org/sample-collection) and then visualized on a map to facilitate the selection of genotypes of interest. The contribution of each climatic layer in the score was optimized by systematically varying the weight of each layer and observing the changes in the effective water availability distribution. Future work involves screening the accessions identified by our model for waterlogging resilience, as well as analyzing pennycress waterlogging transcriptome data, to identify genetic and phenotypic variation contributing to waterlogging resilience. We plan to apply our model to additional climate variables to identify natural pennycress populations with resilience to diverse abiotic conditions. As domesticated pennycress (aka Covercress) is adopted as a crop across the Midwest, this research will aid the development of elite varieties with enhanced tolerance to waterlogging.

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