# Interrogating pennycress natural and induced variation to improve abiotic stress tolerance and oilseed bioenergy crop resilience

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**Project Goals: This project will employ 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, will guide 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.**

Abstract: Pennycress (*Thlaspi arvense*; field pennycress) is under development as a winter annual oilseed bioenergy crop for the 80 million-acre U.S. Midwest Corn Belt and other temperate regions including the Pacific Northwest. Pennycress has unique attributes such as extreme cold tolerance and rapid spring growth. Off-season integration of domesticated pennycress varieties into existing corn and soybean acres would extend the growing season on established croplands, avoid displacement of food crops, and yield up to 3 billion gallons of seed oil annually. Pennycress oil has a fatty acid composition well-suited for conversion to biodiesel and biojet fuel that meets the U.S. Renewable Fuels Standard. Academic, governmental, and industrial stakeholders are working closely to commercialize domesticated pennycress varieties by 2022 that can yield over 1680 kg $ha^{-1}$ (1500 lb $ac^{-1}$) of seeds producing 600 liters $ha^{-1}$ (65 gal $ac^{-1}$) of oil annually. However, these first-generation varieties have limited genetic variation, which hampers their adaptability to and resilience against abiotic and biotic challenges. Therefore, crucial work remains to identify genetic variants conferring stress tolerance and resilience for incorporation into next generation elite pennycress varieties. Future pennycress varieties will also require optimized lifespans for a range of latitudes and cropping systems, and improved root architectures and physiologies to maximize water and nutrient scavenging as well as carbon sequestration. To attain these goals, interdisciplinary teams employing eco-evolutionary computational genomics are identifying key genetic variants that have enabled pennycress to locally adapt and colonize all temperate regions of the world. Knowledge of metabolic and cellular networks derived from first principles are being generated to guide precise laboratory efforts aimed at identifying superior abiotic stress resilience gene variants. Candidate gene variants are being identified by screening large sequence-indexed mutant populations and by employing cutting-edge CRISPR genome editing strategies. Advanced high-throughput

phenotyping and in-field analytical methods will be employed to validate pennycress lines exhibiting superior abiotic stress tolerance resulting in consistent and higher seed yields. This project will deliver speed-breeding methods to facilitate the introduction of superior allelic variants into a wide range of commercial pennycress varieties to precisely adapt them to the desired local environments. Many of the findings from this work will be translatable to improving other Brassica crops important for bioenergy including camelina, carinata, rapeseed, and canola.

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