Tolerance to Heat Stress in Natural Variants and CRISPR Gene-Edited High Oleic Acid Lines of the Oilseed Plant Pennycress (*Thlaspi arvense*).

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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, combined with knowledge of metabolic and cellular networks derived from first principles, guides 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 into a wide range of commercial pennycress varieties to precisely adapt them to the desired local environments.

Abstract: Pennycress (*Thlaspi arvense* L.; Field Pennycress) is a cold-temperate and boreal-zone winter annual currently being rapidly domesticated for use as an oilseed crop with winter cover crop benefits. In contrast to its extreme cold tolerance, pennycress is susceptible to heat stress, particularly during flowering. To assess pennycress responses to heat stress across developmental stages, we tested the responses of seedlings and flowering plants to elevated temperatures being experienced more frequently due to climate change. We found that pennycress seedlings elongate hypocotyls at 28 °C, displaying variation between natural populations originally collected from different latitudes and altitudes. This natural variation to heat stress was also observed in plants during reproductive growth as Minnesota-collected MN106 was significantly more tolerant to heat stress than Montana-collected Spring32. In addition, we tested pennycress natural populations (wild-type) and lines CRISPR-edited to produce high-oleic triacylglycerides for lipid peroxidation and seed yield following a heat stress treatment, as well as pollen viability at a series of progressively increasing temperatures. We hypothesized natural lines from low latitudes as well as high-oleic lines could show improved tolerance to heat stress, the latter due to greater oxidative stability and reduced membrane fluidity. This hypothesis appeared correct; for example, high-oleic pennycress showed improved pollen viability at 28 – 30 °C compared to the corresponding wild type, but not at lower or higher temperatures. High-oleic pennycress lines also exhibited higher seed yields following a one-week heat stress at 32 °C. This effect was most likely not due to changes in lipid peroxidation levels, which did not differ between the lines. In a separate experiment, high-temperature stress was found to affect both male and female fertility, as well as seed fitness in the next generation. These results indicate that natural variants can be investigated to discover heat-tolerant varieties and that lines with reduced fatty acid desaturation, in addition to improved seed oil quality, could confer improved heat stress tolerance as an additional benefit. The causative variation is being explored.

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