

## **Genomics and Phenomics to Identify Yield and Drought Tolerance Alleles for Improvement of Camelina as a Biofuel Crop**

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**Project Goals: Plant oils represent renewable sources of energy-dense hydrocarbons that can be used for biofuels, but a major challenge is to produce these oils in non-food crops that have high yields and can grow under marginal conditions. Our goal is to improve the suitability of camelina as a non-food bioenergy crop. The major objectives are to: 1) Develop and apply automated, non-destructive high-throughput phenotyping (HTP) protocols to evaluate the phenotypic diversity of a camelina panel consisting of 250 accessions, grown under well-watered and water-limited conditions; 2) Discover alleles/genes controlling morphological, physiological, seed, and oil yield traits using genome-wide association studies (GWAS); and 3) Identify, test, and validate useful germplasm under diverse environments and marginal production areas. These studies will enable the discovery of new genes associated with crop yield and stress tolerance, and identify high-yielding cultivars that are suitable for certain geographical regions.**

The aim of many breeding programs is to increase seed yield under stress conditions. Abiotic stress tolerance and seed yield are complex quantitative traits that are controlled by genetic, physiological and environmental factors making the identification of the underlying genetic structure difficult. Genomics-based approaches, including GBS and genome-wide association studies, offer great potential for elucidating the networks that underpin these complex traits. A major challenge, however, is that hundreds to thousands of closely related plant lines need to be phenotyped to have the statistical power to identify marker/trait associations. Therefore, these approaches require robust methods for high-throughput phenotyping, which is currently recognized as a major bottleneck for genomics-based crop improvement.

Several research groups, including ours, are developing and advancing the use of cutting-edge, high-throughput phenotyping technologies in both the field and greenhouse (Andrade-Sanchez et al., 2014; Fahlgren et al., 2015; Pauli et al., 2016). In this project, we will use both approaches to study a large and diverse population of camelina accessions grown under well-watered and water-limited conditions. GBS and GWAS will then be used to discover useful gene/alleles that influence crop performance and seed yields in both favorable and water-limited environments.

Greenhouse-based HTP will be conducted at the Donald Danforth Plant Science Center using a custom controlled-environment Conviron growth house that is fully integrated with a LemnaTec

Scanalyzer 3D<sup>HT</sup> conveyor and imaging system that provides quantitative, non-destructive, and time-series phenotyping of up to 1,140 small to medium-sized plants. Environmental controls include temperature, relative humidity, light intensity, and water stress treatments. Imaging systems include visible (VIS) spectrum (color RGB cameras) and near-infrared (NIR) reflectance, along with a stand-alone saturating pulse-induced chlorophyll fluorescence (PSII) system. VIS imaging is used to measure leaf and shoot area to estimate above ground biomass, plant color, leaf morphology and shoot architecture, and time-series enable calculation of growth rates. NIR reflectance is used to measure the water status of plant shoot tissues. The PSII imaging system is used to measure the maximum quantum efficiency of photosystem II, which can be a useful measure of plant stress and as an estimate of photosynthetic performance.

The Danforth Center already has significant experience in analyzing camelina using this system. For example, the camelina panel was phenotyped using four replicates of each camelina accession (1,000 plants), from 5-45 days after planting. Imaging was done starting at day 5 after planting and continued for 40 days, resulting in ~279,000 images, which were analyzed using PlantCV, an open-source image analysis software developed at the Danforth Center. To determine conditions suitable for water stress treatments, a pilot experiment was conducted for two accessions, Licalla (high oil content genotype) and SRS933 (genotype used as sequence reference), with water treatments from 0-100% field capacity (10% steps), from 8-45 days after planting. The tested genotypes were able to survive at 20% field capacity or higher.

The camelina panel will also be grown in two different field locations including Maricopa, AZ and Scottsbluff, NE, under well-watered and water limited conditions. In preparation for these studies, the camelina panel was recently planted in Maricopa for seed multiplication. A pilot study is also being conducted using 10 genotypes, cultivated under well-watered and water-limited conditions, and will be analyzed using both conventional and HTP methodologies. The HTP system in Maricopa features a LeeAgra AvengerPro spray rig carrying various sensors for measuring canopy temperature, canopy height, spectral reflectance, and RGB and infrared imaging. LiDAR technology is also being developed for 3D canopy reconstruction. The Nebraska platform includes a manually operated cart-based system with complimentary sensors to those in Maricopa. Both phenotyping platforms include GPS systems and onboard weather stations. Data will be georeferenced and analyzed using custom algorithms and pipelines.

## References

1. Andrade-Sanchez et al., (2014). Development and evaluation of a field-based high-throughput phenotyping platform. *Functional Plant Biology*. 41:68-79.
2. Fahlgren et al., (2015). A versatile phenotyping system and analytics platform reveals diverse temporal responses to water availability in setaria. *Molecular Plant*. 8:1520-1535.
3. Pauli et al., (2016). Field-based high-throughput plant phenotyping reveals the temporal patterns of quantitative trait loci associated with stress-responsive traits in cotton. *G3 (Bethesda)*. 6:865-879.

*This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2016-67009-25639.*