The project is conducting a genome-wide investigation of the genes that control regenerability and transformation (RT) in Populus, which is one of the best studied crop species with respect to these traits. The project will identify genetic elements that control RT, develop novel phenomic methods based on image analysis, and develop new social science and education methods for teaching about genetic engineering to diverse high school students and teachers.

Our specific objectives are to (1) explore a variety of RT methods to maximize variation in RT responses; (2) develop new phenomic tools, including an image capture and generalizable machine-vision system, to precisely determine in vitro phenotypes; (3) use GWAS, map sets of alleles that are associated with variation in RT frequency; and (4) study cognitive processes with respect to GE crops, develop case studies and new teaching materials, deliver them to rural and underserved communities in the Pacific Northwest—and through publications, social media, and conferences share the project's insights and teaching modules internationally.

### In vitro Optimization Experiments

The goal of this phase of work is to identify a series of treatments that maximize genetic variance and minimize environmental variance in the GWAS population—in other words to identify treatments that give maximal heritability and thus enhance GWAS accuracy. In a factorial experiment, we tested 36 basal media types and a number of other key culture factors on a small number of genotypes, then a few selected treatments on ten to 16 genotypes. Factors tested included nitrogen and meso-nutrients, sucrose (1.5, 2.0, 2.5, and 3.0%), light spectra, and shoot formation concentrations. We have included two examples below. High sucrose concentration (3%) promotes shoot regeneration on most genotypes, and blue/red and full spectrum lighting give the best organogenesis.

### Image Acquisition System

The ability to obtain large amounts of quantitative, precise in vitro data that reflect the complex developmental biology of regenerating tissues is challenging, but critical for GWAS of regeneration traits. The project set out to design a new phenomics system that will yield precise and physiologically sensitive measurements.

We have partnered with Middleton to design and build a high-throughput plant imaging scanner for the needs of this project. The system will be equipped with one high-sensitivity fluorescence/UV/visible camera as well as one high-resolution RGB digital camera. Both of these cameras will be on a motorized stage that can be programmed to move into any position in the grid that the user defines to capture the image of the Petri dish. This system will be able to house a custom designed tray holding up to 21 Petri dishes enabling high-throughput imaging. The tray is being engineered to enable rapid transfer and return to our growth chambers. The images will then be subject to machine vision analyses to identify and quantify different developmental stages.

- Below we show schematic of the instrument as now planned, scanning Petri dishes (A), and test runs of GFP fluorescence in transformed poplar tissues scanned at a variety of hyperspectral wavelengths to maximize GFP, chlorophyll, and innate fluorescence (B) as determined a parent instrument produced by Middleton, the MacroPh.

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**Summary**

- Preliminary in vitro optimization studies showed that optimal shoot formation resulted from lower nitrogen salt concentrations in media, higher sucrose concentration, and full spectrum and high light intensities.
- Preliminary in vivo optimization studies showed that optimal callus formation followed by shoot formation resulted from being grown in R:G:blue/red 1:2:1; steams capped with Eppendorf tube to maintain moisture, temperature moderated at 4 C, room temperature, and for shoot formation TO2 being applied directly after cutting tips and planting.
- Currently in development is a web-based annotation interface that allows ow to quickly annotate various organs types via machine vision which will incorporate a deep learning algorithm.

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