Analysis of Genes Affecting Plant Regeneration and Transformation in Poplar

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A high throughput visible and hyperspectral imager designed for automated capture of trays of 21 Petri-dishes. Custom designed and produced by Middleton Spectral (Wisconsin) for the project.

Project Objectives

Regeneration of differentiated organisms from single cells is a critical need for functional genomics and for the production of genetically engineered organisms. The project will conduct 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.

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) using 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.

Participants

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Progress to Date

1) We have explored a wide variety of macro- and micronutrients, and hormone conditions, to optimize regeneration responses and their heritability in resequenced test genotypes.
2) We have obtained 9 new growth chambers, both full spectrum fluorescent and spectrum-adjustable LED models, and begun to adapt our regeneration media conditions to insure compatibility with our imaging system under GWAS. Key issues include control of condensation and contamination.
3) We worked closely with Middleton Spectral (Wisconsin) to custom design a high throughput imaging system for *in vitro* materials (see the image). Using their hyperspectral analysis software, and an extension that quantifies spectra for specific components (including GFP and chlorophyll) in designated grid areas, we can quantify transformation rate per explant.
3) We have refined our graphic user interface (GUI) for annotation of RGB images with respect to regenerated callus, shoots, and stems. We are now conducting experiments to determine the numbers of annotated samples, and the required power of neural network algorithms, for efficient detection of these tissues in unknown samples.
4) In pilot surveys and meetings with teachers, we have begun to assess their interest and ability to teach curricula related to GMO literacy and cognition goals of the project. The first case case-study modules will be produced this summer.
5) Cloned developmental genes from poplar have been tested by transformation and found to give divergent responses among genes and genotypes based on GFP shoot regeneration.

Highlights

1. We have identified a variety of highly heritable regeneration treatments suitable for GWAS.
2. We have obtained the needed growth chamber and imaging technology to enable high throughput culture and image acquisition.
3. We have conducted our first annotation and machine vision assignments with a graphic user interface as part of a GWAS experiment that is underway to identify genes for *in vivo* stem regeneration and *in vivo* root regeneration .

Project Website: IVGI

Keywords: *Populus*, *in vitro*, machine vision, differentiation, GWAS, phenomics, transformation