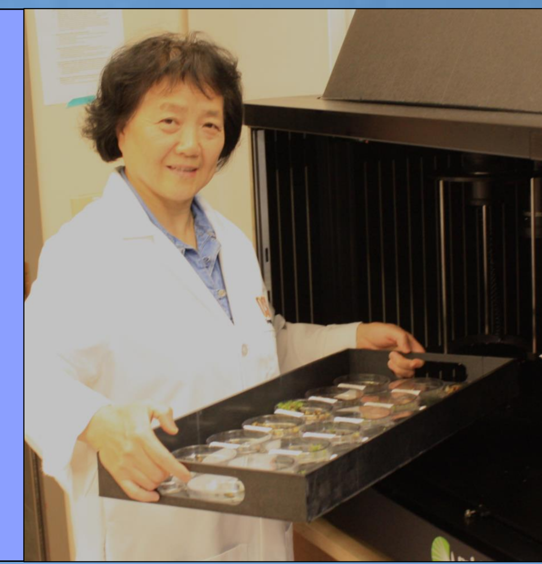
Advanced phenotypic analysis of *in vitro* development and transformation for GWAS in *Populus*: *Machine vision analysis of RGB and hyperspectral images*

Michael Nagle[1], Cathleen Ma[1], Anna Magnuson[1], Jialin Yuan[2], Nihar A. Doshi[2], Fuxin Li[2], Yuan Jiang[3], Peremyslova, Ekaterina[1] and Steven H. Strauss[1]. [1]Department of Forest Ecosystems & Society, Oregon State University, Corvallis OR; [2]Department of Computer Science, Oregon State University, Corvallis OR; [3]Statistics Department, Oregon State University, Corvallis OR



Overview

- Recalcitrance to *in vitro* regeneration: an obstacle to transformation of many species, particularly woody plants
- *Populus trichocarpa* is an ideal model organism for GWAS of regeneration due to variation in transformability, regenerative capacity across genotypes, and diversity across wild populations
- Power and accuracy of GWAS can benefit from new phenotyping methods (high-throughput, hyperspectral, machine vision)
- Through GWAS, we propose that polymorphism in regulation of a known shoot developmental regulator affects shoot regeneration

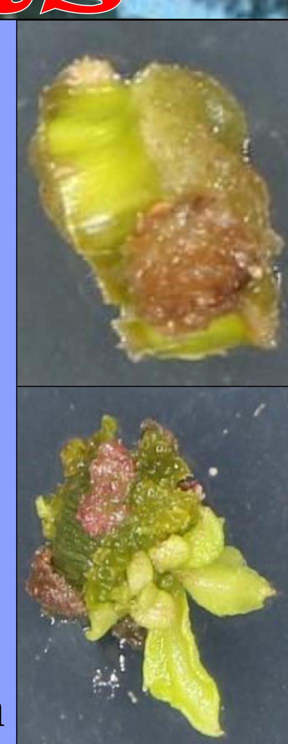
Plant treatments

In vivo

- Cut stem tips placed in water and treated with cytokinin to promote regeneration
- Imaging of stem tips (below) showing various stages of callus and shoot development

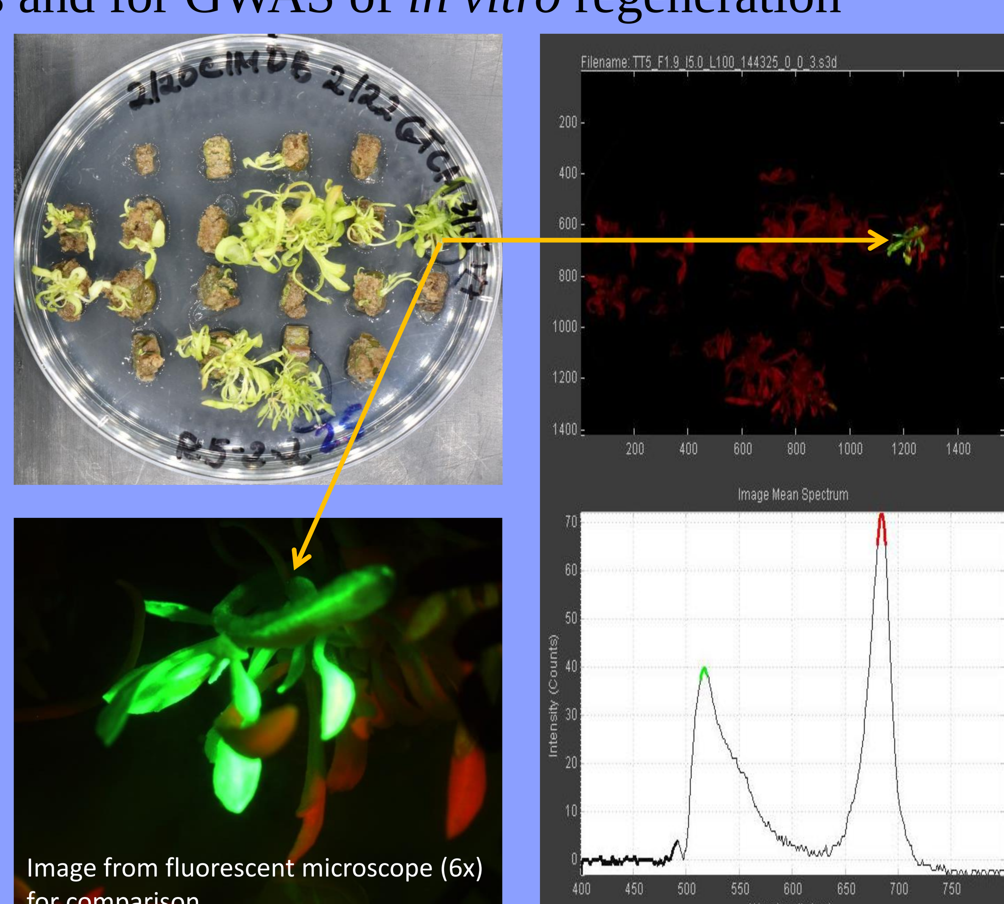
In vitro

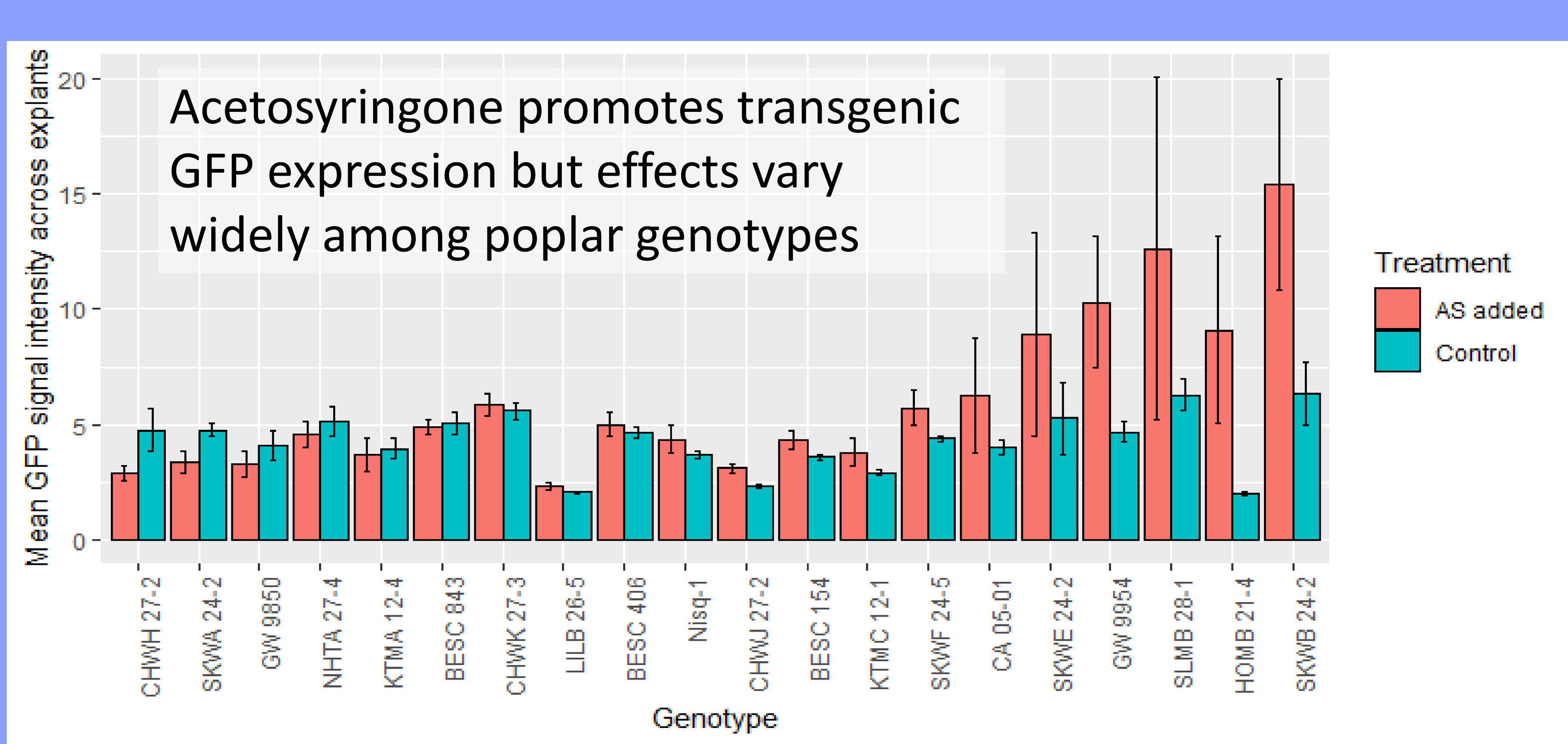
- *In vitro* treatments used in GWAS were selected for high heritability of regeneration
- Imaging of whole plates with each explant at various stages of necrosis/regeneration



Spectral analysis

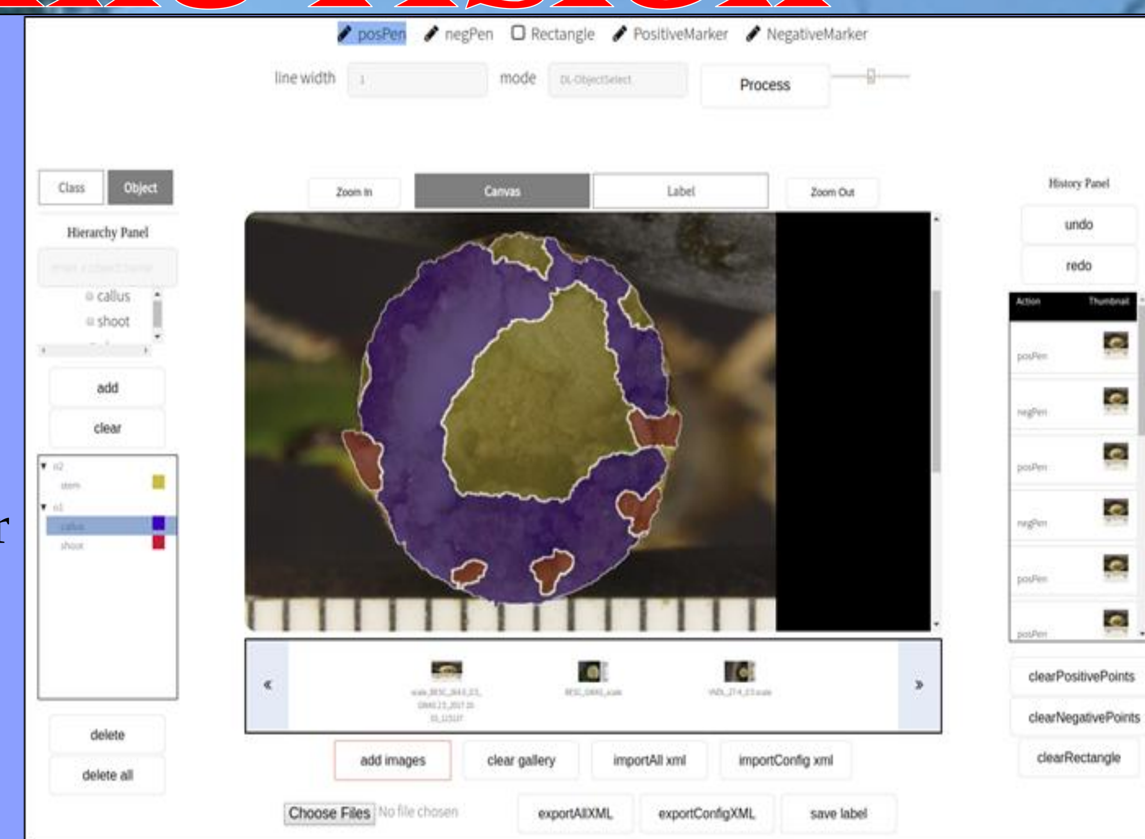
- Laser excites fluorophores including chlorophylls and GFP
- Hyperspectral camera collects visible-IR spectra of fluorescence for each pixel; each fluorophore is identified, quantified
- Statistical analysis determines effects of treatments on GFP signal, an indicator of transformation efficiency
- System currently being used to test effects of particular treatments (e.g., acetosyringone, lipoic acid, *Agrobacterium* strains) on transformation rates and for GWAS of *in vitro* regeneration

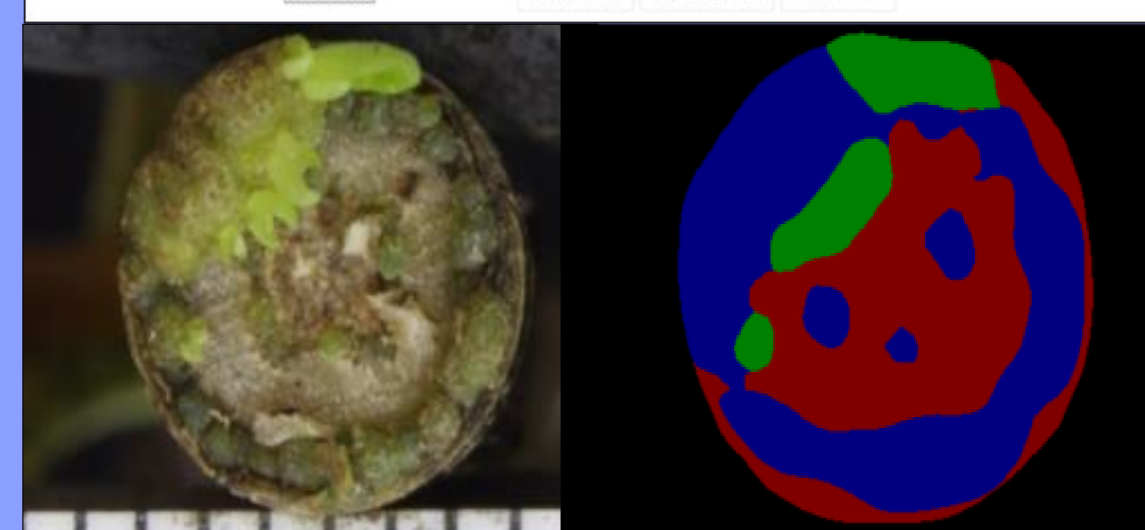




Machine vision

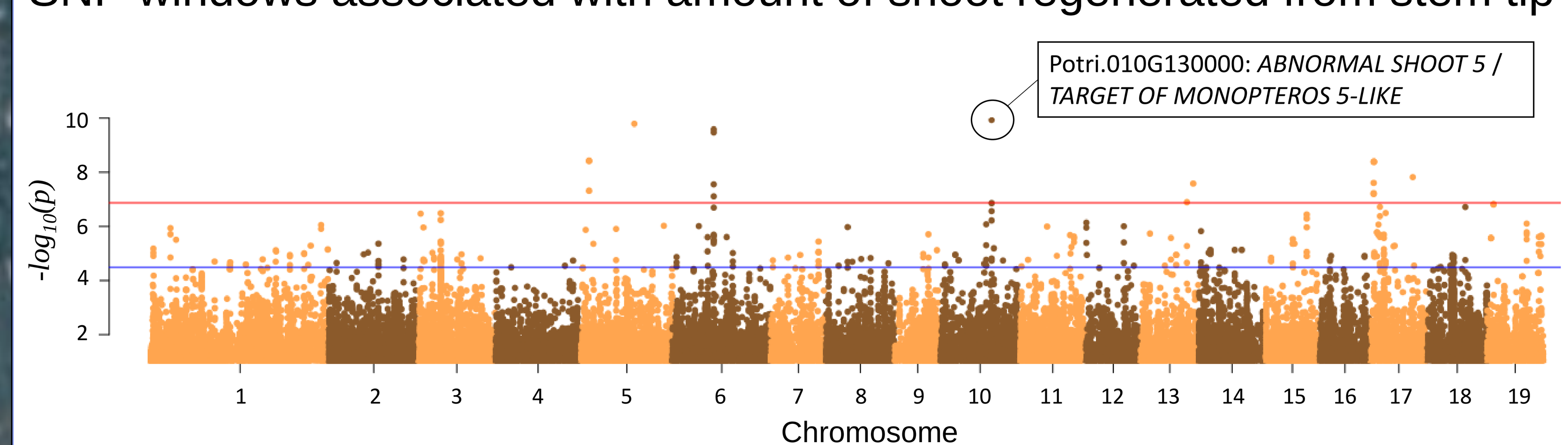
- Through a GUI, different types of tissue (callus, shoot, root) are annotated
- Annotated images are used to train neural networks for segmentation and classification
- Trained networks divide images into callus (blue), shoot (green), stem (red) and background
- Calculations of the relative size of different types of tissues are used as traits for GWAS





Association testing

SNP windows associated with amount of shoot regenerated from stem tip



- The GWAS method Sequence Kernel Association Test (SKAT) was used to collapse SNPs into 3kb windows (shown by dots above) and test for their combined effect on shoot regenerated from cut stem tips
- Shoot area was significantly associated with a homolog of a known shoot regulator that mediates auxin-cytokinin crosstalk in *Arabidopsis*
- Epistasis analysis (BOOST method in PLINK) indicates that the effect of this gene depends on a homolog of *LONESOME HIGHWAY (LHW; p = 3.109e-08*, Bonferroni-significant with 545,490 pairwise tests for epistasis between SNPs of interest*)*. This suggests conservation of function from Arabidopsis, in which *ABNORMAL SHOOT 5 (ABS5)* and *LHW* must interact with one another to regulate transcription in pluripotent cells of the procambium (Rybel et al., *Dev. Cell* 2013)

Outlook

- Convolutional neural networks and hyperspectral imaging provide new opportunities for *in vitro* optimization and genetic discovery by enabling precise, high-throughput phenotyping of complex traits
- Characterization of the genetic basis of regeneration offers opportunities for converting poor responders to regeneration into efficient responders via overexpression or knockdown/out of developmental regulators
- Efficient transformation of recalcitrant genotypes may involve a combination of *in vitro* treatments including with acetosyringone, as well as genetic treatments such as overexpression of *ABS5* and other developmental regulators

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