Next-generation phenomics in support of GWAS to Identify Genes Controlling Regeneration and Transformation in *Populus trichocarpa*

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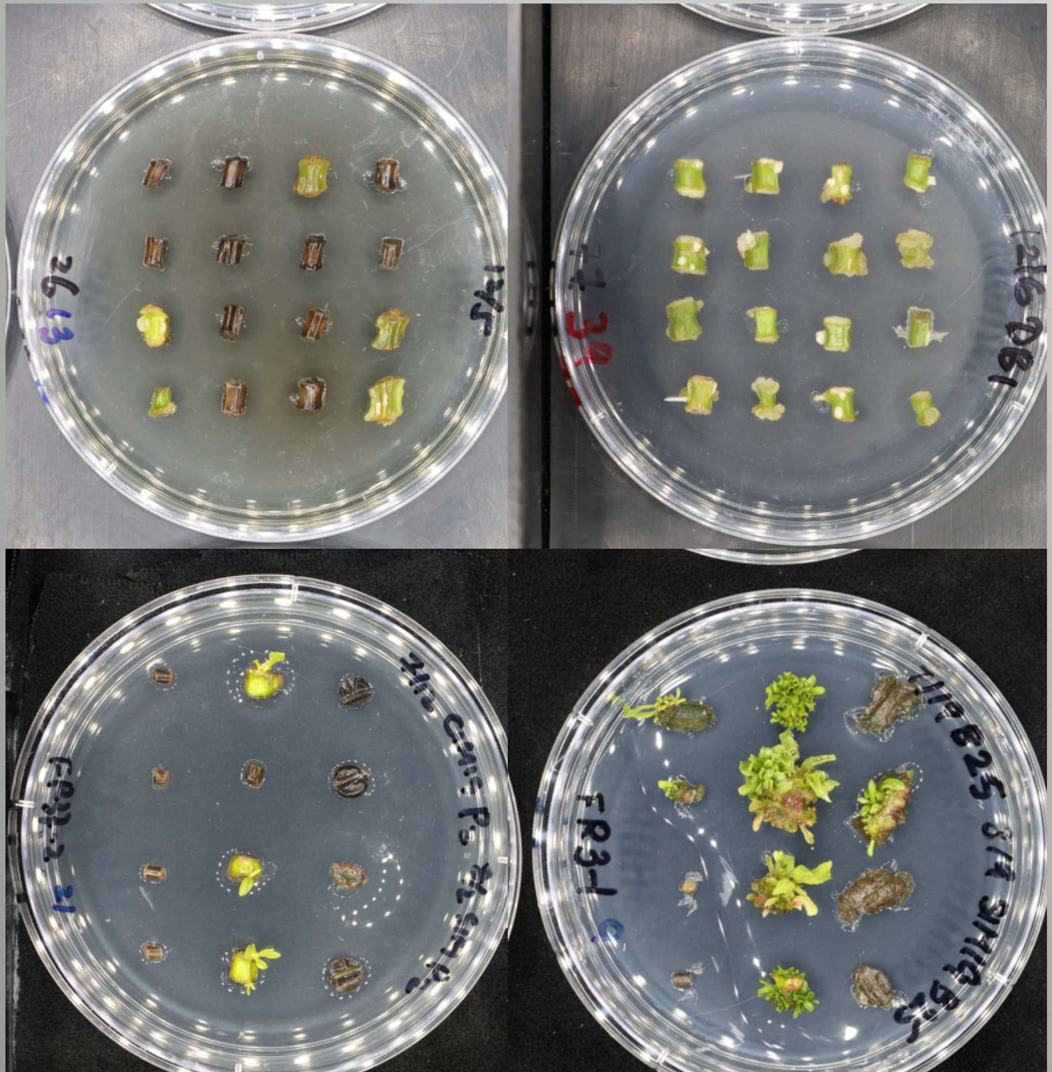
PAG XXVII, Forest Tree Workshop

Jan. 13, 2019

Presentation Overview

- **I. New opportunities in phenotyping**
    - **I. Need for innovation**
    - **II. Machine vision**
    - **III. Hyperspectral imaging**
- II. Case study: GWAS of regeneration in *Populus trichocarpa*
    - I. Efficient regeneration as a roadblock in plant improvement
    - II. Use of phenomic data
    - III. Preliminary GWAS results

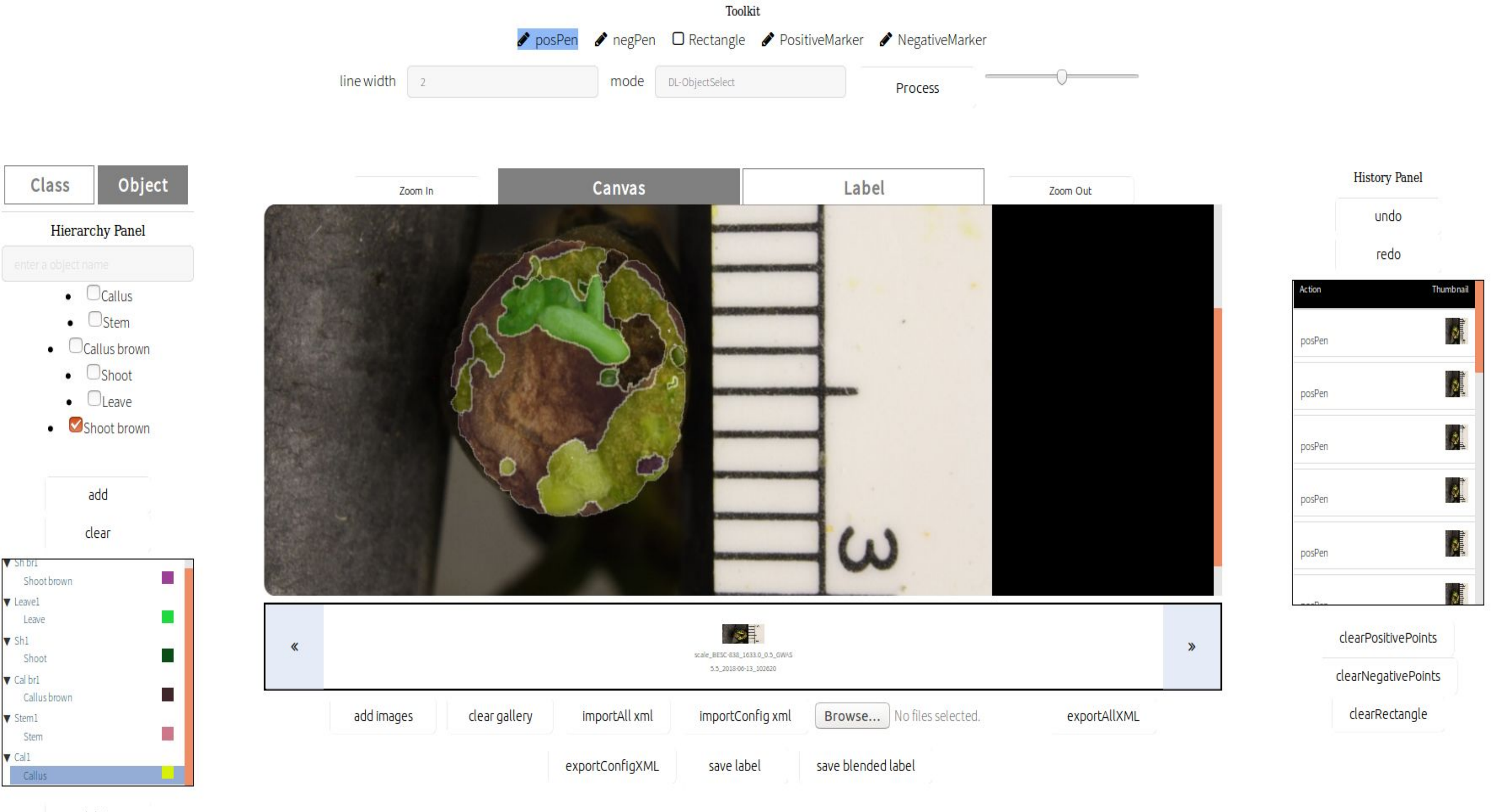
Lack of proven high-throughput phenotyping methods: The primary obstacle to rapid genetic analyses of plants



- Despite advances in genome sequencing, bioinformatics, genetic analysis of plants limited by ability to phenotype.
- Efficient, precise scoring of phenotypes for large numbers of plants, limits experimental design of mutant screens and GWAS[1].

[1] Fahlgren, et al. *Curr. Opin. Plant Biol.* 2015;*24*:93-99

Training machine vision system for precise, high-throughput phenotyping



line width 2

mode DL-ObjectSelect

GUI for annotation to train deep learning models: Poster PO0069

Hyperspectral imaging:
Capturing detail invisible to the naked eye

Conventional (RGB)

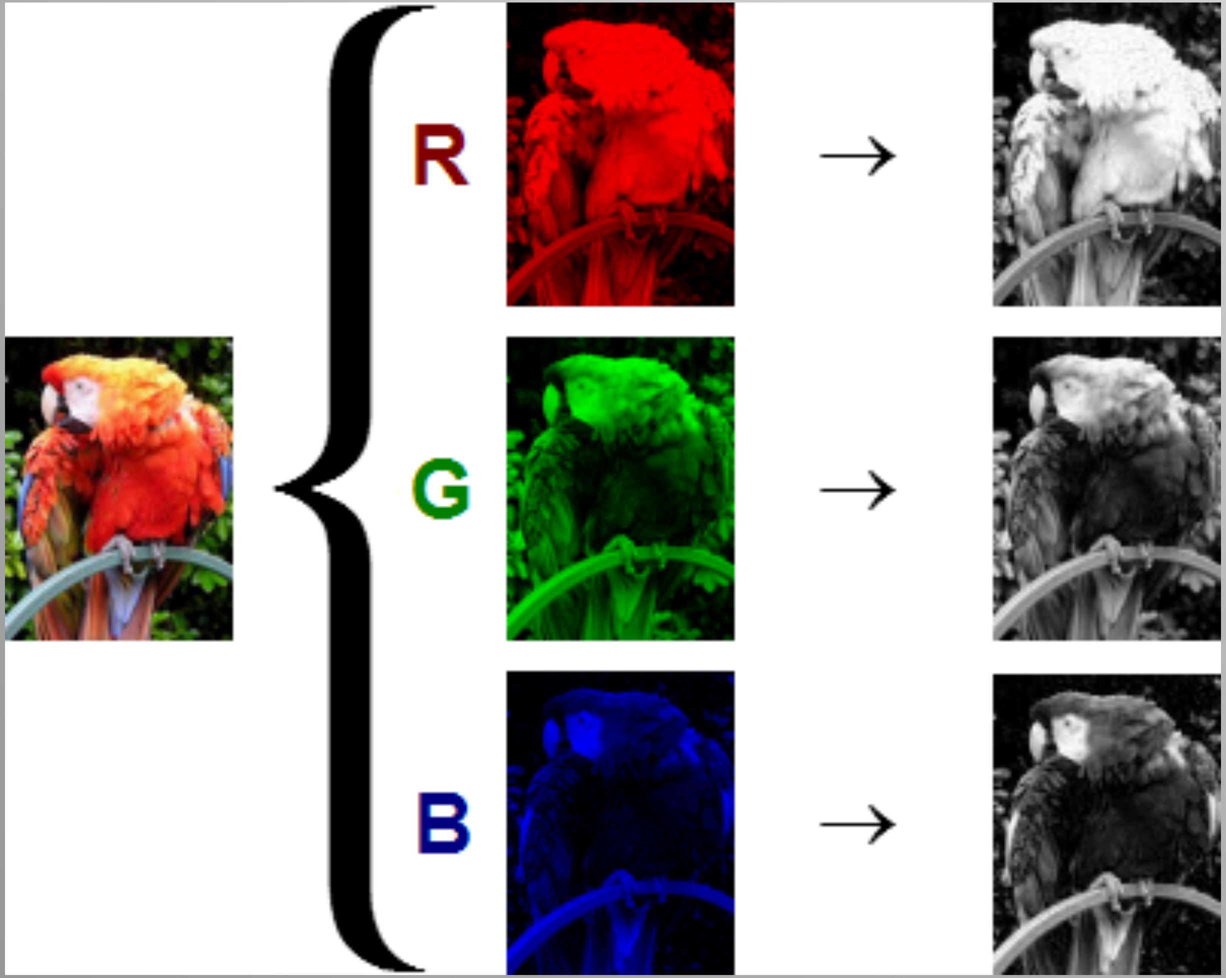


Image from Wikimedia commons

Hyperspectral

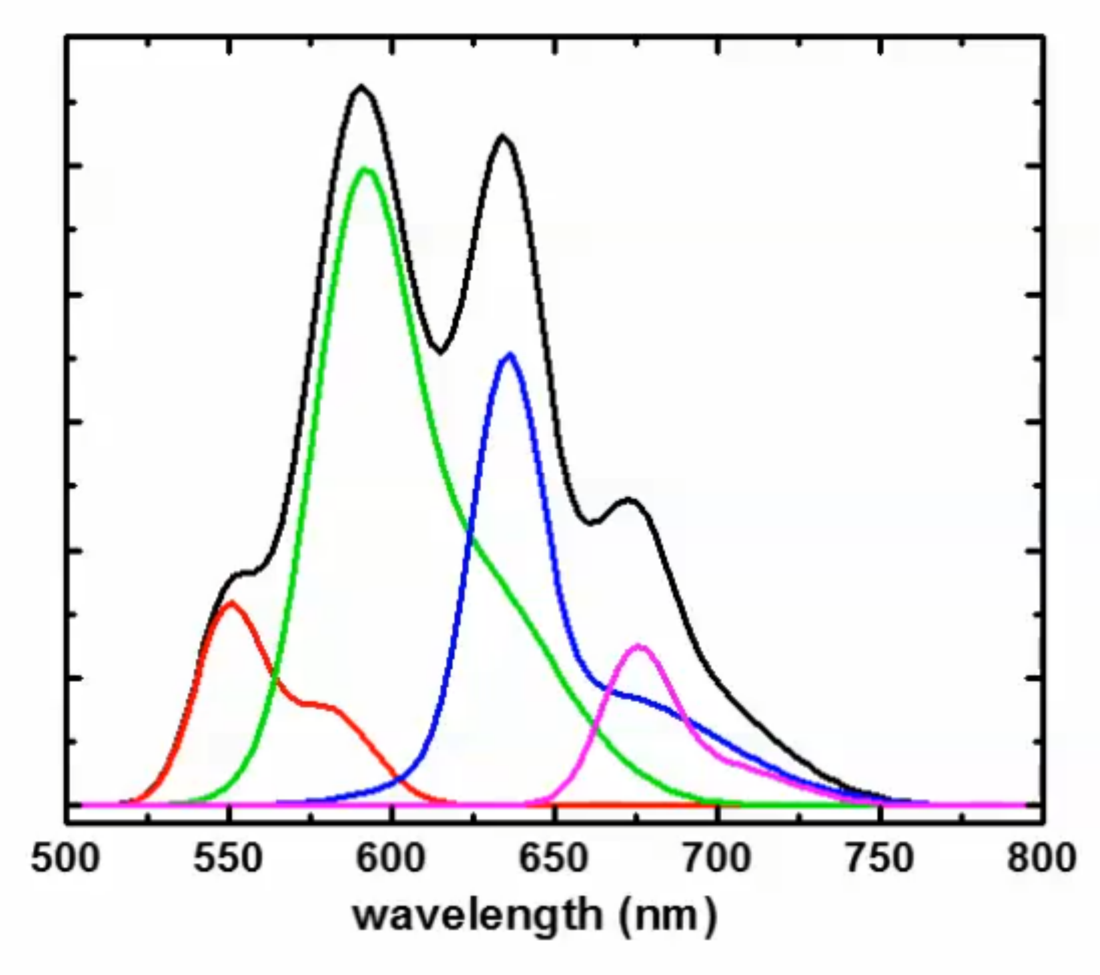
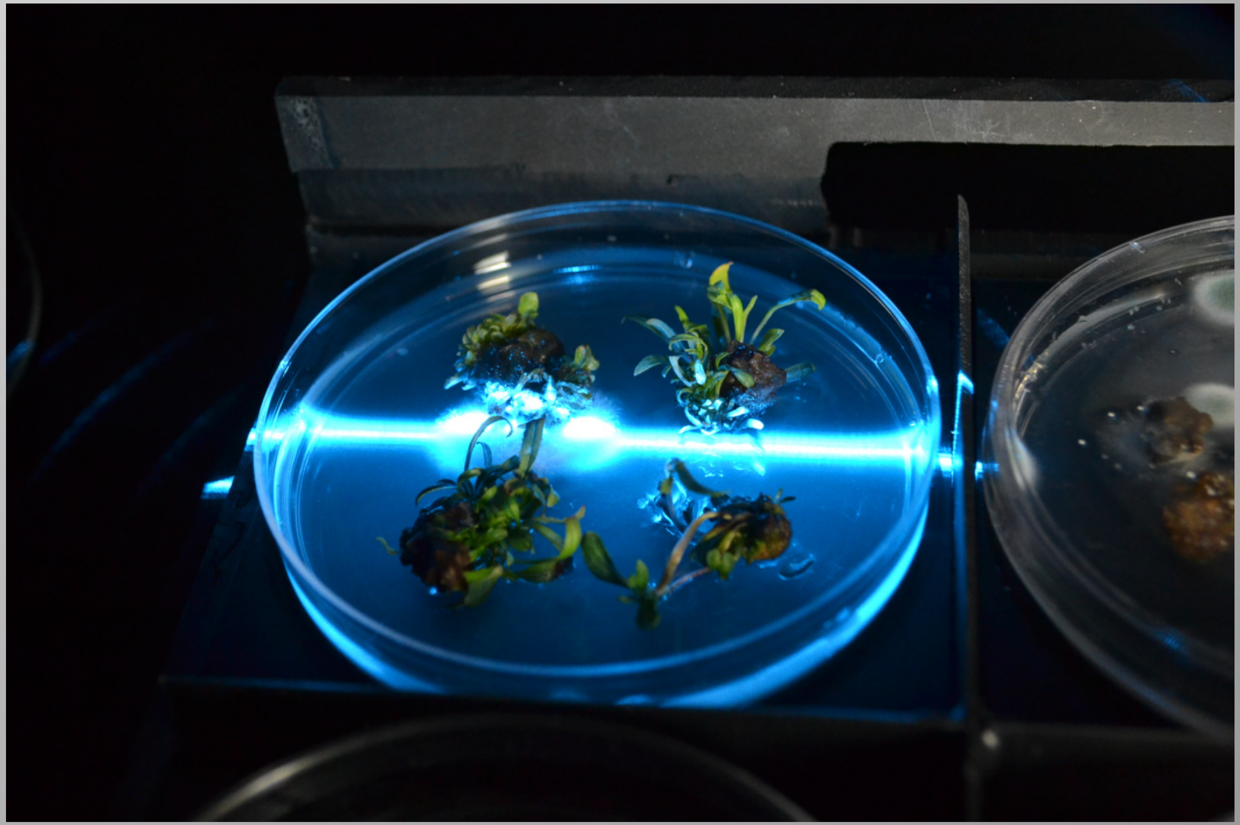


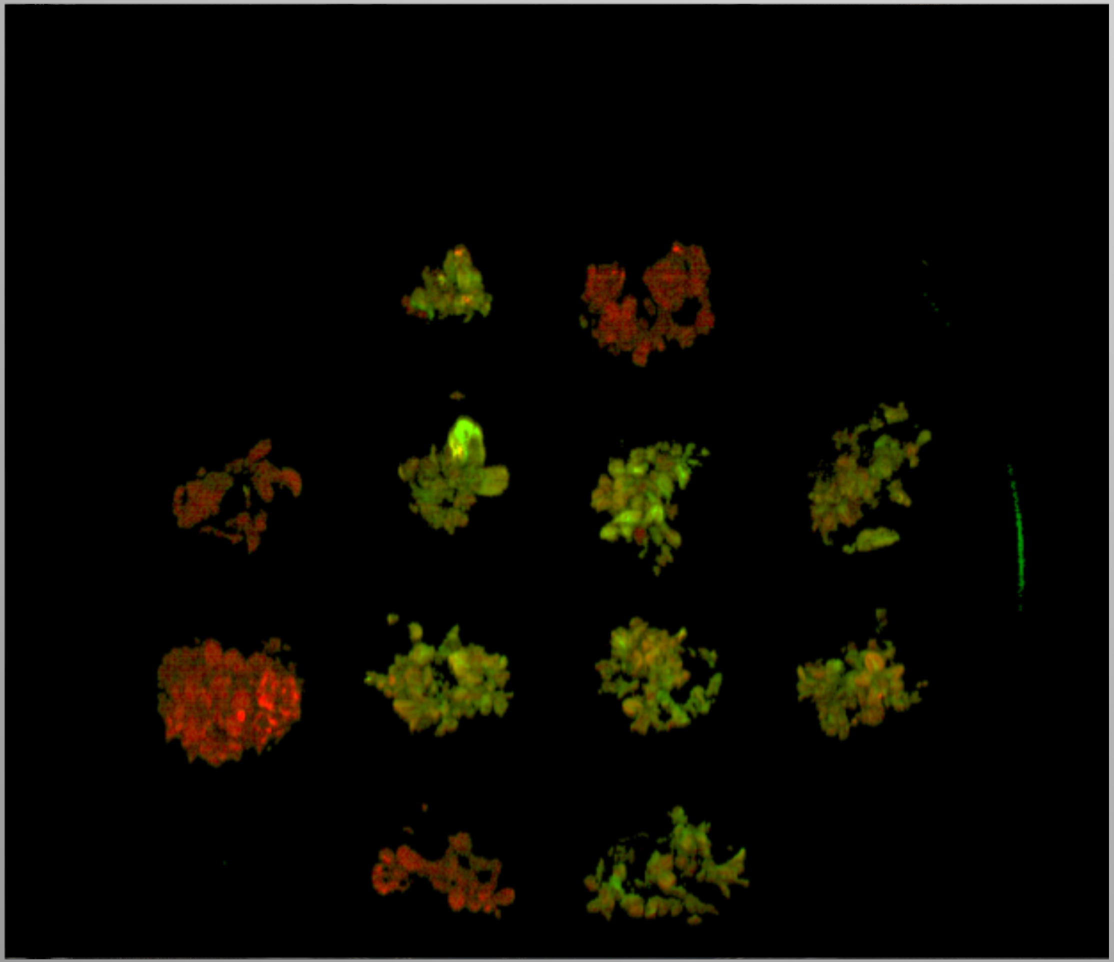
Image from Middleton Spectral Vision

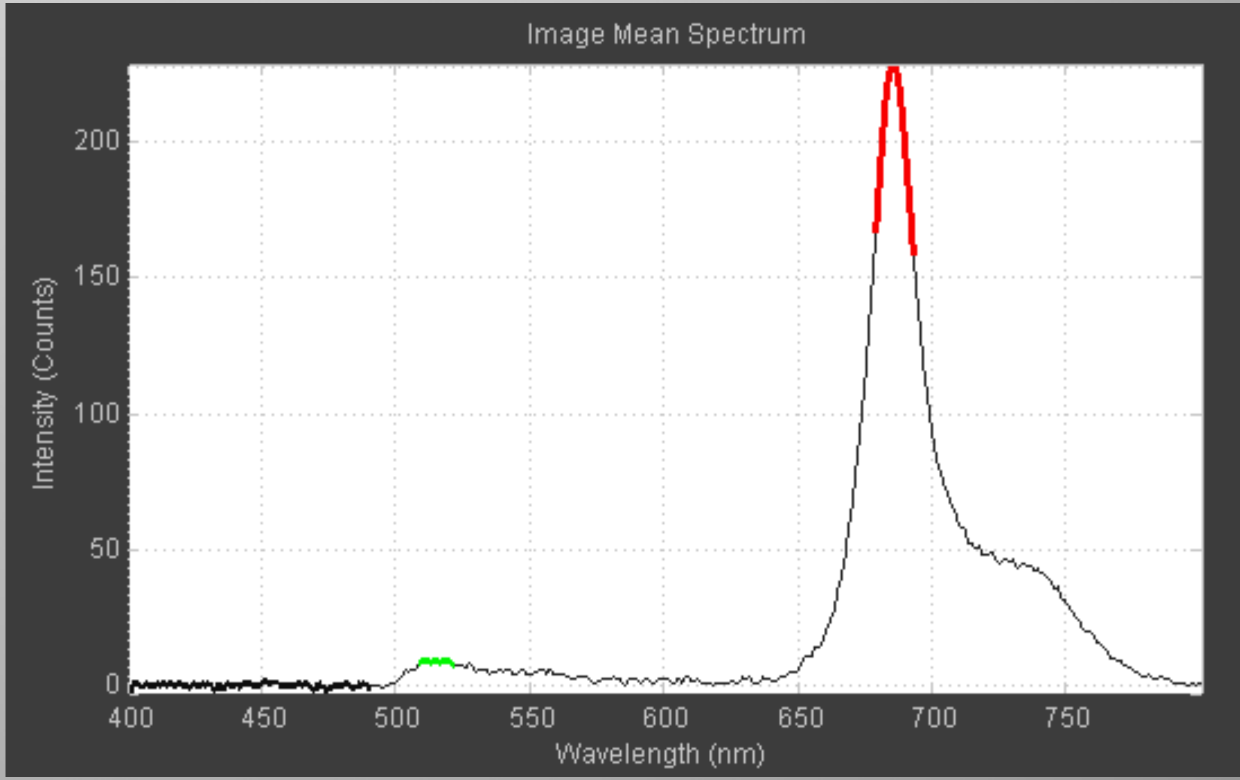
macroPhor ARRAY for high-throughput RGB and hyperspectral imaging



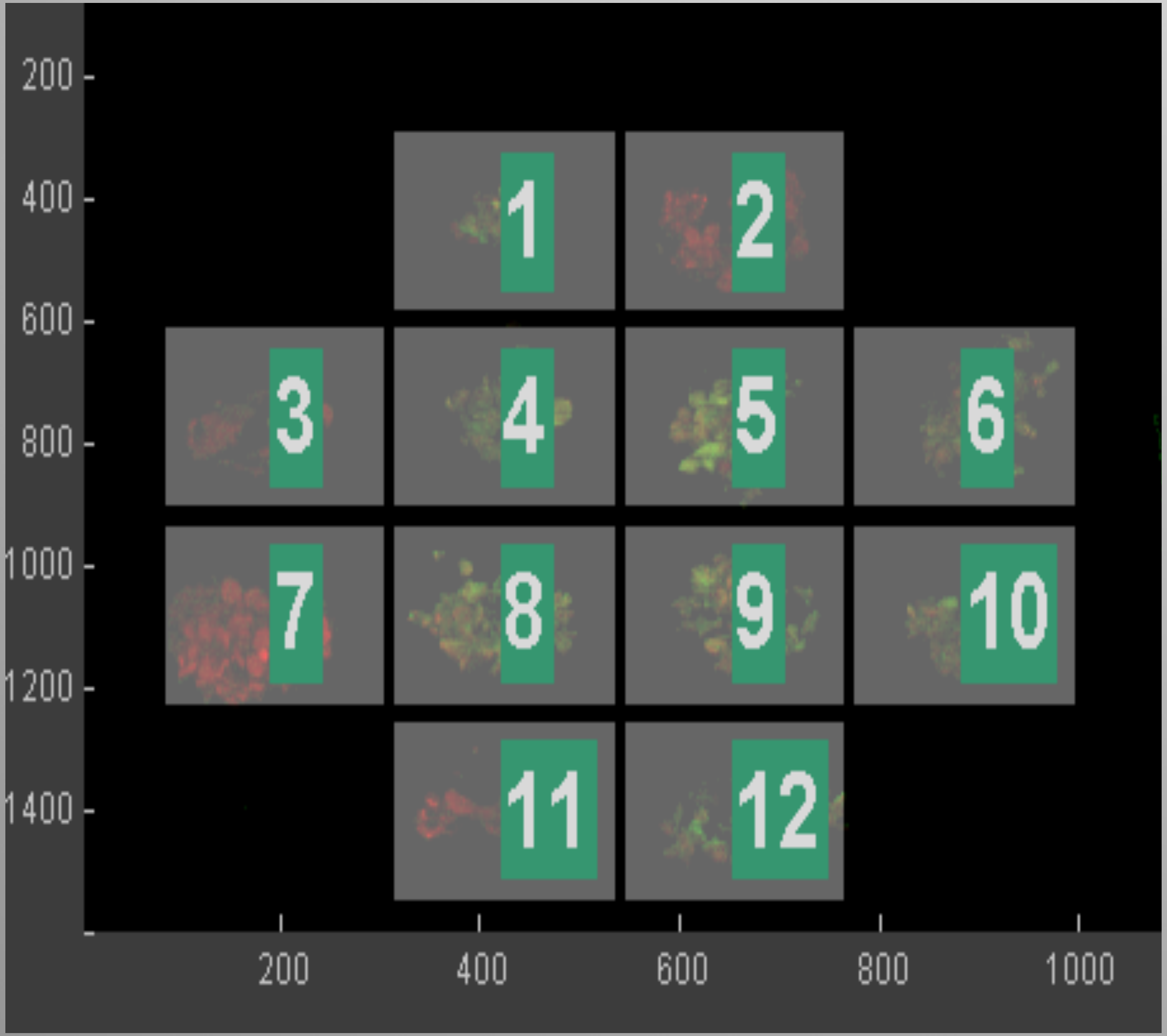
Middleton Spectral Vision, Middleton, WI

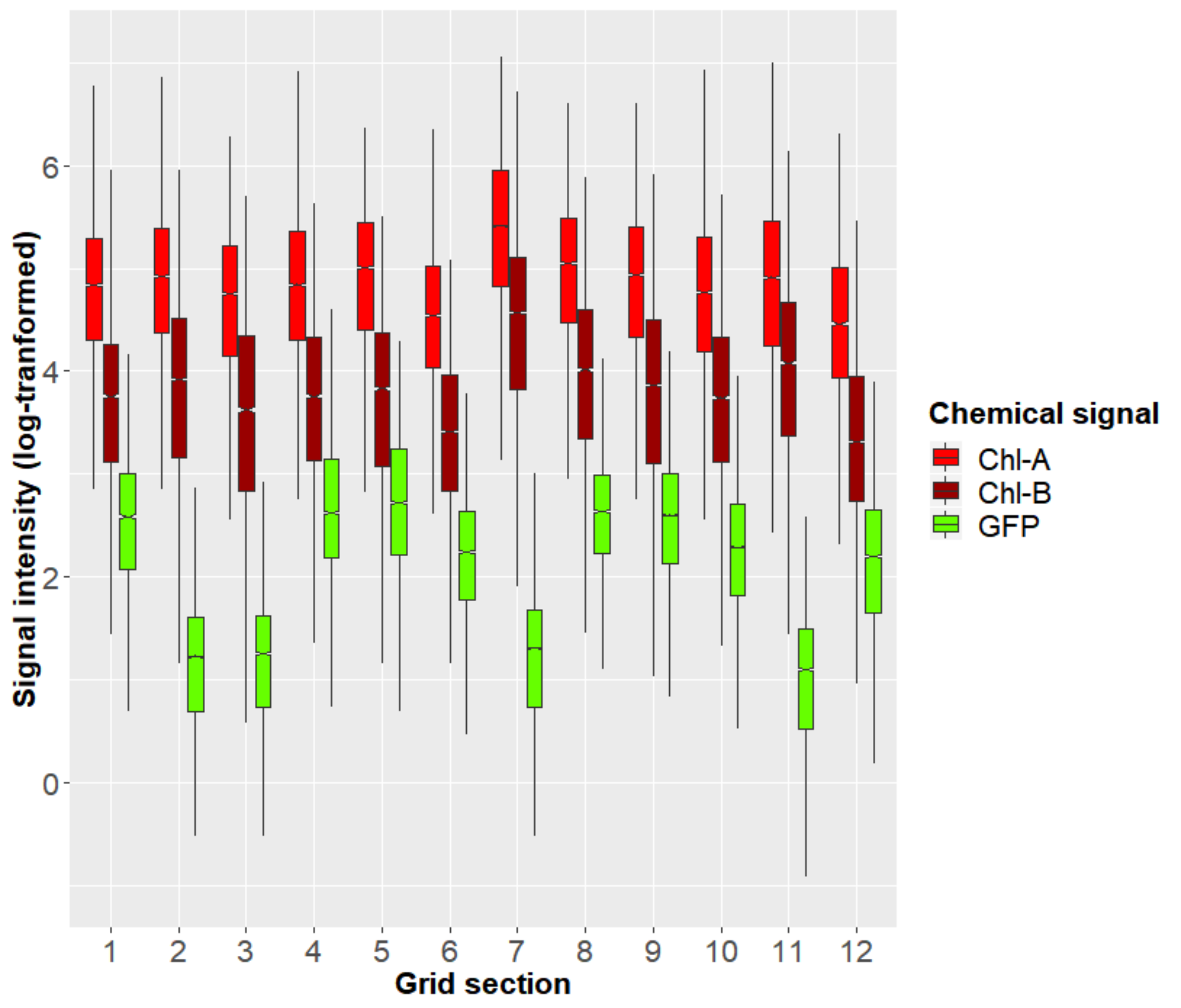
RGB and hyperspectral images are collected for each plate on tray





Signals for fluorescent compounds are quantified by linear regression, compared





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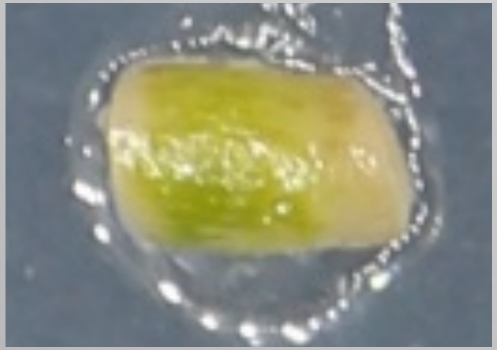
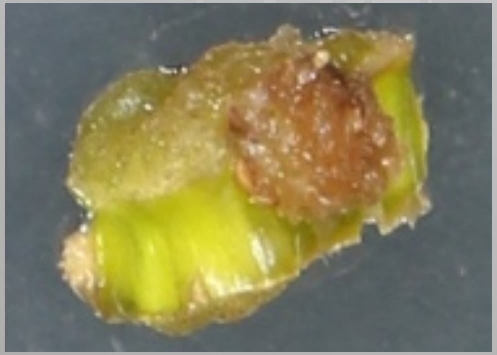
Efficient *in vitro* regeneration of transformed cells: A barrier to transformation of many genotypes

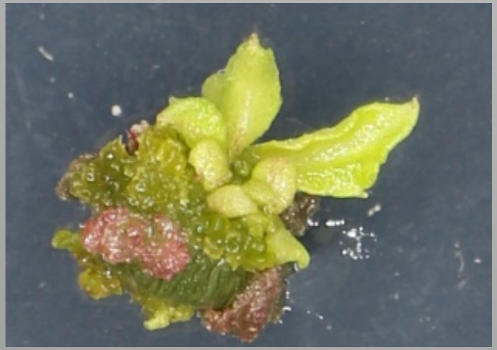
Co-cultivation with *Agrobacterium*



Callus induction



Shoot formation



- Many genotypes, entire species recalcitrant to *in vitro* regeneration
  - Genotype-indendent methods described as "holy grail" (Hofmann Plant Cell 2016)
- "Although considered part of the transformation process, the regeneration step is often a greater bottleneck than is the stable integration of DNA sequences

The Plant Cell, Vol. 28: 1510–1520, July 2016, www.plantcell.org © 2016 American Society of Plant Biologists. All rights reserved.

PERSPECTIVE

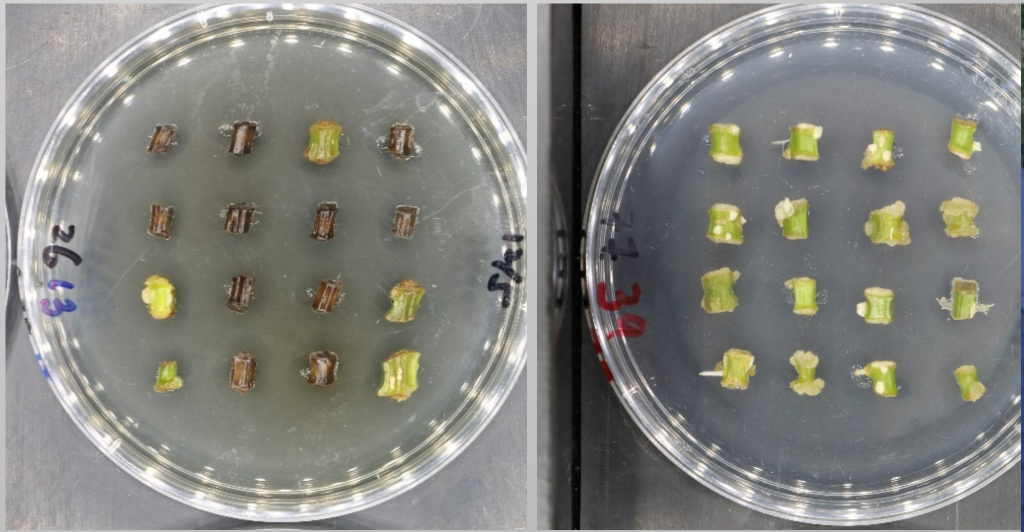
Advancing Crop Transformation in the Era of Genome EditingOPEN

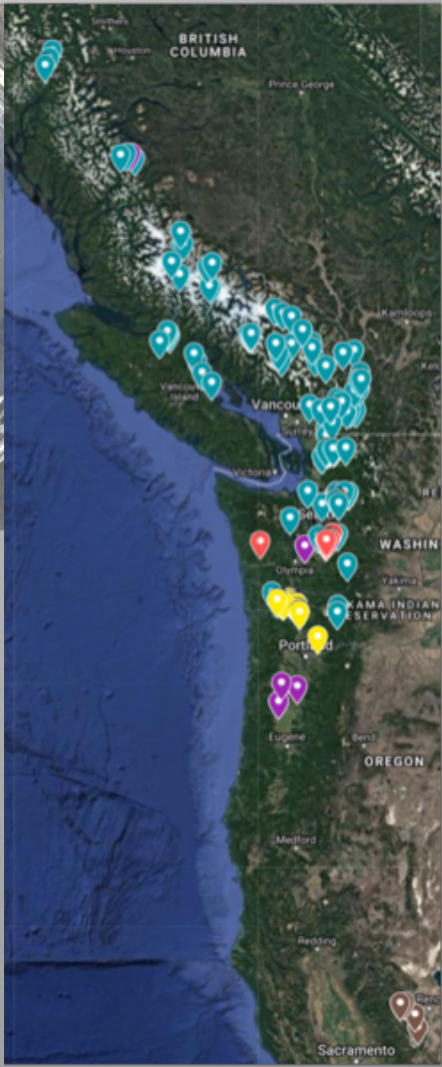
Fredy Altpeter,[a] Nathan M. Springer,[b] Laura E. Bartley,[c] Ann E. Blechl,[d] Thomas P. Brutnell,[e] Vitaly Citovsky,[f] Liza J. Conrad,[g] Stanton B. Gelvin,[h] David P. Jackson,[i] Albert P. Kausch,[j] Peggy G. Lemaux,[k] June I. Medford,[l] Martha L. Orozco-Cárdenas,[m] David M. Tricoli,[n] Joyce Van Eck,[o] Daniel F. Voytas,[p] Virginia Walbot,[q] Kan Wang,[r] Zhanyuan J. Zhang,[s] and C. Neal Stewart Jr.[t,1]

[a] Agronomy Department, Plant Molecular and Cellular Biology Program, University of Florida, IFAS, Gainesville, Florida 32611

Poplar an ideal model species to study regeneration

- Desirable traits:
    - Wide range in regeneration responses across genotypes
    - Rapid linkage disequilibrium decay
- Useful resources:
    - Availability of high-coverage genome data for 917 genotypes from Department of Energy’s Joint Genome Institute
    - Well-established protocols for transformation and regeneration





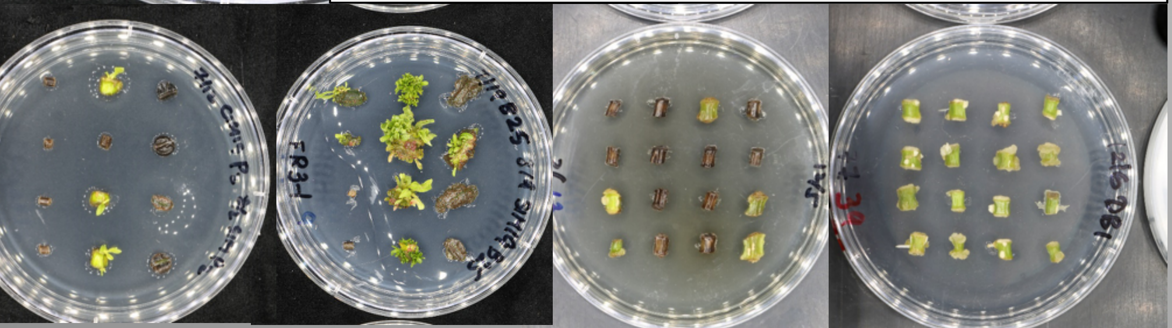
THE CENTER FOR BIOENERGY INNOVATION

bioenergycenter.org/poplargwas

De novo organogenesis in *Populus trichocarpa:* *In vivo* and *in vitro* methods

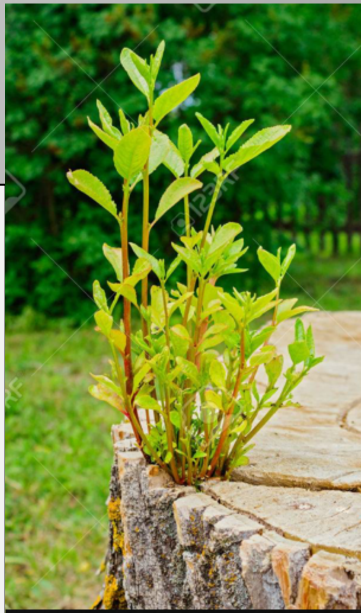
In vitro regeneration

- Standard for transformation and regeneration
- Phytohormones in media



Stem regeneration

- Stem is cut, leading to callus as wounding response, then shoot
- Cytokinin applied to tip of cut stem encourages transitions to callus and shoot

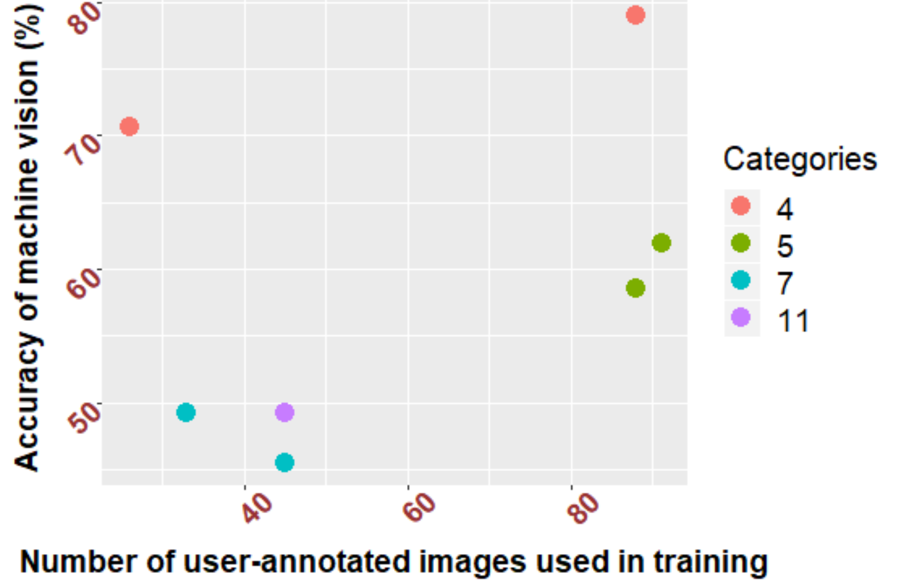


Regeneration GWAS progress and status

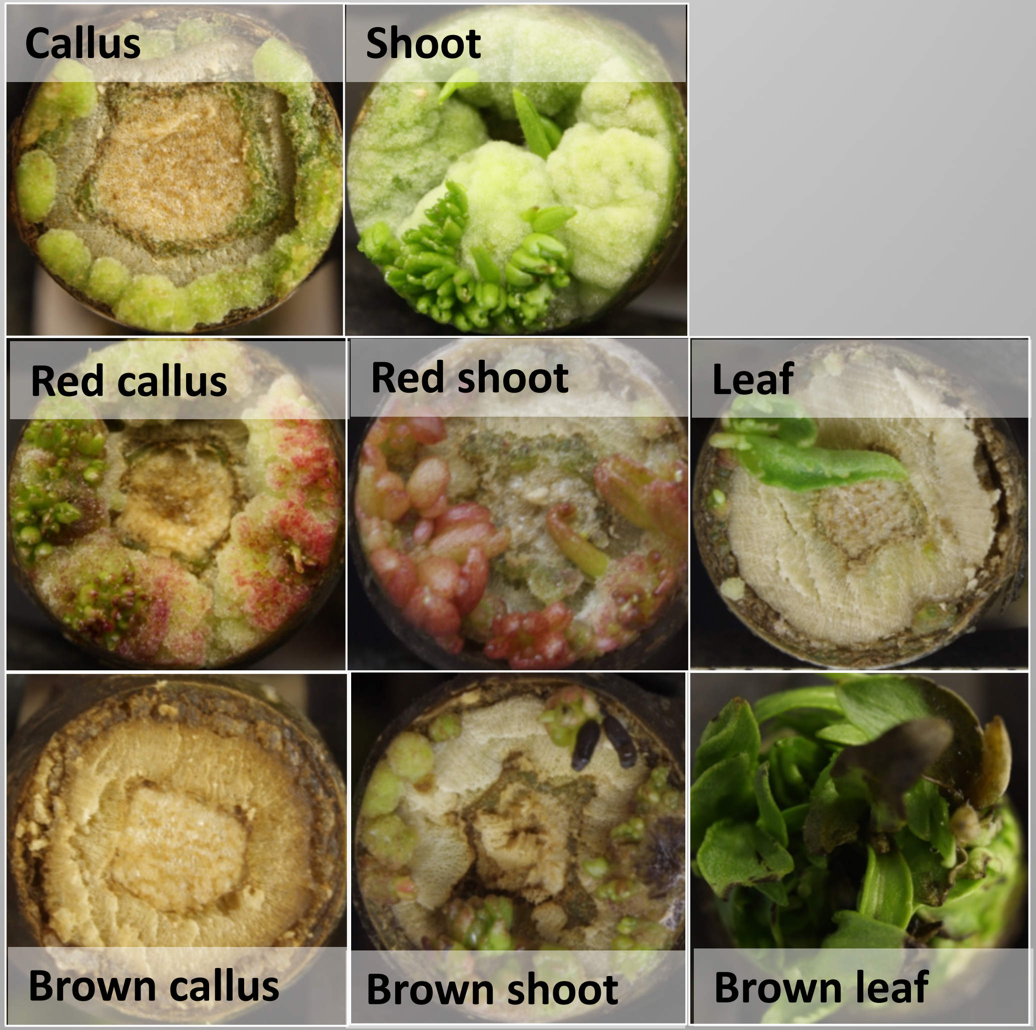
- Stem regeneration:
  - 590/902 genotypes have been phenotyped, included in test GWAS to refine methods
  - Preliminary results for callus, shoot and color of each
  - Machine vision training continues, accuracy still improving
- *In vitro* GWAS:
  - Pilot study ongoing, main phase of phenotyping to be completed in 2020
  - Starting use of hyperspectral imaging

Comparing options to segment images by tissue and color

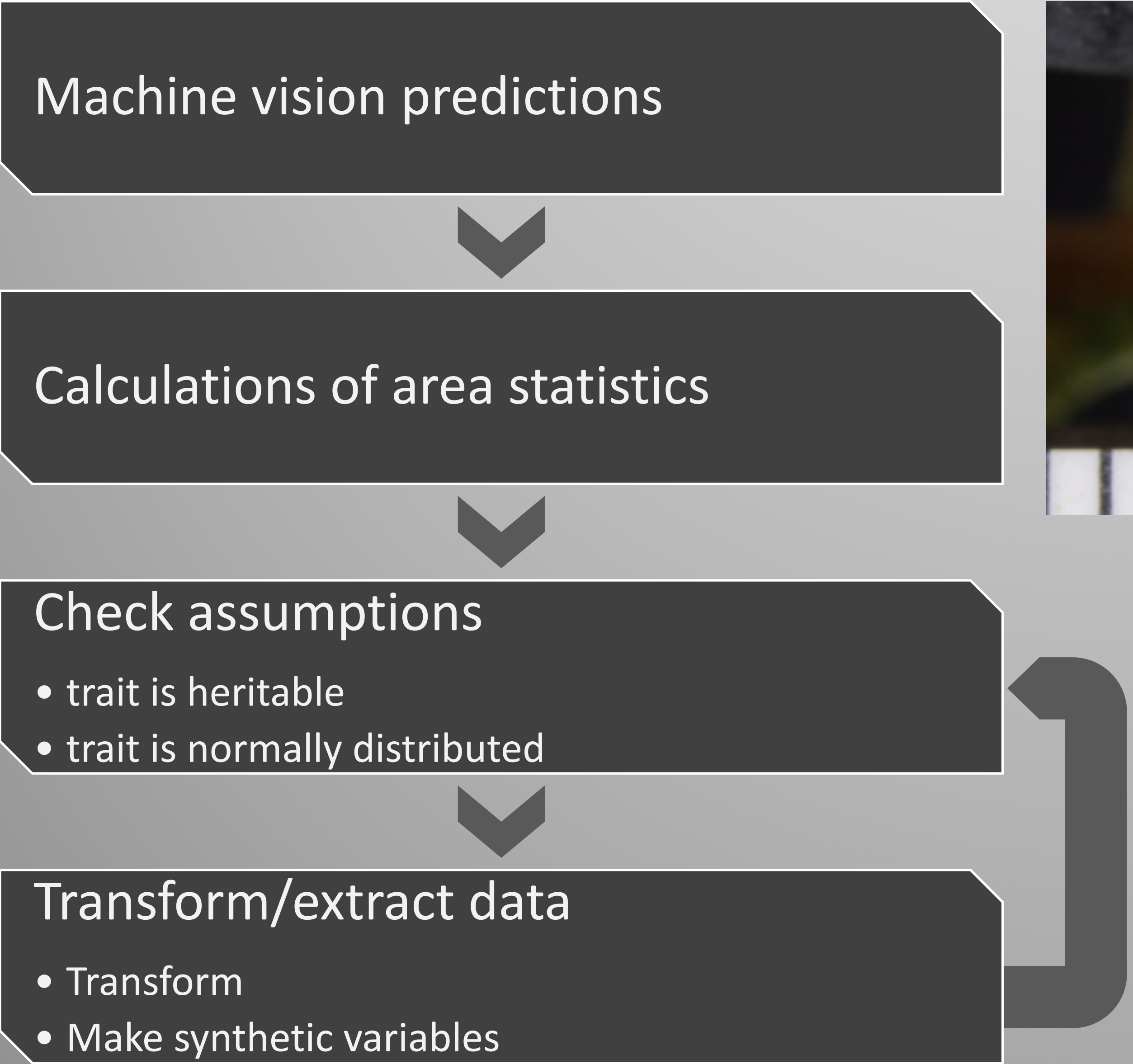
- Separate classes for tissues of each color: reduced accuracy in predicting tissue type



- Now using second neural network to classify callus, shoot *color* based on user examples



Phenotype data from machine vision outputs is processed and fed into GWAS pipeline



Machine vision predictions

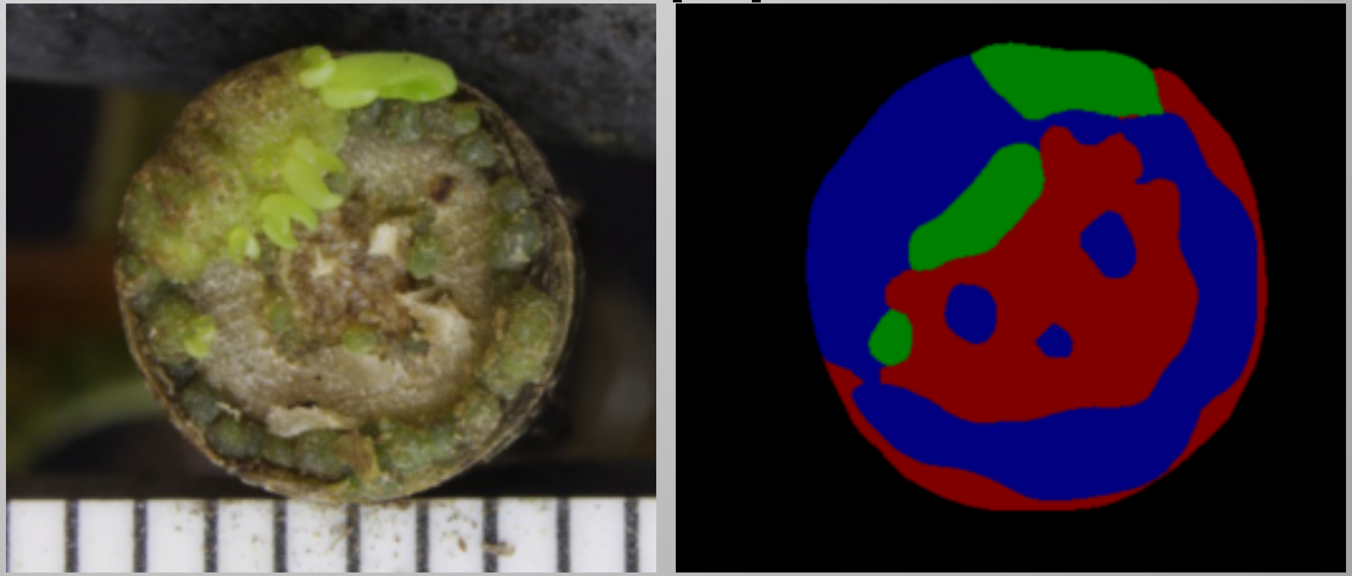
Calculations of area statistics

Check assumptions

- trait is heritable
- trait is normally distributed

Transform/extract data

- Transform
- Make synthetic variables



Tissue class	Percent of total area
stem	45%
callus	43%
shoot	12%

Genome-wide association studies to identify genes behind phenotypes

- Standard linear mixed model tools (i.e. Genome-wide Efficient Mixed Model Association) test one SNP at a time
- SNP-seq sequence Kernal Association Test (SKAT) combines SNPs within windows, tests for combined effect

ARTICLE

2011

Rare-Variant Association Testing for Sequencing Data with the Sequence Kernel Association Test

Michael C. Wu,[1,5] Seunggeun Lee,[2,5] Tianxi Cai,[2] Yun Li,[1,3] Michael Boehnke,[4] and Xihong Lin[2,*]

ARTICLE

2013

Sequence Kernel Association Tests for the Combined Effect of Rare and Common Variants

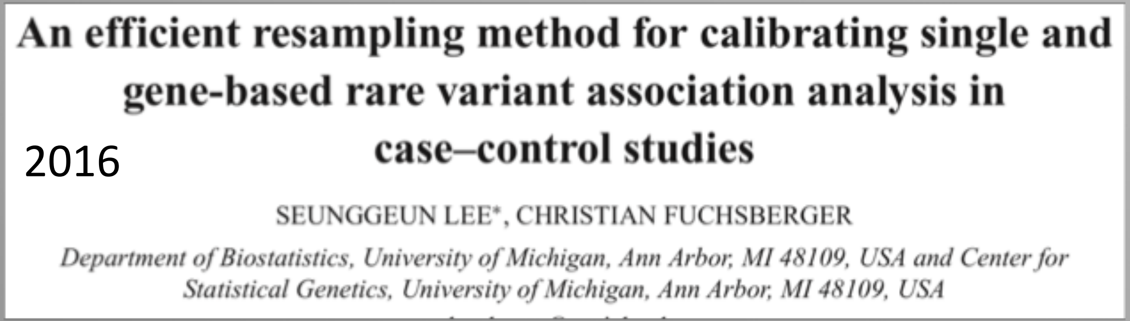
Iuliana Ionita-Laza,[1,6,*] Seunggeun Lee,[2,6] Vlad Makarov,[1] Joseph D. Buxbaum,[3,4,5] and Xihong Lin[2,*]

Multi-SKAT: General framework to test multiple phenotype associations of rare variants

Diptavo Dutta[1,2], Laura Scott[1,2], Michael Boehnke[1,2], and Seunggeun Lee [*1,2]

[1]Department of Biostatistics
[2]Center for Statistical Genetics
University of Michigan
Ann Arbor, Michigan, USA

2018



An efficient resampling method for calibrating single and gene-based rare variant association analysis in case–control studies

2016

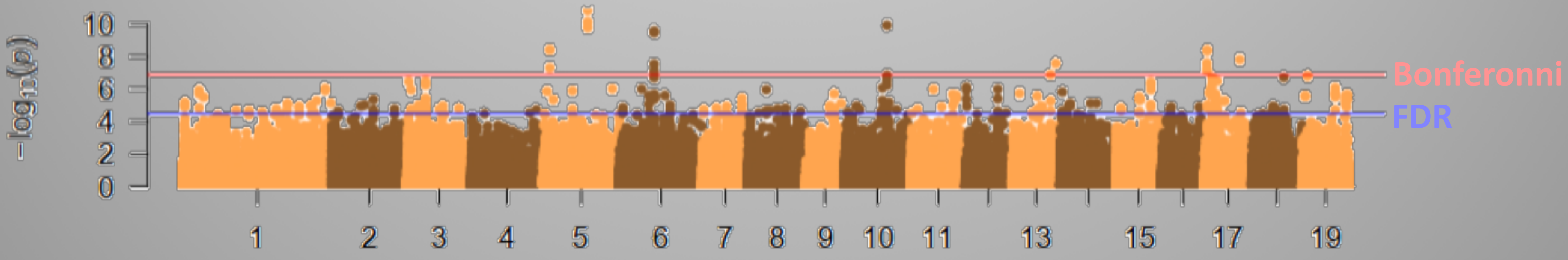
SEUNGGEUN LEE*, CHRISTIAN FUCHSBERGER

Department of Biostatistics, University of Michigan, Ann Arbor, MI 48109, USA and Center for Statistical Genetics, University of Michigan, Ann Arbor, MI 48109, USA

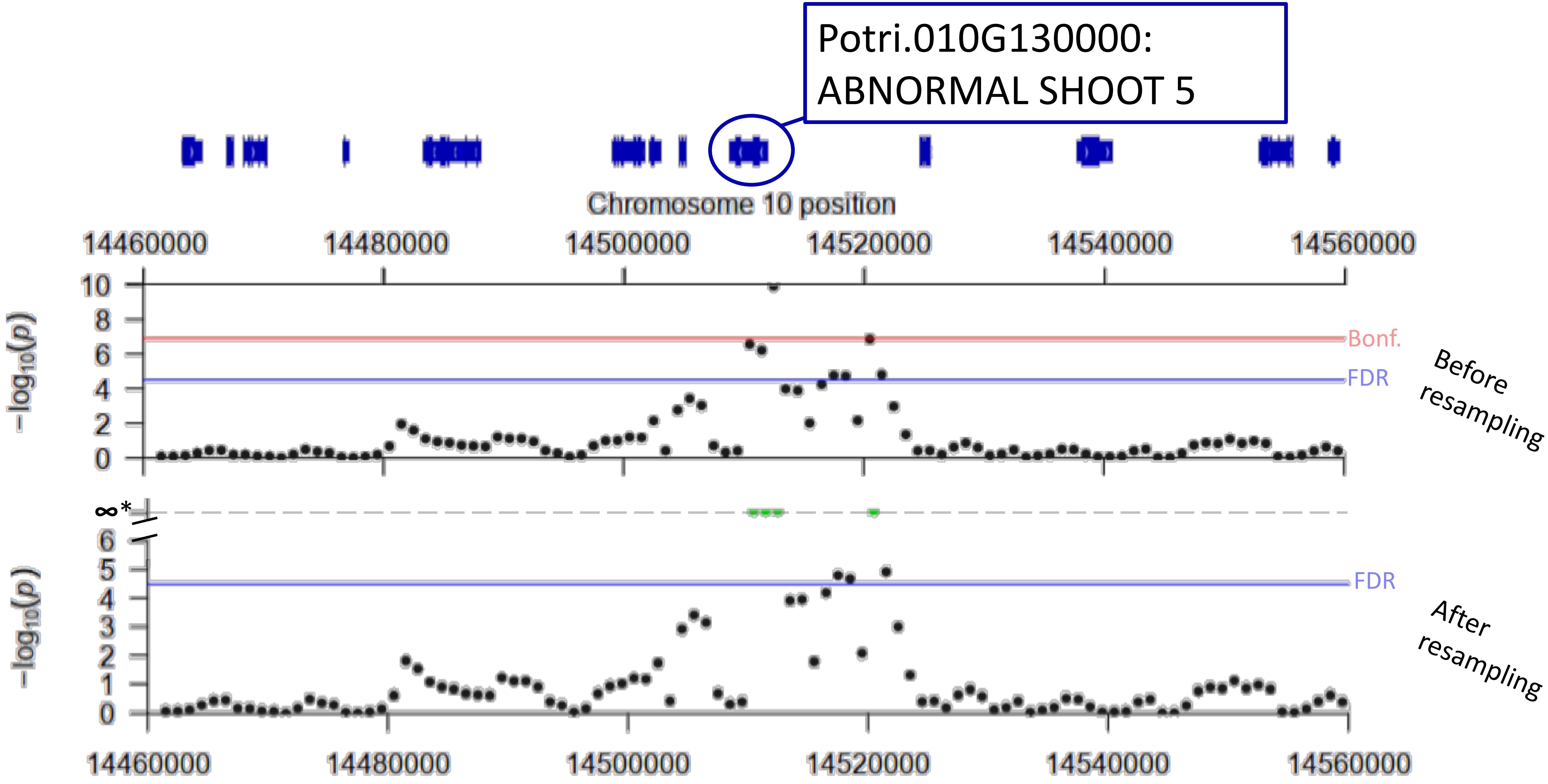
SKAT reveals several SNP windows putatively associated with shoot regeneration

Genetic markers associated with shoot area:

Genome-wide view



View zoomed to chromosome 10 subsection, aligned to gene track



Potri.010G130000:
ABNORMAL SHOOT 5

Before resampling

After resampling

Arabidopsis ABNORMAL SHOOT 5 role in shoot development

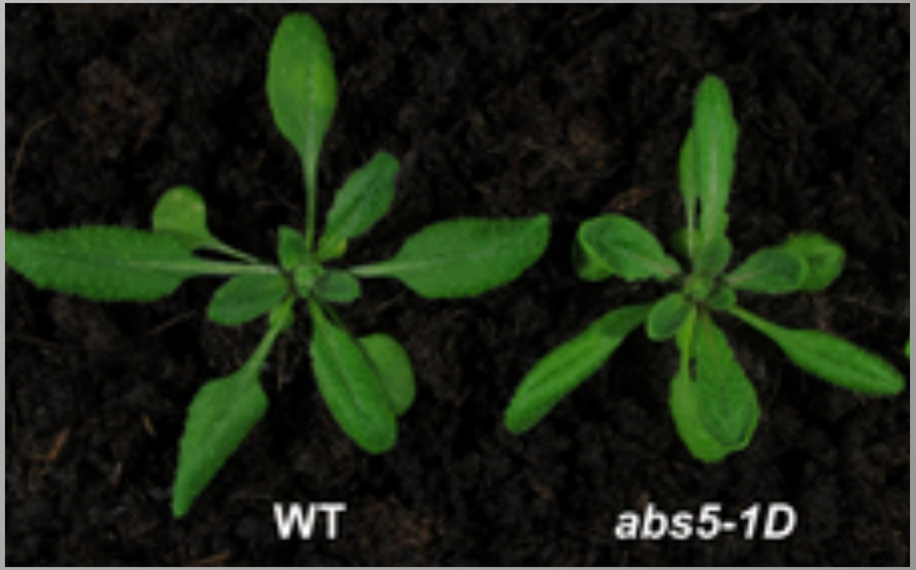
- Gene identified in activation-tagging screen, phenotype replicated by overexpression
- Transcription factor (basic helix-loop-helix)
- Effects of overexpression on vegetative and floral shoots:
    - Short length of epidermal cells on adaxial side of leaf, leading to upward curling of leaves
    - ~50% increase in secondary inflorescences


OPEN ACCESS Freely available online PLOS ONE

The Over-Expression of Two Transcription Factors, ABS5/bHLH30 and ABS7/MYB101, Leads to Upwardly Curly Leaves

Rui An[¶], Xiayan Liu[¶], Rui Wang, Haicui Wu, Shuang Liang, Jingxia Shao, Yafei Qi, Lijun An, Fei Yu[*]

State Key Laboratory of Crop Stress Biology in Arid Areas and College of Life Sciences, Northwest A&F University, Yangling, Shaanxi, People's Republic of China



Summary and prospects for next-generation phenomics in GWAS

- Machine vision and hyperspectral analysis offer unprecedented opportunity to capture high-quality phenotype data on large scale
- To incorporate unsupervised learning and semantic segmentation into machine vision
- Appearance of known shoot regulator as 2nd top hit in test phase of shoot regeneration GWAS suggests methods are effective
- Future GWAS using our system: Transformation ability, Response to several *in vitro* treatments, effects of developmental gene overexpression

Acknowledgements

- **Collaborators:**
  - *Machine vision:* Li Lab, Department of Electrical Engineering and Computer Science, Oregon State University
  - *Hyperspectral imaging:* Middleton Spectral Vision Inc.
  - *Genomics:* Center for Bioenergy Innovation at Oak Ridge National Laboratory (Key collaborators Wellington Muchero, Gerald Tuskan)
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  - Tree Biosafety and Genomics Research Cooperative, Oregon State University

Thank you!