

Analysis of Genes Affecting Plant Regeneration and Transformation

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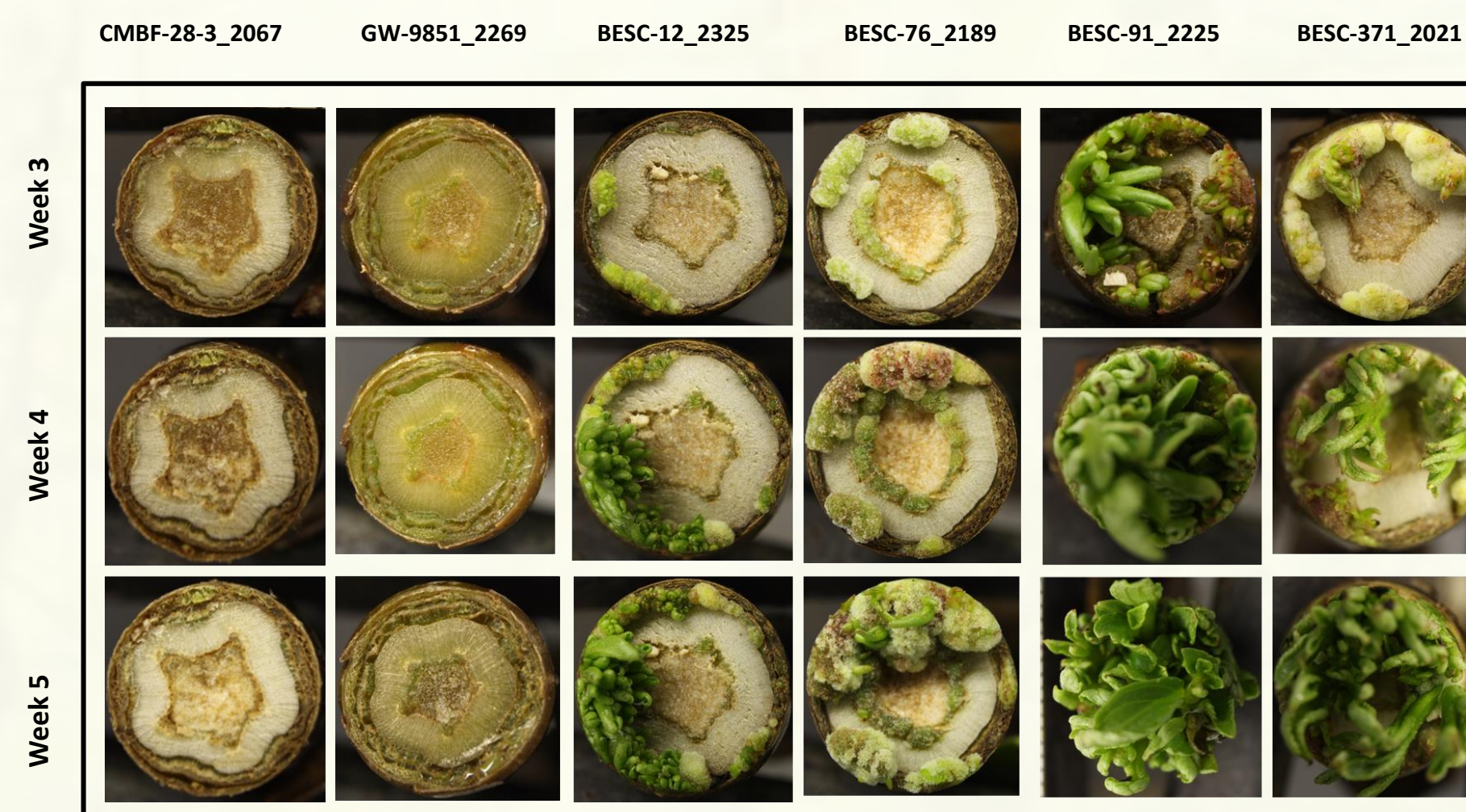


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Summary

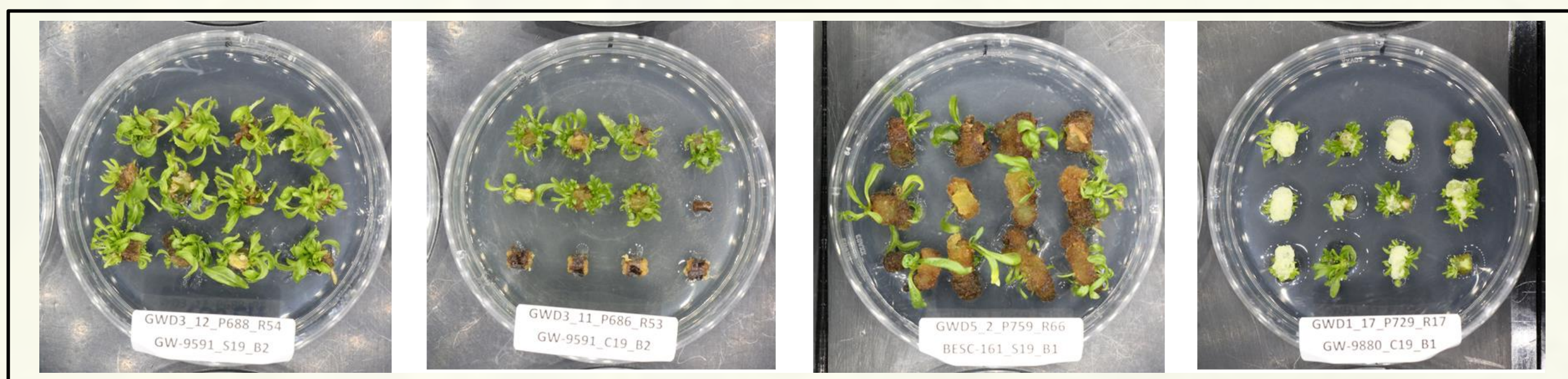
- The goal of this project is to develop high throughput phenomic systems for studying genetic control of regeneration and transformation in poplar using GWAS
- A secondary goal is to conduct social science research, and create new education materials informed by that research, related to understanding GMOs, agriculture, and scientific literacy in the online/social media world
- Key developments to date include: **1)** A customized imaging system for high throughput visible and hyperspectral images of *in vitro* plant materials (produced by Middleton Spectral Vision); **2)** In depth analysis of *in vitro* regeneration conditions suitable for producing highly heritable regeneration and transformation responses is nearly complete; **3)** A machine vision/annotation system for rapid phenotyping based on RGB images is nearly complete; **4)** Phenotyping of *in vivo* and *in vitro* regeneration responses is nearly complete for three GWAS experiments (~1,000 genotypes); **5)** GWAS of preliminary data on *in vivo* shoot regeneration (from machine vision predictions) suggests regeneration-control-genes can be identified.

Extensive natural variation in regeneration responses in wild cottonwoods

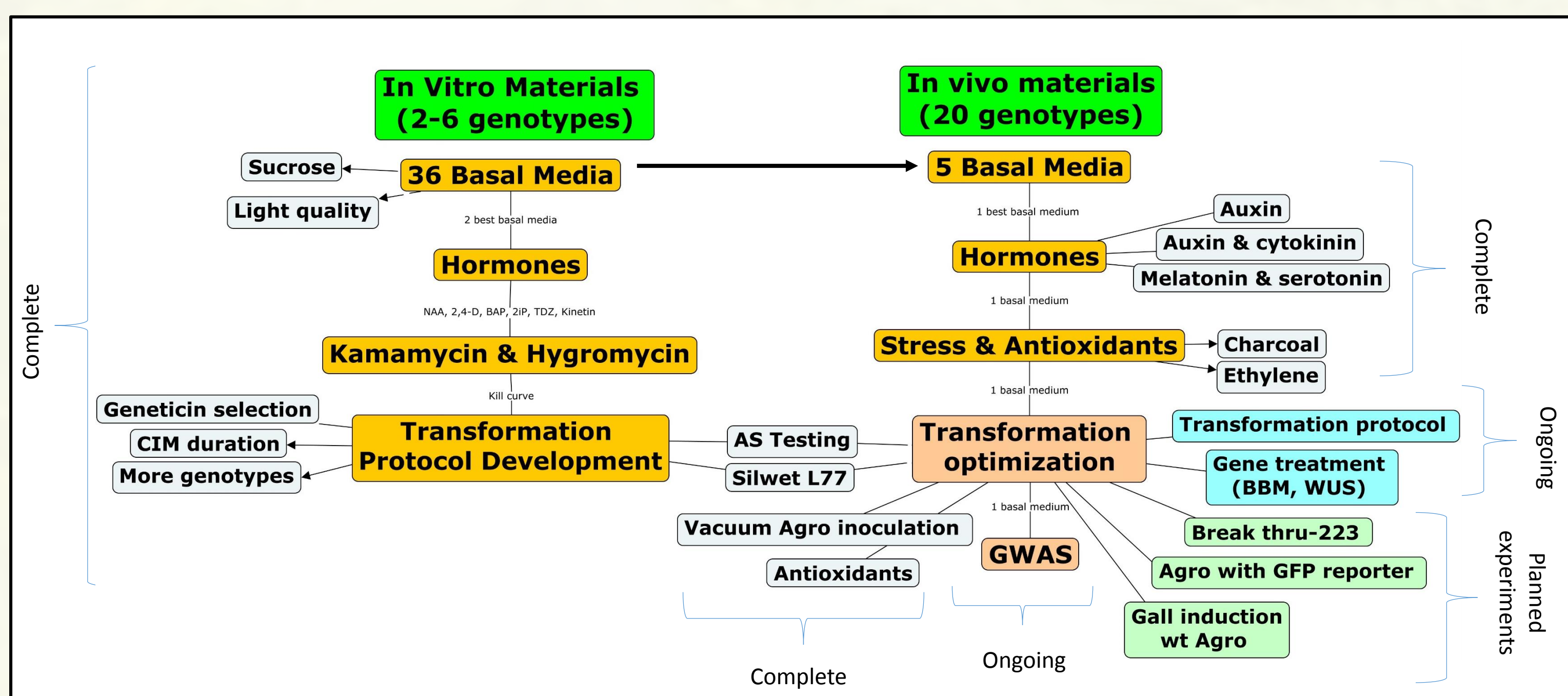


Our GWAS study relies on naturally high levels of SNP and trait polymorphism accompanied by low linkage disequilibrium in wild outcrossed trees. Shown to the left are six wild genotypes (columns) as they regenerate shoots *in vivo* from cut, dormant stem surfaces over a 5 week experiment (rows).

Shown below are four wild genotypes growing *in vitro* as they regenerate shoots after callus induction. Note the variation in callus size, color, and subsequent shoot size and number.

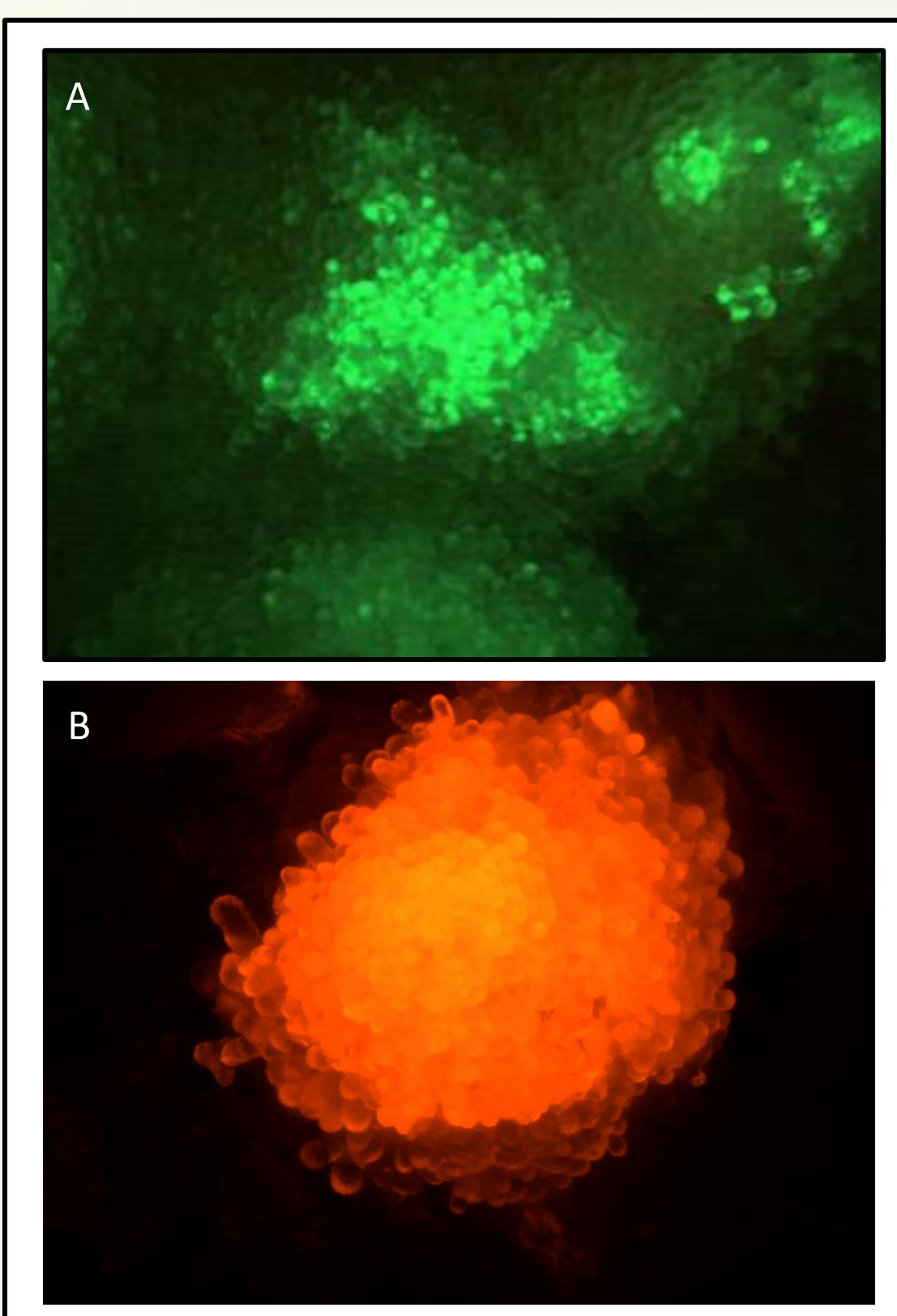


In Vitro Treatment and Heritability Optimization



To identify conditions that will maximize regeneration, and increase our power to detect causative genes via GWAS, we have explored 1,757 unique combinations of *in vitro* medium-hormone-explant treatments x genotypes over 12 distinct experiments. The figure above summarizes the treatments and explant sources studied or under study.

Variation in Phenotypic Responses



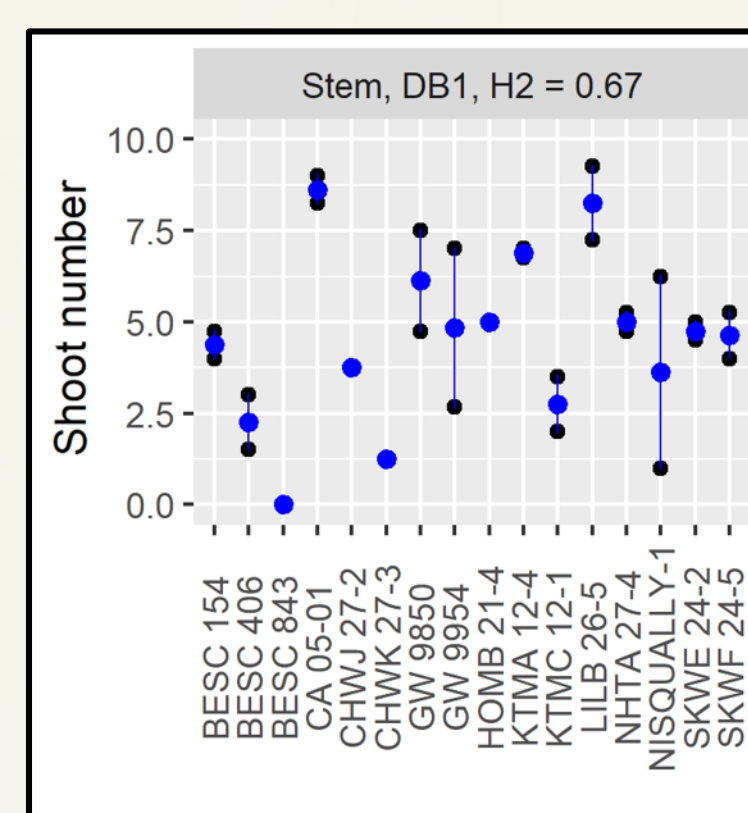
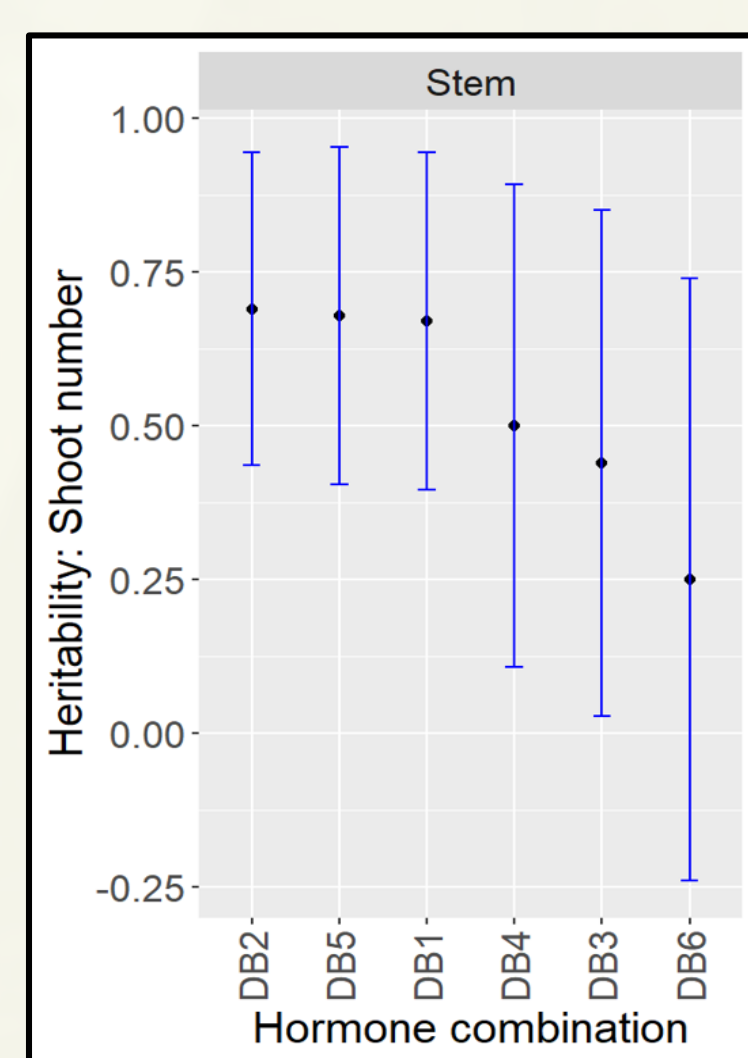
In vitro treatment optimization (above box) has produced a wide range of regeneration phenotypes useful for GWAS.

To the left we see fluorescent reporters are being used to monitor transformation and regeneration response, including unusual embryogenesis (B). The reporters can be readily quantified by hyperspectral image analysis using the macroPhor Array (see next box).

The use of multiple genotypes allows us to estimate heritability and identify the best treatments for maximizing genetic expression for GWAS. DB1 hormones gave high H^2 and much genotype variance.

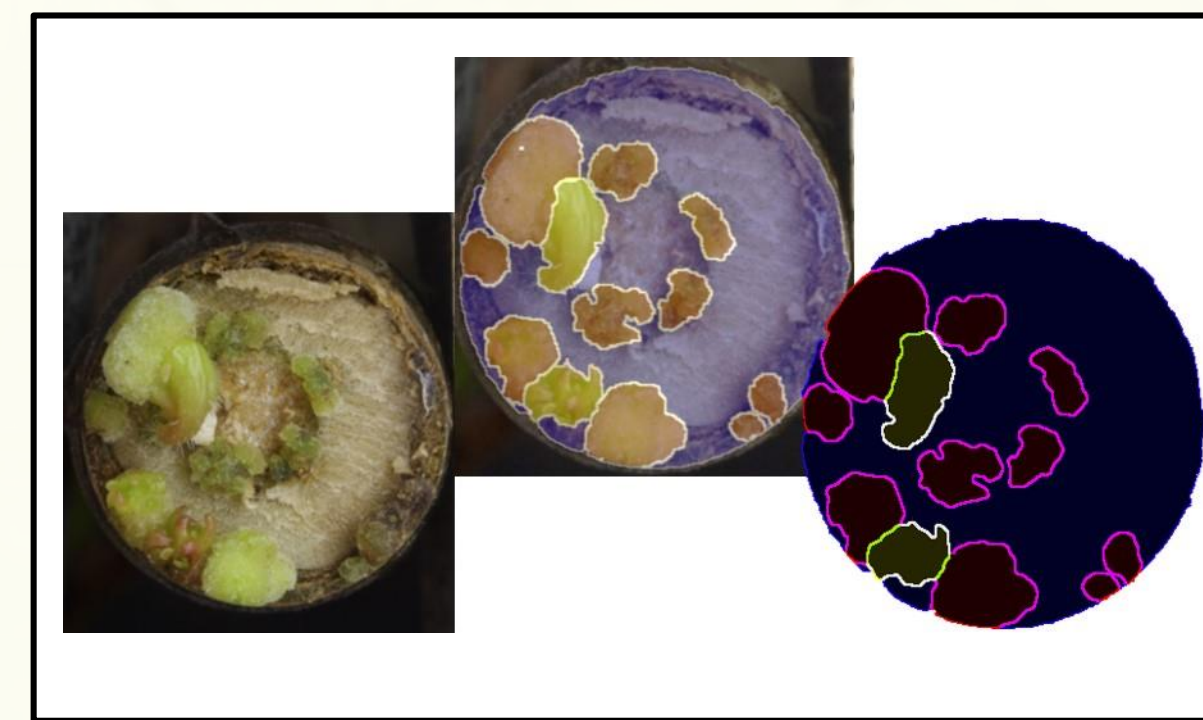
Treatment	DB1	DB2	DB3	DB4	DB5	DB6
2,4-D (mg/L)	0.01	0.05	0.1	0.01	0.05	0.1
BAP (mg/L)	0.5	0.5	0.5	1	1	1

Heritability of shoot regeneration and 95% confidence intervals

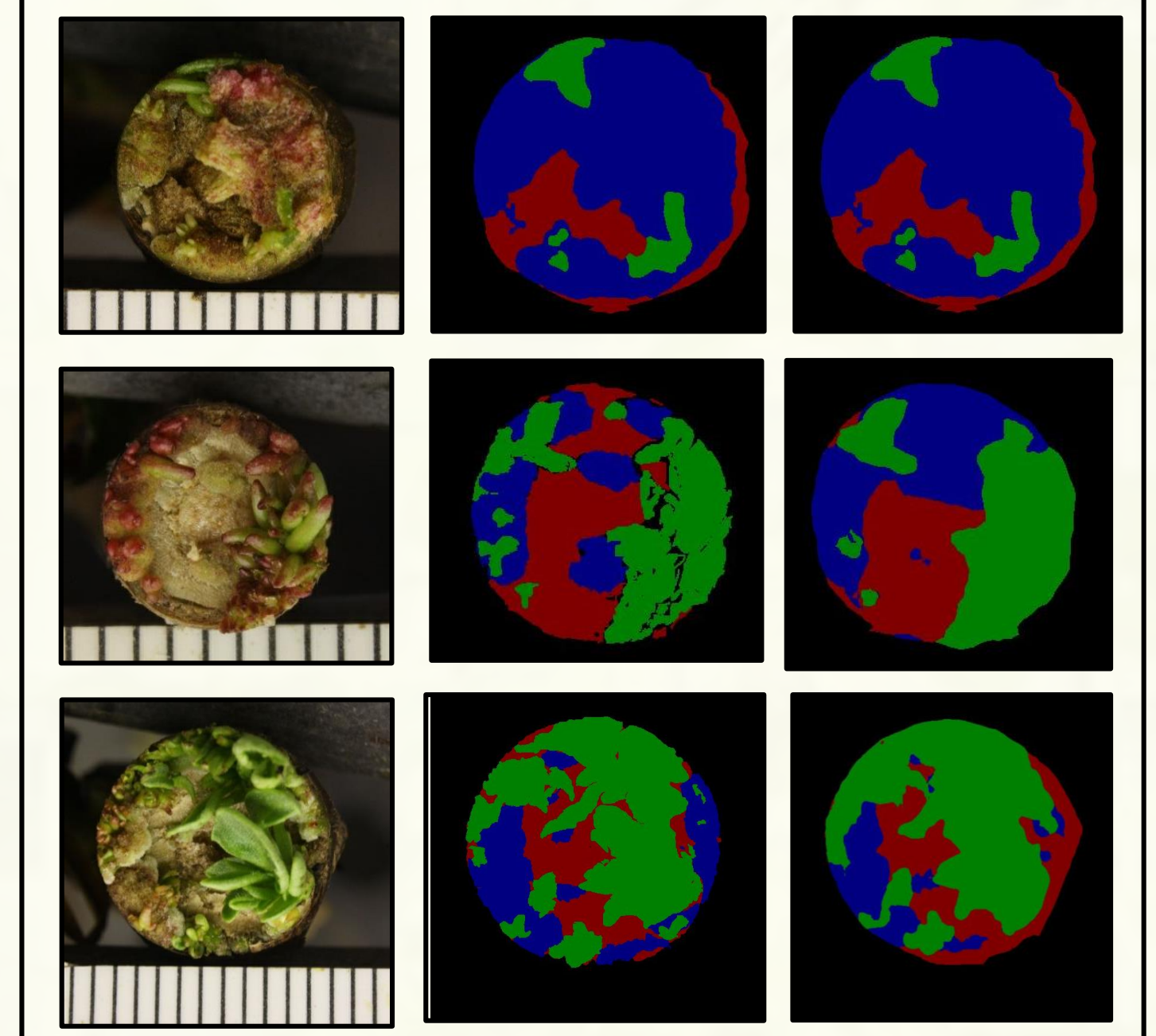


GFP (A) and Ds-Red (B) fluorescent markers indicate the presence of a transgenic shoot meristems or somatic embryos in response to hormone or DEV gene treatments such as *WUSCHEL*.

Machine Vision, Genotype Prediction & Hyperspectral Phenotyping



macroPhor Array™
Custom instrument for high-throughput hyperspectral & RGB imaging



	Background	Stem	Callus	Shoot	Mean IoU
Training	98.71%	87.72%	88.11%	84.48%	89.86%
Validation	98.85%	72.05%	77.60%	77.27%	81.38%

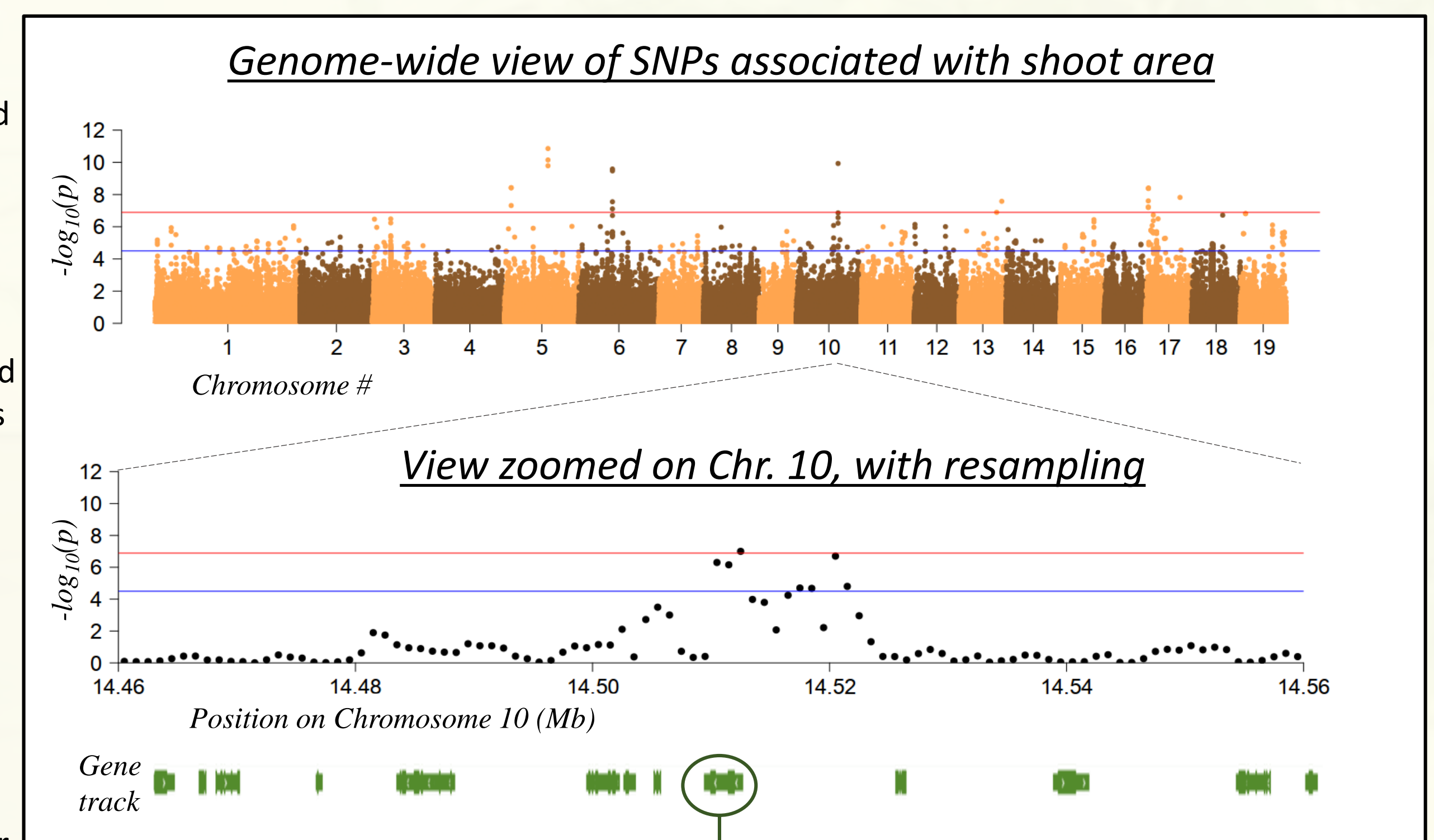
Table 1. Results of the Deeplab 3+ model after it has been trained via transfer learning on our hand-annotated ground truth dataset. The validation dataset percentages represent the accuracy of the current best model in annotating the differences between background, stem, callus, and shoot.

RGB Image **Ground Truth** **Prediction**

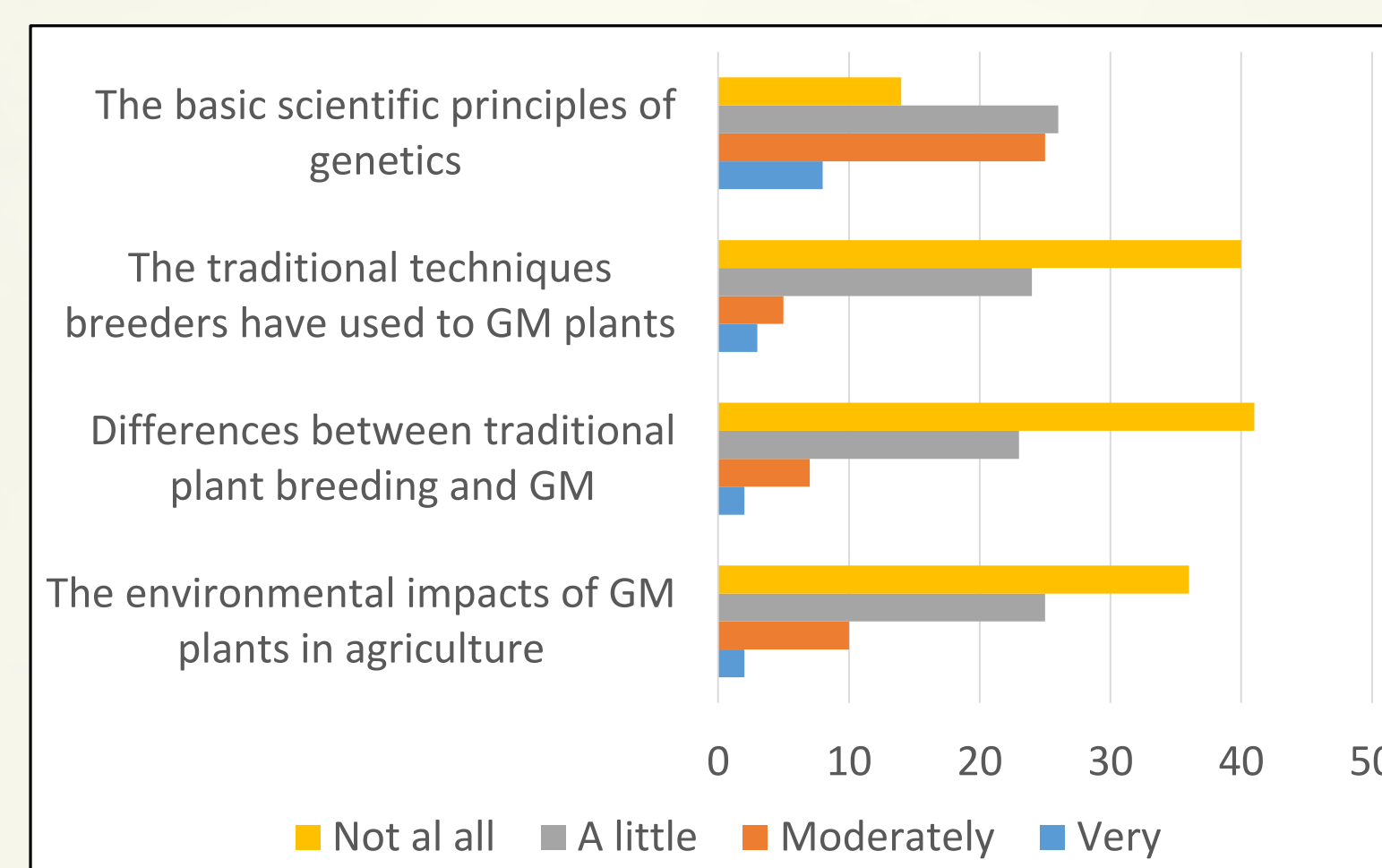
'Ground Truth' images above are the result of the hand annotation of 117 RGB images of explants denoting stem, callus, and shoot growth on an *in vivo* explant, as well as the surrounding background. These images were used to train the model. The images labeled 'Prediction' are a result our model utilizing semantic segmentation in Deeplab 3+. Prediction accuracy (mean IoU) is currently 81.4%

Preliminary GWAS mapping of in vivo shoot regeneration

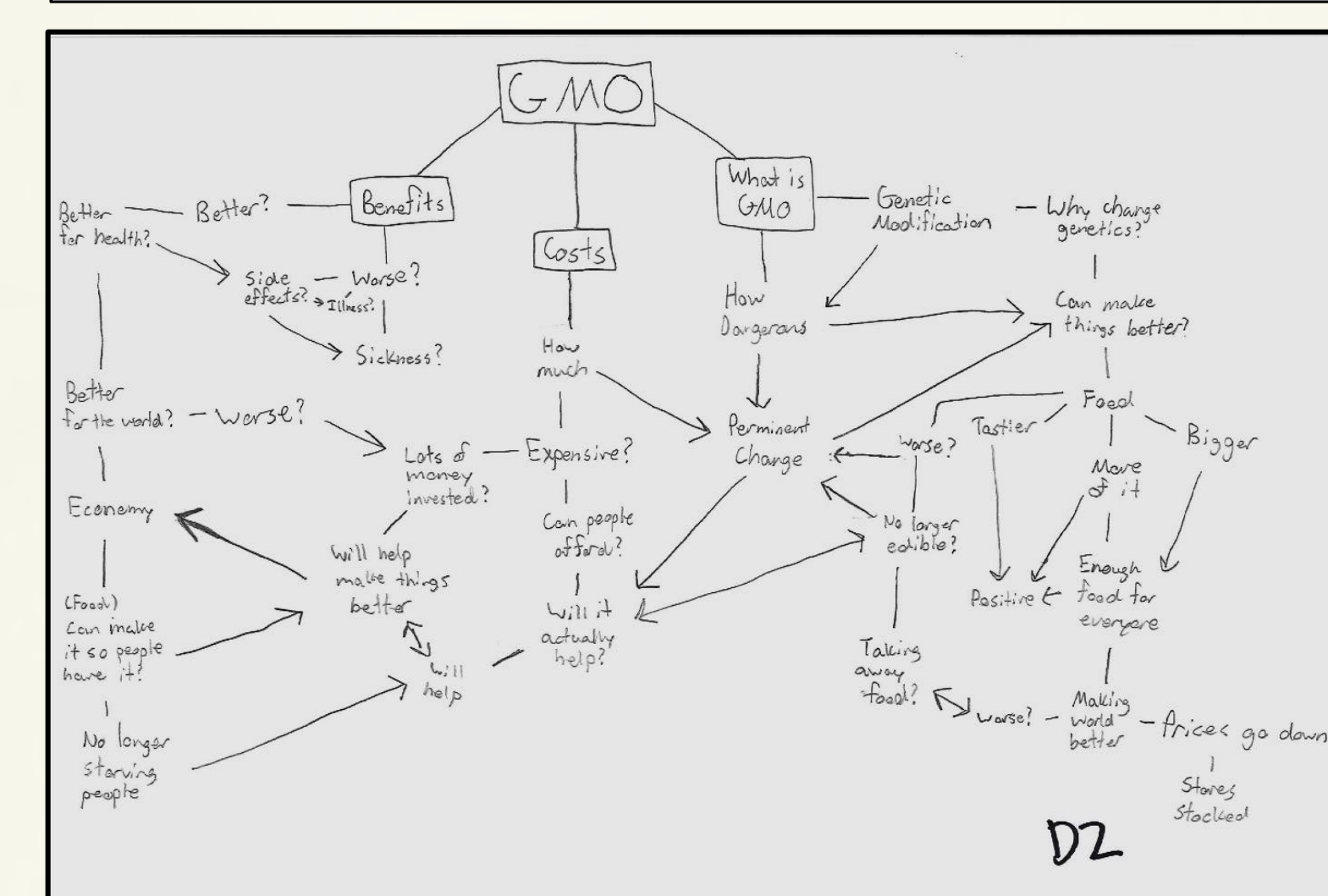
SNP-set Sequence Kernel Association Test (SKAT) was used to identify 3kb windows of SNPs (indicated by dots) associated with the proportion of each plant's area classified by machine vision as shoot. Resampling (up to 10M permutations) corrected for non-normality of the trait. A top association appears in the promoter and 5'UTR of a homolog of a known regulator of shoot development, *AB55*.



Broader Impacts through SMILE



We are working with the SMILE's state wide network of middle and high school programs to study and educate underrepresented students and teachers around the topic of agricultural genetic modification (GM). Survey results (left) of 73 students show that they rated their knowledge about topics related to agricultural GM as mostly low. Other than understanding the basic principles of genetics, they expressed little confidence in their literacy about GM.



To understand how students think about genetic modification and GM foods, 103 students were asked to create their own concept map to display their knowledge about these topics. One of the most complex maps is shown above.



As part of this project, we have developed 8 lessons about the science behind genetic modification and the various perspectives on the technology. These have been used in classrooms across Oregon and can be accessed at smile.oregonstate.edu/view-lessons.

Acknowledgements

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