Extensive Natural Variation in Callus and Shoot Regeneration in relation to Agrobacterium-Mediated Transformation of Wild Black Cottonwood (Populus trichocarpa)

Cathleen Ma and Steven H. Strauss, Oregon State University, Department of Forest Ecosystems and Society, Corvallis OR 97331

Caiping.Ma@OregonState.Edu  Steve.Strauss@OregonState.Edu

Abstract
The capacity for plant regeneration and transformation (RT) is notoriously variable among species and genotypes of plants. In many cases, transformation is impossible or impractical. The reasons for this extraordinary biological variation, however, are largely unknown. As part of a major project to use GWAS (genome-wide association studies) to map genes controlling RT in poplar, we are studying variation in RT among resequenced wild genotypes of black cottonwood—for which low levels of linkage disequilibrium facilitates GWAS-based gene identification.

We tested both direct and indirect regeneration pathways using two different types of explants, petioles and leaves, from 20 genotypes of greenhouse-grown plants based on our previously published protocol (1). We found that indirect regeneration, where callus proliferation preceded shoot induction, strongly promoted shoot regeneration, but that the effect varied widely between petiole and leaf explants.

We also studied various influent factors on transient and stable transformation on 3-5 selected genotypes of in vitro grown plants, using both leaf and stem explants. We discovered pre-culture for one day on callus induction medium (CIM) greatly increased both transient and stable GFP expression. Auxin-rich media and acetosyringone (AS) in CIM during co-cultivation enhanced GFP expression, both during transient and stable transformation phases and in both leaf and stem explants, of all tested genotypes. Further analysis and recovery of transgenic shoots is under study.

Project overview
To explore the diversity of in vitro RT responses in P. trichocarpa to inform GWAS analysis we will:
- Expose a diversity of RT methods to maximize in vitro trait heritability (see right panel for partial list)
- Develop new phenomic tools—generalized machine-vision methods—to rapidly and precisely determine in vitro phenotypes (in progress)
- Using GWAS, precisely map alleles associated with variation in RT frequency

Methods - Shoot regeneration
- Cuttings of genotypes cloned from wild populations from throughout the Pacific Northwest were grown in a greenhouse
- Leaf discs and petiole segments were used
- Two regeneration systems were tested: indirect vs. direct
- 8-15 explant per plate; 3 plates per genotype
- Explants were cultured on callus induction medium (CIM) for 20d in dark (MS supplemented with 2µM 2iP and 10µM NAA)
- Explants were transferred onto shoot induction medium 1 (SIM1) for 20d under light (MS supplemented with 1µM TDZ)
- Then explants were subcultured on SIM2 (MS supplemented with 0.1µM TDZ)

Methods - Transformation
- Three to five genotypes were randomly selected
- Plants were grown in WPM hormone-free medium (example of source plants shown to right)
- Leaf and stem (including petiole) explants
- GMubi1500:eGFP (kan selection) was transformed
- Two to four plates per genotype and 20-30 explants per plate
- Transient and stable GFP expression checked under GFP microscope (3d and 20d on CIM containing 75µg/mL kanamycin
- Four CIMs tested:
  1. CIM1 (WPM + 5.4µM NAA + 22µM BAP)
  2. CIM2 (MS + 2µM NAA + 10µM 2iP)
  3. CIM3 (MS + 2µM NAA + 10µM 2iP + 0.45µM 2,4-D)
  4. CIM4 (MS + 2µM NAA + 10µM 2iP + 0.45µM 2,4-D)
- Pre-culture vs. no pre-culture with CIM
- Acetosyringone (AS) vs. no AS in cultivation medium

Results
Leaf but not petiole explants frequently produced roots on CIM
The incidence of root formation from leaves varied widely among genotypes

For more detailed results and analysis, please refer to the original research papers and publications cited in the references section.

References

Acknowledgements
This project is supported by NSF (UCRC Center for Advanced Forestry (NSF 15-548)) and the TBRG industrial cooperative at Oregon State University. We thank the Biological Energy Sciences Consortium at Oak Ridge National Laboratory for use of their poplar collections.