Excisable gene editing systems:
Generation of dwarf and sterile poplars using a developmental and chemical-controlled CRISPR/recombinase excision system

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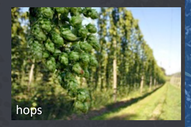
PB21 PLANT BIOLOGY

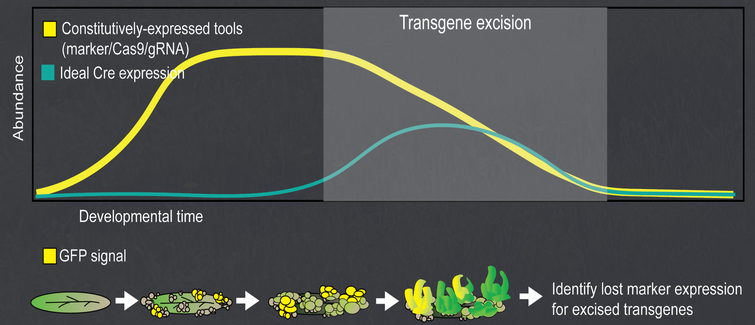
Editing machinery is difficult to remove from trees and clonally propagated plants

- Most CRISPR/Cas9 editing systems in plants remove transgenes through sexual segregation
- This method does not work for clonally propagated or highly heterozygous plants, or in induced or naturally sterile plants, including many forest trees
- Transformation aids like “DEV” genes that help recalcitrant species also need to be removed to recover usable events
- Excision of editing transgenes after insertion through Cre/lox is an attractive method around these roadblocks
- May be categorically accepted under new USDA SECURE regulatory system



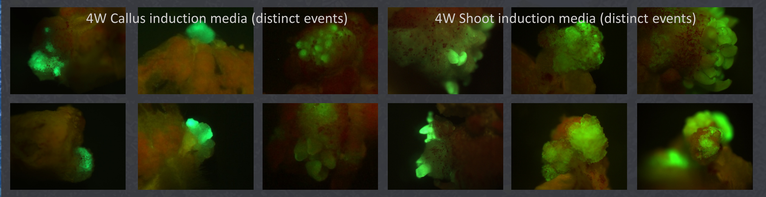
Initial goal: Developmental excision during regeneration using meristem-specific promoters

- Common Cre / FLP recombinase induction systems use stress stimuli like heat shock, which can harm plant health and impede regeneration
- Developmental triggers would be an alternative method to express Cre, when developmental shifts occur as plants are regenerated into shoots
- Ideally, we could then quickly recover edited events with transgenes removed

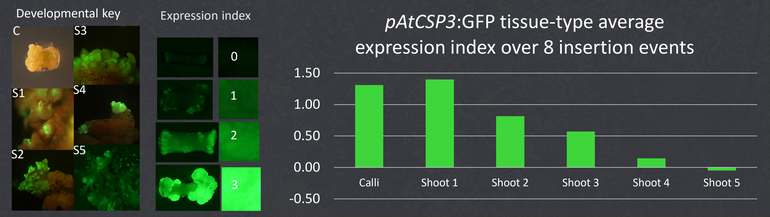


Meristem-dominant promoters tested through promoter:GFP fusions – all strongly callus active

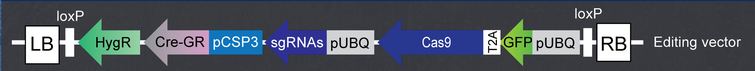
AtCSP3 promoter, 1.3kb fragment, drives strong GFP expression in meristems and callus



Scoring of tissue specificity & expression level showed high callus expression in all events

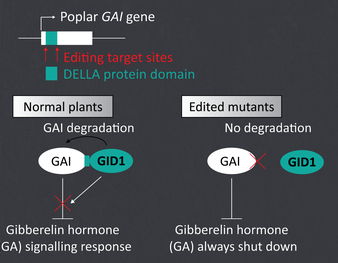


Final vectors included GR/dex induction, tested as in gene edited, dwarf / sterile tree prototype



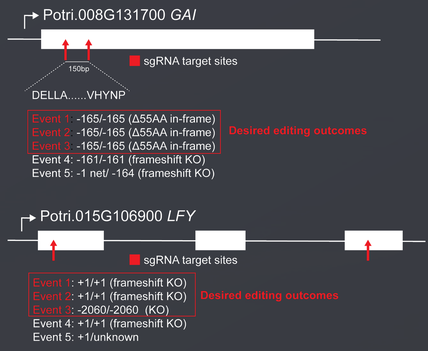
Prior *35S:ΔGAI/RGL1* transgenic poplar field trial showing range of dwarf phenotypes possible

Editing strategy concept / mechanism

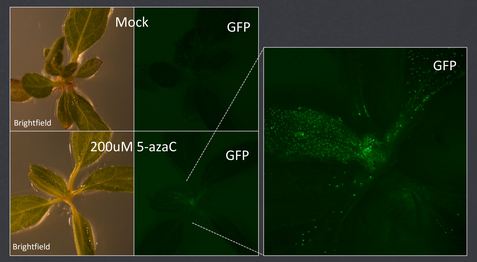


Dwarf / sterile gene-edited poplars were produced, but with low rate of transgene excision, very strong dwarf phenotypes

- Explants treated with DEX during shoot regeneration (3WCIM/3WSIM + 1mo SIM + DEX (20uM)) and removed from selection during dex treatment
- High escape rate: 13/87 (15%) of shoots transgenic
- 5 events had a *gai* deletion detected by PCR
- 3 in-frame deletions had a severe dwarf phenotype



Reawakening of GFP expression with 5-azacytodine suggests that DNA methylation may explain low excision rate



- A recent study showed strong DNA methylation in recombinase target region inhibits excision, but can be relieved by demethylase treatments (Liu et al., New Phytologist 2021)
- In a test event, 8/8 propagated shoots showed a recovery of GFP expression with 5-azacytidine
- Currently studying effects of demethylation chemicals and demethylase induction on excision rates, resolution of transgenes from complex insertions, and plant health and genetic integrity

Conclusions

- A meristem-enriched promoter + GR/Dex system was functional in triggering excision of CRISPR-Cas9 editing transgenes in poplar, but at a low frequency
- CRISPR editing using multiplexed gRNAs was successful at inducing dwarfism and obtaining loss-of-function edits in the key flowering gene *LEAFY*
- Impairment of methylation appears to improve GFP expression and likely excision; this and other improvements to excision efficiency are under study
- Similar approaches could be used for other plant species for control of plant form and elimination of pollen, seeds, or fruits

Acknowledgements

We thank the JF Schmidt Family Foundation, the USDA Biotechnology Research Assessment Grant program (2011-68005-30407 and 2010-33522-21736), the National Science Foundation Plant Genome Research Program (IOS 1546900),and the GREAT TREES industry cooperative based at Oregon State University.

We also thank Corteva Agriscience for technical expertise related to excision vectors and Agrobacterium strains that were used in this study.

USDA NSF J. Frank Schmidt Family Charitable Foundation