Low rates of non-target mutations in field- and greenhouse-grown CRISPR/Cas9 expressing transgenic trees

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Reproductive containment in forest biotechnology

GE trees with advantageous traits might thrive habitat with native trees with which they can interbreed, or encroach on native forests as exotic. Many trees are wind pollinated or have wide dispersal, which produce flowers unable to produce functional male or female organs. We have used CRISPR/Cas9 to produce edited poplars and eucalypts with the goal of complete sterility. By targeting the floral initiation gene LEAFY (LFY), no floral organs should initiate. Other edited trees in this study include those with knock-out mutations in AGAMOUS genes, which should produce flowers unable to produce functional male or female organs.

CRISPR/Cas9: a highly specific editor in plants - but what about when expressed for many years?

Cas9 has a proven record in many plant species for high activity, high specificity, and low rates of non-target mutations. Most studies involved in vitro plant transformation and tissue culture, not due to prolonged expression of Cas9 - future studies will examine the relationship of Cas9/gRNA expression to the rate of non-target editing. Though non-target mutations were frequently found downstream of PAM site in mutagenic potential - still, at least in eucalypts (Elorriaga et al. 2021, Wang et al., 2021, 2017-33522-27098, for financial support).

Methods:

- 353 and 717 hybrid poplars were transformed with Cas9 only, and combinations of one or two gRNAs containing constructs targeting two AGAMOUS homologs or the single copy LEAFY gene. Two ramets were sampled from multiple events containing varied editing outcomes (homozygous/methylation-edited, heterozygous edited, non-edited but transgenic).
- 26 events in poplar were surveyed which contained gRNA-expression cassettes.

Non-target CRISPR mutations found in all species tested - but with only some gRNAs

Non-target CRISPR mutations identified tended to be shared between events or shared within all transgenic events, showing a common donor lineage. Non-targets showed event specific mutations related to target or non-target edit.

Results:

- Non-target mutation rate and editing rate were low over surveyed DNA sequences than was expected.
- We found more off-target mutations than Young et al., 2019. So, Rito, or Wang et al., 2021 Hirt Reses, in maize or grape, respectively.

Conclusions and next steps

Mutations due to CRISPR/Cas9 activity occur at target sites that differ substantially from sgRNA sequences (up to 5 bp). We found more off-target mutations than Young et al., 2019. So, Rito, or Wang et al., 2021 Hirt Reses, in maize or grape, respectively.

Better models of gRNA binding affinity will reduce chances of designing gRNAs with unintended mutagenic potential - still, at least in eucalypts (Elorriaga et al. 2021, Page Biotech A) (TBD in poplar), there were no previous impacts on growth in non-target mutated events even when KOs were found.

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We compared novel somatic mutations against non-target mutations within the bait windows surveyed.

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