


In planta transformation in *Populus* and *Eucalyptus*

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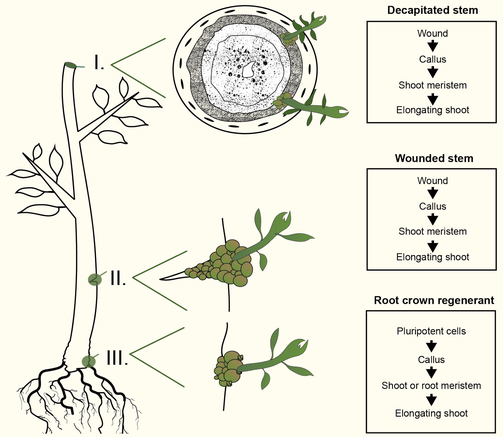
I. *in planta* transformation as an alternative to typical *in vitro* plant transformation

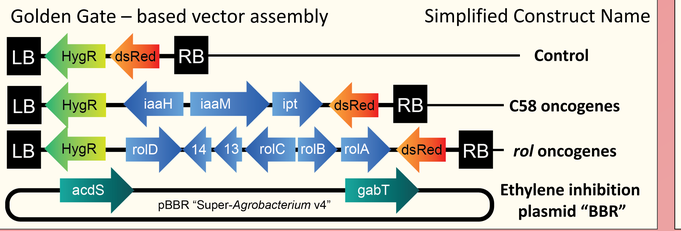
- We applied *Agrobacterium*-containing developmental regulator and fluorescent gene binary vectors on greenhouse grown trees
- We successfully obtained transgenic gall/callus tissue with constructs containing the *iaa/ipt* oncogenic genes from C58 Agro
- We were more successful with older plant decapitated stems compared with younger plants, especially in *Eucalyptus*
- Next steps will develop methods of removing developmental genes through excision and regenerating shoots from these tissues

II. Exploiting regenerative tissues types is important for *in planta* methods



This is coupled with different developmental regulator combinations, hormone treatments, selection treatments, and horticultural methods

III. Developmental regulators are included in the T-DNA

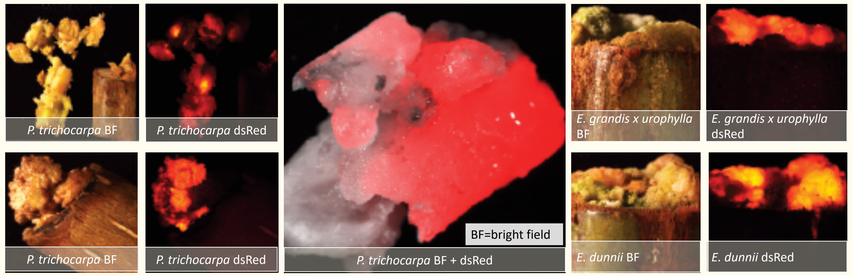


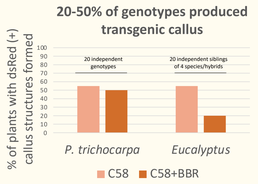
IV. Three populations of poplars and eucalypts used for *in planta* testing

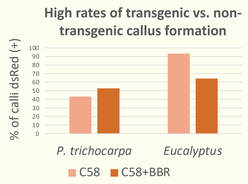
P. tremula x alba | *P. trichocarpa* | *E. grandis x urophylla*

Young plant trial with ~200, 3–5-month-old trees where multiple inoculation points / constructs / methods were attempted

V. C58 oncogenes produced transgenic callus tissue *in planta* on decapitated stems of 1-2 year-old cottonwoods and eucalypts







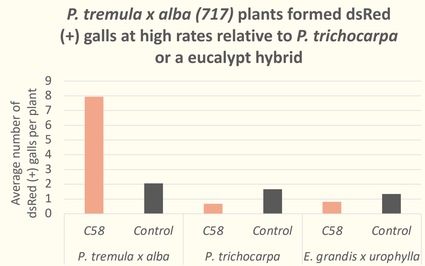
P. trichocarpa	# genotypes	# plants with callus at 10W	# plants with dsRed callus	% calli / construct	% dsRed calli
C58	20	11	9	55	45
C58 +BBR	20	10	8	50	40
Eucalyptus					
C58	20	11	7	55	35
C58 + BBR	20	4	4	20	20

- "Calli" were any circular growth over 2mm

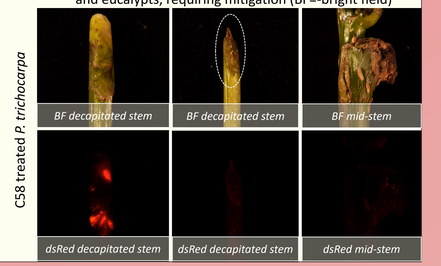
VI. C58 oncogenes produced transgenic callus on younger *P. tremula x alba* plants at multiple inoculation sites

- Plants were treated with control and C58 constructs on independent branches or sides of the root crown
- Easily identifiable galls formed at many sites

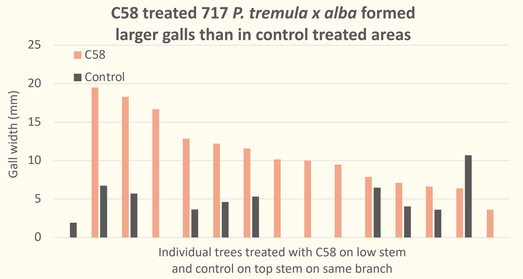
VII. C58 oncogenes also produced transgenic callus at high rates in hybrid poplars but low rates in young cottonwoods and eucalypts

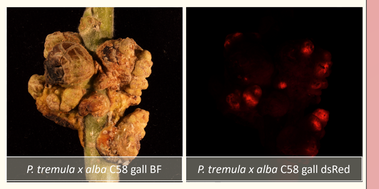


Necrosis was common for transformation in *P. trichocarpa* and eucalypts, requiring mitigation (BF=-bright field)



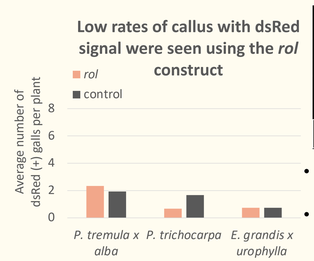
VIII. C58 oncogenes produced large gall tissues in *P. tremula x alba*

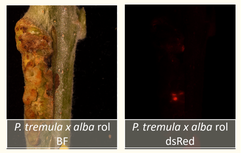




- Many galls had chimeric dsRed expression patterns, thus antibiotic selection will be studied in future work

IX. *rol* constructs were less effective at producing transgenic callus *in planta*





- No clear hairy root formation was seen near root crowns
- *rol* genes are inefficient for larger callus formation *in planta* – may require other regulators or *rol*-related genes

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- We thank Sappi and Futuragene for supplying *Eucalyptus* genotypes for testing