

Supplemental Table 2. Summary of major studies from our laboratory to optimize eucalypt transformation (2020-2024), and definition of associated acronyms.

Goal	Dates	Agrobacterium strains	Number of treatments	Genotypes tested	Brief or salient results	Supplemental figure
Agrobacterium strains	2022	LBA4404 and AGL1	LBA4404 thy and AGL1	Eg18-1, Eug21-1, Eug22-1	AGL1 was superior for all three genotypes	S1, S10
Basal medium	2021	AGL1	WPM, MMS	Eg18-1, Eug21-1, Eug22-1	WPM is beneficial in reducing explant browning and enhanced callus and transgenic callus formation	S2
Break thru (surfactant)	2020	AGL1	BT 0.02, 0.03, 0.04, and 0.05%	Eg18-1, Eug21-1, Eug22-1	0.03% BT seems to help in transformation for Eug22-1	S3
Agrobacterium concentration	2020	LBA4404 thy	OD0.2, 0.4, 0.6, and 0.8	Eg18-1, Eug21-1, Eug22-1	Agro OD 0.6 helped in transformation for Eug21-1	S4
Acetosyringone concentrations in Agrobacterium induction medium	2021	AGL1	AS 0, 25, 50, 75, and 100uM	Eg18-1, Eug21-1, Eug22-1	50 μ M AS seems to help in transformation of Eug22-1	S5, S6
Fipexide (FPX)	2021	AGL1	FPX 0, 15uM	Eg18-1, Eug21-1	FDX promoted large GFP callus formation, but did not help in transgenic shoot production	S7

Types of cytokinin	2022	AGL1	TDZ 0.176; Zeatin 2, 5; Meta-topolin T 2, 5, Brassinolide 0.01, and 0.03mg/L	Eug21-1, Eug22-1	Meta-topline at 5mg/L promoted 25% shoots regeneration for Eug21-1 in 4 months, but lower DS-Red callus (37.5%) formed than medium with TDZ (58.3%);	S8
Forchlorfenuron (CPPU)	2023	AGL1	CPPU 0, 0.12, 0.24, 0.36 mg/L	Eug21-1, Eug22-1, Egu45-1, Egu42-1	Less transgenic callus formed using CPPU, and shoot formation occurred more quickly, but all were not transgenic	S9
Types of sugar	2022	AGL1	glucose, maltose, fructose and sucrose (30g/L each)	Eg18-1, Eug21-1, Eug22-1	Maltose is beneficial for gene delivery but causes explant browning	S10
Spectinomycin kill curve	2022	LBA4404 thy	Spec 0, 25, 50, 100, and 150 mg/L	Eug21-1, Eug22-1	Over 70% explants showing transgenic cells at 100mg/L spec in both clones	S11
Resting vs. no resting	2023	AGL1	0d vs. 7d resting	Eug21-1	More transgenic callus formed in no resting treatment	S12
Preheating explants before transformation	2024	AGL1	Preheating (PH) vs. No	Eug21-1, Eug31-4	Preheating explants enhanced transgenic callus production	S13

Activated charcoal (AC) and vitamin C (VC)	2021	AGL1	Preheating (NPH) AC 0, 0.1, 0.5%; VC100 and 200 mg/L in CIM	Egu45-1, SP7 Eg18-1, Eug21-1, Eug22-1	AC and VC have no beneficial to reduce browning and promote transgenic callus formation	S14
Lipoic acid (LA)	2021	AGL1	LA 0, 5, 10, 50, and 10 uM	Eg18-1, Eug21-1, Eug22-1	LA has no effect on transgenic callus formation for Eug21-1 and Eug22-1, but LA at 5uM helped Eg18-1 in DS-Red callus formation	S15
Proline (PL)	2021	AGL1	PL 0, 1, 5, 10, 25mM	Eg18-1, Eug21-1	5 mM PL promotes transgenic callus production for Eug21-1, but not for Eg18-1; one transgenic shoot regenerated from Eug21-1 at 5mM LA	S16
Polyvinylpyrrolidone PVP10 and PVP40	2023	AGL1	WPCIM1 with PVP10 and WPCIM2 with PVP40	Eug21-1, Egu42-1, Eug22-1, Egu45-1, Ed17-3, Eug32-3, Eug31-4	8.3% and 5.9% transformation efficiency obtained in PVP10 from Egu45-1 and Eug21-1 genotypes, respectively	Fig 4, S17
Silver nitrate (SN)	2024	AGL1	SN0, SN0.1, SN1.0	Egu41-4, Eg2-2, Eug35,	8.2% transformation efficiency obtained in no	S18

				Eug26-1, Eug21-1, Egu45-1, Egu47-2	silver nitrate treatment from Egu45-1	
Genotype screening	2023	LBA4404 thy	SIM0 and SIM7	53 eucalypt genotypes including E. dunnii, E. grandis, and hybrid E.granids x E. urophylla and E. urophylla x E. grandis	Some genotypes give 40- 89% transgenic callus on medium without any hormone	S19

Acronyms

AC (activated charcoal)

Agro (Agrobacterium)

AS (Acetosyringone)

BT (Break-Through commercial surfactant)

CcFT3 (Citrus clementina FT3)

CPPU (Forchlorfenuron)

CIM (callus induction medium)

Eg (Eucalyptus grandis)

Egu (Eucalyptus grandis x urophylla)

Eug (Eucalyptus urophylla x grandis)

FPX (fipexide)

GFP (green fluorescent protein)

LA (lipoid acid)

MMS (modified Murashige-Skoog)

OD (optical density of cultures)

PVP (polyvinylpyrrolidone)

PVPP (Polyvinylpolypyrrolidone)

PL (proline)

SIM (shoot induction medium)

SN (Silver nitrate)

TDZ (thidiazuron)

thy (thymidine)

VC (vitamin C, ascorbic acid)

WPM (Woody Plant Medium)