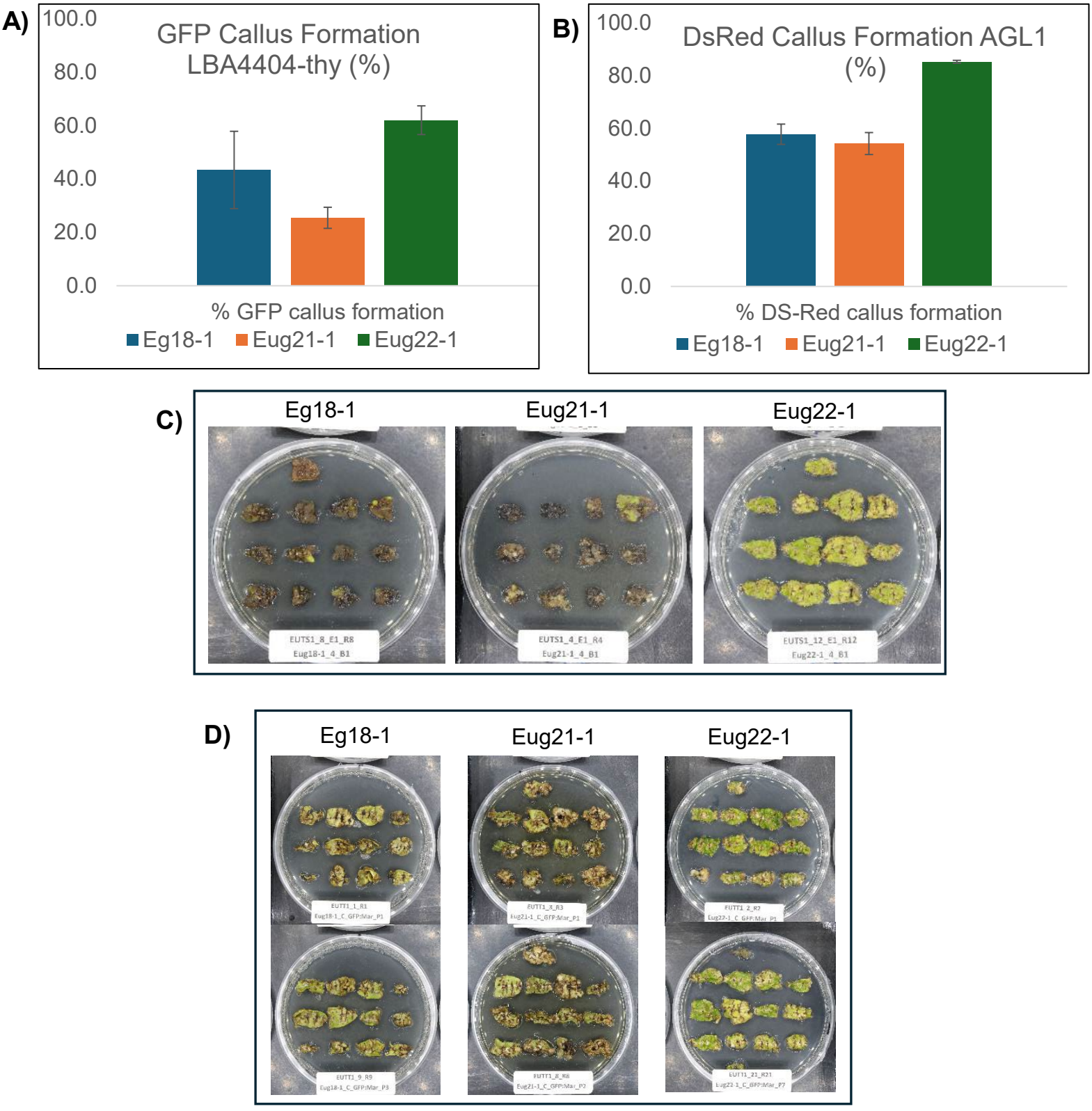


Figure S1. Effects of *Agrobacterium* LBA4404-thy in comparison to AGL1 on *Eucalyptus* genotypes during transformation. Three eucalyptus genotypes (Eg18-1, Eug21-1, and Eug22-1) were tested for transformation efficiency and browning using the Agrobacterium strain LBA4401-THY carrying 35S::eGFP.Mar and AGL1 containing AtSUC2- CcFT3 according to the transformation protocol described in Figure S5. **A)** GFP callus formation across three genotypes as a percentage of total explants 3 weeks after transformation with LBA4404-THY. Error bars represent two standard errors centered about the mean. **B)** DsRed callus formation as a percentage of total explants 3 weeks after transformation with AGL1. **C)** Full plate images of explants transformed with AGL1 after washing and 3 weeks on SIM in the dark. Clockwise, from bottom left: Eg18-1, Eug21-1, Eug22-1. **D)** Images of explants after transformation with LBA4404-THY without washing and three weeks on SIM media in the dark.

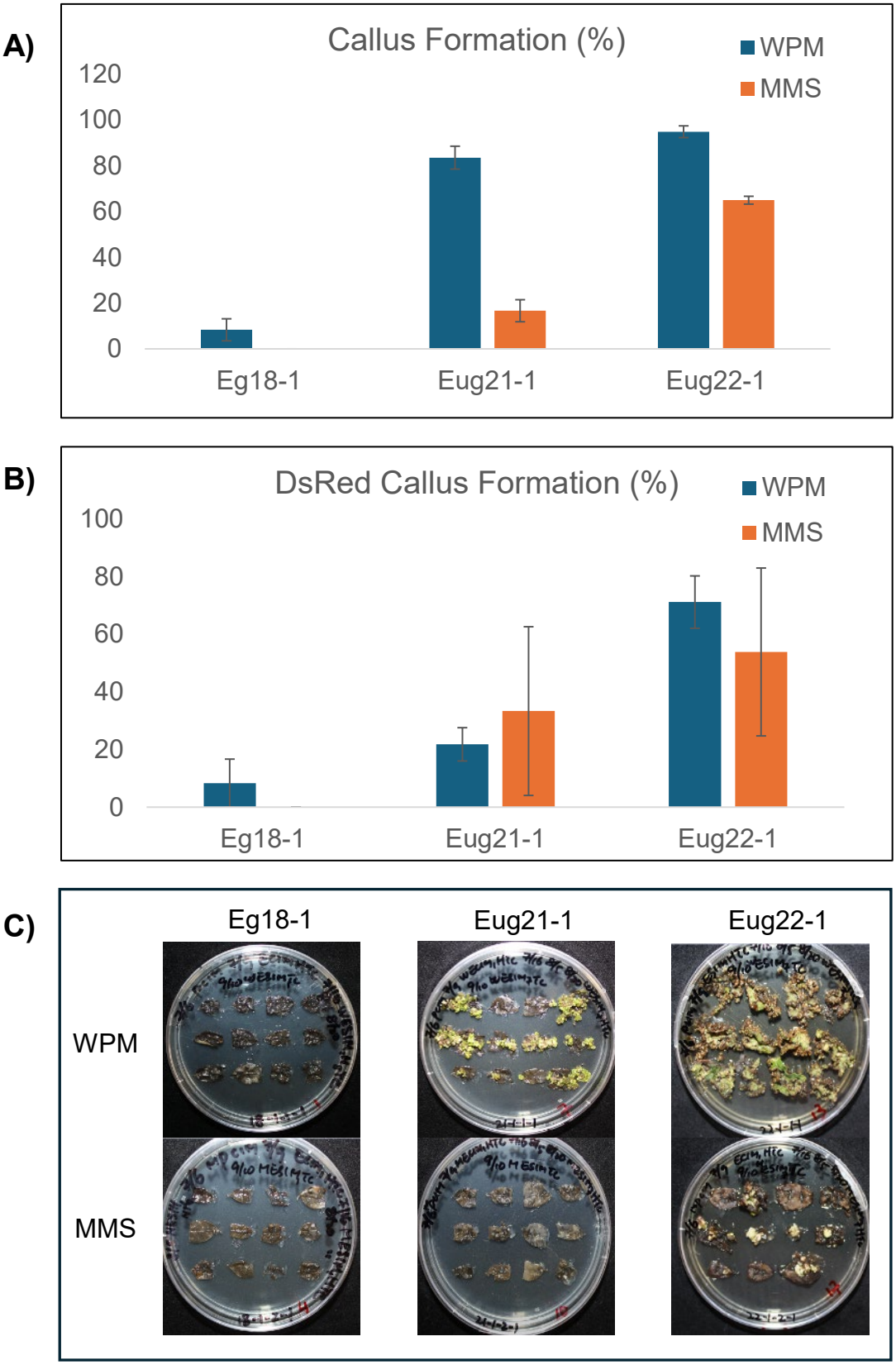


Figure S2. Effects of basal medium on Eucalyptus genotypes during transformation. Basal medium effects were tested on eucalyptus genotypes Eg18-1, Eug21-1, Eug22-1. Explants were transformed as described in Figure S5 A). After transformation, explants were placed on CIMTC with either WPM or MMS basal salts and 2.5 mg/L hygromycin for one week in the dark. Next, explants were transferred to SIMTC with either WPM or MMS basal salts and 5 mg/L hygromycin under light (6-12 µEm-2s-1) for five months. **A) P**ercent callus formation in explants at week 15. Error bars as in Figure S1A. **B)** Percent of transgenic callus formed 18 weeks post-inoculation. **C)** Images of each genotype taken 15 weeks after transformation.

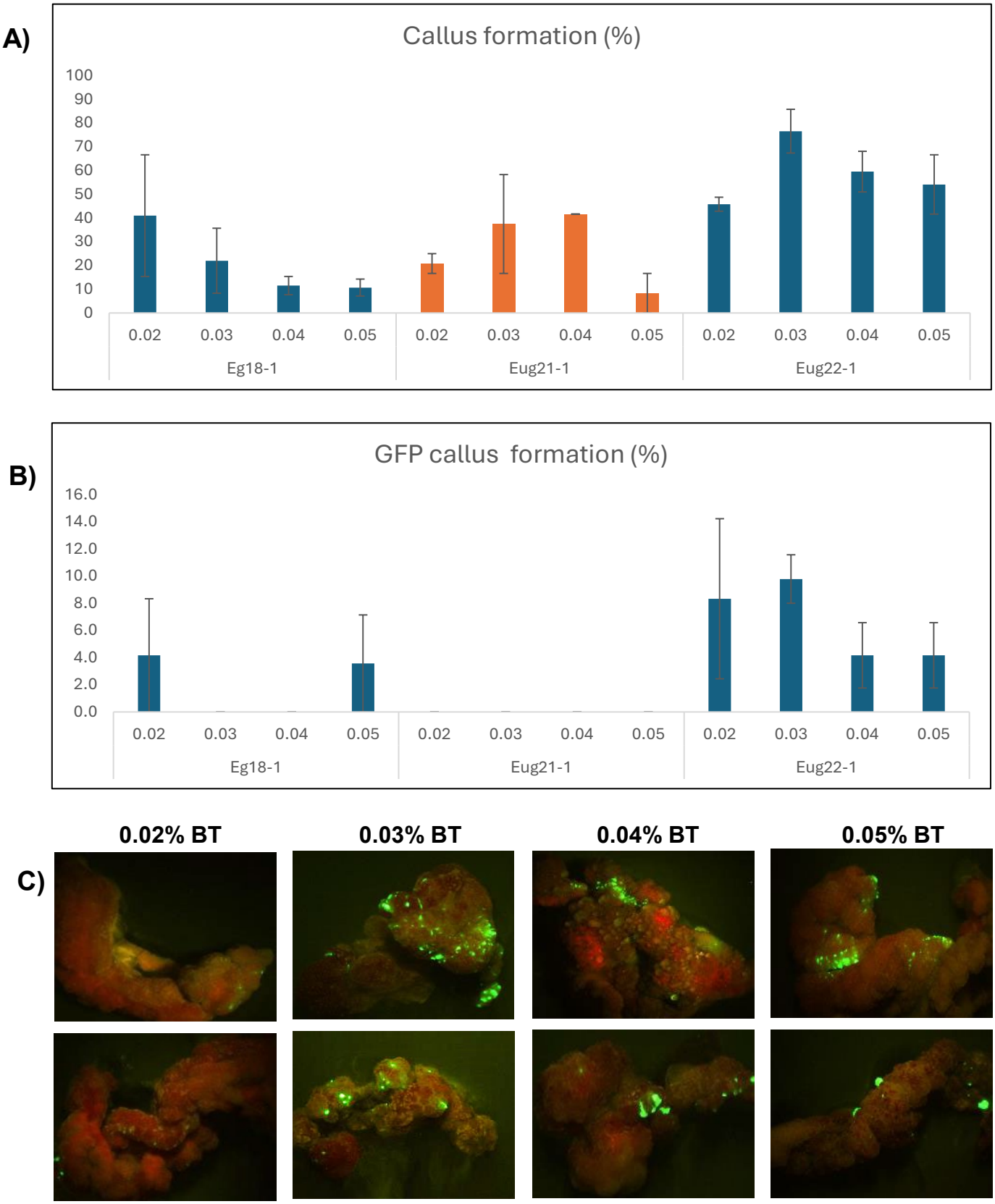
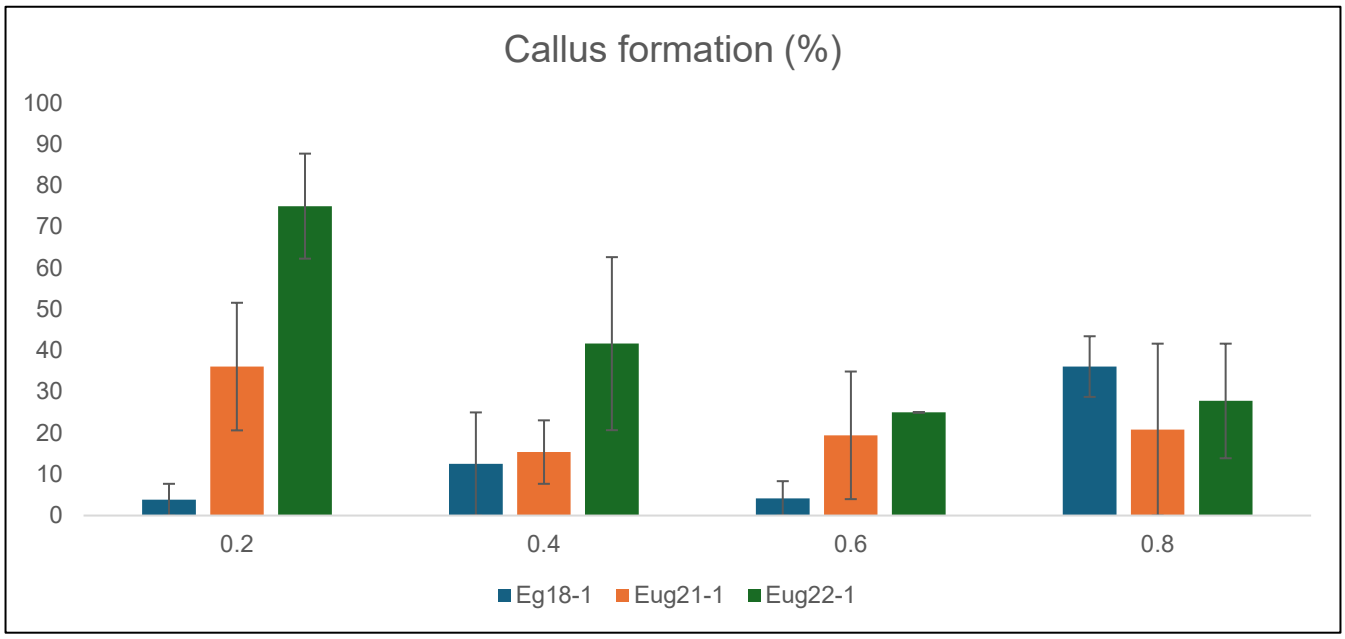


Figure S3. Effects of Break-Thru (BT) on *Eucalyptus* genotypes during transformation. BT was tested in *Eucalyptus* leaf explants from three genotypes, Eg 18-1, Eug 21-1, and Eug 22-1. Explants were inoculated with Agrobacterium strain LBA4404 containing p35S:eGFP-Hspt with Corteva's virulence plasmid (OD_{600} 0.6) in culture with acetosyringone (100μM) and differing levels of BT (0.02%, 0.03%, 0.04%, 0.05%) for 20 minutes. Each treatment had two – four plates with 3-15 explants per plate. Explants were scored for GFP expression, where "expression" was counted as an explant expressing any amount of detectable GFP fluorescence. **A)** Percentage callus formation **B)** Percentage of explants per plate expressing GFP signal. Error bars as in Figure S1A. **C)** Fluorescent microscope images of eucalyptus callus of Eug22-1 with increasing levels of BT.

A)



B)

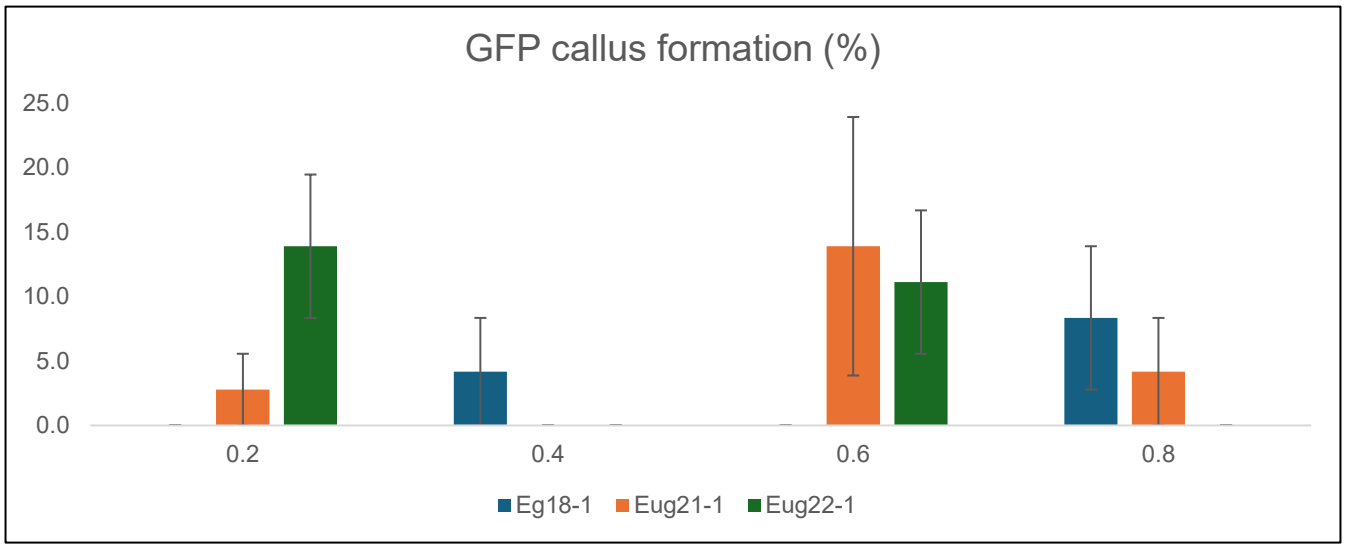


Figure S4. Effects of *Agrobacterium* OD_{600} on *Eucalyptus* transformation. Various OD_{600} levels of *Agrobacterium* LBA4404 containing p35S:eGFP-Hspt with Corteva's virulence plasmid were tested for transformation efficiency. Three different eucalyptus genotypes were used (Eg 18-1, Eug 21-1, Eug 22-1) and four different OD_{600} levels (0.2, 0.4, 0.6, and 0.8), with acetosyringone (50µM) and 0.02% BT during inoculation. Agrobacterium inoculation method same as Figure 3S. Each treatment had two – three plates with 3-12 explants per plate. **A)** Callus formation 4 weeks after inoculation. **B)** Percentage of callus per plate expressing GFP signal. Error bars as in Figure S1A.

A)

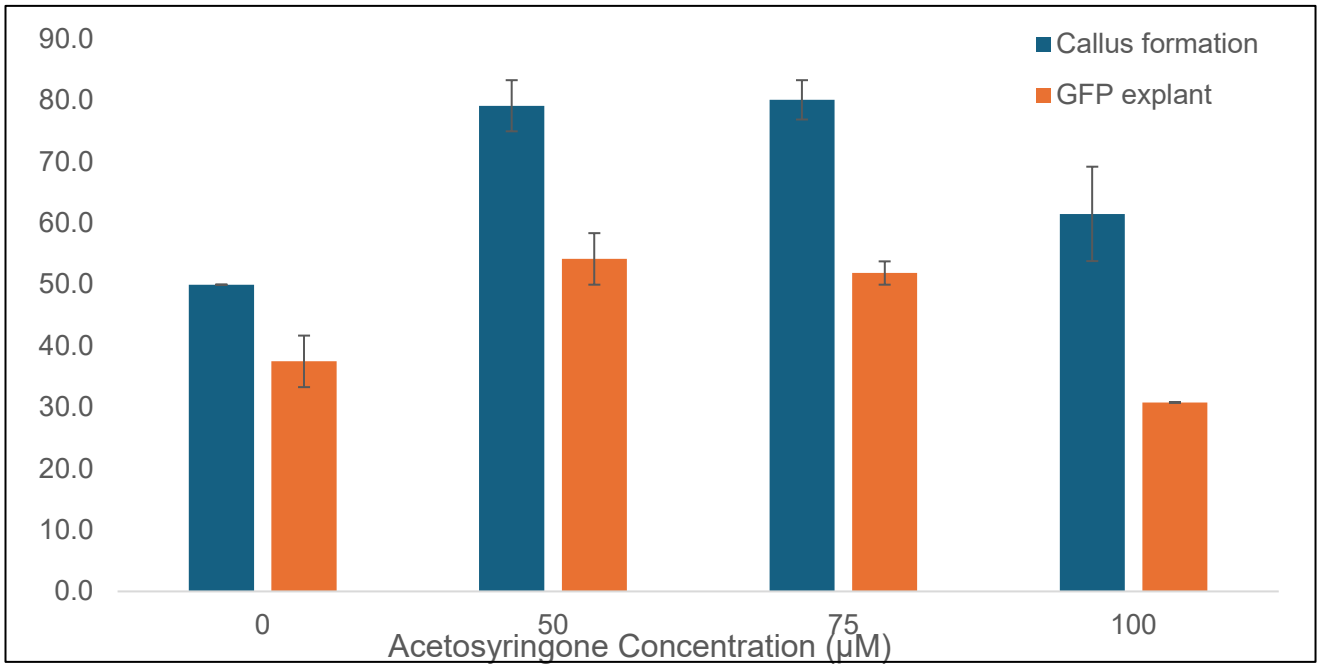
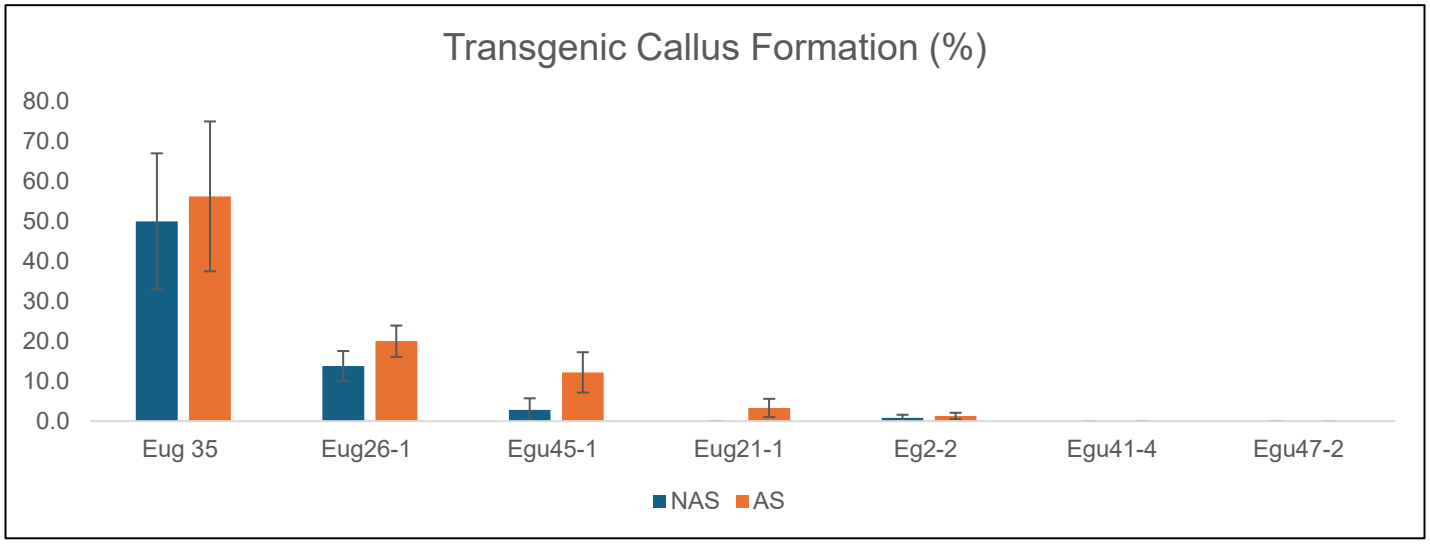


Figure S5. Effects of acetosyringone (AS) concentration on *Eucalyptus* transformation. Eucalyptus leaf explants were precultured for one day on CIM before transformation. Explants were then inoculated with *Agrobacterium* strain AGL1 with GFP and a hygromycin resistance gene in dense liquid cultures (OD_{600} = 3.0) with 50 µM AS and 0.02% Silwet-L77 by dipping a scalpel in culture liquid and wounding each leaf 4 times perpendicular to the midrib. Explants then spent two days on CIM for coculture before being washed four times in sterile water, soaked in antibiotic solution containing cefotaxime and timentin, and subcultured onto CIM plates containing 2.5 mg/L hygromycin as well as timentin and cefotaxime. After one week, explants were subcultured to SIM containing 5 mg/L hygromycin as well as timentin and cefotaxime. Each treatment had two plates with 12 explants per plate. Different levels of acetosyringone were tested during cocultivation (0, 25, 50, and 100 µM) in two transformations. **A).** Callus formation and DsRed expression are shown as a percent of explants for genotype Eug22-1. Error bars as in Figure S1A.

A)



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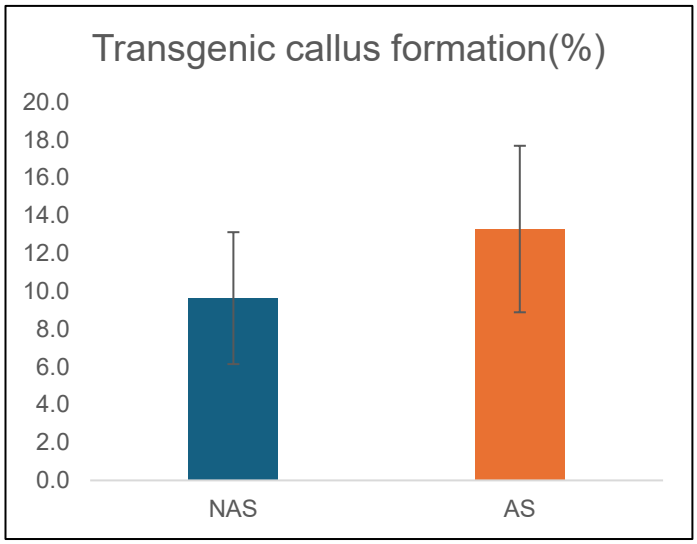


Figure S6. Effect of acetosyringone (AS) on *Eucalyptus* transformation. Leaf explants were transformed as described in Figure S5. Coculture medium either contained no AS or 50μM AS. **A)** Transgenic callus formation as a percentage of callus forming explants per genotype per treatment 32 weeks after transformation. **B)** Overall transgenic callus formation as a percent of callus forming explants per treatment. Error bars as in Figure S1A.

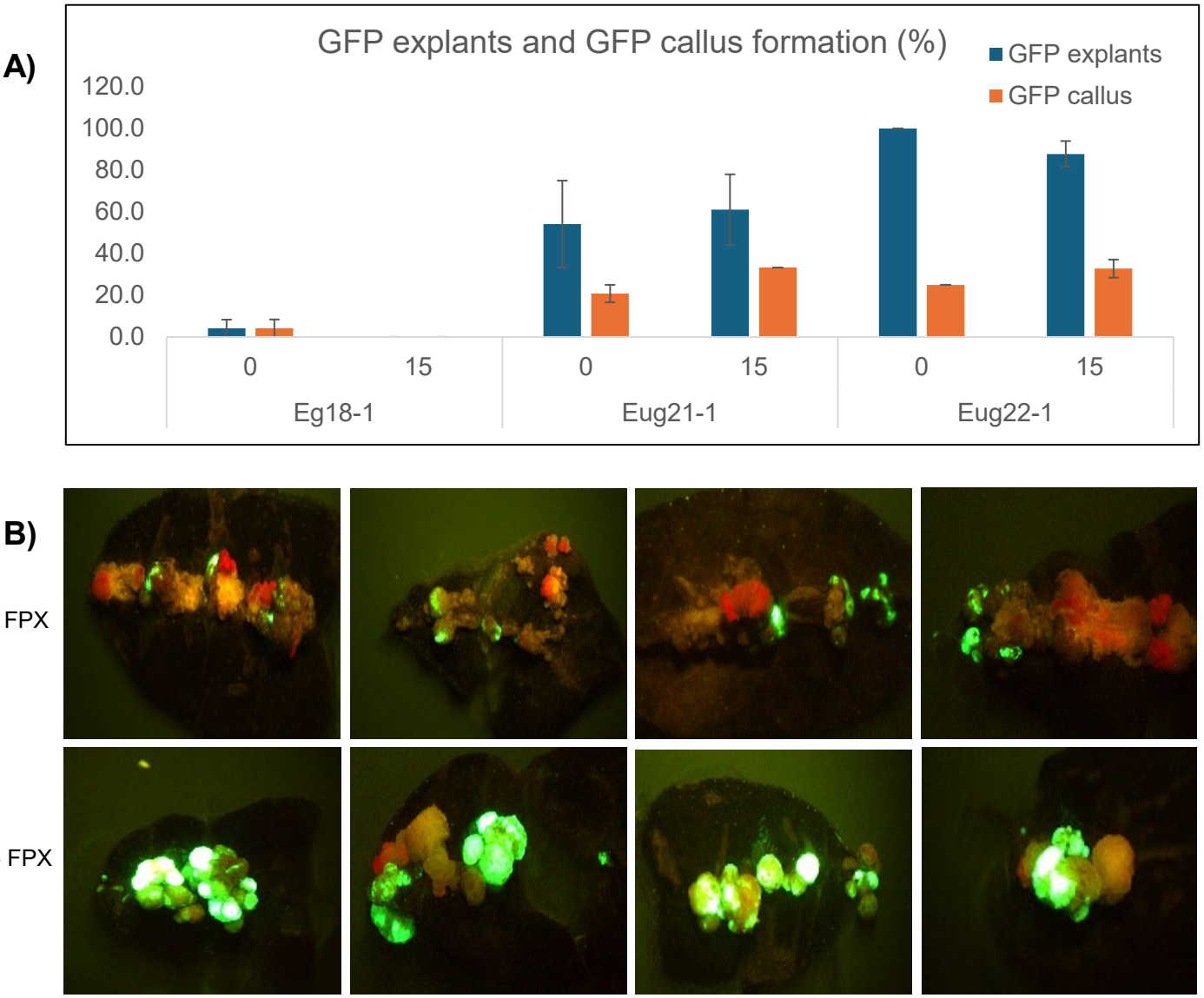


Figure S7. Effects of fipexide (FPX) on *Eucalyptus* genotypes during transformation. Leaf explants from three eucalyptus genotypes (Eg18-1, Eug21-1, Eug22-1) were placed on CIM with (15) or without (0) 15 µM FPX for two weeks, then placed on SIM (MMSIM + BAP and NAA for Eg18-1 & MMSIM + TDZ for all others) for four weeks. Callus, root, and shoot formation data were collected at 3 weeks and 7 weeks. **A)** Percentage of explants or callus expressing GFP across FPX treatments at week 4. Error bars as in Figure S1A. **B)** GFP callus under a fluorescent microscope on Eug21-1 explants 6 weeks after transformation. MMSIM is modified MS.

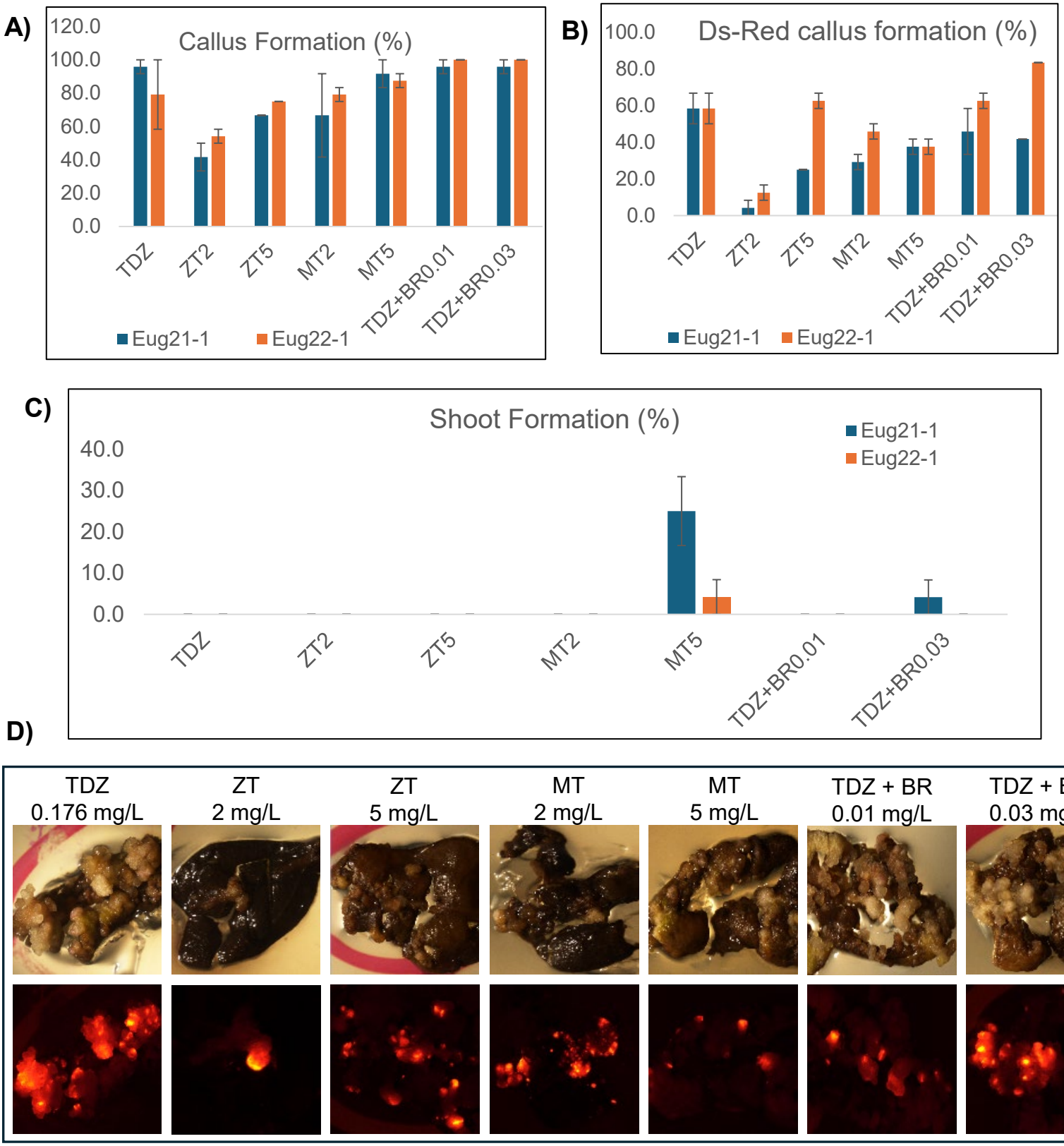


Figure S8. Effects of different cytokinins on *Eucalyptus* genotypes during transformation. Two *Eucalyptus* genotypes, Eug21-1 and Eug22-1 were transformed as described in Figure S5. Different cytokinins were included in SIM. Cytokinin concentrations included: thidiazuron (TDZ) at 0.176 mg/L, zeatin (ZT) at 2 and 5 mg/L, meta-topolin (MT) at 2 and 5 mg/L, and brassinolide (BR) at 0.01 mg/L and 0.03 mg/L in addition to TDZ. **A)** Callus formation as a percent of explants per treatment one month after inoculation. Error bars as in Figure S1A..**B)** DsRed callus formation as a percent of explants per genotype per treatment expressing DsRed one month after inoculation. **C)** Shoot formation as a percent of explants. **D)** Brightfield (top) and fluorescence (bottom) microscope images of DsRed expressing callus across cytokinin treatments in Eug21-1 one month after inoculation.

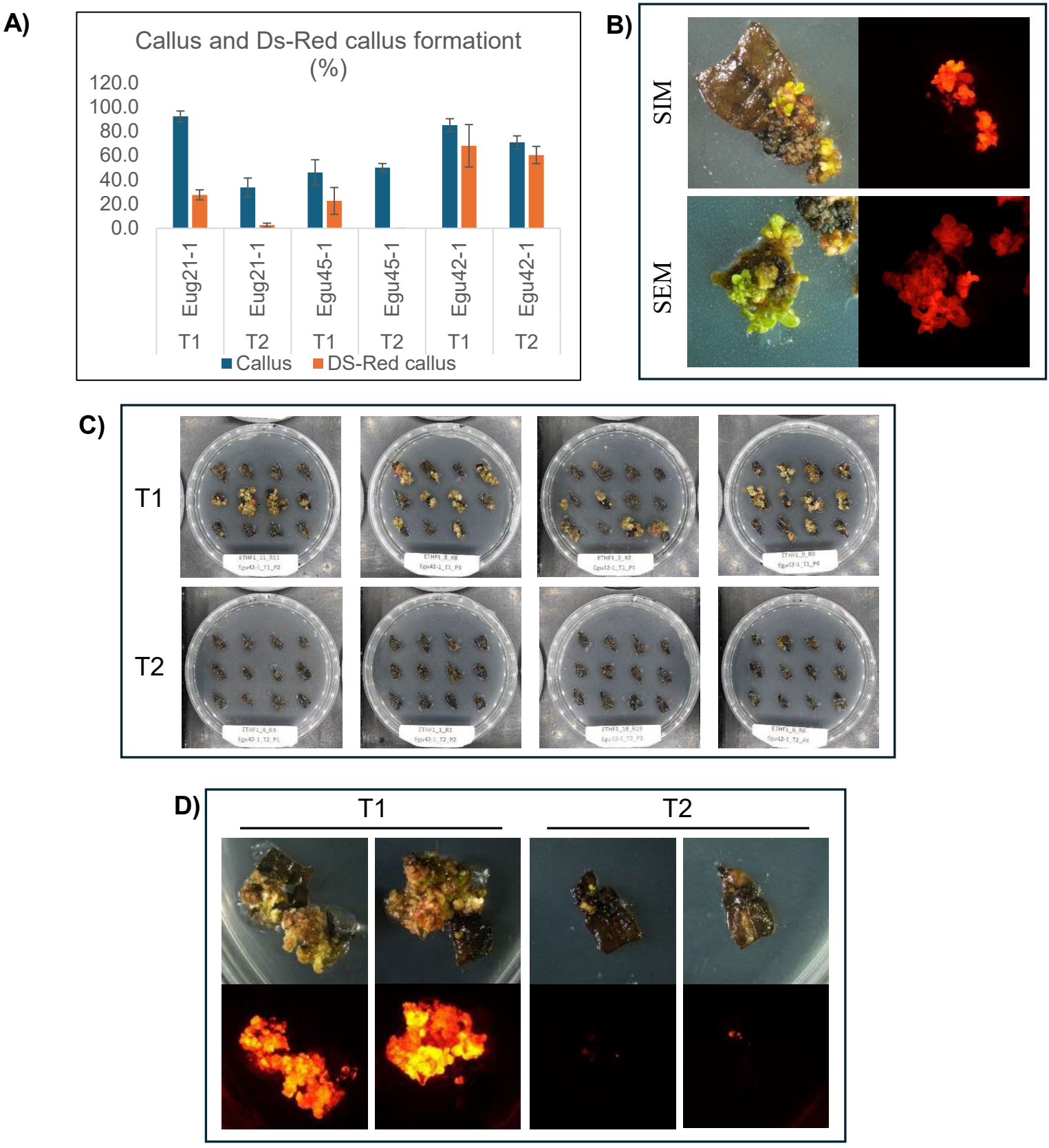


Figure S9. Effects of different cytokinins in during transformation. We tested N-(2-Chloro-4-pyridyl)-N′-phenylurea (CPPU) in CIM and 6-benzylaminopurine (BAP) in SIM. Three *Eucalyptus* genotypes (Eug21-1, Egu42-1, and Egu45-1) were transformed as described in Figure S5. Treatment 1 (T1) included CIM with 1-naphthaleneacetic acid (NAA) and BAP followed by SIM with NAA and TDZ. Treatment 2 (T2) included CIM with NAA and CPPU followed by SIM with NAA and BAP **A)** Formation of callus and DsRed expressing callus as a percentage of explant frequency. Error bars as in Figure S1A. **B)** Brightfield (left) and fluorescence (right) images of a transgenic shoot formed by Eug45-1 28 weeks after inoculation on T1. SEM = shoot elongation medium (Table 1). **C)** Whole plate images of Egu42-1 twelve weeks after inoculation. **D)** Brightfield (top) and fluorescence (bottom) microscope images of Egu42-1 explants 12 weeks after inoculation.

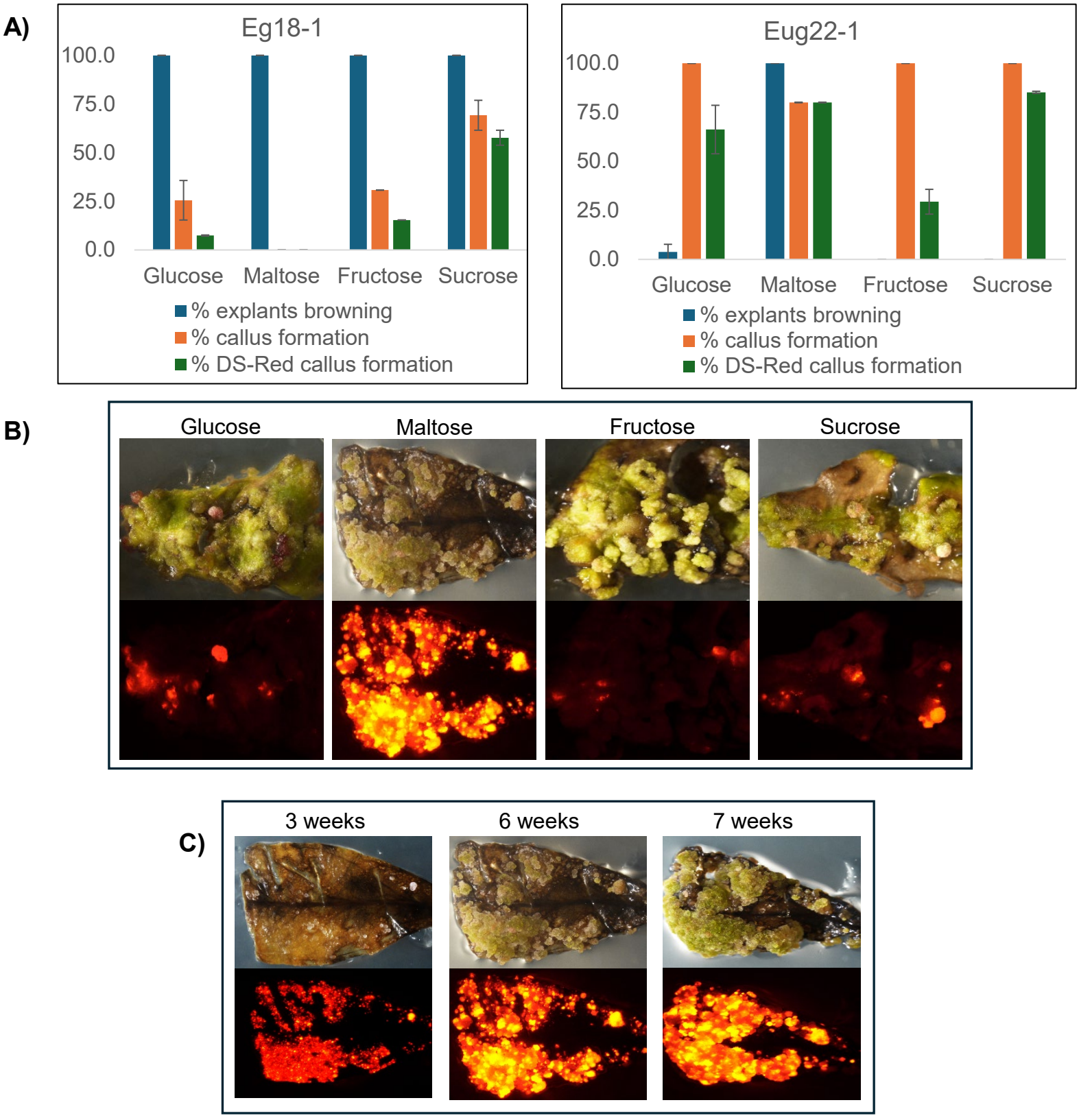


Figure S10. Effects of different carbon sources on *Eucalyptus* genotypes during transformation. Different sources of carbon were tested in PCIM, CIMHTC, and SIMHTC media. Treatments were glucose, maltose, fructose, or sucrose at 30 g/L each. **A)** Data collected on Eg18-1 (left) and Eug22-1 (right) explants after five weeks on SIM media. Includes explant browning, callus formation, and DsRed callus formation as a percentage of explants per treatment. Error bars as in Figure S1A. **B)** Brightfield (top) and fluorescence (bottom) microscopy images of callus expressing DsRed in Eug22-1 explants across carbon source treatments. **C)** Time course images of DsRed-expressing callus from Eug22-1 explants on maltose growing larger over time. From left to right: callus after three weeks on SIMHTC, 6 weeks on SIMHTC, and 1 week on SIMTC without hygromycin (6 weeks with and 1 week without hygromycin).

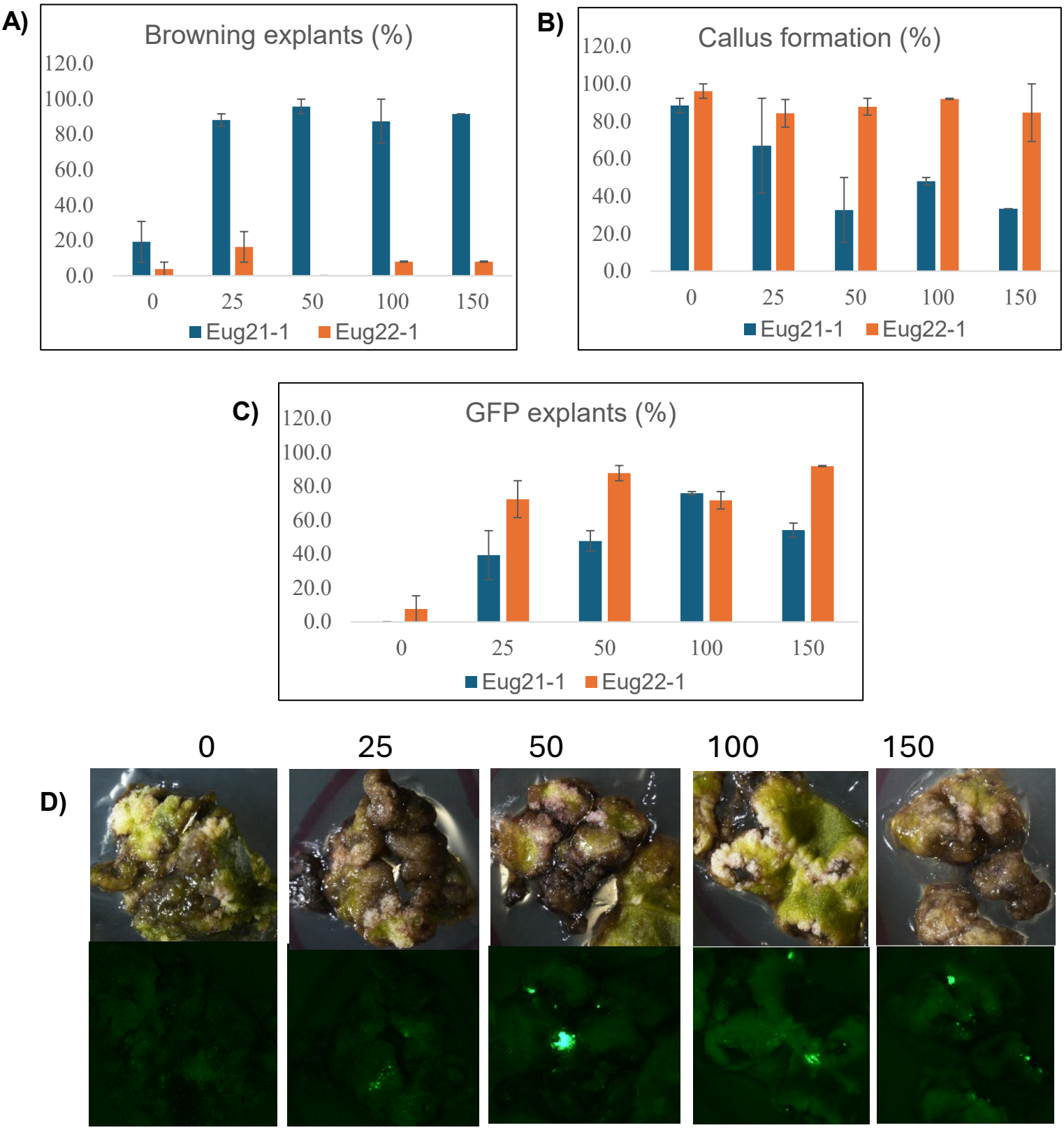


Figure S11. Effects of spectinomycin selection in media on *Eucalyptus* genotypes after transformation. Two *Eucalyptus* genotypes (Eug21-1 and Eug22-1) were inoculated with LBA4404-THY containing 35S::eGFP.Mar as described by the protocol in Figure S5. But 50 uM AS was added in co-cultivation medium. **A)** Browning explants shown as percentage of explants per treatment which exhibited browning. Spectinomycin levels increasing from 0 to 150 mg/L in media. Error bars as in Figure S1A. **B)** Callus formation as a percent of explants per treatment which formed callus. Spectinomycin levels increasing from 0 to 150 mg/L. **C)** GFP callus formation as a percent of explants per treatment showing GFP expression. Spectinomycin levels increasing from 0 to 150 mg/L. **D)** Brightfield (top) and fluorescence (bottom) microscope images of GFP expressing explants across spectinomycin treatments in Eug22-1 two months after inoculation.

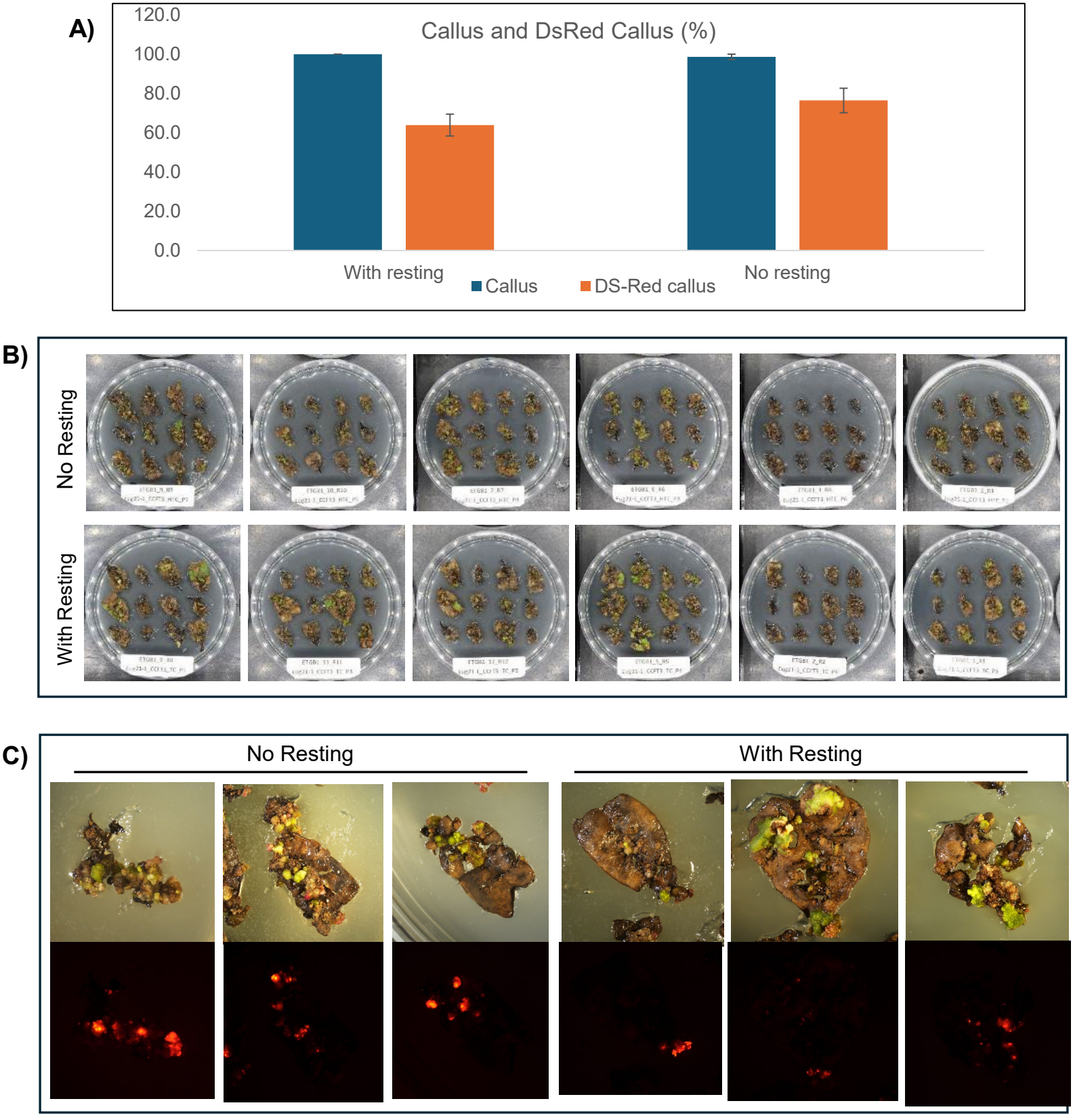


Figure S12. Effects of resting period after transformation on *Eucalyptus* explants. We tested the effects of a resting period between transformation and exposure to hygromycin in eucalyptus genotype Eug21-1 with AtSUC2- CcFT3 construct.in AGL1. Leaves were transformed as described in Figure S5. Explants were either immediately introduced to CIM with 2.5 mg/L hygromycin after transformation or CIM with 0 mg/L hygromycin for 7 days. Then all explants were moved to media with 5 mg/L hygromycin. **A)** Callus and DsRed callus as a percentage of explants per treatment. Error bars as in Figure S1A. **B)** Images of leaves on petri plates 12 weeks post-inoculation. Resting (0 mg/L hygromycin for the first 7 days) on top row, no resting (directly to selection) on bottom row. **C)** Brightfield (top) and fluorescence (bottom) microscope images of explants from resting and no resting treatments showing DsRed expression in callus after 16 weeks of inoculation

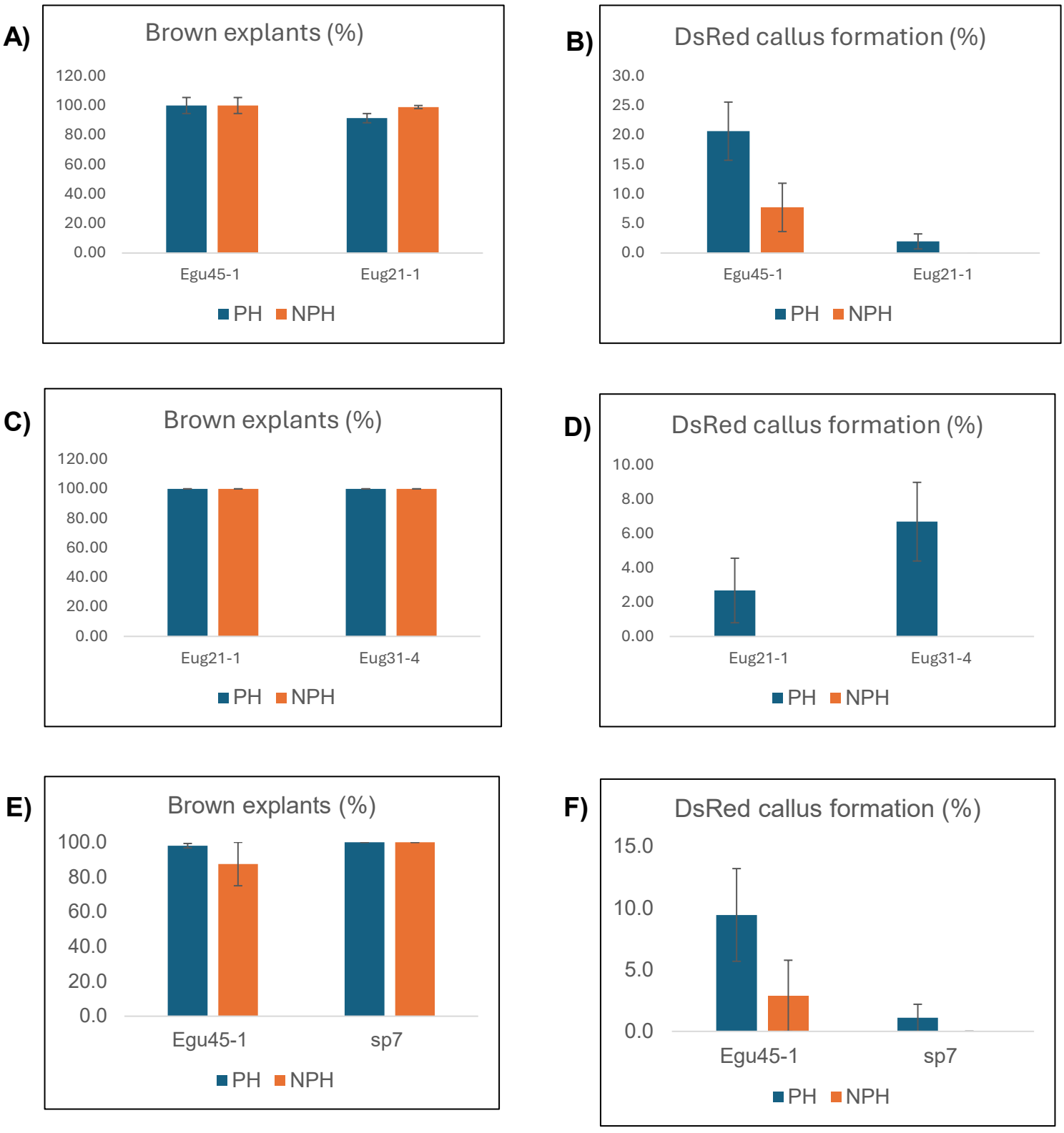
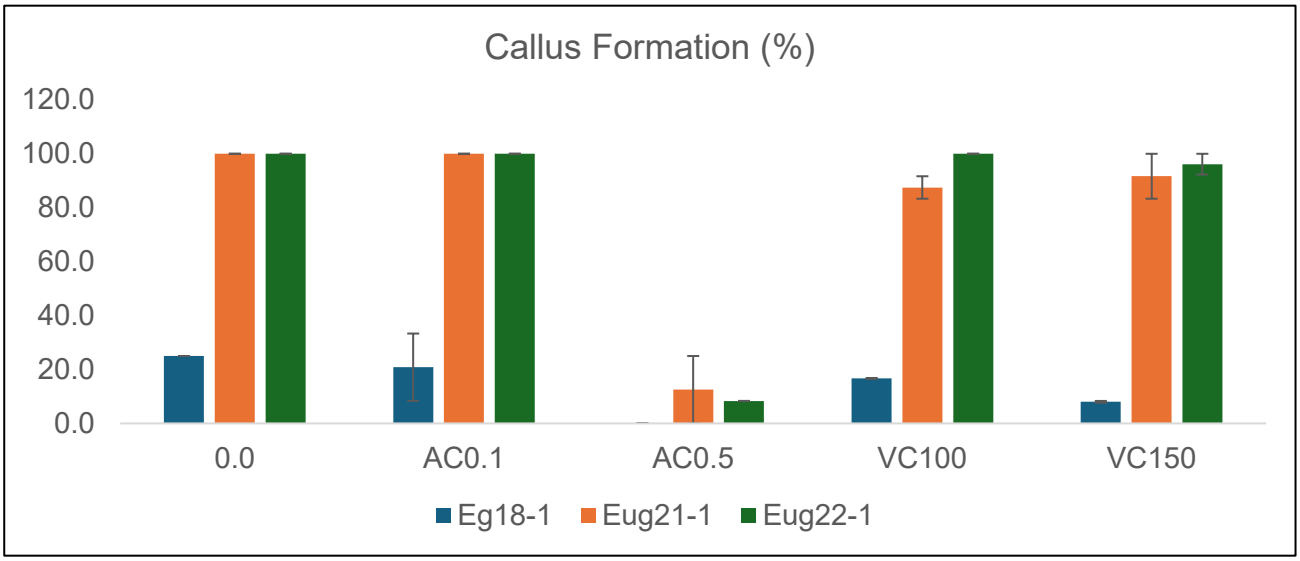


Figure S13. Effect of explant preheating on Eucalyptus transformation. Explants were preheated at 37˚C for 2-4 hours prior to transformation with AGL1 containing a binary vector with Suc2::CcFT3, DsRed, and hygromycin resistance, otherwise following procedure described in Figure S5. Error bars for all charts as in Figure S1A. **A)** Brown explants as a percentage of explants per genotype per treatment, preheating (PH) or no preheating (NPH) in Egu45-1 and Eug21-1. **B)** DsRed callus formation as a percentage of explants forming DsRed callus per genotype per treatment in Egu45-1 and Eug21-1. **C)** Brown explants as a percentage of explants per genotype per treatment in Eug21-1 and Eug31-4. **D)** DsRed callus formation as a percentage of explants forming DsRed callus per genotype per treatment in Eug21-1 and Eug31-4. **E)** Brown explants as a percentage of explants per genotype per treatment in Egu 45-1 and sp7. **F)** DsRed callus formation as a percentage of explants forming DsRed callus per genotype per treatment in Egu 45-1 and sp7 at 12 weeks post inoculation.

A)



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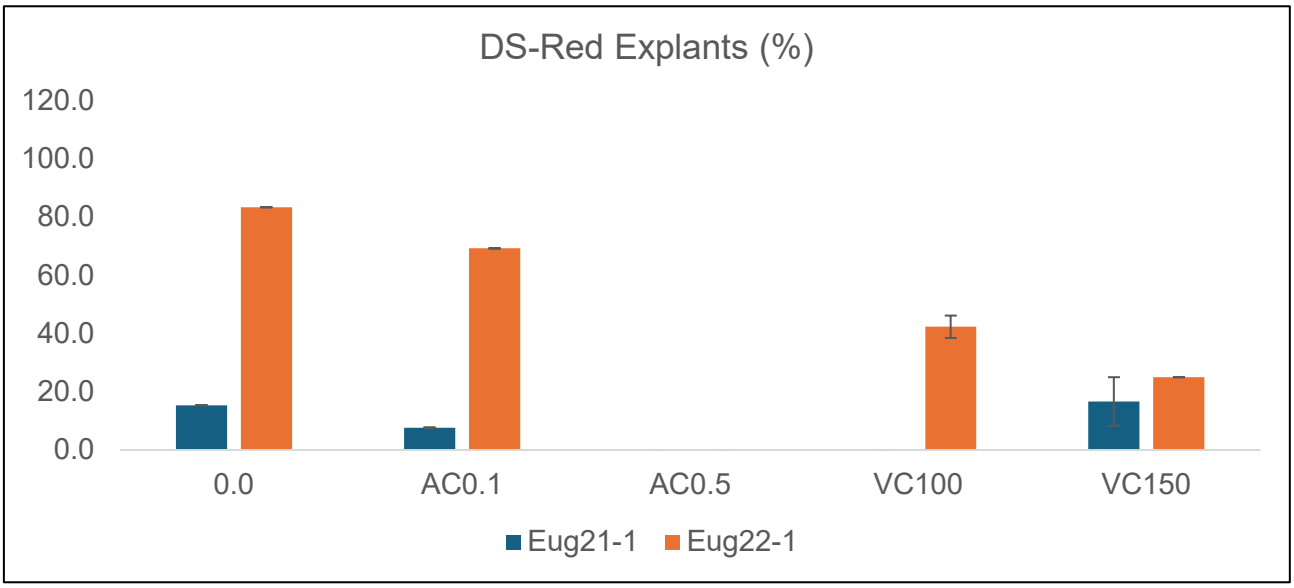

Figure S14. Effect of activated charcoal (AC) and vitamin C (VC) on Eucalyptus genotypes during transformation. Three eucalyptus genotypes (Eg18-1, Eug21-1, Eug22-1) were transformed as described in Figure S5. CIM and SIM media contained differing levels of AC from 0-0.5% and VC from 0-150 mg/L. **A)** Effects of AC and VC on percent callus formation 7 weeks (Eug21-1 and Eug22-1) or 9 weeks (Eug18-1) post-inoculation. **B)** Effects of AC and VC on DsRed expression by percent of explants 7 months post-inoculation. Error bars as in Figure S1A.

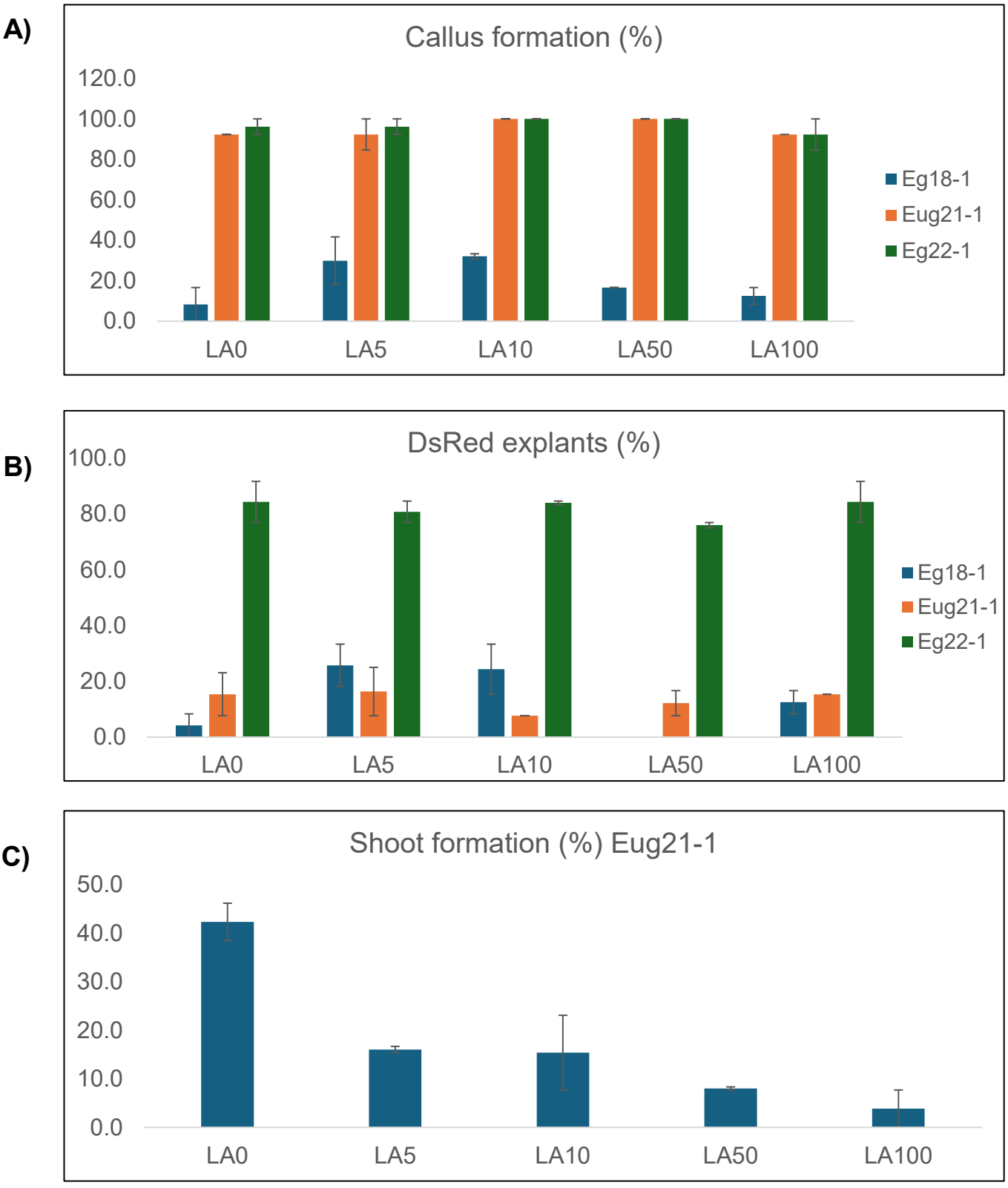

Figure S15. Effects of lipoic acid (LA) on *Eucalyptus* genotypes during transformation. Young leaf explants of Eg18-1, Eug21-1, and Eug22-1 were transformed as described in Figure S5. Varying levels of LA were included in CIM and SIM. **A)** Effects of LA (0, 5, 10, 50, 100μM) on percent callus formation in three genotypes. **B)** Effect of LA on DsRed expression by percent of explants 6 months post-inoculation. **C)** Effect of LA on shoot formation (escape) by percent of explants 6 months post-inoculation in Eug21-1. Error bars for all charts as in Figure S1A.

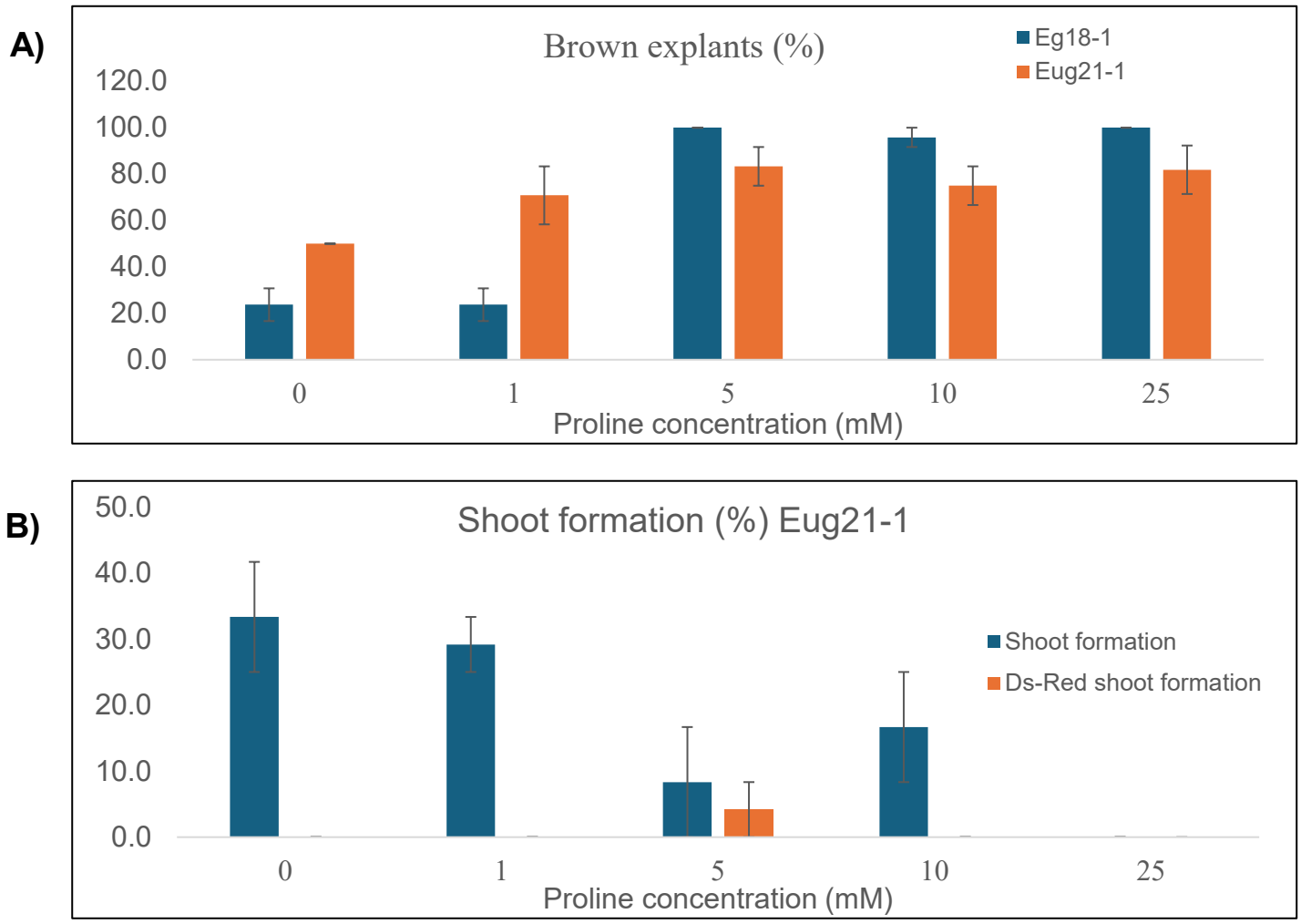

Figure S16. Effects of proline (PL) on *Eucalyptus* genotypes during transformation. Two eucalyptus genotypes (Eg18-1 and Eug21-1 were transformed as described in Figure S3. Effects of PL were tested by adding concentrations of 0, 1, 5, 10, and 25 mM PL to CIM and SIM media. **A)** Effect of PL on browning by percent of explants after 2 weeks on CIM with PL. Panel C includes an example of 100% browning in Eg18-1 at 5 mM PL, and an example of 23.7% browning in Eg18-1 at 0 mM PL. Error bars for all charts as in Figure S1A. **B)** Effect of PL on shoot and transgenic shoot formation in Eug21-1 at 5 months post-inoculation. **C)** Effects of proline on leaf explants from Eg18-1 (top) and Eug21-1 (bottom) after 7 days on CIM.

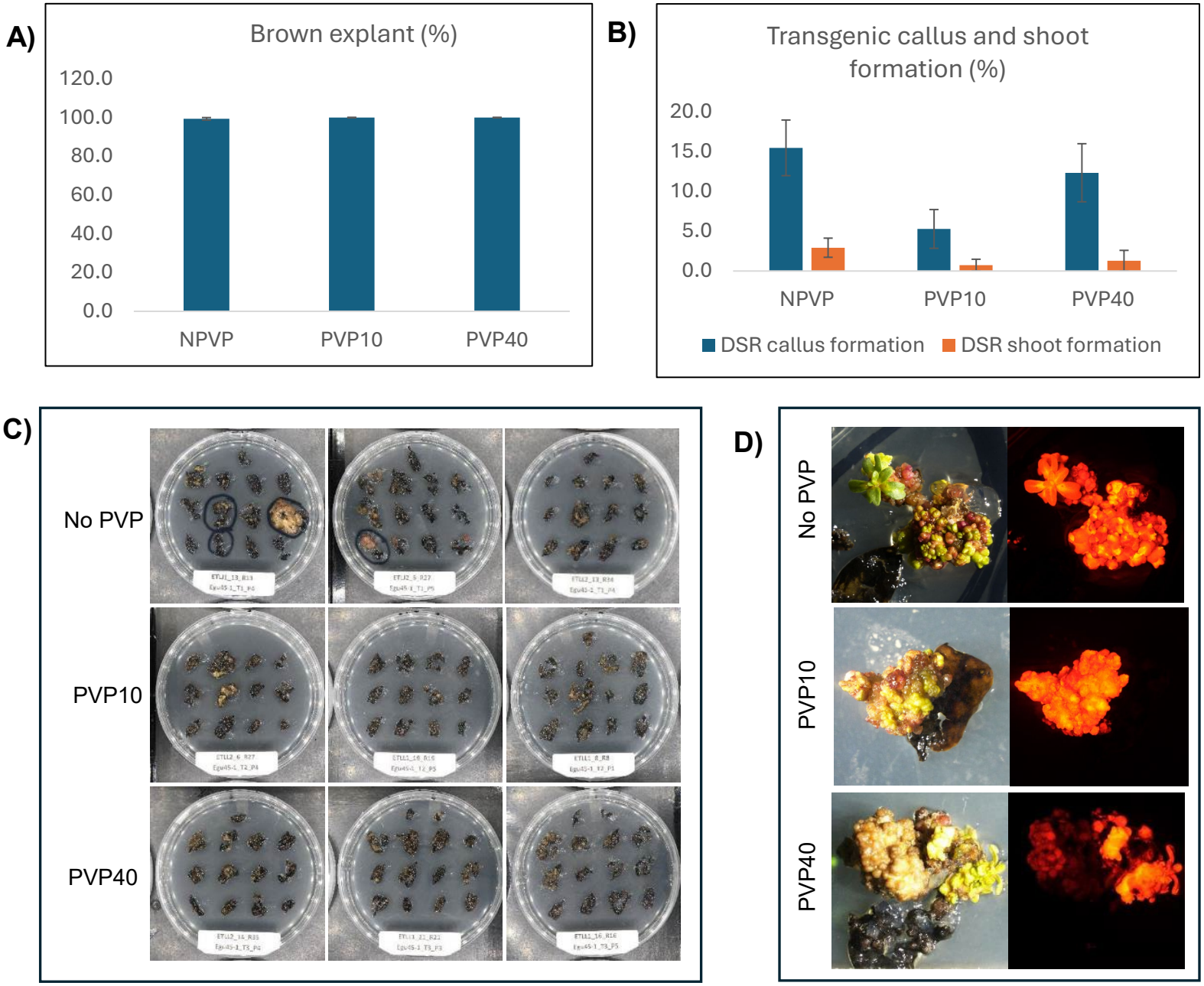

Figure S17. Effects of polyvynilpyrrolidone (PVP) on *Eucalyptus* genotypes during transformation. Leaf explants from *Eucalyptus* genotypes Egu45-1 were transformed as described in Figure S5. Varying molecular weights of PVP (PVP-10 and PVP-40) were added to cocultivation media and CIM. **A)** Brown explants as a percentage of explants per treatment per genotype. **B)** DsRed callus and shoots as a percentage of total explants per treatment per genotype. Error bars as in Figure S1A. **C)** Whole plate images of Egu45-1 24 weeks after inoculation. **D)** Brightfield (left) and fluorescence (right) microscope images of transgenic callus and shoots in Egu45-1 across all treatments.

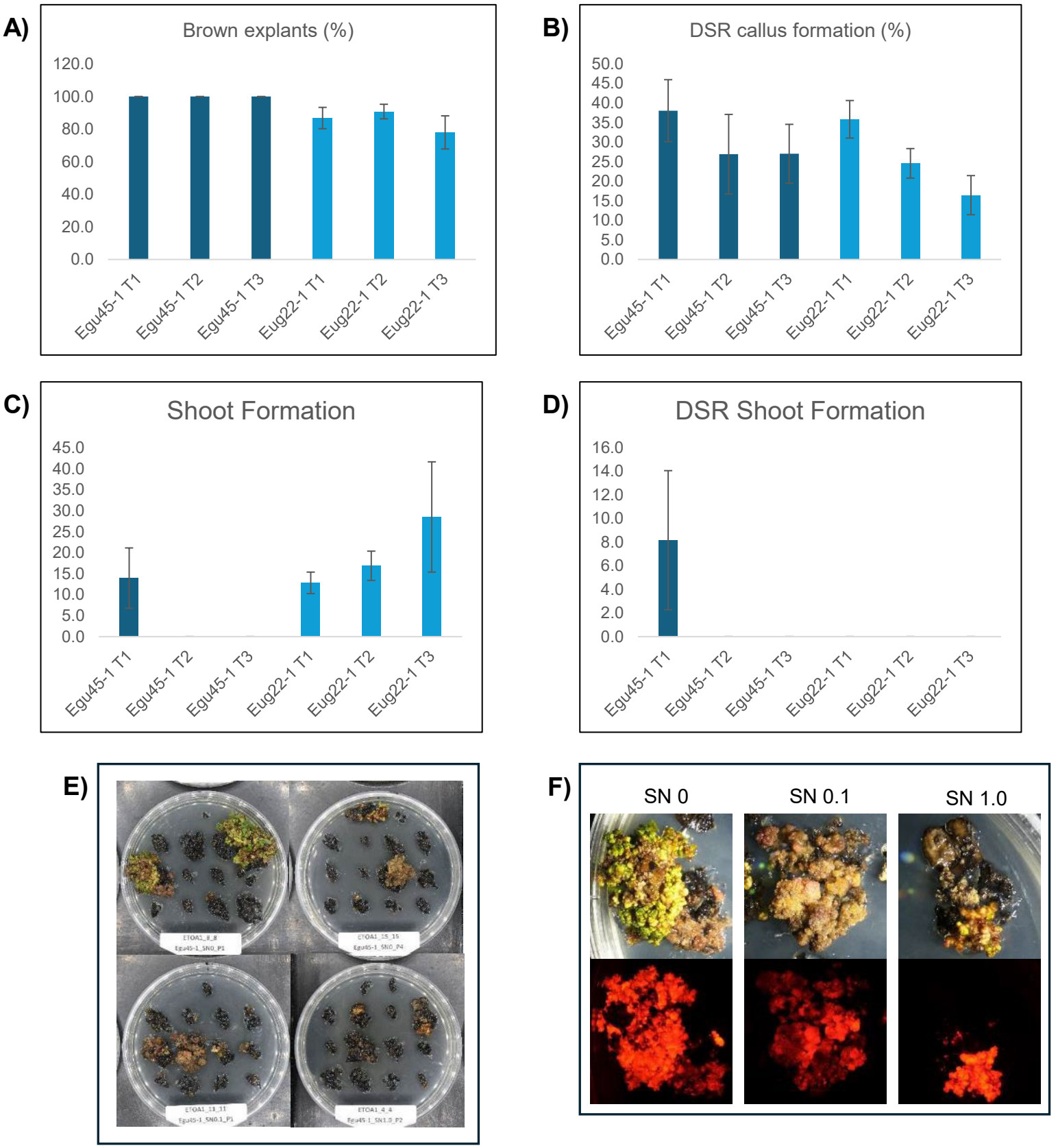

Figure S18. Effect of silver nitrate (SN) on *Eucalyptus* transformation. Two *Eucalyptus* genotypes, Eug22-1 and Egu45-1, were transformed as described in Figure S5. Three SN treatments were applied to CIM and SIM media. Treatment one (T1) had 0 mg/L SN, treatment two (T2) had 0.1 mg/L SN, and treatment three (T3) had 1.0 mg/L SN. **A)** Brown explants as a percent of explants per genotype per treatment 12 weeks after inoculation. **B)** DsRed callus formation as a percent of explants per genotype per treatment 12 weeks after inoculation. **C)** Shoot formation as a percentage of explants per genotype per treatment. **D)** DsRed shoot formation as a percentage of explants per genotype per treatment. Error bars for all charts as in Figure S1A.**E)** Whole plate images of Eug45-1 explants in T1 (top), T2 (bottom left) and T3 (bottom right) at 32 weeks post-inoculation. **F)** Brightfield (top) and fluorescence (bottom) microscope images of Egu45-1 explants at 32 weeks post-inoculation.

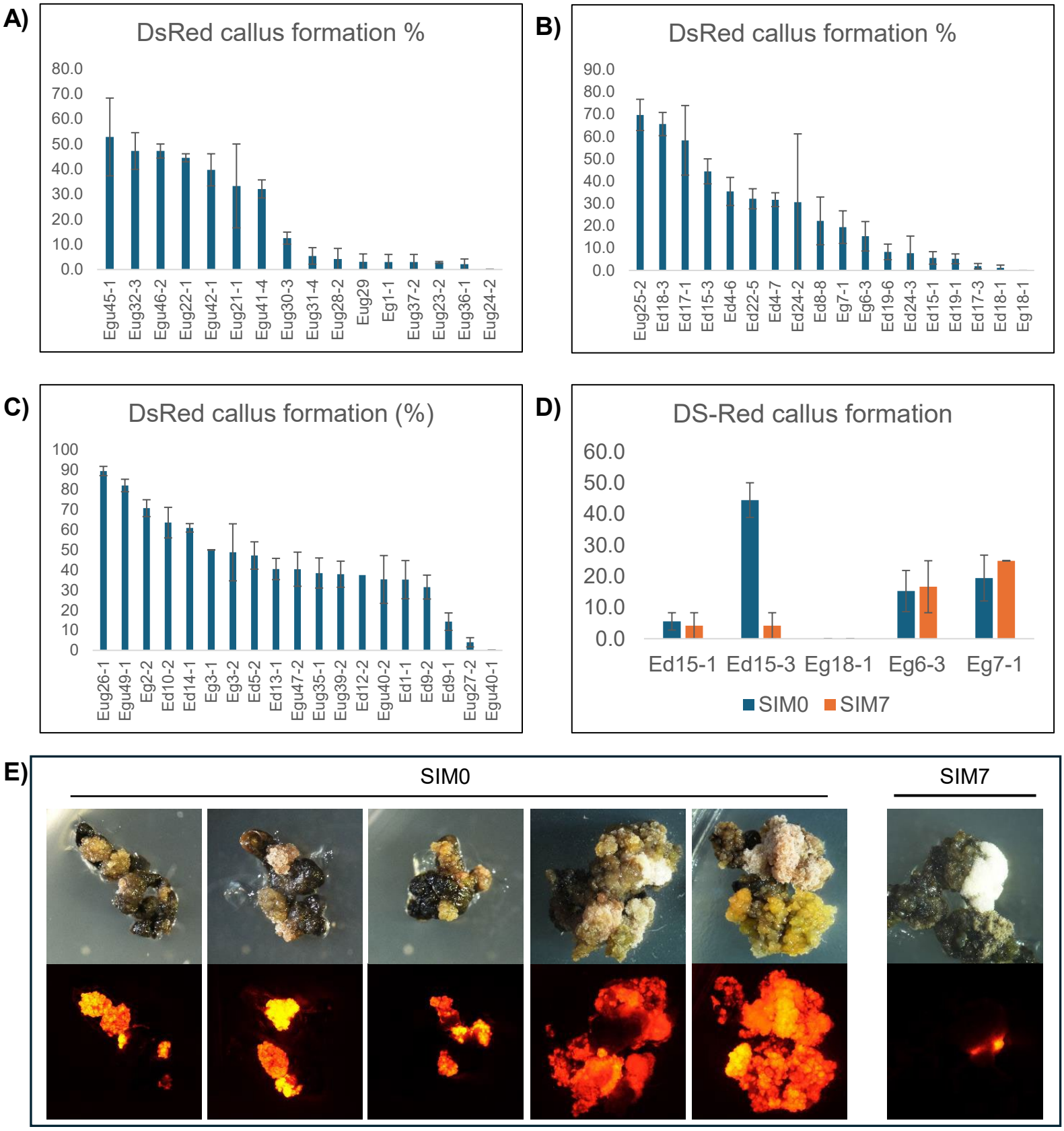

Figure S19. Screening Eucalyptus genotypes for response to transformation. Fifty-three eucalyptus genotypes were tested for Agrobacterium susceptibility using the C58 oncogene in auxotrophic LBA4404. . Explants were transformed as described in Figure S5. Two media types were tested, SIM0 (no hormone) and SIM7 (with 0.176 mg/L TDZ). **A)** DsRed callus formation (on SIM0) as percentage of total explants across 16 genotypes tested in 2022. Error bars as in Figure S1A. **B)** DsRed callus formation (on SIM0) as percentage of total explants across 18 genotypes tested in 2023. Error bars as in Figure S1A. **C)** DsRed callus formation (on SIM0) as percentage of total explants across 19 genotypes tested in 2023 and 2024. Error bars as in Figure S1A. **D)** DsRed callus formation as a percentage of total explants tested on two different media (SIM0 and SIM7) and five eucalyptus genotypes. Error bars as in Figure S1A. **E)** Brightfield (top) and fluorescence (bottom) microscope images of callus expressing DsRed on SIM0 and SIM7 in Ed15-3 at 24 weeks post-inoculation.