

## **Supporting Information file for:**

Transformation of European ash (*Fraxinus excelsior* L.) callus as a starting point for understanding the molecular basis of ash dieback.

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## **Supporting information figure and table list and short legends:**

Table S1 – Primer sequences and RT-PCR conditions used.

Figure S1 – Selection conditions evaluated for *F. excelsior* callus.

Figure S2 – Further images of GUS activity in callus eight weeks after transformation.

Figure S3 - PCR analysis of *A. tumefaciens* cultures

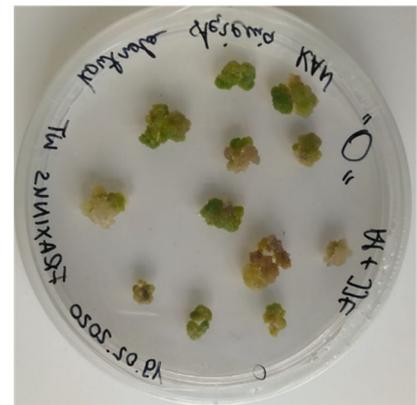
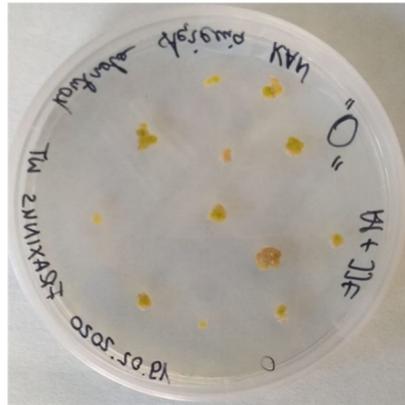
**Table S1. Primer sequences used in this study.** RT-PCR was performed with 10 uM forward and reverse primer concentrations using PCR-Mix Plus (A&A Biotechnology). For each reaction the initial melting was performed for 2 minutes at 95 °C. PCR was performed for 35 cycles with 30 seconds melting (95 °C), 45 seconds annealing (60 °C) and 90 seconds extension (72 °C). Final extension was performed for 5 minutes.

Primer name and target	Primer sequence (5'-3')
Fraxinus_excelsior_UBIQUITIN_For	GACCAGCAGCGATTGATCTTT
Fraxinus_excelsior_UBIQUITIN_Rev	GAGGACAAGATGGAGGGTAGAC
GUS_For	CAACGAACTGAACTGGCAGA
GUS_Rev	AGAGGTAAAGCCGACAGCA

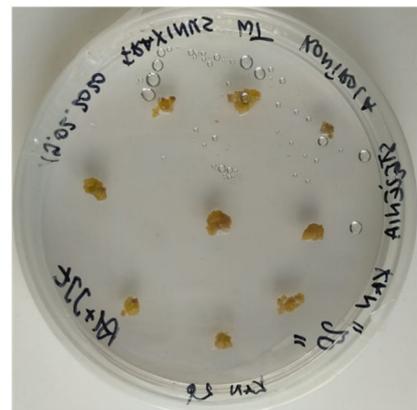
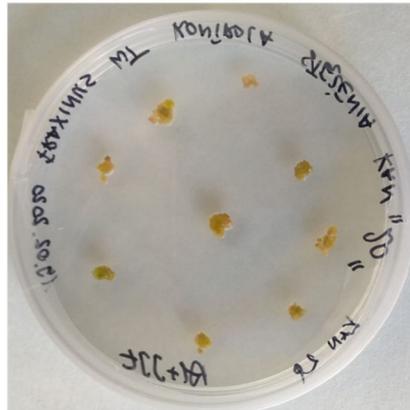
Start of selection

After 8 weeks of selection

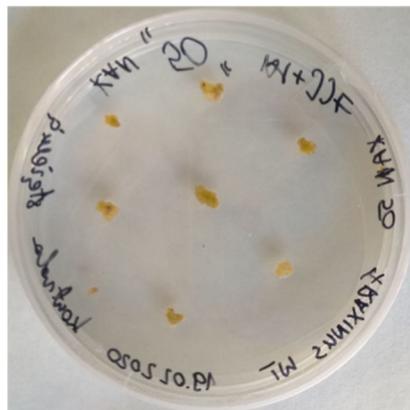
Kanamycin 0 ug/mL  
Timentin 100 ug/mL



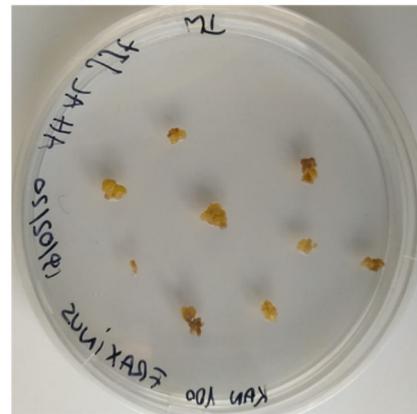
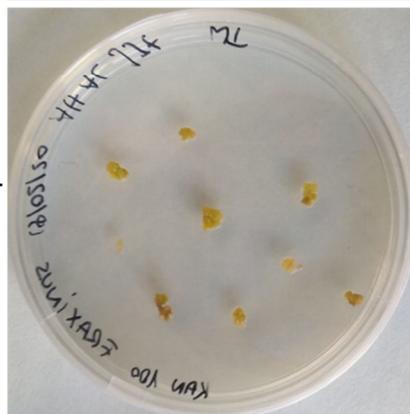
Kanamycin 20 ug/mL  
Timentin 100 ug/mL



Kanamycin 50 ug/mL  
Timentin 100 ug/mL



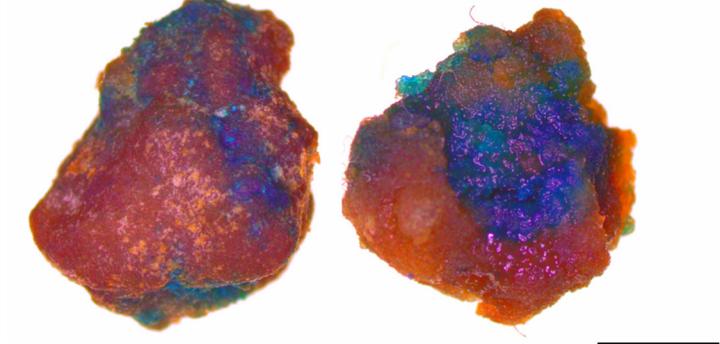
Kanamycin 100 ug/mL  
Timentin 100 ug/mL



**Figure S1. Selection conditions evaluated for *F. excelsior* callus.** Kanamycin and timentin concentrations are provided for each image.



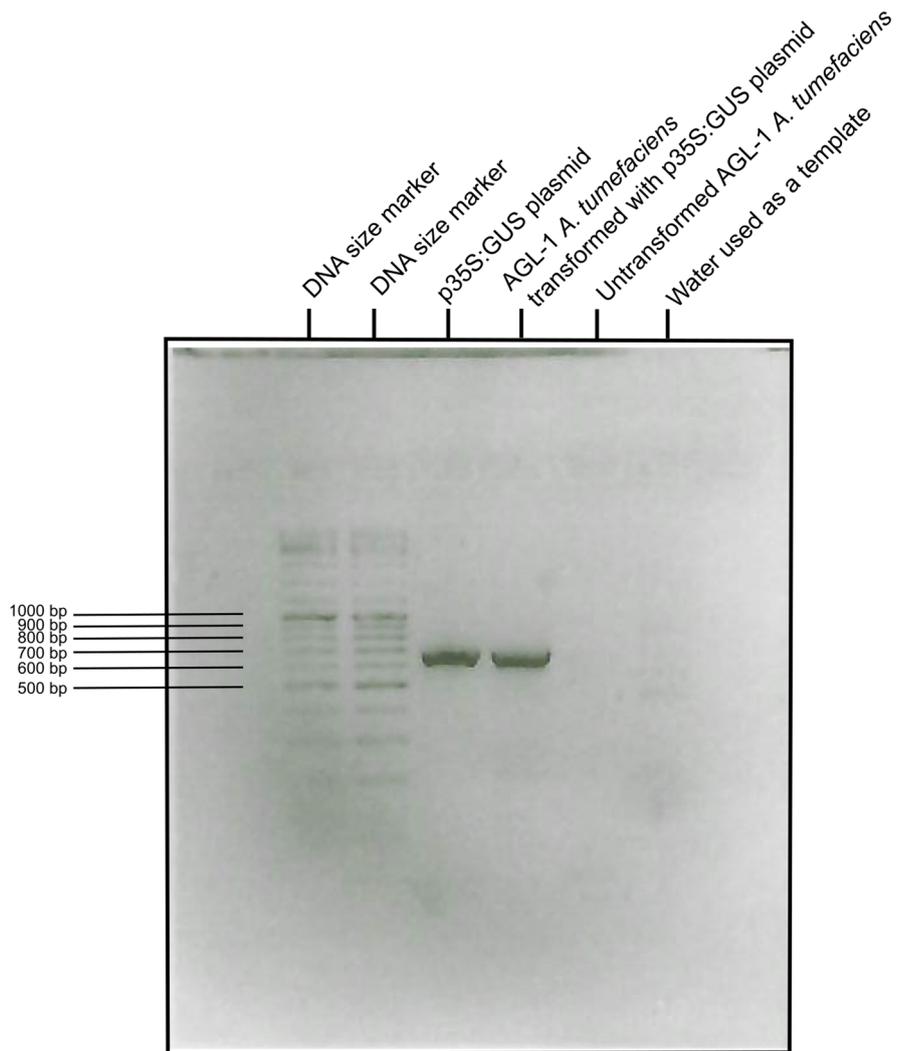
Wild type *F. excelsior* callus



p35S:GUS *F. excelsior* callus

**Figure S2. Further images of GUS activity in callus eight weeks after transformation.**

Black size bar corresponds to 1 mm.



**Figure S3. PCR analysis of *A. tumefaciens* cultures.** Agarose gel electrophoresis of PCR products obtained with *GUS* specific primers (SI Table 1, same conditions used for amplification) using plasmid DNA (0.3 ng), transformed and untransformed AGL-1 *A. tumefaciens* cultures (10  $\mu$ L of full-grown culture preheated to 98°C to release DNA, 1  $\mu$ L of that used in PCR) or water as a template. Product of same size is observed when plasmid and transformed *A. tumefaciens* are used.