

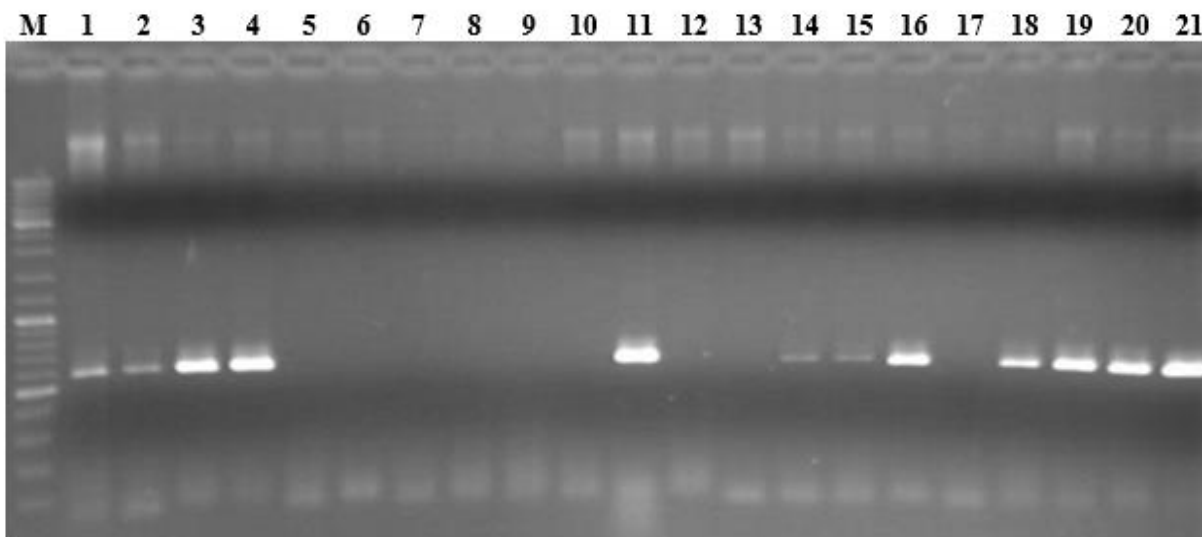


Fig. S1. Colony PCR screening for selection of transformed *Agrobacterium* LBA4404 colonies harboring the pCaDRRG-Pro-Gi construct. PCR was performed using the primer pair PSh4-F2/PSh4-R, which amplifies the expected 1287 bp fragment spanning the *CaDRRG-Pro* promoter and the intron-containing *GUS* reporter gene. Lanes 1–20: Transformed *Agrobacterium* LBA4404 colonies grown on LB medium containing kanamycin (50 mg/L) and rifampicin (30 mg/L). Lane 21: Positive control, *Agrobacterium* GV3101 harboring the CaDRRG-Pro:GUSi construct, as reported by Shokouhifar et al. (2025). M: 1 kb DNA size marker (CinnaGen, Iran). Colonies in lanes 5–10, 13, and 17 did not show amplification of the expected band, indicating they did not harbor the target construct. The remaining colonies showed successful amplification of the expected 1287 bp fragment. Negative controls (PCR reaction without template DNA and non-transformed *Agrobacterium*) were checked in earlier steps and consistently showed no amplification. Colonies that did not show amplified bands could also be considered as additional negative controls, confirming that the strain and the PCR reactions themselves are not able to produce the expected band. Selected confirmed colonies were further re-tested using both primer pairs (PSh4-F2/PSh4-R and PKa-F/PSh4-R), and the results are presented in Fig. 1 of the main manuscript.

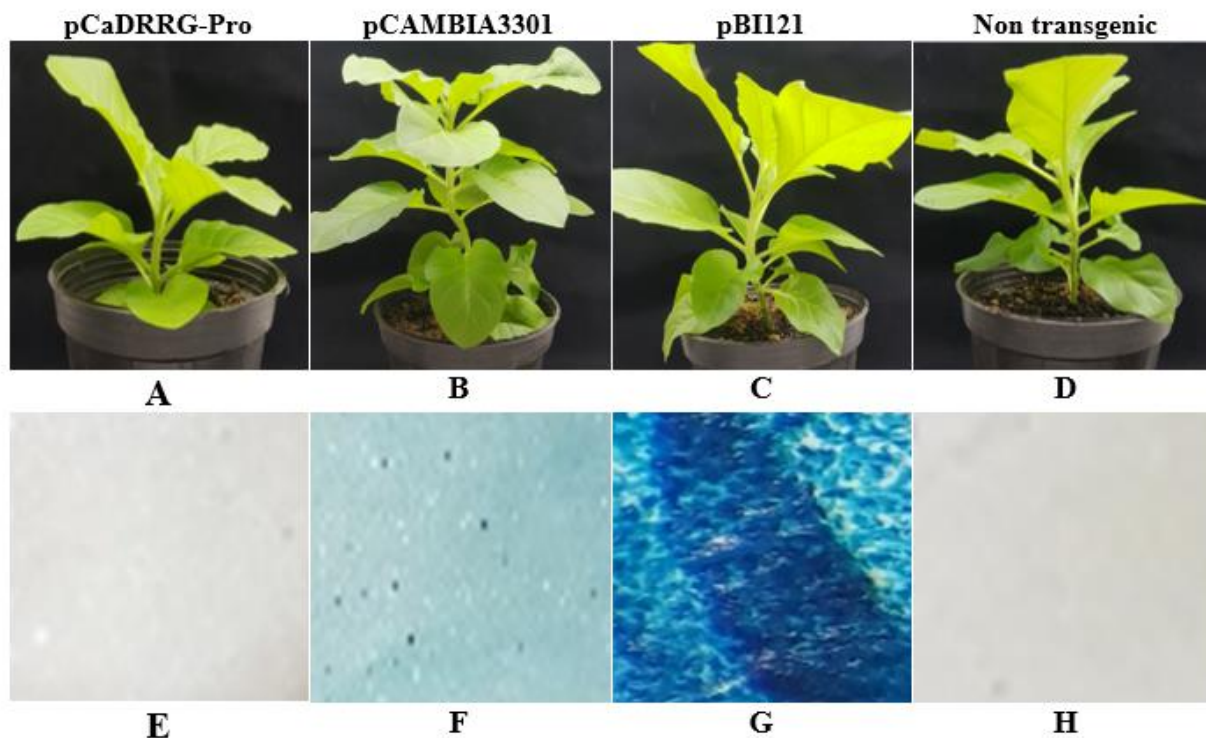


Fig. S2. Basal GUS expression analysis of transgenic and non-transgenic tobacco plantlets without hormonal treatment. (First row, A–D): Potted T0 plantlets. (Second row, E–H): Corresponding leaf samples assayed for histochemical GUS activity. (A, E): Transgenic plantlet harboring pCaDRRG-Pro (code 1-5) showing no blue coloration in the absence of MeJA treatment, confirming no basal GUS expression. (B, F): Transgenic plant harboring pCAMBIA3301 (constitutive CaMV 35S promoter driving intron-containing *GUS*) obtained from previous studies (Shokouhifar et al., 2025), showing clear blue coloration as a positive control. (C, G): Transgenic plant harboring pBI121 (constitutive CaMV 35S promoter driving intron-less *GUS*) obtained from previous studies (Shokouhifar et al., 2025), showing intense blue coloration due to the absence of an intron. (D, H): Non-transgenic regenerated plantlet from the current study, showing no blue coloration, confirming the absence of endogenous GUS activity. The difference in blue intensity between pCAMBIA3301 and pBI121 may be attributed to the presence of the intron in the *GUS* coding sequence of pCAMBIA3301, which may affect expression levels. However, as segregation analysis was not performed, other factors such as transgene copy number or positional effects may also contribute to the observed differences. All plantlets were maintained for seed collection.