

Figure 1. Flavonol biosynthesis in plants (redrawn from [4], [8], [16]). CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavone 3-hydroxylase; F3'H, flavonoid 3'-hydroxylase; F3'5'H, flavonoid 3'5'-hydroxylase; FLS, flavonol synthase; FST, flavonol 4-sulfotransferase.

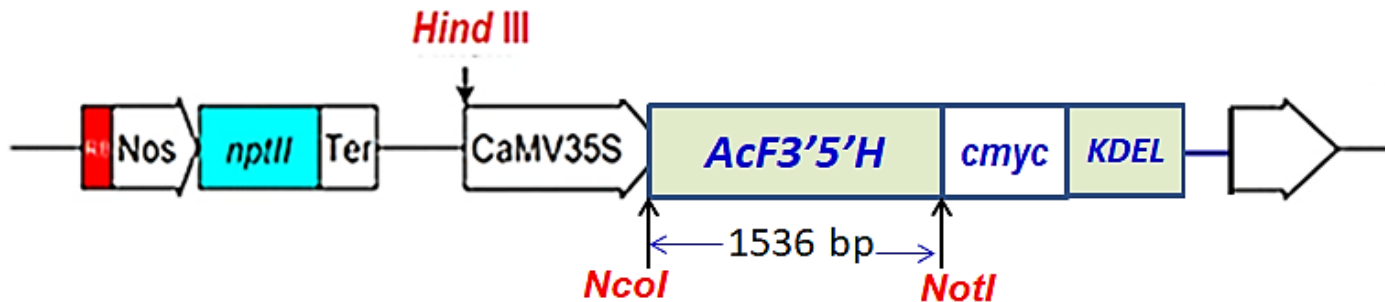


Figure 2. Structure diagram of *35S_AcF3'5'H_cmyc_KDEL* in the transgenic vector pCB301_AcF3'5'H. *nptII*: The gene coding for the kanamycin-resistant protein; CaMV35S: 35S promoter; *AcF3'5'H*: The *F3'5'H* gene of *A. carmichaelii* containing the cut points *NcoI* and *NotI*; *cmyc*: The nucleotide sequence encoding the peptide c-myc; *KDEL*: The nucleotide sequence encoding the KDEL peptide.

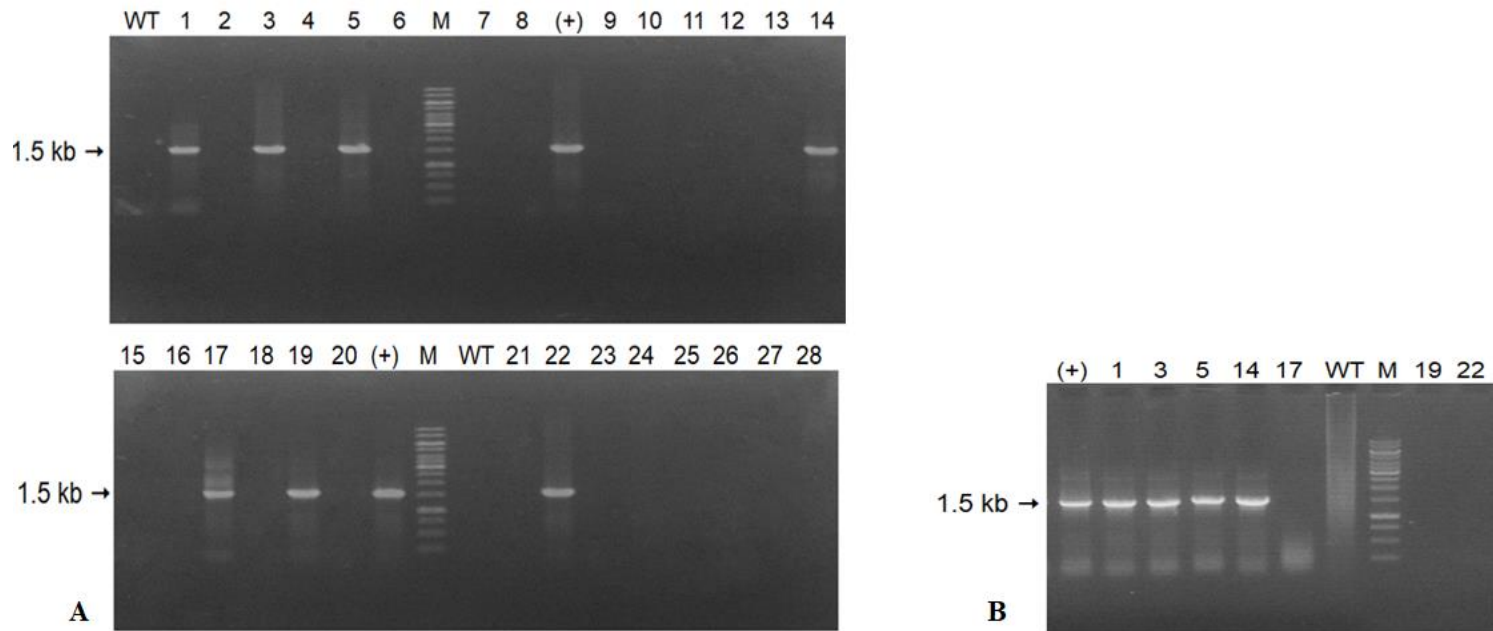


Figure 3. Electrophoresis results confirming the presence and transcription of the *AcF3'5'H* transgene in the transgenic plants. A- Results of electrophoresis analysis of PCR products that amplified the *AcF3'5'H* transgene. M: 1.0 kb DNA ladder; (+): plasmid pCB301_*AcF3'5'H*; WT: non-transgenic tobacco plants. Electrophoresis lanes from 1 to 28: transgenic tobacco plants. B- Results of electrophoretic analysis of RT-PCR products, confirming the transcription of the *AcF3'5'H* transgene in transgenic tobacco plants. M: 1.0 kb DNA ladder; (+): plasmid pCB301_*AcF3'5'H*; WT: non-transgenic tobacco plants; electrophoresis lanes from 1, 3, 5, 14, 17, 19, and 22 are the transgenic plants that were PCR-positive, corresponding to T01, T03, T05, T014, T017, T019, and T022.

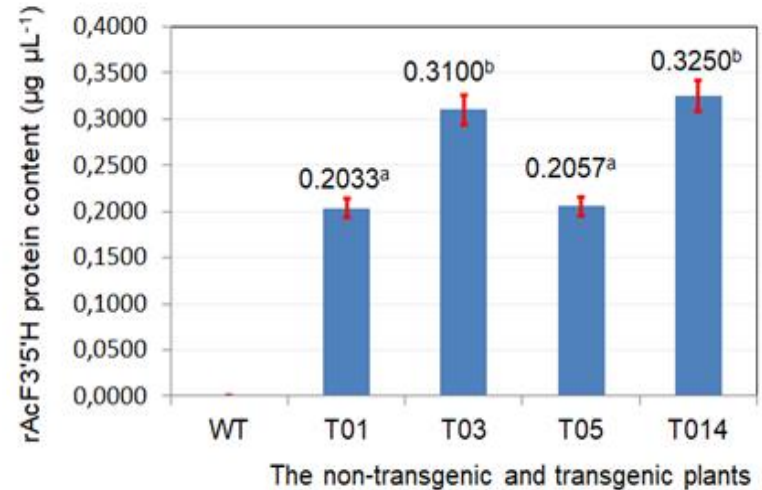
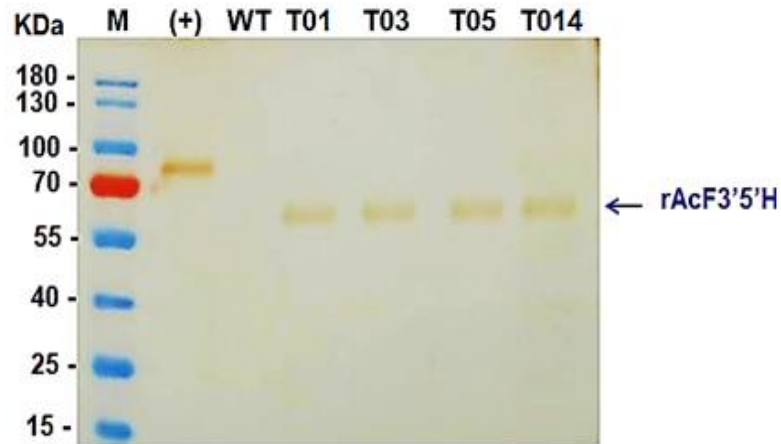


Figure 4. Expression of the rAcF3'5'H protein in transgenic tobacco plants in the T0 generation. A: Analysis of the expression of rAcF3'5'H protein by Western blot. M: Standard protein ladder; (+): H5 protein of influenza A/H5N1 virus with the c-myc tag as a positive control; WT: non-transgenic plants; T01, T03, T05, and T014: transgenic tobacco plants. B: Content of the rAcF3'5'H protein ($\mu\text{g } \mu\text{L}^{-1}$) in the T0 transgenic tobacco plants. WT: non-transgenic plants; T01, T03, T05, T014: T0 transgenic plants. Vertical bars represent the standard error. The different letters (a, b) above the columns indicate statistically significant differences ($P < 0.001$) as measured by Duncan's test, $n = 3$.

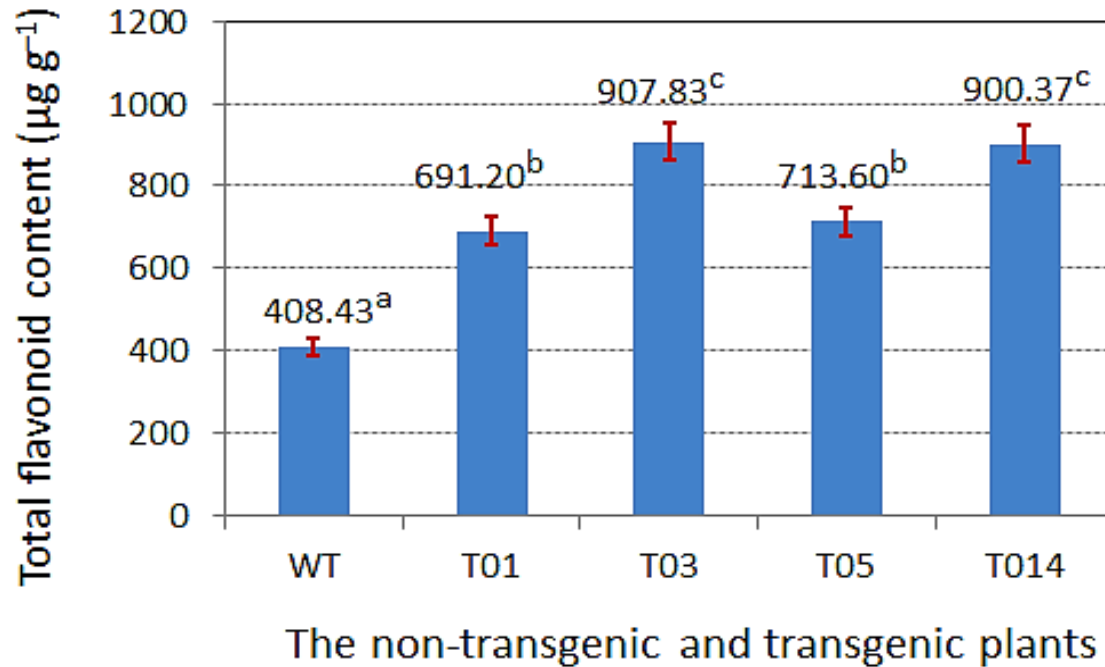


Figure 5. Total flavonoid content ($\mu\text{g g}^{-1}$) of four transgenic tobacco lines, T01, T03, T05, and T014, and non-transgenic plants. WT: non-transgenic plants; T01, T03, T05, T014: the T0 transgenic plants. Vertical bars represent the standard error. The different letters (a, b, c) indicate statistically significant differences ($P < 0.001$) as measured by Duncan's test, $n = 3$.