

Supplementary

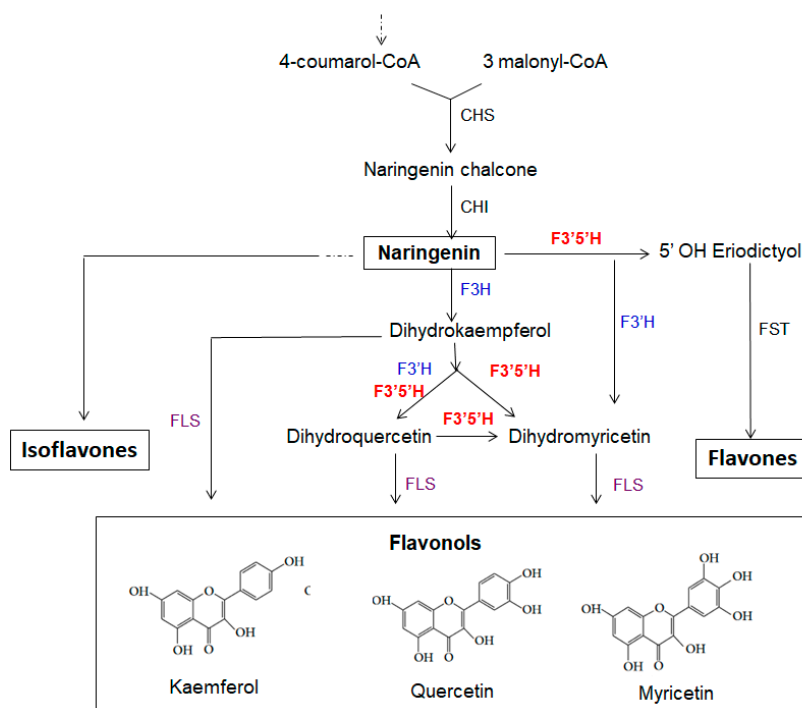


Figure S1. Flavonoid biosynthesis in plants (redrawn from [1-3]. CHS: chalcone synthase; CHI: chalcone isomerase; F3H: flavone 3-hydroxylase; F3'H: flavonoid 3'-hydroxylase; F3'5'H: flavonoid 3',5'-hydroxylase; FLS: flavonol synthase; FST: flavonol 4-sulfotransferase.

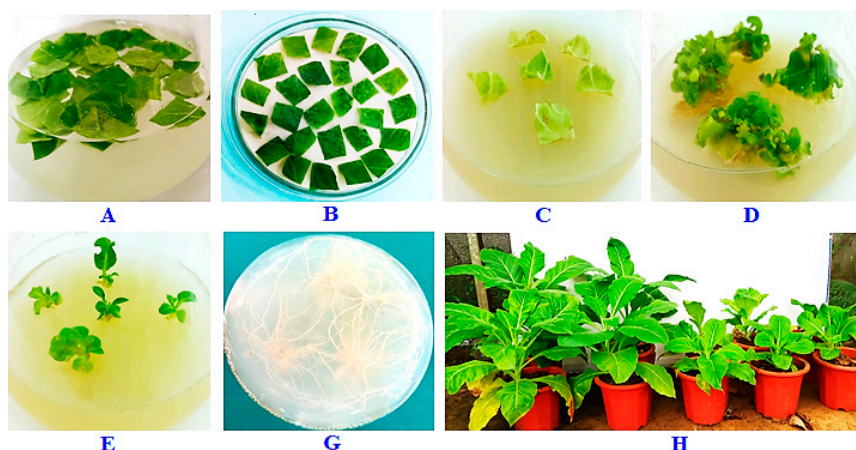


Figure S2. *AcF3'5'H* transgene transformation via *A. tumefaciens* and *in vitro* generation of the transgenic tobacco plants. (A) Leaf fragments infected using *A. tumefaciens* contain a *35S_AcF3'5'H_cmyc_KDEL* structure; (B) Transformed samples were transferred to CCM, in the dark; (C) The transformed samples cultured on SIM, including basic MS, 1.0 mg L⁻¹ BAP, 30 g L⁻¹ sucrose, 9.0 g L⁻¹ agar, with the addition of 500 mg L⁻¹ cefotaxime, and 50 mg L⁻¹ kanamycin; (D) Transformed samples were regenerated multiple shoots on SIM; (E) The shoots were removed from the samples and transferred to shoot-growing medium supplemented with 50 mg L⁻¹ kanamycin. (G) The shoots were root regenerated on RM, including basic MS, MES (1.0 g L⁻¹), sucrose (30 g L⁻¹), agar (9.0 g L⁻¹), coconut water (100 mL L⁻¹), and the addition of 0.5 mg L⁻¹ IBA, and 50 mg L⁻¹ kanamycin; (H) The seedlings were transferred to pots contained a mixture of 2 soil:1 rice husk biochar:2 coir.

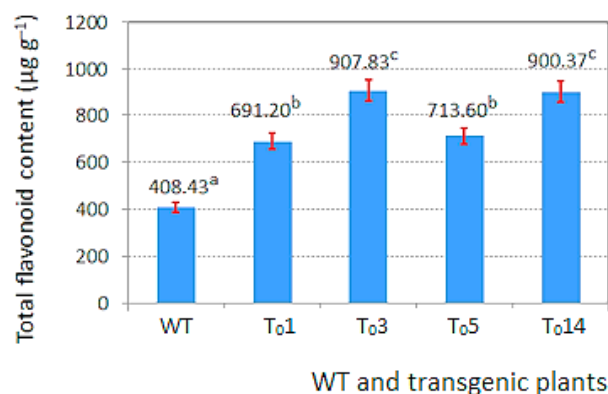


Figure S3. Total flavonoid content ($\mu\text{g g}^{-1}$) of four transgenic tobacco lines, T₀₁, T₀₃, T₀₅, and T₀₁₄, and WT plants. WT: non-transformed plants; T₀₁, T₀₃, T₀₅, T₀₁₄: the T₀ GM tobacco lines. The letters a, b and c on the columns represent difference with $P < 0.001$; $n = 3$.

Table S1. Fifteen plants in the top 100 blast hits of F3'5'H (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>)

Order	Species	Accession	Query Cover	Total Score	% identity
1	<i>Aconitum carmichaelii</i>	Quan Ba, Viet Nam			
2	<i>Aconitum carmichaelii</i>	JN635708	100%	2765	99.47%
3	<i>Aconitum carmichaelii</i> var. <i>truppelianum</i>	KY272865	100%	2699	98.69%
4	<i>Aconitum vilmorinianum</i>	JQ806761	99%	2501	96.38%
5	<i>Delphinium chefoense</i>	KX825847	94%	1701	88.12%
6	<i>Delphinium grandiflorum</i>	AY856345	95%	1698	87.81%
7	<i>D. grandiflorum</i> var. <i>chinense</i>	AB818394	98%	1657	86.59%
8	<i>Delphinium grandiflorum</i>	AB819289	98%	1652	86.52%
9	<i>Clematis patens</i>	LC169756	93%	876	77.90%
10	<i>Epimedium sagittatum</i>	HM011055	91%	512	73.65%
11	<i>Catharanthus roseus</i>	AJ011862	80%	343	72.56%
12	<i>Solanum lycopersicum</i>	EU626067	85%	278	70.88%
13	<i>Solanum lycopersicum</i>	NM_001247911	85%	267	70.77%
14	<i>Solanum lycopersicum</i>	GQ904194	33%	200	73.89%
15	<i>Theobroma cacao</i>	XM_018120633	2%	54.7	92.11%

References

1. Czemmel, S.; Stracke, R.; Weisshaar, B.; Cordon, N.; Harris, N.; Walker, A.; Robinson, S.; Bogs, J. The grapevine R2R3-MYB transcription factor VvMYB1 regulates flavonol synthesis in developing grape berries. *Plant Physiol.* **2009**, *151*, 1513–1530, doi:10.1104/pp.109.142059.
2. Shimada, Y.; Nakano-Shimada, R.; Ohbayashi, M.; Okinaka, Y.; Kiyokawa, S.; Kikuchi, Y. Expression of chimeric P450 genes encoding flavonoid-3', 5'-hydroxylase in transgenic tobacco and petunia plants. *FEBS Lett.* **1999**, *461*, 241–245, doi:10.1016/s0014-5793(99)01425-8.
3. Zabala, G.; Zou, J.; Tuteja, J.; Gonzalez, D.; Clough, S.J.; Vodkin, L. Transcriptome changes in the phenylpropanoid pathway of *Glycine max* in response to *Pseudomonas syringae* infection. *BMC Plant Biol.* **2006**, *6*, doi:10.1186/1471-2229-6-26.