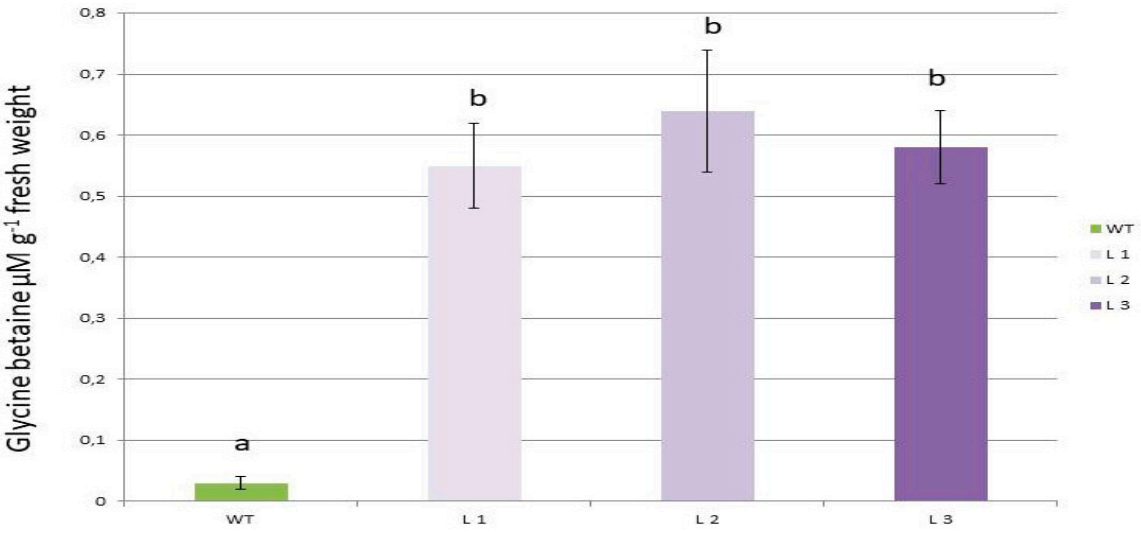
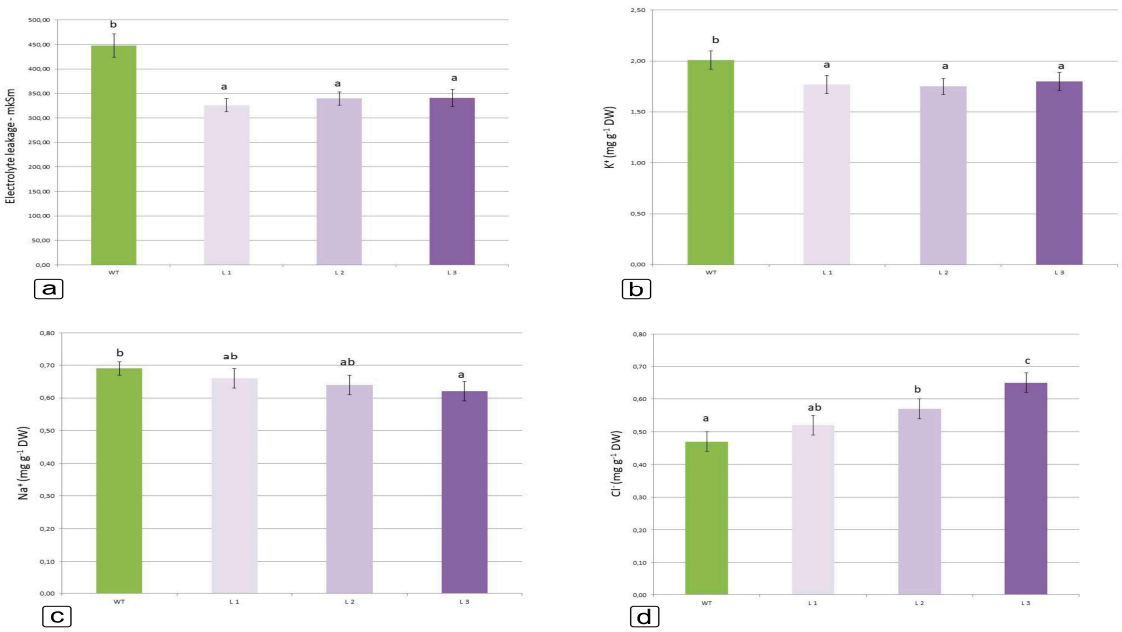
Heterologous *codA* Gene Expression Leads to Mitigation of Salt Stress Effects and Modulates Developmental Processes

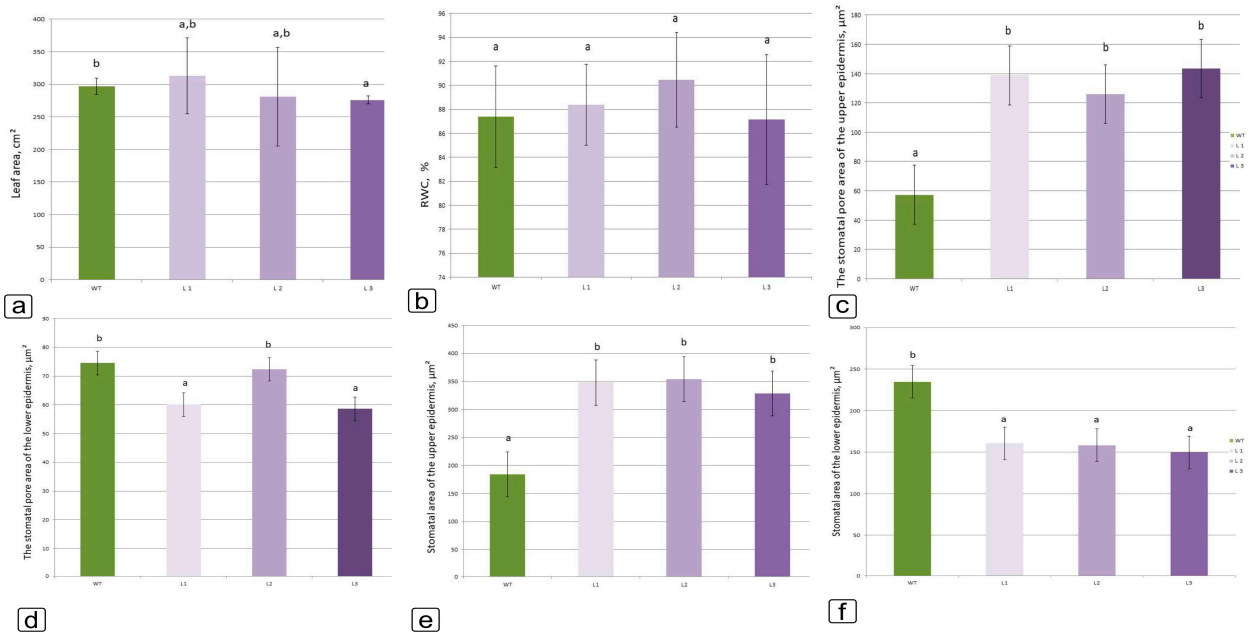
Supplementary Materials



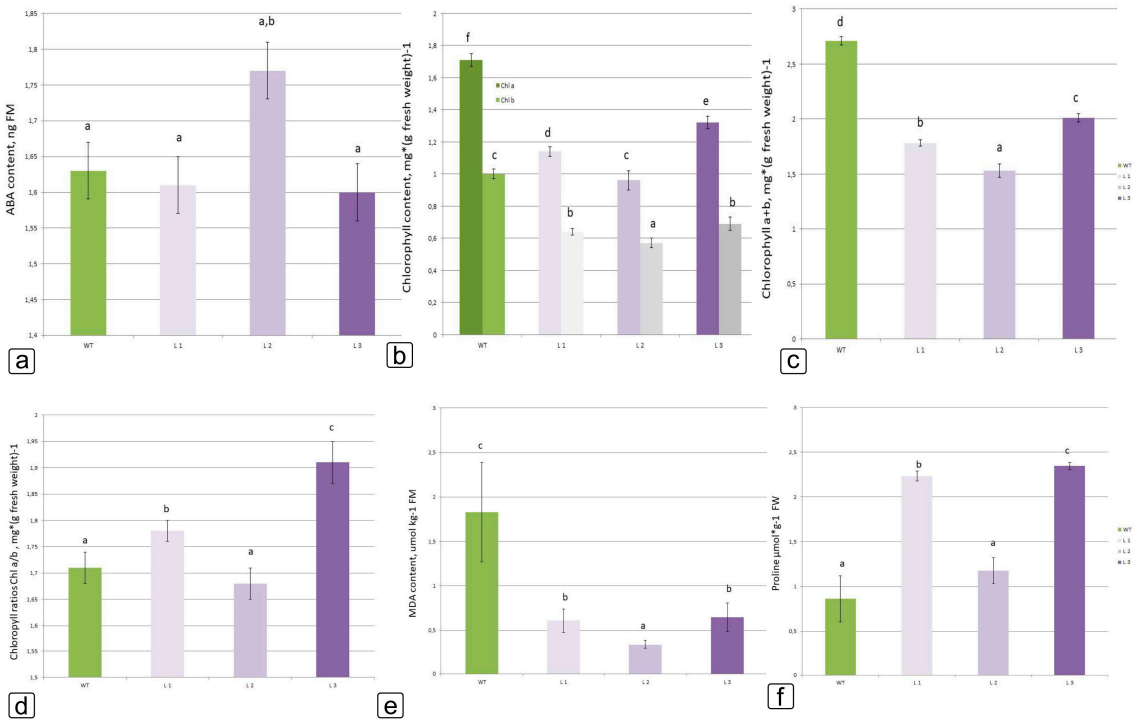
Supplementary Materials Figure S1. Glycine betaine content in transgenic plant lines. Control—WT tobacco plants; L1, L2, and L3—codA transgenic lines. Columns with the same letters are not significantly different ($p \leq 0.05$, Duncan's test).



Supplementary Materials Figure S2. Characterization of the physiological and biochemical features of electrolyte outflow (a), the content of K (b), Na (c), and Cl (d) ions in wild-type plants and *codA* transgenic tobacco plants with constitutive expression of choline oxidase gene.

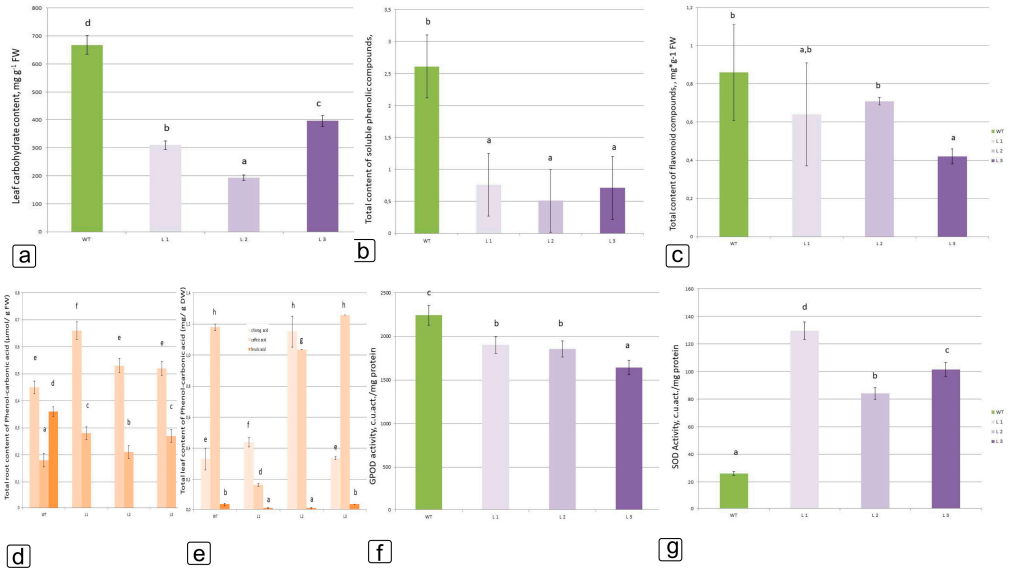


Supplementary Materials Figure S3. Characterization of the physiological and biochemical features of leaf area (a), hydration (b), size of stomata pore (c,d) and stomata cells (e,f) of upper (c,e) and lower(d,f) epidermis in wild-type plants and *codA* transgenic tobacco lines with constitutive expression of choline oxidase gene.

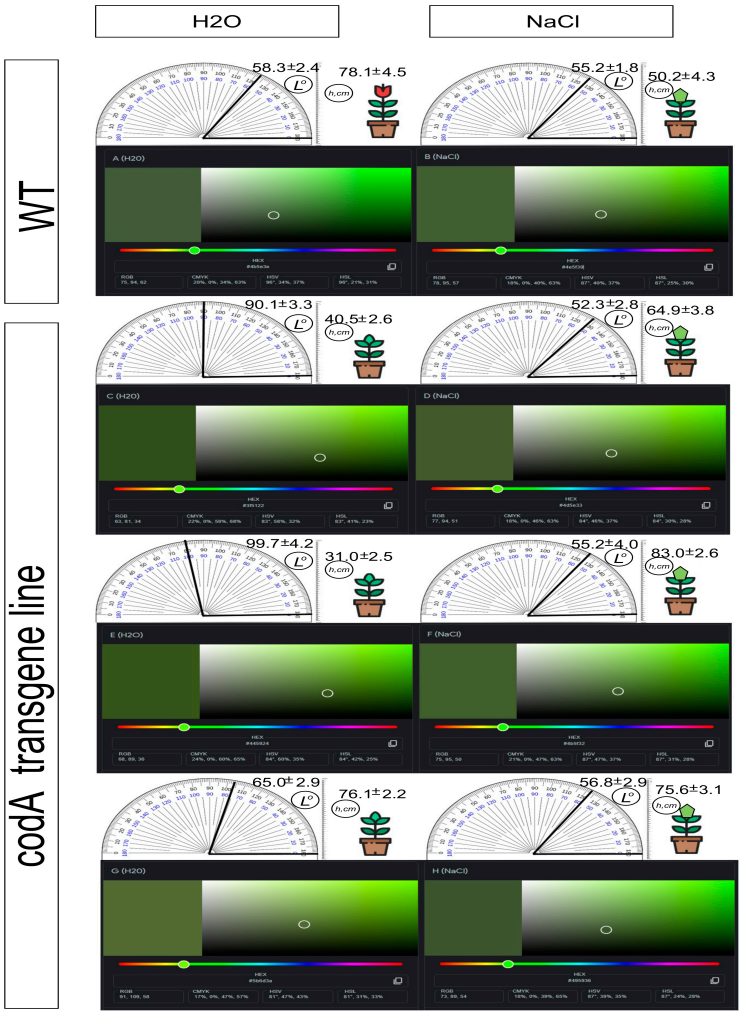


Supplementary Materials Figure S4. Characterization of the physiological and biochemical features of ABA (a), chlorophylls a and b (b), a+b sum (c) and their ratios (d), as well as MDA (e) and proline (f) in wild-type plants and *codA* transgenic tobacco lines with constitutive expression of choline oxidase gene.

Supplementary Materials Figure S5. Characterization of the physiological and biochemical



features of free sugar (a), phenolic compounds (b), flavonoids (c) and phenolic acids (chloric, caffeic and ferulic) in root (d) and leaf (e), as well as gPOD (f) and SOD (g) activity in wild-type plants and *codA* transgenic tobacco lines with constitutive expression of choline oxidase gene.



Supplementary Materials Figure S6. Results of phenotyping of control and *codA* transgenic lines of tobacco plants with assessment of plant height, angle of deviation of leaf petioles in the central part of plants, transition of plants to budding and flowering, green mass color parameters. Averaged data are given based on the results of measuring 5

independent plants of each variant during cultivation using water and NaCl solution (up to a final concentration of 200 mM NaCl based on the total moisture capacity of the substrate).

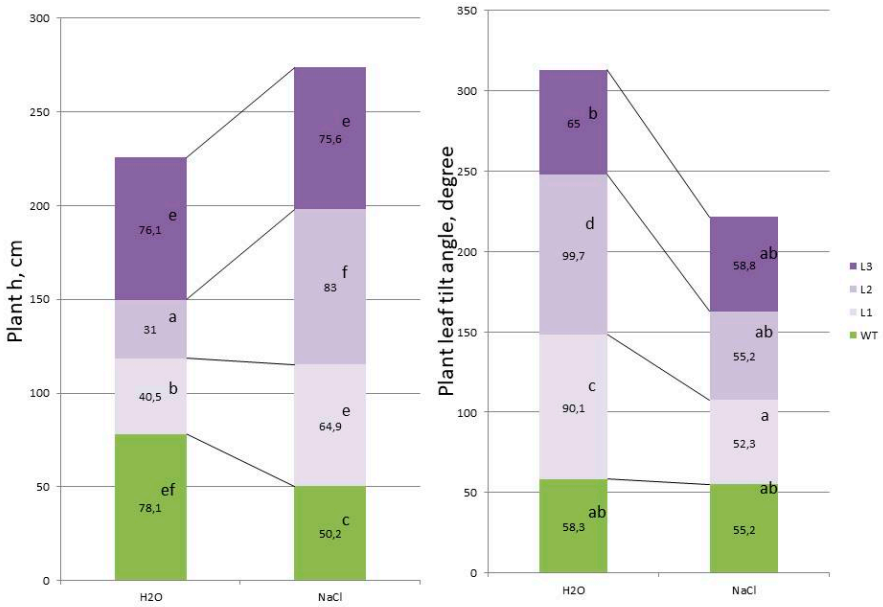


Figure S7. Characterization of the plant height and degree of deviation of leaf petioles from the stem in response to 200 mM NaCl salinity in WT plants and *codA* transgenic tobacco plants.

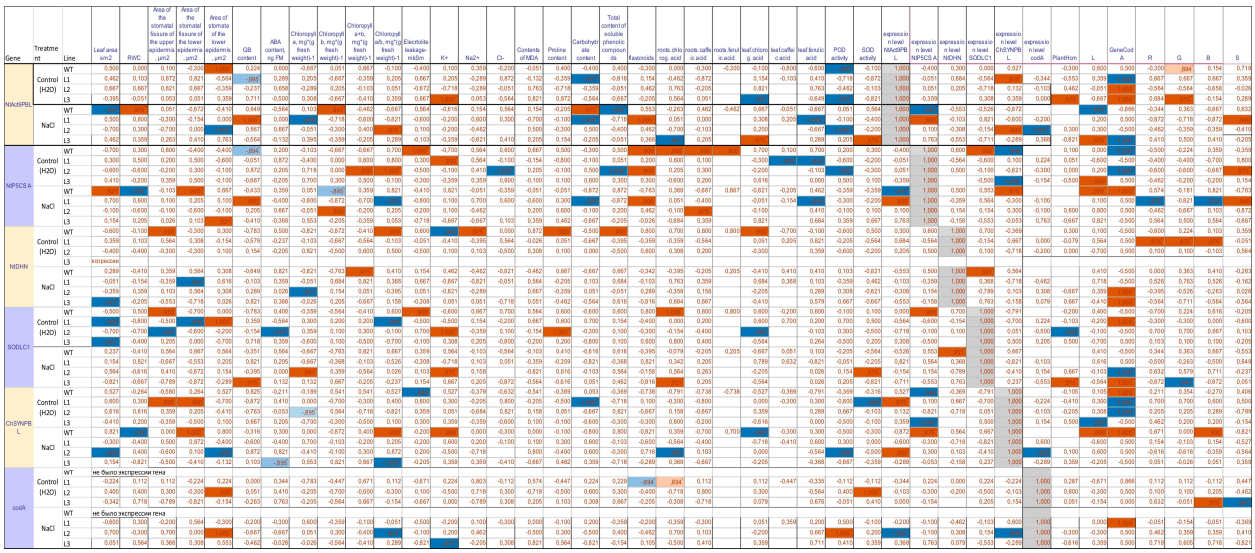


Figure S8. Spearman's correlation coefficients between indicators of gene expression and the main morphometric and biochemical parameters. Legend: only significant correlation coefficients are highlighted, * - correlation is significant at p=0.05, ** - correlation is significant at p=0.01; dark blue - very strong inverse (negative) correlation (r<-0.9), blue -

strong inverse (negative) correlation (r<-0.8), dark orange - very strong direct (positive) correlation (r>0.9), orange - strong direct (positive) correlation (r>0.8).

Figure S9. The choline oxidase expression cassette. Обозначения: RB and LB—right and left border sequences of T-DNA; codA—choline oxidase gene with transit plastid targeted peptide sequence (pls); NPTII—neomycin phosphotransferase gene; NOS pro—nopaline synthase promoter; NOS ter—nopaline synthase terminator; 35S pro—promoter of cauliflower mosaic virus CaMV 35S RNA.

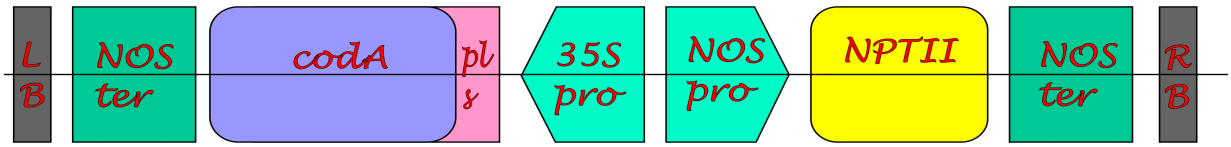


Table S1. The primers used in the gene expression analysis

	Title	Sequence 5` → 3`	Primer length	T°
codA	codA_F	TGCACCTGATGAACGTGCC	19	58-60
	codA_R	GGTGGAGAGCACGACTTCAT	20	

	Title	Sequence 5` → 3`	Primer length	T°
Actin	NtAct2_F	TGCACCTGATGAACGTGCC	19	58-60
	NtAct2_R	GGTGGAGAGCACGACTTCAT	20	
	NtAct9PBL_F	TCGCTGATCGAGTCCAATCTG	20	60
	NtAct9PBL_R	GCATGAACAACGACGTGCAA	20	

	Title	Sequence 5` → 3`	Primer length	T°
P5CS A	NtP5CS A_F	TGCACCTGATGAACGTGCC	19	60
	NtP5CS A _R	GGTGGAGAGCACGACTTCAT	20	

	Title	Sequence 5` → 3`	Primer length	T°
ERD10B	DehF2_F	ATGGACAAGGCGGAAGAAG	20	60
	DehR2_R	GTTGTTGCAGTTGAATGAGT	19	

	Title	Sequence 5` → 3`	Primer length	T°
Zn/CuSOD	SODLC1_F	CACAGTTACTGGAAATGTCTCT	22	60

	SODLC1_R	GATTGTAATGTGGTCCCGTTGA	22	

	Title	Sequence 5` → 3`	Primer length	T°
ChSyn	ChSYNPBL1F	TCTCTGCAGCCCAAACTCTT	20	58-60
	ChSYNPBL1R	GTTGGAATGCTTCCACAAGGC	22	

Figure S10.

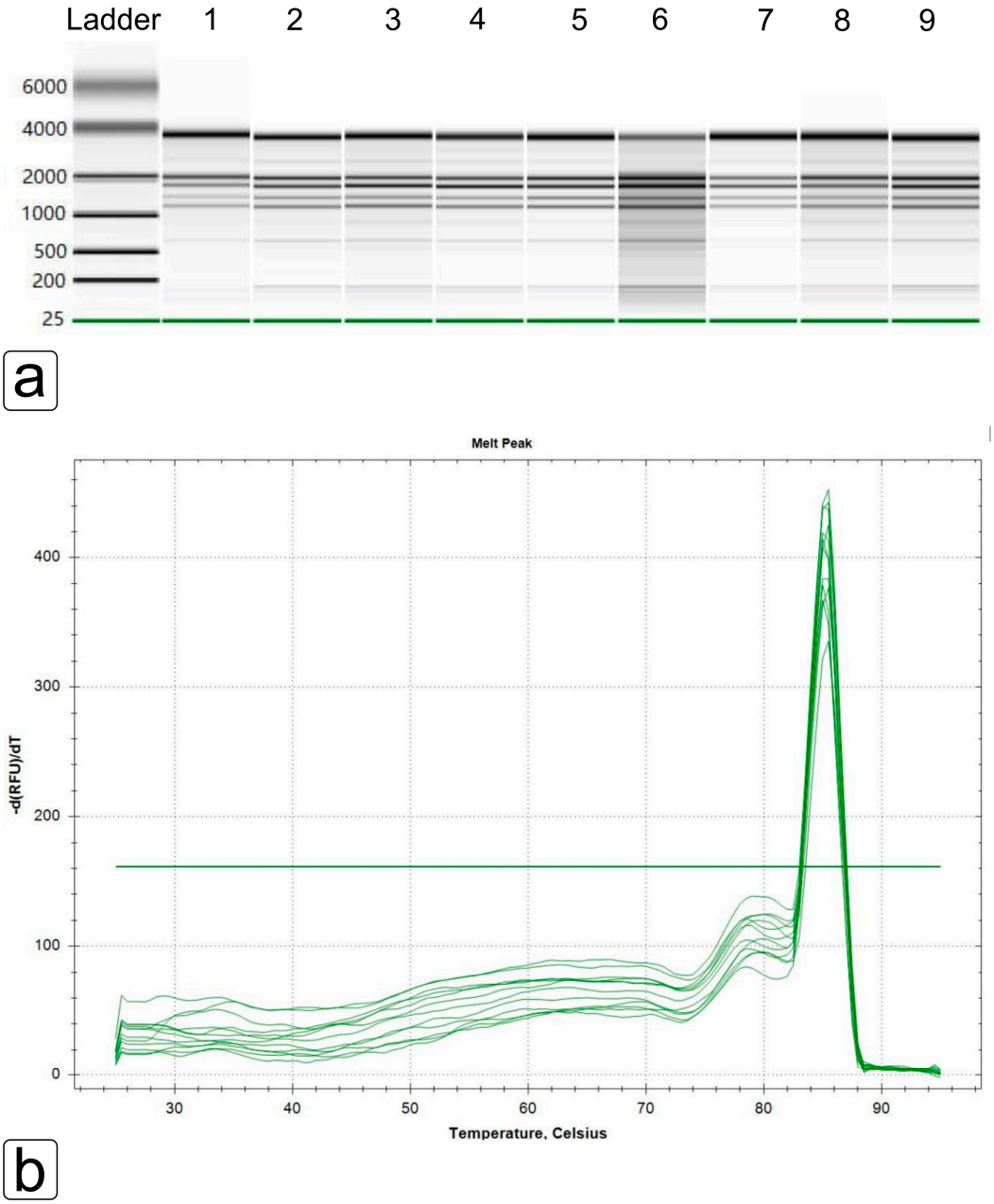


Figure S10. Integrity of RNA samples isolated from leaf tissue of tobacco plants.

(a) Electrophoregram of RNA ladder and total RNA isolated by trizol method.

(b) Melt curve analysis. The SYBR Green qPCR reactions contained 10 ng RNA-equivalent cDNA from tobacco leaf tissue. The reactions were performed with CFX96 Touch real-time amplifier. The reaction mixtures contained variable primer pair concentrations.