

The title page

The manuscript title:

Overexpression of the Eggplant (*Solanum melongena*) NAC Family Transcription Factor, *SmNAC*, Suppresses Resistance to Bacterial Wilt.

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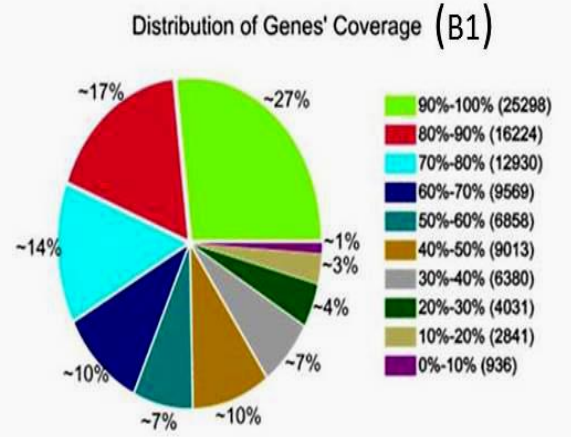
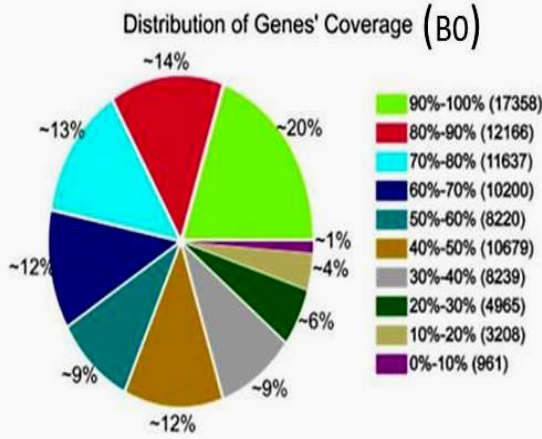
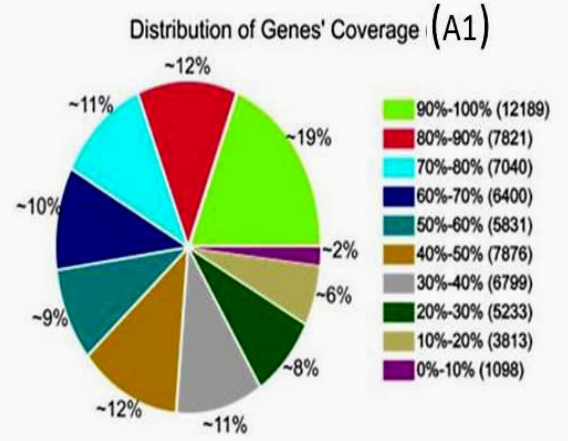
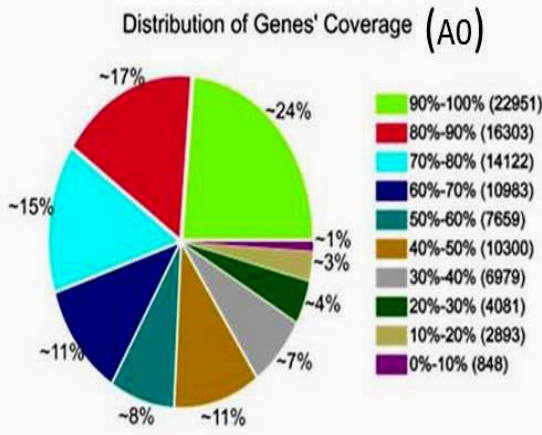
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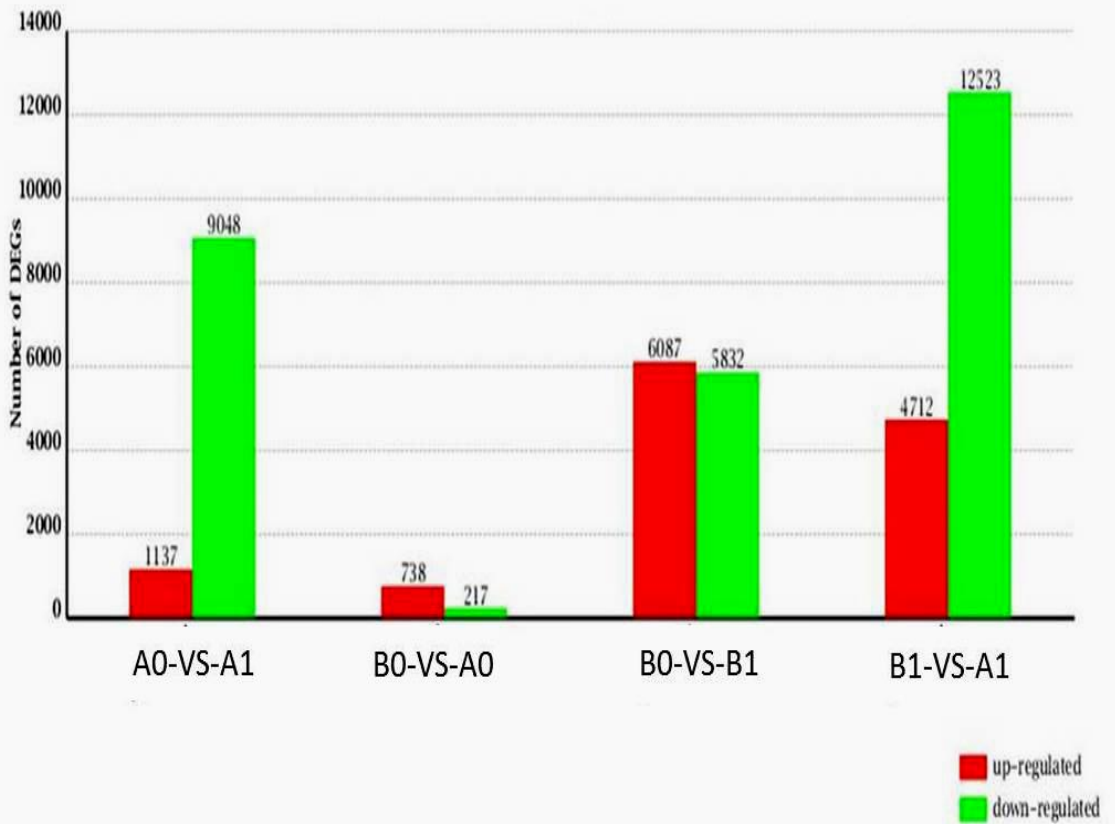
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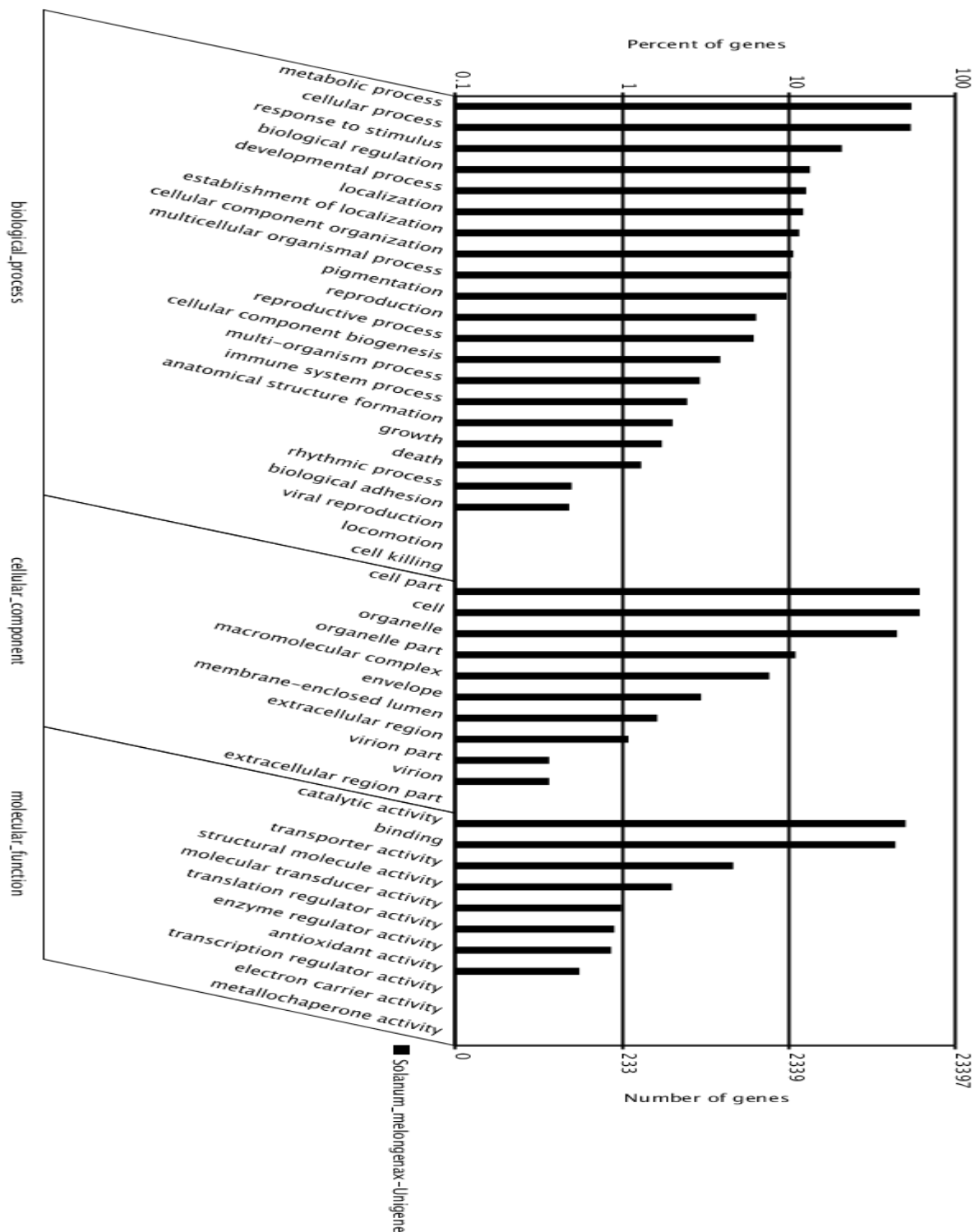


Fig.S1. Distribution of gene coverage in each RNA-seq library, Differential gene expression analysis and GO-terms.

RNA-Seq analysis was performed on resistant (E-31) and susceptible (E-32) eggplant lines. Lines A0 and A1 corresponded to mock-inoculated and pathogen-inoculated E-31 plants at 7 days post-inoculation (dpi). Similarly, B0 and B1 correspond to mock-inoculated and pathogen-inoculated E-32 plants at also 7 dpi. RNA was extracted from ten plants stems of each of these four lines and used for RNA-Seq library construction, with 2 replicates for each treatment.

(a) Distribution of gene coverage of each library. (b) Differential gene expression analysis.

(c) Frequency of sequences with assigned Gene Ontology (GO) terms (slimmed) for the category biological process found in the stem tissue assemblies.

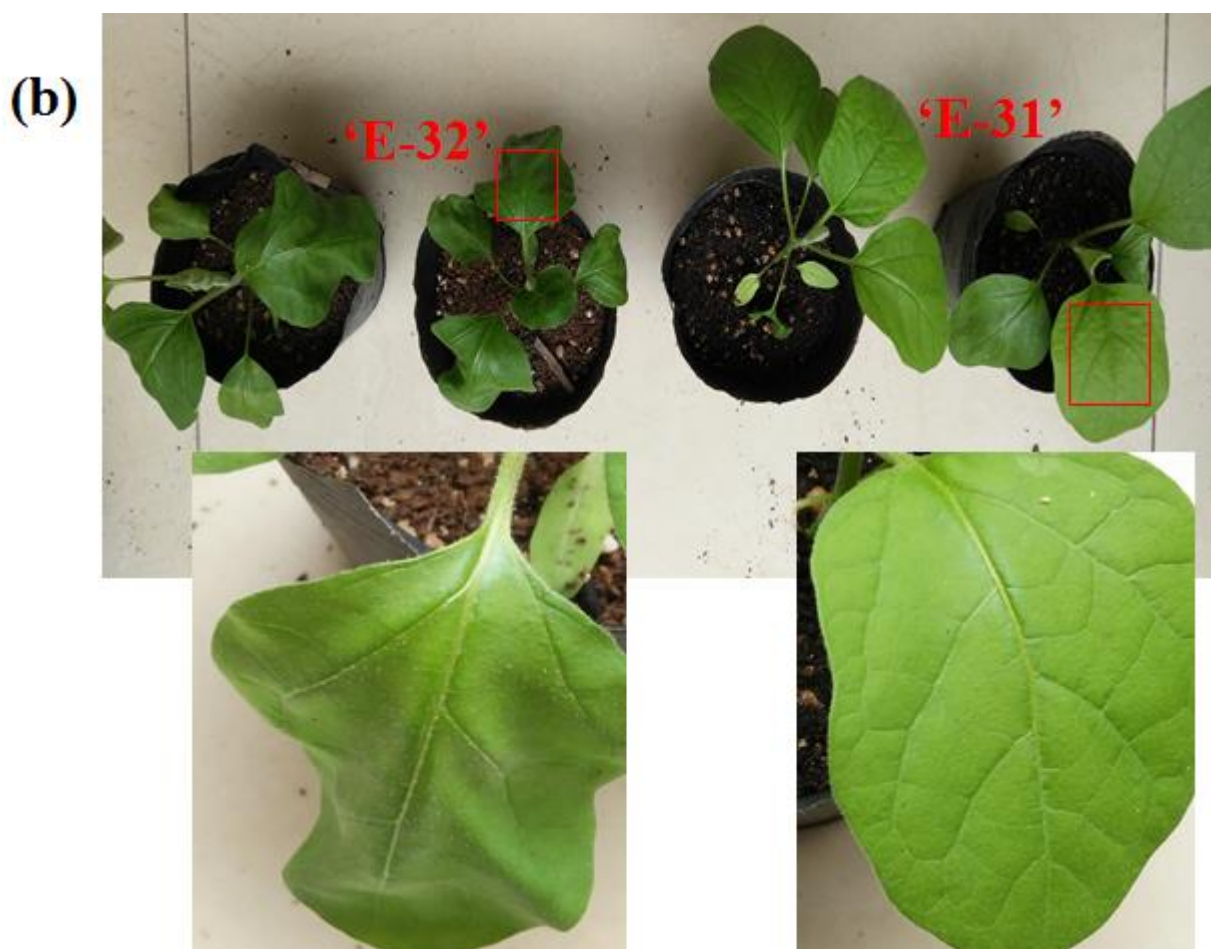
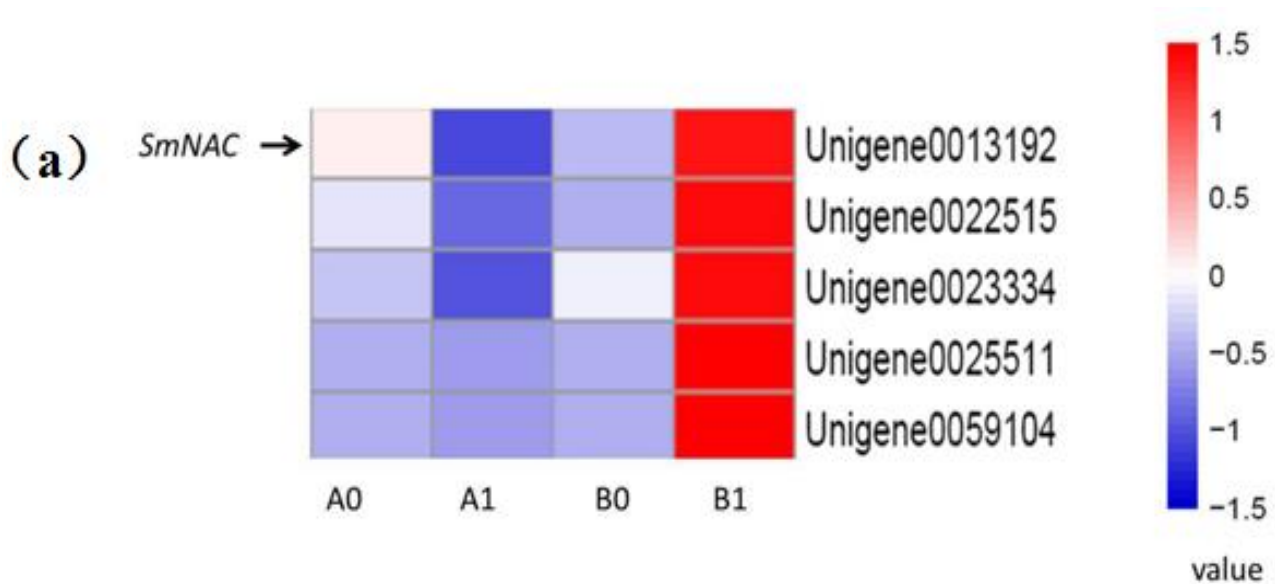


Fig.S2. The heat maps depict the expression patterns of *SmNAC* in resistant plant and susceptible plant, and the phenotypic symptoms of susceptible plants after inoculation with *Ralstonia solanacearum*.

(a): The heat maps depict the expression patterns of *SmNAC* in resistant plant and susceptible plant. Heat-map values represent log-transformed relative expression values.

A0 and A1 corresponded to mock-inoculated and pathogen-inoculated E-31 plants, 7 days post-inoculation (dpi). B0 and B1 correspond to mock-inoculated and pathogen-inoculated E-32 plants, also 7 dpi. Arrow showing the expression level of *SmNAC*.

(b): The phenotypic symptoms of eggplants after 15 days inoculation with *Ralstonia solanacearum*. 'E-32': susceptible plant; 'E-31': resistant plant.

Fig.S3. A phylogenetic tree including *SmNAC* and related proteins.

Phylogenetic tree of NAC proteins (black circles). Protein sequences were aligned with those of the *A. thaliana* and rice NAC families. A multiple sequence alignment was carried out using CLUSTALW, and the phylogenetic tree was constructed in MEGA6.0 using the minimum evolution test and default parameters. Bootstrap values indicate the divergence of each branch and the scale indicates branch length. The full-length amino acid sequences of the ANAC proteins were obtained from the Plant Transcription Factor Database (<http://planttfdb.cbi.pku.edu.cn/family.php?sp=Ath&fam=NAC>) and the full length amino acid sequences of the ONAC proteins were obtained from the Rice genome annotation project (<http://rice.plantbiology.msu.edu>). Full length sequences were used in the analysis.

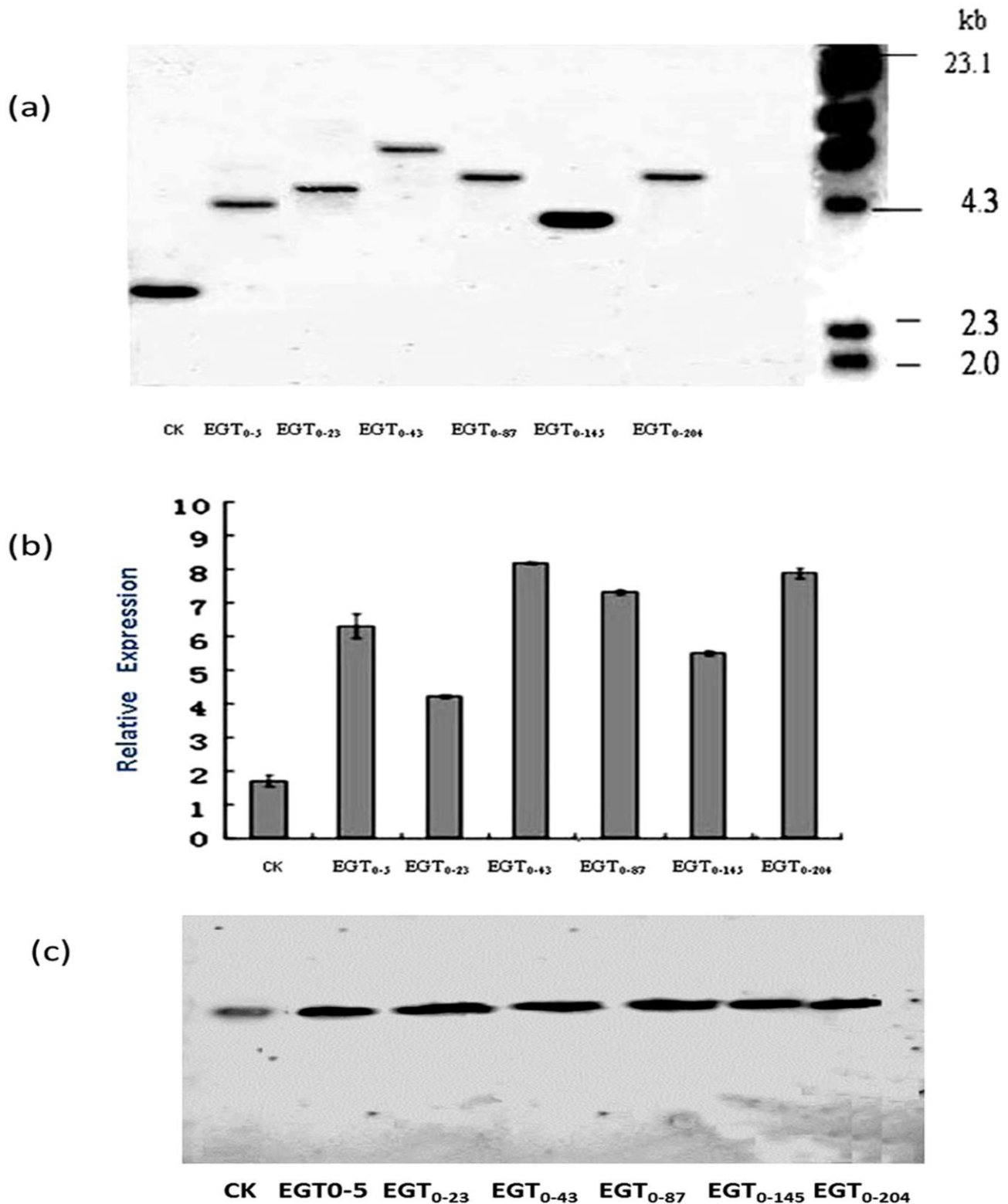


Fig.S4. Southern blot, qRT-PCR and Western blot analyses of *SmNAC* in *SmNAC*-overexpressing plants.

(a) Genomic DNA from transgenic Km-resistant plants was digested with *Hind*III, and a *NPT-II* gene fragment was used as a probe. CK: pBI-*SmNAC*. (b) qRT-PCR analysis of *SmNAC* expression in transgenic plants using the primers: F-P1 5'-GAAAATTAGAAAGATTACAAC-3' and R-P1 5'-TAAAAGAATATACATGTCCCT-3'. CK: Non-transgenic plant from the 'E-31' line. (c) Western blot detection of *SmNAC* in overexpression transgenic plants, anti-*SmNAC* was used as the probe. CK: Non-transgenic plant from the 'E-31' line; EGT₀₋₅, EGT₀₋₂₃, EGT₀₋₄₃, EGT₀₋₈₇, EGT₀₋₁₄₅, and EGT₀₋₂₀₄ represent the *SmNAC*-overexpression plants.

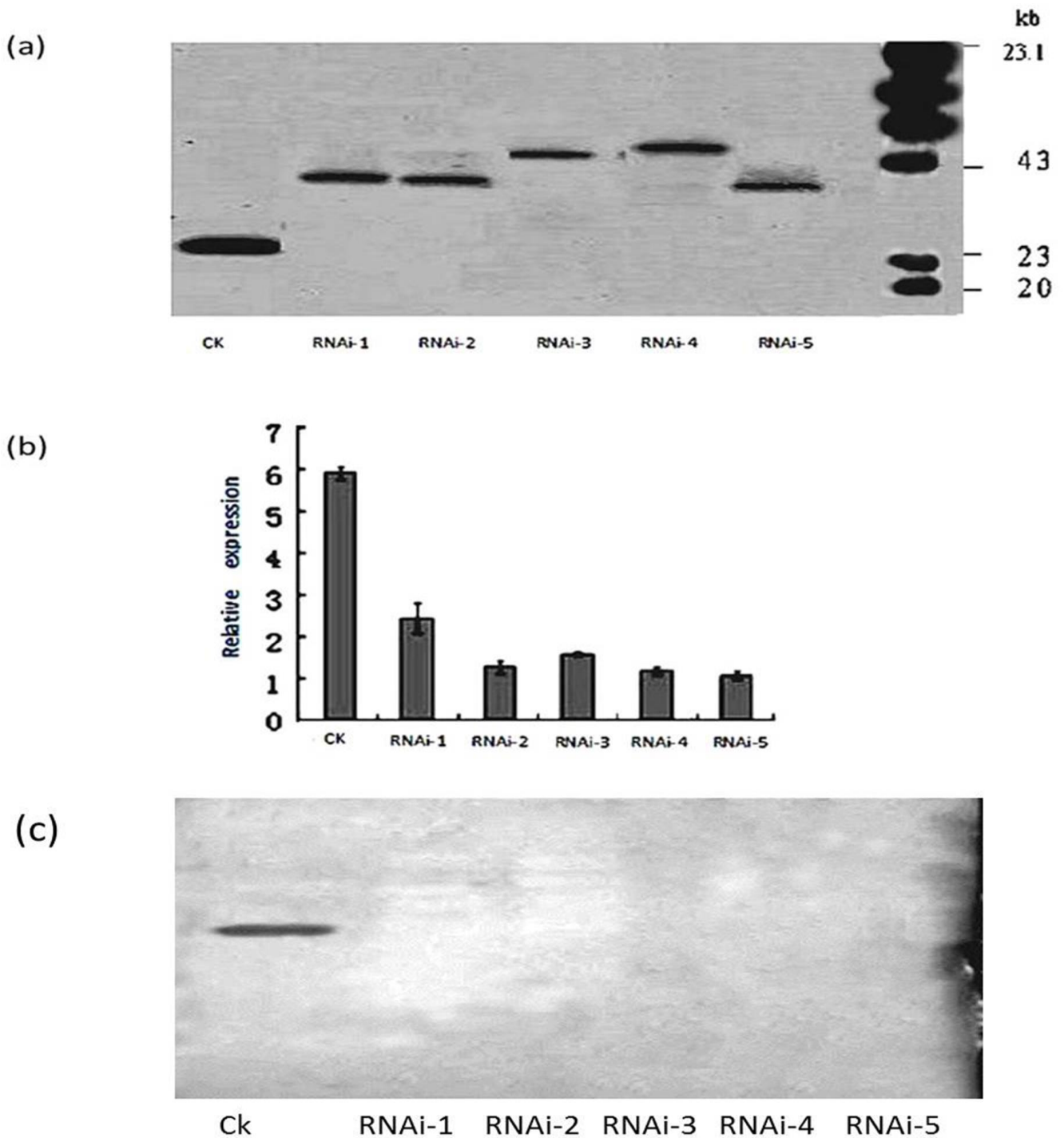


Fig.S5. Southern blot, qRT-PCR and Western blot analysis of expression of *SmNAC* in RNAi-*SmNAC* plants.

(a) Genomic DNA from the resistant transgenic plants was digested with *Hind* III, and *Bar* gene fragments were used as a probe. CK: pFGC5941-*SmNAC*. (b) qRT-PCR analysis of *SmNAC* in transgenic plants using the primers: F-P1 5'-GAAAATTAGAAAGATTACAAC-3' and R-P1 5'-TAAAAGAATATACATGTCCT-3'. CK: Non-transgenic plants from the 'E-32' line. (c) Western blot detection of *SmNAC* RNAi transgenic plant, anti-*SmNAC* was used as the probe. CK: Non-transgenic plants from the 'E-32' line; RNAi-1, RNAi-2, RNAi-3, RNAi-4, and RNAi-5 represent the *SmNAC*-RNAi transgenic plants.

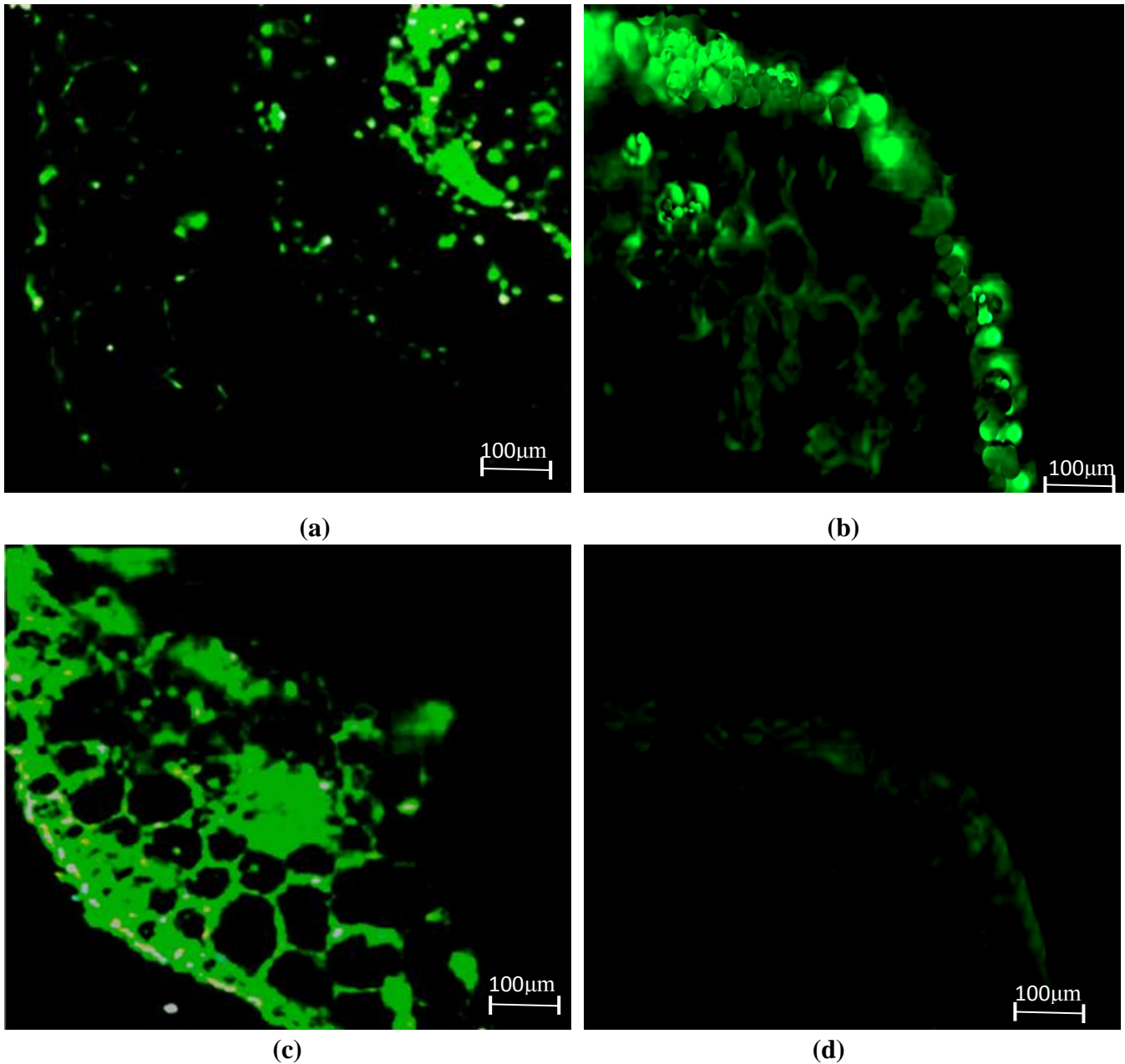


Fig.S6. Immunohistochemical localization analysis of *SmNAC* in *SmNAC*-overexpressing and *SmNAC*-RNAi transgenic plants.

Fresh *S. melongena* stem tissues were used, and cross-sections of eggplant tissue labeled with purified *SmNAC* antibodies and visualized using an anti-rabbit IgG conjugated to green fluorescent protein. Scale bars, 100µm. Each experiment repeated three times.

- (a) *SmNAC* protein distribution in the stem of a non-transgenic plant (E-31);
- (b) *SmNAC* protein distribution in the stem of a *SmNAC*-overexpressing plant (EGT₁₋₈₇);
- (c) *SmNAC* protein distribution in the stem of a non-transgenic plant (E-32);
- (d) *SmNAC* protein distribution in the stem of a *SmNAC*-RNAi transgenic plant (RNAi-2-1).

TableS1 primer sequence of qRT-PCR

Gene name	Forward(5'-3')	Reverse(5'-3')	Gen Bank accession
<i>Pin2</i>	TGATGCCAAGGCTTGTACTAGAGA	AGCGGACTTCCTTCTGAACGT	AY129402
<i>JAR1</i>	GCAGCTATGGGGTTTGAATGGCAGA	AAGAGGTCAGGAATCAAGCCGTACC	DQ359730.1
<i>LoxA</i>	GACCAAAACGCTCGTCTCTC	TGGTAGACCACCAACACGAA	U09026
<i>EDS1</i>	GTTCGCAGACAAGTTGAGCC	CTCTGTGTGAACCGATAACGC	AY679160.1
<i>GluA</i>	GCCGACTGGGTGAGATGGTAA	ACATTGTTGTGCCCGTGGAC	M80604
<i>NPR1</i>	CTTGGACTGGGTGTTGCTAATG	TGCCCATCCAATGTAATGTCTG	NM_001247633.1
<i>TGA</i>	GCAAGTGACCCTGAACTACGAAG	GGGTTTTCCACATCCCTGACAAG	GQ386946.1
<i>SGT1</i>	TTCTCGGTTTTGAGGAAGGG	GCAGATACCAAGTGATGTCTACCA	NM_001247758.1
<i>PAD4</i>	ACATCGGCTGAAACCTCCTTATT	TTTGATAAGTGGTGGGGAAATGA	AY753546.1
<i>PR-1a</i>	GAGGGCAGCCGTGCAA	CACATTTTTCCACCAACACATTG	M69247
<i>EIN2</i>	GGGACGAACGGGCACTGCAGCA	GTGATGAGGCAGACAGCGTTACT	AY566238
<i>EIL1</i>	TTGAACGACAGCCCTGAGTATG	AGGTGTCCAGTTCTTTCTTTGTTTCG	NM_001247612.1
<i>ICS1</i>	GCATGGGACAATGCTGCTGCCTCATGGA	TCTGGTGCTACGAGCAAGTACCACCT	NM_001247865.1
<i>PBS3</i>	GTTTGCACTATGTATGCTTCCTCTG	GCTCATACTCCTGACCAATCTTgtcGACA	XM_004251437.2
<i>BSMT1</i>	TATGAGTGGGAACCTTTTTGGCTACGACGC	GCCATATCGTATTTTCATCATCACAAAG	XM_004237341.1
<i>SAGT1</i>	GCCTTCGTTTATTACGTACATGGAACG	CCACATGGAATAGATTGAGACCATAC	XM_004245012.1
<i>ACTIN</i>	TATTGTGGGTCGTCCTCG	TCTCTCTGTTGGCCTTGG	XM_016658880.1

TableS2 Primer sequences used for promoter isolation

Primer	Sequence of primer
LAD1	5'-ACGATGGACTCCAGAGNTCGA(G/C)T(A/T)T(G/C)G(A/T)GTT-3'
LAD2	5'--ACGATGGACTCCAGAG NGTCGA(G/C)(A/T)GANA(A/T)GAA-3'
LAD3	5'--ACGATGGACTCCAGAG (A/T)TCTGNCT(A/T)ANTANCT-3'
LAD4	5'--ACGATGGACTCCAGAG AG(A/T)GNAG(A/T)ANCA(A/T)AGG-3'
AC1	5'-ACGATGGACTCCAGAG-3'
SP1	5'-AGGATCGGACTTAATCATATCGGAGATGGC-3'
SP2	5'-GCAGGGCTAGAAACTGCAGGCAAAGT-3'
SP3	5'-CCAATAGGAGCTCTTGAATCGCCTTGGCA-3'

TableS3 Main regulatory motifs found within the promoter sequence of *ICS1* in eggplant

Factor or site name	Site	Signal sequence	Function
ABRE	167(+),1350(+)	CACGTG	cis-acting element involved in the abscisic acid responsiveness
AE-box	881(-)	AGAAACAT	part of a module for light response
ARE	1134(-)	TGGTTT	cis-acting regulatory element essential for the anaerobic induction
ATCT-motif	501(+)	AATCTAATCT	part of a conserved DNA module involved in light responsiveness
Box-4	343(+),265(+)	ATTAAT	part of a conserved DNA module involved in light responsiveness
Box -I	9(-),846(-), 615(+),1075(+)	TTTCAAA	light responsive element
ERE	125 (-) ,1389(-)	ATTTCAAA	ethylene-responsive element
CAAT-box	109(+),316(+)	CAAT	common cis-acting element in promoter and enhancer regions
CAT-box	471(+),936(+), 1138(+),1323(+) 1336(+)	GCCACT	cis-acting regulatory element related to meristem expression
EIRE	125(-),1389(-)	TTCGACC	elicitor-responsive element
G-Box	167(+), 956(+), 1350(+)	CACGTG, CACGTT	cis-acting regulatory element involved in light responsiveness
HD-Zip 1	37(+)	CAAT(A/T)ATTG	element involved in differentiation of the palisade mesophyll cells
HSE	73(+),998(+), , 1150(+)	AAAAAATTTC	cis-acting element involved in heat stress responsiveness
Skn-1motif	136(+),495(-)	GTCAT	cis-acting regulatory element required for endosperm expression
TATA-box	69(+),145(+), 242(+),386(+),489(+), 642(+),789(-),908(-), 116(-),1163(+)	TATAA	core promoter element around -30 of transcription start
TC-rich repeats	928(+)	ATTTTCTTCA	cis-acting element involved in defense and stress responsiveness
WUN-motif	808(-)	AAATTCCT	wound-responsive element
Circadian	618(+)	CAAAGATATC	cis-acting regulatory element involved in circadian control
CACG	364(+),427(+), 681(+),1152(+), 1190(+), 1547(+)	CACG	core binding site of NAC factor domain