

Supplementary Materials: Overexpression of *Arabidopsis AnnAt8* Alleviates Abiotic Stress in Transgenic *Arabidopsis* and Tobacco

Deepanker Yadav, Israr Ahmed, Pawan Shukla, Prasanna Boyidi
and Pulugurtha Bharadwaja Kirti

Table S1. Primers used in the amplification of *AnnAt8* for the construction of recombinant vectors.

Cloning	Primer Name	Primer Sequence
pRT100	<i>AnnAt8</i> (ORF1) <i>Nco</i> I F	5'-CCCATGGCCACCATTGTTTCTCC-3'
	<i>AnnAt8</i> (ORF1) <i>Xba</i> I R	5'-CTCTAGACTAAAGTTGTTTTCCATGTCCT-3'
pEGAD	<i>AnnAt8</i> (ORF2) <i>Eco</i> RI F	5'-AAGAATTCATGGCCACCATTGTTTCTCC-3'
	<i>AnnAt8</i> (ORF2) <i>Sma</i> I R	5'-AACCCGGGCTAAAGTTGTTTTCCATGTCCT-3'

Table S2. Primers used in the amplification of *AnnAt8* and *NptII* for the confirmation of the transgene presence in the putative transformants.

Gene	Primer Sequence
<i>AnnAt8</i>	F-5'-CCCATGGCCACCATTGTTTCTCC-3'
	R-5'-CTCTAGACTAAAGTTGTTTTCCATGTCCT-3'
<i>NptII</i>	F-5'-GAGGCTATTCGGCTATGACTG-3'
	R-5'-ATCGGGAGCGGCGATAACCGTA-3'

Table S3. Primers used for semi quantitative and real time PCR analysis of various genes expressed under stress.

Gene	Gene Bank ID	Primer Sequence
<i>NtDREB3</i>	EU727157.1	F-5'-ATGGCTTGGCACTTTCCTT-3'
		R-5'-ATATTCTTGGCGTCGGAGGA-3'
<i>NtSAMDC</i>	U91924.1	F-5'-CAGTCGTTTCCTCACCGTCA-3'
		R-5'-ATAGGTCCTGCAGAGGCAGA-3'
<i>NtAPX</i>	U15933.1	F-5'-GTTTGGGCTTTTCTCCTCGAC-3'
		R-5'-GGAGCATAAGAGGAGCGCAA-3'
<i>NtMnSOD</i>	AB093097.1	F-5'-TCCCCTACGACTATGGAGCA-3'
		R-5'-CGGTATGCAATTTGGCGACG-3'
<i>NtERF5</i>	AY655738.1	F-5'-GGATTGTCTCCTGCTGCTGT-3'
		R-5'-GCTCTTCTAATAACTCAGCACCC-3'
<i>NtERD10D</i>	AB049338.1	F-5'-GCACGAGGGAAGAAGAGAAGG-3'
		R-5'-TGGAGGCGCCACTTCCTC-3'
<i>NtERD10C</i>	AB049337.1	F-5'-AAAGCCAACTCATGCCCAAG-3'
		R-5'-AGAGCTGCTACTTGATCGATGG-3'
<i>NtNCED3</i>	JX101472.1	F-5'-TGTCTGAAATGATCCGGGGC-3'
		R-5'-AGTTTCCGGCTCTTCCCAAG-3'
<i>NtSUSY</i>	AB055497.1	F-5'-CACGGATATTTGCCCCAGGA-3'
		R-5'-GCAGCAGCCGAGTAGCAATA-3'
<i>NtP5CS</i>	HM854026.1	F-5'-GCTGCTCAACAGGCTGGATA-3'
		R-5'-CCATCAGCAACCTCCGTTCT-3'
<i>NtSOS1</i>	XM_009789739.1	F-5'-CAAATGTTATCCCCCGAAAGC-3'
		R-5'-CGGAGAACCTGAGGAAATGTGA-3'

Table S3. Cont.

Gene	Gene Bank ID	Primer Sequence
<i>NtActin</i>	EU938079.1	F-5'-TTTCCGATGCCCTGAAGTCC-3' R-5'-CATAGTCGAACCGCCACTGA-3'
<i>NtCAT</i>	U93244.1	F-5'-GGCCGCTACAACCTCTCTTT-3' R-5'-ACAGGACCTCTTGCACCAAC-3'
<i>Nt18S</i>	AJ236016.1	F-5'-CCAGGTCCAGACATAGTAAG-3' R-5'-GTACAAAGGGCAGGGACGTA-3'

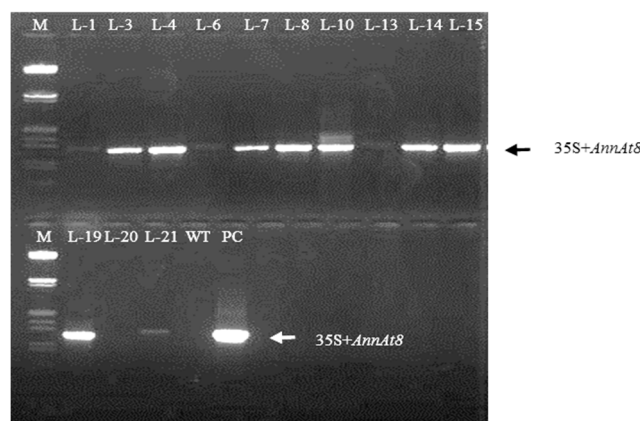


Figure S1. Confirmation of putative transgenic lines by PCR using *Arabidopsis* genomic DNA. A representative PCR gel picture showing amplified product for 35S+AnnAt8 from thirteen putative *AnnAt8* transgenic (T_i) *Arabidopsis* plants. Letter 'PC' corresponds to positive control for the PCR using plasmid (pCAMBIA2300::AnnAt8) as a template. Letter 'WT' corresponds to negative control for the PCR using DNA from untransformed plant. Letter 'M' correspond to λ HindIII/EcoRI DNA Marker.

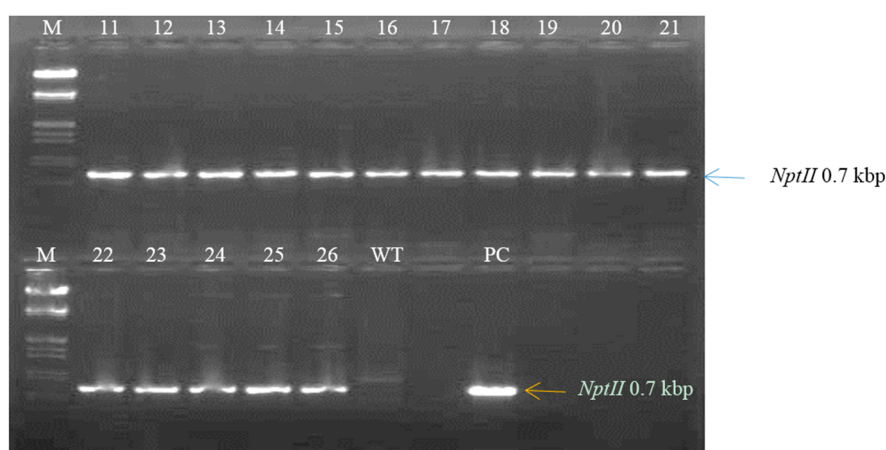


Figure S2. A representative PCR gel picture showing 0.7 Kb amplified product for *NptII* from sixteen putative *AnnAt8* transgenic (T₀) tobacco plants. Letter 'PC' corresponds to positive control for the PCR using plasmid (pCAMBIA2300::AnnAt8) as a template. Letter 'WT' corresponds to negative control for the PCR using DNA from untransformed plant. Letter 'M' correspond to λ HindIII/EcoRI DNA Marker.