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The *AhDREB* transgene expression activates *NtP5CS* and *NtSUSY*, promoting osmotic adjustment in transgenic tobacco under salt stress

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Electronic Supplementary Material (ESM)

The authors are fully responsible for both the content and the formal aspects of the electronic supplementary material. No editorial adjustments were made.

Table S1. Composition of bacterial recovery and plant tissue culture media used for *Agrobacterium*-mediated transformation of tobacco

Medium	Purpose	Composition
YEP	bacterial recovery culture	10 g/L yeast extract + 10 g/L peptone + + 5 g/L NaCl + 15 g/L agar; pH 7.0
MS1	bacterial resuspension medium	1/2 MS + 200 μM AS + 30 g/L sucrose; pH 5.8
GM1	*in vitro* germination of the seed medium	MS + 30 g/L sucrose + 8 g/L agar, pH:5.8
GM2	*in vitro* growth of plants and storage medium	MS + 30 g/L sucrose + 8 g/L agar, pH:5.8
CCM	co-cultivation medium	MS + 1 mg/L BAP + 200 μM AS + + 30 g/L sucrose + 8 g/L agar; pH 5.8
SRM1	shoot regeneration and selection, and bacterial elimination medium	MS + 1 mg/L BAP + 100 mg/L kanamycin + + 500 mg/L cefotaxime + 30 g g/L sucrose + 8 g/L agar; pH 5.8
SRM2	shoot growth and selection, and bacterial elimination medium	MS + 1 mg/L BAP + 150 mg/L kanamycin + + 500 mg/L cefotaxime + 30 g/L sucrose + 8 g/L agar; pH 5.8
RM	rooting and selection medium	MS + 50 mg/L kanamycin + 500 mg/L cefotaxime + + 30 g/L sucrose + 8 g/L agar; pH 5.8

MS – Murashige and Skoog (1962) basal medium; BAP – 6-benzylaminopurine; AS – acetosyringone

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Table S2. Electrophoresis results of PCR products of transformed samples with primer pairs XB-AhDREB-F/ SacAhDREBHis-R and 35S-F/Nos-R

Ordinal number	Transformed samples	AhDREB primer	35S primer
1	2.1	+	+
2	2.2	+	+
3	2.3	+	+
4	6.1	+	+
5	6.2	+	+
6	7.2	+	+
7	8.1	+	+
8	9.1	+	+
9	9.2	–	–
10	10.1	–	–
11	11.1	–	–
12	11.3	+	+
13	12.1	+	+
14	13.1	+	+
15	13.2	+	+
16	14.1	–	–
17	15.1	+	+
18	16.1	+	+
19	17.1	–	–
20	18.1	–	–
21	19.1	+	+
22	20.2	+	+
23	21.1	+	+
24	23.1	–	–
25	23.2	+	+
26	23.3	–	–
27	24.1	–	–
28	26.1	+	+
29	27.1	+	+
30	27.2	+	+
31	28.1	+	+
32	29.1	+	+
33	30.1	+	+
34	31.1	–	–
35	32.1	–	–
36	32.2	+	+
37	33.1	+	+
38	34.1	–	–
39	36.1	+	+
40	37.1	+	+
41	37.2	+	+
42	38.1	+	+
	Total	30/42 positive	

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(A)

(a) (b)
tctagaggatccATGGACGTGGACCCGCCGCAAAAACCCCTTGTCTCTCCCATCATTGTCAACTCCGCCGCT
TCCTCATCTTCCCCCGATCAATCTTCACCTCCACCACCCAAACCACGCGCCAAAAAGAGGGCCGCTG
CTAGTGGATACTGCGGAGTACGCATGCGCCAATGGGGCAAATGGGTCTCTGAAATTAGAGAGCCCAA
GAAGAGAAACAGAATCTGGCTCGGAACTTACGCCACTGCTGAAATGGCGGCTCGAGCCCACGACGC
CGCGGCTCTAGCAATCAAAGGCCGCGCTGCTATCCTTAACTTCCCCGAACTCGGCCCGCACCTTCCAC
GTCCGCCGACCAATTCCCCTAAGGACATACAAGCCGCCGCAGCCAAGGCAGCCGCATTGGATTACTTT
CCAAGCCATGAAGCCGTAGCCAATCCCAGCCGAATCGTGTCCGCGTCGTCCTCATCGTCCTCCTCATC
CTCTTCCTCCCACCCTAAGGACAAGGAAGAATCACCGAATTCATCGATGGACAAGGATGATGACATGT
TTGTGGACCTCCCTGACCTGATAATTGACTTGGATCACGGTGGTCGAGGAAGTGAATTTGATTACTCG
ACGCGTTGGCTTGTAAACGAAGCCGACCAACTCGACTCGGCTTTCCAGCTCGCAGAGCCTTTCCAGT
GGGAGTCAGTTCCCTACCATCATCACCATCATCACTAAgagctc
(c) (d)

(B)

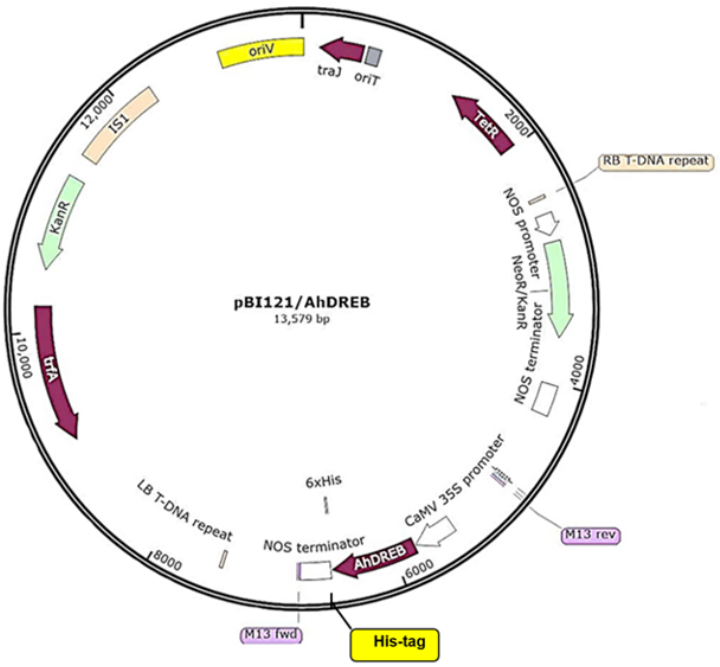

Figure S1. Schematic representation of the synthetic *AhDREB* gene and the recombinant expression vector pBI121_*AhDREB*: (A) sequence map of the engineered *AhDREB* fragment highlighting introduced elements: a – *XbaI* site (tctaga), b – *BamHI* site (ggatcc), c – His-tag coding sequence (CATCATCACCATCATCAC), d – *SacI* site (gagctc); (B) circular map of the binary vector pBI121_*AhDREB*; key features include the CaMV 35S promoter, the *AhDREB* coding region fused to a C-terminal His-tag, the NOS terminator, the kanamycin resistance cassette (KanR), and the T-DNA border sequences (LB, RB) required for *Agrobacterium*-mediated plant transformation

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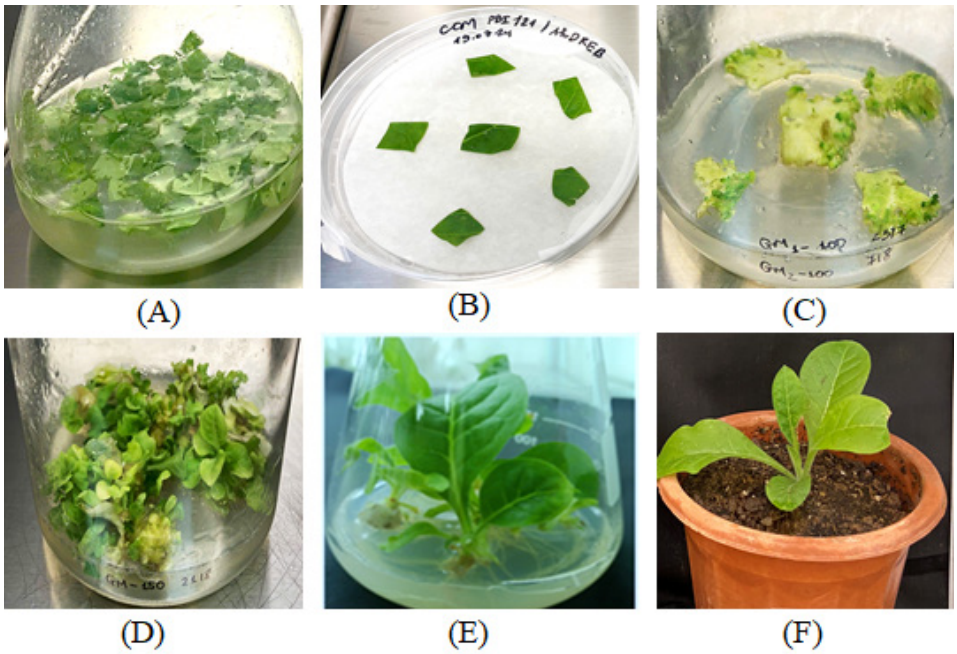

Figure S2. Stages of tobacco (*Nicotiana tabacum*) transformation with the recombinant construct pBI121_AhDREB: leaf explants immersed in *Agrobacterium tumefaciens* suspension harbouring the pBI121_AhDREB vector (A), co-cultivation of infected leaf explants on solid medium (B), induction of multiple shoots from infected explants (C), regeneration of antibiotic-resistant shoots on kanamycin-containing selection medium (D), rooting of regenerated shoots on kanamycin--supplemented medium (E), acclimatized transgenic tobacco plants grown in substrate under greenhouse conditions (F)

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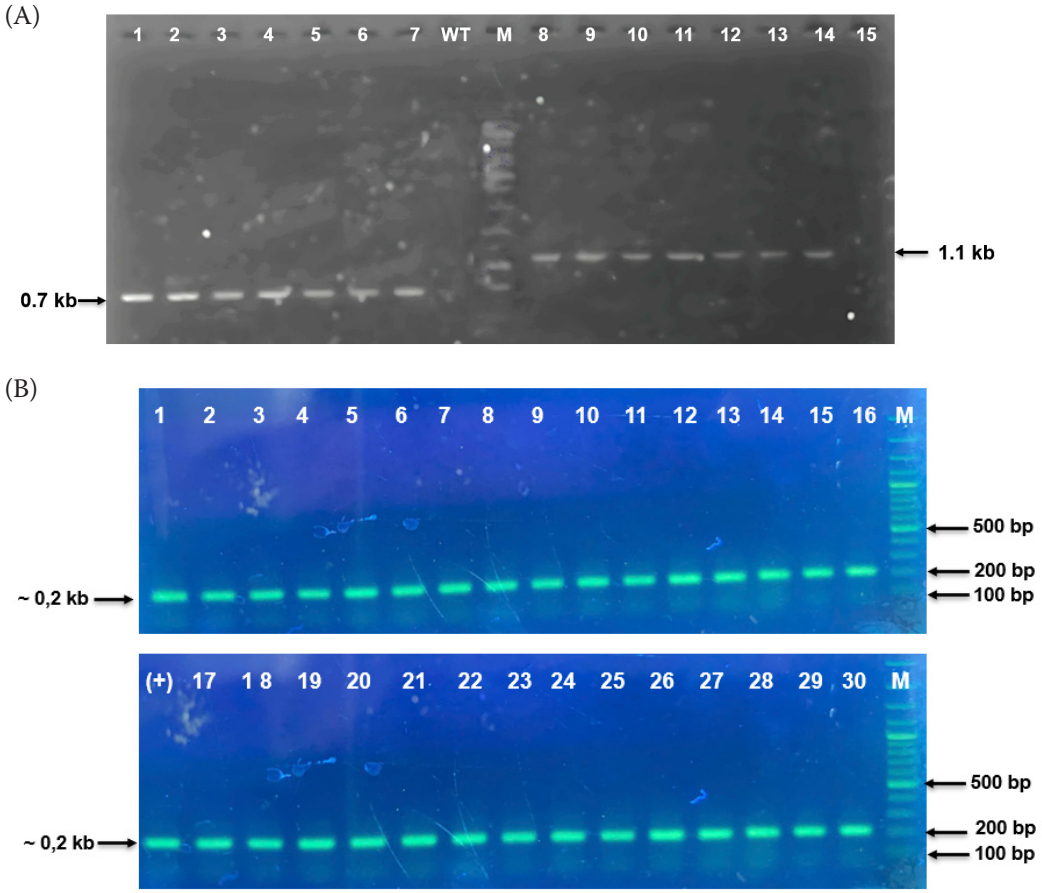

Figure S3. Full image of PCR and RT-PCR product electrophoresis confirmation of *AhDREB* transgene integration and expression in transgenic tobacco plants: (A) agarose gel electrophoresis of PCR products confirming the presence of the *AhDREB* transgene in tobacco transformants; lanes 1–7: amplification using primer pair XB-AhDREB-F/SacAhDREBHis-R (expected fragment size ~ 0.7 kb); lane M: 1 kb DNA ladder; lanes 8–15: amplification using primer pair 35S-F/Nos-R (expected fragment size ~1.1 kb); (B) RT-PCR analysis using primer pair qAhDREB F/ qAhDREB-R showing *AhDREB* transcript expression in transgenic tobacco lines; lanes 1–30: individual transgenic lines; M: 100 bp DNA ladder; (+): positive control (PCR product from pBI121_*AhDREB* construct)

Figure S4. Images of two transgenic tobacco lines, L32.2 line and L37.2 line, and wild-type (WT) plant