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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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Abstract: Soil salinity is a major environmental constraint that limits the growth and productivity of peanut (*Arachis hypogaea* L.), a legume adapted to mildly acidic soils but highly sensitive to saline–alkaline conditions. Dehydration-Responsive Element Binding (DREB) transcription factors are key regulators of plant responses to abiotic stresses. In this study, the *AhDREB* gene from peanut was introduced into tobacco (*Nicotiana tabacum*) to examine its functional role under salt stress. The transgenic lines (L32.2 and L37.2) exhibited strong induction of *AhDREB* expression upon exposure to 150 and 250 mM NaCl, with transcript levels increasing up to 2.34-fold compared with untreated controls ($P < 0.001$). Quantitative RT-PCR analysis revealed that AhDREB enhanced the transcription of two osmolyte-related genes, *NtP5CS* and *NtSUSY*. Under saline conditions, the expression of these genes was 1.20–1.89-fold higher in transgenic lines than in wild-type (WT) plants and 4.74–7.66-fold higher than in non-stress conditions ($P < 0.001$). Consistently, both lines accumulated greater amounts of proline and soluble sugars, showing 2.09–2.30-fold and 2.40–4.70-fold increases, respectively, compared with the WT. Relative to non-stress conditions, proline and sugar contents increased by 3.59–5.47 fold and 3.75–7.65-fold, respectively. Line L37.2 accumulated higher proline levels, whereas L32.2 exhibited greater sugar content, indicating distinct osmolyte regulation patterns. Overall, the *AhDREB* gene enhances salt tolerance in tobacco by transcriptionally activating osmolyte biosynthetic pathways and improving cellular osmotic adjustment, providing molecular evidence for its potential application in developing stress-tolerant peanut cultivars.

Keywords: abiotic stress; *Arachis hypogaea*; downstream genes; osmolyte regulation; transcription factor

Peanut (*Arachis hypogaea* L.) is one of the most critical oilseed legumes cultivated globally. Its seeds are rich in proteins, essential fatty acids, vitamins, and minerals, serving as a vital source of nutrition for humans and livestock. Besides its dietary value, peanut consumption contributes to public health by reducing cardiovascular risks and alleviating malnutrition. The crop also contains bioactive compounds such as resveratrol and flavonoids, along with micronutrients including zinc and iron, which

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enhance its therapeutic and functional food properties (Chukwumah et al. 2009; Arya et al. 2016). Given this dual nutritional and economic significance, peanut is considered a key target in global programs for sustainable agriculture and food security.

However, the productivity of peanuts is being increasingly threatened by soil salinisation, a significant abiotic stress that restricts plant growth and yield. It is estimated that over 8 million hectares of farmland are affected by salinity, representing about 6% of the world's arable land. This proportion is projected to rise substantially by mid-century (Rengasamy 2006; Qadir et al. 2014). The problem is especially critical in arid and semi-arid regions where climate change, poor irrigation management, and water scarcity accelerate soil salt accumulation (Machado & Serralheiro 2017). Soil salinisation adversely affects peanut production by suppressing vegetative growth and yield, reducing both pod size and number, and diminishing nutritional quality. Elevated salt levels impair photosynthetic activity, decrease plant water status, and negatively influence seed germination as well as total biomass accumulation (Heuer et al. 1994; Sousa et al. 2023). Conventional approaches, such as chemical amendments or irrigation control, are costly and often unsustainable. In contrast, the development of salt-tolerant cultivars through molecular breeding and genetic engineering offers a practical and environmentally sound alternative (Munns & Tester 2008; Flowers et al. 2010).

Transcription factors that regulate stress-responsive pathways are key components in engineering abiotic stress tolerance. Among them, dehydration-responsive element binding (DREB) proteins of the AP2/ERF superfamily have been widely recognised for their central roles in plant adaptation to stress. These proteins specifically bind to dehydration-responsive element (DRE/CRT) motifs in gene promoters and activate downstream genes associated with osmotic regulation, antioxidant defence, and hormone signalling (Sakuma et al. 2002). The functional versatility of DREB transcription factors makes them attractive candidates for strategies aimed at increasing drought, salinity, and cold resilience in plants.

The DREB family is divided into two main subgroups. Members of the DREB1/CBF subgroup primarily participate in cold stress responses. In *Arabidopsis thaliana*, ICE1 activates CBF1, CBF2, and CBF3, triggering cold-responsive gene networks (Chinnusamy et al. 2003). Similar mechanisms have been reported in *Saussurea involucrata* and *Citrullus lanatus*, where homologous transcription factors enhanced freezing tolerance by activating downstream *CBF* or *DREB* genes (Jiang et al. 1996). Conversely, DREB2-type proteins mainly function in drought response. For example, StDREB2 from potato improved drought tolerance in cotton (Bouaziz et al. 2012), while *OsDREB2A* expression increased drought resistance in rice (Dubouzet et al. 2003). The constitutively active *AtDREB2Ac*a variant driven by the Rab17 promoter enhanced drought tolerance in sugarcane without growth inhibition (Morran et al. 2011). In maize, allelic variation in the *ZmDREB2.7* promoter contributes to drought resilience (Qin et al. 2007).

Beyond drought and cold, several DREB members have been implicated in salinity tolerance. In *Arabidopsis*, the expression of *ScDREB5*, *ScDREB8*, and *ScDREB10* from the desiccation-tolerant moss *Syntrichia caninervis* improved salt resistance through osmotic regulation and jasmonic acid signalling (Li et al. 2011, 2019; Liang et al. 2017; Liu et al. 2022). Likewise, *LcDREB3a* from *Leymus chinensis* and *PeDREB2a* from *Populus euphratica* enhanced drought and salt tolerance in transgenic systems (Chen et al. 2007, 2009). In soybean (*Glycine max*), overexpression of *GmDREB* genes improved tolerance to drought, cold, and salinity in both *Arabidopsis* and tobacco (*Nicotiana tabacum*) (Zhang et al. 2008). More recently, *GmDREB5*, *GmDREB6*, and *GmDREB7* were shown to regulate downstream stress-related genes in transgenic tobacco (Tu et al. 2021; Nguyen et al. 2023, 2025b). Collectively, these studies highlight the pivotal role of DREB transcription factors in strengthening plant resilience under adverse environmental conditions.

Despite increasing evidence from other species, information on the functional roles of *DREB* genes in peanut remains limited. Although genomic analyses have identified members of the AP2/ERF family in *Arachis* species, the regulatory mechanisms of peanut-derived DREBs have not been experimentally validated. In particular, the involvement of peanut *DREB* genes in salt tolerance is still poorly understood, leaving a gap in our understanding of the molecular basis of stress adaptation in this species.

To address this, the present study investigates the function of a peanut-derived *DREB* gene (*AhDREB*) in response to salinity. Tobacco was used as a heterologous system for functional characterisation because of its reliability in testing transcription factors across species. The potential of *AhDREB* to enhance salt tolerance was evaluated through its effects on key stress-responsive genes involved

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in osmotic adjustment, including Δ^1-pyrroline-5-carboxylate synthetase (P5CS), a rate-limiting enzyme in proline biosynthesis, and sucrose synthase (SUSY), a central enzyme in sucrose metabolism and carbon allocation. Proline and soluble sugars are major osmolytes contributing to osmotic adjustment under salt stress, and both P5CS and SUSY have been widely reported as important targets in abiotic stress adaptation. Therefore, these two genes were selected as representative molecular markers to assess the regulatory role of *AhDREB* in osmotic adjustment.

By elucidating the regulatory function of *AhDREB* under salinity stress, this work provides new evidence for its role in osmotic regulation. It contributes to understanding the molecular mechanisms of stress tolerance in peanut. The findings establish a foundation for future applications of DREB transcription factors in molecular breeding programs aimed at developing salt-tolerant peanut cultivars, thereby supporting sustainable production of this nutritionally and economically important crop.

MATERIAL AND METHODS

Plant materials. *In vitro*-grown tobacco (*Nicotiana tabacum* cv. K326) plants were obtained from aseptically germinated seeds and are currently being preserved *in vitro* at the Biotechnology Laboratory, Tay Bac University. Young leaf segments of approximately 1 cm^2 were excised and used as explants for *Agrobacterium*-mediated transformation. The composition of the *in vitro* tobacco plant culture medium is shown in Table S1 in the Electronic Supplementary Material (ESM). The binary vector pBI121, containing the CaMV 35S promoter, together with *Agrobacterium tumefaciens* carrying the recombinant construct pBI121_*AhDREB*, was also supplied by the same laboratory. Primer sequences employed for gene amplification and verification are listed in Table 1.

Construction of the *AhDREB* expression vector. The *AhDREB* gene from peanut was designed and chemically synthesised based on the coding sequence of the *DREB1* gene (GenBank accession No. FM955398.1; Liu 2010), which is 687 bp in length. To facilitate cloning, restriction sites for *Xba*I and *BamH*I were incorporated at the 3' end of the sequence, and an 18-bp His-tag sequence was fused to the 5' end, followed by a *Sac*I restriction site. These modifications extended the total length of the synthetic *AhDREB* fragment to 723 bp (Figure S1A in ESM).

The synthesised fragment was first cloned into the pUC57 vector, generating the intermediate plasmid pUC57_*AhDREB*. The *AhDREB* insert was then excised from pUC57_*AhDREB* using *Xba*I and *Sac*I. In parallel, the *GUS* reporter gene was removed from the binary vector pBI121_*GUS* using the same restriction enzymes. The *AhDREB* fragment was subsequently ligated into the digested pBI121 backbone, resulting in the recombinant expression vector pBI121_*AhDREB* (Figure S1B in ESM).

The structural integrity of pBI121_*AhDREB* was verified after transformation into *Escherichia coli* strain G10. Positive clones were identified by colony PCR. The validated plasmid was then introduced into *A. tumefaciens*, and the presence of the *AhDREB*

Table 1. List of primers and their sequences used for PCR, RT-PCR, and quantitative real-time RT-PCR

Primers	Sequences (5'–3')	Expected size of amplified DNA fragment (bp)
35S-F	CACTGACGTAAGGGATGACGC	1 100
Nos-R	GTTGTAAAACGACGGCCAGT	
XB-AhDREB-F/	aaaTCTAGAGGATCCATGGACGTGGACCCGC	720
SacAhDREBHis-R	aaagagctcTTAGTGATGATGGTGATGATG	
qNtSUSY-F	CATAGCTCATGCGTTGGAGA	200
qNtSUSY-R	CCTGGAAAGTGCTGGTGATT	
qNtP5CS-F	TGTGACACGGACTGATGGAA	194
qNtP5CS--R	GCTCACTTTGGGGCTTTTGA	
qAhDREB F	CTCTAGCAATCAAAGGCCGC	211
qAhDREB R	TGGGAGGAAGAGGATGAGGA	
qNtactin_F	CTATTCTCCGCTTTGGACTTGGCA	153
qNtactin_R	AGGACCTCAGGACAACGGAAACG	

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insert was reconfirmed by colony PCR using the same primer set.

***Agrobacterium*-mediated transformation of tobacco.** Genetic transformation of *N. tabacum* was performed using *A. tumefaciens* harbouring the pBI121_*AhDREB* construct, according to a modified protocol of Topping (1998). Healthy *in vitro* leaves were excised into fragments of approximately 1 × 1 cm and immersed in an *Agrobacterium* suspension supplemented with 200.0 µM acetosyringone. The explants were gently agitated for 20 min to induce vir gene activation. Following inoculation, the tissues were blotted dry and co-cultivated for 48 h in the dark on MS medium containing 1.0 mg/L 6-benzylaminopurine (BAP) (Duchefa Biochemie, Haarlem, Netherlands), 30.0 g/L sucrose (Duchefa Biochemie), and 8.0 g/L agar (Duchefa Biochemie).

After co-cultivation, the explants were transferred to shoot induction medium (MS supplemented with 1.0 mg/L BAP, 100.0 mg/L kanamycin, 500.0 mg/L cefotaxime, 30.0 g/L sucrose, and 8.0 g/L agar). Two weeks later, surviving explants were subcultured onto a second selection medium containing a higher concentration of kanamycin (Duchefa Biochemie) (150.0 mg/L) to enhance selection efficiency. Regenerated shoots appearing within 30 days were excised and placed onto root induction medium (MS supplemented with 0.3 mg/L α-naphthaleneacetic acid (NAA) (Duchefa Biochemie), 50.0 mg/L kanamycin, 500.0 mg/L cefotaxime, 30.0 g/L sucrose, and 8.0 g/L agar). Rooted plantlets were maintained under sterile conditions before acclimatisation. The composition of all culture media used in this study is summarised in Table S1 in ESM.

Tobacco transformants harbouring the pBI121_*AhDREB* vector were identified as T_0 plants. After *in vitro* growth produced three to four internodes, the T_0 individuals were multiplied through vegetative propagation to obtain sufficient material for experimentation. Each plant was sectioned into stem pieces bearing at least one lateral bud, and these explants were placed on regeneration medium to promote shoot initiation and rooting. The resulting plantlets were subsequently acclimated and *in vitro* preserved as distinct transgenic lines for downstream evaluations

Salt stress treatment. PCR-confirmed transgenic *N. tabacum* plants expressing *AhDREB*, along with WT controls, were transferred from *in vitro* culture to a hydroponic system and acclimatised for 10 days. Hydroponic culture was performed in plastic containers (110 × 90 × 100 mm) filled with INAPONIC nutrient solution (Institute of Agricultural Biology, Vietnam National University of Agriculture), prepared according to the manufacturer's instructions.

Salt stress was imposed by adding NaCl to final concentrations of 0 mM (control), 150.0 and 250.0 mM. Plants were exposed to these treatments for seven days, after which salt application was discontinued. Samples were collected for physiological and molecular analyses. All experiments were conducted under greenhouse conditions at 25 °C, 80% relative humidity, and a 16-h light and 8-h dark photoperiod.

Detection of the *AhDREB* transgene by PCR. Integration of the *AhDREB* transgene was confirmed by PCR using two primer pairs (35S-F/Nos-R and XB-AhDREB-F/SacAhDREBHis-R; sequences listed in Table 1). Each 20.0 µL reaction contained 1.0 µL genomic DNA (25.0 ng/µL), 9.0 µL Dream*Taq* Master Mix (Thermo Fisher Scientific, Waltham, USA), 0.5 µL of each primer (50.0 ng/µL), and nuclease-free water. Thermal cycling was performed with the following conditions: initial denaturation at 95 °C for 3.0 min; 40.0 cycles of 95 °C for 10 s, annealing at 55–65 °C (primer-dependent) for 20 s, and extension at 72 °C for 20 s; followed by a final melting curve analysis from 65 °C to 95 °C with 5 °C increments. PCR products were separated on a 2.0% agarose gel.

Reverse transcription-PCR (RT-PCR) analysis. To evaluate *AhDREB* expression, RT-PCR was carried out on transgenic lines verified by PCR. Total RNA was extracted, and first-strand cDNA synthesis was performed using the FastQuant RT Kit (Tiangen Biotech, Beijing, China). The primer pair AhDREB-F/SacAhDREBHis-R (Table 1) was used for amplification of *AhDREB* cDNA. Each 15.0 µL reaction contained 7.5 µL of 2× PCR Master Mix (Thermo Fisher Scientific), 0.5 µL of each primer (10.0 pmol), cDNA template, and nuclease-free water. The cycling program consisted of the following steps: initial denaturation at 94 °C for 3 min, followed by 27 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 1 min, and a final extension at 72 °C for 5 min. Products were visualized on a 2.0% agarose gel under UV illumination.

Western blot analysis. Total protein was extracted from 0.5 g of leaf tissue using a Retsch NM400 homogeniser (Retsch GmbH, Germany) (24 Hz, 90 s). Protein extracts were denatured at 95 °C for 10 min in 1× loading buffer and separated by SDS-PAGE (Laemmli 1970) at 100 V for two hours. Proteins were electrotransferred onto nitrocellulose membranes (Bio-Rad, Hercules, USA) at 100 V for two

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hours. Membranes were blocked in 5% skim milk (phosphate-buffered saline, PBS; pH 7.4) for two hours and washed three times with 0.5% skim milk in PBS.

Primary antibody (Abcam, Cambridge, UK) incubation was performed with 6×His Tag Monoclonal Antibody (Invitrogen, Waltham, US) diluted 1 : 1 000 in 5% skim milk for two hours, followed by incubation with HRP-conjugated Goat Anti-Mouse IgG (Abcam, Cambridge, UK; 1 : 5 000) for 2 hours. After five washes with PBS (10 min each), detection was performed using Clarity Western ECL substrate (Bio-Rad), and signals were captured with an Amersham Imager 680 system (GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

The recombinant M-pII-ELP-His protein of porcine epidemic diarrhea virus (PEDV) was used as a positive control in Western blot analysis.

Quantitative real-time PCR (qRT-PCR). Expression levels of *AhDREB, SUSY*, and *P5CS* were quantified using quantitative real-time PCR (qRT-PCR) with the primers listed in Table 1. Each 20.0 µL reaction contained 10 U SYBR Premix Ex *Taq* II (Thermo Fisher Scientific), 1.5 µL of each primer (10.0 µM), 1 µL of cDNA template, and nuclease-free water. The program consisted of the following steps: initial denaturation at 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, annealing at 60–62 °C for 20 s, and extension at 72 °C for 20 s. Each reaction was performed in triplicate. Data analysis was carried out using Q-Rex v1.0.0 (QIAGEN, Hilden, Germany). Relative gene expression was determined by the 2^−ΔΔCT method as described by Livak and Schmittgen (2001).

Growth rate evaluation under salt stress. WT and transgenic tobacco plants were exposed to NaCl at final concentrations of 0, 150, and 250 mM. Fresh weights were identified before treatment and again after seven days of salt exposure. Growth rate (H%) was calculated according to the formula:

$$H = (\text{fresh weight after seven days/initial fresh weight}) \times 100.$$

Proline content determination. Free proline content in leaf tissue was quantified following the procedure of Bates et al. (1973) and expressed as µmol/g fresh weight.

Sugar content determination. Total soluble sugars were extracted from fresh leaves and determined colourimetrically using a glucose standard curve. The concentration of soluble sugars (mg/g FW) was calculated using the formula:

$$\text{Sugar content} = K \times \text{dilution factor} \times \times (\text{absorbance/sample weight})$$

where:
K = 20, following the method of Khan and Bano (2019).

Statistical analysis. All statistical analyses were performed using GraphPad Prism (GraphPad Software, San Diego, USA). One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was used to evaluate differences among treatment groups. Differences were considered statistically significant at $P < 0.001$.

RESULTS

Generation and molecular verification of transgenic tobacco plants carrying the pBI121_*AhDREB* construct

The final pBI121_*AhDREB* construct (Figure S1B in ESM) comprised the CaMV 35S promoter for regulation of *AhDREB* expression, the *NPTII* gene conferring kanamycin resistance, the *AhDREB* coding sequence fused to a C-terminal His-tag, and a modified multiple cloning site harbouring the *Xba*I, *BamH*I, and *Sac*I restriction sites.

Following *Agrobacterium*-mediated transformation using the pBI121_AhDREB vector, a total of 50 *in vitro*-derived tobacco leaf explants were subjected to kanamycin selection. After the first round of selection, 38 explants survived and continued to grow, whereas all negative-control explants exhibited severe chlorosis and necrosis, confirming the effectiveness of the selection procedure. During the second selection step, these surviving explants regenerated 42 green and morphologically normal shoots. The regenerated shoots were subsequently transferred to rooting medium, and well-rooted plantlets were successfully acclimatised and transferred to soil (Figure S2 in ESM). In contrast, all explants used as negative controls failed to survive under kanamycin selection. In addition, ten plants regenerated on non-selective medium were maintained as non-transgenic (WT) controls for subsequent experiments.

To confirm the presence of the transgene in the regenerated plants, genomic DNA was extracted from leaf tissues of 42 putative transformants and analysed by PCR using two primer sets: XB-*AhDREB* -F/Sac*AhDREB* His-R, designed to amplify a 720 bp fragment of the *AhDREB* coding region, and 35S-F/Nos-R, targeting a 1 100 bp

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fragment spanning the CaMV35S promoter to the NOS terminator. Agarose gel electrophoresis of the PCR products (Figure S3A in ESM) revealed that 30 of the 42 regenerated plants produced amplification with both primer pairs, yielding fragments of the expected sizes (approximately 0.7 and 1.1 kb, respectively). The remaining 12 lines showed no detectable amplification, suggesting the absence of the transgene. Representative PCR profiles are presented in Figure S3A in ESM, where clear DNA bands corresponding to the predicted fragment sizes are observed. The confirmed transgenic lines were subsequently used for further molecular and physiological analyses.

To evaluate the transcriptional activity of the *AhDREB* gene in transgenic tobacco, reverse transcription PCR (RT-PCR) was performed. Total RNA was isolated from the leaves of 30 T_0 plants that had previously been confirmed as PCR-positive (Table S2 in ESM). The RNA was subsequently reverse-transcribed into cDNA, which served as the template for RT-PCR using the primer pair for real-time RT-PCR (qAhDREB-F/R, Table 1), and an expected 211 bp fragment was amplified. Agarose gel electrophoresis of the RT-PCR products (Figure S3B in ESM) revealed that all 30 lines produced a distinct band of approximately 0.2 kb, matching both the predicted size and the positive control (PCR product derived from the pBI121_*AhDREB* construct). These results demonstrate that the *AhDREB* transgene was not only successfully integrated but also actively transcribed in all analysed transgenic tobacco plants. These results served as the basis for real-time RT-PCR analysis of the *AhDREB* gene.

Western blot analysis of recombinant AhDREB protein

Based on the design of the pBI121_*AhDREB* construct, the recombinant protein was predicted to have a molecular mass of approximately 25 kDa due to the addition of a C-terminal His-tag.

Among the 30 transgenic lines propagated *in vitro* with sufficient plantlets and roots, two representative lines (L32.2 and L37.2) were selected for western blot analysis, together with the non-transgenic WT control (Figure S4 in ESM). Western blot analysis revealed distinct bands at ~25 kDa in transgenic tobacco lines, L37.2 and L32.2 (Figure 1), consistent with the theoretical size of the recombinant AhDREB protein. In contrast, no signal was detected in the WT. These findings demonstrate that the *AhDREB* gene was successfully translated in transgenic tobacco, confirming the functionality of the pBI121_*AhDREB* construct. Therefore, western blotting served as a qualitative confirmation of recombinant protein expression of the *AhDREB* transgene.

Results of *AhDREB* transcriptional expression in transgenic tobacco

Among the 30 transgenic lines propagated *in vitro* with sufficient plantlets and roots, two representative lines (L32.2 and L37.2) were selected for further analysis, together with the non-transgenic WT control (Figure S4 in ESM). These plants were first acclimatised in a hydroponic culture system for 7 days before being subjected to salt treatments. After the acclimatisation phase, salt stress was applied at concentrations of 0, 150, and 250 mM NaCl (Figure 2A, B).

The relative transcription levels of the *AhDREB* transgene in two transgenic tobacco lines (L32.2 and L37.2) and WT plants under both non-stress and salt stress conditions are presented in Figure 4C. Under non-stress conditions, the expression of *AhDREB* in lines L32.2 and L37.2 was significantly lower than that in WT plants ($P < 0.001$). In contrast, salt treatment markedly

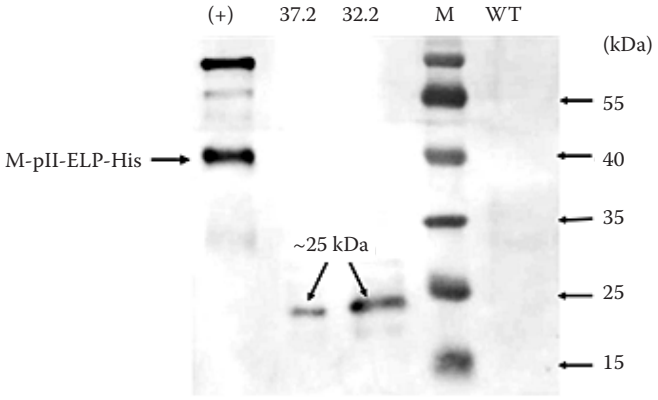

Figure 1. Western blot detection of recombinant AhDREB protein in transgenic tobacco lines

M – protein marker (Thermo Scientific, 10–180 kDa); 32.2, 37.2 – proteins from transgenic tobacco lines, L32.2, L37.2; (+) – positive control, the recombinant M-pII-ELP-His protein (~40 kDa) of porcine epidemic diarrhea virus was used as a positive control; WT – negative control, proteins from wild-type plants; specific bands of ~25 kDa were detected in lines 32.2 and 37.2, corresponding to the predicted size of the recombinant AhDREB protein

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induced *AhDREB* transcription in the transgenic lines, with levels exceeding those of WT plants ($P < 0.001$).

At 150 mM NaCl, lines L32.2 and L37.2 exhibited 1.41–1.70-fold higher expression relative to WT, while at 250 mM NaCl, the increase ranged from 1.28- to 1.49-fold. When compared with their own untreated controls, the transcription level of *AhDREB* in line L32.2 increased 2.14-fold and 2.34-fold under 150 mM and 250 mM NaCl, respectively; line L37.2 showed increases of 1.89- and 2.16-fold, respectively. In contrast, WT plants exhibited only slight increases of 1.09- and 1.37-fold under the same treatments. These results demonstrate that the *AhDREB* transgene is strongly upregulated in transgenic lines in response to salt stress, whereas WT plants show only limited induction.

Relationship between *AhDREB* transgene and the transcriptional expression of *NtP5CS* and *NtSUSY* genes in transgenic tobacco

The regulatory association of the AhDREB transcription factor with the expression of *NtP5CS* and *NtSUSY* was examined in transgenic tobacco lines using qRT-PCR (Figure 3).

As shown in Figure 3A, *NtP5CS* expression was significantly upregulated in both transgenic lines (L32.2 and L37.2) and WT plants under salt stress compared with non-stress. In line L32.2, *NtP5CS* expression increased 4.77-fold at 150 mM NaCl and 7.66-fold at 250 mM NaCl, while in line L37.2, the increases were 4.53-fold and 5.60-fold, respectively ($P < 0.001$). Compared with WT plants, *NtP5CS* transcript levels in lines L32.2 and L37.2 were 1.57- and 1.48-fold higher under 150 mM NaCl, and 1.65- and 1.20-fold higher under 250 mM NaCl, respectively. Meanwhile, under non-stress conditions, the transcriptional level of the *NtP5CS* gene in WT plants and the two transgenic lines did not show a significant difference. These results indicate that AhDREB positively regulates *NtP5CS* transcription in response to salt stress.

For the *NtSUSY* expression, Figure 3B demonstrates a similar trend. Transcript levels in lines L32.2 and L37.2 were significantly higher than in non-stress plants ($P < 0.001$), with increases of 4.76- and 4.74-fold under 150 mM NaCl, and 7.12- and 7.53-fold under 250 mM NaCl, respectively. Compared with WT, *NtSUSY* expression in L32.2 and L37.2 was elevated 2.04- and 1.62-fold at 150 mM NaCl, and 2.24- and 1.89-fold at 250 mM NaCl, respectively. In contrast, under non-stress conditions, the difference in transcription levels of the *NtSUSY* gene between WT plants and the two transgenic lines was not significant.

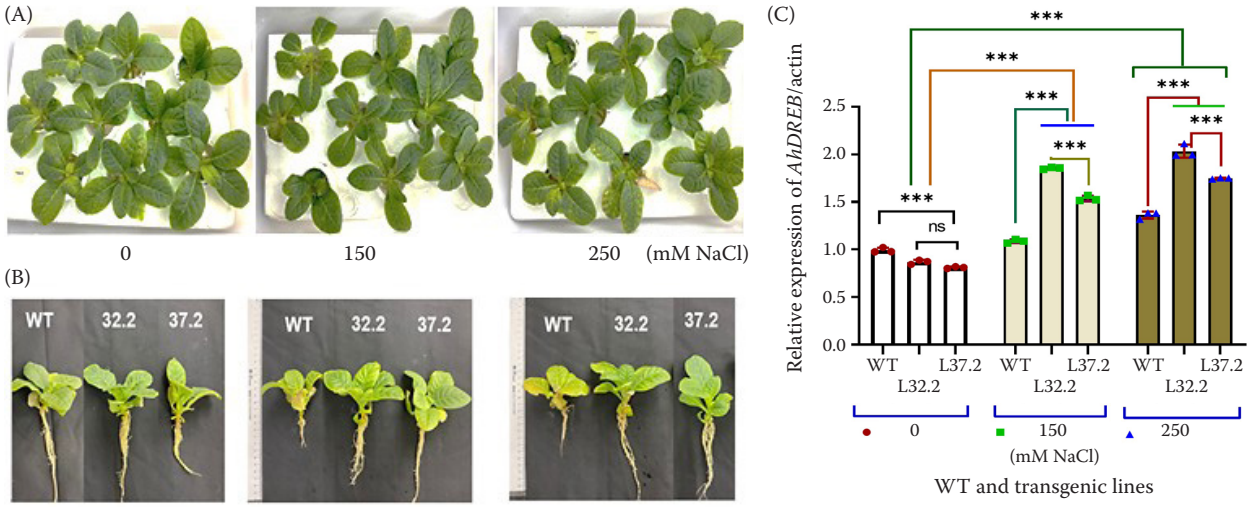

Figure 2. Phenotype and transcriptional expression of WT plants and the *AhDREB* transgene in tobacco lines under salt stress: WT plants and transgenic lines were grown under non-stress and salt stress conditions at concentrations of 150 and 250 mM NaCl (A); morphological characteristics of WT and transgenic lines (L32.2 and L37.2) after salt treatment (B); qRT-PCR quantified relative expression levels of *AhDREB* in WT and transgenic tobacco lines (L32.2 and L37.2, T0 generation) under control and salt-treated conditions (150 and 250 mM NaCl, seven days) (C)

WT – wild-type, non-transgenic plants; 32.2 and 37.2 – *AhDREB* transgenic tobacco lines, L32.2 and L37.2; the *Actin* gene was used as the internal reference; bars indicate geometric means, error bars represent geometric standard deviations (SD), and dots correspond to individual samples; ***statistically significant differences at $P < 0.001$

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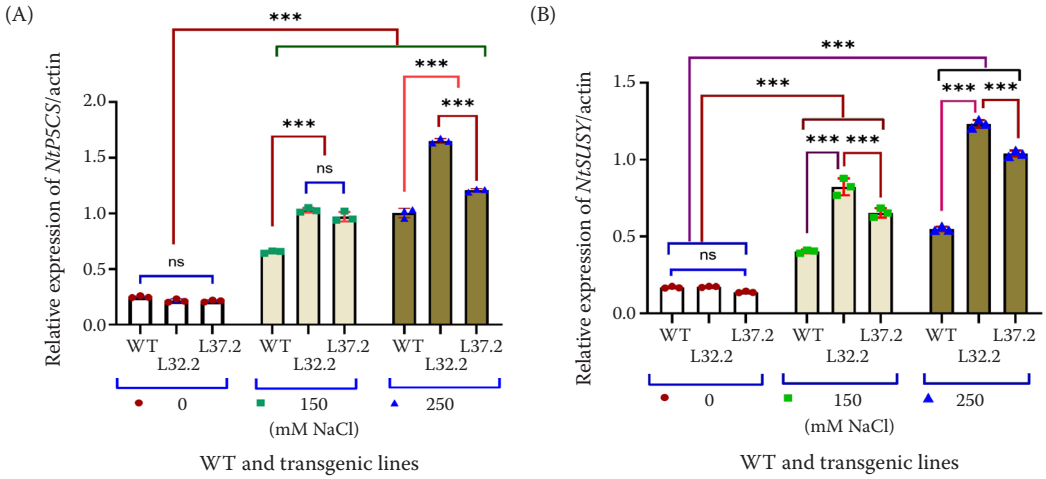

Figure 3. Transcriptional levels of *NtP5CS* and *NtSUSY* genes in T_0 transgenic tobacco lines and WT plants under control and salt stress conditions: relative expression of *NtP5CS* in transgenic lines compared with WT plants ($P < 0.001$) (A), relative expression of *NtSUSY* in transgenic lines compared with WT plants ($P < 0.001$) (B)

Actin was used as the internal reference gene; WT – wild-type, non-transgenic plants; L32.2 and L37.2 – T_0 *AhDREB* transgenic lines; ***statistically significant differences at $P < 0.001$; ns – non-significant differences ($P > 0.001$); bars represent the geometric mean ± geometric SD, and dots correspond to individual samples

These findings confirm a transcriptional regulatory relationship between AhDREB and *NtSUSY* under salt stress conditions.

Growth characteristics and proline, sugar contents of *AhDREB* transgenic tobacco lines responding to salt stress

Before and after seven days of salt exposure, plants were weighed to determine their growth rate (H%). Concurrently, leaf samples collected from WT and transgenic lines L32.2 and L37.2 were used to quantify proline and total soluble sugar contents. The comparative results of growth coefficient, proline, and sugar levels between transgenic and WT plants are summarised in Figure 4.

Growth characteristics analysis. Under the three tested conditions, non-saline control (0 mM NaCl), moderate salinity (150 mM NaCl), and severe salinity (250 mM NaCl), no visible difference in leaf morphology was observed among the plants (Figure 2A). In contrast, noticeable variations in root growth were detected (Figure 2B). The growth rate varied significantly between the transgenic lines and the WT plants (Figure 4A-1). Both WT and transgenic plants exhibited a reduction in growth coefficient under salt stress (150 and 250 mM NaCl) compared with the control ($P < 0.001$). However, line L37.2 maintained a relatively higher growth rate under 250 mM NaCl, suggesting enhanced salt tolerance compared with WT and L32.2 (Figure 4A-2).

Proline accumulation. As shown in Figure 4B, the proline content of the transgenic tobacco lines L32.2 and L37.2 was consistently higher than that of the WT plants across all experimental treatments, with highly significant differences ($P < 0.001$) (Figure 4B-1). Under non-stress conditions, proline levels in lines L32.2 and L37.2 were 3.41- and 2.85-fold higher, respectively, than in WT plants. When exposed to 150 mM NaCl, the proline content increased to 2.09-fold in L32.2 and 1.68-fold in L37.2 compared to WT plants, while at 250 mM NaCl, the respective increases were 2.30-fold and 1.81-fold ($P < 0.001$).

When comparing proline accumulation within each transgenic line across the three salt treatments (0, 150, and 250 mM NaCl), an apparent increase in proline content was observed (Figure 4B-2). In transgenic line L32.2, the proline concentration increased by 3.59-fold at 150 mM NaCl and 4.65-fold at 250 mM NaCl compared with the untreated control (0 mM). In line L37.2, the corresponding increases were 4.65-fold and 5.47-fold ($P < 0.001$). Overall, both transgenic lines accumulated significantly higher levels of proline than the WT under all salinity treatments. Among them, line L37.2 exhibited the most significant increase, suggesting a stronger osmoprotective capacity in response to salt stress.

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Sugar content. As shown in Figure 4C, soluble sugar levels differed between WT and transgenic tobacco plants depending on salt concentration. Under non-saline conditions (0 mM NaCl), no significant difference in sugar content was observed between WT and the transgenic lines ($P > 0.001$). When subjected to salt stress (150 and 250 mM NaCl), both transgenic lines accumulated markedly

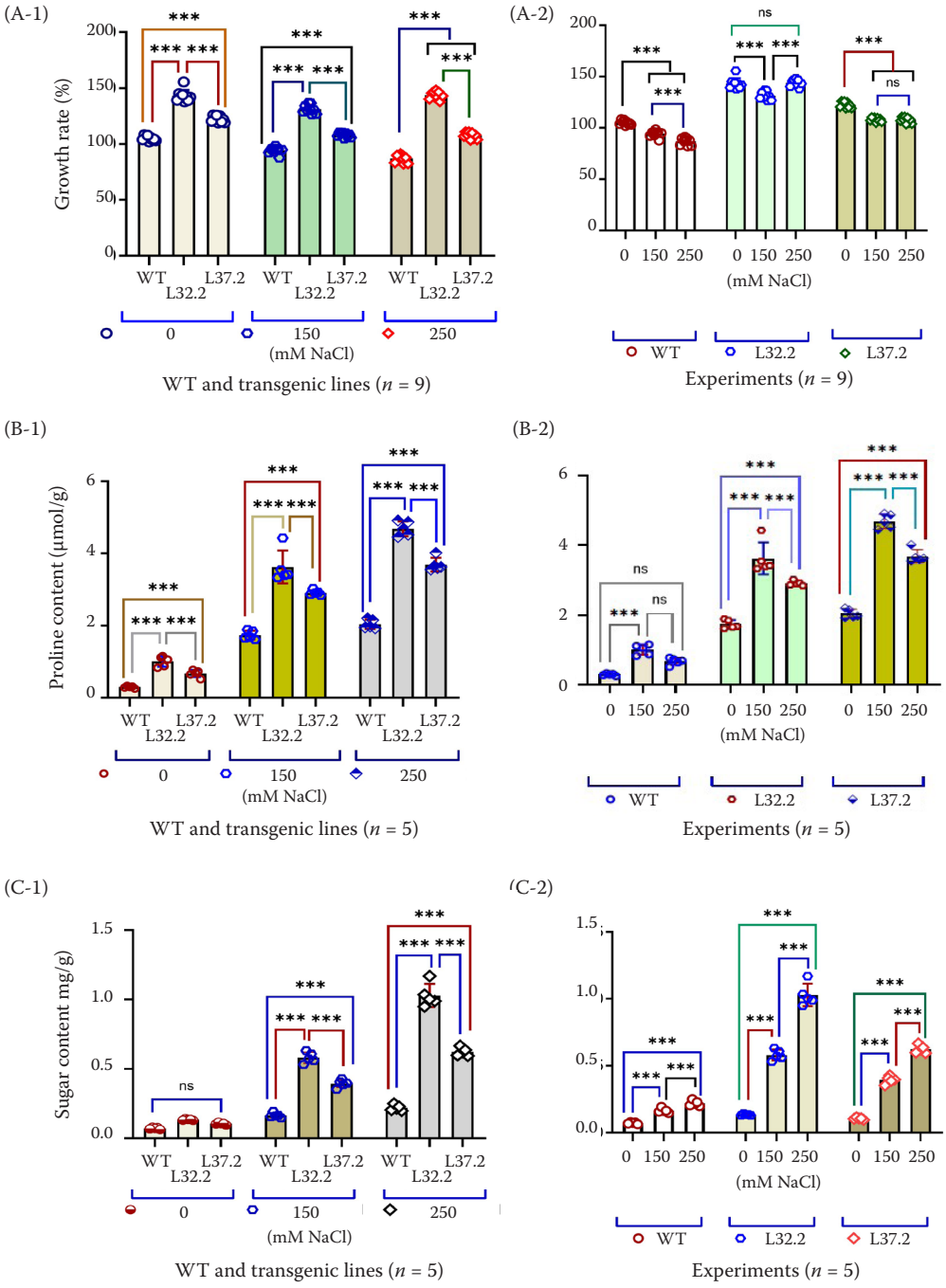

Figure 4. Growth coefficient, proline content, and total soluble sugar content of transgenic tobacco lines and WT plants under salt stress: growth coefficient (H%) (A-1, A-2), proline content (B-1, B-2), total soluble sugar content (C-1, C-2)
WT – wild-type, non-transgenic plants; L32.2 and L37.2 – T_0 *AhDREB* transgenic lines; error bars indicate geometric mean ± geometric SD; ***statistically significant differences at $P < 0.001$; ns – indicates non-significant differences ($P > 0.001$); each dot represents an individual biological replicate

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higher amounts of soluble sugars than WT plants ($P < 0.001$) (Figure 4C-1). At 150 mM NaCl, sugar levels increased 3.53-fold in L32.2 and 2.40-fold in L37.2 relative to WT. At 250 mM NaCl, these increases reached 4.70-fold and 2.85-fold, respectively.

Within each transgenic line, soluble sugar accumulation rose consistently with increasing salt concentration (Figure 4C-2). In L32.2, sugar levels increased 4.31-fold at 150 mM and 7.65-fold at 250 mM compared with the control. Similarly, in L37.2, the corresponding increases were 3.75-fold and 5.92-fold ($P < 0.001$). Both transgenic lines exhibited a clear enhancement in soluble sugar accumulation under saline conditions compared with WT plants. Notably, line L32.2 showed the highest sugar content across treatments, reflecting a more effective osmotic adjustment and a stronger metabolic response to salt stress.

DISCUSSION

Peanut (*A. hypogaea*) is a salt-sensitive legume that grows best in slightly acidic soils (pH 5.8–6.5) but shows limited growth and productivity under alkaline or saline conditions (pH > 8.0) (Sparks 2003). Salinity and alkalinity negatively affect seed germination, vegetative development, and pod formation, leading to substantial reductions in yield and seed quality. Improving the salt tolerance of peanut and related legumes has therefore become an essential objective in current crop stress physiology and breeding research.

Among transcriptional regulators, the DREB family is well known for mediating plant tolerance to abiotic stresses such as salinity, drought, and cold by activating multiple downstream stress-responsive genes (Lata & Prasad 2011; Sadau et al. 2024). Members of this family function through AP2/ERF DNA-binding domains that recognise specific *cis*-acting DRE/CRT motifs within promoters of target genes involved in osmotic regulation and detoxification pathways. In legumes, the *DREB* gene family has been extensively characterised in soybean (*GmDREB2A*, *GmDREB5*, *GmDREB6*, *GmDREB7*), where several members were shown to improve salt and drought tolerance in transgenic systems (Nguyen et al. 2019, 2025a, b). However, in peanuts, studies have so far been limited to cloning and sequence identification of *AhDREB1* (Hong et al. 2013), with only preliminary evidence that overexpression of *AhDREB1A* enhances drought resistance. The molecular function of this transcription factor in salinity tolerance remains largely unexplored.

In this context, our study designed and constructed an expression cassette for the *AhDREB* gene based on the previously cloned sequence, which is deposited in GenBank (GenBank accession No. FM955398.1). In this study, the peanut-derived *AhDREB* gene was introduced into tobacco to evaluate its heterologous function in a non-leguminous system. DREB transcription factors are key regulators of plant responses to abiotic stress, as they bind to DRE/CRT in stress-inducible promoters (Agarwal et al. 2017). The transcriptional activity of *AhDREB* and its regulatory relationship with the downstream target genes, *NtP5CS* and *NtSUSY*, were analysed in transgenic tobacco under salt stress. The results revealed that *AhDREB* expression was markedly upregulated in both transgenic lines (L32.2 and L37.2) under salinity conditions, increasing 2.14- to 2.34-fold in line L32.2 and 1.89- to 2.16-fold in line L37.2 at 150 mM and 250 mM NaCl, respectively (Figure 2C). Line L32.2 exhibited a markedly higher level of *AhDREB* transcripts compared with line L37.2 and the WT. This pattern suggests that the *AhDREB* transgene is highly responsive to salt stress and likely acts as a primary transcriptional activator regulating stress-inducible genes in transgenic tobacco. The pronounced increase in *AhDREB* expression at both 150 and 250 mM NaCl further supports its strong induction by salinity. Such transcriptional activation agrees with earlier observations for *AtDREB1A* and *GmDREB2A*, which are rapidly upregulated under osmotic and ionic stress (Liu et al. 1998).

To investigate downstream regulatory effects, *NtP5CS* and *NtSUSY* were examined as representative genes involved in osmotic adjustment. *NtP5CS* encodes Δ^1-pyrroline-5-carboxylate synthetase, a key enzyme in proline biosynthesis, whereas *NtSUSY* encodes sucrose synthase, which participates in carbohydrate metabolism. Both proline and soluble sugars act as compatible osmolytes that help maintain cell turgor and stabilise macromolecules under saline conditions (Szabados & Savouré 2010; Gupta & Huang 2014). The elevated expression of *NtP5CS* and *NtSUSY* in *AhDREB* transgenic lines under salt stress indicates that AhDREB functions as a positive regulator, enhancing osmolyte biosynthetic pathways.

The transcriptional activation of these genes corresponded well with the observed physiological performance of the transgenic lines. Compared with WT plants, both L32.2 and L37.2 maintained higher

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growth rates and accumulated greater amounts of proline and soluble sugars under moderate and severe salinity. Line L37.2 exhibited the highest proline levels, while L32.2 accumulated more soluble sugars, suggesting that *AhDREB* expression promotes distinct osmoprotective mechanisms that contribute to maintaining energy balance and cellular homeostasis during salt stress. However, in this study, T_0 lines were vegetatively propagated by *in vitro* cuttings for analysis, which represents a limitation of the present work. In future studies, seed-derived T_1, T_2 plants and subsequent generations will be more appropriate for genetic analysis of transgenic plants.

The successful heterologous expression of *AhDREB* in tobacco supports the evolutionary conservation of DREB-mediated stress signalling. The ability of a peanut-derived transcription factor to activate orthologous genes in a Solanaceae host demonstrates its broad regulatory potential across plant taxa. These findings extend current understanding of DREB functional networks and position *AhDREB* as a promising candidate for developing stress-tolerant crops through transcription factor–based genetic engineering approaches.

CONCLUSION

In this study, a DREB transcription factor gene (*AhDREB*) derived from peanut (*Arachis hypogaea* L.) was cloned, constructed, and successfully expressed in transgenic tobacco to assess its function in salinity tolerance. The *AhDREB* transgene showed strong induction under salt stress, demonstrating its sensitivity to osmotic changes. Expression analyses revealed a positive regulatory relationship between AhDREB and the downstream stress-responsive genes *NtP5CS* and *NtSUSY* at both 150 and 250 mM NaCl. The upregulation of these genes was accompanied by elevated levels of proline and soluble sugars, which contributed to osmotic adjustment and improved growth in transgenic plants compared with the wild type. Enhanced salt tolerance in tobacco was thus achieved through coordinated activation of osmolyte biosynthetic pathways regulated by AhDREB. These results provide molecular and physiological evidence that AhDREB acts as a transcriptional activator promoting osmotic regulation under salinity stress. The findings further underscore the functional conservation of peanut-derived DREB transcription factors and their potential utility in developing salt-tolerant crop varieties through molecular breeding and genetic engineering approaches.

REFERENCES

Agarwal P.K., Gupta K., Lopato S., Agarwal P. (2017): Dehydration responsive element binding transcription factors and their applications for the engineering of stress tolerance. Journal of Experimental Botany, 68: 2135–2148.

Arya S.S., Salve A.R., Chauhan S. (2016): Peanuts as functional food: A review. Journal of Food Science and Technology, 53: 31–41.

Bates L.S., Waldren R.P., Teare I.D. (1973): Rapid determination of free proline for water-stress studies. Plant and Soil, 39: 205–207.

Bouaziz D., Pirrello J., Ben Amor H., Hammami A., Charfeddine M., Dhieb A., Bouzayen M., Gargouri-Bouzid R. (2012): Ectopic expression of dehydration responsive element binding proteins (StDREB2) confers higher tolerance to salt stress in potato. Plant Physiology and Biochemistry, 60: 98–108.

Chen J., Xia X., Yin W. (2009): Expression profiling and functional characterization of a DREB2-type gene from *Populus euphratica*. Biochemical and Biophysical Research Communications, 378: 507–512.

Chen M., Wang Q.Y., Cheng X.G., Xu Z.S., Li L.C., Ye X.G., Xie L.Q., Ma Y.Z. (2007): GmDREB2, a soybean DRE-binding transcription factor, conferred drought and high-salt tolerance in transgenic plants. Biochemical and Biophysical Research Communications, 353: 299–305.

Chinnusamy V., Ohta M., Kanrar S., Lee B.H., Hong X., Agarwal M., Zhu J.K. (2003): ICE1: A regulator of cold-induced transcriptome and freezing tolerance in *Arabidopsis*. Genes & Development, 17: 1043–1054.

Chukwumah Y., Walker L.T., Verghese M. (2009): Peanut skin color: A biomarker for total polyphenolic content and antioxidative capacities of peanut cultivars. International Journal of Molecular Sciences, 10: 4941–4952.

Dubouzet J.G., Sakuma Y., Ito Y., Kasuga M., Dubouzet E.G., Miura S., Seki M., Shinozaki K., Yamaguchi-Shinozaki K. (2003): OsDREB genes in rice encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. The Plant Journal, 33: 751–763.

Flowers T.J., Gaur P.M., Gowda C.L.L., Krishnamurthy L., Samineni S., Siddique K.H.M., Turner N.C., Vadez V., Varshney R.K., Colmer T.D. (2010): Salt sensitivity in chickpea. Plant, Cell & Environment, 33: 490–509.

Gupta B., Huang B. (2014): Mechanisms of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. International Journal of Genomics, 2014: 701596.

Heuer B., Schaffer A., Meiri A., Badani H., Ben-Dor B., Fogelman M., Kapulnik Y. (1994): Saline water irrigation and gypsum amendment effects on the quality of peanut seeds, European Journal of Agronomy, 3: 169–174.

https://doi.org/10.17221/124/2025-CJGPB

Hong L., Hu B., Liu X., He C.Y., Yao Y., Li X.L., Li. L. (2013): Molecular cloning and expression analysis of a new stress-related *AREB* gene from *Arachis hypogaea*. Biologia Plantarum, 57: 56–62.

Jiang C., Iu B., Singh J. (1996): Requirement of a CCGAC cis-acting element for cold induction of the BN115 gene from *Brassica napus*. Plant Molecular Biology, 30: 679–684.

Khan N., Bano A. (2019): Exopolysaccharide-producing rhizobacteria and their impact on growth and drought tolerance of wheat grown under rainfed conditions. PLoS ONE, 14: e0222302.

Laemmli U.K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227: 680–685.

Lata C., Prasad M. (2011): Role of DREBs in regulation of abiotic stress responses in plants. Journal of Experimental Botany, 62: 4731–4748.

Li C., Yue J., Wu X., Xu C., Yu J. (2019): An AP2/ERF transcription factor gene from *Syntrichia caninervis* confers salt tolerance in transgenic *Arabidopsis*. Plant Physiology and Biochemistry, 139: 45–53.

Li X., Zhang D., Li H., Wang Y., Zhang Y., Wood A.J. (2011): EsDREB2A, a novel transcription factor from *Eremopyrum sibiricum*, enhances tolerance to multiple abiotic stresses in transgenic tobacco. Plant Molecular Biology, 75: 433–453.

Liang Y., Li X., Zhang D., Zhang D., Gao B., Yang H., Wang Y., Guan K., Wood A.J. (2017): ScDREB8, a novel A-5 type of DREB gene in the desert moss *Syntrichia caninervis*, confers salt tolerance to *Arabidopsis*. Plant Physiology and Biochemistry, 120: 242–251.

Liu J., Yang R., Liang Y., Wang Y., Li X. (2022): The DREB A-5 transcription factor ScDREB5 from *Syntrichia caninervis* enhanced salt tolerance by regulating jasmonic acid biosynthesis in transgenic *Arabidopsis*. Frontiers in Plant Science,13: 857396.

Liu Q., Kasuga M., Sakuma Y., Abe H., Miura S., Yamaguchi-Shinozaki K., Shinozaki K. (1998): Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression in *Arabidopsis.* The Plant Cell, 10: 1391–1406.

Liu W. (2010): *Arachis hypogaea* mRNA for Putative DREB Transcription Factor (DRE-Binding Domain Gene), Isolated from Leaf. GenBank: FM955398.1. Bethesda, National Center for Biotechnology Information. Available at https://www.ncbi.nlm.nih.gov/nuccore/FM955398.1 (accessed May 20, 2025).

Livak K.J., Schmittgen T.D. (2001): Analysis of relative gene expression data using real-time quantitative PCR and the 2(-ΔΔCT) method. Methods, 25: 402–408.

Machado R.M.A., Serralheiro R.P. (2017): Soil salinity: Effect on vegetable crop growth. Horticulturae, 3: 30.

Morran S., Eini O., Pyvovarenko T., Parent B., Singh R., Ismagul A., Eliby S., Shirley N., Langridge Lopato S. (2011): Improvement of stress tolerance of wheat and barley by modulation of expression of DREB/CBF factors. Plant Biotechnology Journal, 9: 230–249.

Munns R., Tester M. (2008): Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59: 651–681.

Nguyen G.T., Nguyen Y.T.H., Nguyen H.D., Chu M.H., Nguyen Q.H. (2025b): The DREB7 transcription factor enhances salt tolerance in soybean plants under salt stress. Open Life Science, 20: 20251153.

Nguyen Q.H., Vu L.T.K., Nguyen L.T.N., Pham N.T.T., Nguyen Y.T.H., Le S.V., Chu M.H. (2019): Overexpression of the *GmDREB6* gene enhances proline accumulation and salt tolerance in genetically modified soybean plants. Scientific Reports, 9: 19663.

Nguyen Y.T.H., Tu T.Q., Nguyen N.H., Nguyen D.V., Tran H.T., Do P.T., Lo T.T.M., Pham N.T.T., Nguyen L.T.N., Vu T.T.T., Nguyen Q.H., Chu M.H. (2023): A novel soybean transcription factor, DREB7, regulates *RD29A* and *SODFe* gene expression in transgenic tobacco plants. In Vitro Cellular & Developmental Biology – Plant, 59: 275–284.

Nguyen Y.T.H., Nguyen G.T., Nguyen H.D., Vu T.T.T., Nguyen L.T.N., Nguyen Q.H., Chu M.H. (2025a): The GmDREB5 from soybean enhanced salt tolerance by upregulating the transcription levels of NtP5CS and NtSODFe in transgenic tobacco. Molecular Biology Reports, 52: 656.

Qadir M., Quillérou E., Nangia V., Murtaza G., Singh M.., Thomas R.J., Drechsel P., Noble A.D. (2014): Economics of salt-induced land degradation and restoration. Natural Resources Forum, 38: 282–295.

Qin F., Kakimoto M., Sakuma Y., Maruyama K., Osakabe Y., Tran L.S.P., Shinozaki K., Yamaguchi-Shinozaki K. (2007): Regulation and functional analysis of ZmDREB2A in response to drought and heat stresses in maize. The Plant Journal, 50: 54–69.

Rengasamy P. (2006): World salinization with emphasis on Australia. Journal of Experimental Botany, 57: 1017–1023.

Sadau S.B., Liu Z., Ninkuu V., Guan L., Sun X. (2024): DREB transcription factors are crucial regulators of abiotic stress responses in *Gossypium* spp. Plant Stress, 11: 100350.

Sakuma Y., Liu Q., Dubouzet J.G., Abe H., Shinozaki K., Yamaguchi-Shinozaki K. (2002): DNA-binding specificity of the ERF/AP2 domain of *Arabidopsis* DREBs. Biochemical and Biophysical Research Communications, 290: 998–1009.

https://doi.org/10.17221/124/2025-CJGPB

Sparks D.L. (2003): Environmental Soil Chemistry. Cambridge, Academic Press.

Sousa H.C., Viana T.V.A., Sousa G.G., Azevedo B.M., Lessa C.I.N., Freire M.H.C., Baldé B.G.F. (2023): Productivity in the peanut under salt stress in soil with a cover of plant mulch. Revista Ciência Agronômica, 54: e20228513.

Szabados L., Savouré A. (2010): Proline: A multifunctional amino acid. Trends in Plant Science, 15: 89–97.

Topping J.F. (1998): Tobacco transformation. In: Foster G.D., Taylor S.C. (eds): Plant Virology Protocols. Methods in Molecular Biology, Vol 81, Humana Press: 365–372.

Tu T.Q., Vaciaxa P., Lo T.T.M., Nguyen N.H., Pham N.T.T., Nguyen Q.H., Do P.T., Nguyen L.T.N., Nguyen Y.T.H., Chu M.H. (2021): GmDREB6, a soybean transcription factor, notably affects the transcription of the *NtP5CS* and *NtCLC* genes in transgenic tobacco under salt stress conditions. Saudi Journal of Biological Sciences, 28: 7175–7181.

Zhang G., Chen M., Chen X, Xu Z., Guan S., Li L.C., Li A., Guo J., Mao L., Ma Y. (2008): Phylogeny, gene structures, and expression patterns of the ERF gene family in soybean (*Glycine max* L.). Journal of Experimental Botany, 59: 4095–4107.

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