



Novel inducible promoter DREB1G cloned from date palm exhibits high fold expression over AtRD29 to drought and salinity stress

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Abstract

Right and timely expression of the stress regulatory genes is required for plants to compete against abiotic stresses; it necessitates the isolation and characterization of stress-responsive promoters for improving crops' tolerance to abiotic stresses. Dehydration Responsive Element Binding (DREB) regulates the expression of numerous stress-responsive genes in plants and leads an inevitable role in the adaptation of plants to abiotic stresses. In this study, the promoter region of *Phoenix dactylifera* (Date palm, a major fruit crop of the arid region) *PdDREB1G* gene was isolated and characterized for the first time. A comparison of the activity of two promoter fragments, 880 bp (DS) and 1.6 kb (DF) of *PdDREB1G* to *AtRD29A* was performed. Histochemical assay displayed remarkable GUS staining and RT-qPCR analysis confirmed the induction of *GUS* expression in T₃ plants of transformed tobacco subjected to different abiotic stresses. Furthermore, compared with the widely used *AtRD29A* promoter, the relative expression of GUS in leaves by DS and DF was three and twofold higher under salt stress, respectively, while it was twofold in polyethylene glycol (PEG) and abscisic acid (ABA) for DS. Under SA stress, DF and DS displayed 1.5 and onefold expression in leaves, respectively. In the root, DS showed a fourfold increased expression in salt, threefold in PEG and ABA, and twofold in SA. Hence, the DS promoter characterized in the present study becomes a choice over *RD29A* for abiotic stress responses and is useful to develop stress-tolerant transgenic plants by inducing the expression of stress-inducible genes on stress.

Key message

Novel inducible promoter DREB1G cloned from the Date palm, a major fruit crop of the arid region, exhibiting high fold expression over *AtRD29A* to drought and salinity stress is useful to develop stress-tolerant transgenic plants by inducing the expression of stress-inducible genes on stress.

Keywords Abiotic stress · GUS · *PdDREB1G* · Promoter · Salinity · Stress-inducible

Abbreviations

ABA Abscisic acid
DF DREB full (1.6 kb)
DS DREB short (880 bp)
GUS β-Glucuronidase

IAA Indole-3-acetic acid
PEG Polyethylene glycol
SA Salicylic acid

Introduction

Plants are constantly challenged by various environmental stresses especially drought, salinity, and temperature. These abiotic stresses hinder plant growth by affecting biochemical, physiological, and molecular activities from germination onwards causing severe loss to crop productivity. Nevertheless, some plant species are adapted to compete against the stress through an integrated network of various mechanisms like the activation of genes by inducible promoters.

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Promoters are crucial regulators of gene expression due to the presence of important *cis*-acting elements. Promoters are upstream gene regulatory sequences recognized by transcription factors (TFs) involved in controlling transcription initiation and progression. In modern crop improvement strategies, the design of efficient gene constructs relies on promoter efficiency, tissue specificity, and other characteristics that allow the introgression of agronomically relevant traits to overcome biotic and abiotic stresses (de Melo et al. 2021). The widely used promoter for the development of transgenic plants, pCaMV35S is reported to have other effects like gene silencing and metabolic penalties impacting plant fitness (de Melo et al. 2021). The constitutive promoters cause unnecessary high gene expression all the time which leads to interference with other cellular pathways of plant development (Zhang et al. 2016; Jiang et al. 2018). Variability in the expression pattern of genes under CaMV 35S promoter across different plant species, among and within tissues, and under different environmental conditions has been exemplified (Schnurr et al. 2000). Post-transcriptional and translation silencing by overexpression of genes under 35S promoter has been documented well (Rajeevkumar et al. 2015). The pros and cons of 35S promoter with its limitation to use it as a preferred promoter are also reviewed (Amack and Antunes 2020). To circumvent the negative impacts of constitutive promoters, the identification and functional characterization of plant-derived stress-inducible promoters be on focus as an alternative to drive the expression of the genes on stress with no negative impact on plant growth. The inducible promoters that cause temporal expression of a gene based on certain stimuli are able to eradicate the negative effects of the constitutive promoters. They are used to express stress-tolerant genes under certain stressful conditions such as drought, heat, cold, dehydration, and oxidative environment (Rai et al. 2009; van Essen et al. 2010). Overexpression of *AtDREB1A* under stress-inducible *AtRD29A* promoter in transgenic *Arabidopsis* and rice showed improved tolerance to abiotic stress with no abnormalities in plant growth (Kasuga et al. 2004; Kong et al. 2016). Besides, the right expression of the stress regulatory genes in a spatiotemporal fashion is required to target diverse abiotic stresses (Kasuga et al. 1999), and it mandates a well-characterized inducible promoter. The review on the impact of inducible promoters in transgenic plant production and crop improvement (Misra and Ganesan 2021) signifies the importance to look for act-on stress promoters. High-level expression by inducible promoters has been reported under different stresses: salinity and osmotic stress-inducible GAPP promoters from maize (Hou et al. 2016), heat-inducible *Apx*, *Dhn* and *Hsc70* from pearl millet (Divya et al. 2019), osmotic and cold stress-inducible BBX24 promoter from chrysanthemum (Imtiaz et al. 2015), drought stress-inducible promoters RD29A and RD29B

from *Arabidopsis* (Bihmidine et al. 2013), and osmotic stress-inducible *DREB2*, *DREB6*, and *Wdreb2* from wheat (Wang et al. 2021). Among various abiotic stress promoters characterized, the *AtRD29A* promoter is the prime one with stronger activity under drought stress (Yamaguchi-Shinozaki and Shinozaki 1993, 1994), therefore, it has been successfully used to drive the expression of drought-tolerant genes in different plant species (Kasuga et al. 2004; Bihmidine et al. 2013).

Dehydration Responsive Element Binding (DREB) gene has been widely gaining attention as these are important plant transcription factors involved in pathways for enhancing abiotic stress tolerance by regulating the expression of many stress-inducible genes. However, the functionally characterized promoters of DREB transcription factors are relatively limited: DREB3 from soybean (Xiao et al. 2008), DREB1B from rice (Gutha and Reddy 2008), DREB2C from *Arabidopsis* (Chen et al. 2012), DREB1 from buckwheat (Fang et al. 2015) and *DREB2*, *DREB6* and *Wdreb2* from wheat (Wang et al. 2021). The studies on *DREB1G* are limited to the expression of the *OsDREB1G* gene of rice which is documented as a cold stress-responsive *DREB* gene (Moon et al. 2019).

Date palm (*Phoenix dactylifera* L.), an important fruit crop of the *Arecaceae* family is a perennial and dioecious monocot widely cultivated in arid and semi-arid regions of North Africa and the Middle East. Its adaptability to arid and semi-arid regions, especially to withstand temperature fluctuations, ranging from 56 to 60°C to a few degrees below zero (Safronov et al. 2017) makes it a choice to explore the genome to design strategies like cloning of stress-inducible promoters as well as genes imparting tolerance and thereby the development of resilient crops.

We have investigated the promoter potential of the upstream region of the date palm *DREB1G* gene for the first time. The activity of the promoter fragments cloned from Date palm was evaluated in transgenic tobacco plants under different stress conditions viz., salinity, drought, cold, abscisic acid (ABA), and salicylic acid (SA). Further, in the present study, the promoter activity was also compared to the widely used stress-inducible promoter *AtRD29A*.

Materials and methods

Plant material and cloning of the promoter fragments

Leaf tissues of Date palm (*Phoenix dactylifera* L.) cv. Khalas were collected from the Date Palm Research Center, UAE University, Al Ain. DNA was extracted from the leaf tissues ground in liquid nitrogen using the Bioline DNA extraction kit (Meridian Biosciences, TN, USA)

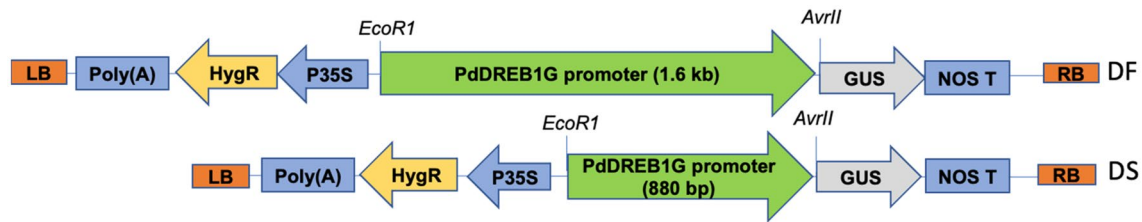


Fig. 1 Schematic drawing of the PdDREB1G promoter expression cassette in the pCambia1391Z::GUS vector

following the kit protocol. The DNA was quantified using Nanodrop 2000 (ThermoScientific, USA). The primers for the DREB1G promoter were designed from the upstream sequence of the translational start codon of the *PdDREB1G* gene sequence retrieved from the date palm genome database (NW_008246748.1:c253756-251297 *Phoenix dactylifera* cultivar Khalas unplaced genomic scaffold, DPV01 pdS000242) using Primer3 (Koressaar and Remm 2007; Untergasser et al. 2012; <https://bioinfo.ut.ee/primer3-0.4.0/>). The two promoter fragments (1.6 kb and 880 bp) were amplified using the primers DREB-F1: *CGGAATTCCCGTGCTATGGCATGATTA*, DREB-R: *TGCCTAGGGTTTCTCGGGGACTGATTGG*, and DREB-F2: *CGGAATTCAATGGTGCCA TGAATTGGAT* (DREB-F1 and R for 1.6 kb and F2 and R for 880 bp (the bases in italics represents the restriction sites of *EcoRI* and *AvrII* for the forward and reverse primers respectively). The PCR was performed with the primers using Phusion High-Fidelity Taq polymerase (NEB, USA) following the program: initial denaturation at 98 °C for 30 s, and 30 cycles of denaturation (at 98 °C for 10 s), annealing (at 55 °C for 20 s) and extension (at 72 °C for 40 s) with a final extension at 72 °C for 10 min. The RD29A promoter (730 bp, AB019226.1) from *Arabidopsis thaliana* DNA (extracted using DNeasy Plant Mini kit, Qiagen, Hilden, Germany) using the primers RD29A-F (*CGGAATTCGTGAATTAAGAGGAGAGAGGAGG*) and RD29A-R (*TGCCTAGGTTTCCAAAGATTTTTTTTCTTTCC*) by following the above PCR program. The amplified PCR products were electrophoresed on 1% (w/v) Agarose gel and the fragments were purified using a QIAquick PCR gel extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The purified amplicons were cloned into the pCR®-Blunt II-TOPO® vector using the Zero Blunt TOPO PCR Cloning Kit (ThermoFisher, MA, USA) following the kit protocol. The cloned fragments were confirmed by sequencing (Macrogen, Seoul, South Korea).

Analysis and comparison of *Cis*-elements

The sequence of PdDREB1G promoter fragments was scanned for the presence of *cis*-acting elements using the program PlantCARE (Lescot et al. 2002; [\[tics.psb.ugent.be/webtools/plantcare/html/\]\(http://tics.psb.ugent.be/webtools/plantcare/html/\)\). The regulatory elements were analyzed using the PLACE \(Higo et al. 1999; <http://www.dna.affrc.go.jp/PLACE/>\) database. The upstream region 1.6 kb and 880 bp from the start codon of the *PdDREB1G* gene were analyzed and compared to AtRD29A promoter.](http://bioinforma-</p>
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Construction of the promoter-GUS reporter vectors

The promoter fragments were cloned upstream of the *gusA* reporter gene of the plant transformation plasmid vector pCambia1391Z. The *gusA* gene has a 5'-extension with a catalase intron to ensure the expression in plants but not in bacteria. The plasmid has genes for selecting kanamycin (*nptII*) resistance in bacteria and hygromycin (enhanced 35S:*hpt*) resistance in plants. The promoter fragments digested from the pCR®-Blunt II-TOPO® vector with *EcoRI* and *AvrII* restriction enzymes were ligated into the pCambia1391Z vector containing *gusA* gene digested with the same enzymes using T4 DNA ligase (NEB, USA) to construct the plant transformation vector pCambia1391Z-*P_{DREB1G-1.6}*::*GUS* named as DF and pCambia1391Z-*P_{DREB1G-880}*::*GUS* named as DS (Fig. 1). The plasmid vector pCambia1391Z-*P_{RD29A}*::*GUS* (as RD29A hereafter) was developed by ligating the digested fragment of the RD29A promoter as above. The ligated plasmids were transformed into DH5α by the chemical transformation method. The plasmids of the positive clones confirmed by PCR using the promoter-specific primers as described previously were extracted using QIAprep spin Miniprep kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quantity of the plasmids was measured by Nanodrop 2000 and the sequence was confirmed by sequencing (Macrogen, Seoul, South Korea). The recombinant plasmids of DS, DF, RD29A, and also pCambia1391Z-35S::*GUS* were mobilized into disarmed hypervirulent *A. tumefaciens* strain EHA105 (Hood et al. 1993) through electrotransformation. The positive colony (confirmed by PCR with specific primers) selected was used for tobacco transformation.

Agrobacterium-mediated transformation of tobacco

The binary vector constructs in *A. tumefaciens* strain EHA105 streaked on solid LB medium (10 g l⁻¹ Tryptone; 5 g l⁻¹ Yeast extract; 5 g l⁻¹ NaCl; 15 g l⁻¹ bacteriological grade agar) plates containing 25 mg l⁻¹ rifampicin (Phytotechnology Laboratory, KS, USA) and 50 mg l⁻¹ kanamycin (Phytotechnology Laboratory, KS, USA). Single colonies of the bacteria were transferred into 25 ml liquid LB medium containing antibiotics in a 50 ml sterile Falcon tube and incubated overnight horizontally on a shaker (250 rpm) at 28 °C. The cells were pelleted by centrifugation at 8000 *xg* for 7 min after reaching the culture at 0.5–0.6 OD (OD₆₀₀). The LB medium was replaced with an equal volume of liquid MS (Murashige and Skoog 1962; Phytotechnology Laboratory, KS, USA) medium (3% w/v sucrose) containing 200 µM acetosyringone (Acros Organics, Geel, Belgium). After dispersing the pellet, the bacterial culture was incubated at 50 rpm for 1–2 h at room temperature (22–23 °C).

Nicotiana tabacum cv. SR1 was transformed for promoter characterization. Leaf segments of tobacco (1.5 to 2.5 cm segments excised from in vitro grown tobacco plants on half-strength MS medium with 2% (w/v) sucrose were immersed in the Agrobacterial suspension for 20 min and was followed by vacuum infiltration of 10 min. The infected leaf segments were blot-dried on sterile filter paper and co-cultivated on MS medium (with 3% sucrose) supplemented with 9.4 µM Kinetin (Sigma, USA), 5.7 µM Indole-3-acetic acid (IAA; Sigma, USA), and 200 µM acetosyringone. Co-cultivated plates were incubated in dark at 25 ± 2 °C for 2 days. The co-cultivated leaf segments were washed with 300 mg l⁻¹ timentin (Phytotechnology Laboratory, KS, USA) solution, blot dried, and cultured on a regeneration medium with the selection agent *i.e.*, MS medium with 9.4 µM Kinetin, 5.7 µM IAA, 300 mg l⁻¹ timentin, and 25 mg l⁻¹ hygromycin (Phytotechnology Laboratory, KS, USA). The shoots grown were transferred on MS basal medium supplemented with 100 mg l⁻¹ timentin, and 25 mg l⁻¹ hygromycin in 3–4 weeks. The well-grown shoots were rooted on half-strength MS medium (with 2% sucrose) containing 100 mg l⁻¹ timentin, and 25 mg l⁻¹ hygromycin. The pH of all plant tissue culture media was adjusted to 5.8 before the addition of 0.8% (w/v) agar (PlantMedia, Ohio, USA) in the case of solid media and was autoclaved at 121 °C (15 lb) for 20 min. Antibiotics and filter-sterilized plant growth regulators dissolved in respective solvents were added to the media after autoclaving. All the plant cultures were incubated in a growth room at 25 ± 1 °C (60–80% humidity) in 16 h light: 8 h dark photoperiod under white fluorescent tubes (40 µmol m⁻² s⁻¹) unless otherwise mentioned.

Molecular confirmation of transgenic plants

Genomic DNA extracted from leaves of rooted shoots growing on half-strength MS medium (with 2% sucrose) containing 100 mg l⁻¹ timentin, and 25 mg l⁻¹ hygromycin using the cetyltrimethylammonium bromide (CTAB; Sigma, St. Louis, USA) method as described by Dutta et al. (2013). Putatively transformed plants (9 independent lines) were confirmed by the presence of DS and DF with respective primers as described earlier and the *gusA* gene using PCR. PCR reactions were performed in a volume of 20 µl containing 1 × reaction buffer, 100 ng of DNA template, 1.5 mM MgCl₂, 0.2 mM of each of the dNTPs, 0.2 µM of each primer, and 2.5 unit of HotStar Taq DNA polymerase (HotStar Taq DNA polymerase kit, Qiagen, Hilden, Germany). The PCR conditions of *gusA* gene-specific primers (GUS-F: ATGAACATGGCATCGTGGTGATTG; GUS-R: GAGATC GCTGATGGTATCGGTGTG) consist of 95 °C for 15 min, 25 cycles of 94 °C for 20 s, 60 °C for 20 s, 72 °C for 30 s and a final extension of 72 °C for 5 min. The plasmid DNA (pCAMBIA1391Z) was used as a positive control, whereas DNA isolated from untransformed plants (WT) was used as the negative control.

Acclimatization of transgenic (T₀) plants

PCR confirmed plantlets after washing were planted in a soil mix (sand and peat; 1:1) in small pots (8 cm w × 8.5 cm ht). The pots were initially covered by a polyethylene bag for 10 days to retain moisture during the acclimatization in the plant room (25 ± 2 °C, 70% humidity, 16 h light: 8 h dark photo-period, 400 µmol/m²/s—Heliospectra LED Lights). The well-growing plantlets were transplanted into 5 L pots (21 cm w × 19 cm ht) and grown till seed harvest (5–6 months).

Generation of T₂ and T₃ plants

The transgenic T₁ tobacco seeds with promoters and *gusA* gene collected from different T₀ plant lines were surface sterilized separately by treating with 10% (v/v) Clorox solution in 2 ml Eppendorf tubes for 10 min with inversion. After washing three times with sterile water, the blot-dried seeds were cultured on half-strength MS (2% sucrose) containing 25 mg l⁻¹ hygromycin for germination. The healthy plants derived from T₁ seeds after 20 days were selected based on segregation and were transplanted into the soil as described previously. T₂ transgenic tobacco seeds germinated on hygromycin containing half-strength MS were subsequently transplanted into soil and collected the seeds from the dried pods. The T₃ plantlets were used for the promoter analysis studies.

Abiotic stress treatments

For characterization and elucidating the response of PdDREB1G promoter to different abiotic stresses, 25-day-old transgenic T₃ plantlets (three lines each) of DS, DF, RD29A, 35S, and untransformed plantlets (WT) were subjected to 150 mM NaCl, 10% (w/v) PEG6000, 20 μM ABA, 10 μM SA, 4 °C for 24 h. After 24 h some of the treated plantlets were collected for GUS staining; the remaining plantlets (leaves and roots) were frozen in liquid nitrogen and stored at -80 °C for the real-time qPCR analysis. All experiments were repeated three times.

Histochemical GUS assay

Seedlings (15- and 25-days) of WT and transgenic plants of DS, DF, RD29A, and 35S were collected for GUS expression analysis in response to various abiotic stresses. Histochemical staining was performed following the procedure described by Jefferson et al. (1987). The samples collected from transgenic plantlets and WT with and without stress were transferred into the GUS assay buffer (1 mg m⁻¹ X-Gluc, 100 mM sodium phosphate buffer pH 6.8, 0.5 mM potassium ferrocyanide, 0.5 mM potassium ferricyanide, 100 μM Na₂EDTA) and after applying vacuum (10 min) were incubated overnight at 37 °C. The chlorophyll was removed by repeated washing with absolute alcohol: acetic acid (3:1, v/v) solution. After destaining, the samples were stored in 5% (v/v) acetic acid and photographed (Nikon D5300). For each construct, samples were collected from at least three different transgenic lines.

RNA isolation and RT-qPCR analysis

Total RNA from the leaves and roots of the transformed tobacco seedlings and control was extracted using Maxwell® RSC Plant RNA Kit (Promega, Madison, USA). The quantity and quality of RNA were checked by Nanodrop 2000 and gel electrophoresis, respectively. cDNA was synthesized from 1 μg total RNA using a QuantiTect Reverse Transcription kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The qRT-PCR reactions were carried out in a total of 10 μl volume containing 2 μl of diluted cDNA (1:5), 200 nM of *gusA* gene primers (GUS-qF: GAATACGGCGTGGATACGTTAG; GUS-qR: GATCAAAGACGCGGTGATACA) and 5 μl of 2×Fast SYBR Green PCR Master Mix (Applied Biosystems, CA, USA) in an optical 96 well plate using a StepOnePlus™ Real-Time PCR System (Applied Biosystems, CA, USA). qRT-PCR temperature cycle was set up as 95 °C for 10 min, 40 cycles of 95 °C for 10 s, 58 °C for 15 s, and 72 °C for 20 s. The transcript level of the *gusA* gene under the control of different promoters between samples was compared

by the 2^{-ΔΔCt} method (Livak and Schnittgen 2001). Actin (LOC_107821481) tobacco (NtACT-qF: AGGCTGTCC TTCCTTGATG; NtACT-qR: CAAAGCATGACCCTC GTAGAT) was used as an endogenous reference gene for normalization and calculating the fold change of transgene. Three technical replicates were analyzed for each biological replicate.

Statistical analysis

For all the stress experiments, the relative expression of the genes and standard error values were analyzed using the Microsoft Excel program. The data values represent means ± SE from three independent experiments. Student t-test was performed to assess the significant differences between control and treatment conditions. Differences of P < 0.05 were considered significant.

Results

Bioinformatic analysis of the PdDREB1G promoter sequence

A 1.6 kb fragment (DF) and 880 bp fragment (DS) located upstream of the start codon (ATG) of *PdDREB1G* were isolated from the genomic DNA of the date palm *cv.* Khalas. The analysis of the promoter sequences using PLACE and PlantCARE showed many abiotic stress-responsive *cis*-elements. The frequency of *cis*-elements related to abiotic stress in the DF, DS fragments, and RD29A was compared (Table 1). The results revealed that the promoter sequences contain several water-deficit responsive *cis*-acting elements such as EBOXBNNAPA (CANNTG), DRE1COREZMRAB17 (ACCGAGA), ABRELATERD1 (ACGTG), ACG TATERD1 (ACGT), MYB2AT (TAAGT), MYBCORE (CNGTTR), MYB1AT (WAACCA), MYB2CONSENSU-SAT (YAACKG). G-box (CACGTG) and TC-rich repeats (GTTTCTTAC) are responsible for dehydration and salinity tolerance. The LTRECOREATCOR15 (CCGAC) represented low-temperature tolerance. The DF fragment contained one SA-responsive TCA (CCATCTTTT) element. The numbers of ABRE elements accountable for the activation of genes under early dehydration were 11 and 10 in DF and DS fragments, respectively. EBOXBNNAPA was intended for dehydration, and the ABA-responsive elements were nine in the DF region and three in the DS fragment. Two G-box regions in DF and one in DS were responsible for dehydration, high salinity, and ABA response. There were two DRE1COREZMRAB17 and TC-rich repeats in the DF fragment and one each in the DS fragment which is significant for drought and salinity tolerance. The promoter sequence showed two cold-responsive LTR elements which

Table 1 Comparison of *Cis*-regulatory elements related to abiotic stresses in PdDREB1G promoter fragments and AtRD29A (DF-DREB Full; DS – DREB Short)

<i>Cis</i> -regulatory elements	Core sequence	Number of <i>cis</i> -regulatory elements			Description
		DF	DS	AtRD29A	
MYBCORE	CNGTTR	2	0	0	Dehydration
MYB2AT	TAACGTG	1	0	0	Dehydration
MYB1AT	WAACCA	1	1	0	ABA, Dehydration
MYB2CONSENSUSAT	YAACKG	1	0	0	Dehydration
EBOXBNNAPA	CANNTG	9	3	2	Dehydration, Cold, ABA
DRE1COREZMRAB17	ACCGAGA	2	1	0	Drought, ABA
ABRELATERD1	ACGTG	7	6	1	ABA, Dehydration
ACGTATERD1	ACGT	11	10	5	Early dehydration
ACGTTBOX	AACGTT	1	1	1	Dehydration
LTRECOREATCOR15	CCGAC	2	2	4	Cold, ABA, Drought
GBOX/CACGTGMOTIF	CACGTG	2	1	0	Dehydration, high salinity, ABA
TC-rich repeats	GTTTTCTTAC	2	1	0	Salinity
TCA-element	CCATCTTTTT	1	0	0	SA responsiveness
DRE2COREZMRAB17	ACCGAC	0	0	3	ABA, drought
DRECRCOREAT	RCCGAC	0	0	4	ABA, drought, cold
CBFHV	RYCGAC	0	0	4	Cold
DREDR1ATRD29AB	TACCGACAT	0	0	2	Drought, Cold, Salt

were on the DS region. There were two MYBCORE and one MYB2AT region in DF and one each MYB1AT region in DF and DS fragments which are important for dehydration stress response (Fig. 2 & 3).

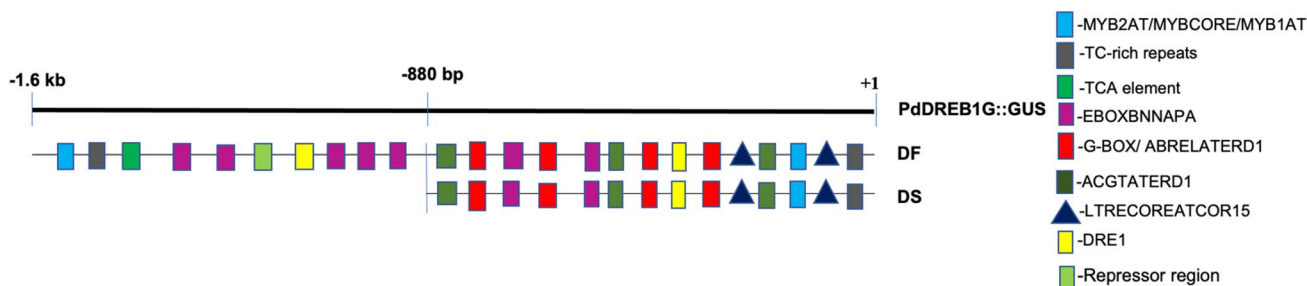
PCR confirmation, GUS assay, and characteristics of the PdDREB1G promoter in response to various abiotic stresses

Putatively transformed plants (9 independent lines) were confirmed by the presence of DS and DF (with respective primers) with an amplicon of 880 bp and 1.6 kb, respectively (Fig. 4a). All transgenic plants were also confirmed by the *gusA* gene (an amplicon of 353 bp) using PCR (Fig. 4b). The GUS assay of the transgenic seedlings with the *gusA* gene under the promoter fragments of the present study (DF and DS), under AtRD29A and 35S, showed GUS activity. The GUS staining of the transgenic plants with the *gusA* gene

under the control of DF and DS exposed to salt and PEG treatment showed intense blue staining (Fig. 5). Treatment with ABA showed comparatively less staining (Fig. 5). The staining after exposure to SA and cold was more intense in DS compared to DF (Fig. 5). The positive controls with 35S promoter displayed staining in all the stresses, but was less intense to non-stressed of the same (Fig. 5). AtRD29A deep blue staining in all stress conditions except cold. No GUS activity was detected in non-transgenic control (WT) plants (Fig. 5).

Quantitative *gusA* gene expression levels

Quantitative *gusA* gene expression levels of the DS and DF fragments measured via real-time PCR analysis after exposure of T₃ tobacco seedlings to 150 mM NaCl, 10% polyethylene glycol 6000 (PEG 6000), 20 μM ABA, and 10 μM SA revealed high transcript levels of *gusA* compared

**Fig. 2** Diagram of the main *cis*-acting elements in the full-length PdDREB1G (DF; 1.6 kb) and short promoter PdDREB1G (DS; 880 bp)

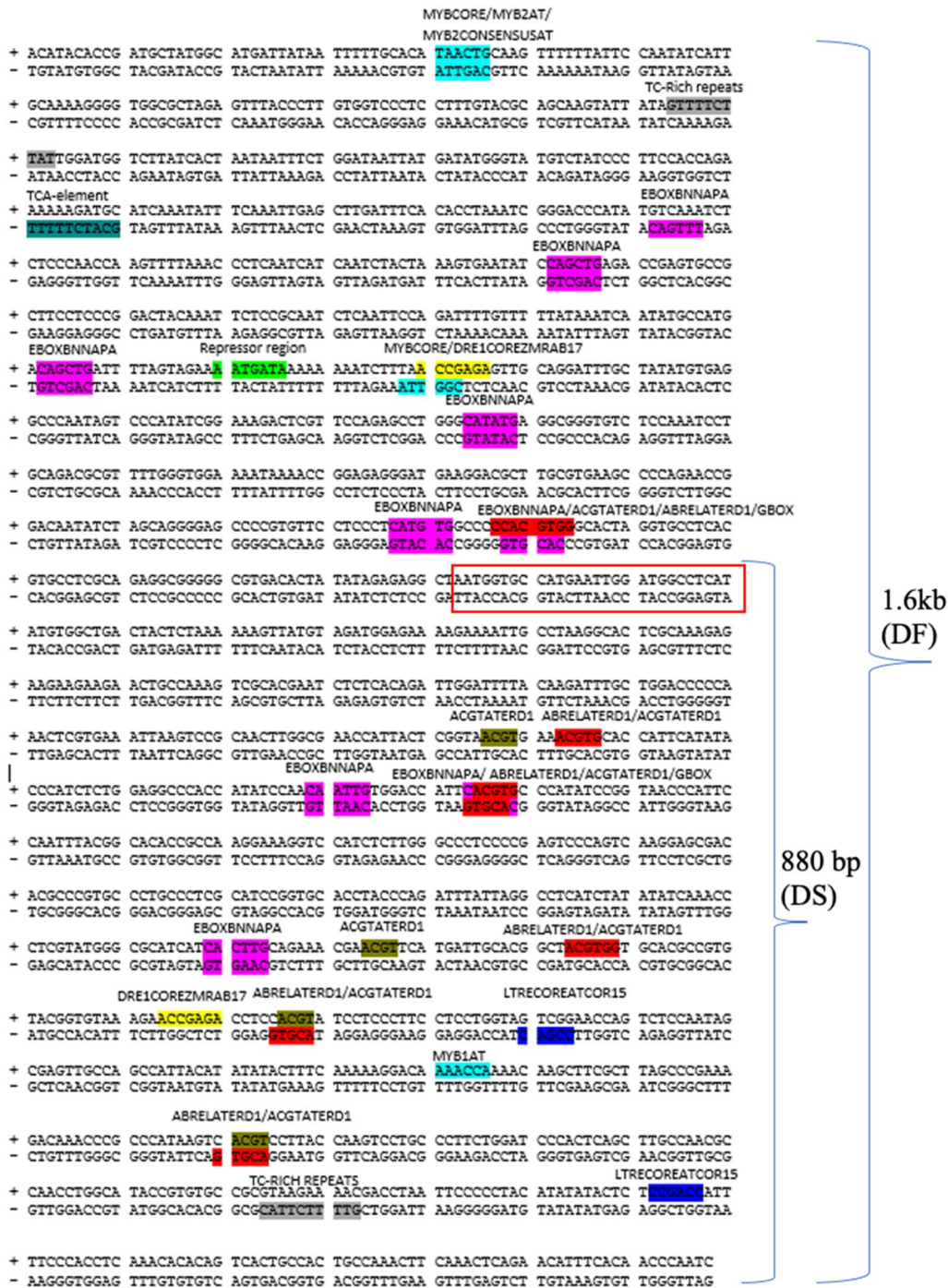


Fig. 3 DNA sequence of PdDREB1G promoter showing *cis*-elements for abiotic stress induction (DS-DREB Short; DF-DREB Full). Red square shows the start of the DS

to control (WT) indicated high promoter activity (Fig. 6a-e). The transcript levels were high in leaves compared to the root (Fig. 6a-e). Among these stresses, treatment with NaCl caused the most substantial changes in *gusA* gene expression, followed by ABA, PEG, and SA (Fig. 6a-e). The expression level was lowest under cold treatment (Fig. 6a-e).

The DS showed an increased expression of *gusA* gene in all the given stresses in the leaf and root compared to DF (Fig. 6a-e). The only exception was in cold stress where there was no difference between DF and DS even though there was a significant increase in *gusA* gene expression level compared to the control (Fig. 6e).

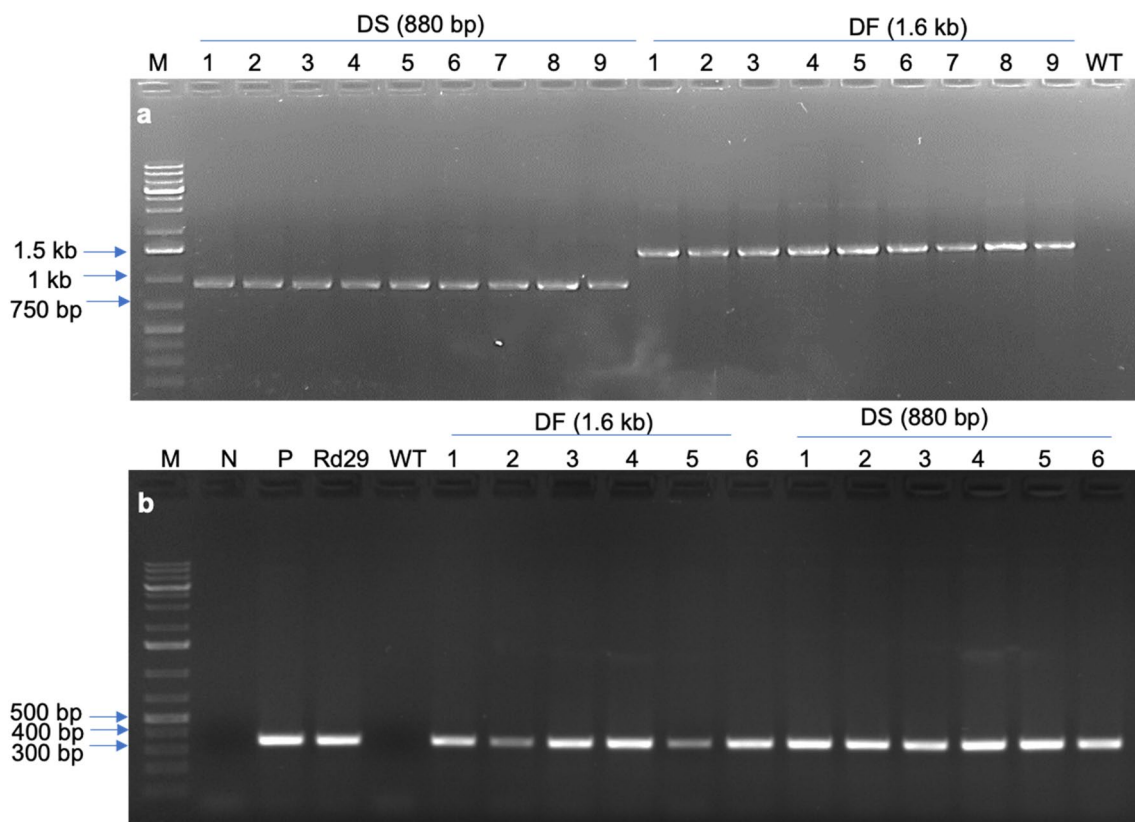


Fig. 4 PCR confirmation of transgenic plants **a.** PdDREB1G promoter (DF and DS) fragments; **b.** *gusA* gene (N-Negative control, P-Positive control (pCAMBIA1391Z vector), RD29-AtRD29A::*GUS*, WT-Untransformed, M-1 kb Plus Marker)

When compared with the RD29A promoter, in the case of leaves the expression by DS was threefold higher and DF was twofold under salt stress (Fig. 7a). The DS was two times higher in PEG and ABA. The DS, DF, and RD29A showed no significant difference under SA and cold stress (Fig. 7a). In the case of root, expression of the *gusA* gene in DS was fourfold higher in salt, threefold higher in PEG and ABA, twofold higher in SA, and no significant difference under cold stress (Fig. 7b). Expression of the *gusA* gene by DF displayed no significant difference compared to the RD29A promoter (Fig. 7b).

Spatiotemporal expression of the PdDREB1G promoter

Since GUS staining with DS promoter was the highest under salt stress and more prominent than DF, DS transgenic plants were used to determine whether promoter fragment DS is developmentally regulated. The patterns of the GUS histochemical staining were monitored during different plant developmental stages (15-, 25-day-old and mature plants), and in tissues such as roots, leaves, stems, flowers, pods, and seeds under salt stress (Fig. 8a-h). In 2 week-old tobacco seedlings, the intensity of GUS staining was weak (Fig. 8a).

In a 25-day-old seedling, there was GUS staining in almost all tissues, but intense in the vascular region (Fig. 8b). In well-grown plants, GUS activity was prominent in leaves, roots, and stems (Fig. 8c-h). GUS staining was higher in leaves than in roots (Fig. 8c). In the case of the root, GUS staining was intense in the elongation zone of the root and root tips (Fig. 8d). The stem hairs and vascular tissues exhibited intense blue staining (Fig. 8e). There was considerable staining on petals and pods (Fig. 8f, g), but was negligible in seeds (Fig. 8h). The results showed that the inducible promoter potential of the DS fragment in driving gene expression was in a spatiotemporal manner under stress.

Discussion

Stress tolerance of plants is materialized by inducing the expression of genes by the promoters which control the binding of RNA polymerase to DNA. The selection of the promoter *i.e.*, a stretch of DNA comprising the core-promoter region and multiple repeats or combinations of heterologous upstream regulatory elements (*cis*-motifs or TF-binding sites) is significant in designing a transformation cassette that would enable the precise control of transgene

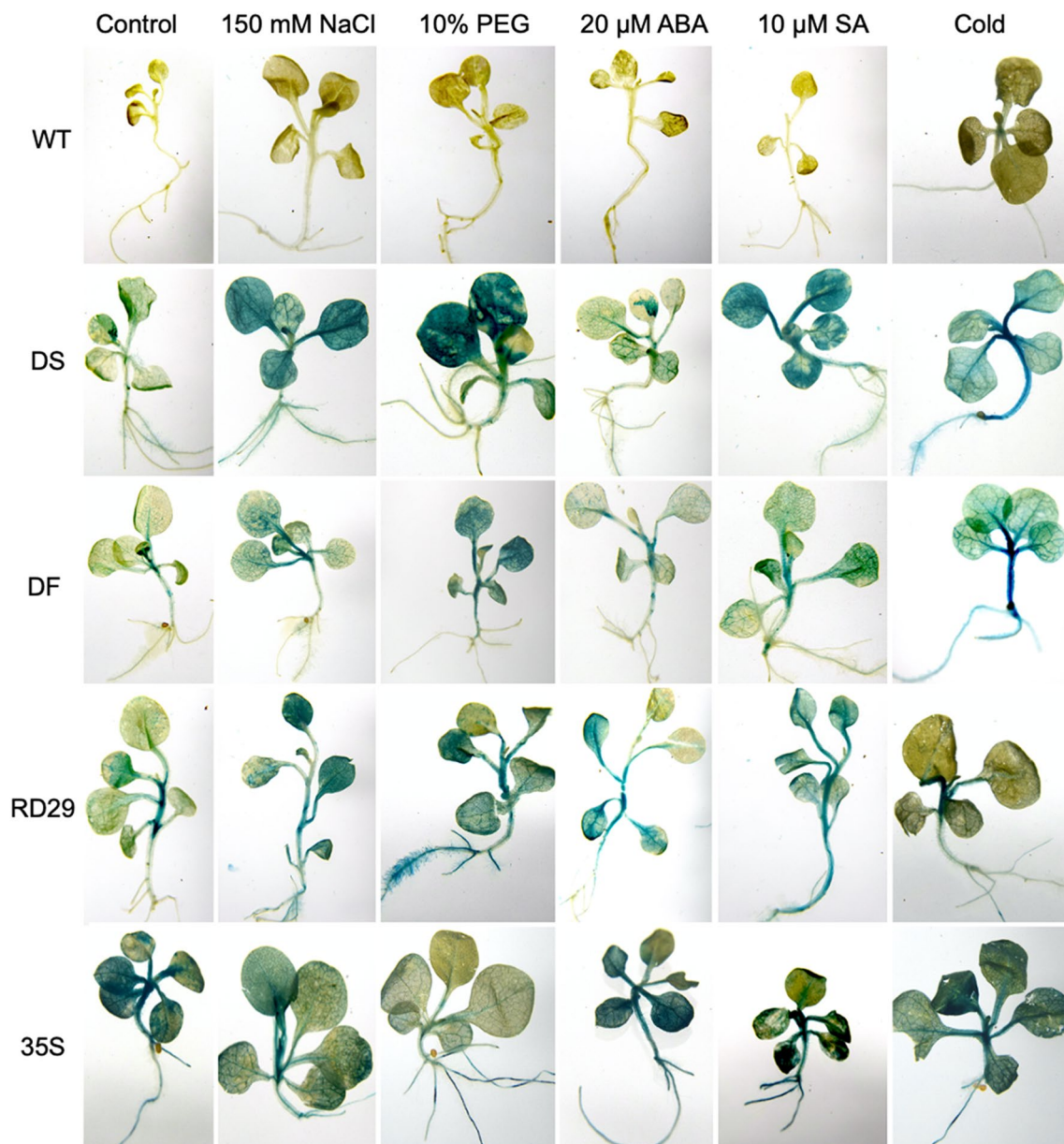


Fig. 5 GUS histochemical staining of 25-day-old transgenic tobacco seedlings with different promoters (DF-DREB Full; DS-DREB Short; RD29-RD29A, 35S) under various abiotic stress treatment (WT-

Untransformed; NaCl-sodium chloride; PEG-polyethylene glycol; ABA-abscisic acid; SA-salicylic acid)

activity (Ali and Kim 2019). The selection of a promoter, to confer constitutive, spatial, and/or temporal transgene expression, is one of the vital components for the development of genetically modified plants. Of the promoters, constitutive promoters are of wide use but are reported to have undesirable effects such as gene silencing due to methylation, etc. on the expression of transgenes (Okumura et al. 2016; Amack and Antunes 2020). Validation of promoters for precise spatial and temporal control of transgene expression contributes to the improvement of crop productivity and sustainable agriculture. The process of transcription is

essential for gene regulation and is accomplished through sequence-specific binding of transcription factors to their target promoters (Ali and Kim 2019). The pivotal role in controlling processes is not played by encoding sequences, but by regulatory elements which dynamically enhance or restrict gene expression levels within an organism (Venter 2007). Targeted activation of promoters by environmental stresses inducing the expression of genetic information leading to the final product has great significance, and the review by Misra and Ganesan (2021) exemplifies its importance in transgenic plant production and crop improvement. The

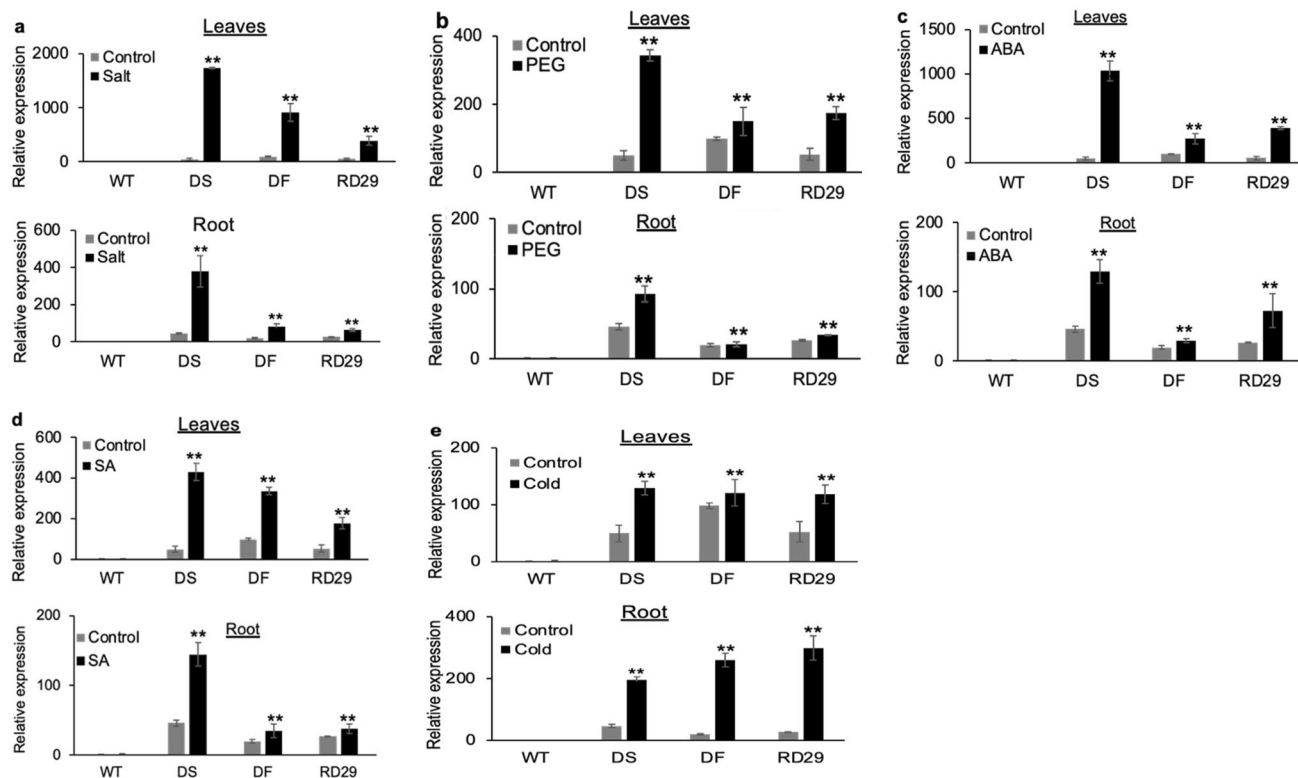


Fig. 6 Transcriptional expression levels of *gusA* in leaves and roots of transgenic tobacco plants under different promoters (DS, DF, and RD29A) and WT seedlings after 24 h of exposure to abiotic stresses. **a.** Salt, **b.** PEG, **c.** ABA, **d.** SA, **e.** Cold. The actin gene was used as an endogenous control for normalization. The relative expression was calculated with respect to WT plants. The relative gene expression

was calculated by the $2^{-\Delta\Delta Ct}$ method. In all cases, data values represent means \pm SE from three independent experiments. Asterisks indicate statistical significance ($**P < 0.01$, Student's *t*-test) of differences between transgenic lines and WT seedlings. (WT-Untransformed; NaCl-sodium chloride; PEG-polyethylene glycol; ABA-abscisic acid; SA-salicylic acid)

understanding of the regulation of plant gene expression at the *cis*-acting elements level of a promoter cloned from a native plant gene of date palm, an arid fruit crop tailored to *gusA* gene and its functional validation at the plant level is an add-on to the development of stress competent crops.

Date palm is one of the widely cultivated fruit crops in arid regions, the genes and the mechanism involved in its tolerance to various abiotic stress are relatively unknown. The functional analysis of the promoter fragments of the *DREB1G* gene from the Date palm in the present study revealed multiple *cis*-acting elements that modulate transcription in a stress-inducible and tissue-specific manner. DREBs are important transcription factors belonging to the family of AP2/ERF transcription factors containing conserved AP2/ERF domain and are further subdivided into six subgroups of A-1 to A-6. These DREBs bind to CRT/DRE *cis*-elements (A/GCCGAC) in the promoters which regulate genes playing a pivotal role in plants' tolerance to biotic and abiotic stresses (Zhou et al. 2010). It is proven now that most DREB genes are regulated by abiotic stresses, and this induction may be ABA-dependent or ABA-independent. (Yoshida et al. 2014). Further, the DREB1/CBF-type TFs

are activated by four or fewer major abiotic stresses (cold, heat, drought, and salinity), although the pattern of expression of orthologous genes in different species varied (Yang et al. 2020; Li et al. 2021).

The efficacy of the date palm promoters of the present study and the *Arabidopsis*-derived stress-inducible promoter RD29A in driving the expression of the *gusA* reporter gene in transgenic tobacco was analyzed. In this study, we have compared long (1.6 kb) named DF and short (880 bp) named DS of the PdDREB1G promoter fragments to find out the optimum promoter length for higher activity under abiotic stress. The well-known drought AtRD29A promoter was kept for comparison as the positive control. The screening of *cis*-elements showed that the PdDREB1G promoter fragments were enriched in stress-related *cis*-elements related to salinity, dehydration, ABA, and temperature (Fig. 3). This result directly correlated with an excellent performance of the promoter under salinity, dehydration and ABA treatment (Fig. 5 and Fig. 6a-e). The DF and DS showed similar or greater GUS activity than the AtRD29 promoter. Compared to the DF promoter and AtRD29, the performance of DS was significantly high in salinity, PEG, and ABA treatment

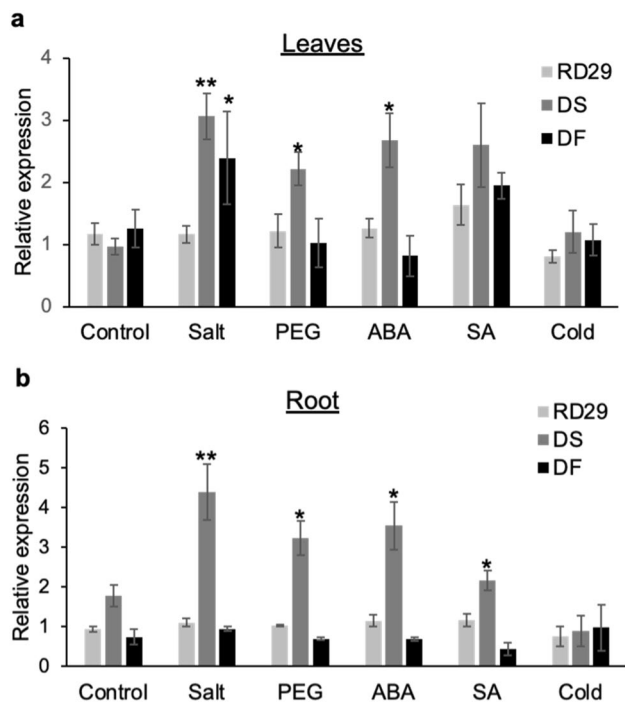


Fig. 7 Transcriptional expression levels of *gusA* in leaves and roots of 25-day-old transgenic tobacco plants under different promoters (DF, DS, and RD29A) after 24 h of exposure to abiotic stresses (Salt, PEG, ABA, SA, and Cold). **a.** Leaves. **b.** Roots. The actin gene was used as an endogenous control for normalization. Relative expression of DS and DF were calculated with respect to RD29A plants. The relative gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method. In all cases, data values represent means \pm SE from three independent experiments. Asterisks indicate statistical significance (* $P < 0.05$ and ** $P < 0.01$, Student's *t*-test) of differences of DS and DF with respect to the RD29A line. (WT-Untransformed; NaCl-sodium chloride; PEG-polyethylene glycol; ABA-abscisic acid; SA-salicylic acid)

(Fig. 7a, b). The expression level was comparatively less in SA and cold treatment (Fig. 7a, b). Most of the *cis*-elements related to drought, salinity, and cold like ACGTAT ERD1(ACGT), ABRELATERD1(ACGTG), EBOXBN-NAPA (CANNTG), GBOX (CACGTG), LTR (CCGAC) are concentrated in the DS (880 bp) fragment (Fig. 2). For example, the occurrence of 10 ACGTATERD1 regions and 6 ABRELATERD1 and 3 EBOXBN-NAPA regions in the DS fragment could be the reason for the high GUS expression under salinity, PEG and ABA (Guiltinan et al. 1990). ABA signaling is also known to play an important role in salinity and drought stress tolerance. ABRE functioned in response to low-temperature, high-salinity, and dehydration treatments, but not to ABA. Studies showed that the G-box family core (ACGT) is the most conserved element among different plant species and responds to abiotic stresses especially water-deficit and salt stresses (Mehrotra et al. 2013). Other *cis*-elements are also present in DF and DS such as MYBs, which are known to respond to water deficit and

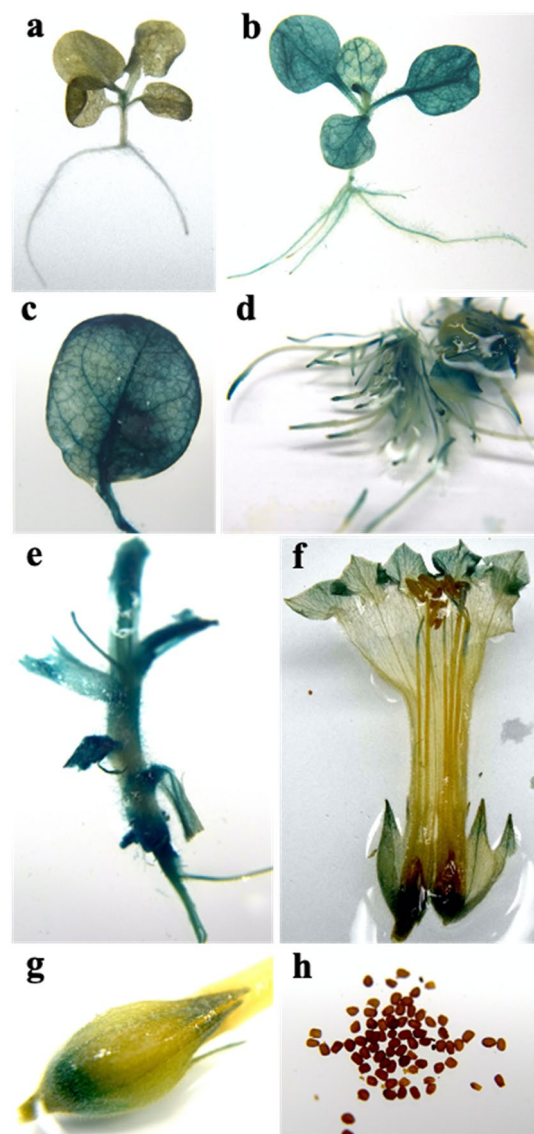


Fig. 8 Spatiotemporal expression pattern of PdDREB1G promoter in transgenic tobacco plants under salt stress: **a.** 15-day-old, **b.** 25-day-old, **c.** leaf, **d.** root, **e.** stem, **f.** flower, **g.** pod, **h.** seed

ABA (Hussain et al. 2021). Functional characterization of the TkSRPP promoter in response to hormones and wounding stress in transgenic tobacco has also been reported (Dong et al. 2023). Increased expression of GUS in DS compared to positive control AtRD29A could be because of fewer number *cis*-elements in its promoter region *e.g.*, five ACGTAT ERD1 regions, one ABRELATERD1, and two EBOXBN-NAPA regions (Table 1). Responsiveness to SA indicates that PdDREB1G has a role in oxidative stress and disease response in plants as had been documented in OsDREB1B (Gutha and Reddy 2008). The *cis*-acting element (TACCGA CAT), namely the dehydration-responsive element (DRE), is absent in the DF and DS promoters of the present study

but has been present in the promoter regions of many dehydration and low-temperature stress-inducible genes (Shinozaki and Yamaguchi-Shinozaki 2000). Fang et al. (2015) notified high GUS activity under drought stress conditions by the FeDREB1 promoter of common buckwheat. Further, in coffee plants, three different promoter haplotypes of *CcDREB1D* consist of different *cis*-elements that are involved in the tissue-specific expression, ABA and light regulation has been reported (Alves et al. 2018).

The differences in GUS expression between DS and DF under different types of abiotic stresses are probably related to the distribution pattern of specific *cis*-regulatory sequences and the co-localization and aggregation of motifs nearby as documented in rice and sorghum (Srivastav et al. 2010). The activation of genes depends not only on the *cis*-elements in the promoter but also on their positions and the presence of enhancers, regulatory sequences, repressors, and other synergistic *cis*-elements (Sawant et al. 2005). In spite of the presence of a high number of stress-responsive *cis*-elements in DF, the expression of GUS under salinity, PEG and ABA are significantly less in DF compared to DS. A recent study showed the presence of repressor region 5'-AAT GATA-3' region in the promoter could negatively affect the expression of genes under salt stress or hypoxia (Seok et al. 2022). The DF fragment, which is not a part of the DS fragment contains one repressor region (Fig. 3). The reduced activity of the DF promoter compared to DS may be due to the presence of the repressor elements in the distal region of the promoter *i.e.*, specific to the DF fragment. Though there are more *cis*-elements in the DF, their relative distance of them from the transcription start site may also be a reason behind the reduced activity over DS. The upstream region of several gene promoters was found to contain positive or negative regulatory elements, some of which were characterized as enhancers or silencers (Timko et al. 1985; Tyagi 2001). The DS region may also contain an uncharacterized new element crucial for salinity stress response because of its ability to respond to salt stress in high profoundness.

GUS staining revealed that the DS promoter was active in different tissues and organs of the tobacco plants, such as roots, stems, leaves, flowers, and pods except in seeds (Fig. 8a-h). This result was similar to the PsDREB2 promoter in *Paeonia suffruticosa* (Liu et al. 2019), and the GmPRP2 promoter in soybean (Chen et al. 2014). PdDREB1G showed stress-specific and organ-specific expression as reported by the OsDREB1B promoter (Gutha and Reddy 2008). The results proved that the promoter PdDREB1G is highly activated under salinity than cold stress which is different from the result obtained with overexpression of *OsDREB1G* which was highly activated only with cold stress and not with drought and salinity or ABA (Moon et al. 2019). In line with it, the expression in the present study was high in the leaf. High low-temperature activation has also been demonstrated by OsDREB1B (Gutha

and Reddy 2008). The differences in the result with respect to our study are due to the promoter modules enriched in *cis*-acting elements which drive more consistent gene expression and it reinforces the idea of a synergistic effect of *cis*-elements in gene promoter sequences.

Very specific expression patterns of transgenes enable elucidation of the cellular regulation mechanisms; in such cases, inducible promoters are an open choice as they switch on or off the gene of interest under certain conditions or at certain developmental stages. The comparison of the DS promoter to well-studied stress inducible promoter RD29A in the present study establishes that DS is a strong inducible promoter over RD29A except in cold stress and will be a choice in designing transformation-cassettes in the development of genetically modified crops for stress tolerance.

Conclusions

Validation of promoters with respect to the reflexes of agronomically important plants like Date palms growing well in arid regions is of great significance in the development of genetically modified plants with improved stress competence. The present study for the first time places a strong stress-inducible promoter cloned from the arid fruit crop, Date palm is an open choice for the development of stress-tolerant crops.

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Author contributions MK Conceived the project, PK designed and carried out the experiment mostly, drafted the Manuscript, KW carried out initial experiments, SS, GL, SK, and SRHA assisted with the experiments, and KA overlook the experiment. All authors reviewed and corrected the Manuscript.

Data availability The data are available from the corresponding author on request.

Declarations

Conflict of interest The authors have no conflict of interest, financial or otherwise.

Ethical approval The results in the Manuscript are the author's original work. The authors confirm that this manuscript has not been previously published and is not currently under consideration by any other journal. Additionally, all of the authors have approved the contents of this paper and have agreed to the journal's submission policies.

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References

- Ali S, Kim W-C (2019) A fruitful decade using synthetic promoters in the improvement of transgenic plants. *Front Plant Sci* 10:1433. <https://doi.org/10.3389/fpls.2019.01433>
- Alves GSC, Torres LF, de Aquino SO et al (2018) Nucleotide diversity of the coding and promoter regions of DREB1D, a candidate gene for drought tolerance in *Coffea* species. *Tropical Plant Biol* 11:31–48. <https://doi.org/10.1007/s12042-018-9199-x>
- Amack SC, Antunes MS (2020) CaMV35S promoter – A plant biology and biotechnology workhorse in the era of synthetic biology. *Curr Plant Biol* 24:100179
- Bihmidine S, Lin J, Stone JM et al (2013) Activity of the *Arabidopsis* RD29A and RD29B promoter elements in soybean under water stress. *Planta* 237:55–64. <https://doi.org/10.1007/s00425-012-1740-9>
- Chen H, Je J, Song C, Hwang JE, Lim CO (2012) A proximal promoter region of *Arabidopsis* DREB2C confers tissue-specific expression under heat stress. *J Integr Plant Biol* 54:640–651. <https://doi.org/10.1111/j.1744-7909.2012.01137.x>
- Chen L, Jiang B, Wu C et al (2014) GmPRP2 promoter drives root-preferential expression in transgenic *Arabidopsis* and soybean hairy roots. *BMC Plant Biol* 14:1471–2229. <https://doi.org/10.1186/s12870-014-0245-z>
- de Melo BP, de Moura SM, Morgante CV et al (2021) Regulated promoters applied to plant engineering: an insight over promising soybean promoters under biotic stress and their *cis*-elements. *Biotechnol Res Innov* 5:e2021005. <https://doi.org/10.4322/biori.202105>
- Divya K, Kavikishor PB, Bhatnagar-Mathur P et al (2019) Isolation and functional characterization of three abiotic stress-inducible (*Apx*, *Dhn*, and *Hsc70*) promoters from pearl millet (*Pennisetum glaucum* L.). *Mol Biol Rep* 46:6039–6052. <https://doi.org/10.1007/s11033-019-05039-4>
- Dong G, Fan M, Wang H et al (2023) Functional characterization of TksRPP promoter in response to hormones and wounding stress in transgenic tobacco. *Plants* 12:252
- Dutta I, Kottackal M, Tumimbang E, Tajima H, Zaid A, Blumwald E (2013) Sonication-assisted efficient *Agrobacterium*-mediated genetic transformation of the multipurpose woody desert shrub *Leptadenia pyrotechnica*. *Plant Cell Tiss Organ Cult* 112:289–301. <https://doi.org/10.1007/s11240-012-0236-4>
- Fang ZW, Xu XY, Gao JF et al (2015) Characterization of FeDREB1 promoter involved in cold- and drought-inducible expression from common buckwheat (*Fagopyrum esculentum*). *Genet Mol Res* 14:7990–8000. <https://doi.org/10.4238/2015.July.17.7>
- Guiltinan MJ, William R, Marcotte J, Quatrano RS (1990) A plant leucine zipper protein that recognizes an abscisic acid response element. *Science* 250:267–271. <https://doi.org/10.1126/science.2145628>
- Gutha LR, Reddy AR (2008) Rice DREB1B promoter shows distinct stress-specific responses, and the overexpression of cDNA in tobacco confers improved abiotic and biotic stress tolerance. *Plant Mol Bio* 68:533–555. <https://doi.org/10.1007/s11103-008-9391-8>
- Higo K, Ugawa Y, Iwamoto M, Korenaga T (1999) Plant *cis*-acting regulatory DNA elements (PLACE) database 1999. *Nucleic Acids Res* 27:297–300
- Hood EE, Gelvin SB, Melchers LS, Hoekema A (1993) New *Agrobacterium* helper plasmids for gene transfer to plants. *Transgenic Res* 2:208–218. <https://doi.org/10.1007/BF01977351>
- Hou J, Jiang P, Qi S et al (2016) Isolation and functional validation of salinity and osmotic stress inducible promoter from the maize type-11 H⁺-pyrophosphatase gene by deletion analysis in transgenic tobacco plants. *PlosOne* 11:e0154041. <https://doi.org/10.1371/journal.pone.0154041>
- Hussain Q, Asim M, Zhang R et al (2021) Transcription factors interact with ABA through gene expression and signaling pathways to mitigate drought and salinity stress. *Biomol* 11:1159. <https://doi.org/10.3390/biom11081159>
- Imtiaz M, Yang Y, Liu R et al (2015) Identification and functional characterization of the BBX24 promoter and gene from chrysanthemum in *Arabidopsis*. *Plant Mol Biol* 89:1–19. <https://doi.org/10.1007/s11103-015-0347-5>
- Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO* 6:3901–3908. <https://doi.org/10.1002/j.1460-2075.1987.tb02730.x>
- Jiang P, Zhang K, Ding Z et al (2018) Characterization of a strong and constitutive promoter from the *Arabidopsis* serine carboxypeptidase-like gene *AtSCPL30* as a potential tool for crop transgenic breeding. *BMC Biotechnol* 18:59. <https://doi.org/10.1186/s12896-018-0470-x>
- Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1999) Improving plant drought, salt and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat Biotechnol* 17:287–291. <https://doi.org/10.1038/7036>
- Kasuga M, Miura S, Shinozaki K, Yamaguchi-Shinozaki K (2004) A combination of the *Arabidopsis* DREB1A gene and stress-inducible promoter improved drought and low temperature stress tolerance in tobacco by gene transfer. *Plant Cell Physiol* 45:346–350. <https://doi.org/10.1093/pcp/pch037>
- Kong X, Zhou S, Yin S et al (2016) Stress-inducible expression of an F-box gene TaFBA1 from wheat enhanced the drought tolerance in transgenic tobacco plants without impacting growth and development. *Front Plant Sci* 7:1295. <https://doi.org/10.3389/fpls.2016.01295>
- Koressaar T, Remm M (2007) Enhancements and modifications of primer design program Primer3. *Bioinformatics* 23:1289–1291
- Lescot M, Déhais P, Thijs G et al (2002) PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. *Nucleic Acids Res* 30:325–327
- Li Z, Wang G, Liu X et al (2021) Genome-wide identification and expression profiling of DREB genes in *Saccharum spontaneum*. *BMC Genom* 22:456. <https://doi.org/10.1186/s12864-021-07799-5>
- Liu H, Zhu K, Tan C et al (2019) Identification and characterization of PsDREB2 promoter involved in tissue-specific expression and abiotic stress response from *Paeonia suffruticosa*. *Peer J* 7:7052. <https://doi.org/10.7717/peerj.7052>
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2^{(-Delta Delta C(T))} method. *Methods* 25:402–408. <https://doi.org/10.1006/meth.2001.1262>
- Mehrotra R, Sethi S, Zutshi I, Bhalothia P, Mehrotra S (2013) Patterns and evolution of ACGT repeat *cis*-element landscape across four plant genomes. *BMC Genom* 14:1471–2164. <https://doi.org/10.1186/1471-2164-14-203>
- Misra S, Ganesan M (2021) The impact of inducible promoters in transgenic plant production and crop improvement. *Plant Gene* 27:100300
- Moon S-J, Min MK, Kim J-A et al (2019) Ectopic expression of *OsDREB1G*, a member of the *OsDREB1* subfamily, confers cold

- stress tolerance in rice. *Front Plant Sci* 10:297. <https://doi.org/10.3389/fpls.2019.00297>
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 5:473–497
- Okumura A, Shimada A, Yamasaki S et al (2016) CaMV-35S promoter sequence-specific DNA methylation in lettuce. *Plant Cell Rep* 35:43–51. <https://doi.org/10.1007/s00299-015-1865-y>
- Rai M, He C, Wu R (2009) Comparative functional analysis of three abiotic stress-inducible promoters in transgenic rice. *Transgenic Res* 18:787–799. <https://doi.org/10.1007/s11248-009-9263-2>
- Rajeevkumar S, Anunanthini P, Sathishkumar R (2015) Epigenetic silencing in transgenic plants. *Front Plant Sci* 6:693. <https://doi.org/10.3389/fpls.2015.00693>
- Safronov O, Kreuzwieser J, Haberer G et al (2017) Detecting early signs of heat and drought stress in *Phoenix dactylifera* (Date palm). *PLoS ONE* 12:e0177883. <https://doi.org/10.1371/journal.pone.0177883>
- Sawant SV, Kiran K, Mehrotra R et al (2005) A variety of synergistic and antagonistic interactions mediated by *cis*-acting DNA motifs regulate gene expression in plant cells and modulate stability of the transcription complex formed on a basal promoter. *J Exp Bot* 56:2345–2353. <https://doi.org/10.1093/jxb/eri227>
- Schnurr JA, Guerra GA (2000) The CaMV-35S promoter is sensitive to shortened photoperiod in transgenic tobacco. *Plant Cell Rep* 19:279–282
- Seok H-Y, Tran HT, Lee S-Y, Moon Y-H (2022) AtERF71/HRE2, an *Arabidopsis* AP2/ERF transcription factor gene, contains both positive and negative *cis*-regulatory elements in its promoter region involved in hypoxia and salt stress responses. *Int J Mol Sci* 23:5310. <https://doi.org/10.3390/ijms23105310>
- Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. *Curr Opin Plant Biol* 3:217–223. [https://doi.org/10.1016/s1369-5266\(00\)80068-0](https://doi.org/10.1016/s1369-5266(00)80068-0)
- Srivastav A, Mehta S, Lindlof A, Bhargava S (2010) Over-represented promoter motifs in abiotic stress-induced DREB genes of rice and sorghum and their probable role in regulation of gene expression. *Plant Signal Behav* 5:775–784. <https://doi.org/10.4161/psb.5.7.11769>
- Timko MP, Kausch AP, Castresana C et al (1985) Light regulation of plant gene expression by an upstream enhancer-like element. *Nature* 318:579–582. <https://doi.org/10.1038/318579a0>
- Tyagi AK (2001) Plant genes and their expression. Transgenic rice: a valuable monocot system for crop improvement and gene research. *Curr Sci* 80:161–169
- Untergasser A, Cutcutache I, Koressaar T et al (2012) Primer3 - new capabilities and interfaces. *Nucleic Acids Res* 40:e115
- van Essen D, Zhu Y, Saccani S (2010) A feed-forward circuit controlling inducible NF- κ B target gene activation by promoter histone demethylation. *Mol Cell* 39:750–760. <https://doi.org/10.1016/j.molcel.2010.08.010>
- Venter M (2007) Synthetic promoters: genetic control through *cis* engineering. *Trends Plant Sci* 12:118–124. <https://doi.org/10.1016/j.tplants.2007.01.002>
- Wang H, Zhu Y, Yuan P et al (2021) Response of wheat DREB transcription factor to osmotic stress based on DNA methylation. *Int J Mol Sci* 22:7670. <https://doi.org/10.3390/ijms22147670>
- Xiao S, Jian-Hu D, Ming C et al (2008) Isolation and regulative region analysis of promoter of stress-related gene GmDREB3 from soybean. *Acta Agron Sin* 34:1475–1479. <https://doi.org/10.3724/SP.J.1006.2008.01475>
- Yamaguchi-Shinozaki K, Shinozaki K (1993) *Arabidopsis* DNA encoding two desiccation-responsive *rd29* genes. *Plant Physiol* 101:1119–1120. <https://doi.org/10.1104/pp.101.3.1119>
- Yamaguchi-Shinozaki K, Shinozaki K (1994) A novel *cis*-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low temperature, or high-salt stress. *Plant Cell* 6:251–264. <https://doi.org/10.1105/tpc.6.2.251>
- Yang Y, Al-Baidhani HHJ, Harris J et al (2020) DREB/CBF expression in wheat and barley using the stress-inducible promoters of HD-Zip I genes: impact on plant development, stress tolerance and yield. *Plant Biotechnol J* 18:829–844. <https://doi.org/10.1111/pbi.13252>
- Yoshida T, Mogami J, Yamaguchi-Shinozaki K (2014) ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. *Curr Opin Plant Biol* 21:133–139. <https://doi.org/10.1016/j.pbi.2014.07.009>
- Zhang H, Hou J, Jiang P et al (2016) Identification of a 467 bp promoter of maize phosphatidylinositol synthase gene (*ZmPIS*) which confers high-level gene expression and salinity or osmotic stress inducibility in transgenic tobacco. *Front Plant Sci* 7:42. <https://doi.org/10.3389/fpls.2016.00042>
- Zhou M-L, Ma J-T, Pang J-F et al (2010) Regulation of plant stress response by dehydration responsive element binding (DREB) transcription factors. *Afr J Biotechnol* 9(54):9255–9279

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