

Isolation, characterization and expression of transcription factor *ScDREB2* from wild, commercial and interspecific hybrid sugarcane in salinity condition

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Abstract Dehydration Responsive Element Binding (*DREB*) gene is one of the essential transcription factors plants use for responding to stress conditions including salinity, drought, and cold stress. The purpose of this study was to isolate the full length and characterize the *DREB* gene from three different genotypes of sugarcane, wild, commercial cultivar, and interspecific hybrid sugarcane. The length of the gene, designated *ScDREB* was 789 bp, and coding for a putative polypeptide of 262 amino acid residues. Sequences of the gene were submitted to the GenBank database with accession numbers of KX280722.1, KX280721.1, and KX280719.1 for wild sugarcane, commercial cultivar (KPS94-13), and interspecific hybrid (Biotec2), respectively. *In silico* characterization indicated that the deduced polypeptide contains a putative nuclear localization signal (NLS) sequence, and a conserved AP2/ERF domain of the DREB family, at 82-140 amino residues. Based on multiple sequence alignment,

sequences of the gene from the three sugarcane genotypes were classified in the *DREB2* group. Gene expression analysis indicated, that *ScDREB2* expression pattern in tested sugarcane was up-regulated by salt stress. When the plants were under 100 mM NaCl stress, relative expressions of the gene in leaves was higher than those in roots. In contrast, under 200 mM NaCl stress, relative expressions of the gene in roots was higher than those in leaves. This is the first report on cloning the full length and characterization, of *ScDREB2* gene of sugarcane. Results indicate that *ScDREB2* is highly responsive to salt stress.

Keywords *DREB2*, Hydroponic, Sugarcane, Salt stress, Gene expression

Introduction

Salt stress is one of the most common stresses in agricultural regions worldwide. This abiotic stress adversely affects crop productivity and crop quality (Chung et al. 2012; Mahajan et al. 2013). In particular, sugarcane is affected by the salt stress condition. The plant is a moderately sensitive to salts and has a salinity threshold of 1.7 dS m⁻¹ (Maas and Hoffmam 1977). There is no sugarcane cultivar presently that shows high productivity accompanied by a tolerance to salt stress (Passamani et al. 2017). Santana et al (2007) stated that sugarcane yield can be reduced by 50% in soils with electrical conductivity of 10.4 dS m⁻¹. Wahid et al. (1997) reported some characters of sugarcane such as waxy-coated stem, large number and area of green leaves, greater root and shoot yield, high-tillering and ratooning potential have positive correlation with salt tolerance trait. The reduction of sugarcane yield by salinity due to the restrictions in the assimilation of CO₂ (Vasantha

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et al. 2010), decrease in chlorophyll content (Silva et al. 2010), reduction in turgor pressure, limited elongation and cell division (Taiz and Zeiger 2013) and accumulation of reactive oxygen species (Willadino et al. 2011). Salt accumulation in soils occurs when the quantity of salts accumulated due to irrigation water is higher than the quantity removed by the drainage water (Armas et al. 2010). Globally, data from the FAO showed that about 22% of agricultural land is saline. (Guo et al. 2014). In Thailand, the majority of the sugarcane planting areas are in the Northeast of the country (Office of the Cane and Sugar Board, 2017) where more than 2.8 million hectares are affected by salinity (Arunin 1984).

There are numerous genes associated with salt stress. However, due to the complex genome structure and inheritance, the genetic and molecular basis of biomass yield in sugarcane is still largely unknown (Singh et al. 2018). Crop tolerance to salinity is not only related to the quantity and types of salt, but also to plant genetics, as well as the external factors such as climate, soil nutritional availability, irrigation management and others (de Lira et al. 2018). Dehydration responsive element binding (*DREB*) gene is an important transcription factor which play a crucial role in plant abiotic stress tolerances such as salt, drought, cold and heat (Huang et al. 2018). *DREB* belongs to a large subfamily of the AP2/EREBP superfamily which is a large group of plant-specific transcription factors that comprises of two groups, *DREB1* and *DREB2* based on the sequence similarities of the AP2/EREBP domain and conservation of others specific motif present in the ERF protein (Agarwal et al. 2007; Cheng et al. 2007; Nakano et al. 2006). *DREB1* is induced in drought and freezing stress. In contrast, *DREB2* is induced in dehydration and high salt stress (Bouaziz et al. 2012; Zhao et al. 2007). Bouaziz et al (2012) over-expressed *StDREB2* in transgenic potato plant resulting in enhanced tolerance to salt stress while the expression of the *BpDREB2* gene from *Broussonetia papyrifera* was remarkably induced by dehydration and high-salt treatments (Sun et al. 2014). The *DREB2* transcription factor has been isolated and characterized for many plant species such as wheat (*TaDREB2*) (Shen et al. 2003), *Arabidopsis thaliana* (*AtDREB2*) (Nakashima et al. 2006), soybean (*GmDREB2*) (Chen et al. 2007), and *Pennisetum glaucum* (*PgDREB2*) (Agarwal et al. 2007).

To gain more understanding of how sugarcane respond to salt stress at molecular level, cloning of the genes involve the salt stress responsive mechanism is the first step to be accomplished. The objective of this study were to isolate and do the *in silico* characterization of *DREB2* from three

sugarcane genotypes and to analyze the relative expression of the gene under difference salt concentrations and plant parts.

Materials and Methods

Plants material and growth conditions

One-inch long stalks of three sugarcane genotypes, wild sugarcane (*Saccharum spontaneum* L.), commercial cultivar (*S. officinarum* L.) cv. KPS94-13, the drought tolerant cultivar and interspecific hybrid (*S. officinarum* L. x *S. spontaneum* L.) cv. Biotec2 were used as the plant material. It has to be noted that KPS94-13 and the wild sugarcane are not the parent of Biotec2. The female parent of the interspecific hybrid was the commercial cultivar cv. Chinat 1. Each piece of stalk contained 1 bud. They were germinated in a sand:rice ash 1:1 mixture. The 1.5 month old seedlings were transferred to and grown hydroponically in 1/10 Hoagland's nutrient solution (Hoagland and Arnon, 1950) containing 0, 100 and 200 mM NaCl (Fig. 1). Leaf and root samples were collected at 0, 24, 48, 72, 96, and 120 h after salt stress for RNA extraction.

RNA extraction, cDNA synthesis and sequence analysis of *DREB*

The total RNA was extracted from 0.1 g of the leaves and roots collected at various time of salt-stress by using the method described previously by Laksana and Chanprame (2015). The RNA quality was determined through PCR

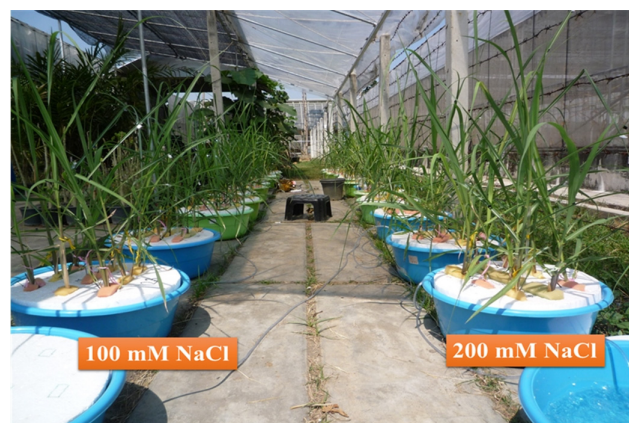


Fig. 1 The 1.5-month-old seedlings of commercial sugarcane cultivar (KPS 94-13), wild species, and interspecific hybrid were grown hydroponically in 1/10 Hoagland's nutrient solution containing 0, 100, and 200 mM NaCl

using *GADPH* specific primers, while the RNA quantity was determined by using a Thermo Scientific™ NanoDrop™ One Spectrophotometer. The total RNA was used as a template for first-stranded cDNA synthesis by using a SuperScript III One-Step RT-PCR System with Platinum *Taq* DNA Polymerase (Invitrogen).

Amplification of the *DREB* gene from three genotypes of sugarcane

In order to clone the full length of the *DREB* gene, first strand cDNA synthesized from the total RNA extracted from the leaves of 1.5 months old seedling of the three genotypes of sugarcane was used as the template. A pair of degenerated primer (*DREB-F1* 5'- ATGGAGCTSGGA GACGCC -3' and *DREB-R1* 5'- CTAATATGMRAAAAAGRCT -3') was designed based on the nucleotide sequences of the *DREB* gene of numerous plant species from the NCBI database, such as *Sorghum bicolor* (XM_002457244.2), *Hemarthria compressa* (KC203598.1), *Saccharum arundinaceum* (KJ670161.1), *Zea mays* (NM_001158997.1), *Setaria italica* (XM_004968237.4), and *Oryza brachyantha* (XM_006643723.2). The PCR reaction was carried out in a volume of 20 μ L containing 300 ng of first-stranded cDNA template, 50 μ M dNTPs, 1U of Platinum® *Taq* DNA Polymerase High Fidelity (Invitrogen), 2 mM MgCl₂, 1x buffer (Invitrogen), 0.125 μ M of each forward and reverse degenerated primer. The amplification was performed under the following conditions: initial denaturation at 95°C for 3 min; then 30 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, extension at 68°C for 1 min; then a final extension at 68°C for 5 min. The amplification products were resolved on a 0.7% (w/v) agarose gel electrophoresis at 100 V for 25 min.

The PCR products were cloned into pGEM®-T Easy Vector (Promega), and transformed to *Escherichia coli* strain DH5 α , then sequenced at 1st BASE Company, Malaysia. The sequences were compared with the sequences in the GenBank database (Nucleotide BLAST) (www.ncbi.nlm.nih.gov/BLAST/) using default parameters, and then translated into amino acid sequences by using Genetyx 5.0 (<http://genetyx.software.informer.com/>). The derived DREB amino acid sequences were aligned with the DREB amino acid sequences of other plant species by using the Clustal Omega (<https://www.ebi.ac.uk/Tools/msa/clustalo/>). After which, the alignment results were imported into GeneDoc 2.7 (<http://genedoc.software.informer.com/2.7/>) to generate a picture of multiple alignment. A phylogenetic tree was constructed based on DREB1 and DREB2 protein members by using the MEGA

program (v6.0) running the Neighbor - joining algorithm.

Quantitative real time PCR (qRT-PCR) analysis

Analysis of the *DREB* gene expression of the three sugarcane genotypes under mimicking saline soil conditions *via* quantitative RT-PCR was performed. The accuracy of quantification was confirmed through normalization of the *ScDREB* expression to reference transcripts encoding glyceraldehydes-3-phosphate dehydrogenase: *GAPDH* (GenBank accession no. CA254672) and eukaryotic elongation factor 1-alpha: *Eef-1a* (GenBank accession no. EF581011.1). Both of the reference genes were identified as suitable references gene for the normalization of the gene expression under salinity/drought-treatment in sugarcane (Guo et al. 2014). Specific primers for qRT-PCR (Table 1) were designed from conserve sequences found in the full length *DREB* cDNA of three sugarcane genotypes by using Primer3 ver 0.4.0 (<http://simgene.com/Primer3>)

PCR reactions were performed in a total volume of 20 μ L containing 500 ng of first strand cDNA template, 1x SensiFAST SYBR No-ROX mix buffer (Bioline Reagent Ltd.), 0.4 μ M forward primer, and 0.4 μ M reverse primer. The amplification was performed under the following conditions: initial denaturation at 95°C for 30 sec; then 45 cycles of denaturation at 94°C for 5 sec, annealing at 58°C for 15 sec, and extension at 72°C for 10 sec in a CFX96 Touch™ Real-Time PCR from BIO-RAD®. The expressions of the gene were compared with the control condition (0 h). For each sample, the reactions were carried out in three biological replicates and three technical replicates. The relative expression of each gene was calculated according to the method of $2^{-\Delta\Delta Cq}$ (Livak and Schmittgen 2001).

Statistical analysis

Statistical analysis was performed by using the R program (R Core Team 2013). The results were performed as mean \pm SE (standard error of the mean; n=9). The differences

Table 1 Specific primers used, for real-time quantitative PCR

Primer name	Primer sequence
DREB-RT F	GCTCCTTCCCTACTGCTGTG
DREB-RT R	CACTAGATGCCAGCAACGAA
Sc-GADPH F	CACGGCCACTGGAAGCA
Sc-GADPH R	TCCTCAGGGTTCCTGATGCC
Sc-Eef-1a F	TTTCACACTTGGAGTGAAGCAGAT
Sc-Eef-1a R	GACTTCCTTACAATCTCATATAA

in the data were compared by ANOVA followed by using the Duncan's multiple range test. The differences were investigated significant at $p < 0.05$.

Results

Cloning of full length DREB cDNA from three genotypes of sugarcane

The full length of the DREB gene from three genotypes of sugarcane were cloned using a pair of degenerated primer. The sequences of the putative DREB gene exhibited one

open reading frame (ORF) composed of 789 bp and encoded a putative polypeptide of 262 amino acid residues. The predicted protein had a calculated molecular mass of 28.6 kDa and a theoretical isoelectrics point of 5.42. A putative nuclear localization signal (NLS) sequence was detected by using NLSstradamus (<http://www.moseslab.csb.utoronto.ca/NLSstradamus/>) (Ba et al., 2009). The deduced protein contained a conserved AP2/ERF domain of the DREB family at 82-140 amino residues (Fig. 2). The sequence show a high similarity to the DREB2 gene from many plant species including *Sorghum bicolor* (XM_002457244.2; 94%), *Hemarthria compressa* (KC203598.1; 96%), *Saccharum arundinaceum* (KJ670161.1; 97%), *Zea mays* (NM_001158997.1;

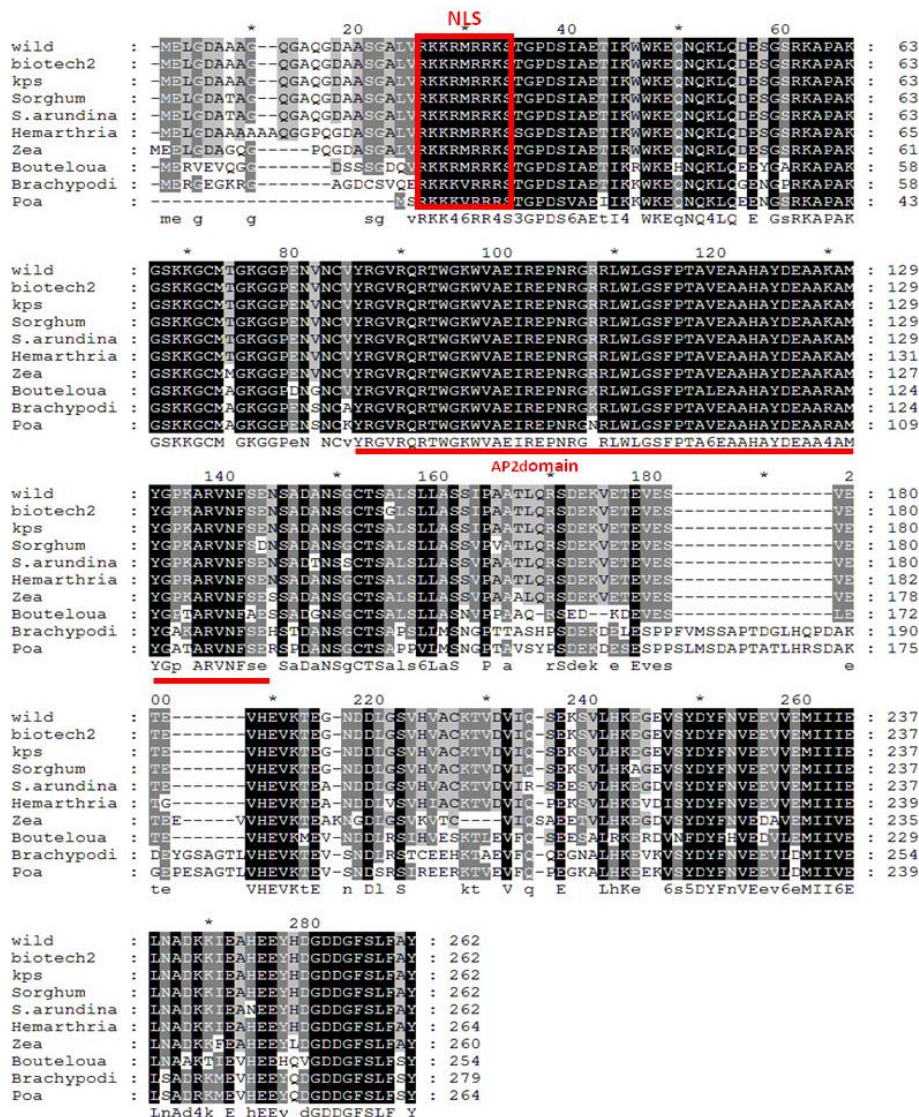


Fig. 2 Multiple alignment of the deduced amino acid sequence of ScDREB2 from wild sugarcane, commercial cultivar cv. KPS 94-13, and interspecific hybrid cv.Biotech2, *Sorghum bicolor* (XP_002457289.1) *Saccharum arundinaceum* (AIU40168.1), *Hemarthria compressa* (AGG20202.1), *Setaria italica* (P_001296982.1), *Zea mays* (NP_001152469.1), *Bouteloua dactyloides* (ABP52086.1), *Dichanthelium oligosanthes* (OEL14136.1), *Brachypodium distachyon* (XP_024314715.1), *Oryza sativa Indica* (EAY72665.1), and *Poa pratensis* (AAS59530.1)

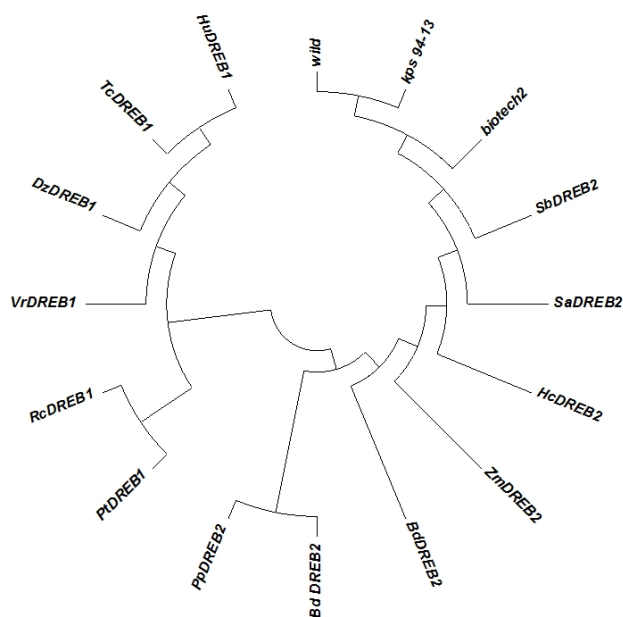


Fig. 3 Phylogenetic tree of ScDREB2 protein from wild sugarcane (wild), commercial cultivar (KPS94-13), interspecific hybrid (Biotech 2), and other different plant species. It was constructed based on deduced amino acid sequences using MEGA 5.0 software. GenBank accession numbers of amino acid sequences used: *SbDREB2 Sorghum bicolor* (XP_002457289.1), *SaDREB2 Saccharum arundinaceum* (AIU40168.1), *HcDREB2 Hemarthria compressa* (AGG20202.1), *Setaria italica* (P_001296982.1), *ZmDREB2 Zea mays* (NP_001152469.1), *BdDREB2 Bouteloua dactyloides* (ABP52086.1), *BdDREB2 Brachypodium distachyon* (XP_024314715.1), *PpDREB2 Poa pratensis* (AAS59530.1), *TcDREB1 Theobroma cacao* (XP_007015927.2), *HuDREB1 Herrania umbratica* (XP_021277951.1), *DzDREB1 Durio zibethinus* (XP_022730283.1), *VrDREB1 Vitis rubra* (AIL00538.1), *PtDREB1 Populus trichocarpa* (XP_024455149.1), *RcDREB1 Ricinus communis* (XP_002532187.1)

88%), *Setaria italica* (XM_004968237.4; 86%), and *Oryza brachyantha* (XM_006643723.2; 79%). Based on this similarity, we named it *ScDREB2*. The sequence data from the three genotypes of sugarcane have been submitted to the NCBI database. The accession number of *ScDREB2* from wild sugarcane, commercial cultivar (cv. KPS94-13) and interspecific hybrid (cv. Biotech2) are KX280722.1, KX280721.1 and KX280719.1, respectively. The phylogenetic analysis of the deduced ScDREB protein from the three sugarcane genotypes showed some genetic distance among them. The protein from wild sugarcane was close related to that of KPS94-13 while the protein from the interspecific hybrid showed more distance to the early mentioned protein. However, these proteins were very similar to DREB2 protein from *Sorghum bicolor* (SbDREB2) and *Saccharum arundinaceum* (SaDREB2) (Fig. 3).

Expression analysis of *ScDREB2* in the leaves and roots of the three sugarcane genotypes under different salt stress conditions

The analysis of the *ScDREB2* expression was done under different NaCl-stress levels using semi-quantitative PCR. The sugarcane seedlings were subjected to 1/10 Hoagland solution together with 100 and 200 mM NaCl for 0, 24, 48, 72, 96, and 120 h. Under 100 mM NaCl-stress condition, relative expression of *ScDREB2* in the leaf of KPS 94-13 reached the highest level at 72 h of stress and decreased sharply later on. When compared with the control (0 h) this was about a 50-fold increase. For the wild species and interspecific hybrid (Biotech2) at 24–72 h of stress the relative expressions of the gene also increased but at lower levels than KPS 94-13. At 96 and 120 h of stress the wild sugarcane and Biotech2 showed higher expression level than KPS 94-13 (Fig. 4A). In the root, KPS 94-13 had the highest relative expression at 24 h of stress at 24-fold higher than the control and decreased sharply later on. Whereas the expression of the gene of wild sugarcane gradually increased starting at 24 h and reached the highest expression level at 96 h of stress (Fig. 4B). For the 200 mM NaCl condition, in the expression of the gene in the leaf KPS 94-13 was the highest at 24 h of stress with about 63-fold higher than the control and then it gradually decreased, whereas the wild sugarcane and Biotech2 were substantially increased (Fig. 4C). In the root the expression of *ScDREB2* reached the highest induction of 120-fold of the control at 24 h. The expression of the gene in Biotech2 reached the highest level of about 80-fold of the control at 72 h of stress, while a lower and slowly increasing expression level was found in the wild sugarcane (Fig. 4D). The results indicated that under salt stress the *ScDREB2* expressions in all tested genotypes were up-regulated when compared with the control. When compared the expression level of the gene in leaf or root at 100 mM NaCl with 200 mM NaCl it was found that the 200 mM NaCl induced higher expressions of the gene than those of the 100 mM NaCl. The results suggested that the higher level of salt stress could induce more expression of the *ScDREB2* than lower level of stress did. When compared the expression level of the gene in leaf to root it revealed that the tendency of the expression level of the gene in leaf was higher than in root. This inferred that this transcription factor might be involved in the expression of more genes in leaves than the genes in roots. The pattern of gene expression in KPS 94-13 was difference from wild and interspecific hybrid. The expression of *ScDREB2* in KPS 94-13 increased

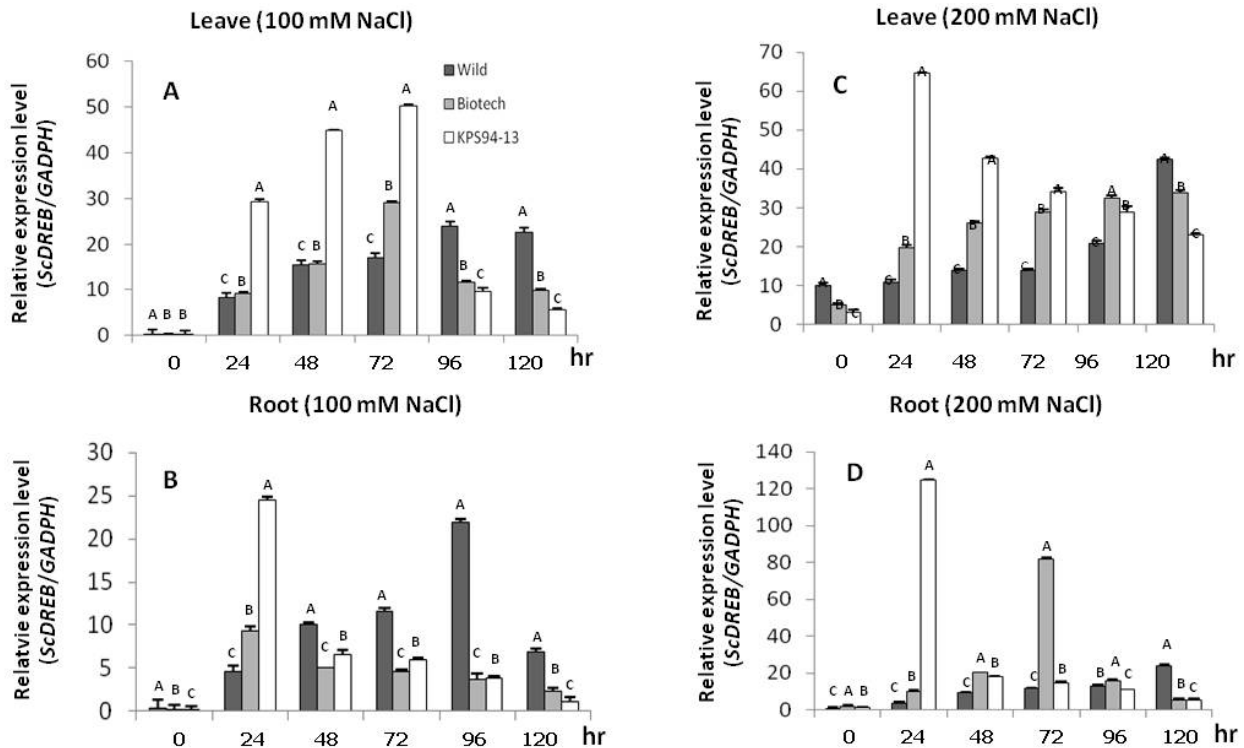


Fig. 4 Relative expression level of *ScDREB2* in leaves (A, C) and roots (B, D) of wild sugarcane, interspecific hybrid (Biotech2), and commercial cultivar (KPS 94-13) subjected to 100 mM NaCl (A and B) and 200 mM NaCl (C and D) for 0–120 hours. Data are means \pm SE of the three biological replicates. Different letters on the boxes indicate significant differences between genotype, under the same period of stress at $P < 0.05$ according to DMRT

immediately after receiving salt stress and then decreased when the stress was prolonged while the expressions of the gene in wild and interspecific hybrid were gradually increased when the times of stress were extended. These results indicated that the commercial sugarcane responded to salt stress faster than the wild and interspecific hybrid sugarcane. However, the physiological study of these sugarcane genotypes under salt stress in combination with the expression pattern of the *ScDREB2* should be done to confirm the relationship of the gene expression pattern and the sensitivity or tolerance to salt stress.

Discussion

Salt stress is a seriously problem on the growth and production of most economic crops. Understanding the adaptive mechanisms to salinity stress is an important prerequisite for crop improvement and sustainable production. Physiological traits and biochemical processes that plants use for coping salt stress are developed from gene expressions. Transcription factor is one of the important factors controlling the transcription level of the genes. DREB is one of the transcription factors that participates in plant response

to salt stress in several plant species and is an important prerequisite for use of stress-inducible gene in crop improvement (Sun et al. 2014). Numerous studies have demonstrated that DREB protein is involved in the improvement of stress tolerance of plants. DREB belongs to a subfamily of the AP2/EREBP superfamily which comprises of two groups, DREB1 and DREB2 which are major regulators and functions of DREBs (Agarwal et al. 2007; Nakano et al. 2006). DREB1 is induced by cold while DREB2 is induced by dehydration and high salinity (Liu et al. 1998). *DREB2A* is a salinity-inducible transcription factor and have been isolated and characterized from many plant species such as *Sophora moorcroftiana* (Yao et al. 2016), rice (Cui et al. 2011), *Buchloe dactyloides* (Zhang 2014), soybean (Chen et al. 2007), *Populus euphratica* (Chen et al. 2009), potato (Bouaziz et al. 2012) and paper mulberry (Sun et al. 2018). However, there has been no publication on the cloning and characterization of the *DREB2* gene in sugarcane under salt stress. This is the first report describing the cloning and characterization of *ScDREB2* gene in sugarcane. In this study, we described the identification and characterization of *ScDREB2* from the commercial sugarcane cultivar cv. KPS 94-13, wild sugarcane and interspecific hybrid sugarcane cv. Biotech2.

ScDREB2 was successfully isolated by using the PCR technique with primers designed from the *DREB* gene of various plants species. The result of phylogenetic analysis indicated that the ScDREB belonged to the DREB2 group and was closely related with DREB2 from sorghum (Fig. 3) because the sugarcane and sorghum genomes are mostly collinear in the genic regions (Wang et al. 2010). The sequence of ScDREB2 contains NLS and AP2/ERF domain position. The entrance of DREB proteins is arbitrated to NLS. The transcription factor having no NLSs enter into the nucleus through protein–protein interaction with the transcription factors that have NLSs (Stockinger et al. 1997). The AP2/ERF family is a large group of plant-specific transcription factors that include four major subfamilies: AP2, RAV, ERF and DREBs (Sakuma et al. 2002). DREB is a major transcription factor involved in plant abiotic stress response by regulating gene expression *via* the cis-acting dehydration-responsive element/C-repeat (DRE/ CRT) element. (Yamaguchi-Shinozaki and Shinozaki 2006). The AP2 domain of ScDREB2 consists of approximately 60 amino acid residues which is the same as the AP2/ERF domain reported by Weigel (1995). Furthermore, the sequences of the AP2 domain show high conservation among the 3 genotypes of sugarcane and also among other plant species (Fig. 1). This indicates the important role of this domains as a DNA-binding domain (Weigel 1995).

The relative expression study indicated that the expression of *ScDREB2* was up-regulated under salt stress both in leaf and root when compared with the normal condition. Similar results were also observed in *BdDREB2* of buffalograss (Zhang et al. 2014), *GmDREB2* of soybean (Chen et al. 2007), *StDREB2* of potato (Bouaziz et al. 2012) and *BpDREB2* of *Broussonetia papyrifera* (Sun et al. 2014). Under 100 mM and 200 mM NaCl conditions, the expression of *ScDREB2* in KPS94-13 was high in the early period of stress and then sharply decreased the next day, whereas the expressions of the gene in wild sugarcane gradually increased and reached the highest level at late period of stress (96–120 h after stress induction). This phenomenon might correlate with the characteristic of wild species of plant that more adaptive to unfavorable conditions than the commercial cultivar, in this case when salt stress was prolonged. In contrast, the expression pattern of the gene in wild sugarcane was similar to the expression pattern of the interspecific hybrid. This may be due to the genetic back ground concerning salt stress responsive traits of the interspecific hybrid derived from wild species of sugarcane. As mentioned in the material and method section that the commercial cultivar and the wild sugarcane were not the

parent of the interspecific hybrid used in this study. So, it is very difficult to elucidate the relationship of the expression patterns among them.

The relative expressions of *ScDREB2* in root and leaf of sugarcane subjected to 200 mM NaCl were higher than those of the 100 mM NaCl. This result is very similar with the expression of *PeDREB2* in *Populus euphratica* which showed a higher expression in high salt treatment (300 mM NaCl) (Chen et al. 2009). Yang et al (2017) also reported the expression of *ThDREB* from *Tamarix hispida* was highly induced by NaCl and the highest expression level was 369.2-fold over the control plant. The expression level of the gene in leaf was higher than that in root at both concentrations of NaCl. This result is accordant with Laksana and Chanprame (2017) and Kaewjiw et al (2018) who reported that *MIPS* and *SOS1* gene in leaves of sugarcane expressed at higher levels than those of the roots in salt stress condition. These two genes also involve in the salt responsiveness mechanism and somehow the transcription factor *ScDREB2* may regulate the expression of them. These evidences point out that *ScDREB2* is a salt stress responsive gene and it might play a crucial role in providing tolerance to multiple stresses. However, we found some variations in the nucleotides among the sugarcane studied. These variations may or may not relate to different patterns of gene expression in salt stress condition. These variations may relate to the difference isoforms of the gene and protein as well. To gain more understanding and to confirm the role of this gene relates to salt tolerance, we will produce transgenic tobacco containing different *ScDREB2*. We will determine and compare the expression levels of the transgenes and the degree of salt tolerance of the transgenic tobacco. When the correlation between the expression pattern and salt tolerance is proven this will also be a benefit for the development of a molecular marker for selection of salt tolerant sugarcane in the breeding program.

Conclusion

Salt stress reduces the potential yield in crop plants including sugarcane. The development of crops that are better adapted to salt stresses is important for sustainable production. In this work, we cloned and characterized the *ScDREB2* from three different genotypes of sugarcane subjected to salt stress. The deduced amino acid sequence of ScDREB2 had a strong similarity to DREB2 of many other plant species and contained the AP2 domain which is an important site for DNA binding. The expression of *ScDREB* gene in

sugarcane showed up-regulation pattern when compared with the control plant in salt stress condition. Based on the result, we concluded that *ScDREB2* is an important transcription factor that regulated under salt stress and has considerable potential for improving salt stress tolerance in sugarcane.

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Conflict of interest The authors declare that they have no conflict of interest.

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