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Full Length Article

Differential Expression of Stress Responsive *Scdr1* Gene in Indigenous Sugarcane Genotypes

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Abstract

Advancements in molecular approaches have helped to develop desired crop plants through transgenic technology. In addition to exogenous genes, endogenous plant genes may also be tailored for the betterment of particular traits. Here we report the comparative analysis of physiological performance parameters and differential expression of an endogenous stress responsive gene, Sugarcane drought responsive 1 (*Scdr1*) in indigenous elite sugarcane genotypes while growing on different levels of salt stress. Six sugarcane genotypes (CPF-247, CPF-248, CPF-246, CP77-400, S2006-US-272 and S2003-US-127) were grown in pots and exposed to salt stress ranging from 30 m*M* to 170 m*M* NaCl. Quantitative expression analysis revealed that *Scdr1* is a stress inducible gene as elevated level of expression was observed in all genotypes after exposure to salt stress. Its expression was highest in genotype CPF-77400 and lowest in genotype CPF-246 under salt stress. Further, genotypes with higher expression of *Scdr1* appeared to be more competent when assessed for physiological performance. Hence, retreived results may be employed for the selection and screening of stress tolerant sugarcane genotypes. Phylogenetic analysis revealed that homologues of this gene are present in sorghum, wheat, rice, maize and barley so, these results can be of great value for the improvement of other monocots as well.

Keywords: Sugarcane; Salinity; *Scdr1*; Expression analysis; Physiological parameters

Introduction

Changing climatic conditions in combination with rapidly increasing population may result in increased malnutrition particularly in developing countries (Heger *et al*., 2018). Producing more food under worsening climatic conditions is really a big challenge. Developing smart crop varieties having ability to produce more with less input is a major component of this intervention (Tester and Langridge, 2010; Caine *et al*., 2019). Sugarcane flowers only in typical climatic conditions, as a result its breeding is limited to certain global regions. Long breeding cycle and complexity of the genome are other major limitations in the development of climate smart varieties by conventional breeding (Mustafa and Khan, 2012a). At the same time, nature has blessed this grass with a range of valuable genes which are supposed to play critical role in biotic and abiotic stress tolerance (Su *et al*., 2014; Li *et al*., 2018). These endogenous plant genes may be tamed through molecular approaches to improve this sweet grass (Mustafa and Khan, 2012b; Khan *et al*., 2013). Sugarcane is a typical glycophyte and more prone to abiotic stresses which may cause 50% decline in crop yield than its actual potential (Suprasanna *et al*., 2011). Salinity is appearing as a drastic problem in our country owing to changing climatic conditions, water disputes, poor irrigation practices and human induced soil erosion (Kausar *et al*., 2012). In Pakistan 6174.5 thousand hectares of the land is salt affected, 16.795 million hectares is under irrigation of which 10% is slightly saline, 4% is moderately saline, 7% is highly saline, 6% is miscellaneous and 73% is considered as non-saline (Haq *et al*., 2010). Salt stress increases soil osmotic potential (Horneck *et al*., 2007; Farooq *et al*., 2015) and affects cellular life at various levels (Zhu, 2002) by affecting cell size, reduced CO_2 assimilation rate, transpiration rate, stomatal opening and water potential, so directly or indirectly affect photosynthesis and ultimately lead to plant death (Affenzeller *et al*., 2009; ; Farooq *et al*., 2017).

Advancements in next generation sequencing and functional genomics have opened new eras to understand complex genomes. Integration of transcriptomics, metabolomics and proteomics approaches in the presence of systems biology simplified the complex signaling cascades to serve humanity by developing smart crop varieties that can better tolerate environmental stresses. More than 1670 sugarcane genes have been recognized to be differentially expressed under water deficit conditions (Rodrigues *et al*., 2011). Functional characterization of these genes may lead to explore their role in the physiological performance of this plant. Sugarcane drought responsive 1 (*Scdr1*) is an

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endogenous sugarcane gene which encodes for a valuable protein engaged in protecting plant against drought, salinity and oxidative stresses (Begcy *et al*., 2012). In our country, variety development is dependent on the import of fuzz, its germination and screening of competent clones. Long breeding cycle is a major impediment in the screening of stress tolerant genotypes with improved agronomic performance. Developing molecular markers or linking expression of particular genes with particular trait can be of great help to speed up this selection and screening process of promising elite sugarcane lines (Pastina *et al*., 2010). Considering this, expression analysis of a stress inducible gene as well as physiological performance was assessed in indigenous elite sugarcane genotypes growing at different levels of salt stress.

Materials and Methods

Establishment of Sugarcane Plants in Pots and Application of Salt Stress

Six indigenous sugarcane (*Saccharum officinarum* L.) genotypes (CPF-247, CPF-248, CPF-246, CP77-400, S2006-US-272 and S2003-US-127) were collected from Sugarcane Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan, on the basis of better agronomic performance. Single budded setts were sown in plastic pots (36.576 × 85.344 cm with 15 kg soil). Three single budded setts were sown in each pot and were allowed to grow for 60 days under greenhouse conditions. Six pots were sown for each genotype to have three biological replicates. The two months old plants (at 5–6 leaf stage) were exposed to salinity stress. Concentration of 170 m*M* NaCl was attained in the pots by applying commercially available table salt dissolved in tap water. The water was applied multiple times in order to avoid osmotic shock. After 10 days of salinity stress, leaf samples were collected from control and treated plants, immediately wrapped in aluminum foil and frozen in liquid nitrogen for RNA extraction.

Determination of Physiological Parameters

Chlorophyll estimation is a rapid way to determine plant's response to osmotic stresses. After 10 days of salt stress, chlorophyll content was determined by chlorophyll meter (SPAD 502 Plus) during 9–10 a.m. in the morning. While young fully expanded 3rd leaf at the same position was used to estimate the net photosynthesis (A), stomatal conductance (GS), transpiration rate (E) and internal leaf CO_2 (Ci) with Infrared Gas Analyzer (IRGA, LCA-4) at 360 μL L^{-1} CO2 concentration, 1000 μmol m^{-2} s^{-1} saturating light intensity and at 200 mL min^{-1} rate of gas flow (Guo *et al*., 2008).

Molecular Characterization of *Scdr1*

Genomic DNA was isolated by CTAB method and was subjected to PCR by using gene specific primers (Table 2). The resultant amplicons were cloned in T/A cloning vector (ThermoFisher Scientific, U.S.A.) and were sequence characterized. Physicochemical properties (molecular weight, aliphatic index and isoelectric point) of the *Scdr1* were predicted by ProtParam (http://web.expasy.org/protparam/). SOPMA (Geourjon and Deleage, 1995) was used to predict secondary structure of *Scdr1*. Other homologues of *Scdr1* protein were found by BLASTP. Phylogenetic tree of *Scdr1* amino acid sequence and its homologs from 15 other species was constructed by using the neighbor joining (NJ) method with 1000 bootstrap replicates in the PAUP (http://paup.phylosolutions.com/).

RNA Extraction and Synthesis of cDNA

Total cellular RNA was extracted from leaves of sugarcane plants growing under salt stress and under control conditions (without salt stress) by using trisolution reagent (GeneMark, Bio) following the manufacturer's instructions. Quality of total RNA was checked on 2% agarose gel and was quantified by using nanodrop spectrophotometer (ThermoFisher Scientific, USA). Then it was treated with DNase followed by ethanol precipitation. Then it was quantified prior to synthesis of cDNA. ThermoFisher Scientific Revert Aid cDNA synthesis kit was used for synthesis of cDNA from 1 μg total cellular RNA following the manufacturer's instructions. All incubations were performed in a thermal cycler (Bio-Rad, USA) and synthesized cDNA was immediately stored at -80ºC.

Expression Analysis by Quantitative Real Time PCR (Q-PCR)

Real time qPCR allows to monitor amplification of a specific DNA molecule. So, real time qPCR was performed by following the protocol developed by Rocha *et al*. (2007). Primers were designed by using OligoAnalyzer Tool (https://eu.idtdna.com/calc/analyzer) and were used for the comparative expression analysis. Reaction conditions were normalized by using *25S rRNA* reference gene (Table 2). Relative gene expression (control/experimental) was determined by using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) which was normalized by using plants grown under normal/controlled conditions. Syber green supermix (Bio-Rad, USA) was used in the reaction and reaction cycles were as follows: 95°C for 3 min and 40 cycles at 95°C for 1.5 min, 55°C for 1.5 min and extension at 72°C for 2 min.

Results

Molecular Characterization of *Scdr1* Gene in Indigenous Sugarcane Genotypes

Scdr1 is a novel stress responsive gene with putative role in

Table 1: Physicochemical properties (molecular weight, aliphatic Index and isoelectric point) of the *Scdr1*, predicted by ProtParam

Number of amino acids	247	
Molecular weight	27644.04	
Theoretical pI	8.66	
Amino acid composition		
Amino acid	Number	Percentage
Ala (A)	8	3.2%
Arg (R)	3	1.2%
Asn (N)	4	1.6%
Asp (D)	7	2.8%
Cys (C)	33	13.4%
Gln (Q)	9	3.6%
Glu (E)	18	7.3%
Gly (G)	6	2.4%
His (H)	4	1.6%
Ile (I)	6	2.4%
Leu (L)	7	2.8%
Lys (K)	35	14.2%
Met (M)	5	2.0%
Phe (F)	4	1.6%
Pro (P)	49	19.8%
Ser (S)	7	2.8%
Thr (T)	10	4.0%
Trp (W)	7	2.8%
Tyr (Y)	3	1.2%
Val (V)	22	8.9%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%
(B)	0	0.0%
(Z)	0	0.0%
(X)	0	0.0%
Total number of negatively charged residues (Asp + Glu)		
Total number of positively charged residues (Arg + Lys)		38
Atomic composition		
Carbon C	1225	
Hydrogen H	1933	
Nitrogen N	319	
Oxygen O	331	
Sulfur S	38	
Instability index		
The instability index (II) is computed to be 47.05		
This classifies the protein as unstable.		
Aliphatic index: 49.60		

Table 2: Sequences of different primer pairs used for the molecular characterization and expression analysis of *Scdr1* gene. Primers 1, 2, 3 and 4 were used for comparative expression analysis through qPCR whereas primers 5 and 6 were used for the amplification of *Scdr1* gene through conventional PCR. All of the primers were designed using OligoAnalyzer Tool

Sr. #	Primer name	Primer Sequence
1	25S rRNA Forward	5'-GGATTGGCTCTGAGGGTTG-3'
2	25S rRNA Reverse	5'-CAGGAGCATGGGTCATATCC-3'
3	*Scdr1*Q Forward	5'-GCAGAGCCAAGATCACCAAG-3'
4	*Scdr1*Q Reverse	5'-GCAGAGCTTCTCAGCGTC-3'
5	*Scdr1* Forward	5'-CCATGGGTATACTGGTGATTACGG-3'
6	*Scdr1* Reverse	5'-TCACATGACAGAGCAGGAG-3'

scavenging reactive oxygen species (ROS). But very limited information is available about its role in abiotic stress tolerance. Hence it was amplified and characterized in indigenous sugarcane genotypes. While using genomic DNA as template, a 1266 bp fragment was amplified (Fig. 1A) whereas 744 bp fragment was amplified from cDNA template (Fig. 1B). PCR amplified fragments from genomic DNA and cDNA were different in size. This led us to predict prevalence of introns in the native *Scdr1* sugarcane gene. The resultant amplicons were cloned in TA cloning vector and were sequence characterized. Genomic sequence of *Scdr1* was characterized to have one intron of 492 bp. The resultant 247 amino acids appeared to constitute a 27.6 kDa protein with theoretical pI of 8.66 (Table 1). *Scdr1* secondary structure was predicted by SOPMA and according to prediction protein consists of 15.95% alpha helix, 3.98% beta turn, 14.79% extended strand and 67.35% random coils. According to the software predictions, almost 65% of *Scdr1* is constituted by non-structured random coils. Most of the proteins are non-structured and they can change their structure easily. Such proteins are ideal for protein-protein interaction studies and it was predicted that *Scdr1* may act as a hub for protein complex assembly. When BLASTP was performed for *Scdr1* protein, it did not appear to contain putative conserved domains. It shared homology with numerous monocotyledonous species like *Sorghum bicolor* Pi21 (XP_021318068.1, XP_002447741.1, XP_021319356.1, XP_021318404.1, XP_002447739.2, XP_002466443.1), *Saccharum officinarum* (AFH41561.1, AOZ57105.1, AOZ57127.1), *Triticum urartu* (EMS54667.1), *Zea mays* (NP_001335666.1, ACG30511.1, NP_001147655.1, NP_001152552.1, NP_001149523.1, NP_001183152.1, KMZ58897.1), *Hordeum vulgare* (BAJ94226.1, BAJ92937.1), *Oryza sativa* (CAH66465.1), *Oryza sativa japonica* (XP_015635345.1, XP_015635064.1, XP_015634829.1, EAZ30605.1), *Oryza sativa indica* (EAY93962.1, EAY93961.1, EAY93960.1, BAG72124.1), *Oryza brachyantha* (XP_015691435.1, XP_006653354.1, XP_015691436.1), *Panicum hallii* (PAN37906.1, PAN37908.1, PAN44952.1, PAN15474.1), *Aegilops tauschii* (XP_020188880.1, XP_020188875.1), *Dichanthelium oligosanthes* (OEL36920.1), *Setaria italica* Pi21 (XP_004975477.1, XP_004975474.1, XP_004975475.1, XP_014661056.1, XP_004981747.1), *Phoenix dactylifera* (XP_008780993.1) and *Brachypodium distachyon* (XP_003581156.1, XP_003579640.1). Among highly homologous proteins, conserved sequences were highlighted by Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) (Fig. 2). Phylogenetic tree was constructed by PAUP to demonstrate *Scdr1* relationship with other homologues. Phylogenetic tree depicted the presence of this gene prior to speciation and revealed that it is highly conserved among various monocots (Fig. 3).

Determination of Physiological Parameters

Physiological parameters are quick indicators of stress tolerance (Fghire *et al*., 2017). So, physiological performance was evaluated by determining the total chlorophyll contents and photosynthetic parameters *i.e*., net photosynthesis (A), transpiration rate (E), stomatal

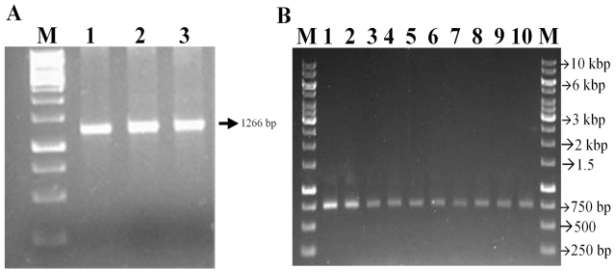

Fig. 1: PCR amplification of *Scdr1* gene from indigenous sugarcane genotypes. **A**) Amplification of 1266 bp fragment from genomic DNA of sugarcane. **B**) Amplification of 744 bp fragment from cDNA confirmed presence of introns in native *Scdr1* gene. M stands for 1 kb DNA ladder

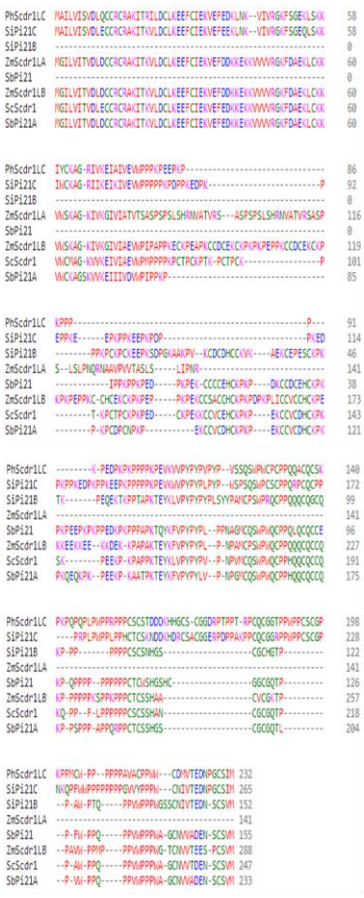

Fig. 2: Sequence analysis of *Scdr1* protein. The alignment of Scdr1 was performed with other highly homologues proteins by Clustal Omega

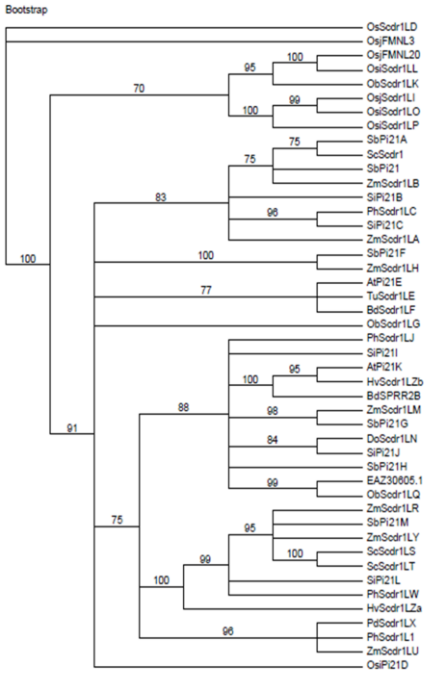

Fig. 3: Phylogenetic analysis of *Scdr1* protein. BLASTP was performed to find all possible homologues of *Scdr1* and neighbor joining tree from 15 different plant species. The tree was constructed by using Paup whereas 1000 Bootstrap values were used and expressed as percentage above each node

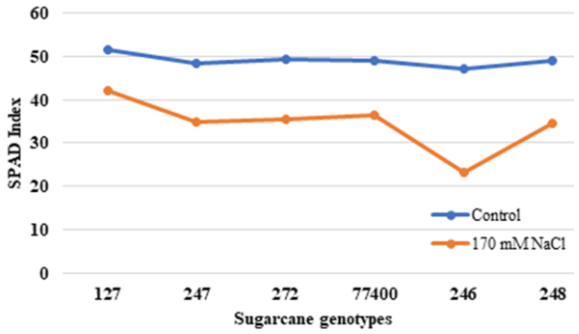

Fig. 4: Determination of chlorophyll content in indigenous sugarcane genotypes under salinity stress. Chlorophyll content was measured in the leaves of sugarcane plants irrigated with normal water (30 m*M* NaCl) and plants growing under salt stress conditions (170 m*M* NaCl). $P < 0.001$ at each time point while n=3

conductance (GS) and internal leaf CO_2 concentration (Ci). Under salt stress chlorophyll contents appeared to be reduced sharply (Fig. 4) as compared with control plants. Among the selected genotypes, chlorophyll content was highest in genotype S2003-US-127 and lowest in genotype CPF-246. Genotypes S2003-US-127 and CP77-400 were able to retain more photosynthetic ability after exposure to salt stress as compared with other genotypes. Overall, physiological parameters were most affected in genotype CPF-246 (Fig. 5).

Expression Analysis of *Scdr1* in Response to Salinity Stress

Scdr1 expression was evaluated by quantitative real

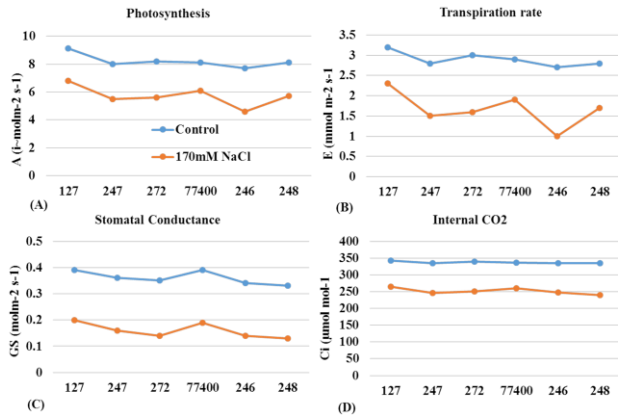

Fig. 5: Effect of salt stress on physiological parameters of indigenous sugarcane. 60 days old plants were exposed to 170 m*M* NaCl stress for 10 days, then were allowed to recover by watering with normal water fit for irrigation. (**A**) represents net photosynthesis (A), (**B**) transpiration rate (E), (**C**) stomatal conductance (GS), (**D**) Internal leaf CO_2 concentration (Ci), $P < 0.001$ at each time point while n=3

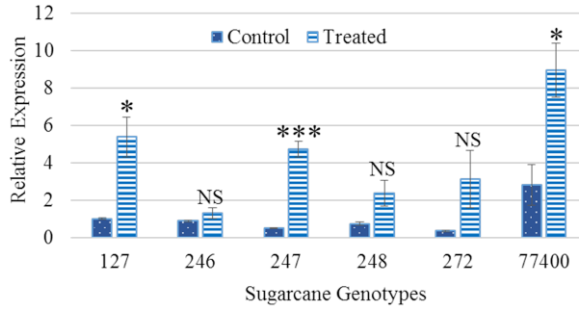

Fig. 6: Comparative expression analysis of *Scdr1* gene in indigenous sugarcane genotypes growing in control conditions and salt stress (170 m*M* NaCl). The dark blue bars are comparable with dark blue bars whereas light blue bars are comparable with each other. Differential expression revealed that CPF-77400 has highest level of expression as compared with other genotypes. Results from three biological replicates were analysed with Students t-test; *=$P \leq 0.05$, **=$P \leq 0.01$, ***=$P \leq 0.001$. Columns in dark blue colour with dots represent control samples while columns in light blue colour with lining represent samples under stress condition

time PCR by extracting mRNA from leaves of six indigenous sugarcane genotypes. Before expression analysis, cDNA as well as primer concentrations were optimized. Primers worked best at a concentration of 0.2 μM with cDNA 0.3 μL. Specificity of primers was also evaluated by introducing melt curve. Each primer pair exhibited a unique peak of fluorescence, indicating that a single fragment was amplified during amplification. Same concentration of cDNA and primers were used for *Scdr1* and *25S rRNA* for all genotypes. To evaluate stress inducibility of the *Scdr1*, relative expression analysis was carried out in indigenous sugarcane genotypes (Fig. 6) growing in salt stress conditions and without salt stress. Expression of the gene appeared to be increased in all of the genotypes after exposure to salt stress (170 m*M* NaCl). Under control conditions (without salt stress) *Scdr1* expression was maximum in genotype CP77400 and minimum in S2006-US-272. While under salt stress, it appeared to be maximum in genotype CP77400 followed by S-2003-US-127, CPF-247, S2006-US-272 and CPF-246. Nevertheless, CP 77400 showed prominent level of expression as compared with other genotypes and appeared to be more promising as far as stress tolerance is concerned. These results suggest that *Scdr1* is a stress inducible gene and may be an indicator of level of tolerance.

Discussion

Abiotic stresses particularly salinity and drought adversely affect plant developmental processes by inducing morphological, physiological and biochemical changes (Parida and Das, 2005). They are responsible for increased gap between actual and potential yield. To assure sustainable crop production, it is necessary to develop improved crop varieties with better tolerance to the continuously changing environmental conditions (Liu *et al*., 2018; Xie *et al*., 2018). Under stress conditions several genes act synergistically to produce changes at physiological, biochemical and molecular level. So, identification and functional characterization of stress inducible genes is critical to develop smart crop varieties with improved tolerance to environmental stresses. Rodrigues *et al*. (2011) reported differential expression of 1670 genes in sugarcane plants in response to drought stress. Transcriptome of 1545 genes exhibited differential expression in sugarcane plants growing under drought, abscisic acid, methyl jasmonate, herbivory, phosphate starvation and nitrogen fixing bacteria (Rocha *et al*., 2007). These biotic and abiotic factors influenced wide array of metabolic pathways by modulating the expression of multiple genes. Therefore, differential expression of genes under stress conditions serves as a tool to identify potential candidate genes for crop improvement. Begcy *et al*. (2012) studied expression of *Scdr1* gene in different sugarcane genotypes under drought stress and its overexpression enabled tobacco plants to withstand abiotic stresses (Begcy *et al*., 2012). Considering its potential role in abiotic stress tolerance, it was characterized in indigenous elite sugarcane genotypes. Amplification of 1266 bp fragment from genomic DNA and 744 bp fragment from cDNA template lead to conclude that native *Scdr1* gene has introns. Sequence analysis revealed presence of one major intron of 492 bp. *Scdr1* is a non-structured protein with 65% random coils and can change its structure easily. Phylogenetic analysis of *Scdr1* revealed out prevalence of its homologues in other monocotyledonous species including wheat, rice, sorghum and maize. The protein is predicted to be present even prior to speciation and genus *Oryza* was grouped in a separate clade from other monocotyledonous species. So,

characterization of this novel protein can be helpful in functional characterization of other proteins that belong to the same family.

Therefore, for functional characterization of *Scdr1* gene, relative expression analysis was performed in six indigenous sugarcane genotypes (CPF-247, CPF-248, CP77-400, S2006-US-272, CPF-246 and S2003-US-127) growing under control conditions and under 170 m*M* NaCl stress. *Scdr1* exhibited positive response to stress conditions as its expression was higher in plants exposed to stress as compared with the ones growing in control conditions. The outcomes of this experiment were in agreement with Begcy *et al*. (2012) who first documented the differential expression of *Scdr1* in drought tolerant and sensitive genotypes of sugarcane. Sugarcane has complex genome and in most of the cases abiotic stress tolerance is a multigenic trait. We observed differential expression of *Scdr1* in different elite genotypes, a general trend was observed that genotypes with comparatively higher expression of *Scdr1* are better tolerant to salinity stress, though exceptions were there. Chlorophyll contents and photosynthesis parameters are significant indicators of plant's potential to tolerate stress conditions (Zlatev and Yordanov, 2004; Li *et al*., 2006; Errabii *et al*., 2007). Silva *et al*. (2007) reported the degradation of chlorophyll and carotenoids in sugarcane under water deficit conditions. These pigments reduced sharply, depending on the level of stress, post treatment days and also on sugarcane genotypes (susceptible or tolerant). Tolerant genotypes of sugarcane were able to retain more chlorophyll content than susceptible ones (Jangpromma *et al*., 2010). Li *et al*. (2018) evaluated physiological parameters *i.e*., net photosynthesis (A), stomatal conductance (GS), transpiration rate (E) and internal leaf CO_2 in cold sensitive and tolerant cultivars of sugarcane. Therefore, in the current study physiological parameters such as total chlorophyll content, net photosynthesis (A), transpiration rate (E), stomatal conductance (GS) and internal leaf CO_2 concentration (Ci) were recorded to evaluate the response of different indigenous sugarcane genotypes under salt stress. Genotypes CP77-400 and S2003-US-127 appeared more competent regarding their physiological performance under salinity stress whereas genotype CPF-246 was most affected. These outcomes are in line with Ashraf *et al*. (2007) who reported that CP77-400 is the most tolerant genotype having potential to perform better under salt stress. *Scdr1* expression was highest in genotype CP77-400 and was minimum in genotype CPF-246. These results indicate that genotypes with better physiological parameters have higher expression of *Scdr1* gene under salt stress. This indicates that *Scdr1* has some crucial role in stress tolerance. In our country where there is no viable flowering in sugarcane. Variety development program is dependent on the import of fuzz, its germination and screening. All this consumes 10–15 years owing to long breeding cycle of this grass. The retrieved results are of pivotal importance in this context as by employing molecular tools, stress tolerant genotypes can be screened out in time proficient manner.

Conclusion

Differential expression of stress responsive gene *Scdr1* was observed in indigenous sugarcane genotypes. Highest level of expression of *Scdr1* in genotype CP77-400 and its physiological competence led us to propose that the gene plays some critical role in stress tolerance. Phylogenetic analyses of *Scdr1* led us to conclude that the gene is present in monocotyledonous species including wheat, rice, sorghum and maize so these results can not only be employed for the improvement sugarcane but also for other monocots.

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References

Affenzeller, M.J., A. Darehshouri, A. Andosch, C. Lutz and U. Lutz-Meindl, 2009. Salt stress-induced cell death in the unicellular green alga *Micrasterias denticulata. J. Exp. Bot*., 60: 939–954

Ashraf, M., S. Kanwal, M. Tahir, A. Sarwar and L. Ali, 2007. Differential salt tolerance of sugarcane genotypes. *Pak. J. Agric. Sci*., 44: 85–89

Begcy, K., E.D. Mariano, A. Gentile, C.G. Lembke, S.M. Zingaretti, G.M. Souza and M. Menossi, 2012. A novel stress-induced sugarcane gene confers tolerance to drought, salt and oxidative stress in transgenic tobacco plants. *PLoS One*, 7: 1–14

Caine, R.S., X. Yin, J. Sloan, E.L. Harrison, U. Mohammed, T. Fulton, A.K. Biswal, J. Dionora, C.C. Chater and R.A. Coe, 2019. Rice with reduced stomatal density conserves water and has improved drought tolerance under future climate conditions. *New Phytol*., 221: 371–384

Errabii, T., C.B. Gandonou, H. Essalmani, J. Abrini, M. Idaomar and N.S. Senhaji, 2007. Effects of NaCl and mannitol induced stress on sugarcane (*Saccharum* spp.) callus cultures. *Acta Physiol. Plantarum*, 29: 95

Farooq, M., M. Hussain, A. Wakeel and K.H.M. Siddique, 2015. Salt stress in maize: effects, resistance mechanisms and management. A review. *Agron. Sustain. Dev*., 35: 461–481

Farooq, M., N. Gogoi, M. Hussain, S. Barthakur, S. Paul, N. Bharadwaj, H.M. Migdadi, S.S. Alghamdi and K.H.M. Siddique, 2017. Effects, tolerance mechanisms and management of salt stress in grain legumes. *Plant Physiol. Biochem*., 118: 199–217.

Fghire, R., F. Anaya, O.A. Issa and S. Wahbi, 2017. Physiological and growth response traits to water deficit as indicators of tolerance criteria between quinoa genotypes. *J. Mater. Environ. Sci*., 8: 2084–2093

Geourjon, C. and G. Deleage, 1995. SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *Bioinformatics*, 11: 681–684

Guo, Q., J. Zhang, Q. Gao, S. Xing, F. Li and W. Wang, 2008. Drought tolerance through overexpression of monoubiquitin in transgenic tobacco. *J. Plant Physiol*., 165: 1745–1755

Haq, I., A.A. Khan, F.M. Azhar and Ehsanullah, 2010. Genetic basis of variation for salinity tolerance in okra (*Abelmoschus esculentus* L.). *Pak. J. Bot*., 42: 1567–1581

Heger, M., G. Zens and M. Bangalor, 2018. *Does the Environment Matter for Poverty Reduction*? The role of soil fertility and vegetation vigor in poverty reduction. The World Bank, Washington DC, USA

Horneck, D.A., J.W. Ellsworth, B.G. Hopkins, D.M. Sullivan and R.G. Stevens, 2007. *Managing Salt Affected Soil for Crop Production*, pp: 1–22. A Pacific Northwest extension publication Oregon State University, University of Idaho, Washington State University, USA

Jangpromma, N., S. Kitthaisong, K. Lomthaisong, S. Daduang, P. Jaisil and S. Thammasirirak, 2010. A proteomics analysis of drought stress-responsive proteins as biomarker for drought-tolerant sugarcane cultivars. *Amer. J. Biochem. Biotechnol*., 6: 89–102

Kausar, A., M.Y. Ashraf, I. Ali, M. Niaz and Q. Abbass, 2012. Evaluation of sorghum varieties/lines for salt tolerance using physiological indices as screening tool. *Pak. J. Bot*., 44: 47–52

Khan, S.A., Z. Hanif, U. Irshad, R. Ahmad, M. Yasin, M.F. Chaudhary, A. Afroz, M.T. Javed, U. Rashid and H. Rashid, 2013. Genetic transformation of sugarcane variety HSF-240 with marker gene GUS. *Intl. J. Agric. Biol*., 15: 1258–1264

Li, J., T.T. Phan, Y.R. Li, Y.X. Xing and L.T. Yang, 2018. Isolation, transformation and overexpression of sugarcane *SoP5CS* gene for drought tolerance improvement. *Sugar Tech*, 20: 464–473

Li, R.H., P.G. Guo, B. Michael, G. Stefania and C. Salvatore, 2006. Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. *Agric. Sci. Chin*., 5: 751–757

Liu, F., N. Huang, L. Wang, H. Ling, T. Sun, W. Ahmad, K. Muhammad, J. Guo, L. Xu, S. Gao, Y. Que and Y. Su, 2018. A novel L-ascorbate peroxidase 6 gene, *ScAPX6*, plays an important role in the regulation of response to biotic and abiotic stresses in sugarcane. *Front. Plant Sci*., 8: 1–13

Livak, K.J. and T.D. Schmittgen, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*, 25: 402–408

Mustafa, G. and M.S. Khan, 2012a. Reproducible *in vitro* regeneration system for purifying sugarcane clones. *Afr. J. Biotechnol*., 11: 9961–9969

Mustafa, G. and M.S. Khan, 2012b. Prospecting the utility of antibiotics as lethal selection agents for chloroplast transformation in sugarcane. *Intl. J. Agric. Biol*., 14: 307–310

Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: A review. *Ecotoxicol. Environ. Saf*., 60: 324–349

Pastina, M.M., L.R. Pinto, K.M. Oliveira, A.P. Souza and A.A.F. Garcia, 2010. Molecular mapping of complex traits. *In: Genetics Genomics and Breeding of Sugarcane*, pp: 117–148. Henry, R.J. and C. Kole (Eds.). CRC Press, Boca Raton, Florida, USA

Rocha, F.R., F.S. Papini-Terzi, M.Y. Nishiyama-Jr, R.Z.N. Vencio, R. Vicentini, R.D.C. Duarte, V.E.D. Rosa-Jr, F. Vinagre, C. Barsalobres, A.H. Medeiros, F.A. Rodrigues, E.C. Ulian, S.M. Zingaretti, J.A. Galbiatti, R.S. Almeida, A.V.O. Figueira, A.S. Hemerly, M.C. Silva-Filho, M. Menossi and G.M. Souza, 2007. Signal transduction-related responses to phytohormones and environmental challenges in sugarcane. *BMC Genomics*, 8: 71

Rodrigues, F.A., J.P.D. Graça, M.L.D. Laia, A. Nhani-Jr, J.A. Galbiati, M.I.T. Ferro, J.A. Ferro and S.M. Zingaretti, 2011. Sugarcane genes differentially expressed during water deficit. *Biol. Plantarum*, 55: 43–53

Silva, M.D.A., J.L. Jifon, J.A.D. Silva and V. Sharma, 2007. Use of physiological parameters as fast tools to screen for drought tolerance in sugarcane. *Braz. J. Plant Physiol*., 19: 193–201

Su, Y., J. Guo, H. Ling, S. Chen, S. Wang, L. Xu, A.C. Allan and Y. Que, 2014. Isolation of a novel peroxisomal catalase gene from sugarcane, which is responsive to biotic and abiotic stresses. *PloS One*, 9: e84426

Suprasanna, P., M.V. Patade, N.S. Desai, R.M. Devarumath, P.J. Kawar, M.C. Pagariya, A. Ganapathi, M. Manickavasagam and K.H. Babu, 2011. Biotechnological developments in sugarcane improvement: an overview. *Sugar Tech*, 13: 322–335

Tester, M. and P. Langridge, 2010. Breeding technologies to increase crop production in a changing world. *Science*, 327: 818–822

Xie, G., Z. Li, Q. Ran, H. Wang and J. Zhang, 2018. Over-expression of mutated Zm DA 1 or Zm DAR 1 gene improves maize kernel yield by enhancing starch synthesis. *Plant Biotechnol. J*., 16: 234–244

Zhu, J.K., 2002. Salt and drought stress signal transduction in plants. *Annu. Rev. Plant Biol*., 53: 247–273

Zlatev, Z.S. and I.T. Yordanov, 2004. Effects of soil drought on photosynthesis and chlorophyll fluorescence in bean plants. *Bulg. J. Plant Physiol*., 30: 3–18

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