Differential gene expression profiling through transcriptome approach of *Saccharum spontaneum* L. under low temperature stress reveals genes potentially involved in cold acclimation

Dharshini Selvarajan¹ · Chakravarthi Mohan¹ · Vignesh Dhandapani³ · Gauri Nerkar¹ · Ashwin Narayan Jayanarayanan¹ · Manoj Vadakkancherry Mohanan¹ · Naveenarani Murugan¹ · Lovejot Kaur¹ · Mahadevaiah Chennappa¹ · Ravinder Kumar² · Minturam Meena² · Bakshi Ram¹ · Appunu Chinnaswamy¹

Received: 21 November 2017 / Accepted: 2 March 2018 / Published online: 23 March 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Sugarcane (*Saccharum* sp.) is predominantly grown in both tropics and subtropics in India, and the subtropics alone contribute more than half of sugarcane production. Sugarcane active growth period in subtropics is restricted to 8–9 months mainly due to winter's low temperature stress prevailing during November to February every year. Being a commercial crop, tolerance to low temperature is important in sugarcane improvement programs. Development of cold tolerant sugarcane varieties require a deep knowledge on molecular mechanism naturally adapted by cold tolerant genotypes during low temperature stress. To understand gene regulation under low temperature stress, control and stressed (10 °C, 24 h) leaf samples of cold tolerant *S. spontaneum* IND 00-1037 collected from high altitude region in Arunachal Pradesh were used for transcriptome analysis using the Illumina NextSeq 500 platform with paired-end sequencing method. Raw reads of 5.1 GB (control) and 5.3 GB (stressed) obtained were assembled using trinity and annotated with UNIPROT, KEGG, GO, COG and SUCEST databases, and transcriptome was validated using qRT-PCR. The differential gene expression (DGE) analysis showed that 2583 genes were upregulated and 3302 genes were down-regulated upon low temperature stress. A total of 170 cold responsive transcriptional factors belonging to 30 families were differentially regulated. CBF6 (C-binding factor), a DNA binding transcriptional activation protein associated with cold acclimation and freezing tolerance was differentially upregulated. Many low temperature responsive genes involved in various metabolic pathways, viz. cold sensing through membrane fluidity, calcium and lipid signaling genes, MAP kinases, phytohormone signaling and biosynthetic genes, antioxidative enzymes, membrane and cellular stabilizing genes, genes involved in biosynthesis of polyunsaturated fatty acids, chaperones, LEA proteins, soluble sugars, osmoprotectants, lignin and pectin biosynthetic genes were also differentially upregulated. Potential cold responsive genes and transcriptional factors involved in cold tolerance mechanism in cold tolerant *S. spontaneum* IND 00-1037 were identified. Together, this study provides insights into the cold tolerance to low temperature stress in *S. spontaneum*, thus opening applications in the genetic improvement of cold stress tolerance in sugarcane.

Keywords Low temperature stress · *S. spontaneum* · Transcriptome · Cold sensor · Signaling · qRT-PCR · Cold responsive genes

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s13205-018-1194-2>) contains supplementary material, which is available to authorized users.

✉ Appunu Chinnaswamy
cappunu@gmail.com

Extended author information available on the last page of the article

Introduction

Sugarcane is the second most important commercial crop in India. It is grown over 5 M ha in varied agro-ecological climatic conditions with an annual cane production of more than 350 million tonnes (Solomon 2016). India is the second largest sugar producers in the world. Sugarcane growing regions are broadly classified under two climatic conditions commonly referred to as tropical and subtropical regions in India. The major sugarcane producing area lies in the

subtropical regions comprising the states, viz. Uttar Pradesh, Bihar, Uttarakhand, Haryana, Punjab, Assam, West Bengal, Rajasthan and other Northeastern states of India, which accounts for 55% of total area and 50% of total production (Solomon 2016). Sugarcane is quite an unusual plant with polyploidy genome, which introduces difficulties and hurdles in genetical and physiological approaches of crop improvement (Azevedo et al. 2011). The sugarcane productivity and quality of juice are influenced by weather conditions and optimum temperature for germination is 26–32 °C and growth requires 30–33 °C. Temperatures above 38 °C reduce photosynthesis rate and increase respiration. Rapid cane elongation during grand growth period is favored by high humidity (80–85%). In subtropical regions in India, chilling conditions is severe during November to February every year. Severe cold weather inhibits bud sprouting in ratoon crop and arrests cane growth of sugarcane. The critical temperature for chilling-sensitive tropical plants is 10–12 °C and winter's low temperature (LT) affects stalk growth more than the sugar production (Ram et al. 2001).

LT stress induces several abnormalities in physiological and morphological characters, which include changes in reduction in water/mineral uptake and photosynthetic rate, changes in membrane system, ion homeostasis disturbance and induction of ROS. An early response to low temperature is inhibition of photosynthesis by changing pigment composition, decreased quantum efficiency (Fryer et al. 1995), modified thylakoid membranes, and impaired chloroplast development (Nie et al. 1995). The reduction of pyruvate, phosphate dikinase (PPDK) and *NADP*-malate dehydrogenase (*NADP*-MDH) activities may be the primary cause of the reduction of the photosynthetic rate in sugarcane leaves at chilling temperature (Du et al. 1999). The membrane systems are primary sites of injury in plants during cold exposure, which in turn disturbs the fluidity, causing water and soluble materials to leak out into the intercellular spaces, where water is lost through evaporation and is the primary cause of wilting (Wright 1974). During LT stress, Na^+ and Cl^- levels increase (Blumwald et al. 2000) and this imbalance of ion homeostasis leads to cellular damage and cell death. Another important effect of low temperature is induction of oxidative stress in tissues leading to generation of reactive oxygen species (ROS), including superoxide radicals, hydroxyl radicals and hydrogen peroxide resulting in cell death (Dhingra 2015).

Tolerant plants show responsive mechanism towards LT stress in a genotype, organ and environment-specific manner through various complex networks of metabolic pathways (Zhang et al. 2014). Sessile plants sense the LT stress through membrane fluidity changes and accumulation of calcium signatures that leads to downstream activation of cold signaling pathways (Virdi et al. 2015). Fatty acid composition of cellular membrane with more than 40% of unsaturated fatty acid, desaturation of membrane lipids by glycerol 3-phosphate

acyltransferase and biosynthesis of polyunsaturated fatty acid by fatty acid desaturase 3 (FAD3) confers tolerance to LT stress (Murata et al. 1992; Li et al. 2016). Many transcriptional factors like bHLH, CAMTA, MADS, WRKY, NAC, TRAF, C3H, AP2 were associated with cold responsive mechanisms (Shi et al. 2014). MAP Kinases are highly conserved and involved in cascades of signal transduction and activation of cold responsive mechanism (Zhao et al. 2017). ICE-CBF-DREB1 dependent and independent (ABA-dependent) pathways are regulating the LT response mechanism in plants (Thomashow 1999; Nogueira et al. 2003). ICE-CBF-DREB1 dependent cold responsive genes (COR) are reported to play a significant role in signaling, thermoregulation and cold acclimatization (Viswanathan and Zhu 2002). Phytohormones confer tolerance to LT through modulating cold pathways and enhancing plant growth and development (Shi et al. 2014). ABA is central regulator and vital stress hormone expressed during LT stress (Sah et al. 2016). Antioxidative enzymes involved in scavenging of ROS and non-enzymatic antioxidants cum secondary metabolites such as anthocyanin, phenylpropanoid, terpenoids (Vanlerberghe and McIntosh 1997; Maxwell et al. 1999; Arnholdt-Schmitt et al. 2006), cellular membrane stabilizers, viz. higher proportion of unsaturated fatty acid (Murata et al. 1992; Li et al. 2016), chaperones, late embryogenesis abundant (LEA) proteins, osmotin and proline protect the cells from LT-induced cellular damage (Al-Whaibi 2011). Accumulation of soluble sugars during cold stress acts as stabilizer of cellular components and plasma membrane (Tarkowski and Van den Ende 2015). Differential transcriptome profiling through NGS approaches emerged as robust, efficient and sensitive for both low and high level gene expression (Wang et al. 2010). This tool also facilitates rapid identification of stress-responsive genes and deciphering metabolic pathways associated with various biotic and abiotic stresses (Zhang and Huang 2010).

Recently, we reported for the first time a transcriptome analysis of a low temperature tolerant *S. spontaneum* clone (Dharshini et al. 2016). In the present study, assembled transcriptome was blasted against SUCEST database (<http://sucet-fun.org>) and performed an extensive differential gene expression (DGE) analysis to identify the low temperature responsive novel genes and metabolic pathways. Also, this study illustrates the expression pattern of genes overviewed in cellular regulations, like cell wall modifications, receptor-like kinases, transcription factors and phytohormones upon low temperature stress conditions.

Materials and methods

Plant material and RNA isolation

Saccharum spontaneum L. IND 00-1037 clone was raised under glass house conditions (at 28 ± 2 °C) at ICAR—Sugarcane Breeding Institute, Coimbatore, Tamil Nadu, India. Three months-old seedlings were selected for the experiment and leaf tissues were collected from three pots (three replicates, one pot per replicate) of stressed sample which pooled together as one biological replicate after being exposed to low temperature (10 °C) for 24 h. Total RNA was isolated from both control (C) and stressed (S) samples using TRIzol reagent (Invitrogen, USA). Purified RNA was quantified using Nanodrop (Thermo Scientific, USA) and agarose gel electrophoresis was also performed to check the quality of RNA.

cDNA library, sequencing and de novo assembly

The cDNA library was constructed using the NextFlex Rapid Directional RNASeq kit as per the manufacturer's instructions (Bioo Scientific, USA) and sequenced using Illumina Nextseq 500 platform with paired-end technology. Illumina yielded raw reads quality was checked using FastQC tool to obtain processed reads. The high-quality clean reads were assembled using Trinity tool (<http://trinityrnaseq.sourceforge.net/>) with K-mer value 25.

Sequence annotations

The de novo assembled transcripts annotated against UNIPROT, KEGG, GO and COG databases were described elsewhere (Dharshini et al. 2016). In the present study, the assembled transcripts were blasted against SUCEST database (<http://sucet-fun.org>) using mapping parameters of minimum length fraction = 0.7 (70%); minimum similarity fraction = 0.8 (80%); maximum number of hits for a read = 1 (Park et al. 2015).

Gene function analysis using SUCEST data

Gene ontology information was generated for all plant RefSeq (NCBI) by finding common NCBI gene ID's in gene2refseq (<ftp://ftp.ncbi.nih.gov/gene/DATA/gene2refseq>) and gene2go (<http://ncbi.nih.gov/gene/DATA/gene2go>). Gene functional information and enzyme information were found in database of gene info (ftp://ftp.ncbi.nih.gov/gene/DATA/gene_info) and ec2go (<http://www.geneontology.org/external2go/ec2go>) files. Blastx of the NCBI RefSeq database were also used to identify gene ontology and gene functional information for each contig by their RefSeq IDs.

Differential gene expression (DGE) analysis

DGE analysis for control and stress samples were performed as described elsewhere (Dharshini et al. 2016). In brief, generation of master control transcriptome data was performed and used as reference for DGE analysis by employing a negative binomial distribution model with DESeqv1.8.1 package (<http://www-huber.embl.de/users/anders/DESeq/anders/DESeq/>). The expression profile for those transcripts expressed only in C, expressed in both C and S, and expressed only in S was generated. P and Q significant of transcripts were calculated for those transcripts expressed in both C and S samples.

Quantitative real-time polymerase chain reaction (qRT-PCR)

A total of 18 genes representing different cold responsive pathways, viz. membrane fluidity, cold sensor, signaling, transcription factors, osmolytes like simple sugars and sugar alcohols, photosynthesis and ubiquitination were chosen for validation using qRT-PCR. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an endogenous reference (Park et al. 2015). qRT-PCR was performed with a Step-One plus Real-Time PCR system (Applied Biosystems, Canada). Each reaction was carried out in triplicates. The primers used were designed based on the transcriptome data and are listed in supplementary table S1. The nucleotide sequence of 18 genes used for qRT-PCR experiment are given in supplementary file S1. For qRT-PCR experiments, RNA of stress and control samples were used as template and cDNA synthesis was done using RevertAid first Strand cDNA synthesis kit (Thermo Scientific, USA). The cDNA concentration was standardized for each sample and to check primer specificity dissociation curve analysis was performed. In brief, each qRT-PCR reaction consists of 50 ng cDNA, 2.5 pmol primers, 12.5 µl of 2X MESAGREEN Master Mix (Eurogentec, Belgium) and the final volume was made up to 25 µl with sterile water. qRT-PCR reaction conditions used were as follows: denaturation for 10 min at 95 °C followed by annealing and extension at 1 min for 60 °C (40 cycles). The fold change of the target genes was determined by $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001).

MapMan analysis

Transcripts were annotated using BLAST against *Zea mays* and TAIR (The *Arabidopsis* Information Resources) database with parameters like CDS and E value of 0.0005. Functional mapping was created for the differentially expressed genes upon low temperature in *S. spontaneum*. The functional mapping generated a reference file that was uploaded in MapMan software v3.5.1R2 to visualize the metabolic pathways, cell regulation, secondary metabolites, and other processes in the datasets.

Results and discussion

Transcriptome summary

In brief, the Illumina platform generated raw reads of 5.1 Gb (Control) and 5.3 Gb (Stress). Processed reads were obtained after a quality check of raw reads and de novo assembly was made using the trinity program. A summary of raw data and QC were given in supplementary table S2 (a) and de novo assembly statistics were given in S2 (b). Information on contigs [S2 (c)] and unigenes statistics [S2 (d)] were tabulated. All clean reads have been submitted as sequence read archive (SRA) in NCBI with the BioProject ID-PRJNA307641.

SUCEST analysis and gene function

All the transcripts of control (2195974) and stress (2145528) were annotated against SUCEST, about 18,336 hits for control and 17,867 hits for stress samples were obtained. The SUCEST outfile is given in supplementary file S2. The SUCEST statistics were given in Table 1.

Table 1 Statistics of SUCEST report

Sample	Total	SUCEST hits	Unique hits
Control	2,195,974	18,336	12,750
Stress	2,145,528	17,867	12,355

Differential gene expression (DGE) analysis

The DGE analysis showed that 21,283 unigenes were expressed in the stress sample and 19,506 unigenes were expressed in the control sample and 10,823 unigenes were neutrally expressed with $FDR \leq 0.001$ and $\text{Log}_2 \geq 2$. The distribution of DGE pattern is represented as scatter plot (Fig. 1). The DGE analysis revealed that a total of 2583 genes were upregulated and 3302 genes were down-regulated upon LT (Dharshini et al. 2016). Herein, we present information on differentially expressed genes that are potentially involved in cold acclimation in *S. spontaneum* under LT conditions.

Membrane fluidity

The plant cell membrane gives mechanical strength and acts as primary defense machinery. Sugarcane cell wall is composed largely of celluloses (48.6%), hemicelluloses, mainly by galactose and xylose (31.1%), lignin (19.1%), and 1.2% of extractives (Masarin et al. 2011). During LT stress, most of the genes that play a major role in cell wall composition have been upregulated as a defense mechanism. Arabinoxylan arabinofuranohydrolase (plasticizing of cell), expressed only in stress, is a structural component of cell, which serves as a phenolic reservoir, activates antioxidant production and maintains membrane fluidity by rehydration (Moore et al. 2013). In this data, xyloglucan endotransglucosylase/hydrolase (XTH) genes were upregulated by 4- Log_2 fold and expansions expA1,

Fig. 1 Scatter plot representation of differentially expressed genes

expB5, expB7 were upregulated by 2.4, 5.5 and 4.7 Log₂ fold, respectively. XTH along with expansins are recognized as wall-modifying proteins, which participate in cold acclimation (Cho et al. 2006).

LT stress strengthens plant cell wall by lignin and pectin synthesis and decreases the cell wall pore size. Upon LT stress, pectin methyl esterase was 3.5 Log₂ fold upregulated, thereby proving that pectin acts as a key element in plant response to cold stress and also regulates pore size (Solecka et al. 2008). Lignin synthesis was enhanced by upregulation of cinnamyl alcohol dehydrogenase I (2.5 Log₂ fold). Cell wall-related proteins, like wall-associated kinases, were 4-Log₂ fold upregulated. Cold acclimation includes changes in polysaccharides, lipid composition and reshuffling to maintain cell membrane fluidity. Fatty acid desaturase (FAD8) gene was 2.1 Log₂ fold upregulated which helps in inserting double bonds to hydrocarbon chains of fatty acids to produce unsaturated fatty acid (Pang et al. 2013). Phospholipase D was 2.9 Log₂ fold enhanced which anchors the microtubules to plasma membrane and causes rearrangement in cytoskeleton confirmation, thereby activating the calcium channels (Fowler and Thomashow 2002). Also, other genes upregulated in cell wall study during low temperature are represented in table S3.

Restoration of cellular homeostasis

Low temperature leads to increase in Na⁺ ions and the excessive Na⁺ has to be effluxed or compartmentalized in the vacuole to maintain the cellular homeostasis. In plant cell, H⁺ ATPase and H⁺ pyro-phosphatases create proton motive force and transport ions like Na⁺ (Conde et al. 2011). DGE analysis revealed that ATPases were upregulated by 3.7 Log₂ fold and a vacuolar proton pyro-phosphatase, which is involved in hydrogen-translocating pyro-phosphatase activity and Na⁺ ion transport to vacuole, was 2.1 Log₂ fold upregulated. This leads to a decrease in intracellular Na⁺ level in the cytosol. Thioredoxin, Grx_S16-glutaredoxin subgroup II, glutaredoxin, and 68 putative proteins involved in restoration of ion homeostasis imbalance caused by cold stress, were upregulated. Multi-drug and toxic compound extrusion proteins (MATEs) are active secondary transporters with H⁺/Na⁺ antiporter function. MATEs were upregulated during stress in rice seedlings (Tiwari et al. 2014). Seventeen MATEs transcripts were upregulated in our transcriptome (3.4 Log₂ fold).

Cold sensors and receptor protein kinases

Cold sensors detect the changes in membrane fluidity caused due to low temperature and initiate signal transduction

machinery. The calcium channel and two-component histidine kinases are potential low temperature sensors in plants (Xiong et al. 2002). The reorganization of the membrane results in elevation of cytosolic calcium levels and transmit primary signal through Ca²⁺ regulated protein like calmodulin, calcineurin (sensor relays) and protein kinases (responders). Sensor relay changes phosphorylation status of different proteins and regulate the cold responsive gene expression. Responders, like phosphatase, calcium-dependent protein kinase, have effector domains through which they relay the message to downstream targets (Reddy and Reddy 2004). Ca²⁺ dependent protein kinase (CDPK) and calmodulin are Ca²⁺ dependent multifunctional proteins that could be activated by influx of calcium upon LT (Nogueira et al. 2003). In this study, calmodulin and calcineurin were upregulated by 2.8 and 2.3 Log₂ folds, respectively. Ca²⁺ binding calmodulin-like receptor protein kinase can enhance cold tolerance by regulating CBF regulons (Yang et al. 2010). In addition, calcium-dependent protein kinases (CDPK) and protein phosphatase (PP2C) were also upregulated. Ca²⁺ ATPase act as calcium pumps by pumping Ca²⁺ out of the cytosol as signaling and thereby restore cell homeostasis (Sze et al. 2000).

Our data revealed 13 calcium transporting ATPases, of which one gene was highly upregulated (3.7 Log₂ fold). The two-component histidine kinase (HK) is another type of cold sensor in plants. In *Arabidopsis*, AtHK1 has been found to be upregulated upon LT (Urao et al. 2000). A total of 41 putative HK genes involved in phosphor relay signaling were upregulated in this study. Importantly, histidine-containing phosphor transfer protein 4 was upregulated by 4.6 Log₂ fold upon cold stress and transduces the signal to the nucleus through a mitogen-activated protein kinase (MAPK) phosphorylation cascade.

Receptor protein kinases (RPK) are putative cold sensors that contain membrane spanning domains that function in cold signaling (Solanke and Sharma 2008). DGE analysis shows that RPK1 and RPK4 were upregulated by 2.6 and 2.2 Log₂ fold, respectively, by cold stress, which was similar to that reported in *Arabidopsis* (Hong et al. 1997). Receptor-like kinases (RLKs) contain serine/threonine kinase as a cytosolic domain and convey signals to their target proteins in the cytoplasm by catalytic processes of protein kinase activity (Osakabe et al. 2013). Figure 2 represents various receptor-like kinases, viz. LRR, DUF28, PERK, L-lectin and receptor-like cytoplasmic kinases that are differentially expressed during LT stress. Our data shows that 264 RLKs were upregulated and 33 were down-regulated upon cold exposure. Different types of receptor-like kinase genes expressed in response to low temperature in *S. spontaneum* are listed in Table 2.

Fig. 2 Receptor-like kinase families involved in cold *S. spontaneum* response signaling

Signal transduction machinery

Secondary messengers, like calcium and inositol, reactive oxygen species (ROS) and abscisic acid (ABA) are the important role players in cold signal transduction. In plants, MAPK pathways are responsible for the production of compatible osmolytes and antioxidants stimulated during

cold stress. These MAPK pathways are, in turn activated by receptors/sensors, such as protein tyrosine kinase, G-protein-coupled receptors, and two-component histidine kinases (Xiong et al. 2002). The protein phosphatase 2C, which acts as a MAPK phosphatase, has a positive regulation in seed germination, stomatal closure and ABA-inducible gene expression. The Ras gene is a small GTPase, which binds

Table 2 Receptor-like kinase family expressed during low temperature stress in *S. spontaneum*

Transcript ID	Receptor-like kinase family	Gene name
c13911_g1_i1	Leucine rich repeat	Light repressor protein kinase I, FLG-induced receptor-like kinase, Serine/threonine protein kinase, LRR transmembrane protein kinase, STRUBBELIG-receptor-like family, CLAVATA1 receptor kinase, Phytosulfokin receptor, ERECTA, Transmembrane kinase I
c43332_g1_i1	Extensin	Abnormal leaf shape 2 kinase, Protein kinase family
c94275_g1_i1	L-lectin	L-type lectin domain containing protein
c39298_g1_i1	LysM	Chitin elicitor protein kinase I (CERK) Peptidoglycan-binding LYSM domain containing protein
c54171_g1_i1	Crinkly4-like	Transmembrane receptor kinase
c37603_g1_i1	DUF26	Cysteine rich RLK1, 6, 10, Protein kinase
c67713_g1_i1	LRK	Serine/threonine protein kinase
c18643_g1_i1	PERK-like	Proline rich extension-like containing receptor kinase
c78419_g1_i1	S-locus	S-locus protein kinase, S-locus-lectin containing protein kinase\
c34272_g1_i1	Wall-associated kinases	Wall-associated kinase family–WAK1, WAK4, WAK2,WAK5,WAK20, Wall-associated kinase-like family 4
c37963_g1_i1	Thaumatin	Serine/threoinine kinase PR5
c107496_g1_i1	Receptor-like cytoplasmic kinases	Cytoplasmic kinase IX, Connexin 32, protein kinase 2A, Protein kinase 2B, Protein kinase 1A, Constitutive differential growth 1, STRUBBELIG –receptor family 8, Protein kinase family

and activates Ras–MAPK cascade and exhibits regulation of cold-induced gene expression. In alfalfa, MAPK was found to be upregulated in response to cold (Sangwan et al. 2002). In our data, MAPK4 was 3.3 Log₂ fold upregulated and Ras5 expressed only under low temperature treated sample of *S. spontaneum*. Nucleoside diphosphate kinase is a positive regulator of MAPK cascade (Kovtun et al. 2000) and detected enhanced expression upon LT stress, in this study.

Abscisic acid-dependent genes like abscisic acid 8'-hydroxylase 1, abscisic acid receptor PYL10, abscisic acid responsive elements-binding factor 2, abscisic acid responsive elements-binding factor 3 and abscisic acid 8'-hydroxylase 3 were expressed in LT. There are 33 putative abscisic acid-dependent genes involved during cold stress, of which abscisic stress-ripening protein 1 (uncharacterized protein) and putative ankyrin-kinase were upregulated by 3.9 and 2.3 Log₂ fold, respectively. A list of ABA-dependent genes (Table S4a) and ABA-independent genes (Table S4b) were tabulated. Cold stress induces phospholipase C, which in turn triggers inositol-3-phosphate synthase (IP3), which binds to endoplasmic reticulum and triggers specific response during LT. In this study, IP3 was 3.0 Log₂ fold upregulated which substantiates its role as secondary messenger during chilling stress.

Transcription factors (TFs) and LT stress

The low temperature signal is perceived by plasma membrane and transduced by variety of transduction components, which results in stimulating an array of transcription factors of cold responsive genes. There are 170 upregulated cold responsive TFs in our transcriptome, which fall into 30 families (Table S5). The major TFs including CBF, AP2/EREBP, bHLH, MYB, NAC, C2C2-GATA, GNAT, WRKY were actively upregulated under low temperature stress. Cold stress induces APETALA2/ETHYLENE RESPONSE FACTOR family TFs, i.e., CBFs (C-repeat binding factors, also known as dehydration-responsive element-binding protein 1s or DREB1s), which bind to cis-elements in the promoters of COR genes and activate their expression (Chinnusamy et al. 2006, 2007). AP2-EREBP (9 genes) was upregulated by 6.0 Log₂ fold. bHLH of *Poncirus trifoliata* had 62% sequence identity to GmICE2 of soybean and 61% to ICE2 of *Arabidopsis*. PtrbHLH is a stress-responsive TF and plays a positive role in cold response (Huang et al. 2013). ICE1-like bHLH transcription factors are involved in the regulation of CBF1 and/or CBF2 (Van Buskirk and Thomashow 2006). Different types of bHLH such as bHLH36 (3.1 Log₂ fold), bHLH128 (1.9 Log₂ fold), bHLH113 (1.8 Log₂ fold), bHLH161 (3.3 Log₂ fold), bHLH115 (1.8 Log₂ fold), bHLH82 (2 Log₂ fold), bHLH48 (2.4 Log₂ fold) were upregulated in *S. spontaneum* under low temperature conditions.

MYB is a large family that is involved in abiotic stress tolerance. There were different types such as MYB34, MYB15, MYB94, MYB9, MYB111, MYB33, MYB12, MYB4, MYB49, MYB52, MYB59, MYB57, MYB55, MYB70, MYB1 that were upregulated during low temperature stress in *S. spontaneum*. MYB15 protein interacts with ICE1 and binds to MYB recognition sequences in the promoters of CBF genes, thereby imparting freezing tolerance (Agarwal et al. 2006) and OsMYB2 regulates cold and salt tolerance in rice (Yang et al. 2012). OsMYB4 is involved in cold and drought tolerance in transgenic apple (Pasquali et al. 2008). MYB15 and MYB4 were upregulated by 2.7 and 3.7 Log₂ fold, respectively, in *S. spontaneum*.

NAC TFs play a pivotal regulatory role in abiotic stresses like cold and drought (Nakashima et al. 2012). In this study, NAC TFs such as NAC2, NAC3, NAC4, NAC8, NAC9, NAC11, NAC39, NAC70, NAC71, NAC82 and NAC87 were upregulated under LT stress. SNAC2 is a cold stress-responsive NAC TF in rice (Hu et al. 2008). NAC4 was induced upon cold exposure in sugarcane (Nogueira et al. 2003).

WRKY transcription factors are responsive during abiotic stresses like cold and drought. Few of WRKY proteins like GmWRKY21, AtWRKY34, VvWRKY55, PtrWRKY2, TaWRKY10 and BcWRKY46 were enhanced upon cold stress in plants (Mare et al. 2004). In *S. spontaneum* cold treated sample, WRKY92, WRKY78, WRKY13, WRKY11, WRKY30, WRKY34 and WRKY21 were upregulated. Different types of transcription factors expressed differentially upon low temperature stress in *S. spontaneum* are given in Fig. 3.

Enzyme family profiling upon chilling stress

Upon low temperature, *S. spontaneum* transcriptome displays differential expression of a large family of enzymes which are depicted (Fig. 4). There were more than 13 types of enzymes such as peroxidases, glucosidases, oxidases, beta-1, 3 glucan hydrolases, alcohol dehydrogenases, glutathione S-transferases, phosphatases, and acetyltransferases. Superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), glutathione S-transferase (GST), and peroxiredoxin (PRX) enzymes are located in different organs of cells and helps in scavenging of reactive oxygen species (ROS) such as hydrogen peroxide, superoxide anions, hydroxyl radicals and singlet oxygen generated under different abiotic stress conditions in various plants (Gill and Tuteja 2010; You and Chan 2015). Alcohol dehydrogenase (ADH) performs a crucial role during cold stress in catalyzing the acetaldehydes into alcohols and

Fig. 3 Profiling of differentially expressed transcription factors upon LT stress

Fig. 4 Large enzymes families expressed during low temperature stress in *S. spontaneum*

generation of NDPH and ATPs in plants (Drew 1997; Song et al. 2017).

Simple sugars

Cold acclimation is accompanied with accumulation of simple sugars, which defend the membranes against freezing-induced damage (Strauss and Hauser 1986). Soluble sugars can remove hydroxyl radicals, indirectly induce protein synthesis, and improve the cold resisting ability of plants. It is reported that soluble sugars and protein contents showed positive correlation with the cold hardiness of plants (Luo et al. 2002). Several compatible solutes, such as raffinose, trehalose, sucrose, glucose and fructose have been known to frequently accumulate upon low temperature stresses. During chilling stress, synthesis and accumulation of the two most highly induced sugars are maltose and raffinose. In our transcriptome data, the raffinose synthase I gene is upregulated during LT. Also, 14 genes belonging to trehalose sugars were identified of which most of the genes were more than 3.0 Log₂ fold upregulated during cold exposure. We also found 15 sucrose precursor genes, of which many of these recorded more than 2.5 Log₂ fold enhanced expression upon cold stress. Sucrose accumulation may be regulated by energy metabolism and sugar transporters (Ferreira et al. 2016). Genes involved in carbohydrate metabolism, such as lichenase2 and α -amylase, were upregulated by 6-Log₂ fold and 3.7-Log₂ fold in *S. spontaneum*. Interestingly, genes involved in the glycolytic pathway such as hexokinase and 6-phosphofructokinase 2 were also upregulated by 2 Log₂ fold and 3.5 Log₂ fold, respectively.

Sugar alcohols

The accumulation of sugar alcohols are yet another characteristic event during low temperatures, which aids in protecting the membrane and protein complexes during cold exposure. Our transcriptome revealed 7 genes related to phospholipid biosynthesis that belonged to inositol-3-phosphate synthase activity, which was upregulated by 8.3-Log₂ fold upon cold exposure. Another gene belonging to NADP-dependent D-sorbitol-6-phosphate dehydrogenase, involved in oxidoreductase activity, was expressed only during low temperature stress.

Polyamines and ethylene

Proline is a key osmolyte that is actively involved in response to several abiotic stresses. Excessive proline production leads to enhanced osmotolerance in plants (Gubis et al. 2007). The free amino acid content varied among different sugarcane varieties, and the most cold resistant sugarcane variety showed obviously higher free amino acid content

(Huang et al. 2015). Delta-1-pyrroline-5-carboxylate synthetase (P5CS) is a key enzyme in proline synthesis, which participates in the cold stress tolerance (Szekely et al. 2008) by solute accumulation (Pearce 1999). In *S. spontaneum* transcriptome, we found P5CS, the key enzyme known to initiate proline biosynthesis in plants, which is upregulated in stress. Another putative gene (proline) involved in proline transport was also found to be 3 Log₂ fold upregulated.

Ethylene, an important regulator during drought, flooding and biotic stress (Wilkinson and Davies 2010), appears to be complex and species-dependent during cold stress. Increased ethylene biosynthesis has been reported during cold stress in plants, such as tomato (Wang and Adams 1982) and tobacco (Zhang et al. 2010). On the contrary, cold stress has led to suppression of ethylene biosynthetic activity in bean (Collins et al. 1993) and *Arabidopsis* (Shi et al. 2012a). In our data, 1-aminocyclopropane-1-carboxylate oxidase, an enzyme involved in ethylene biosynthesis, was 9.3 Log₂ fold upregulated upon low temperature exposure. In addition, an ethylene responsive factor was upregulated only during cold stress, illustrating that ethylene biosynthesis is enhanced during cold stress in *S. spontaneum*.

Photosynthetic machinery

Photosynthesis is drastically affected upon cold stress which includes light reactions, Calvin cycle and photorespiration (Yan et al. 2004). Studies have shown that cold conditions can affect photosynthesis in sugarcane, thereby hindering the carbon supply for synthesis of carbohydrates, storage and transport (Machado et al. 2013; Sales et al. 2012). Some genes involved in the photosynthetic pathway like chlorophyll *a-b* binding protein, thiamine thiazole synthase2 and α -1,4 glucanphosphorylase were negatively regulated upon cold stress. An uncharacterized protein present in the thylakoid lumen (based on gene ontology terms), which is an extrinsic component of membrane and also oxygen evolving complex in photosystem II, is drastically down-regulated by -3.5 Log₂ fold. ELR1 (early-light induced I) protein inhibiting photosystem II was also greatly down-regulated by -3.3 Log₂ fold, in our data. Several genes involved in photosynthetic machinery were upregulated under low temperature stress in *S. spontaneum* (Fig. 5). The ferredoxin-NADP oxidoreductase (FNR) was upregulated by 4.1 Log₂ fold. The increase in expression of FNR during stress results in concomitant release of FNR from thylakoid membrane which indicates that the induction of PSI cyclic electron transfer (CET) is exclusively involved in ATP synthesis (Lehtimäki et al. 2010). Rubisco activase (RCA) was upregulated by 2.2 Log₂ fold, this higher level of expression of RCA is not only the activate Rubisco enzyme but it also plays an important role in photosynthetic acclimation to multiple stresses (Chen et al. 2015). Similarly, PSII cytochrome b was 3.32

Fig. 5 Gene regulation involved in photosynthetic activity during chilling stress in *S. spontaneum*. **a** Photosystem II—(i) LHCb3 (light-harvesting chlorophyll b binding protein 3), (ii) PSBF (PSII cytochrome b559), (iii) PSAN (calmodulin-binding), (iv) NPQ4 (Nonphotochemical quenching); **b** Photosystem I—(i) LHCA3 (chlorophyll binding), (ii) LHCA5 (pigment binding), (iii) PSAE-2 (photosystem I subunit E-2), (iv) PSAC (Encodes the Psac subunit of

photosystem I), (v) FNR1 (ferredoxin-nadp(+)-oxidoreductase 1), (vi) ATFD1 (ferredoxin 1); **c** Calvin cycle—(i) RPE(catalytic/ribulose-phosphate 3-epimerase), (ii) PGK1 (phosphoglycerate kinase 1), (iii) FBA (fructose-bisphosphate aldolase), (iv) RBCL-Rubisco large subunit; **d** Chloroplast—(i) RBCL (large subunit of RUBISCO), (ii) Rubisco activase; **e** Peroxisome—(i) oxidoreductase, (ii) glycolate oxidase; **f** Mitochondria—(i) & (ii) glycine dehydrogenase

Log₂ fold upregulated and a light-harvesting chlorophyll *b* binding protein 3 (LHCb-3) was upregulated by 4.5 Log₂ fold. Nogueira et al. (2003) identified the cold-inducible sugarcane inorganic phosphate transporter protein (pht1-2), that might have a critical role in readjustment of cellular status of Pi and photosynthetic carbon metabolism recovery. Inorganic phosphate transporter protein (pht2) was found to be 2.2 Log₂ fold upregulated in this data, suggesting that the photosynthetic process maintains even under low temperatures. In chloroplast, the calmodulin-binding gene, which activates the downstream signaling, was upregulated by 2.4 Log₂ fold. The upregulation of these genes may play a vital role in restoring photosynthetic activity. In addition, this upregulation may also occur due to the cold tolerant nature of *S. spontaneum*. Therefore, it indicates that comparative studies are necessary to elucidate the expression of these genes in cold susceptible clones of sugarcane. Figure 5

depicts the DEGs involved in photosynthetic activity upon LT.

Antioxidant systems

In plants, chilling induces oxidative stress (Sato 2001) and accumulates reactive oxygen species (ROS) upon cold exposure. Upon cold acclimation, there is a remarkable upsurge in the antioxidant systems (Baek and Skinner 2003). Copper/zinc SOD is a metalloenzyme that protects cells from superoxide radicals (Wu et al. 1999). In our study, about 95 DEGs representing oxidoreductase activity were recorded. Superoxide produced during stress conditions are quenched by superoxide dismutase (SOD). In our data, SOD was upregulated by 2.5 Log₂ fold and copper/zinc SOD was also upregulated upon chilling stress. Catalase (CAT) splits hydrogen peroxide into water and oxygen. CAT3 was upregulated by

4.7 Log₂ fold, and CAT1 and CAT2 were significantly upregulated under low temperature condition. Glutathione peroxidase is a selenium-containing enzyme, which catalyzes the reduction of H₂O₂ and lipid hydroperoxide to water, using reduced glutathione as substrate (Gupta and Sharma 2006). Glutathione peroxidase, glutathione reductase and glutathione transferase were found to be upregulated upon low temperature stress in our data. Also, several antioxidant enzymes, such as ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), GDP-D-mannose 3′5′-epimerase (GME), and uricase were also upregulated significantly upon chilling stress. Therefore, both enzymatic and non-enzymatic pathways were active during cold acclimation.

Aquaporins

Aquaporins are proteins that transport water and small molecules through biological membranes identified in many plant species (Maurel et al. 2015). Several studies have revealed that aquaporins are upregulated under environmental cues, like drought, salinity, cold and reports on overexpressing aquaporins have resulted in tolerance to low temperature stress (Peng et al. 2008; Hu et al. 2012). In our transcriptome analysis, we identified two aquaporins—aquaporin TIP4-4 and aquaporin SIP2.1 were 1.5 Log₂ fold and 1.2 Log₂ fold expressed, respectively, upon LT exposure.

Cold responsive genes

Several genes such as cold regulated (COR), chilling tolerance divergence 1 (COLD1), dehydrins, late embryogenic abundant protein (LEA), C-repeat element (CRT), DRE, temperature induced lipo protein and responsive to abscisic acid (ABF) were significantly upregulated upon LT

in the present study. A list of cold responsive genes with SUCEST ID and NCBI ID are given in Table 3. COLD1 is involved in sensing cold and triggers downstream responses to chilling stress (Ma et al. 2015). Many of these gene products are structural proteins that are involved in protection upon stress (chaperones, osmoprotectants, ice-binding proteins), while others include regulatory genes, such as TFs and protein kinases. AFPs are highly similar to pathogen-related (PR) proteins, based on sequence and are enhanced upon abiotic stresses. PR proteins, such as thaumatin-like protein, glucanase-like protein and chitinase-like protein have direct effects on the stability of cellular membranes, reducing injury due to chilling in sugarcane (Nogueira et al. 2003). In our transcriptome, several PR proteins were found to be upregulated, viz. β 1, 3-glucanase (7.3 Log₂ fold), chitinase (4.6 Log₂ fold) and thaumatin-like PR5 (3.8 Log₂ fold). Dehydrins are late embryogenesis abundant (LEA) proteins that are produced in plant cells in response to abiotic stresses, like cold, drought and salinity. The COR15 protein was accumulated during cold acclimation in *Arabidopsis* (Thalhammer and Hinch 2014). Nogueira et al. (2003) suggested that sugarcane has cold-inducible genes, which are CBF regulon members. In this study, CBF6 was found to be upregulated in *S. spontaneum* upon 24 h chilling stress. WCOR413 is directly involved in chilling tolerance (Nogueira et al. 2003). Cold responsive genes, such as COR413, CSP 1 (cold shock protein) and CBF6, were found to be upregulated both in transcriptome data, as well as in qRT-PCR, signifying their role in protection during LT stress (Dharshini et al. 2016). A CSD (cold shock domain) gene was expressed only during stress in our analysis. Highly conserved amino acids within the CSDs in

Table 3 List of cold responsive genes with SUCEST ID and NCBI ID

Cold responsive genes	Transcript ID	SUCEST ID	NCBI ID
Chilling tolerance divergence 1 (COLD1)	c52148_g1_i1	SCPIRT3023D12.g	XP_015633398.1
Late embryogenic abundant protein (LEA)	c49241_g1_i1	SCRFS3145F08.g	NP_171654.1
LEA5	c43998_g1_i3	SCVPRT2082F07.g	XP_015624701.1
C-repeat element (CRT)	c47945_g1_i2	SCUTSB1076A04.g	NP_200445.1
DRE	c67793_g1_i1	SCACAM1072C03.g	NP_191814.1
Responsive to abscisic acid (ABF)	c40199_g1_i2	SCCCCL5001A10.g	NP_849777.1
Temperature-induced lipocalin TIL1	c52142_g1_i2	SCAGLV1040D05.g	NP_200615.1
β-1,3-glucanase	c50245_g1_i3	SCEQSD1075A09.g	NP_193361.4
Chitinase	c36174_g1_i1	SCRFS2023E02.g	XP_015614506.1
Thaumatin-like PR5	c31407_g1_i1	SCRLST3163F06.g	NP_568046.1
CBF6	c27680_g1_i2	Not available	Not available
Cold acclimation protein (COR413)	c50615_g2_i1	SCACSB1124E06.g	NP_564328.1
Cold shock protein 1 (CSP)	c73005_g1_i1	Not available	Not available
Cold shock domain (CSD)	c68782_g1_i1	Not available	Not available

plants are capable of binding to nucleic acids and respond to cold adaptation (Karlson and Imai 2003).

Phytohormones under cold stress

In cells, phytohormones like abscisic acid (ABA), auxin (IAA), cytokines (CKs), ethylene (ET), gibberellins (Gas), brassinosteroids (BRs), jasmonates (Jas), salicylic acid (SA) and strigolactones (SL) are diverse groups of signaling molecules that mediate the external stimuli response (Wani et al. 2016). These trigger phosphoprotein cascade pathways leading to expression of genes associated with cold stress tolerance (Kolaksazov et al. 2013). ABA, a stress hormone, is an essential messenger of plants and increases the levels as an adaptive mechanism that responds to abiotic stress (O'Brien and Benkova 2013). In this study, the abscisic stress-ripening protein was upregulated by 3.3 Log₂ fold under cold stress conditions. Auxin is an important hormone, which defends biotic and abiotic stress responses through regulation of many genes (Fahad et al. 2015). Auxin responsive protein (ARP), auxin responsive factor (ARF) and auxin induced protein (AIP) were upregulated as a cold stress-responsive protein in *S. spontaneum*. Endogenous CKs level alteration in stress conditions indicates their responsive act during abiotic stress (O'Brien and Benkova 2013). During cold stress, cytokinin rapidly induces *Arabidopsis* response regulator 6 (ARR) gene (Vogel et al. 2005). In this transcriptome data, RR6 gene was upregulated by 2.5 Log₂ fold and also isopenyltransferase 2 was upregulated by 3.5 Log₂ fold.

Ethylene is a gaseous phytohormone whose level is altered by low temperature and salinity stresses. Higher ethylene concentration shows enhanced tolerance (Shi et al. 2012b). In this case, the ethylene responsive element-binding protein 2 was upregulated by 8.3 Log₂ fold which indicates that ethylene plays an important role in freezing tolerance mechanism in *S. spontaneum*. However, the process of tolerance remains unclear. The Gibberellins show their vital roles in response to abiotic stress (Colebrook et al. 2014). The Gibberellin receptor recorded 2.0 Log₂ fold upregulation in cold transcriptome. As brassinosteroids are involved in chilling tolerance (Wang et al. 2014), genes like brassinosteroid insensitive1-associated receptor kinase 1 and brassinosteroid biosynthesis-like protein were upregulated by 3.6 and 2.2 Log₂ fold change, respectively, in this study.

Kosova et al. (2012) found that the content of JA and SA increased upon cold stress in wheat. Also, the expression of LOX (one of the first enzymes in JA biosynthetic pathway) in kiwi (*Actinidia delicosa*) and *Caragana jubata* was positively regulated during cold stress (Zhang et al. 2006; Bhardwaj et al. 2011). Kolaksazov et al. (2013) suggested that jasmonate may be the principle mediator of cold stress in *Arabidopsis*. Genes like lipoxygenase 1 (LOX1), jasmonate-induced regulator like and jasmonate-zim-domain

protein 1 were upregulated in our study. Gharib and Hegazi (2010) experimented that seeds treated with SA show increased germination under chilling stress. In our data, proteins involved in SA like S-adenosylmethionine synthetase 1 family and UDP-glucuronosyl transferase protein were expressed in high levels, indicating synthesis of SA upon low temperature stress. The present *S. spontaneum* transcriptome data gives the list of upregulated phytohormones under low temperature. Apart from these genes, few of them are tabulated in Table S6.

Secondary metabolites

Secondary metabolites, such as flavonoids, terpenoids, carotenoids, betaines, wax, lignin and tocopherol play an important role in low temperature cold tolerance. Chalcone synthases are upregulated under LT in sugarcane (Nogueira et al. 2003) and it helps in formation of flavonoids (Grace and Logan 2000). Low temperature stress induces the production of several flavonoids, among which anthocyanins are prominent and can inhibit all types of ROS. Recently, it was reported that anthocyanins play an important role in *S. officinarum* leaves by acting as antioxidants upon cold exposure (Zhu et al. 2013). Our analysis revealed the upregulation of three genes involved in anthocyanin biosynthesis. Two of them that belonged to anthocyanin 5, 3-*o*-glucosyltransferase family were 1.5 Log₂ fold upregulated under stress. The leuco-anthocyanidin dioxygenase (LDOX) gene, which is involved in synthesis of pro-anthocyanin, was 3.3 Log₂ fold upregulated. Also, 1-deoxy-D-xylulose 5-phosphate synthase, which is involved in terpenoid metabolism and an uncharacterized protein orthologous to *Sorghum bicolor* involved in flavonoid metabolism were also upregulated by 2 Log₂ fold and 2.8 Log₂ fold, respectively, in *S. spontaneum*. In addition, gamma tocopherol methyl transferase, laccase8 and chalcone synthase were also upregulated. Different types of secondary metabolites expressed under low temperature stress in *S. spontaneum* are given in Fig. 6.

Regulation of cell under LT

Upon low temperature stress, cell undergoes several cellular regulations such as activation of transcription factors, calcium signaling, redox signaling, protein degradation and induction of phytohormones that were found differentially expressed in our study. Similar mechanism of activating downstream cold metabolic pathways by LT sensors, viz. calcium channel and two-component histidine kinases was reviewed by Xiong et al. (2002). Phytohormones, like abscisic acid (ABA) and jasmonic acid also play a crucial role in plant chilling stress responses (Verslues and Zhu 2005). A

Fig. 6 A detailed representation of differentially regulated genes related to secondary metabolite synthesis in *S. spontaneum*. MVA (mevalonate) pathway, Non MVA (non-mevalonate) pathway

rapid induction of phytohormones, such as abscisic acid and jasmonic acid in cold tolerant rice accelerates the stomatal closure to prevent excessive water loss (Yang et al. 2015).

Chaperones

Chaperones or heat shock proteins (HSPs) are produced in cells in response to adverse environments. A range of HSPs (HSP70, HSP90 and small HSPs) have been found to accumulate upon low temperature stress (Timperio et al. 2008). HSPs were found in abundance in rice (Yan 2005), maize (Kollipara et al. 2002), poplar (Renaut et al. 2004), chicory (Degand et al. 2009) under low temperature stress. Of these, HSP70 family is most predominant. These HSPs act as molecular chaperones and thus prevent aggregation of the denatured proteins and facilitate protein refolding (Lee et al. 2009). In our study, HSP70 was 2.8 Log₂ fold upregulated and HSP90 was expressed only during stress, implicating their pivotal roles in protection against cold stress. Furthermore, two genes HSP60 alpha and HSP60 beta were also enhanced upon cold exposure in our analysis.

Ubiquitination

In recent times, ubiquitin conjugation has been identified to be a major regulator of stress-responsive transcription factors and other regulatory proteins. Ubiquitination plays a significant role in regulating transcriptional processes essential for adaption to abiotic stresses (Lyzenga and Stone 2012). In our transcriptome analysis, genes like ubiquitin thioesterase 16, SUMO conjugating enzyme, ubiquitin conjugating enzyme

9 and polyubiquitin 3 were found to be upregulated. There were two uncharacterized genes involved in protein ubiquitination, which were found to be 4 Log₂ fold upregulated in *S. spontaneum*. Regulation of abiotic stress responses by SUMOylation was suggested by the observation that SUMO conjugates increase when plants are exposed to adverse environmental conditions including low temperature (Miura and Hasegawa 2010). Very recently, overexpression of a pepper U-box E3-ubiquitin ligase conferred enhanced cold tolerance in transgenic rice (Min et al. 2016). A total of 15 genes encoding E3-ubiquitin protein ligases were found upregulated in our transcriptome data, signifying the role of E3-ubiquitin ligases upon LT stress. RUB (Related to ubiquitin) are ubiquitin-like modifiers, which involves in regulation of plant stress response (Miura and Hasegawa 2010). RUB1 is upregulated upon chilling stress in *S. spontaneum*. The transcripts ID for the genes described in this manuscript is tabulated in supplementary file S3.

Validation using quantitative real-time PCR (qRT-PCR)

A total of 18 genes were chosen for validation using qRT-PCR which were involved during LT stress tolerance. Overall, a similar trend was observed in both transcriptome data, as well as qRT-PCR. During cold acclimation, upregulation of phospholipase D (PLD) and fatty acid desaturase 8 (FAD8), which serve as membrane fluidity proteins, were observed. Histidine-containing phospho-transfer protein 4 (HPT4), which acts as cold sensor in plants, was enhanced upon LT stress (7.1 Log₂ fold). MAPK and calmodulin,

Fig. 7 Relative expression analysis using qRT-PCR; each bar represents the average of three replicates, error bars indicate SE

which play a major role in signaling of cold stress, were upregulated by 18.1 Log₂ fold and 5.8 Log₂ fold, respectively. The expression of thioredoxin was enhanced by 5.7 Log₂ fold. Transcription factors, such as ARF, FAR, C3H, NAC28, WRKY9 and BHLH, were quantitatively validated and their respective Log₂ fold changes were 8.7, 1.7, 9.7, 10.8, 2.3 and 1.2, respectively. Osmolytes, like trehalose-6-phosphate synthase (2.6 Log₂ fold) and IP3 (1.5 Log₂ fold) were upregulated. E3 ubiquitin protein ligase, which is involved in ubiquitination, was upregulated by 4.0 Log₂ fold. Relative expression profile of the genes in qRT-PCR with that of the transcriptome data is shown in Fig. 7. Transcript transcriptome ID and its category of genes used for qRT-PCR is given in supplementary table S7.

Conclusion and future perspectives

Our cold transcriptome analysis revealed a preliminary model for the tolerance of *Saccharum spontaneum* to cold stress. Based on the potential genes expressed in our cold transcriptome, we hypothesize a pathway by which cold acclimation may occur in the LT tolerant *S. spontaneum* and is pictorially represented in supplementary fig S1. In nature, several morphological, physiological and biochemical changes occur in *Saccharum* sp., due to low temperature stress. In *S. spontaneum*, exposure to low temperature stress leads to activation of cold sensors, such as Ca²⁺, two-histidine kinase, receptor kinase, which activate signal transduction and trigger the maintenance of membrane fluidity by producing unsaturated fatty acids, through FAD8 and rearrangement of cytoskeleton structure by PLD. PLD opens the calcium channel and increases cytosolic Ca⁺ levels upon LT. PP2C induces IP3 to bind to endoplasmic reticulum and calcium release from endoplasmic reticulum is triggered by IP3. Calcium ions bind with calmodulin,

calcineurin that induce the MAPK cascade. Also, protein kinase C, calcium-dependent protein kinases (CDPK), receptor-like kinases and lectin class of receptor kinases activate the MAPK cascade. Homeostasis is restored by efflux of sodium ions and influx of potassium ions across cell membrane, through K⁺/Na⁺ channels. MATEs, H⁺ATPases and a vacuolar proton pyro-phosphatase are involved in hydrogen-translocating pyro-phosphatase activity and thus cytosolic Na⁺ ions are transported to the vacuole, thereby restoring the K⁺/Na⁺ levels in cytosol. Upregulation of genes, like LHCB-3 and RIBISCO activate in chloroplast, suggests that these genes play a crucial role in photosynthesis under cold tolerance mechanism. ABA-dependent and -independent pathways regulate the expression of cold responsive transcriptional factors, like CBF, AP2, C3H, bHLH and NAC. As a result, accumulation of CSPs, COR413, osmoprotectants, aquaporins, antioxidants, antifreeze proteins, secondary metabolites and chaperones altogether may confer cold tolerance in *S. spontaneum*. This study provides insights into the cold tolerance to low temperature stress in *S. spontaneum*, thus opening applications in the genetic improvement of cold stress tolerance in sugarcane.

Data availability statement All relevant data are within the paper and its supporting information files.

Acknowledgements The authors thank ICAR-Sugarcane Breeding Institute, Coimbatore for providing the necessary infrastructure. We would like to thank Dr. G. Hemaprabha, Head, Division of Crop Improvement and Dr. N. Subramonian, Emeritus Scientist, ICAR-SBI for their critical comments on the content. Thanks to Mr. K. Selvamuthu for his technical assistance to carry out the work.

Author contributions DS and AC designed and performed the experiments. DS, CM, ANJ, MVM, NM CM, RK and MM wrote the

manuscript. VD did the MapMan analysis of the data. GN did the artwork for figures. AC and BR revised the manuscript. All authors read and approved the final manuscript.

Funding The authors thank the Science and Engineering Research Board (SERB), Department of Science and Technology for financial support (Grant No.SB/YS/LS-165/2013). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Compliance with ethical standards

Conflict of interest The authors have declared that no competing interests exist.

References

- Agarwal M, Hao Y, Kapoor A, Dong CH, Fujii H, Zheng X, Zhu JK (2006) A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance. *J Biol Chem* 281:37636–37645
- Al-Whaibi MH (2011) Plant heat-shock proteins: a mini review. *J King Saud Univ Sci* 23(2):139–150
- Arnholdt-Schmitt B, Costa JH, de Melo DF (2006) AOX—a functional marker for efficient cell reprogramming under stress? *Trends Plant Sci* 11:281–287
- Azevedo RA, Carvalho RF, Cia MC, Gratao PL (2011) Sugarcane under Pressure: an overview of biochemical and physiological studies of abiotic Stress. *Trop Plant Biol* 4:42–51
- Baek KH, Skinner DZ (2003) Alteration of antioxidant enzyme gene expression during cold acclimation of near-isogenic wheat lines. *Plant Sci* 165:1221–1227
- Bhardwaj PK, Kaur J, Sobti RC, Ahuja PS, Kumar S (2011) Lipoxigenase in *Caragana jubata* responds to low temperature, abscisic acid, methyl jasmonate and salicylic acid. *Gene* 483:49–53
- Blumwald E, Aharon GS, Apse MP (2000) Sodium transport in plant cells. *Biochim Biophys Acta* 1465:140–151
- Chen Y, Wang XM, Zhou L, He Y, Wang D, Qi YH, Jiang DA (2015) Rubisco activase is also a multiple responder to abiotic stresses in rice. *PLoS One* 10(10):e0140934
- Chinnusamy V, Zhu J, Zhu JK (2006) Gene regulation during cold acclimation in plants. *Physiol Plant* 126:52–61
- Chinnusamy V, Zhu J, Zhu JK (2007) Cold stress regulation of gene expression in plants. *Trends Plant Sci* 12:444–451
- Cho SK, Kim JE, Park JA, Eom TJ, Kim WT (2006) Constitutive expression of abiotic stress-inducible hot pepper CaXTH3, which encodes a xyloglucan endotransglucosylase/hydrolase homolog, improves drought and salt tolerance in transgenic *Arabidopsis* plants. *FEBS Lett* 580:3136–3144
- Colebrook EH, Thomas SG, Phillips AL, Hedden P (2014) The role of gibberellin signaling in plant responses to abiotic stress. *J Exp Biol* 217:67–75
- Collins GG, Nie X, Saltveit ME (1993) Heat shock increases chilling tolerance of mung bean hypocotyl tissue. *Physiol Plant* 89:117–124
- Conde A, Chaves MM, Geros H (2011) Membrane transport, sensing and signaling in plant adaptation to environmental stress. *Plant Cell Physiol* 52:1583–1602
- Degand H, Faber AM, Dauchot N, Mingeot D, Watillon B, Cutsem PV, Morsomme P (2009) Proteomic analysis of chicory root identifies proteins typically involved in cold acclimation. *Proteomics* 9:2903–2907
- Dharshini S, Chakravarthi M, Ashwin Narayan J, Manoj VM, Naveenarani M, Kumar R, Meena M, Ram B, Appunu C (2016) De novo sequencing and transcriptome analysis of a low temperature tolerant *Saccharum spontaneum* clone IND 00-1037. *J Biotechnol* 231:280–294
- Dhingra M (2015) Physiological responses and tolerance mechanisms of low temperature stress in plants. *Int J Adv Res* 3:637–646
- Drew MC (1997) Oxygen deficiency and root metabolism: injury and acclimation under hypoxia and anoxia. *Annu Rev Plant Physiol Plant Mol Biol* 48:223–250
- Du YC, Nose A, Wasano K (1999) Effects of chilling temperature on photosynthetic rates, photosynthetic enzyme activities and metabolite levels in leaves of three sugarcane species. *Plant Cell Environ* 22:317–324
- Fahad S, Hussain S, Bano A, Saud S, Hassan S, Shan D, Khan FA, Khan F, Chen Y, Wu C, Tabassum MA, Chun MX, Afzal M, Jan A, Jan MT, Huang J (2015) Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. *Environ Sci Pollut Res* 22:4907–4921
- Ferreira SS, Hotta CT, de Carli Poelking VG, Leite DCC, Buckeridge MS, Loureiro ME, Barbosa MHP, Carneiro MS, Souza GM (2016) Co-expression network analysis reveals transcription factors associated to cell wall biosynthesis in sugarcane. *Plant Mol Biol* 9:15–35
- Fowler S, Thomashow MF (2002) Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. *Plant Cell* 14:1675–1690
- Fryer MJ, Oxborough K, Martin B, Ort DR, Baker NR (1995) Factors associated with depression of photosynthetic quantum efficiency in maize at low growth temperature. *Plant Physiol* 108:761–767
- Gharib FA, Hegazi AZ (2010) Salicylic acid ameliorates germination, seedling growth, phytohormone and enzymes activity in bean (*Phaseolus vulgaris* L.) under cold stress. *J Am Sci* 6:675–683
- Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 48:909–930
- Grace SC, Logan BA (2000) Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. *Philos Trans R Soc Lond B Biol Sci* 355:1499–1510
- Gubis J, Vankova R, Cervena V, Dragunova M, Hudcovicova M, Lichtnerova H, Dokupil T, Jurekova Z (2007) Transformed tobacco plants with increased tolerance to drought. *S Afr J Bot* 73:505–511
- Gupta V, Sharma S (2006) Plants as natural antioxidants. *Indian J Nat Prod Resour* 5:326–334
- Hong SW, Jon JH, Kwak JM, Nam HG (1997) Identification of a receptor-like protein kinase gene rapidly induced by abscisic acid, dehydration, high salt and cold treatments in *Arabidopsis thaliana*. *Plant Physiol* 113:1203–1212
- Hu H, You J, Fang Y, Zhu X, Qi Z, Xiong L (2008) Characterization of transcription factor gene SNAC2 conferring cold and salt tolerance in rice. *Plant Mol Biol* 67:169–181
- Hu W, Yuan Q, Wang Y, Cai Deng X, Wang J, Zhou S, Chen M, Chen L, Huang C, Ma Z, Yang G, He G (2012) Overexpression of a wheat aquaporin gene, *TaAQP8*, enhances salt stress tolerance in transgenic tobacco. *Plant Cell Physiol* 53:2127–2141
- Huang X, Wang W, Zhang Q, Liu J (2013) A basic helix-loop-helix transcription factor, *PtbHLH*, of *Poncirus trifoliata* confers cold tolerance and modulates peroxidase-mediated scavenging of hydrogen peroxide. *Plant Physiol* 162:1178–1194
- Huang X, Chen MH, Yang LT, Li YR, Wu JM (2015) Effects of exogenous abscisic acid on cell membrane and endogenous hormone contents in leaves of sugarcane seedlings under cold stress. *Sugar Tech* 17:59–64

- Karlson D, Imai R (2003) Conservation of the cold shock domain protein family in plants. *Plant Physiol* 131:12–15
- Kolaksazov M, Laporte F, Ananieva K, Dobrev P, Herzog M, Ananiev ED (2013) Effect of chilling and freezing stresses on jasmonate content in *Arabidopsis thaliana*. *Bulg J Agric Sci* 19:15–17
- Kollipara KP, Saab IN, Wych RD, Lauer MJ, Singletary GW (2002) Expression profiling of reciprocal maize hybrids divergent for cold germination and desiccation tolerance. *J Plant Physiol* 129:974–992
- Kosova K, Prasil IT, Vitamvas P, Dobrev P, Motyka V, Flokova K, Novak O, Tureckova V, Rolcik J, Pesek B, Travnickova A, Gaudinova A, Galiba G, Janda T, Vlasakova E, Prasilova P, Vankova R (2012) Complex phytohormone responses during the cold acclimation of two wheat cultivars differing in cold tolerance, winter Samanta and spring Sandra. *J Plant Physiol* 169:567–576
- Kovtun Y, Chiu WL, Tena G, Sheen J (2000) Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proc Natl Acad Sci USA* 97:2940–2945
- Lee DG, Ahsan N, Lee SH, Lee JJ, Bahk JD, Kang KY, Lee BH (2009) Chilling stress-induced proteomic changes in rice roots. *J Plant Physiol* 166:1–11
- Lehtimäki N, Lintala M, Allahverdiyeva Y, Aro EM, Mulo P (2010) Drought stress-induced upregulation of components involved in ferredoxin-dependent cyclic electron transfer. *J Plant Physiol* 167(12):1018–1022
- Li SB, Xie ZZ, Hu CG, Zhang JZ (2016) A Review of auxin response factors (ARFs) in plants. *Front Plant Sci* 7:47. <https://doi.org/10.3389/fpls.2016.00047>
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25:402–408
- Luo HL, Weili X, Jianzhong L (2002) Study on the acceptance of *Acacia mangium* to low temperature stress. *J South China Agricul Univ (China)* 23:51–53
- Lyzenga WJ, Stone SL (2012) Abiotic stress tolerance mediated by protein ubiquitination. *J Exp Bot* 63:599–616
- Ma Y, Dai X, Xu Y, Luo W, Zheng X, Zeng D, Pan Y, Lin X, Liu H, Zhang D, Xiao J, Guo X, Xu S, Niu Y, Jin J, Zhang H, Xu Li L, Wang W, Qian Q, Ge S, Chong K (2015) COLD1 confers chilling tolerance in rice. *Cell* 160:1209–1221
- Machado DFSP, Ribeiro RV, Silveira JAG, da Filho JRM, Machado EC (2013) Rootstocks induce contrasting photosynthetic responses of orange plants to low night temperature without affecting the antioxidant metabolism. *Theor Exp Plant Physiol* 25:26–35
- Mare C, Mazzucotelli E, Crosatti C, Francia E, Stanca AM, Cattivelli L (2004) Hv-WRKY38: a new transcription factor involved in cold- and drought-response in barley. *Plant Mol Biol* 55:399–416
- Masarin F, Gurgilhaes DB, Baffa DC, Barbosa MH, Carvalho W, Ferraz A, Milagres AM (2011) Chemical composition and enzymatic digestibility of sugarcane clones selected for varied lignin content. *Biotechnol Biofuels* 4:55
- Maurel C, Boursiac Y, Luu DT, Santoni V, Shahzad Z, Verdoucq L (2015) Aquaporins in plants. *Physiol Rev* 95:1321–1358
- Maxwell DP, Wang Y, McIntosh L (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. *Proc Natl Acad Sci USA* 96(14):8271–8276
- Min HJ, Jung YJ, Kang BG, Kim WT (2016) CaPUB1, a hot pepper U-box E3 ubiquitin ligase, confers enhanced cold stress tolerance and decreased drought stress tolerance in transgenic rice (*Oryza sativa* L.). *Mol Cells* 39:250–257
- Miura K, Hasegawa PM (2010) Sumoylation and other ubiquitin-like post-translational modifications in plants. *Trends Cell Biol* 20:223–232
- Moore JP, Nguema-Ona EE, Vicre-Gibouin M, Sorensen I, Willats WGT, Driouch A, Farrant JM (2013) Arabinose-rich polymers as an evolutionary strategy to plasticize resurrection plant cell walls against desiccation. *Planta* 237:739–754
- Murata N, Ishizaki-Nishizawa O, Higashi S, Hayashi H, Tasaka Y, Nishida I (1992) Genetically engineered alteration in the chilling sensitivity of plants. *Nature* 356(6371):710
- Nakashima K, Takasaki H, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) NAC transcription factors in plant abiotic stress responses. *Biochim Biophys Acta Gene Regul Mech* 1819:97–103
- Nie GY, Robertson EJ, Fryer MJ, Leech RM, Baker NR (1995) Response of the photosynthetic apparatus in maize leaves grown at low temperature on transfer to normal growth temperature. *Plant Cell Environ* 18:1–12
- Nogueira FT, De Rosa VE, Menossi M, Ulian EC, Arruda P (2003) RNA expression profiles and data mining of sugarcane response to low temperature. *Plant Physiol* 132:1811–1824
- O'Brien JA, Benkova E (2013) Cytokinin cross-talking during biotic and abiotic stress responses. *Front Plant Sci* 4:451. <https://doi.org/10.3389/fpls.2013.00451>
- Osakabe Y, Yamaguchi-Shinozaki K, Shinozaki K, Tran LSP (2013) Sensing the environment: key roles of membrane-localized kinases in plant perception and response to abiotic stress. *J Exp Bot* 64:445–458
- Pang T, Ye CY, Xia X, Yin W (2013) De novo sequencing and transcriptome analysis of the desert shrub, *Ammopiptanthus mongolicus*, during cold acclimation using Illumina/Solexa. *BMC Genom* 14:488
- Park JW, Benatti TR, Marconi T, Yu Q, Solis-Gracia N, Mora V, da Silva JA (2015) Cold responsive gene expression profiling of sugarcane and *Saccharum spontaneum* with functional analysis of a cold inducible saccharum homolog of NOD26-Like intrinsic protein to salt and water stress. *PLoS One* 10:e0125810
- Pasquali G, Biricolti S, Locatelli F, Baldoni E, Mattana M (2008) Osmyb4 expression improves adaptive responses to drought and cold stress in transgenic apples. *Plant Cell Rep* 27:1677–1686
- Pearce RS (1999) Molecular analysis of acclimation to cold. *Plant Growth Regul* 29:47–76
- Peng Y, Arora R, Li G, Wang X, Fessehaie A (2008) Rhododendron catawbiense plasma membrane intrinsic proteins are aquaporins and their overexpression compromises constitutive freezing tolerance and cold acclimation ability of transgenic *Arabidopsis* plants. *Plant, Cell Environ* 31:1275–1289
- Ram B, Sreenivasan TV, Sahi BK, Singh N (2001) Introgression of low temperature tolerance and red rot resistance from *Erianthus* in sugarcane. *Euphytica* 122:145–153
- Reddy VS, Reddy AS (2004) Proteomics of calcium-signaling components in plants. *Phytochemistry* 65:1745–1776
- Renaut J, Lutts S, Hoffmann L, Hausman JF (2004) Responses of poplar to chilling temperatures: proteomic and physiological aspects. *Plant Biol* 7:81–90
- Sah SK, Reddy KR, Li J (2016) Abscissic acid and abiotic stress tolerance in crop plants. *Front Plant Sci* 7:571. <https://doi.org/10.3389/fpls.2016.00571>
- Sales CRG, Ribeiro RV, Machado DFSP, Machado RS, Dóvis VL, Lagôa AMMA (2012) Gas exchange and carbohydrate balance in sugarcane plants under root stressful conditions. *Bragantia* 71:319–327
- Sangwan V, Orvar BL, Beyerly J, Hirt H, Dhindsa RS (2002) Opposite changes in membrane fluidity mimic cold and heat stress activation of distinct plant MAP kinase pathways. *Plant J* 31:629–638
- Sato Y (2001) Heat shock-mediated APX gene expression and protection against chilling injury in rice seedlings. *J Exp Bot* 52:145–151
- Shi HT, Li RJ, Cai W, Liu W, Wang CL, Lu YT (2012a) Increasing nitric oxide content in *Arabidopsis thaliana* by expressing rat

- neuronal nitric oxide synthase resulted in enhanced stress tolerance. *Plant Cell Physiol* 53:344–357
- Shi Y, Tian S, Hou L, Huang X, Zhang X, Guo H, Yang S (2012b) Ethylene signaling negatively regulates freezing tolerance by repressing expression of CBF and Type-A ARR genes in *Arabidopsis*. *Plant Cell* 24:2578–2595
- Shi Y, Ding Y, Yang S (2014) Cold signal transduction and its interplay with phytohormones during cold acclimation. *Plant Cell Physiol* 56(1):7–15
- Solanke AU, Sharma AK (2008) Signal transduction during cold stress in plants. *Physiol Mol Biol Plants* 14:69–79
- Solecka D, Zebrowski J, Kacperska A (2008) Are pectins involved in cold acclimation and de-acclimation of winter oil-seed rape plants? *Ann Bot* 101:521–530
- Solomon S (2016) Sugarcane production and development of sugar industry in India. *Sugar Tech* 18(6):588–602
- Song Y, Liu L, Wei Y, Li G, Yue X, An L (2017) Metabolite profiling of adh1 mutant response to cold stress in *Arabidopsis*. *Front Plant Sci* 7:2072
- Strauss G, Hauser H (1986) Stabilization of lipid bilayer vesicles by sucrose during freezing. *Proc Natl Acad Sci USA* 83:2422–2426
- Sze H, Liang F, Hwang I, Curran AC, Harper JF (2000) Diversity and regulation of plant Ca^{2+} pumps: insights from expression in yeast. *Annu Rev Plant Biol* 51:433–462
- Szekely G, Abraham E, Cseplo A, Rigo G, Zsigmond L, Csiszar J, Ayaydin F, Strizhov N, Jasik J, Schmelzer E, Koncz C (2008) Duplicated P5CS genes of *Arabidopsis* play distinct roles in stress regulation and developmental control of proline biosynthesis. *Plant J* 53:11–28
- Tarkowski LP, Van den Ende W (2015) Cold tolerance triggered by soluble sugars: a multifaceted countermeasure. *Front Plant Sci* 6:203. <https://doi.org/10.3389/fpls.2015.00203>
- Thalhammer A, Hincha DK (2014) A mechanistic model of COR15 protein function in plant freezing tolerance: integration of structural and functional characteristics. *Plant Signal Behav* 9:e977722
- Thomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. *Annu Rev Plant Biol* 50(1):571–599
- Timperio AM, Egidi MG, Zolla L (2008) Proteomics applied on plant abiotic stresses: role of heat shock proteins (HSP). *J Proteom* 71:391–411
- Tiwari M, Sharma D, Singh M, Tripathi RD, Trivedi PK (2014) Expression of *OsMATE1* and *OsMATE2* alters development, stress responses and pathogen susceptibility in *Arabidopsis*. *Sci Rep* 4:3964 (PMCID: PMC3912489)
- Urao T, Miyata S, Yamaguchi-Shinozaki K, Shinozaki K (2000) Possible His to Asp phosphorelay signaling in an *Arabidopsis* two component system. *FEBS Lett* 478:227–232
- Van Buskirk HA, Thomashow MF (2006) *Arabidopsis* transcription factors regulating cold acclimation. *Physiol Plant* 126:72–80
- Vanlerberghe GC, McIntosh L (1997) Alternative oxidase: from gene to function. *Annu Rev Plant Biol* 48(1):703–734
- Verslues PE, Zhu JK (2005) Before and beyond ABA: upstream sensing and internal signals that determine ABA accumulation and response under abiotic stress. *Biochem Soc Trans* 33:375–379
- Virdi AS, Singh S, Singh P (2015) Abiotic stress responses in plants: roles of calmodulin-regulated proteins. *Front Plant Sci* 6:809. <https://doi.org/10.3389/fpls.2015.00809>
- Viswanathan C, Zhu J (2002) Molecular genetic analysis of cold-regulated gene transcription. *Phil Trans R Soc Lond B* 357:877–886
- Vogel JT, Zarka DG, Van Buskirk HA, Fowler SG, Thomashow MF (2005) Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of *Arabidopsis*. *Plant J* 41:195–211
- Wang CY, Adams DO (1982) Chilling-induced ethylene production in cucumbers (*Cucumis sativus* L.). *Plant Physiol* 69:424–427
- Wang B, Guo G, Wang C, Lin Y, Wang X, Zhao M, Guo Y, He M, Zhang Y, Pan L (2010) Survey of the transcriptome of *Aspergillus oryzae* via massively parallel mRNA sequencing. *Nucleic Acids Res* 38(15):5075–5087
- Wang XH, Shu C, Li HY, Hu XQ, Wang YX (2014) Effects of 0.01% brassinolide solution application on yield of rice and its resistance to autumn low-temperature damage. *Acta Agric Jiangxi* 26:36–38
- Wani SH, Kumar V, Shriram V, Sah SK (2016) Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *Crop J* 4:1–15
- Wilkinson S, Davies WJ (2010) Drought, ozone, ABA and ethylene: new insights from cell to plant to community. *Plant, Cell Environ* 33:510–525
- Wright M (1974) The effect of chilling on ethylene production, membrane permeability and water loss of leaves of *Phaseolus vulgaris*. *Planta* 120:63–69
- Wu G, Wilen RW, Robertson AJ, Gusta LV (1999) Isolation, chromosomal localization, and differential expression of mitochondrial manganese superoxide dismutase and chloroplastic copper/zinc superoxide dismutase genes in wheat. *Plant Physiol* 120:513–520
- Xiong L, Schumaker KS, Zhu JK (2002) Cell signaling during cold, drought, and salt stress. *Plant Cell* 14:165–183
- Yan SP (2005) Comparative proteomic analysis provides new insights into chilling stress responses in rice. *Mol Cell Proteom* 5:484–496
- Yan J, He C, Wang J, Mao Z, Holaday SA, Allen RD, Zhang H (2004) Overexpression of the *Arabidopsis* 14-3-3 protein GF14 lambda in cotton leads to a “stay-green” phenotype and improves stress tolerance under moderate drought conditions. *Plant Cell Physiol* 45:1007–1014
- Yang T, Shad Ali G, Yang L, Du L, Reddy SN, Poovaiah BW (2010) Calcium/calmodulin-regulated receptor-like kinase CRLK1 interacts with MEKK1 in plants. *Plant Signal Behav* 5:991–994
- Yang A, Dai X, Zhang WH (2012) A R2R3-type MYB gene, *OsMYB2*, is involved in salt, cold, and dehydration tolerance in rice. *J Exp Bot* 63:2541–2556
- Yang YW, Chen HC, Jen WF, Liu LY, Chang MC (2015) Comparative transcriptome analysis of shoots and roots of TNG67 and TCN1 rice seedlings under cold stress and following subsequent recovery: insights into metabolic pathways, phytohormones, and transcription factors. *PLoS One* 10:0131391
- You J, Chan Z (2015) ROS regulation during abiotic stress responses in crop plants. *Front Plant Sci* 6:1092
- Zhang Z, Huang R (2010) Enhanced tolerance to freezing in tobacco and tomato overexpressing transcription factor TERF2/LeERF2 is modulated by ethylene biosynthesis. *Plant Mol Biol* 73:241–249
- Zhang B, Chen K, Bowen J, Allan A, Espley R, Karunairatnam S, Ferguson I (2006) Differential expression within the LOX gene family in ripening kiwifruit. *J Exp Bot* 57:3825–3836
- Zhang G, Guo G, Hu X, Zhang Y, Li Q, Li R, Zhuang R, Lu Z, He Z, Fang X, Chen L, Tian W, Tao Y, Kristiansen K, Zhang X, Li S, Yang H, Wang J, Wang J (2010) Deep RNA sequencing at single base-pair resolution reveals high complexity of the rice transcriptome. *Genome Res* 20(5):646–654
- Zhang BQ, Huang LT, Li YR (2014) Comparison of physiological and biochemical characteristics related to cold resistance in sugarcane under field conditions. *Sugar Tech* 17:496–505
- Zhao C et al (2017) MAP kinase cascades regulate the cold response by modulating ICE1 protein stability. *Dev Cell* 43:618–629
- Zhu JJ, Li YR, Liao JX (2013) Involvement of anthocyanins in the resistance to chilling-induced oxidative stress in *Saccharum officinarum* L. leaves. *Plant Physiol Biochem* 73:427–433

Affiliations

Dharshini Selvarajan¹ · Chakravarthi Mohan¹ · Vignesh Dhandapani³ · Gauri Nerkar¹ · Ashwin Narayan Jayanarayanan¹ · Manoj Vadakkancherry Mohanan¹ · Naveenarani Murugan¹ · Lovejot Kaur¹ · Mahadevaiah Chennappa¹ · Ravinder Kumar² · Minturam Meena² · Bakshi Ram¹ · Appunu Chinnaswamy¹

¹ Division of Crop Improvement, ICAR-Sugarcane Breeding Institute, Coimbatore, India

² Division of Crop Improvement, ICAR-Sugarcane Breeding Institute Regional Centre, Karnal, India

³ Molecular Genetics and Genomics Laboratory, Department of Horticulture, Chungnam National University, Daejeon 305764, South Korea