Regulation of stress related transcriptional factors in Sugarcane

Plants face two types of stresses, biotic stress and abiotic stress. Salinity, drought, high temperature, oxidative stress and chemical toxicity are included in abiotic stress, while viruses, bacteria fungi and herbivores are included in biotic stress. Both stresses are a threat to agricultural crops. Sugarcane is a perennial crop and belongs to the family, Poaceae. It is used in producing sugar and ethanol. It has been studied that different types of transcriptional factors were found to be differentially expressed in response to stresses in sugarcane. In salt tolerant ecotypes, SuSK, SUT, P5CS, NHX1 and CAT2 were up-regulated under salt stress while in salt sensitive ecotype (K92-80) P5CS was down-regulated. Similarly, under drought stress, SoDip22 was found to be up-regulated. Another study revealed that nine hundred and eighty-seven (987) genes were differentially expressed in response to drought stress in sugarcane. In another study, it was found that eighteen (18) families of miRNA were differentially expressed after applying stress on different days. RPM1, RPP13, RGA2, RGA4 and chitinase (I-VII) were activated due to biotic stress in sugarcane. In cold stress, 20 out of 34 genes were expressed while in another study 600 out of 35340 genes were differentially expressed and 61 out of 74 genes were up-regulated while 13 genes were down-regulated. PScMYBAS1 were up-regulated in response to cold, salinity, wounding, water deficit and hormones (Gibberellic acid, jasmonic acid and salicylic acid). Thus, this information may lead to a rise in the transgenic sugarcane which could exhibit better growth and yield under abiotic and biotic stresses.

Key words: Sugarcane, salinity, drought, SoDip22, Chitinase, MYB.

INTRODUCTION

Plants stresses are categorized biotic stresses and abiotic stresses. Drought, salinity, oxidative stress and high temperature are main abiotic stress that causes the crop loss worldwide. It is estimated that more than 50% crops are lost by the abiotic stresses (Bray et al., 2000). These stresses cause different changes in plants like biochemical, molecular, physiological and morphological changes. It is estimated that 2,050 drought and salinity will reduce more than 50% crops (Wang et al., 2003). These stresses are interlinked to each other and cause similar damage to the plant. For example, salinity and drought first resulted in disturbance to osmotic balance in the plant as a result of it disturbance occurs in ion homeostasis (Serrano and Rodriguez-Navarro, 2001). Salinity, high temperature or drought stress leads to oxidative stress. This oxidative stress stimulates denaturation of proteins and altered the structure or function of the enzymes (Smirnoff, 1998).

Biotic stresses are mainly caused by bacteria, viruses, fungi and herbivores. These bacteria and fungi produce different kinds of proteins and enzymes which are harmful to the cell and cause cellular damage. The herbivores are directly or indirectly harmful to plant (Thordal-Christensen, 2003). Sugarcane belongs to family, Poaceae and is of Andropogoneae tribe. It is a perennial grass which is worldwide cultivated in tropical and sub-tropical regions (Park et al., 2015). Brazil is the highest producer of
sugarcane in the world. In the world, about 70% sugar is obtained from sugarcane. Sugarcane is used in the production of ethanol (Poonsawat et al., 2015).

SALT TOLERANCE RELATED GENE

Under salt stress condition different kinds of genes are up-regulated and others down-regulated. The transcriptional factors like SuSK, SUT, P5CS, NHX1, CAT2 and GAPDH were studied under salt stress (200 mM) and normal condition in sugarcane. These transcriptional factors encode sucrose transporter shaggy-like kinase, pyrroline-5-carboxylate synthetase, Na+/H+ antiporter and catalase. The expression levels of all these genes were up-regulated in salt-tolerant genotype except K92-80 in which the expression of P5CS was low due to ecotype being salt sensitive. In A19 mutant species, SuSk and Nhx1 expression were unchanged, while the expression Sut1 and Cat2 were low under salt stress condition.

GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) was utilized as a house-keeping gene or reference gene. This gene was up-regulated under stress condition (Poonsawat et al., 2015). Similarly, in another study, GAPDH was also used as normalization relative gene expression in sugarcane (Patade et al., 2014). About Cat there are against the report in tobacco plant and the level of Cat gene increases when salt stress (150 mM NaCl for five days) was applied to the plant (Savouré et al., 1999). In another study in sugarcane it was up-regulated in sugarcane when 200 mM NaCl was applied for one day (Patade et al., 2012). In ecotype, K88-92 the expression of Nhx1, SuSK, P5CS, Sut1 and Cat2 were up-regulated. Comparing with another study these transcriptional factors were up-regulated in ecotype Co86-032 of sugarcane (Patade et al., 2011).

DROUGHT TOLERANCE RELATED GENE

Water deficit or drought stimulates the upregulation or downregulation of certain genes or transcriptional factors. The most important example is LEA protein family. SoDip22 transcriptome was studied under drought stress condition in sugarcane. It was studied at the transcriptional level as well as the proteomic level. The length of transcript was 0.9 kb which was present under stress condition and protein molecular weight was 15 kDa. There was no difference between the stress and normal condition in the transcriptome. The protein was expressed and amino acids sequence of this protein was found to be similar to ABA stress protein. To conform to this gene, osmotic potential was determined and then water potential of leaf examined, which increase from -0.3 to -0.9 MPa, respectively. This showed that under water deficit condition this gene was regulated. When the leaves were treated with ABA, the expression of this gene increased.

Similarly, the leaves were treated with gibberellic acid, jasmonic acid, sucrose and proline. These leaves were not shown to not increase the transcriptional level of SoDip22. This transcript was found in bundle sheath cell and the nature of protein of this transcriptional factor is hydrophilic. SoDip22 is closely related to Bsi (Sugiharto et al., 2002). Similarly, another study was carried out on maize in which Bsi transcript was present in bundle sheath (Furumoto et al., 2000).

Another study was carried out about the drought to sugarcane in which 987 transcript factors were differentially expressed when drought stress was applied for 24, 72 and 120 h. Fifty-nine (59) transcripts were antisense, while other transcript factors were sense. Among these transcripts, some transcripts (RNA Metabolism Signal, Transduction and Carbohydrate Metabolism) were conformed to the help of qPCR. Some of the antisense transcript factors were expressed with sense transcriptional factors. Drought stress differentially regulated the only 24 transcripts (that were both antisense and sense). 22 transcripts had a similar pattern of expression under drought stress condition, this means that when antisense are regulated or expressed then sense was also regulated and vice versa (Lembke et al., 2012). This was also studied by Henz et al. (2007) in Arabidopsis.

The two cultivars of sugarcane (RB867515, which was drought tolerance and RB855536 which was less tolerance) were grown for three months in the greenhouse and drought tolerance was given 2, 4, 6 and 8 days. The early symptoms such as leaf curling and senescence appeared on the second day of stress. On the sixth day, all plants were severely affected by drought. By applying small RNAs sequencing method 18miRNA families (miR156, miR160, miR164, miR166, miR167, miR169, miR171, miR172, miR319, miR390, miR393, miR394, miR396, miR397, miR399, miR528, miR529 and miR1432) were identified. The names of these miRNAs were given by resembling the homology of sorghum miRNAs. In these 18 families, seven families had differential expression under drought stress condition, while the other six were differentially expressed when subjected to a two-day stress. The other five miRNA were differentially expressed when subjected to a four-day drought stress oncondit. After two days of stress, sspmir164 miRNA was differentially expressed. In RB867515 cultivar three miRNAs: ss-pmir164, ss-pmir397 and ss-pmir528 were up-regulated and in these miRNAs were not down-regulated under drought stress condition. sspmir164, sspmimr394, sspmimr399-seq 1 and ss-pmir1432 were down-regulated when subjected to drought stress in RB855536 cultivar while ss-pmir397 was up-regulated. The ss-pmir397 was induced after two days of water stress and has the same pattern of expression in both cultivars. After four days of drought stress, ss-pmir393 was differentially expressed.

In HT (high tolerance) cultivar, the four miRNA (sspmir394, ss-pmir397, ss-pmir399-seq 1 and ss-pmir528)
were down-regulated. In the LT (low tolerance) cultivar ssp-miR399-seq 1 and ssp-miR528 were down-regulated, while ssp-miR393 was upregulated. In both cultivars, the ssp-miR399-seq 1 and ssp-miR528 were down-regulated (Ferreira et al., 2012). In Arabidopsis, miR164 regulate the five protein NAM/ATAF/CUC (NAC). These transcriptional factors have an important role in the growth and stress like cool, drought and pathogen attack (Kikuchi et al., 2000; Collinge and Boller, 2001; Hegedus et al., 2003; Ooka et al., 2003; Ditt et al., 2011).

In another study, NAC gene family was over-expressed in the tobacco plant when subjected to salt (Liu et al., 2011). In Arabidopsis, Medicago truncatula, rice and Pinguicula vulgaris miR393 were up-regulated commonly during water deficit (Sunkar, 2010). In both cultivars of sugarcane, ssp-miR394 was down-regulated in drought stress indicating that this miRNA is important for plant independently of its phenotype. The target of this gene is to encode the glyceraldehyde-3-phosphate dehydrogenase (GAPDH). In the sixth step of glycolysis, GAPDH catalyzes the oxidation of D-glyceraldehyde-3-P (D-G3P) to 3-phosphoglycerate (3-PGA) with the production of NADPH. Under drought stress condition, the need of ATP and NADH2 is an increase, therefore, it is necessary.

In another study on rice, OsGAPDH transcripts level was increased under water deficit condition. From the previous report, it was found that ssp-miR397 encode laccases. It was reported that laccase is involved in cell wall modification. Laccase transcript was increased in maize under salt stress condition. The ssp-miR397 was down-regulated under drought stress in four days. The reason for this may be that cell has enough quantity of lignin. It was reported that miR399 is a negative regulator of inorganic phosphatase concentration. Its target is pyrophosphatase. Arabidopsis vacuolar pyrophosphatase gene (AVPP) were over-expressed under drought stress in transgenic Arabidopsis. The ssp-miR528 targets a gene encoding a UBX domain-containing protein.

Arabidopsis genome encodes 15 UBX containing proteins. The ssp-miR528 was down-regulated in drought stress at the fourth day suggesting that miRNA is involved in the reduction of growth under this stress. A ssp-miR1432 was determined by the gene encoding a BZIP transcription factor. Several bZIP has been known to play an important role in stress tolerance adaptation by regulation of gene expression in plant. In both cultivars of sugarcane, ssp-miR1432 was down-regulated under drought stress indicating that BZIP activate the gene that tolerates the drought stress (Ferreira et al., 2012).

**COLD TOLERANCE RELATED GENE**

Under cold stress the yield of sugarcane becomes low. The effect of cold was on bud. When the cold comes it decreases the germination of ratoon and regrowth of plants (Yang et al., 2016). The sugarcane ecotype, CP72-1210 was grown in normal condition for eight weeks and then cold stress applied for twenty hours at 34.7°F. After that transcriptional (35, 340 genes) analysis was done. 600 genes were differentially expressed than the other genes when cold stress was applied. Further investigations showed that at transcriptome level 74 genes out of 311 in sugarcane were differentially expressed. In these genes, 13 genes were down-regulated when cold stress was applied. Similarly, 61 genes (74) were up-regulated in response to chilling temperature (Park et al., 2015). Similar to this ecotype, another study was carried out, in which 51% genes were differentially expressed in response to chilling stress (Dugas et al., 2011).

When sugarcane was grown under normal and cold stress for 3 h to one day, 1, 536 expressed sequences were analyzed. In these, 34 EST were reviewed and 20 out of 34 were expressed under cold stress. Except these 25 EST were identified which were down-regulated when cold stress was applied. In this study, it was found that polyubiquitin protein was encoded by EST and this protein positively responds to cold or chilling stress (Nogueira et al., 2003).

**PATHOGEN RELATED GENE**

In sugarcane, different transcripts factor which were related to leaf abscission as well as a pathogen were studied. In these transcripts, 1, 202 were up-regulated in sugarcane varieties (leaf abscission) as compared to other varieties in which leaf was closely packed. Functional analysis revealed that 6238 and 10 transcripts factors were up-regulated. These transcripts were related to pathogen stress response and ABA-related pathways respectively. In these transcripts which were up-regulated, 4 transcripts encode disease resistance proteins like RPM1, RPP13, RGA2, RGA4 and 6 transcripts encode abscisic acid transporter G family members, other 16 transcripts encode WRK33 and heat stress. Thus, these transcripts may be used to make transgenic sugarcane plant (Li et al., 2016).

In barley plant, programmed cell death occurred due to powdery mildew fungi and the plant was a wild type (Peterhansel et al., 1997). In Arabidopsis plant, RPM1 interacts with RIN4 and this interaction is important in plant defense against plant pathogen. When the tomato is infected by yellow leaf curl viruses then the RPP13 was up-regulated in tomato tolerance ecotype (Mackey et al., 2002). WRK33 transcriptome acted against the bacteria pathogen (*P. syringae*) (Zheng et al., 2006).

It was reported that in sugarcane that transcripts of chitinase were also up-regulated in response to pathogen attack. Ten genes were analyzed using sensitive and non-sensitive ecotype of sugarcane during the *Sporisorium scitamineum* attack. Seven genes (ScChI1, ScChI2, ScChI3, ScChI1I1, ScChI1I2, ScChI1V1 and ScChI1V1) were up-regulated and maintained the amount of transcript in the
incompatible genes, but in the compatible this amount of transcript was low. Three genes (ScChil1, ScChV1 and ScChVIII) showed no difference between the compatible and incompatible genes (Su et al., 2015). Chitinase gene family (I-VII) was found in rice and Arabidopsis. In these plants, these transcriptional produced protein which showed antifungal activity (Xu et al., 2007).

**SALINITY, DROUGHT, COLD, WOUND AND HORMONES RELATED GENES**

Salinity (200 mM NaCl) and drought (Polyethylene glycol) stresses were separately applied for short time to sugarcane and thereafter, transcriptome analysis was done; SuSK was identified. Under drought stress condition, its expression level increased 1.5 fold as compared to the control plant in 120 min. While in salt stress it increased 1.5 fold as compared to the control plant. Thus, it was indicated that this transcriptome responded to drought and salt stress condition (Patade et al., 2011). When MYB transcript factors were analyzed, 1033bp PscMYBAS1 regions were isolated and characterized in sugarcane. The deletion was done in PscMYBAS1 at the transcriptional start site (-56, -152, -303, -442, -777, -613, -843 and -1,033) and these regions were fused with GUS (reporter gene). These were then transformed into Agrobacterium and the Agrobacterium allowed to infect the tobacco leaves. These tobacco plants were already facing different types of stress like water deficit, salinity, chilling stress and wounding. Giberellic acid, jasmionic acid and salicylic acid impact on the plant was shown by spraying them.

It was indicated that promoter region which was about 303bp was essential for basal expression. The other promoter region like 777bp or longer than it showed 2 to 4 fold response to all the aforementioned abiotic factor and hormones. The -777 and -613 regions of transcriptional start site upstream was essential for the response to salinity and drought stress. These results were matched with Arabidopsis transcriptional factor RD29 (A, B) and MYB promoters. Similarly -777 and -843 responded to cold stress. These were up-regulated to 2.03 and 2.80 fold when chilling stress was applied. The tobacco leaves were treated with methyl jasmionic acid for the identification of phytohormone related with cis-acting transcriptional elements. Jasmonic acid engendered the GUS countenance in -303, -442, -613, -777 and -1033 promoters and salicylic acid induced the -777 and -1033 promoters, while there was no response when the leaves were treated with giberellic acid (Wang et al., 2011; Prabu and Prasad, 2012).

Jasmonic acid and methyl jasmonate (ester of jasmionic acid) were used for the communication interlay in the plant against pathogen or wound. Methyl jasmonate responded to gene expression against pathogen and wound (Hu et al., 2009). The -442, -303, -613, -1033, -843 and -777 were induced by methyl jasmonate and wounding. This region contained w-box consisting of -303 to -256 transcriptional regions. 3.05 fold GUS expression was induced by wounds. W-box was connected to WRKY transcriptional factors and involved in the trans-activation of hormones related or pathogen-responsive genes (Zahur et al., 2009; Prabu and Prasad, 2012). The -843 and -613 transcriptional regions have Box E and TCA elements (Reinbothe et al., 1994). Basic helix–loop–helix (bHLH) proteins recognized the E sequence which was present in -843 to -613 regions. This is involved in plant-pathogen interaction (Jung et al., 2006).

**REFERENCES**


family genes in Oryza sativa and Arabidopsis thaliana. DNA Res. 10:239-247.


Cite this article as:

Submit your manuscript at
http://www.academiapublishing.org/ajsr