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Recent developments in sugarcane transcriptome

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Abstract

Hybrid sugarcane is one of the major industrially important cash crops. Cultivated in the tropical and subtropical regions, it is a C4, tall-stalked plant of the Poaceae family which provides 80% world sugar and bioethanol. The genome of hybrid represents polyploidy originating from two *Saccharum* species *Saccharum officinarum* L. and *Saccharum spontaneum* L. The complexity of the polyploid genome remains a challenge for researchers to analyze the whole genome sequence of sugarcane. The recent, more sophisticated DNA sequencing technologies have made studying the genomes of the closest species possible. Once the whole genome of sugarcane is available, it becomes easier to understand the hybrid. transcriptome sequencing by High Throughput Illumina sequencing technology has a great role in studying an organism's total transcriptome at different developmental stages, in different tissues, and under environmental stimuli. Large-scale expression profiling techniques of hybrid *Saccharum* involving generating sequence tags or hybridizing RNA samples with nucleotide probes have been used. In this review, we mainly focused on the recent developments in the transcriptome analysis of sugarcane.

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Introduction

Sugarcane (*Saccharum* spp.) is one of the important commercial crops harvested mainly in the tropical and subtropical areas. The hybrid *Saccharum* is a tall perennial true grass with sweet stalk rich in sucrose content. It belongs to the family Poaceae (family with seed plants like maize, sorghum, rice, wheat and many other important grasses), genus *Saccharum* and tribe Andropogoneae. The modern complex *Saccharum* is derived by the interbreeding of *Saccharum* species. Sugarcane is the major source of sugar. All the modern sugarcane crop species are interbreed (Vilela *et al.,* 2017). The sugarcane production in the year 2018/19 estimated at 21 million tonnes and around 19.5 million next year. Brazil is the largest producer with yield at about 28.6 million tonnes of sugar in year 2018/19 (Anonymous, 2019).

According to Food and Agriculture Organization in 2018 reported that the crops are cultivated on about 64 million acres (26million hectares) in more than 100 countries. Sugarcane accounts for 79% of world sugar. It was cultivated almost in all tropical and subtropical parts of the globe (sugar beets grown in cold regions). The products of sugarcane other than sugar are molasses, rum, falernum, cachaça (Brazil traditional spirit), ethanol, and bagasse. People use sugarcane reeds to make mats, pens, thatch and screens. In some regions like south, southeast Asia, Fiji and some island communities in Indonesia the young unexpended inflorescence of *Saccharum edule* (tebu telor or duruka) is eaten raw, toasted or steamed and prepared and eaten in some way (Dahlia *et al.,* 2010).

The sugarcane crop is the incent crop of the Papuan and Austronesian people. This crop was introduced to Madagascar, island Melanesia and Polynesia by Austronesian sailors. In southern china and India the crops was introduced by Austronesian traders round about 1000 to 1200 BC (Daniels and Menzies, 1996).

The Greeks followed the Persian encountered the famous “reeds that produce honey without bees” in India round about in 4th or 6th centuries BC. They spread and adopted the sugarcane agriculture (Food and Agriculture Organization, United Nations. 2009). Dealers began to start trading of sugar from India, which is known as an expensive and luxurious spice. The sugarcane crop was introduced to South America, Caribbean, Indian Ocean and Pacific Ocean in 18th century AD. The need of laborers becomes a major driver of large human migration, both the voluntary in indentured servants (The National Archives, Government of the United Kingdom, 2010) and the involuntary migrations, in the form of slave labor (Sidney, 1986).

Fig. 1. The representative figure showing the different parts of the sugarcane plant (adopted from Perez, 1997)

The sugarcane plant form lateral shoots at the base yield to multiple stems, typically the stems is 3 to 4 m (10 to 13 feet.) (Fig. 1) high and the diameter is 5cm (2 in). The sugarcane stalk grown from stem, the mature stalk of *Saccharum* hybrid mostly consists of 75% of the entire plants. The sugarcane crop is mainly composed of 63 to 73% of water, 12 to 16% soluble sugar, 11 to 16% fiber and 2 to 3% non-sugars. The sugarcane plant is mostly sensitive to stresses (biotic and abiotic stresses), the response to stress varies among the cultivars, and also it depends on time period of harvesting, fertilizer and climate. The yield may vary between 30 and 180 tons/hectare,

depending on management and crop cultivation techniques. It is also used as a fodder for livestock (Perez, 1997).

According to botanical description, six species of genus *Saccharum* namely *S. officinarum*, *S. spontaneum*, *S. edule*, *S. barberi*, *S. robustum* and *S. sinense* have been reported worldwide (D'Hont *et al.,* 1998) (Table 1). The modern *Saccharum* hybrid cultivars are derived from introgression among *S. spontaneum*, *Miscanthus sinensis* and *Erianthus arundinaceus* (Daniels and Roach, 1987), although some data supports it originating from *S. robustum* (Amalraj and Balasundaram, 2006). However, Irvine (1999) has proposed that grouping six *Saccharum* species should be reduced to only two major species as *S. officinarum* and *S. spontaneum* on the basis of inter-fertility grouping of species and insufficient discriminative traits to nominate separate species. Moreover, it has been discussed in few reports that *Erianthus* is synonym of *Saccharum* and therefore *Erianthus* spp. should be incorporated into *Saccharum* genus (Burner and Webster, 1994). It is thought that the word *Saccharum* derived from Sanskrit Sharkara (Daniels and Roach, 1987).

Table 1. A summary of species belonging to genus *Saccharum* (Daniels and Roach, 1987; Buzacott, 1965)

Species	Classification	Sugar content	Chromosome number
S. spontaneum	Wild species	Nil	2n= 40-128
S. robustum	Wild species	Nil	2n= 60-~200
S. officinarum	Noble canes	High	2n= 80
S. barberi	Ancient hybrid	Low	2n= 111-120
S. sinense	Ancient hybrid	Low	2n= 80-124
S. edule	Wild species	Compacted inflorescence eaten as a vegetable	2n= 60-80 with aneuploid forms

The chromosome numbers (2n) of *S. officinarum* are more than 80 with a basic chromosome nomenclature of X=10, which is responsible for polyploidy (more than 2 sets) of this species. Furthermore, *S. officinarum* L. is a hybrid of different species and therefore it has not simple polyploidy but an autopolyploid (set of chromosomes from a single species is more than 2 set) and also an allopolyploid (unlike 2 or more sets of chromosomes) (Sreenivasan *et al.,* 1987). It is predicted that the chromosomes of *S. officinarum* are homologous to the chromosomes of the genera *Miscanthus* and *Erianthus* (Daniels and Roach 1987; Besse *et al.*, 1997).

Other distinct species of genus *Saccharum* is *S. spontaneum* and it is described as shorter, polymorphic in texture, smaller, resistant to disease, high vigorous and high fibrous species and it indicates a complex polypliod species with basic 2n chromosome numbers of 40 to 128 and X=8 to 10 (Sreenivasan *et al.,* 1987; D'Hont *et al.,* 1996). It can be differentiated from the cultivated sugarcane by thinner cane and a narrow panicle (Pursglove, 1972). The traits such as spikelets at end of the tertiary branches of inflorescence are the useful key for taxonomists to identify and distinguish various species of *Saccharum* spp. The wild species of *Saccharum* are *S. barberi* and *S. sinense* and it is thought these have been cultivated since prehistoric times in Northern India and China. The considerable interbreeding of these species with other genera and species are reported and these are thought to be ancient intergenic hybrids (Daniels and Roach, 1987). Further, *S. barberi* is originated as a result of *S. officinarum* × *Erianthus* (sect. *Ripidium*) introgression, while *S. sinense* is supposed to be derived from *S. officinarum* × *Miscanthus* introgression. It is further explored that each species containing chromosomes homologous to *S. officinarum* and *S. spontaneum* as well as to those from members the genera *Erianthus* and *Miscanthus* and it is again indicating the complex origins and inter-relationships within the *Saccharum* genus (Daniels and Roach, 1987; D'Hont *et al.,* 1996).

Another wild species, *S. robustum* is supposed to be an intermediary in the evolutionary pathway between *S. spontaneum* and *S. officinarum*. Two major groups with the species are known, those that have 2n=60 and 2n=80 chromosomes respectively. The sixth species of genus *Saccharum* which has similar morphological traits to *S. robustum* flower spike is *S. edule* and its cultivation has been recorded as a vegetable in the island of the Pacific and Papua New Guinea. It is believed that. *S. edule* is derived from introgression of *S. officinarum* or *S. robustum* with other genera (Daniels and Roach, 1987). A summary of the characteristics of species belonging to genus *Saccharum* is shown in Table 1.

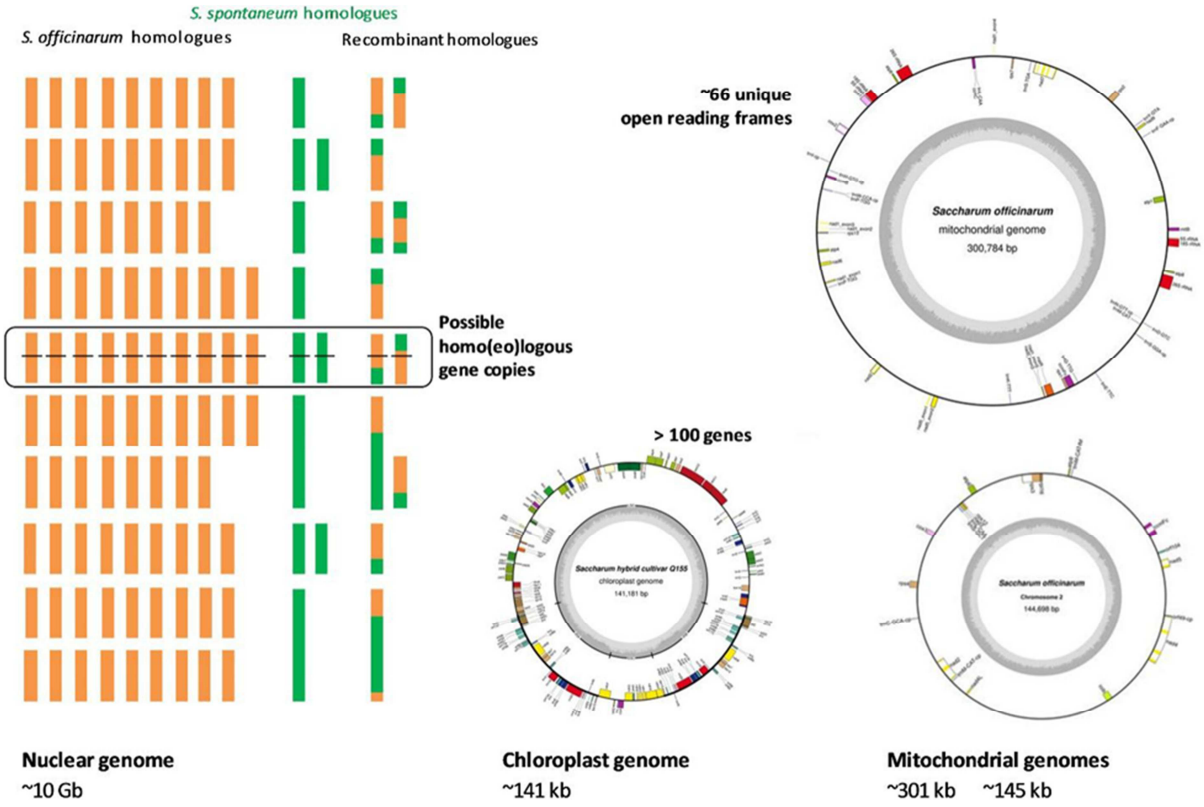

Fig. 2. The representative scheme of nuclear sub genome and genomic contribution of each *Saccharum* species along with the description of genomes of chloroplast and the mitochondrion. Adapted from D'Hont *et al.* (1996), (1998); Piperidis *et al.* (2010); Hoang *et al.* (2015b); *Aitken et al.* (2016); Shearman *et al.* (2016); Garsmeur *et al.* (2017).

Nuclear genome of modern sugarcane cultivar is composed of two sub genome originated from two species, a female *S. officinarum* L. and a wild male *S. Spontaneum* L. (Daniels and Roach, 1987; D'Hont and Glaszmann, 2001). *S. officinarum* L. has monoploid (2n=80) chromosome with ~1 GB octoploid genome size and total genome size is ~7.88GB (D'Hont *et al.*, 1996, 1998; Zhang *et al.*, 2012) (Fig. 2). While the *S. spontaneum* L. is composed of monoploid 2n=40-128 with genome size 750 to 843 Mb and total genome size is ranged between 3.36 to 12.64 Gb (Panje and Babu, 1960; Daniels and Roach, 1987; Sreenivasan *et al.*, 1987; da Silva *et al.*, 1995; D'Hont *et al.*, 1998; Ha *et al.*, 1999; Zhang *et al.*, 2012) (Fig. 2). These species have 5 to 16 homologous copies of each gene in their genome because the ploidy level of the species. Moreover it is estimated that the total number of chromosomes are from 100 to 130 (Simmonds, 1976; Sreenivasan *et al.,* 1987) with 8 to 14 homologous copies of genes at a given locus in the genome (Heinz, 1987; Grivet and Arruda, 2002; Rossi *et al.,* 2003; Aitken *et al.,* 2004, 2016; Souza *et al.,* 2011). The variation number of chromosomes genotypes to genotypes or cross to cross has been reported in different, due the random organization of chromosomes in each cross genome,

like in cultivar Q117, there are 110 and 107 for cultivar Q200 chromosomes number are reported, 115 and 124 are reported for Co128 and Co453 respectively (Heinz, 1987; Piperidis *et al.,* 2010; Aitken *et al.,* 2016). The sugarcane genome actual size is about 10Gb, if we consider haploid genome the size is range ~1Gb (D'Hont and Glaszmann, 2001; Le Cunff *et al.,* 2008). The detail of sugarcane genome and sub genome are given below in Fig. 2.

Sugarcane transcriptomics

The large scale array based studies have been performed in sugarcane cultivars in the last decade (Manners and Casu, 2011; Rehman *et al.,* 2021; Rehman *et al.,* 2023). The microarray investigation of leaf and stalk sugarcane have been performed for understanding differentially expressed genes necessary to high sucrose and revealed high sucrose metabolism transcript in tissue which accumulates high concentration of sucrose (Carson *et al.,* 2002). Similar technique is being extensively used during studying novel cold tolerance genes and their signal pathways in sugarcane (Nogueira *et al.,* 2003). The sugarcane transcriptome profiling s based on signal transduction related genes using microarray have investigated 3500 genes and these genes are further reported as encoding 600 transcription factors for signal transduction, 477 receptors, 114 calcium and inositol metabolism proteins, 510 protein kinases, 107 protein phosphatases, 17 G proteins and 75 small GTPases (Papini-Terzi *et al.,* 2005).

Expression pattern study of sugarcane stem has provided the evidence on association between gene and maturation and also provided information on differentially expression of genes involved in cellulose synthase (Casu *et al.,* 2007). The microarray analysis of cDNA of sugarcane leaves exposed to elevation of CO_2, have shown 22 genes up regulated and 14 down regulated, which are mostly linked to development and photosynthesis and at last have shown linkages to enhance 29% sucrose content (De Souza *et al.*, 2008). Another microarray study has been reported the 70 differentially expressed transcripts, when ethanol is applied to sugarcane leaf and this gene expression pattern is known for gene regulation in abiotic stress (Camargo *et al.*, 2007). Therefore, the use of arrays for gene expression profiling to identify the gene specific to a tissue like stem have been reported (Damaj *et al.,* 2010).

Sugarcane cultivars producing both high and low sucrose, microarray transcriptomics study have been conducted to investigate the signal transduction pathway involved during sucrose synthesis and this study revealed 24 genes involved in differential expression among and them 19 genes are involved in low sucrose producing plants, 3 genes are involved in reducing sucrose phosphate synthase (de Maria Felix *et al.,* 2009). Further transcriptome analysis of the sugarcane plant exposed to polyethylene glycol stress for 2 to 4 hours have been carried out and the results have revealed that the up regulation of sucrose transporter 1, proline dehydrogenase, catalase-2 and sodium proton antiporter, while results during salt stress have revealed the different down regulated response to different stress (Patade *et al.*, 2012). It is further reported that during stress condition the plant accumulate osmoprotectants and 56 group of genes and these osmoprotectants small organic molecule help organism during extreme osmatic stress) and genes were up regulated (Dos Santos *et al.,* 2011).

Transcriptome sequencing and its application in sugarcane

Transcripts or RNA sequencing technique could be used to investigate the transcriptome profile in any organism. Illumina Seq technology for RNA-sequencing has commercial importance in development of sequence library in population and also has great application in microarray analysis. Total transcriptome studies of the organism explain the different stages of development and response to stress and treatments (Schnable *et al.*, 2004; Brady *et al.*, 2006; Galbraith, 2006: Rehman *et al.,* 2021).

For *Saccharum* hybrid large scale expression profiling was used involving generation of sequence tags or hybridization of RNA samples with nucleotide probe. The sugarcane multiple EST database was

developed by different research groups around the world, and there are more than 300.000 ESTs in these databases.

Transcriptome analysis in sugarcane in response to biotic stress

Sugarcane mutant obtained by the cultivar B4362 susceptible to chemical mutagenesis used by Oloriz *et al.* (2012) showed post-haustorial hypersensitivity response (HR) and mediated resistance to pathogen and was used to identify differentially expressed genes in response to *P. melanocephala* with suppression subtractive hybridization (SSH).The up regulation of genes involved in glycolysis and C4 carbon fixation were found in both interactions, although nascent polypeptide complex genes, post translational proteome modulation and autophagy were translated at higher levels in the compatible reaction. In the incompatible reaction genes coding for a putative no apical meristem, nonspecific lipid transfer protein, s-adenosyl methionine decarboxylase and GDP-1- galactose phosphorylase involved in ascorbic acid biosynthesis were up regulated. High-throughput tag-sequencing based on Solexa technology was used by Wu *et al.* (2013) to analyze sugarcane plants infected with *Sporisorium scitaminea* L. and the results revealed the 2015 differentially expressed genes, among these genes 1125 up regulated genes and 890 down regulated genes and these findings were obtained after mapping to sugarcane EST databases in NCBI.

Fungus *Sporisorium scitamineum* causes smut diseases in sugarcane which led to huge loss. A transcriptome analysis was performed by Que *et al.* (2014) to study the response of sugarcane plant response to fungus *S. scitamineum*. Sugarcane genotypes (ROC22 and YC05-179) were exposed to *S. scitamineum* at 24, 48 and 120 houres. The RNA seqqunce was performed using illumina and confiremed through qrtPCR. The sequence data generate 65,852 unigenes, mostly the two genotypes showed common biological pathways to smut infection. Although, the resistant genes expression in genotype YC05-179 was found earlier at (24-48 hours) than those of genotype ROC22 at (48 – 120 hours), suggesting resistance specificity and early timing of these genes in non-affinity sugarcane and *S. scitamineum* interactions. The obtained unigenes were linked to molecular function, cellular components and biological processes. The functional annotation of unigenes associated with signal transduction, ion transport, metabolism, energy conversion and production and defense mechanism. Further, enrichment analysis evolved the differential expression of genes associated with hormone signal transduction of plant, plant and pathogen intersection, cell wall modification, and other metabolic pathways linked with resistance.

The libraries from undifferentiated and young plants had lower redundancy levels than libraries constructed from mature plant tissue. A study was conducted by Su *et al.* (2015) to find out the rule of a chitinase genes family of sugarcane, 10 genes were found deferentially expressed during RNA Seq analysis of both compatible and incompatible sugarcane cultivars during infection of *S. scitamineum*. Moreover, 7 chitinases genes showed more response and maintain an increased transcript during an incompatible interaction than those of compatible interaction ones. The results of sugarcane chitinases family genes showed show differential response during biotic and abiotic stresses.

A de novo transcriptome assembly (TR7) was produced by Santa Brigida *et al.* (2016) from sugarcane plant infected with *Acidovorax avenae* subsp. *avenae* pathogen and treated with drought to develop RNA-seq libraries (247 million of raw reads resulting in 168,767 reference transcripts). Further, the mapping of de novo transcriptome reads obtained from infected libraries revealed 798 differentially expressed transcripts, of which 723 were annotated and corresponded to 467 genes. Differential analysis revealed up regulated genes involved in the biosynthetic pathways of ET and JA PRRs, oxidative burst genes, NBS-LRR genes, cell wall fortification genes, SAR induced genes, and pathogenesis-related genes (PR).

Bedre *et al.* (2019) performed isoform level transcriptome analysis of sugarcane infected with smut fungus (*Sporisorium scitamineum*) using sorghum bicolor reference genome and trinity de novo mapping tools. The results showed 16,039 and 15,379 transcripts (≥2 FPKM) after 5 and 200 day of infection. Further, a conservative estimate of isoforms expression level showed that approximately 5000 sugarcane genes (41%) undergo AS. The differential expression analysis of alternative spliced genes in sugarcane (healthy and infected) showed 896 AS events at various stages of infection. The functional enrichment and gene ontology analysis of differentially spliced genes showed the functional categories related to defense, cell wall and redox homeostasis pathways.

The transcriptome analysis of sugarcane cultivars susceptible to sugarcane mosaic disease was studied by Meng *et al.* (2017). The RNA sequences were analyzed by high throughput paired-end RNA sequencing technology and total 63,025 unigenes were generated, among these 14,384 unigenes were measured more than 1Kb each. Further, 38,505 unigenes were annotated using COG, KEGG, KOG, GO, Pfam, Swiss-Prot and Nr. Total 4,982 differentially expressed genes (DEGs) were screened, including 3,841 annotated DEGs, among these 3,791 DEGs were Upregulated, and 50 DEGs were downregulated. Differentially expressed genes in the three KEGG pathways, unbiquitin proteolytic system, proteasome and translational pathways in endoplasmic reticulum (ER) was investigated in response to stress and unfolded protein response (UPR). Interestingly, the DEGs in the 3 pathways were up-regulated. In the data analysis, they investigated eight differentially expressed genes in response to ER stress and UPR and also found Upregulation of these genes in sugarcane in response to Sugarcane Steak Mosaic Virus (SCSMV). Some of the results are shown in the figure.3 and this figure is included in the article with the Journal permission.

Plant mosaic virus causes a huge disease in plants, among these sorghum mosaic virus affects the can production and sugar content. Ling *et al.* (2018) performed a deep sequencing of sugarcane cultivar ROC22 leaves infected with SrMV and a leaves with no infection. The transcriptome analysis were performed and confirmed by qrtPCR, to study the interaction and mechanism of SrMV and sugarcane. The data generate 89,338 unigenes among these 481 unigenes were differentially expressed and 51 genes of potyvirus host interactor (PHI) homologus sequences were obtained. The analysis showed that, increase of SrMV replication from ER (Endoplasmic reticulum) to chloroplast led to damage of chloroplast, resulting the expression of genes associated with Ca2+, cytokinin, auxin, ethylene signaling, ROS and also the transcription of some defense related genes. Further, among the 51 PHIs genes the upregulation of gene linked with ethylene inducible transcription factor and genes related to calmodulin releated protein, in two SrMV resistant and two SrMV susceptible sugarcane cultivars under SrMV infection sowed that these two genes could be used as a target genes for creating resistant germplasm to SrMV. Moreover, 70 genes encoding heat shock protein, reticulon homology domain protein, chloroplastic rieske Fe/S protein and salicylic acid binding protein-3 might be used as markers for identifying resistance or susceptible cultivars in sugarcane. This transcriptomic study was a useful tool for identifying resistance or susceptible cultivars of sugarcane.

Huang *et al.* (2018) performed a transcriptome study of sugarcane in response to *S. scitamineum*, suppression subtractive hybridization (SSH) with reverse northern blotting was conducted on sugarcane cultivar ROC22. The data analysis revealed a total of 155 differentially expressed genes after alignment of reads from SSH libraries with expressed sequence tag (EST).

Out of 155 DEG 26 unigenes were confirmed by qrtPCR in both resistant cultivar (YC05-179) and susceptible cultivars (ROC22). These genes encoded serine/threonine kinase (Q2), fiber protein (Q3), translation initiation factor of eukaryotic 5A (Q23), Sc14-3-3-like protein (Q24)

and two unknown protein (Q1 and Q11) and were induced in YC05-179 (smut Resistant cultivar) while inhibited in susceptible cultivar (ROC22). From the data analysis Ling *et al.* (2018) found that, Ca^{2+} sensor, serine/threonine kinases, genes linked with nitrogen activated protein and some NBS-LRR genes might be involved in the signal transduction and recognition of smut fungus infection in sugarcane. Further, genes related to abscisic acid, ethylene, auxin, and salicylic acid in the plant hormone signaling pathways were more apparently in response to smut fungus infection.

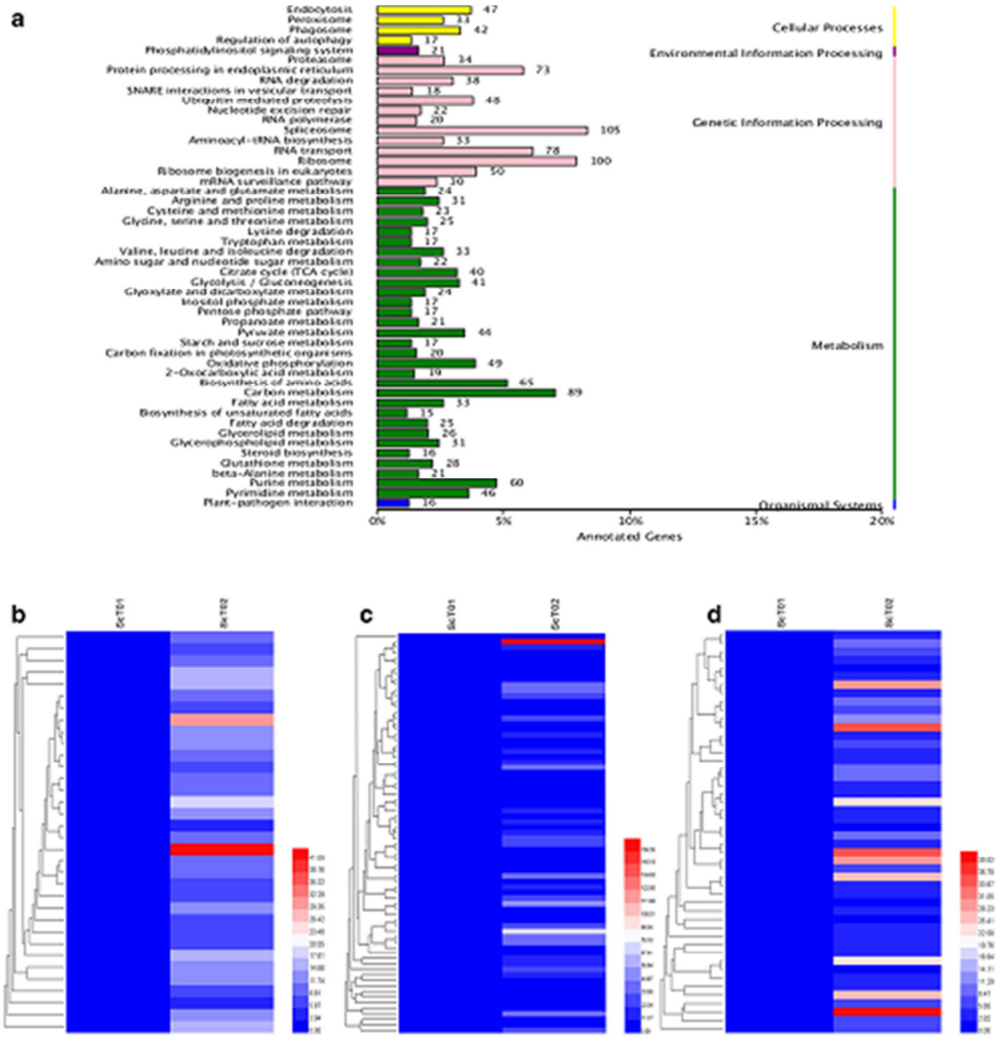

Fig. 3. The KEGG pathway analysis of differentially expressed genes (DEGs) in sugarcane: A classification of DEGs based on the annotation in KEGG pathway. b-d Heat map showing log2 FPKM values of DEGs in the three pathways of proteasome, protein processing in endoplasmic reticulum, ubiquitin mediated proteolysis using HemI (Heatmap Illustrator, version 1.0) (Meng *et al.*, 2017)

In sugarcane twisted leaf disease caused by *Phoma* sp, which is one of the largest genera of fungi, a transcriptome study was performed by Wei *et al.* (2019) to study the response of modern sugarcane to this fungal infection and also detect the mechanism of twisted leaf disease which is caused by *Narenga porphyrocoma*. Two sugarcane cultivars H3-8 natural infected select as susceptible and the unsusceptible cultivar (H3-19) was used as control. Two biological repeats and three stages were adjusted and 65,780,017,250bp data were obtained, the reads were mapped with reference genome *Saccharum spontaneum* L (Zhang *et al.*, 2018). The annotation revolved that there are 91,386 genes were differentially expressed among these 65,391 known and 25,995 novel genes expressed. While in 96,101

annotated transcripts, 53,481 were novel alternative splicing subtypes encode known protein, 27, 151 novel protein coding genes and 15, 469 long non coding RNAs. The deferentially expressed genes including hormone pathways and R genes between the two cultivars, the comparison between susceptible cultivar and the control identified that 16, 242, 12,892 and 34,924 DEGs in pre-onset, early stage and serious symptom stage respectively (Fig. 3).

Transcriptome analysis in sugarcane in response to abiotic stresses

Four super SAGE libraries were constructed by Kido *et al.* (2012) to develop differentially expressed gene panel responsive in different stresses, by using bulked root tissue from 4 drought resistance and 4 sensitive sugarcane genotypes. The BlastN matches mostly included 567,420 tags, 75,404 uni tags with 164,860 different ESTs. The transcriptome analysis of *Saccharum hybrid* CP72_1210 (cold susceptible) and *S. spontaneum* TUS05_05 (cold tolerant) were investigated by Park *et al.* (2015) using Sugarcane Assembled sequences (SAS) from SUCEST_FUN database. Their studies reported total 35,340 and 34,698 SAS expressed genes before chilling and after chilling stress, respectively. In each genotype, nearly 600 differentially expressed genes were further observed. Moreover, the Blast2GO annotation showed the major difference between CP72_1210 and TUS05_05 gene expression profile after chilling stress and also explored association of genes with the transmembrane transporter activity.

A customized microarray experiment were conducted by Zeng *et al.* (2015) to analyses the level of sugarcane genes transcripts change after 8, 24, and 72hours when exposed to low potassium conditions. They identified total of 4153 differentially expressed genes for at least one of the each stress time points. Gene response to low K stress at 72 hours was reported twofold more than the number of genes at 8 and 24 hours. Analysis of gene ontology (GO) showed the involvement of many genes in developmental, metabolic and biological regulatory process display changes in the transcript level in response to low-k stress. Further, the differential expression of transcription factors, kinases, transporters, gene in Ca+, ethylene signaling pathways and oxidative stress related genes were detected. Ferreira *et al.* (2016) studied the comparative expression profiling of sugarcane ancestral genotypes; *S. spontaneum, S. officinarum, S. robustum* and commercial hybrid cultivar RB867515 to explore the involvement of genes at expression level for sugarcane development. The oligoarray experiments were conducted and 12,621 sense and 995 antisense transcripts were detected in immature and intermediate internodes of leaves. Further, the study revealed the expression analysis of all tissue samples revolved 831, 674 and 648 differentially expressed genes in *S. spontaneum*, *S. officinarum* and *S. robustum* respectively using hybrid RB867515 as a reference. Eighteen transcription factors were identified in co expression network analysis and cis element was detected in silico analysis involved in cell wall biosynthesis.

Dharshini *et al.* (2016) studied the transcriptome profiling of low temperature (10oC) tolerant *S. spontaneum* clone IND 00-1037 collected from the area of high altitude Arunachal Pradesh, North Eastern India. The high-throughput Illumina Nextseq500 technology generated a total of 47.63 and 48.18 million reads corresponding to 4.7 and 4.8 GB reads of cold and as well as control samples (10oC for 24 hours). These reads were assembled de novo into 214,611 unigenes with an average length of 801Bp. Final result of the study analysed that during cold stress about 2583 genes were up regulated and 3302 genes were down regulated.

Yang *et al.* (2017) performed high throughput illumina sequencing method for analyzing the rule of miRNAs in sugarcane under low temperature. Initially 4 small RNA libraries were generated through illumina seq. the result generated total of 412 sugarcane miRNAs among these 261 were known while 261 were novel miRNAs. Among these miRNAs 62 were significantly expressed during cold, 34 were significantly upregulated and 28 were downregulated. The miRNAs were also validated by RT-qPCR.

The gene ontology and KEGG pathways revolved that these miRNAs were expressed in many stress related biological pathways.

Transcriptomics analysis of *S. Spontaneum* cultivar GX83-10 leaves under normal watering (SS_CK) and drought stress (SS_T) were performed by Kai-Chao *et al.* (2018) using Illumina high throughput sequencing technology. Data generated from RNA Seq were assembled de novo, and functional annotation, differential expression of genes and enrichment analysis obtained 54,499640 and 56,440692 clean reads from the treated group and as well as control group.

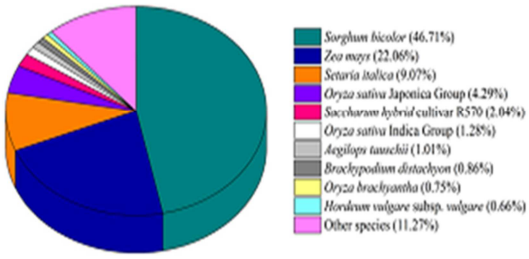

Fig. 4. The species distribution of the top BLAST hit of the assembled unigenes against NR database (E-value ≤ 1.0 × 10−5). Nearly 46.71% of unigenes showed significant homology with that of *Sorghum bicolor* and 22.06% showed significant similarity with that of *Zea mays* (Shiqiang *et al.*, 2018)

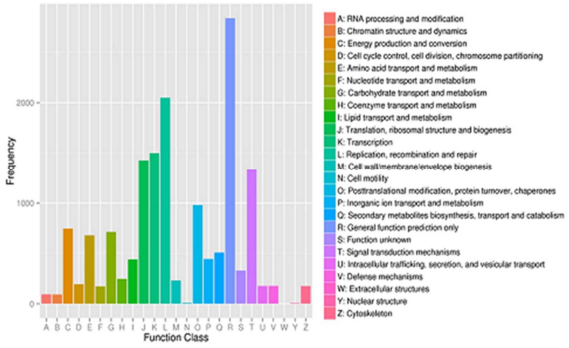

Fig. 5. COG classification of sugarcane assembled unigenes with an E-value threshold of 1.0 × 10−5 against COG databases. Total 10,575 unigenes were grouped into 25 COG classifications, and the cluster of general function prediction represented the largest group, accounting for 18.22% (Shiqiang *et al.*, 2018)

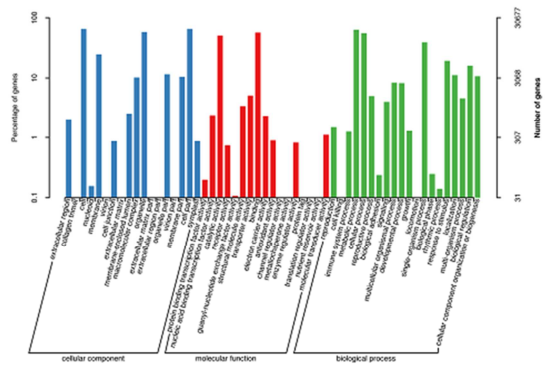

Fig. 6. Gene ontology (GO) classification of the assembled unigenes. A total of 30,677 unigenes with BLASTx matches were assigned to three main categories: biological processes (76,711; 39.94%), cellular components (77,191; 40.19%), and molecular functions (38,159; 19.87%) (Shiqiang *et al.*, 2018)

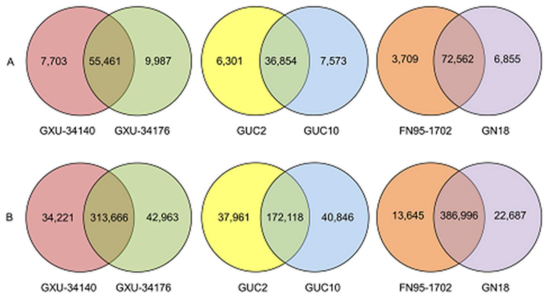

Fig. 7. Venn diagram showing the number of unique and shared unigenes and SNPs among the contrasting sugarcane genotypes. (A) The number of unique and shared unigenes determined based on RSEM analysis. (B) The number of identified unique and shared putative SNPs based on GATK2 analysis. Only SNPs with distance >5 were retained (Shiqiang *et al.*, 2018)

Further GO enrichment analysis revealed 88,941 unigenes and 1325 significantly differentially expressed genes among the functional gene grouped. During KEGG enrichment analysis, 5 metabolic pathways were obtained from plant hormonal signal transduction, starch and sucrose metabolism, phenylpropanoid biosynthesis and ascorbate and aldarate metabolism. For qRT-PCR analysis, 7 significantly Upregulated genes were selected and the result confirmed that all the seven genes were upregulated at varying degree under drought stress. The study provides information about the *S. spontaneum* leaf in response to drought stress, and

would reference for plant researcher and breeder for future breeding of new sugarcane resistance cultivars.

Another transcriptomics study was performed by Shiqiang *et al.* (2018) using six sugarcane cultivars involved in leaf abscission, tolerance to drought stress and pokkah boeng disease. The results from the experiment generated 465 million high quality reads and these reads were de novo assembled into 93,115 unigenes. The similarity search of unigenes against the public databases revealed 43,526 (47.7%) functionally annotated unigenes, which are involved in a wide range of metabolic pathways. Further, the transcriptome comparative analysis of the unigenes explored many upregulated genes in response to ethylene and abscisic. The unigenes associated with response to jasmonic acid and salicylic acid were upregulated in response to pokkah boeng disease in tolerance genotypes, and other unigenes like peroxidase, antioxidant activity and signal transduction were also upregulated in the tolerance genotypes in response to drought stress. Finally, 8,630 simple sequence repeats (SSR) and 44,2152 single nucleotide polymorphisms (SNPs) were recorded and these were used to identify a numbers of putative marker including the data are important resource for future analysis like gene discovery, molecular marker development and sugarcane genomic studies. The summary of the results are shown in Fig. 4, 5, 6, 7 and Table 2.

Table 2. Summary of functional annotation of assembled unigenes (Shiqiang *et al.*, 2018)

Database	Annotated number	Percentage	300–1000 bp	≥1000 bp
NR	42,042	45.15%	14,329	20,611
Swis-Prot	22,660	24.34%	6,727	13,238
GO	30,677	32.95%	9,751	16,179
Pfam	25,853	27.76%	7,251	15,563
KOG	21,108	22.67%	6,427	11,403
COG	10,575	11.36%	2,742	6,517
KEGG	12,367	13.28%	3,872	6,571
All annotated	43,526	46.74%	15,083	20,738
Total unigenes	93,115	—	—	—

Transcriptome analysis of two chines sugarcane cultivars namely, ROC22 and Gt08-1108 treated with low and normal temperature were performed by Tang *et al.* (2018). The 57.41 GB data were generated from the RNA sequence. The total 183,515 unigenes were annotated and among these 110,021 unigenes were functionally annotated using Nr, NT, SWISS-Port, PFAM, KOG/COG, KEGG and GO. In GT08-1108 under cold stress, 16, 145 genes were differentially expressed among these 8,965 were upregulated (55%) and 7,180 genes were differentially down regulated (44%). In ROC22, there are 20,317 genes were upregulated (53%) and 9,419 were down regulated (46%). The gene enrichment analysis showed that the two cultivars shown same mechanism GT08-1180 is more enriched in DNA integration, RNA polymerase, ADP binding and metabolic enzymes, while ROC22 is involved in the synthesis, transport and transporter activity of organic compounds.

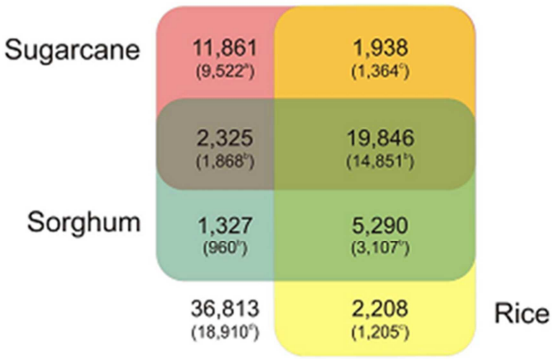

Fig. 8. Sugarcane transcripts homology to sugarcane unigenes, rice and sorghum proteins. The best BLASTX/N hit against nucleotide of protein sequences of the reference organisms is employed for annotation with a cut-off E-value of #1026. In the Figure a, b and c indicates number of different proteins/unigenes in sugarcane, sorghum and rice respectively. The no-hit transcripts are indicated outside of Venn diagram while (d) shows transcripts mapped to the sorghum genome.

Transcriptome analysis of sugarcane for other biological pathways

Cardoso-Silva *et al.* (2014) performed a transcriptome annotation and de Novo assembly of six genotypes using high throughput Illumina sequence technology. They generated more than

400 million short reads which were then assembled into 72,269 unigenes. These unigenes showed significant similarity to 28,788 *Sorghum* protein coding genes. Furthermore, 5272 unigenes not found in Public sugarcane EST databases; this indicated that these are putative sugarcane genes which were not described earlier.

The authors identified a large number of molecular markers like more than 5,000 single sequence repeats (SSR) and around 708,000 single nucleotide polymorphisms (SNPs). The data from their work is useful for future plant breeders and genetic researchers. Their findings are depicted in Fig. 8.

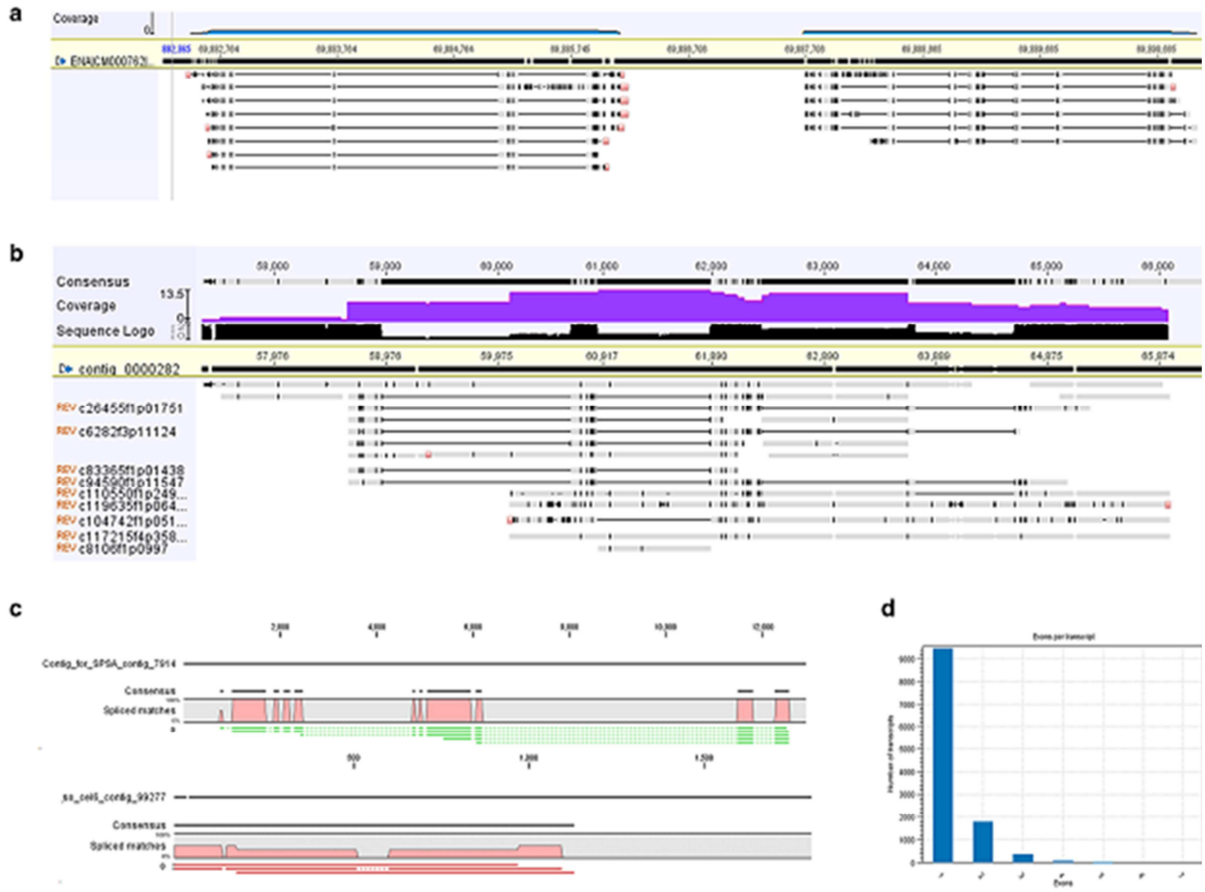

Fig. 9. Transcript isoforms of sugarcane presented in PacBio dataset a, Isoforms aligned against the *Sorghum* chromosome. b, Isoforms aligned to contigs of sugarcane whole genome de novo assembly. c, Different transcript isoforms aligned to sucrose phosphate synthase gene and cellulase 6 gene contigs. d, Average exons per transcript estimated based on the transcript isoforms aligned against sorghum genome (Nam *et al.*, 2017)

Lignin contrasting was performed through a high-throughput transcriptome evaluation of two sugarcane genotypes by Vicentini *et al.* (2015). They have generated a set of 85,000 transcripts of sugarcane by using de novo assembly and RNA-Seq. more than 2000 transcripts were expressed differentially between the studied genotypes which include many genes involved in the biosynthesis of lignin. Their results showed important information about the lignin biosynthesis and its interaction with other metabolic pathways in the genome of *Sacchaum* hybrids. Casu *et al.* (2015) studied the tissue-specific expression pattern in stalk for analyzing the special development pathways responsible for fiber synthesis and sucrose accumulation. They have examined different tissues like vascular bundles, internodes, storage parenchyma and rind dissected from mature stalk for expression profile and identified 10 genes of cellulose synthase subunit and found difference in their expression. They have inferred that there is a

special separation for elevated expression of these significant targets both in cell wall synthesis and cellulose accumulation.

In another study, Sternes and Moyle (2015) investigated the role of small RNA transcriptome complexity in development of sugarcane plant. They have obtained about 50 million small RNA reads from cells suspension, apex, leaf, stem internodes and embryonic calli. They found that complexity of the small RNA components of the transcriptome between tissues is significantly varied.

The comparative expression profiling of sugarcane ancestral genotypes viz *S. spontaneum, S. officinarum, S. robustum* and commercial hybrid cultivar RB867515 was studied by Ferreira *et al.* (2015) for identification of genes responsible for development. Oligoarray experiments were conducted on leaves, immature and intermediate internodes. They detected more than 12,000 sense and 900 antisense transcripts. Expression analysis of all tissue samples revealed 83, 648 and 674 differentially expressed genes in *S. spontaneum, S. robustum* and *S. officinarum* respectively using RB867515 as reference. Furthermore, 18 transcription factors along with *cis-elements* were also identified in co-expression network analysis which was related to cell wall synthesis.

Transcriptome analysis of GT35 a high sucrose cultivar was performed by Huang *et al.* (2016) through Solexa sequencing technology. They identified more than 30 pathways in the sugarcane transcriptome by analysis of 30,000 unigenes using KEGG pathway analysis tools. They also found about 3500 SSRs in more than 3000 unigenes.

Leaf tissue were used in transcriptome dataset from Thai sugarcane cultivar Khon kaen 3 (KK3) was used by Prriyapongsa *et al.* (2018) through Iso-Seq Method. Approximately 120,000 transcript reads were generated with a length of 3,600 bp and N50. These were on average longer than other reported datasets in sugarcane transcritome. About 92% sequences contained an ORF of at least 300 to 1400 bp. There were 4,774 putative novel transcripts which did not matched with other Iso-Seq studies of sugarcane. Furthermore, they also annotated the function of 68,962 putative full length transcripts with at least 90% coverage by comparing with orthologues sequences in other plants.

Similarly, using the Iso-Sequence method PacBio sequence, the transcriptomics study was performed by Nam *et al.* (2017). RNA was extracted from 22 cultivars using leaf, root and internode tissues at different stages of development. They analyzed the full length transcripts isoforms of 107,598 unique transcripts which were 71% of the total sugarcane predicted genes. A major portion (92%) of the transcripts were mapped as plant proteins, 2% were novel transcripts while 2% were long non-coding putative RNAs. 56 and 23%of the total sequences were annotated against gene ontology and KEGG pathways databases. It was also inferred that Iso-Seq method can recovered more full length transcripts as compared to others. A greater diversity of gene and transcripts were captures in RNA sequence. It was also observed that about 69% of PacBio transcripts and 41% de novo contigs mapped with sorghum genome which showed high sharing of orthologs in generic regions of the two genomes (Fig. 9).

Conclusion

Sugarcane (*Saccharum* hybrid) is one of the major important cash crops, cultivated on almost all the regions of the globe. This crop provides 70 to 80% of world sugar and also an important source of biofuels (bioethanol). The crop is complex, have polyploidy genome, and have more than two numbers of copies of each chromosome. So that's why it is difficult to analyze the transcripts and isoforms of sugarcane. However, with the emergence of RNA sequence technology or High throughput Illumina and other sequencing technologies, now it is easy to analyze the transcriptome of sugarcane. Transcriptome sequencing has a wide application in genomic research; provide information about the expression,

function of genes at different stages, tissues and stresses. From this review article and data we concluded that more work is needed in sugarcane transcriptome. Drought, high temperature, rust, smut, water logging, high sucrose contents and cold stress are major issues for sugarcane crop as well as negatively affecting the industrial quality of sugarcane. In this review article we mainly focused on the recent development in transcriptome sequencing of sugarcane. Recently a large number of studies were conducted and performed on the sugarcane transcriptome in response to different biotic and abiotic stresses, biological and biochemical pathways. Study on transcriptome of sugarcane will lead to investigate and identify more resistant cultivars and mechanism of sugarcane in response to these stresses.

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