Chapter 2
Sugarcane Genomics and Transcriptomics

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Abstract Sugarcane is an important commercial crop belonging to Poaceae family and is a major source of sucrose and ethanol production worldwide. Sugarcane's large genome size, aneuploidy of commercial cultivars, and polyploidy of interspecific hybrids have always imposed a challenge for generation of genomic and transcriptomics resources for crop improvement. Despite of these hurdles, linkage maps based on different segregating populations has been constructed. Efforts to map QTLs controlling various traits are being carried out and map-based cloning has also been tried. Available EST data can now be used for SNP mining, expression profiling, discovering new genes, etc. The comparative analysis of sugarcane and sorghum genome revealed high similarity between the two genomes. This information will further expedite sugarcane improvement initiatives. The advent of high-throughput sequencing technologies such as Roche/454 and Illumina/Solexa is being used to gain knowledge on transcriptome of the cell under different stress conditions. RNA-seq can provide the sequences of all RNA molecules, including mRNA, rRNA, tRNA, and noncoding RNAs, produced in one or a population of cells. The data generated can be used to measure transcript levels, to find novel genes, fusion transcript, and splice junctions. Knowledge of the sugarcane transcriptome can provide information about synthesis of various biomolecules and their interactions with other metabolic pathways in the complex sugarcane genome. Both genomic and transcriptome resources of sugarcane are immensely important for improving yield as well as quality of sugarcane; this will help sugarcane farming community to a great extent.

Keywords Genome • Microarray • Polyploid • RNA-seq • *Saccharum* • Transcriptome

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2.1 Introduction

Sugarcane is a complex crop species with each gene represented by a number of alleles in the genome, high polyploidy and aneuploidy. About 70–80% of genome composition of modern sugarcane cultivars has been derived from *S. officinarum* and 10–20% from *S. spontaneum.* It has been reported by Berkman et al. (2014) that proportion of repeated sequences in sugarcane hybrids ranged from 63.74 to 78.37% and that increased proportion may be because of ploidy level of sugarcane genome rather than repetitive content. Since it has been reported that it has synteny with grasses like sorghum and maize, the future of sugarcane improvement seems promising. The sorghum genome is the closest genome to sugarcane which has been fully sequenced and annotated and is widely accepted as reference genome for comparative analysis of sugarcane sequences.The origin of modern sugarcane cultivars raises issues not only related to the extent and nature of the divergence of the sugarcane and sorghum genomes, but also about the relationships (meiosis and expression dosage) among hom(e)ologous loci (De Setta et al. 2014). Association mapping experiments are being carried out in sugarcane to detect marker-trait associations as well as validating the position of different important genes. Sugarcane transcriptomic experiments have led to identification of large number of genes which are involved in controlling important biological functions. Further, various international efforts are concentrating on studying the effect of complex genome on transcript expression in sugarcane. The genes identified through transcriptomic studies can be used either as DNA markers or to develop transgenic sugarcane. Even if sugarcane is a crop of immense importance its genetics has lagged behind and plant scientists has shown little interest in studying it. One of the major reason being the complexity of the sugarcane genome, which exceeds that of any other important crop. This chapter focuses on recent advances in sugarcane genomics and transcriptomics that will further enhance our knowledge of the challenges faced in its improvement.

2.2 Molecular Cytogenetics

Sreenivasan et al. (1987) made initial attempts to elucidate sugarcane genome and its taxonomy by using classical cytogenetic methods. It indicated that basic chromosome set of *Saccharum* sp. could be $x = 5, 6, 8, 10$, or 12 and chromosome number of *S. officinarum* is considered to be $2n = 80$. Bremer (1923, 1961) reported the occurrence of $2n$ gamete transmission in hybrids of *S. officinarum* and *S. spontaneum.* This study further supported the hypothesis that modern sugarcane cultivars are derived from crosses between *S. officinarum* ($2n = 80$) and *S. spontaneum* ($2n = 36–128$). *S. officinarum* clones that had a nuclear DNA content different from those having 80 chromosomes, i.e., outliers were relisted as hybrids (Aitken et al. 2006a). *S. spontaneum* has five major cytotypes: $2n = 64, 80, 96, 112$, or 128 (Panje and Babu 1960) and basic chromosome number of $x = 8$ (hypothesized) (D'Hont et al.

1996, 1998; Ha et al. 1999). The FISH (florescent in situ hybridization) experiments conducted physically mapped the 45S rRNA and the 5S genes on chromosomes of *S. officinarum* and *S. robustum.* Classical cytogeneticists thought that no exchange of chromosomes has occurred between *S. officinarum* and *S. spontaneum* (Price 1963, 1965; Berding and Roach 1987). But, further studies (D'Hont et al. 1996) using FISH of complete genomic DNA of *S. officinarum* and *S. spontaneum* in sugarcane cultivar R570 showed that 10% of the chromosomes appeared to be contributed by *S. spontaneum* and 80% from *S. officinarum* and remaining 10% were recombinants. These results were further confirmed by molecular mapping of cultivar R570 (Grivet et al. 1996; Hoarau et al. 2002). Molecular cytogenetic techniques have also been used to study other members of *Saccharum* complex, especially *Erianthus* and *Miscanthus*. FISH along with DNA markers has been used to identify true hybrids formed with *E. arundinaceus* and to track *E. arundinaceus* genes introgressed into *Saccharum* (D'Hont et al. 1995; Piperidis and D'Hont 2001; Jing et al. 2009). Specific repeated sequences from *Erianthus* and *Miscanthus* were cloned and FISH was used to analyze their chromosome distribution; this analysis revealed two subtelomeric, one centromeric, and one apparently dispersed family along the genome (Alix et al. 1998, 1999). FISH was also used to find out the chromosome composition of fertile *S. officinarum* × *E. arundinaceus* hybrids in F_1, BC_1, and BC_2. Recombinants were not observed in either BC1 or BC2 clones (Piperidis et al. 2010).

2.3 Genetic Diversity Analysis

Present day sugarcane cultivars have been derived from interspecific hybridizations of domesticated species *S. officinarum* known for high sugar and the wild species *S. spontaneum* characterized for resistant to biotic and abiotic stresses. The genome complexity in *Saccharum* spp. has made sugarcane and energy cane breeding cumbersome. Use of only few limited genotypes of *S. spontaneum* and *S. officinarum* clones in earlier breeding experiments has resulted in a narrow genetic base of present sugarcane cultivars. Characterization of genetic variation among different *Saccharum* genotypes has been carried out in both the organelle genome and nuclear genomes (D'Hont et al. 1993; Sobral et al. 1994). Diversity in chloroplast genome was initially studied by Takahashi et al. (2005); it was reported that analysis of genomic sequence of 26 regions in the chloroplast clearly distinguished *S. spontaneum* from the other five species of *Saccharum.* To study genetic variability among the chloroplast genomes of sugarcane (*Saccharum* spp.) and its wild progenitor species *Saccharum spontaneum* L. (Zhu et al. 2014), 19 primer pairs were designed targeting various chloroplast DNA (cpDNA) segments with a total length ranging from 4781 to 4791 bp. Ten out of 19 cp DNA segments were polymorphic, with 14 mutation sites. This demonstrated that the chloroplast genome of *S. spontaneum* was maternally inherited. Also, comparative sequence homology analyses clustered sugarcane cultivars into a distinctive group away from *S. spontaneum* and its progeny.

Large numbers of DNA markers have been used to assess the genetic diversity in the nuclear genome of *Saccharum* species. In recent years, genetic diversity has been investigated for sugarcane cultivars or ancestral species by using several molecular methods, such as restriction fragment length polymorphism (RFLP) (Lu et al. 1994; Besse et al. 1997), random amplified polymorphic DNA (RAPD) (Huckette and Botha 1995; Nair et al. 2002), amplified fragment length polymorphism (AFLP) (Aitken et al. 2005), inter simple sequence repeats (ISSR) (Virupakshi and Naik 2008), sequence-related amplified polymorphism (SRAP) (Li and Quiros 2001; Chang et al. 2012), target region amplification polymorphism (TRAP) (Alwala et al. 2006; Que 2009), genomic in situ hybridization (GISH) (D'Hont 2005; D'Hont et al. 2002), fluorescence in situ hybridization (FISH) (D'Hont 2005; D'Hont et al. 1996; Jenkin et al. 1995), simple sequence repeats (Aitken et al. 2005), and expressed sequence tag-SSR (EST SSR) markers (Cordeiro et al. 2001).

Nayak et al. (2014) evaluated all the 1002 accessions in World Collections of Sugarcane and Related Grasses (WCSRG) germplasm using SSR markers. The population structure analysis and principal coordinate analysis revealed three clusters with all *S. spontaneum* in one cluster, *S. officinarum* and *Saccharum* hybrids in the second cluster, and non-*Saccharum* spp. in the third cluster. A core collection of 300 accessions was selected that represented the majority of diversity in the WCSRG.

2.4 Molecular Genetic Maps

Aneuploidy, double genome structure, and homologous and homoeologous chromosomes of sugarcane have hindered the progress of constructing linkage maps with large coverage. For last 25 years different researchers from various labs have been involved in mapping sugarcane genome. Earlier maps made utilized the single dose (SD) markers generated mostly using RFLP, while the more recent maps used AFLP and SSRs. But the coverage provided is not satisfactory enough.

RFLP markers were used to map AP85-0068 and SES 208 (Silva et al. 1993). Same population was used (Al-Janabi et al. 1993) to map using arbitrary primed PCR. Integration of the data from these studies lead to construction of linkage map with 64 linkage groups (LGs) assembled in eight homologous groups. RAPD and AFLP markers were also used subsequently. Mudge et al. (1996) used LA-Purple and Molokai for linkage map construction using RAPD markers. A total of 160 RAPD markers and a morphological marker were assembled into 51 linkage groups. AFLP markers along with RFLP markers and arbitrary primers were used (Guimaraes et al. 1997, 1999) to generate maps. RFLP markers were further used to map R570; this map contained 96 linkage groups and ten putative HGs (Grivet et al. 1996). The extensive use of RFLP markers were made by Ming et al. (1998), the group generated four different maps using four different parents and the number of linkage groups varied from 69 to 72. Earliest, most extensive maps were constructed by Aitken et al. (2005) and Garcia et al. (2006). AFLP, randomly amplified DNA fingerprints (RAF), and SSR markers were mapped into 136 linkage groups (Aitken

et al. 2005). A linkage map was constructed using RFLP, SSR, and AFLP markers (Garcia et al. 2006); this map comprised 131 linkage groups with larger proportion of markers unlinked. Hybrid cultivar R570 and an old Australian clone MQ76-53 were used for linkage map construction. These maps contained AFLP, SSR, and RFLP markers, which were assembled into 86 linkage groups for R570 and 105 linkage groups for MQ76-53 (Raboin et al. 2006). Segregating F2 population of a hybrid cultivar LCP 85-384 was used for linkage map construction with AFLP, SSR and TRAP markers; in this map nine HGs contained 108 linkage groups (Andru et al. 2011).

In a major stride in sugarcane mapping Aitken et al. (2014) generated a comprehensive sugarcane genetic map of Q165; this map contained 2267 markers generated from Diversity Array Technology (DArT) markers, amplified fragment length polymorphism (AFLP), simple sequence repeats (SSR), single nucleotide polymorphism (SNP), restriction fragment length polymorphism (RFLP), and random amplified polymorphism (RAPD) markers. Usage of large numbers of different markers allowed most of the LGs to be placed into the eight homology groups; this number is consistent with the basic chromosome number of the ancestral species of *Saccharum* and so far the lowest basic chromosome number reported in the *Saccharum* genus. The use of DArT markers will allow the development of consensus genetic maps in sugarcane which would improve genome coverage and allow integration with other genomic resources (Aitken et al. 2014).

2.5 Mapping of QTLs

As discussed in previous section, molecular markers have been used to develop genetic maps; these are being used to trace the position of genes valuable for sugarcane improvement and also to identify quantitative trait loci (QTL) associated with different traits so as to speed up marker-assisted selection efforts. These efforts are slow since multiple QTLs control most of the traits of agronomic importance and individual contribution of these QTLs is very small. Sugarcane QTL mapping is mostly based on single marker analysis or interval mapping.

One of the first studies was done by Sills et al. (1995). Seven different traits namely stalk number, tasseled stalks, smutted stalks, stalk diameter, POL%, fiber content, and plot weight. Of the traits studied, epistatic interaction between two markers associated with stalk diameter was found to be significant. Daugrois et al. (1996) used self-fertilized population of R570, single marker analyses was performed and found a marker linked with brown rust resistant gene (at 10 cM). Extensive work has been done by Ming et al. (2001, 2002a, b, c). The group has used different mapping populations, with RFLP as a marker of choice, and studied different traits like sugar content, sugar yield, fiber content, POL, ash, stalk number, stalk weight, flowering time, and plant height. The analyses were performed using single marker analysis and interval mapping approach. Significant associations between markers and QTLs were identified for these traits. Marker-trait association for stalk number and sucker number identified seven and six RFLP markers, respectively

(Jordan et al. 2004). Aitken et al. (2006b) identified 37 marker–QTL associations for brix and POL. In this study phenotypic variation explained varied from 3 to 9%. In another study Aitken et al. (2008) used AFLP and SSR markers and found that 46% of the marker–QTL associations were consistent across different years of evaluation. This study identified 27 genomic regions significantly associated with traits like cane yield, stalk weight, stalk number, stalk length, and stalk diameter. Pinto et al. (2010) identified putative QTLs as well as their epistatic interactions for fiber content, cane yield, POL, and tonnes of sugar per hectare. A total of 120 associations were found and 50 digenic epistatic marker interactions were identified for the four traits evaluated. Singh et al. (2013) constructed linkage maps to identify QTLs for seedling, brix, sucrose percent, stalk number, stalk length, stalk diameter, internodes, and number of green leaves, at three crop cycles across seven environments in a segregating population with 207 individuals derived from a biparental cross of sugarcane elite cultivars. Thirty-one QTLs were identified, out of these 7 QTLs had stable effect across crop year and locations. Racedo et al. (2016) tried to establish an appropriate genome-wide association analysis (GWAS) tool in a sugarcane breeding population. In this study clones were genotyped with DArT (Diversity Array Technology) and TRAP (Target Region Amplified Polymorphism) markers, and evaluated for cane yield and sugar content. A total of 43 and 38 markers significantly associated with cane yield and sugar content, respectively.

2.6 Comparative Genomics

Sugarcane and sorghum share the same subtribe, i.e., *Saccharinae*, and reported to have diverged from a common ancestor approximately 8 million years ago. The sorghum genome, the closest related fully sequenced and annotated genome to sugarcane, is considered as reference genome for comparative analysis. Ten pairs of chromosomes has been sequenced; this effort has covered 90% genome of sorghum and 99% of protein coding region (Paterson et al. 2009). Sugarcane is highly complex and polyploid. Despite of this fact it shares high degree of synteny with diploid *Sorghum bicolor.* Also, sugarcane and sorghum genomes share extensive microcollinearity with each other, thus strengthening the fact that sorghum genome can be used as reference for assembly of sugarcane genome (Ming et al. 1998; Okura et al. 2012). Sugarcane genome analysis can use the sorghum genomic resources like sorghum gene indices/models (Hoang et al. 2015). BLASTp searches against the NCBI nonredundant database have confirmed that most of the sugarcane protein sequences are most similar to those of sorghum (Setta et al. 2014). Also, using the chromosomal locations of the 935 sorghum–sugarcane orthologous, the group was able to localize 265 sugarcane BACs onto sorghum chromosomal arms. Sequences of four DArT markers (analysis of sugarcane DArT marker sequences associated to important traits) showed high similitude and e-value with coding sequences of *Sorghum bicolor* (Racedo et al. 2016). This study further confirms that *Sorghum bicolor* share high gene microcollinearity between sorghum and sugarcane.

2.7 Sugarcane as an Important Source of Biomass

Tremendous phenotypic variation in *Saccharum* germplasm and advances in sugarcane genomic tools has assisted in characterization of traits important for use of sugarcane as biofuel. The fast growth and high yield of sugarcane makes it a suitable candidate for production of second generation biofuels. For use as biofuel, the genetic potential of sugarcane can be improved by screening the germplasm for biofuel characters, cell wall composition modifications, and utilizing the potential of next generation sequencing technologies (NGS). These approaches will help to pin point the important genes involved in biomass production in sugarcane and hence these genes can be manipulated to enhance sugarcane's potential as biofuel. Sugarcane along with other grasses such as *Miscanthus* species (*Miscanthus giganteus*), *Erianthus* species (*Erianthus arundinaceus* Retz.), and switch grass (*Panicum virgatum*) is an efficient converter of solar energy into chemical energy and biomass accumulation (Tew and Cobill 2008; Furtado et al. 2014). Soluble sugar as well as residues in sugarcane production (bagasse and trash) can be used for biofuel production (Seabra et al. 2010; Alonso Pippo et al. 2011; Macrelli et al. 2012). In sugarcane germplasm, along with other traits, variation can be seen in biomass yield and fiber content within species and within genera. More variations can be seen in wild sugarcane species as compared to the domesticated sugarcane. Moreover, the genetic diversity of *S. officinarum* has been used in sugarcane improvement programs but the diversity of *S. spontaneum* have not been used much (Aitken and McNeil 2010). The cell wall of sugarcane and other grasses are categorized as type II (Souza et al. 2013). This type of cell wall is usually characterized as having little pectin and lesser lignin and structural proteins (Carpita 1996; Henry 2010; Saathoff et al. 2011). Understanding the detailed composition and fine structure of sugarcane cell wall will help in optimizing the tissue pretreatment and cell wall hydrolysis protocol (Hoang et al. 2015). Altering the carbohydrates of the cell walls is the key of improving the biomass composition for biofuel production (Harris and DeBolt 2010). Use of biotechnology can help in producing sugarcane plants genetically modified to have favorable cellulose to non-cellulose content. Different studies have indicated that the efforts for improving sugarcane biomass is impeded by highly complex genome, low transformation efficiency, transgene inactivation, somaclonal variations, and problems during backcrossing (Ingelbrecht et al. 1999; Hotta et al. 2010; Arruda 2012; Dal-Bianco et al. 2012). About 25% of the total lignocellulosic biomass in sugarcane is composed of lignin; this high percentage affects the efficiency of saccharification during conversion to ethanol (Canilha et al. 2012, 2013). Altering sugarcane biomass composition for biofuel production can be done by downregulating some genes involved in lignin pathway. In sugarcane, a minimum of ten enzymes have been reported to be involved in lignin pathway (Higuchi 1981; Whetten and Ron 1995). Jung et al. (2012) reported that when RNA interference (RNAi) suppression was used to downregulate caffeic acid O-methyltransferase (COMT) lignin content and lignin S/G ratio was reduced. Species like *Miscanthus, Erianthus*, *S. officinarum*, and *S. spontaneum* have lot of allelic diversity and can be exploited for improving sugarcane biomass (Hoang et al. 2015).

2.8 Sugarcane Transcriptomics

In the past decade many large scale array-based studies of gene expression have been performed in sugarcane (Manners and Casu 2011). Differentially expressed genes study using microarray during sugarcane leaf and culm development provides information that it is not necessary to observe high abundance of sucrose metabolism transcript when high concentration of sucrose accumulates in tissue (Carson et al. 2002). Identification of novel genes associated with cold tolerance mechanism in sugarcane is extensively studied using microarray experiments (Nogueira et al. 2003). Sugarcane transcriptome profiling based on signal transduction-related genes using microarray were determined and it revealed that, in sugarcane, 3500 genes are reported to be involved in signal transduction including genes coding for 600 transcription factors, 477 receptors, 114 calcium and inositol metabolism proteins, 107 protein phosphatases, 510 protein kinases, 75 small GTPases, and 17 G proteins (Papini-Terzi et al. 2005). The study on expression pattern of sugarcane culm is done and information on genes associated with maturation shows differential gene expression in cellulose synthases and cellulose synthase-like genes (Casu et al. 2007). cDNA microarray analyses revealed by elevation of CO_2 on sugarcane leaves, 22 genes were upregulated and 14 genes were downregulated which mainly related to photosynthesis and development and finally showed an increase of about 29% in sucrose content (De Souza et al. 2008). Using microarray technique the identification of genes when ethanol is applied on sugarcane leaf is studied and reported that 70 transcripts show differential gene expression pattern which comes under categories like gene regulation and abiotic stress (Camargo et al. 2007). Gene expression profiling using arrays were used to identify genes specific to a tissue, e.g., stems (Damaj et al. 2010).

For both low and high sucrose producing cultivars, transcriptome microarray studies on signal transduction pathway involved during sucrose synthesis were done and the study revealed differential expression of 24 genes and of them 19 were reported in low sucrose producing plants. Three of these genes are involved in reducing sucrose phosphate synthase (de Maria Felix et al. 2009). Sugarcane plants were subjected to polyethylene glycol stress for 2–4 h and transcriptome analysis was done, results show upregulation of sucrose transporter 1, sodium proton antiporter, proline dehydrogenase, and catalase-2. When the salt stress were given to plants there was a downregulation in all these genes and indicating that sugarcane response differently to different kind of stress (Patade et al. 2012). Transcriptome studies of sugarcane reported that during stress conditions plant accumulate osmoprotectants and 56 clusters of candidate gene classified to osmoprotectant were upregulated (Dos Santos et al. 2011).

2.9 Next Generation Sequencing Technologies

Next generation sequencing (NGS), also known as high-throughput sequencing, is the catch-all term used to describe a number of different modern sequencing technologies including Illumina sequencing, Roche 454 sequencing, Ion torrent

sequencing, and SOLiD sequencing. Illumina NGS utilizes a fundamentally different approach from the classic Sanger chain-termination method. Illumina sequencing instruments and reagents support massively parallel sequencing using a proprietary method that detects single bases as they incorporated into growing DNA strands using sequencing by synthesis (SBS) and it has many applications such as whole genome sequencing, de novo sequencing, candidate region targeted resequencing, DNA sequencing, RNA sequencing for applications such as transcriptome and small RNA analysis, methylation analysis and protein-nucleic acid interaction analysis (ChIP-seq).

2.9.1 RNA-Seq Technology and Its Application in Sugarcane

RNA sequencing (RNA-Seq) technology allows you to discover and profile the transcriptome in any organism. Illumina RNA-Seq technology records the numerical frequency of sequences in a library population, and offers a number of advantages compared to other technologies such as microarrays.

Analysis of the total RNA complement of the cell is included in transcriptomic studies. This helps in making meaningful comparisons between tissues of the same organism at different developmental stages and tissues exposed to various stresses or treatments (Schnable et al. 2004; Brady et al. 2006; Galbraith 2006). In sugarcane, for large-scale expression profiling techniques involving hybridization of RNA samples with nucleotide probes or generation of sequence tags are used. Different research groups worldwide have developed multiple sugarcane EST databases; these databases collectively contain more than 300,000 ESTs.

With an aim to generate a panel of differentially expressed stress responsive genes Kido et al. (2012) generated four Super SAGE libraries, using bulked root tissues from four drought tolerant accessions as compared with four bulked sensitive genotypes. Most relevant BlastN matches comprised 567,420 tags, 75,404 uni tags with 164,860 different ESTs. Oloriz et al. (2012) used a sugarcane mutant, obtained by chemical mutagenesis of the susceptible variety B4362; it showed a post-haustorial hypersensitive response (HR)-mediated resistance to the pathogen and was used to identify genes differentially expressed in response to *P. melanocephala* via suppression subtractive hybridization (SSH). It was also found that genes involved in glycolysis and C4 carbon fixation were upregulated in both interactions while genes related with the nascent polypeptide associated complex, post-translational proteome modulation, and autophagy were transcribed at higher levels in the compatible interaction. Genes coding for a putative no apical meristem protein, S-adenosyl methionine decarboxylase, nonspecific lipid transfer protein, and GDP-L-galactose phosphorylase involved in ascorbic acid biosynthesis were upregulated in the incompatible interaction. Wu et al. (2013) used high-throughput tag-sequencing (tag-seq) analysis by Solexa technology on sugarcane infected with *Sporisorium scitaminea*, 2015 genes expressed differentially, of these 1125 were upregulated and 890 downregulated were obtained after mapping to sugarcane EST

databases in NCBI. To study small RNA transcriptome complexity and explore their roles in sugarcane development, Sternes and Moyle (2015) obtained almost 50 million small RNA reads from suspension cells, embryogenic calli, leaf, apex, and a developmental series of stem internodes. The complexity of the small RNA component of the transcriptome varied between tissues. The undifferentiated and young tissue type libraries had lower redundancy levels than libraries generated from maturing and mature tissues. Su et al. (2015) made a study to find out the role of sugarcane chitinase gene family. Ten differentially expressed chitinase genes were obtained from RNA-seq analysis of both incompatible and compatible sugarcane genotypes during *Sporisorium scitamineum* infection. Seven chitinases showed more positive with early response and maintained increased transcripts in the incompatible interaction than those in the compatible one. Their results suggest that sugarcane chitinase family exhibit differential responses to biotic and abiotic stress. Park et al. (2015) performed transcriptome analysis of sugarcane hybrid CP72-1210 (cold susceptible) and *Saccharum spontaneum* TUS05-05 (cold tolerant) using Sugarcane Assembled Sequences (SAS) from SUCEST-FUN Database and showed that a total of 35,340 and 34,698 SAS genes, respectively, were expressed before and after chilling stress. The analysis revealed that more than 600 genes are differentially expressed in each genotype after chilling stress. Blast2GO annotation revealed that the major differences in gene expression profile between CP72-1210 and TUS05-05 after chilling stress are present in the genes related to the transmembrane transporter activity.

Vicentini et al. (2015) performed a high-throughput transcriptome evaluation of two sugarcane genotypes contrasting for lignin content. This study generated a set of 85,151 transcripts of sugarcane using RNA-seq and de novo assembly. More than 2000 transcripts showed differential expression between the genotypes, including several genes involved in the lignin biosynthetic pathway. This provided important information on the lignin biosynthesis and its interactions with other metabolic pathways in the complex sugarcane genome. Casu et al. (2015) examined tissue-specific expression patterns to explore the spatial deployment of pathways responsible for sucrose accumulation and fiber synthesis within the stalk. They performed expression profiling of different tissues (storage parenchyma, vascular bundles and rind dissected from a maturing stalk, internode of sugarcane). They identified ten cellulose synthase subunit genes and examined significant differences in the expression of their corresponding transcripts and those of several sugar transporters. Overall, their study indicates that there is spatial separation for elevated expression of these important targets in both sucrose accumulation and cell wall synthesis. Zeng et al. (2015) used a customized microarray to analyze the changes in the level of transcripts of sugarcane genes 8, 24, and 72 h after exposure to low-K conditions. The group identified a total of 4153 genes that were differentially expressed in at least one of the three time points. The number of genes responding to low-K stress at 72 h was almost twofold more than the numbers at 8 and 24 h. Gene ontology (GO) analysis revealed that many genes involved in metabolic, developmental, and biological regulatory processes displayed changes in the level of transcripts in response to low-K stress. Also, differential expression of transcription factors,

transporters, kinases, oxidative stress-related genes and genes in Ca+ and ethylene signaling pathways was detected. Ferreira et al. (2016) performed a comparative expression profiling of sugarcane ancestral genotypes: *S. officinarum, S. spontaneum*, and *S. robustum* and a commercial hybrid: RB867515, linking gene expression to phenotypes to identify genes for sugarcane improvement. Oligoarray experiments of leaves, immature and intermediate internodes, detected 12,621 sense and 995 antisense transcripts. For all tissues sampled, expression analysis revealed 831, 674, and 648 differentially expressed genes in *S. officinarum, S. robustum*, and *S. spontaneum*, respectively, using RB867515 as reference. Co-expression network analysis identified 18 transcription factors possibly related to cell wall biosynthesis and in silico analysis detected cis-elements involved in cell wall biosynthesis in their promoters. Dharshini et al. (2016) performed transcriptome profiling of the low temperature (10 °C) tolerant *S. spontaneum* clone IND 00-1037 collected from high altitude regions of Arunachal Pradesh, North Eastern India. The Illumina Nextseq500 platform yielded a total of 47.63 and 48.18 million reads corresponding to 4.7 and 4.8 gigabase pairs (Gb) of processed reads for control and cold stressed (10 °C for 24 h) samples, respectively. These reads were de novo assembled into 214,611 unigenes with an average length of 801 bp. The study revealed that about 2583 genes were upregulated and 3302 genes were downregulated during the stress.

Huang et al. (2016) performed the transcriptome analysis of a high-sucrose sugarcane variety, GT35, using high-throughput Solexa technology. A KEGG pathway analysis of 30,756 unigenes revealed more than 30 pathways in the sugarcane transcriptome and 3420 simple sequence repeats were identified in 3185 unigenes. Santa Brigida et al. (2016) have produced a de novo transcriptome assembly (TR7) from sugarcane RNA-seq libraries submitted to drought and infection with *Acidovorax avenae* subsp. *avenae*. The libraries presented 247 million of raw reads and resulted in 168,767 reference transcripts. Mapping in de novo transcriptome assembly of reads obtained from infected libraries revealed 798 differentially expressed transcripts, of which 723 were annotated and corresponded to 467 genes. Differential analysis revealed that genes 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) were upregulated (Table 2.1).

2.10 Different Databases and Sequence Sources

For bioinformatics analysis major available resources include expressed sequence tags (ESTs); more than 2.5 lakh sequences, genome survey sequences (GSS); approximately 9500 bacterial artificial chromosome sequences. The chloroplast genome of sugarcane has been sequenced and is of 141,182 bp (Asano et al. 2004; Junior Tercilio Calsa et al. 2004).

Since the complete genome sequencing of sugarcane is going to take few more years, comparative databases can prove to be excellent tools for sugarcane genome analysis. Bioinformatics portals like Gramene and portal at J. Craig Venter Institute

Table 2.1 List of transcriptomic studies in sugarcane

S. No.	Trait studied for transcriptome analysis	Author and year of publication
1	Infection caused by *Audovorax avenae* sub sp. *avenae*	Santa Brigida et al. (2016)
2	Low temperature tolerance gene profiling	Dharshini et al. (2016)
3	Genes associated with leaf abscission	Li et al. (2016)
4	Cell wall biosynthesis	Ferreira et al. (2016)
5	High-sucrose sugarcane varieties	Huang et al. (2016)
6	Maturing sugarcane stalk	Casu et al. (2015)
7	Genotypes contrasting for lignin content	Vicentini et al. (2015)
8	Biotropic interaction with *Sporisorium scitamineum*	Taniguti et al. (2015)
9	Response to low potassium stress	Zeng et al. (2015)
10	Cold responsive gene profiling	Park et al. (2015)
11	*Sporisorium scitamineum* challenge in sugarcane	Que et al. (2014)
12	Contrasting sugarcane varieties	Cardoso-Silva et al. (2014)
13	Sugarcane response to *Sporisorium scitamineum*	Wu et al. (2013)
14	Small RNA transcriptome analysis	Bottino et al. (2013)
15	Drought stress tolerance	Kido et al. (2012)

have genomic resources from different grasses that can be utilized for comparative genome analyses. As described earlier, sugarcane shares maximum similarity with *Sorghum bicolor*. Sorghum has a genome size of approximately 736 bp, similar to the monoploid genome of *Saccharum*. Paterson et al. (2009) sequenced *S. bicolor* genome and its chromosome-based assembly and annotation can be found in Phytozome (http://www.phytozome.org) and Joint Genome Institute (http://genome.jgi-psf.org/Sorbi1/Sorbi1.info.html).

The second most related crop to sugarcane is maize (*Zea mays*). Its sequencing has been completed and information about Maize Genome Sequencing Project can be found out on http://www.maizesequence.org/. The Maize Genome Database (Lawrence et al. 2004) available at http://www.maizegdb.org/ contains information like maps, QTLs, genetic stocks, cytogenetic and variations for alleles and polymorphisms, molecular markers, probes, gene products, images, metabolic pathways, and mutant phenotypes. The SUCEST initiative generated largest collection of ESTs from 26 different cDNA libraries (Vettore et al. 2001, 2003). Additionally, other groups (Casu et al. 2003, 2004; Bower et al. 2005; Ma et al. 2004) also generated >10,000 ESTs. Center for Genomic Research (TIGR) clustered these ESTs as Sugarcane Gene Index 2.1, while Sugarcane Gene index 2.1 was released by Computational Biology and Functional Genomics Laboratory at Dana-Farber Cancer Institute. The updated Sugarcane Gene Index contains theoretical contigs (formed by clustering ESTs and expressed transcripts), singleton ESTs, and singleton expressed transcripts. For transcript expression analysis, data from sugarcane high-throughput profiling experiments have been deposited in Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) (Edgar et al. 2002; Barrett et al. 2007, 2008).

An important Gene Ontology enrichment tool for sugarcane is EasyGO-http://bioinformatics.cau.edu.cn/easygo/ (Zhou and Su 2007). EasyGo can identify enriched GO terms for gene models, gene loci, protein coding genes, Ensembl, RefSeq and Uniport gene products, Gene Index entries and microarray oligonucleotides or probe sets for up to 17 organisms, also including the Sugarcane Gene Index and the Sugar Cane Affymetrix Genome Array probe sets. Efforts have been made to develop molecular marker and genetic Map Databases in sugarcane. TropGENE-DB (http://tropgenedb.cirad.fr/) is publicly available mapping resource (http://tropgenedb.cirad.fr/) (Ruiz et al. 2004). Nine different modules are included in this database. In addition to sugarcane, information about banana, cocoa, coconut, coffee, cotton, oil palm, rice, and rubber tree is contained in this database. Information of six genetic maps, with all maps sharing at least one parent is included in sugarcane module. Also, database can be searched for molecular markers, QTL, and clones. As far as metabolome of sugarcane is concerned KNApSAcK (http://kanaya.naist.jp/KNApSAcK/) tool contains information on different metabolites. This database provides information about the biological origins of the compounds and provides a tool for mass spectrum data.

2.11 Conclusion and Future Perspectives

To summarize, although several significant advancements in sugarcane genomics have been made, there exists a small incompleteness due to the unavailability of complete sugarcane genome information. However, the day is not so far as several research groups from Brazil and Australia are striving hard to sequence this complex polyploid for several years. Future researches would be greatly improved once the genome is sequenced. In addition, functional genomics will largely benefit leading to a great improvement in genetic engineering of sugarcane for value-added traits.

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