Review of Literature
CHAPTER 2

REVIEW OF LITERATURE

Sugarcane is a major industrial crop contributing to over 80% of the total white sugar production in the world. Sugarcane is also considered as an energy crop in view of its high sucrose and biomass potential which has been exploited for ethanol (biofuel) and electricity (bioenergy) production respectively. Sorghum, particularly sweet sorghum, another member of the tribe *Andropogoneae* to which sugarcane belongs, also have similar potential as an energy crop in view of its sucrose potential. Many plant storage organs that accumulate high levels of sugar accumulate primarily sucrose (Glaszious & Hatch, 1963). Previous assessments of the bio-physiological capability of the sugarcane stem to accumulate sucrose have estimated that the *Saccharum* complex is potentially capable of storing approximately 30% sucrose on a fresh weight basis (Jackson, 2005). This limit has recently been challenged (Wu & Birch, 2007), indicating that sugarcane culm may be able to attain even higher sugar contents.

2.1 Sugarcane as a commercial crop

The main product of sugarcane is sucrose, which contributes greatly to the calorie intake of the average person. Sucrose is used largely as a sweetening agent for foods, in the manufacture of cakes and candies, preservatives, alcohol, soft drinks and numerous other foods (Braun, 1999). By-products like molasses and bagasse from sugar industry are used for many different purposes. Molasses is the major feedstock for ethanol production in India is also sold as syrup, used to flavour liquor and other foods and also as an additive in animal feed (Harris & Staples, 1998).

Sugarcane bagasse is a complex material and is comprised of 50% cellulose, 25% hemicelluloses and 25% lignin (Pandey *et al*., 2000a). It is used as a boiler fuel for the generation of steam and power required to operate the sugar mills. Sugarcane bagasse is also used in paper and particle board industry and for the production of several chemicals (Patarau, 1989) or fermentation products, such as the production of protein enriched cattle feed and enzymes (Pandey *et al*., 2000a). But the most important use of sugarcane biomass in the coming years will be as a feedstock for the production of cellulosic ethanol (Bolling & Suarez, 2001).
2.1.1 Origin and evolution of sugarcane

Understanding the sugarcane genome with respect to its evolution and organisation is necessary for making informed decisions on sugarcane breeding. The cultivated species *S. officinarum* probably originated from *S. robustum* (D’Hont et al., 1998; Brown et al., 2007; Irvine, 1999) in the New Guinea-Indonesia region. The other two cultivated species viz., *S. barberi* and *S. sinense* are thought to be natural hybrids of *S. spontaneum* and *S. officinarum*. The wild species included in the genus *Saccharum* are *S. spontaneum*, *S. robustum*, *S. edule* and *S. spontaneum* has a wide distribution along the tropical and sub tropical regions of the world and exhibits substantial variation in morphology and cytotypes. *S. robustum* is largely confined to the New Guinea-Indonesia region with a possible origin through *S. spontaneum*. *S. edule*, has an intergenic origin between *S. officinarum* or *S. robustum* and the Miscanthus species (Daniels & Roach, 1987).

Current cultivated clones are essentially derived from interspecific hybridisation involving *S. officinarum* (2n = 80) and *S. spontaneum* (2n = 40-128). *S. officinarum* is characterized by its high sucrose content and soft stalks compared to other *Saccharum* species. *S. spontaneum* contributed stress tolerance, vegetative vigor and disease resistance to the hybrid varieties (Buterfield et al., 2001). The progenies from these hybridizations were backcrossed with *S. officinarum* (noble cane), a process called nobilisation, to recover the high sucrose phenotype (Bremer, 1961b). The varieties at present cultivated have a chromosome number of 2n= ~100-120.

2.1.2 Taxonomy of sugarcane

Sugarcane belongs to the genus *Saccharum* L., of the tribe *Andropogoneae* of the grass family (Poaceae). The word *Saccharum* is thought to have been derived from the Sanskrit word ‘sharkara’ (Daniels & Roach, 1987). The tribe *Andropogoneae* includes tropical and subtropical grasses and the cereal genera sorghum and maize. The taxonomy and phylogeny of sugarcane is complicated as plants belonging to five genera share common characteristics and form a closely related interbreeding group known as the ‘Saccharum complex’ (Mukherjee, 1957; Daniels et al., 1975). The *Saccharum* complex comprises of *Saccharum*, *Erianthus* section Ripidium, *Miscanthus* section Diandra, *Narenga* and *Sclerostachya* (Daniels & Roach, 1987). These genera are characterised by
high levels of polyploidy and frequently unbalanced numbers of chromosomes (aneuploidy) making it difficult to establish clear taxonomic definitions necessitating several revisions of the taxonomic relationships (Daniels & Roach, 1987; Sreenivasan et al., 1987).

2.1.3 The genetic complexity of sugarcane

Many important crops, including banana, canola, coffee, maize, potato, oats, soyabean, sugarcane and wheat are polyploids (Wendel, 2000; Osborn et al., 2003). Polyploidy could result from the duplication of a single genome (autoploidy) or from combining two fully differentiated genomes into a common nucleus (allopolyploidy) in one of the parental cytoplasms. Both *S. officinarum* and *S. spontaneum* are thought to have complex autoploidy genome (D’Hont et al., 1996; Ming et al., 2001). *S. officinarum* and *S. spontaneum* have a basic chromosome number of x = 10 and x = 8 respectively. As a result of the difference in their basic chromosome number two distinct chromosome organizations co-exist in current varieties (Grivet & Arruda, 2002).

Polyploid organisms have multiple copies of a specific chromosome, which means that multiple copies of a gene are therefore present (Wendel, 2000). The fate of redundant genes resulting from genome duplication is not well understood. When two different or related genomes are combined in a single cell they must respond to the consequence of genome duplication, especially multiple copies of genes with similar or redundant functions. One possible outcome of gene duplication might be the silencing of one of the duplicated copies, resulting in the loss or inactivation of the gene (Wendel, 2000). The silenced gene will remain in the genome as a pseudogene, accumulating mutations until it is no longer recognizable (Wendel, 2000). The most detailed description of the formation of a pseudogene from a duplicated gene is that of the PgiC2 gene in *Clarkia mildrediae* (Gottlieb & Ford, 1997). Of the 23 exons 18 were sequenced and 9 of them showed insertion or deletions, causing frame shift mutations and the insertion of stop codons. Some deletions also resulted in the loss of exon-intron splice junction.

Polyploidy might also raise a problem for gene regulation. The expression of most genes is dependent on a network of regulators such as transcription factors (TFs). The number of TFs in a diploid network is high, but in a polyploidy they can multiply several fold and as a result the regulatory network may be modified. One way by which
the organism solves this problem is by turning off or turning down the expression of some copies of some genes (Kellogg, 2003). Thus, a silencing strategy could balance the advantage and disadvantage of having multiple copies of orthologous genes or gene products (example transcription factors) in polyploid cells.

2.1.4 The sugarcane genome

The complexity and size of the sugarcane genome is a major limitation in genetic improvement of the crop. Most of the present day sugarcane cultivars have 2n= ~100-120 chromosomes which can be assigned to eight homology groups (Rossi et al., 2003; Aitken et al., 2005). The polyploid genome size of commercial sugarcane has been estimated to be approximately 10 GB (Le Cunff et al., 2008) which is extremely large compared the basal (1C) genomes of most crop plants. A basal chromosomal set for sugarcane, usually termed the monoploid genome, is expected to be in the range of 750-950 bp (D’Hont et al., 2008). The estimated size of the monoploid genome of *S. officinarum* and *S. spontaneum* is about 926 and 760 Mbp respectively (Butterfield et al., 2001) which is comparable to that of sorghum (760 Mbp) and about twice the size of the rice genome (450 Mbp) (Paterson et al., 2009).

Over the past two decades, studies utilizing various molecular techniques to unravel the complexity of this important crop species have provided a greater understanding of its complex genetic make-up (Bonierbale et al., 1988; Wu et al., 1992; D’Hont, 1994; Sills et al., 1995; Grivet et al., 1996; Ming et al., 2001; Rossi et al., 2003). Large scale EST sequencing projects by SUCEST (Sugar Cane EST Genome Project) involving SASRI-South African Sugar Research Institute, UGA-University of Georgia, USA and CSIRO-Australia’s Commonwealth Scientific and Industrial Research Organization (Vettore et al., 2001; Carson & Botha, 2000a; Casu et al., 2004; Ma et al., 2004) have resulted in huge volumes of data on sugarcane genome organisation. Nearly 300,000 ESTs and have been generated and functional annotation of many of the ESTs have been carried out (Vettore et al., 2003). Unfortunately, despite these achievements, the pace of progress with sugarcane genomics has lagged behind compared to other agricultural crops in view of its large, polyploid genome and the associated cytogenetical complexities (Ramsay et al., 2000; Delseny et al., 2001; Mullet et al., 2002).
2.2 Sorghum - origin and taxonomy

Sorghum (Sorghum bicolor (L.) Moench) is one of the five major cereals of the world, being grown extensively in tropical and subtropical environments (Rajarajan & Ganesamurthy, 2011). Sorghum was originated in the region of the northeast Africa comprising Ethiopia, the Sudan and East Africa. Sorghum taxonomy based on Harlan and deWet, (1972) classifies Sorghum bicolor into five races based on spikelet morphology. These races are Bicolor, Guinea, Caudatum, Kafir, and Durra. Because of the variability that is found in each race and the existence of race intermediates, a classification scheme integrating Harlan and deWet’s (1972) classification with working groups (sub-races) was established (Dahlberg et al., 2004).

2.2.1 Sorghum genome

Sorghum bicolor, a diploid, has a relatively small genome (735 Mbp), which although larger than rice (389 Mbp) is smaller than the other important cereals (wheat 17,000 Mbp, maize 2,500 Mbp). It was observed that the completion of the whole genome sequencing project in 2007 will exponentially increase the sequence data available for sorghum and will provide valuable information on cereal domestication in the African continent, an event that appears to have occurred independently of other continents though by similar reinforced selective pressures (Paterson et al., 2004).

Sorghum [Sorghum bicolor (L.) Moench], a diploid with 2n = 20, is the fifth most important cereal crop worldwide after rice, wheat, maize and barley (FAO, 2011). It is a food security crop providing dietary staple for many people, especially in the semi-arid tropics (SAT). The crop’s wide adaptability to conditions such as drought, water logging and salinity makes sorghum a crop of choice in marginal soils where growth of other cereals such as maize cannot be supported (ICRISAT, 1996). Mostly, local sorghum landraces demonstrate their worth for drought tolerance as they exhibited greater dry root weight, lengthy roots and higher root: shoot ratios (Ali et al., 2009b). It is an important food, feed, forage and provides raw material for producing of starch, fibre, dextrose syrup, biofuels, alcohol and other products (Zahid et al., 2011). Sweet sorghum can be used in jaggery and syrup preparations, which are used in confectionaries. Some sorghum varieties are rich in antioxidants and all sorghums are gluten free.
2.3 Sorghum as a model system for the genome analysis of sugarcane

The extensive similarity in the gene order between these two genomes have been well established which makes intercrosses between sugarcane and sorghum possible (Ming et al., 1998). Besides sorghum is considered as the best model crop for the Andropogoneae tribe in view of its relatively simple and well characterised genome (Price et al., 2005a) and hence used widely for studying the extensive gene rearrangements and assisting the development of genetic maps in sugarcane. Sequencing of sorghum provides another model genome within the grasses, which particularly when utilized in conjunction with rice, will stimulate evolutionary understanding of the entire Poaceae.

Sequencing will stimulate gene and allele discovery and crop improvement in sorghum as it did in rice. Sugarcane genomics will be supported by the sorghum sequence data. The sequences of sorghum genes and to a lesser extent the location of genes in the genome should be useful in genome analysis of sugarcane. Genetic resources for sorghum and sugarcane improvement have been enhanced by the application of genomic tools to analyse the wild relatives of sorghum and sugarcane. Mutant populations (including TILLING populations) of sorghum provide additional options for gene discovery and genetic manipulation. Protocols for EcoTILLING (Cordeiro et al., 2006a) and quantitative SNP analysis in the complex sugarcane genome should be valuable tools for gene mapping, gene discovery and association genetics in sugarcane. The availability of a sorghum genome sequence will further accelerate the application of these techniques in both sorghum and sugarcane. Gene discovery in this germplasm will also be supported by application of advances in expression profiling tools as has been applied to other crop species in the Poaceae (McIntosh et al., 2007).

EST and micro- and macro-array analyses have been used in the search for genes that control sucrose accumulation in sugarcane (Carson & Botha, 2000, 2002a; Casu et al., 2003, 2005; Watt et al., 2005). These studies have yielded an extensive annotated gene list and correlative data (Watt et al., 2005). However, the identities of key regulatory genes remain elusive. As in sugarcane, sucrose is the photoassimilate translocated from the leaves and stored in the stalk of sorghum (Tarpley et al., 1994). Besides, it has been observed that there is considerable variation among the sorghum genotypes for the amount of sucrose
accumulated in their stalk parenchyma (Lingle, 1987; Vietor & Miller, 1990). The simple
diploid genetics of sorghum and its colinearity and synteny with sugarcane therefore makes it
an attractive, potential model system for the identification of differentially expressed genes in
the sink tissue of genotypes that accumulate different amount of sucrose.

2.3.1 Comparative genetics of *Saccharum* and *Sorghum*

Grasses are the members of the family *Gramineae* or Poaceae and are represented
by over 10,000 species including sugarcane, maize and sorghum (Kellogg & Birchler,
1993). Comparative analysis of several grass genome (Doebley *et al*., 1990; Binelli *et al*.,
1992; Ahn *et al*., 1993; Hulbert *et al*., 1990; Bennetzen & Freeling, 1993) including
maize, rice, sorghum, wheat, and barley have also shown extensive conservation of gene
content and order (Gale & Devos, 1998).

The subtribe *Saccharinae* includes three major biofuel crops, sugarcane, *Miscanthus*,
and sorghum. Sugarcane and *Miscanthus* are closely related and belong to the *Saccharum*
complex (Daniels & Roach, 1987). Sorghum is their closest relative outside of the *Saccharum*
complex, sharing a common ancestor about 8-9 million years ago (Jannoo *et al*., 2007).
Molecular systematic studies have revealed that maternally inherited genomes of sugarcane and sorghum diverged more recently (Al-Janabi, 1994).
The sugarcane genome has gone through at least two rounds of genome wide duplication
events to become an octoploid since it has diversified from a common ancestor shared
with sorghum. The two rounds of duplications might have occurred after the speciation
event separated the two wild species *S. robustum* (x = 10) and *S. Spontaneum* (x = 8)
since these two species have different basic chromosome numbers (Ming *et al*., 1998;
Aitken *et al*., 2005; Garcia *et al*., 2006; Paterson *et al*., 2009), within 2 million years
(Jannoo *et al*., 2007). Ming *et al*., (1998) suggested the extensive duplication between
sorghum linkage groups (LGs) B and E. At least 25 (40%) of the 63 incongruous loci in
sorghum were found mapped to chromosomal regions that showed proximal duplication
in the sugarcane genome.

Sorghum and sugarcane genomes share more extensive genome wide colinearity
and less chromosomal rearrangement (Dufour *et al*., 1997; Guimaraes *et al*., 1997;
Ming *et al*., 1998), than either share with any other known grasses. The completion of the
sorghum genome sequence offered unprecedented opportunities for sugarcane genomic research (Paterson et al., 2009). The available genome sequence of sorghum provides an exceptional opportunity to unravel the complex sugarcane genome. The synteny between sugarcane and sorghum genome has been reported before using DNA markers (Ming et al., 1998), but no details of microsynteny are available except one pair of sugarcane bacterial artificial chromosomes (BACs) containing the Adh1 gene (Jannoo et al., 2007). Sequence comparisons revealed that sugarcane genome is mostly collinear in the genic regions with sorghum genome and the coding region of sugarcane and sorghum shared an average of 95.2% sequence identity. The unaligned regions between sugarcane and sorghum sequences were occupied mostly by repeats and non-coding sequences.

Colinearity has been employed to evaluate the correspondence of QTLs affecting related traits in sugarcane and other grasses. Corresponding QTLs controlling plant height and flowering were found in sorghum and sugarcane (Ming et al., 2002a). Sorghum, rice and maize linkage maps and physical maps were used to identify potential markers for fine mapping and chromosome walking towards cloning the rust resistance gene in sugarcane (Asnaghi et al., 2000); sorghum RFLP markers played a key role in mapping this gene to a small interval. The close relationship among these grasses, a high degree of colinearity, and cross-hybridization of DNA probes are compelling reasons for using the more abundant information from the small genome of sorghum to guide molecular mapping and positional cloning in sugarcane.

Comparative genome and transcriptome data analysis is vital for better understanding of genome structure, evolution and gene function. A total of 38,967 sugarcane proteins were predicted using ESTScan (Iseli et al., 1999) with the Zea mays matrix. To estimate the number of putative orthologous to the predicted 36,338 sorghum proteins, InParanoid was used at default parameters (Remm et al., 2001) (Table 1). The predicted number of Saccharum proteins homologous in sorghum was estimated to be 16,444 and the predicted number of sorghum proteins with homologous in Saccharum was 25,363, yielding a total number of orthologous groups of 10,432. A possible explanation of the lower number of Saccharum proteins with homologs in sorghum may be that the sugarcane assembled sequences (SAS) currently may contain copies of similar genes or some SAS are incomplete sequences map to the same sorghum gene.
Table- 1. Prediction of *Saccharum* proteins and orthologs to sorghum (Milton et al., 2013)

<table>
<thead>
<tr>
<th>Description</th>
<th>Total</th>
<th>Average size</th>
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<tr>
<td>Proteins in <em>Sorghum bicolor</em></td>
<td>36,338</td>
<td>202</td>
</tr>
<tr>
<td>Predicted proteins in <em>Saccharum</em></td>
<td>38,967</td>
<td>387</td>
</tr>
<tr>
<td>Predicted <em>Saccharum</em> proteins with homologs in sorghum</td>
<td>16,444</td>
<td></td>
</tr>
<tr>
<td>Sorghum proteins with homologs in <em>Saccharum</em></td>
<td>25,363</td>
<td></td>
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<tr>
<td>Total groups of orthologs</td>
<td>10,432</td>
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### 2.4 Introgression of desirable traits from other genera

Genetic improvement in sugarcane had been largely achieved through interspecific hybridization involving the cultivated and wild species of *Saccharum*. *Saccharum* is crossable with many of the related genera and intergeneric hybrids of *Saccharum* with *Erianthus*, *Miscanthus*, *Narenga* and *Sclerostachya* had been produced in the past (Janakiammal, 1941 & 1942; Li et al., 1948; Piperidis et al., 2000). There is renewed interest in the use of related genera, particularly *Erianthus* in view of its high biomass potential and tolerance to biotic and abiotic stresses. *Saccharum* x *Sorghum* crosses was made since 1930s by breeders to impart earliness in sugarcane varieties (Venkatraman & Thomas, 1932; Janakiammal & Singh, 1936; Nair, 1999). Many hybrids were produced, but they did not achieve commercial status due various factors. The one major issue in intergeneric hybridisation involving *Saccharum* had been the identification of genuine hybrids among the progenies from such crosses. In such crosses there will be a large number of selfs and distinguishing the hybrids from selfs is a difficult task. The hybrids largely resemble sugarcane parent in view of the significantly larger chromosome contribution from *Saccharum* and morphological differentiation of the hybrids from self is difficult.

However the use of species and genus-specific molecular markers has been found to be useful in identifying genuine hybrids among the progeny of interspecific and intergeneric crosses. A number of species and genus specific markers viz., the 5S ribosomal RNA gene marker (D’ Hont et al., 1995; Piperidis et al., 2000) satellite DNAs (Alix et al., 1998; Besse & McIntyre, 1998) and *Erianthus* specific Inter- Alu sequences
(Alix et al., 1999) have been generated for use in the identification of intergeneric hybrids. The 5S ribosomal DNA variation between *S. officinarum* and *E. arundinaceus*, having about 230 and 370 base pairs respectively, was found to be useful in the identification of the hybrids by the presence of the PCR products (Piperidis et al., 2000). RAPD markers were also found to be effective in precisely identifying hybrids involving *Saccharum x Sorghum*, and *Saccharum x Zea mays*, while identification of *Saccharum x Erianthus* hybrids using RAPD markers was found to be relatively difficult (Nair et al., 2006a). There had been several efforts to develop genus specific SSR markers for hybrid identification. Alix et al. (1998), reported characterization of a tandemly repeated DNA family in the *Saccharum* complex which are useful in monitoring sugarcane introgression programmes. By using SSR markers the interspecific hybrids between *S. robustum* and *S. spontaneum* were identified by Hemaprabha et al. (2005). Cai et al. (2005) screened intergeneric hybrids between *S. officinarum* and *E. arundinaceus* and its BC₁ progeny with sugarcane, and true hybrids were identified using microsatellite (SSR) markers. Nair et al. (2006) screened 50 putative hybrids from *S. robustum x Erianthus* and *S. officinarum x Erianthus* crosses, 30 progenies from *S. robustum x S. spontaneum* crosses, one progeny each from *Sclerostachya x S. officinarum* and *Sclerostachya x S. spontaneum* crosses using 10 sugarcane and 5 sorghum microsatellite markers. Parental polymorphism was studied with respect to the markers generated and compared with that of the progenies. Forty-six of the *Saccharum x Erianthus* progenies showed markers specific to *Erianthus*, confirming their hybridity. Among the 30 progenies of *S. robustum x S. spontaneum*, 21 showed markers specific to the *S. spontaneum* parent and were found to be genuine hybrids. The hybridity of the *Sclerostachya x S. officinarum* hybrid was also confirmed based on the presence of the markers representing both parents. The study clearly established the potential use of SSR markers in the identification of interspecific and intergeneric hybrids of *Saccharum*.

AFLP markers also have been proved to be very efficient in identifying interspecific and intergeneric hybrids (Jannoo et al., 2001; Selvi et al., 2003; Selvi et al., 2006a). In a study involving the *Saccharum* and *Erianthus* spp. using AFLP markers, *Erianthus* was found to have a distinct profile and 30% of the total fragments amplified were specific to the genus offering great scope for the identification of intergeneric
hybrids involving *Saccharum* and *Erianthus* (Selvi et al., 2006a). Thus molecular markers are expected to be powerful tools in the identification of interspecific and intergeneric hybrids and in monitoring the introgression of wild species.

### 2.4.1 Potential benefits of *Saccharum*-Sorghum hybrids

The logical interest in developing *Saccharum*-Sorghum hybrids is in combining the distinctive traits of the two crops like high sucrose and biomass yield of sugarcane and the grain formation, shorter duration and drought tolerance of sorghum. Such combination ideally could benefit both sugar and bioenergy sectors through higher output of sugars and alcohol. In India drought is a major factor affecting productivity and production of sugarcane (Nair, 2012). Sorghum is largely a rainfed crop grown in semi-arid conditions showing considerable tolerance to drought and incorporation of this trait in sugarcane can contribute significantly in improving sugarcane productivity in the country. Another potential benefit of sugarcane x sorghum hybrids could be the possibility of introducing the grain formation in such hybrids which will facilitate the planting of true seeds of sugarcane instead of the present practice of planting vegetative cuttings.

### 2.5 Sucrose metabolism and storage

Sugarcane and sweet sorghum are capable of storing large quantities of sucrose in the vacuoles of stem parenchyma cells. Very little of this stored sucrose is hydrolyzed during its transfer into the ripening stem (Lingle, 1989; Tarpley et al., 1996; Tarpley & Vietor, 2007). The important enzymes involved in sucrose metabolism are invertase (beta-D-fructofuranosidase; EC 3.2.1.26), sucrose synthase (SuSy; EC 2.4.1.13), sucrose phosphate synthase (SPS; EC 2.4.1.14), and sucrose phosphate phosphatase (SPP; EC 3.1.3.24). Soluble acid invertase (SAI) enzymes catalyze the cleavage of sucrose into glucose and fructose, and various types of invertase enzymes are found in plants and function in different locations, including the cell wall, vacuole, and the cytoplasm (Ruan et al., 2010; Vargas & Salerno, 2010; Patrick et al., 2013). SuSy is another enzyme that cleaves sucrose into fructose and UDPG in a reversible reaction (Winter & Huber, 2000; Patrick et al., 2013). Finally, SPS and SPP play an important role in sucrose synthesis in the cell. In the cytosol, SPS and SPP are jointly responsible for the irreversible synthesis of sucrose from UDPG and fructose-6-phosphate (Lunn & MacRae, 2003).
2.5.1 Transcriptional expression profiling of sucrose genes in sugarcane and sorghum

The identification of genes that positively regulate sucrose content is important in developing sugarcane varieties better sucrose potential. The relative role of the sucrose metabolising genes in sucrose synthesis and accumulation had been studied both in sweet sorghum and sugarcane (Calviño et al., 2009; Schafer et al., 2004; Grof et al., 2006). Based on the analysis of sugar accumulation and storage in three cultivars of sweet sorghum, Hoffmann-Thoma et al. (1996) attributed relatively less role for the three key sucrose metabolizing enzymes SuSy, SPS, and invertase. Transcriptional profiling experiments to characterize gene expression changes between stem tissues of sweet and grain sorghum have also been conducted (Calviño et al., 2009). Several candidate genes that are associated with high sucrose content in sweet sorghum have also been identified (Calviño et al., 2009).

Studies on sucrose accumulation in sugarcane stem has shown that the process is linked to the differential RNA or protein expression involved in sucrose metabolism (Schafer et al., 2004; Grof et al., 2006). The role of sucrose metabolizing enzymes in regulating sucrose levels in sink tissues has been demonstrated (Zhu et al., 1997; Botha & Black, 2000; Koch, 2004; Grof et al., 2007). Zhu et al. 1997 reported low SAI activity during maturation in varieties that store high levels of sugar. Sucrose synthesis is correlated with SuSy activity and at least three SuSy isoforms are present in sugarcane. Differential expression of SuSy had been reported during different developmental stages in sugarcane. The ratio of sucrose synthesis to breakdown is higher in mature internodes and it also has been suggested that different isoforms of SuSy are expressed in young internodes compared to mature ones (Schafer et al., 2004). A positive relationship between SPS activity and sucrose accumulation has also been demonstrated in sugarcane (Botha & Black, 2000; Grof et al., 2007). However, studies based on enzyme activity and isotope tracers have demonstrated that the expression need not always be exactly correlated with the sucrose accumulation processes in view of the complexities involved in the developmental cycle of the plant. Sucrose can be transferred from phloem tissues into the vacuoles of storage parenchyma cells without being catabolized and then resynthesized in the stem (Lingle, 1989). These data suggest that the enzymes involved in sucrose metabolism are not solely responsible for sucrose storage by the maturing stem and are unlikely to be major
drivers of stem sink strength at later stages of development in sugarcane. Besides the sucrose metabolizing enzymes, there also could be other mechanisms processes which contribute to high level of sucrose accumulation in sugarcane stems.

2.5.2 Improving sucrose content in sorghum and sugarcane

The main objective of sugarcane breeding over the years had been to improve sucrose content of the varieties developed. For this it is necessary to have a clear understanding of the control of carbon flux in stems, the functions of the genes responsible and the factors limiting sucrose accumulation, to evolve suitable approaches to enhance endogenous sugar content in stem tissues. Reducing the competition for the photo assimilates by altering the sink strength of other organs can result in higher sucrose accumulation in stems. In sweet sorghum by reducing the sink strength of grains, increased sucrose storage in the stem could be achieved (Marcelis, 1996; Andersen, 2003). Breeding efforts have led to the generation of sweet sorghum varieties with larger stems and reduced panicle size. One of the approaches had been to select varieties with thicker stalks thereby increasing the surface/unloading area for sugars into stem storage parenchyma cells leading to increased sucrose concentration in the internodes of both sugarcane and sweet sorghum (Glassop et al., 2007; Slewinski, 2012; Patrick et al., 2013).

There is potential to increase sugar levels in the stem of sugar accumulating plants like sugarcane and sorghum through metabolic engineering for which a clear understanding of the sugar metabolism and transport becomes essential (Patrick et al., 2013). Modulating the activity of the sucrose metabolising enzymes showed altered sucrose accumulation pattern in sugarcane. Improvement in stored sucrose levels could be achieved by downregulating soluble neutral invertase (SNI) in transgenic sugarcanes (Rossouw et al., 2010). However there was a consequent reduction in vigour and growth of the plants. Higher accumulation of total sugars was achieved in sugarcane transgenics expressing a sucrose isomerase enzyme (Wu & Birch, 2007 & 2010). However in field trials these transgenics failed to attain the same sugar level as obtained under greenhouse conditions (Basnayake et al., 2012; Patrick et al., 2013).

Information on sugarcane genomic resources have been ever increasing with the advances in high throughput DNA sequencing, developments in gene-expression technologies.
and other related areas. Consequently more and more information on the regulatory processes of C-partitioning in different sinks of sugarcane is becoming more explicit. The present study was an attempt to understand the role of major functional genes involved in the sucrose biosynthetic pathway. For the purpose, differential expression of these genes was studied at various stages in high and low sugar cultivars of sugarcane, grain and sweet sorghum and their hybrids.