

REVIEW

SUGARCANE AND POLYPLOIDY - A REVIEW

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Abstract

Sugarcane, which is an allopolyploid with genome contributions from Saccharum officinarum and S. spontaneum, is having high chromosome number of $2n=100$ to 130 in different cultivars. The 'Saccharum complex' has species with varying ploidy level and the chromosome number ranges from $2n=20$ to ~200. The high polyploidy and heterozygosity due to hybridization has restricted the classical genetic studies in sugarcane. There have been many studies recently on the effect of genome duplication and hybridization in gene expression and evolution of allopolyploids. In newly formed polyploids the diploidization mechanisms operate for stabilizing the genomes. Genome restructuring and gene expression modifications such as gene silencing and subfractionalization occur by which the allopolyploid may even have altered phenotype compared to the parents. Gene redundancy due to polyploidy provides a selective advantage for a wider geographical adaptation, increased vigour, sucrose and fibre content of sugarcane crop. There is increased global demand for alternative fuel sources and sugarcane is gaining importance as a biofuel crop with its high biomass production potential, besides being a major sugar crop. This review is on the significance of polyploidy in conventional and molecular approaches to genetic improvement of sugarcane in view of a large amount of recent literature available on the genomics and gene expression in

allopolyploids. There are many features in sugarcane that makes it a model crop for studying the effects of allopolyploidy and hybridity in plants.

Key Words: sugarcane, polyploidy, Saccharum, allopolyploid, genome modifications, gene expression

Introduction

Polyploids are organisms having more than two genomes in their nucleus. Polyploidy is widespread in wild as well as cultivated plants and is regarded as an important mechanism of speciation and adaptation (Ramanna and Jacobsen 2003). Whole genome duplication or polyploidy played a major role in the evolution of all angiosperms by enabling fertile interspecific hybrids to be generated with multiple gene alleles at each locus, through freeing the duplicated genes to mutate, and through reproductive isolation of new polyploids leading to speciation (Heslop-Harrison and Schwarzhacher 2011). Recent researches on polyploidy especially with regard to mechanisms of polyploid formation and establishment, the frequency of recurrent polyploidization, diploidization mechanisms, effect of polyploidy on gene expression, epigenetic mechanisms and genome restructuring were reviewed by many authors (Bretagnolle and Thompson 1995; Ramsey and Schemske 1998; Comai 2000; Adams and Wendel 2005; Ma and Gustafson 2005; Chen 2007; Soltis and Soltis 2009). In the grass family Poaceae, at least 80% of the species, if not all, are polyploids (Hilu 1993; Levy and Feldman 2002). Polyploids have superior vigour, generally higher vegetative and seed yields, and greater tolerance of environmental stresses. Among the important crop plants wheat, potato, cotton, sugarcane, banana, groundnut, coffee, tobacco, etc.

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and many horticultural crops are polyploids. Many typical diploid plants of today such as *Arabidopsis* (Vision et al. 2000), rice (Yu et al. 2005) and maize (White and Doebley 1998; Gaut 2001) are ancient polyploids, which are derived from at least one event of whole genome duplication followed by massive gene loss and genome organization through diploidization.

Autopolyploids have three or more homologous chromosome sets derived from a single species and are usually characterized by fully homologous chromosomes. The autopolyploids are intraspecific polyploids, having monophyletic origin (Bretagnolle and Thompson 1995). In general, autopolyploids will have multivalents at meiosis, tetrasomic ratios, slower development and reduced fertility (Soltis and Rieseberg 1986). Allopolyploids contain two or more sets of homeologous chromosomes. They were derived as a result of interspecific or intergeneric hybridization between species with diverged genomes. The wide hybridization between closely related species results in bringing in of two or more different genomes in to the same nucleus to form the allopolyploid. In allopolyploids the parental genomes that are homeologous may not undergo intergenomic recombination and hence maintain its integrity for generations (Soltis and Soltis 1995; Otto and Whitton 2000; Comai et al. 2003). In allopolyploids multivalents occur rarely, and will have diploid like cytogenetic behaviour and disomic inheritance (Stebbins 1971).

The two main modes of origin of polyploids are chromosome doubling or asexual polyploidization and the formation of functional $2n$ gametes or sexual polyploidization (Harlan and de Wet 1975; Bretagnolle and Thompson 1995; Ramsey and Schemske 1998). Sexual polyploidization is unique in that it accomplishes the goals of two fundamental evolutionary processes: sexuality and polyploidy (Ramanna and Jacobsen 2003). According to Harlan and de Wet (1975) somatic chromosome doubling is almost rare and all polyploids originated through sexual polyploidization through the action of $2n$ gametes. Production of unreduced or $2n$ gametes was found in almost all plant species studied, but with varying frequencies even at the individual plant level and affected by environmental factors (Veilleux 1985; Bretagnolle and Thompson 1995). Naturally

allopolyploids are derived from fertilization between unreduced male and female gametes from different species. It is a significant factor in the formation of new polyploids in natural populations and in opening pathways for gene transfer between ploidy levels. The recurrent polyploidization involving genetically different diploids can create a series of genetically distinct polyploid populations. The gene flow between such polyploid populations of independent origins might permit recombination and production of additional genotypes (Soltis and Soltis 1999). Polyploid species can maintain high level of segregating genetic variation through the incorporation of genetic diversity from multiple populations of their diploid progenitors. When polyploid genotypes further hybridize, genome reshuffling with more genetic complexity will occur and this genome reshuffling will be an additional source of genetic variability in polyploid populations.

Polyploidy in sugarcane

The commercial sugarcane cultivars are clonal selections from interspecific hybrid derivatives involving species of the genus *Saccharum* L. (Sreenivasan et al. 1987). This genus consists of six species of which two are wild - *S. spontaneum* L. and *S. robustum* Brandes and Jesw. ex Grassl and four are cultivated - *S. officinarum* L., *S. barberi* Jesw., *S. sinense* Roxb. and *S. edule* Hassk (Daniels and Roach 1987). Irvine (1999) proposed to recognize only two species in *Saccharum*, first *S. spontaneum*, the putative ancestral form which has a very wide natural range, morphologically distinct from other *Saccharum* forms, and second, *S. officinarum*, which includes the wild species *S. robustum*, together with the land races *S. officinarum*, *S. edule*, *S. barberi* and *S. sinense*. The 'Saccharum complex' (Mukherjee 1957) includes the genera *Saccharum*, *Erianthus*, *Sclerostachya*, *Narenga* and *Miscanthus* which constitute a closely related inter breeding group involved in the origin of sugarcane (Daniels et al. 1975).

S. spontaneum is a highly polymorphic wild grass widely distributed in the tropics and sub tropics, with wide eco-geographical distribution and wide range of chromosome numbers from $2n = 40$ to 128 (Panje and Babu 1960; Sreenivasan et al. 1987).

Saccharum robustum, with tall and thick cane is seen on the river banks of New Guinea and Indonesia. The chromosome number of *Saccharum robustum* ranges from $2n = 60$ to about $2n=200$. *Saccharum edule* which is being cultivated in Polynesian islands for its edible inflorescence, is a polymorphic species with chromosome numbers $2n = 60, 70$ and 80 . The *S. robustum* forms with very high chromosome numbers such as $2n = 164$ and ~ 194 were considered to be *Saccharum* x *Miscanthus* hybrids (Sreenivasan et al. 1987). The highest chromosome number recorded for a wild *Saccharum* is $2n = 194$ in the clone 51 NG 106 collected from Mendi region of New Guinea (Stevenson 1965), *Saccharum officinarum*, the noble cane, with chromosome number $2n = 80$, is present only under domesticated conditions and large variability is maintained in the native gardens in New Guinea and Indonesia. According to Stevenson (1965), *S. officinarum* is an allopolyploid of amphidiploid origin and behaves essentially as diploid. The meiotic chromosome pairing behaviour of the $2n = 80$ forms were as that of a diploid species with predominant bivalent formation and with normal segregation at anaphase. It is now generally agreed that *S. officinarum* originated from complex introgression between *S. spontaneum*, *E. arundinaceus* and *Miscanthus sinensis*. *S. robustum* is intermediate in the evolution of *S. officinarum* (Daniels and Roach 1987; Daniels et al. 1989), *S. barberi* ($2n = 81-124$) and *S. sinense* ($2n = 111-120$) are the north Indian and Chinese canes that were under cultivation for sugar production (Sreenivasan et al. 1987).

The lowest chromosome number in the 'Saccharum complex' is $2n = 20$ present in the *Erianthus* species such as *E. ravennae*, *E. elephantinus* and *E. hostii* (Sreenivasan et al., 1987). *E. bengalense* and *E. arundinaceus* were reported to have varying chromosome numbers $2n = 20, 30, 40$ and 60 (Daniels and Roach 1987; Sreenivasan et al. 1987). The chromosome numbers found in *Miscanthus* species are $2n = 38, 40, 76, 57, 95$ and 114 (Daniels and Roach 1987). The chromosome number of *Narenga porphyrocoma* is $2n = 30$ and *Sclerostachya fusca* is also having chromosome number $2n=30$ (Sreenivasan et al. 1987).

The basic chromosome number for *S. spontaneum* was postulated to be $x = 8$ by Janaki Ammal (1939) as there are polyploid series in this species with $2n = 40, 48, 56, 64, 72$ and 80 . Bremer (1961a) suggested three basic chromosome numbers for the genus *Saccharum* such as $x = 6, 8$ and 10 . By physical mapping of ribosomal DNA genes using fluorescence *in situ* hybridization technique, D'Hont et al. (1998) determined the basic chromosome number $x = 10$ for *S. officinarum* and $x = 8$ for *S. spontaneum*. Similarly, the basic chromosome number of genus *Erianthus* was found to be $x = 10$ through chromosomal localization of ribosomal DNA genes (Besse and McIntyre 1999). The segregation and linkage studies using molecular markers by Alwala et al. (2008) had shown that *S. officinarum* clone La striped ($2n=80$) is an auto-allopolyploid and the *S. spontaneum* ($2n=64$) clone SES 147B is an autopolyploid.

The *S. officinarum* ($2n = 80$) x *S. spontaneum* ($2n = 64$) crosses produced hybrids with chromosome number $2n = 112$, due to the functioning of the $2n$ female gamete from *S. officinarum* and n pollen of *S. spontaneum* (Dutt and Rao 1933; Bremer 1961b; Kandasami 1961). Similarly when $2n = 80$ and $2n = 112$ forms of *S. spontaneum* were crossed with *S. officinarum*, the hybrids formed were with $2n = 120$ and 136 , respectively, also with $2n + n$ transmission. By selfing of *S. officinarum* clones or *S. officinarum* x *S. spontaneum* hybrid and in crosses of *S. officinarum* x *S. officinarum* or *S. officinarum* x *S. robustum*, only $n + n$ transmission take place. The differences observed in chromosome transmission pattern in interspecific hybrid progeny of *Saccharum* species was explained by Burner and Legendre (1993) based on the endosperm balance number (EBN) concept (Johnston et al. 1980). In wide crosses, the successful endosperm development for seed formation occurs when the endosperm receives two EBNs from female parent and one EBN from male parent. Commercial sugarcane varieties have same EBN number as that of *S. spontaneum* thereby having $n + n$ transmission in progenies of crosses between them. In *S. officinarum* the EBN is half

as that of *S. spontaneum* and hence will have $2n + n$ progeny in *S. officinarum* x *S. spontaneum* crosses and $n + 2n$ in *S. spontaneum* x *S. officinarum* crosses.

In the first back cross of the *S. officinarum* x *S. spontaneum* hybrids with *S. officinarum* as the female parent, the $2n + n$ transmission occurs and in subsequent back crosses only $n + n$ occur (Bremer 1961b). The functioning of diploid gametes of *S. officinarum* in crosses with *S. spontaneum* increase the chromosome number in the progeny than the parental clones and hence the commercial sugarcane clones which are derivatives of *S. officinarum* x *S. spontaneum* hybrids have chromosome number ranging between $2n = 100$ and 130. The chromosome number of the commercial sugarcane clones may be varying and the chromosome constitution also may vary with difference in the chromosomes contributed by *S. officinarum* and *S. spontaneum*.

In light of the knowledge that increase in chromosome number in the nobilization steps involving *S. officinarum* x *S. spontaneum* hybrids results in superior plants with higher cane yield and sucrose content, many attempts were made to increase the chromosome number by hybridization or induced chromosome doubling through chemicals. Sugarcane plants with chromosome number above $2n = 200$ and up to $2n = 225$ were produced either by selective hybridization of *S. officinarum* x *S. spontaneum* or by chromosome doubling using colchicine under tissue culture (Heinz and Mee 1970; Roach 1972; Sreenivasan et al. 1987). These plants with very high chromosome number were very weak and had stunted growth. The induced autopolyploids will have larger cells and plant parts due to chromosome duplication. The increase in chromosome number beyond $2n = 130$ may not have any advantage in terms of plant vigour or quality in sugarcane (Roach 1972). Induced polyploidy has not been successful in developing commercial varieties of sugarcane.

The rapid recovery of high sugared commercial types from the interspecific hybridization of *S. officinarum* with *S. spontaneum* is attributed to the transmission of diploid complement of the *S. officinarum* to the hybrid. Commercial sugarcane

hybrids which are derivatives from such hybrids contain the full complement of *S. officinarum* and a few *S. spontaneum* chromosomes imparting the favourable agronomic characters from both the species. *S. barberi* and *S. sinense* also contributed to the improvement of the sugarcane in the early stages. Similarly, the high fertility of the majority of hybrid sugarcane varieties can be due to the autosyndetic pairing of chromosomes at meiosis. The initial hybrids of the nobilization as well as the recent complex hybrids used as commercial varieties, with high chromosome number than *S. officinarum*, had preponderance of bivalents and very few univalents and rarely multivalents at diakinesis and metaphase I in pollen mother cells (Daniels and Roach 1987; Sreenivasan et al. 1987). The intergeneric hybrids involving *Saccharum* and *Erianthus* also had increased chromosome number due to $2n + n$ transmission. They had bivalent pairing in almost all cases (Lalitha and Premachandran 2007).

The formation of polyploid gametes is possible in sugarcane commercial hybrids and interspecific or intergeneric hybrids of *Saccharum* due to meiotic abnormalities (Burner and Legendre 1993; Lalitha and Premachandran 2007). The formation of syncytes with two to ten nuclei per pollen mother cell were observed in the sugarcane commercial clone CP 61-37, CP 70-1133 and CP 77-1776 by Burner and Legendre (1993). They found that at metaphase chromosomes were paired as bivalents and possibility of $2n$ or $3n$ gametes formation through syncyte formation. In *S. spontaneum* x *Erianthus arundinaceus* hybrid CYM 04-391 ($2n = 80$) frequent occurrence of syncytes was reported by Lalitha and Premachandran (2007), in which certain syncytes at metaphase revealed very high number of chromosomes, even more than 650 bivalents.

Diploidization of polyploids

In polyploids, the possibilities of homeologous pairing are gradually replaced by homologous pairing strictly, due to chromosome differentiation or introduction of a new genetic system controlling pairing. Such a process of allopolyploidization has been suggested to be essential for the initial stabilization and subsequent establishment of polyploids (Liu et al. 2001). Alternatively the changes observed may be selectively inconsequential. Plants may undergo

repeated cycles of polyploidization followed by extensive diploidization (Soltis et al. 2003). Chromosome pairing mechanisms lead to allopolyploidization in many species. The possibility of homeologous pairing is gradually replaced by homologous pairing strictly due to the chromosome differentiation or pairing control genes in an allopolyploid.

The different parental genomes that were brought together in a common nucleus by allopolyploidy will have rapid and extensive modifications as reported in wide ranging genera such as *Avena*, *Brassica*, *Gossypium*, *Hordeum*, *Nicotiana*, *Secale*, *Triticum* and *Zea* (Soltis and Soltis 1999). Subsequent to polyploid formation intragenomic and intergenomic rearrangements occur. The genetic mapping studies in *Zea mays* confirmed the extensive chromosomal rearrangements that made the allopolyploid a diploid. The merger of two distinct but related genomes by allopolyploidy may not result in genomic additivity with respect to parental genomes and will continue to evolve after polyploid formation, thereby obscuring initial conditions (Liu and Wendel 2002). Studies using artificial allopolyploids of *Triticum-Aegilops* had revealed that rapid elimination of chromosome specific sequences and genome specific sequences in newly synthesized amphiploids is non-random, directional and highly reproducible. This sequence elimination helps the initial stabilization and establishment of newly formed allopolyploids as new species. Sequence elimination is a major and immediate response of the wheat genome to wide hybridization and genome doubling and in one particular combination of diploids up to 14 % of the genomic loci of one parent genome was eliminated in a single generation itself (Shaked et al. 2001). The positive correlation between sequence elimination frequency and fertility (Ozkan et al. 2001) suggested the possibility that allopolyploid speciation will be evolutionarily promoted in species groups that evolved a predisposition for molecular interaction mechanisms that underlie sequence elimination (Liu and Wendel 2002).

In a newly formed allopolyploid there are adverse interactions between the nuclear genome contributed by the male parental diploid and both the nuclear and cytoplasmic genomes of the female

parental diploid; genome adjustments must occur to restore nuclear cytoplasmic compatibility (Gill 1991). In Triticale, the rye genome had undergone a much higher degree of genome adjustment than wheat genome and nuclear cytoplasmic interaction is the factor for the high degree of rye genome changes observed. Cytoplasm-caused directional sequence changes were studied in *Brassica* by Song et al. (1995). The 'hostile' environment of maternal cytoplasm makes the paternal genome more vulnerable to change in the newly formed hybrid (Gill 1991). The extent of genome change depended of parental origin and the genome originating from maternal parent, that donates both the cytoplasm and the nucleus to the polyploid, undergoes much less change than the genome from paternal parent, which donates only its nuclear DNA to the polyploid (Leitch and Bennett 1997).

Chromosome eliminations are recurrent in sugarcane interspecific hybrids (Raghavan 1954). It was reported by Alexander (1968) that in *Saccharum* accessory and multipolar spindles led to elimination of chromosomes to produce gametes with unusual chromosome numbers. The chromosomes which remained as univalents in sugarcane clones, mostly from *S. spontaneum*, were eliminated during meiosis in the successive generations of intercrossing (Sreenivasan et al. 1987). Crosses between species with different genomic compositions often result in sterile F₁ hybrids, which frequently lead to cytological instability and low fertility in newly formed amphiploids. High chromosome number and nucleolar oriented chromosomes attached to the sites of nucleolar membrane might increase probability of error in chromosome association (presynapsis), chiasma formation (synapsis), or chiasma terminalization.

D' Hont et al. (1998) reported the reduction in number of 5s rDNA sites in some higher chromosome number *Saccharum* clones. Such modifications like suppression of the activity, reduction of the number of repeats or deletion of genomic sites have been reported and are frequent in polyploids. Recent studies on the consequences of polyploidy on gene and genome evolution, and gene expression revealed that genome duplication due to polyploidy could result in chromosome

rearrangements and gene loss, interlocus concerted evolution of ribosomal repeats, unequal rates of sequences evolution of duplicated genes and changes in DNA methylation (Adams 2007). Caudrado et al. (2004) provided evidences for nuclear and chromosomal remodeling that took place in three modern sugarcane cultivars studied. There could be other effects such as cell volume increase with increase in ploidy or genomic DNA content by which the concentration of gene product within the cell is altered (Veitia 2005). There could be change in the surface to volume ratio of the plasma membrane to the cytosol or the nuclear envelope to the nucleoplasm and cytoplasm. It may be of importance in sugarcane when the gene expression levels vary in the allopolyploids due to chromosome number variations and allelic variations which could affect even the quantum of sucrose synthesized and accumulated.

Chromosome numbers in *S. spontaneum* ($2n = 64$) x *E. arundinaceus* ($2n = 60$) hybrids studied by Lalitha and Premachandran (2007) were higher than $2n = 62$ expected from $n + n$ chromosome transmission, but less than $2n = 92$ or 94 , that was expected from functioning of a $2n$ gamete from either male or female parent. These hybrids with $2n = 78$ to 86 could be the products of n gamete from one of the parents and $2n$ gamete from the other parent and subsequent elimination of few chromosomes from the hybrid. In these hybrids the elimination of certain chromosomes from *S. spontaneum* or *E. arundinaceus* will be negating the genomic imbalances created by coexisting two diverse genomes in the hybrid whereas in the *S. officinarum* x *S. spontaneum* hybrids the $2n + n$ transmission provide the genome balance without any chromosome elimination.

The allopolyploid genomes experience both revolutionary (instant) and evolutionary (accumulating) changes as explained by Feldman and Levy (2005) which involve many genetic and epigenetic interactions. Polyploidization might be a source of genomic stress that facilitates rapid evolution. Factors that favour allopolyploids are the heterosis due to combination of the homeologous genes and the phenotypic variation generated in new allopolyploids that help in adapting to new ecological niches (Comai 2000). The gene and genomic

duplication in allopolyploid hybrids cause genome instabilities, chromosome imbalances, gene regulation imbalances and often sterility (Ma and Gustafson 2008). The compatible relationship between alien cytoplasm and nuclei and between the divergent genomes is essential for the success of such hybrids (Chen 2007).

Gene expression in allopolyploids affecting phenotype

The interspecific hybridization resulting in allopolyploid formation brings in genomic interactions leading to restructuring of the transcriptome, metabolome and proteome as stated by Leitch and Leitch (2008). The novel genetic variation consequent to allopolyploidy brings in evolutionary advantages than the progenitor species by way of modified phenotypes and ecological preferences. In allopolyploids homeologous genes can be expressed at different levels and can respond differently to allopolyploidy in various organs of the plant. Organ specific silencing of homeologs was found in wheat, *Gossypium* and *Arabidopsis* allopolyploids (Adams 2007). Such organ specific gene expression changes have a role in determining the fate of the duplicated genes. The subfunctionalization of duplicated genes occurs when one homolog has been silenced in some organs and the other homolog silenced in other organs. The partitioning of function and/or expression patterns between duplicated copies by subfunctionalization necessitates retention of both the copies of the gene. According to Adams (2007) if duplicated genes are subfunctionalized or reciprocally lost in geographically isolated populations, uniting of individuals from each population can lead to hybrids that lack both copies of a duplicated gene pair, resulting in hybrid inviability, reproductive isolation, and speciation. Even loss of one duplicated gene copy might result in speciation by divergent resolution if the gene product from one copy is insufficient for normal function.

In *Gossypium* allopolyploids the parental sub-genomes did not contribute equally to the transcriptome (Adams et al. 2003). The expression of the homeologous loci varied among organs which could be due to differential developmental regulation. The expression patterns of certain duplicated genes

in the newly synthesized allopolyploids could be similar to that in natural established polyploids. Similarly there could be unequal contribution of two parents to gene expression in allopolyploids and hybrids due to nuclear dominance (Pikaard 2000). It is due to the silencing of one parental set of rRNA genes in the interspecific hybrid or allopolyploid. *Arabidopsis thaliana* rRNA genes are silenced in natural allopolyploid hybrid *A. suecica*. In F_2 plants of *A. thaliana* x *A. arenosa* hybrids silencing of *A. thaliana* genes and dominance of *A. arenosa* rRNA genes was consistent (Chen et al. 1998). The dominance relationship could be reversed by changing the parental genomic ratio from 1:1 (AACC) to 3:1 (AAAC) where A and C represent haploid genome of *A. thaliana* and *A. arenosa* respectively. It was attributed to an epigenetic interaction between the rRNA genes of the two parents that determine the outcome.

Gene dosage effects also provide a selective force preferring homeologous loci. According to Veitia (2005), segmental DNA duplication can result in absolute increase in the quantity of gene products by increased dosage if the proteins act as monomers which are poorly connected within the cellular interaction network. An increase in expression level of one component of the dosage- sensitive gene can lead to the alteration in the amount of functional complex. Molecular studies on allopolyploids revealed that they can exhibit "enzyme multiplicity" (Soltis and Soltis 1993) and can produce all the enzymes of two different parental genomes, and also new hybrid enzymes that will lead to greater biochemical flexibility providing higher adaptability.

Comai (2000) proposed genetic and epigenetic models of genetic instability in allopolyploids, wherein certain genes that are neutral or advantageous in their species of origin become deleterious in a hybrid background due to accumulation of incompatible features since the divergence of these species. The genetic model of instability is based on the mismatch of protein subunits in hybrids resulting in altered structure of the complex macromolecule and its malfunction. Epigenetics refers to the heritable changes in phenotypes, and hence in gene regulation, that are not caused by changes in DNA sequence. Genes that were rapidly silenced upon

allopolyploidization were related to repetitive DNA elements, a common characteristic of genes susceptible to homology dependant gene silencing, an epigenetic phenomenon. The homeologous genes sequestered in different diploid species diverge and accumulate characteristics that would emerge as incompatible when the homeologous genes are united by hybridization and hence the joining of homeologous genes might bring together genes that undergo silencing interaction. In synthetic allopolyploid hybrids, alterations in gene regulation can result in genomic restructuring and phenotypic instability such as sterility and lethality. The allopolyploid hybrids may be vigorous and may show unusual characteristics such as homeotic phenotypes, flower variegation, tumour formation and dominance of the hybrid phenotype by one parent. Studies in synthetic allopolyploids and their parents in *Arabidopsis* by Comai et al. (2000) indicated that rapid gene silencing occurs in synthetic allopolyploids and there is a relationship between silenced genes and repetitive DNA of presumed heterochromatic region.

Genome instability due to transposons and epigenetic mechanisms

It is now established that in synthetic allopolyploids genomic changes occurred in a burst after allopolyploidization which was called 'genomic shock' by McClintock (1984) could be the reason for the genomic alterations in the established allopolyploids compared to their presumed parental genomes (Comai 2000). Two mechanisms that lead to these genomic changes are homeologous recombination and transposon activation. The transposable elements (TE) can facilitate the genome restructuring in recently formed polyploids. According to Matzke and Matzke (1998) polyploidy permits extensive gene modifications by transposable elements as the polyploid genomes contain duplicated copies of all genes. TEs may be the driving force in the evolution of gene silencing mechanisms such as methylation and heterochromatinization. Matzke and Matzke (1998) were of opinion that polyploid genomes will not only contain more TEs than diploid genomes but will also be more methylated. Comparisons of transcriptomes of sugarcane, wheat and maize indicated that TEs

comprised 2.3%, 2.4% and 0.014% of the total transcripts respectively (Araujo et al. 2005).

The gene function observed in a model organism may not be of the same way in another plant species (Udall and Wendel 2006). Several recent studies have demonstrated that wide hybridization and genome doubling could induce rapid epigenetic modifications in both coding and regulatory sequences as well as in or near TEs resulting in gene silencing, novel expression and de-repression of TE (Liu and Wendel 2002). When divergent genomes are united prior to or followed by genome doubling, dormant TEs can be released from suppression and become transcriptionally and even transpositionally activated. Many TEs have strong promoter sequences suggesting the element insertion may lead to altered expression patterns. TEs are capable of generating genetic novelty.

TEs are DNA sequences capable of movement within the genome and are considered to be the important factors responsible for genome maintenance and diversification. These elements cause mutations and the range of 'mutations' induced by TE activity extends from modifications in the size and arrangement of whole genomes to substitutions, deletions, and insertions of a single nucleotide (Kidwell 2002). Their activity produces structural changes in single genes or overall genome that result in altered spatial and temporal patterns of gene expression and gene function in sugarcane (Rossi et al. 2001). In maize TEs represent over 50% of nuclear DNA (Bennetzen 2000). Sugarcane has a more complex genome than maize and the analysis of TEs revealed the presence of a surprising amount and diverse spectrum of expressed transposable elements (Rossi et al. 2001). The analysis of ESTs database showed that in sugarcane more expressed transposons (54%) are present than retrotransposons (46%). A total of 21 different expressing TEs were found to be present in sugarcane (Rossi et al. 2001).

The contribution of TEs to the genomic plasticity and differential genome expression is likely to be enormous and it is very important to understand and evaluate transposable elements in sugarcane. Many TEs that are silenced in a diploid state may be activated in the new genetic environment of the

polyploid since genetic redundancy in the polyploid may buffer the potential deleterious effects of transposition (Voytas and Naylor 1998). Interspecific hybridization, where a merger of diverged genomes takes place, also activates retrotransposons that contribute to chromosomal rearrangements and gene expression (Kashkush et al. 2003). Sugarcane is a perfect example of this genome stress phenomenon since it is undergoing the hybridization process between two polyploid species (Araujo et al. 2005). It is also hypothesized that some of the somaclonal variation events reported in sugarcane can be a result of TEs activity (Rossi et al. 2001).

Gene expression in sugarcane and polyploidy

Several sugarcane promoters have been isolated and used for transgene expression but with little success of expression activity in the mature plants (Mudge et al., 2009). Silencing of the reporter transgenes was observed in mature plants even though the native copy of the gene remained functional in these plants. This silencing of the transgene did not trigger silencing of the native gene that had the same 5'UTR region. Except for maize *Ubi-1* promoter, other heterologous and synthetic promoters show no/little expression in mature sugarcane plants (Mudge et al. 2009). In *Arabidopsis* the upstream regions of the duplicate genes related to stresses were found to possess TATA-box and low levels of methylation which may facilitate expression divergence between duplicate genes through interactions with transcription factors and trans-acting proteins. It is also suggested that there could be protein domain divergence among duplicate transcription factors. This may in turn affect the downstream genes and pathways. Post transcriptional gene silencing of transgene transcripts and associated promoter methylation has been reported in sugarcane. Increased DNA methylation was observed in the transcribed region of the coat protein transgenes in most of these plants (Ingelbrecht et al. 1999). Developmental polyploidy was suggested to be the cause for such unpredictable onset of gene silencing in other crop species.

Neofunctionalization is the acquirement of new functions in one of the duplicated genes. The additional sets of genomes may free some genes from the pressure of natural selection and allow them

to develop separate functions (Bailey et al. 1978). Expression studies of homeologous genes in different tissues of cotton revealed that one copy could become silenced in selected tissues resulting in subfunctionalization of the duplicates (Adams et al. 2003). Interactions of cell-specific regulatory factors with the cis-regulatory regions of one parent, or tissue-specific epigenetic regulation induced by allopolyploidy are suggested to be the reasons behind such preferential gene expression. Such uniparental expression in alternate tissue types favours both maintenance of duplicates as well as changes that optimize the function of each duplicate gene in selected cell types (Comai 2005). Some genes originating from different progenitors are expressed in specific tissues or at different developmental stages, however little is known about when and how the differential expression patterns of progenitor genes are established.

Most gene copies in a polyploid genome of sugarcane cultivars are different when analyzed at the molecular level. Multiple alleles are expressed at diverse levels ranging from 40% to <1% of total transcripts from a single locus for ScR1MYB1 and up to six haplotypes were reported in three different cultivars for a 6-phosphogluconate dehydrogenase gene B and alcohol dehydrogenase *Adh1* and *Adh2* genes (Grivet et al. 2003). Multiple weak alleles could contribute to the cumulative effect of total expression of a gene from medium to high levels which has broader implications in sugarcane breeding (Mudge et al. 2009).

Sucrose synthase (SuS) is a major enzyme of sucrose metabolism in sugarcane. This gene is homologous to the maize gene that produces the *Shrunken-1* (*Sh1*) phenotype and using a probe from the maize *Sh-1* gene, a restriction fragment length polymorphism (RFLP) linked to the sugar accumulation in sugarcane was identified (Ming et al., 2001). Enzyme and northern analysis of developing internodes of diverse genotypes indicated that this gene is differentially expressed among genotypes which may be the result of polymorphism within the promoter region of the gene (Lingle and Dyer, 2004). The differential expression of sucrose phosphate synthase (SPS), SuS and other sucrose related enzymes observed in an expression profiling study of *Saccharum* and related species could be

explained by the above. Despite a detailed model for sucrose metabolism, our understanding of sucrose accumulation in sugarcane is very limited. The analyses of BAC sequences from homo(eo)logous regions of sugarcane suggested that despite polyploidy, the organisation and structure of homo(eo)logous genes, and genic regions, have been well preserved (Jannoo et al. 2007; Garsmeur et al. 2010).

The transcript complexity of stem-specific *Myb* transcription factor in sugarcane was analysed by Mudge et al. (2009). It was found that four variants that contributed about 70% of the total transcripts with one (termed Z1) contributing about 40%, indicating the presence of some variants of this gene that are more highly expressed than others amongst the gene copies. It was suggested that as the highly expressed transcript Z1 was derived from a high copy number gene that was recovered frequently, the high level of Z1 expression may not be due to over-expression of a single gene but, more likely, is the result of an additive expression of several similar multi-copy genes.

In maize, expression of most genes increased with ploidy but some genes showed an inverse relationship to ploidy (Guo et al. 1996). Dosage regulation of gene expression in a euploid series, consisting of monoploid, diploid, triploid and tetraploid maize plants was studied to determine whether such effects are additive or non-additive in general when the collective regions of the genome are varied together. The transcript levels of 18 different genes including 15 random cDNA clones from maize leaf tissue, as well as *Adh1*, *Adh2* and *Sus1* were studied and found that there were no significant expression level changes observed in monoploid and triploid plants (Guo et al. 1996). The magnitude of increase is so extreme that there is nearly a three fold higher mRNA level per genome in the monoploid and 6.7-fold higher in the triploid as compared with diploid and tetraploid. If similar trend is expected in sugarcane, this increased ploidy and gene expression might be a contributing factor for the high sucrose/fibre content of sugarcane.

Diversified homeologous alleles from *S. officinarum* and *S. spontaneum* may be present in a sugarcane clone along with homologous allelic

diversity from within the homologous chromosomes of each species. It raises questions on whether specific alleles are differentially expressed and regulated or whether there is additive gene expression across all alleles (Jackson and Chen 2010). Some studies have used EST sequences of sugarcane to identify SNPs in transcript variants to see whether multiple genes are expressed. The high frequency of SNP haplotypes identified for many genes in a single genotype by Cordeiro et al. (2006) suggested that multiple gene variants were being expressed. However, whether these variants were derived from true alleles at the same locus or closely related members of a multigene family at diverse loci was not clarified (Manners and Casu 2011).

Significance of polyploidy in sugarcane—advantages and disadvantages

Sugarcane is probably the most complex crop genome studied to date (Jannoo et al. 2007). Modern cultivars of sugarcane derived from two highly polyploid species namely *Saccharum officinarum* ($x = 10$, $2n = 8x = 80$) and *S. spontaneum* ($x = 8$, $2n = 5-16x = 40$ to 128) constitute a particularly complex case of polyploidy. They have approximately 10-12 homeologous copies of most loci and up to 12 alleles on average. The lowest chromosome number in close diploid relatives of sugarcane are those in the genus *Erianthus* with $2n = 20$, and in the genus *Saccharum* the lowest number is $2n = 40$ in *S. spontaneum*. The genome size of commercial sugarcane variety is >10,000 Mb with the size of sugarcane monoploid genome ~900 Mb, compared to 760 Mb of sorghum and 390 Mb of rice (D'Hont and Glaszmann 2001). Very high level of genetic redundancy present in sugarcane makes it difficult for molecular approaches. Due to its genetic complexity, sugarcane has received very little research interest despite its economic importance, and molecular resources are being developed only in recent times (Grivet and Arruda 2001). It was also pointed out that in sugarcane the high ploidy and complex genome structure creates challenges for transgene expression and in development of molecular markers (Lakshmanan et al. 2005)

There are three documented advantages of polyploidy such as 1) heterosis that provides

increased vigour, 2) gene redundancy as a result of gene duplication and 3) asexual reproduction that enables the polyploids to reproduce efficiently (Comai, 2005) which are present in allopolyploid sugarcane. Polyploids are more vigorous due to heterosis and gene redundancy protects polyploids from the deleterious effects of mutations. Another advantage of gene redundancy is the ability to diversify gene function by altering redundant copies of important or essential genes (neofunctionalization). In polyploids all genes have a duplicated copy that is available for evolutionary experimentation (Comai 2005). They also have greater chances of acquiring new beneficial alleles and are better poised to evolve novel functions in duplicated gene families (Otto and Whitton 2000) and increased ploidy can provide a selective advantage by masking the deleterious fitness effects of mutations. In this regard, it is not the gene segregation in the hybrid which is important in the phenotype of the hybrids or the potential of the parents, but the genome restructuring and the altered gene expression which could be in a highly unpredictable way in the interspecific and intergeneric hybrids of sugarcane will be of greater importance.

Genetic analysis of agronomic traits using molecular markers has been limited in sugarcane because of its complex polyploidy nature. It is difficult to construct a QTL map covering all homeologous chromosomes in this high polyploid (Hoarau et al. 2002). The genetic maps of *S. officinarum* and *S. spontaneum*, parental species of commercial sugarcane varieties, are colinear and differ only by a small number of rearrangements (Ming et al. 1998). Repulsion and preferential pairing of chromosomes derived from either of the parents were reported earlier (Al Janabi et al. 1994; Ming et al. 1998; Hoarau et al. 2001). The genetic and epigenetic modeling may be due to various factors associated with the merger of two genomes. Markers were used for genotype determinations which were dominant with no consideration for allele dosage. They had several limitations like incomplete genome coverage and lack of accuracy. Due to polyploidy, the RFLP profiles of single-copy loci in sugarcane are typically multiple banded and cannot be clearly separated from moderately repeated sequences (Le Cunff et al. 2008). Expressed

sequence tags from sugarcane indicated the presence of multiple haplotypes (Grivet et al. 2003). The indicated multiple alleles at a locus are likely to contribute to phenotypic traits, which are combinations and ratios of alleles that make application of marker-assisted selection and selection of markers linked to a single favourable allele to be handled with more caution in sugarcane. No reports are available for the differential expression of homeologues in sugarcane but reports from other polyploids like cotton, maize, wheat and *Arabidopsis* gives hints of dosage-dependent gene regulation.

Being a crop with diverse uses and large diversity, the contributions of polyploidy to its biological advantages are many. Polyploidy often show novel phenotypes that are not present in their diploid progenitors or exceed the range of the contributing species. The major thrust of sugarcane variety improvement programs is to increase sugar yield. Sugar yield depends on cane yield and sugar content of the harvested material. Many favourable adaptability characters, stress resistance factors and yield contributing traits are present in such related species which have varying ploidy level. The gene introgression from wild related species to cultivated sugarcane through hybridization is possible due to the fertility of most of the hybrids and their diploid like meiotic behaviour of chromosomes.

Sugarcane as a model organism for studying gene expression in allopolyploids

Sugarcane is a field crop with high economic importance. It contributes to more than 75 % of the sugar requirement across the world, as a sweetener. As a fuel crop also sugarcane is gaining more attention. The annual sugarcane production across the world is nearly 1.74 billion tonnes (Manners and Casu 2011). Besides a major alternative to the geofuels used in motor vehicles, ethanol is a base material in the chemical industry. The high biomass production capability of sugarcane and its interspecific/intergeneric hybrids is now being exploited for developing energy canes. The sugarcane bagasse left out after extraction of juice is being used in paper production. Huge diversity present in the wild related species and its ready availability in the gene banks is helpful in their use in further genetic improvement of sugarcane for its

diversified uses. In India, more than 900 accessions of *S. spontaneum* collected from different parts of the country are being maintained at Sugarcane Breeding Institute. *S. spontaneum* is having a rare distinction among angiosperms having a large number of cytotypes (mostly in the polyploidy series with multiples of $x = 8$), with chromosome number ranging from $2n = 40$ to 128. Similarly, a large collection of *Erianthus* species including accessions of *E. elephantinus* and *E. ravennae* with $2n = 20$ are also being maintained clonally in the field gene banks.

The cultivated sugarcane is an allopolyploid with high ploidy level ($2n > 10x = 100-130$) which contain genomes of *S. officinarum* and *S. spontaneum*. Increase in chromosome number due to $2n+n$ transmission in the hybrids and backcross hybrids of *S. officinarum* x *S. spontaneum* occur when *S. officinarum* is used as the female parent. The sugarcane varieties under commercial cultivation are clonal selections from large populations of inter-varietal hybrid progeny. Even at the higher ploidy level of $10x$ or more, the diploidized meiotic mechanisms in the commercial sugarcane varieties make them amenable to further breeding by hybridization and selection because of good pollen fertility and seed production. Intergeneric hybridization for introgression of genes is being done with genera *Sorghum*, *Sclerostachya*, *Narenga*, *Miscanthus* and *Erianthus*. Intergeneric and interspecific hybrids involving cultivated sugarcane and the related species are perennials and can be maintained for any number of years by vegetative propagation. Most of these hybrids are vigorous and often fertile giving sexual progeny. Because of these, compared to most of the other crop plants which are seed propagated annuals, *Saccharum* allopolyploid hybrids form an ideal material for the study of genome modifications (due to duplicated genomes) in the hybrids as the hybridity can be perpetuated. The parental material belonging to *Saccharum* and related species used can also be clonally maintained for comparative studies. The transgenic protocols using biolistic method and *Agrobacterium* mediated transformation are in place for sugarcane (Arvinth et al. 2010), which can also be useful in the study on duplicated genes. The effect of the complex genome architecture in sugarcane on gene expression patterns and gene

regulation will be better understood with the information on basic genome sequence where allele complexity and gene family composition can be more accurately assessed (Manners and Casu 2011).

Sugarcane could be a model organism for study on genome restructuring consequent to wide hybridization and other related aspects such as function of unreduced gametes, bivalent pairing of chromosomes at high ploidy levels, chromosome elimination in hybrids, altered gene expression due to allopolyploidy, allelic variations and gene dosage effect.

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