THE SUGARCANE GENOME: A SYNTHESIS OF CURRENT UNDERSTANDING, AND LESSONS FOR BREEDING AND BIOTECHNOLOGY

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Abstract

An understanding of the *Saccharum* genome and insight into its evolution and organisation is necessary for making informed decisions in breeding, germplasm introgression and biotechnology. Over the past 80 years, much has been learned about the sugarcane genome from cytological studies, breeding experiments, chemotaxic markers, and more recently from molecular diversity studies, DNA mapping, and molecular cytogenetics. In addition, detailed DNA mapping and sequencing studies in other plants can provide further clues to genome organisation in sugarcane. In the first part of this report, information from published sources is reviewed to produce a synthesis of current knowledge regarding the *Saccharum* genome. It is clear that there are important gaps that require filling before a complete picture of the genome organisation is obtained. In the second part of this report, the information is used to re-assess some of the conceptual structures used in sugarcane breeding and biotechnology.

Introduction

Modern sugarcane cultivars (*Saccharum* spp. hybrids) are essentially advanced generation hybrids between *Saccharum officinarum* L (2n=80), and *S. spontaneum* L (2n=40-128). This hybridisation is characterised by the functioning of 2n gametes in the *S. officinarum* female parent, giving rise to progeny with a 2n+n chromosome complement, although n+n transmission also can occur. Crossing I_1 progeny to *S. officinarum* again results in 2n+n transmission, but in later generations only reduced gametes occur. Commercial sugarcane cultivars (2n=99-130) thus have complex polyploid, aneuploid genomes that make classical genetic, molecular genetic, and breeding studies difficult to interpret, as information on the structure and organisation of the genome has been largely speculative. Recently, molecular studies in sugarcane and relatives such as maize, rice and sorghum, as well as unrelated taxa, have provided new data that can be used to refine our understanding of the genetic architecture of sugarcane and its progenitors. This information can be used to guide research in breeding and biotechnology by providing a framework around which studies can be designed and interpreted. This paper reviews this information and its possible implications for contemporary sugarcane improvement.

Genome organisation of *Saccharum*

Phylogenetic relationships within Saccharum

The genome of modern sugarcane (*Saccharum* spp. hybrids) needs to be understood in relation to the genome composition of its *S. officinarum* and *S. spontaneum* progenitors. Daniels and Roach (1987) gave a detailed review of the taxonomy of *Saccharum* and summarised the main hypotheses regarding the evolution of *S. officinarum*. Briefly, *S. spontaneum* (2n=40-128), is thought to most closely resemble the ancestral *Saccharum* form, due to it wide geographic distribution and wide range in chromosome numbers. *S robustum* EW Brandes & Jeswiet ex Grassl (2n = 60 and 80), and *S. officinarum* (2n=80, plus aneuploid forms) are thought to be derived from *S. spontaneum* through introgression with other taxa, principally *Miscanthus* Anderss, *Sclerostachya* A Camus and *Erianthus* Michx. *S. officinarum* is found only under cultivated conditions, and *S. robustum* is assumed to be its immediate ancestor. *S. spontaneum* from New Guinea with 2n=80 contains leaf flavonoids that are found in *S. robustum* and *S. officinarum*, but that are absent from *S. spontaneum* with 2n≠80. As such, 2n=80 *S. spontaneum* may represent an intermediate in the evolution of *S. robustum* (Daniels et al., 1975). Evidence from RFLP analysis has, however, shown that *S. officinarum* contains some markers present in *S. spontaneum*, *Erianthus* or *Miscanthus*, but absent in *S. robustum*. The direct origin hypothesis of *S. officinarum* from *S. robustum* is thus not entirely satisfactory (Lu et al., 1994). Analysis of satellite DNA and inter-*Alu*-like sequences, has suggested that *Miscanthus* is more closely related to the *Saccharum* genus than *Erianthus* (Alix *et al.*, 1998; 1999).

The basic genome size of Saccharum *is approximately double that of rice*

The haploid genome size (1C value) of a range of anonymous *Saccharum* species has been reported as varying between 2547 and 4183 Mbp (Arumuganathan and Earle, 1991). D'Hont (unpublished data) obtained DNA amounts of 7.68 pg for *S. officinarum* (2n=80) and 6.30 pg for *S. spontaneum* (2n=64), which would translate to 1C values of 3706 and 3040 Mbp respectively (using 1 pg = 965 Mbp). In polyploids, the haploid chromosome number (1C value = n) is not the same as the monoploid number (= x). The monoploid genome size for *S. officinarum* (x = 10) would thus be approximately 926 Mbp, and for *S. spontaneum* (x = 8) 760 Mbp.

At 760-926 Mbp, the size of the *Saccharum* base genome is roughly double the monoploid genome size of rice (415 Mbp), similar to that of *Sorghum bicolor* Moench (760 Mbp), and significantly smaller than that of maize (2500 Mbp). In rice and maize, genes cover about 24 and 17% of the gene space respectively. The difference in gene coverage appears to be due to differences in the amount of repetitive DNA in each species, and is inversely proportional to the size of the genome (Abdelali

et al. 1997, 1998). From this, *Saccharum* genes may cover about 20% of the genome.

The basic chromosome number is x=8 for S. spontaneum *and x=10 for* S. officinarum

The monoploid or basic chromosome number (x) in *Saccharum* has been widely debated (see Sreenivasan *et al.*, 1987 for review). As the monoploid number of the tribe Andropogoneae is x=5 (Gaut and Doebley, 1997), the ancestral *Saccharum* monoploid number also has been proposed as five, and that higher monoploid numbers arose through auto- and allo-polyploidy, accompanied by chromosomal rearrangements (Raghavan and Govindaswamy, 1956b). Wilson *et al.* (1999) suggested an alternate evolutionary pathway, from an x=12 ancestor, as all maize chromosomal rearrangements could be classified as telomeric fusions, nested insertions, intrachromosomal inversions or non-reciprocal translocations of the 12 basic rice linkage groups. In this scheme, the progenitor of *Sorghum* and *Saccharum* has a monoploid number of x=10. Molecular cytogenetics has shown that the monoploid number for *S.officinarum* and *S. robustum* is x=10, and for *S. spontaneum* is x=8 (D'Hont *et al*., 1998). This indicates that genome rearrangement has occurred, irrespective of whether the *Saccharum* ancestor had a monoploid number of five or ten.

Structural differences exist between S. spontaneum *and* S. officinarum *genomes*

The difference in base number implies that structural differences are present between the two genomes. This is supported by mapping studies that have shown that certain independent linkage groups in *S. spontaneum* are represented by a single linkage group in *S. officinarum*. This could be explained by two *S. spontaneum* chromosomes fusing to produce one chromosome in *S. officinarum* (Ming *et al*., 1998; Grivet *et al*., 1996). Ming *et al.* (1998) also identified 11 chromosomal rearrangements that distinguished *S. spontaneum* from *S. officinarum*, and 13 rearrangements differentiating *Saccharum* from *Sorghum*. This may indicate that the divergence of *S. officinarum* from *S. spontaneum* may be nearly as ancient as the divergence of *Sorghum* from *Saccharum*. Physical evidence for structural changes comes from in situ hybridisation of rDNA sequences, which were located at interstitial positions in *S. spontaneum,* and terminally in *S. officinarum* (D'Hont *et al.,* 1996). This also could be interpreted as evidence for chromosome fusion in *S. spontaneum.*

Preferential pairing of chromosomes occurs in S. officinarum *and hybrids*

Preferential pairing between chromosomes observed in both *S. officinarum* and *S. robustum* (Al-Janabi *et al.*, 1994; Grivet *et al.*, 1996; Ming *et al.*, 1998; Hoarau *et al*., 2001), suggests that further structural changes have occurred within the *S. officinarum* genome, although pairing is a complex phenomenon and may be under genetic control as well. Fluorescence *in situ* hybridisation (FISH) on *S. officinarum* clones using satellite DNA as a probe showed dual hybridisation signals on two chromosomes of Crystallina (2n=80), a single signal on 20 chromosomes and no signal on 58 chromosomes. For Black Cheribon (2n=80), no chromosomes showed dual signals, and 28 chromosomes showed a single signal (Alix *et al*., 1998). This is further evidence that the chromosome complement of *S. officinarum* does not consist of eight equal sets of ten homologous linkage groups, and may in fact differ between clones. Ming *et al.* (1998) suggested that the preferential pairing of *S. robustum* and *S. officinarum*, and a monoploid number of 10 for ancestral *Saccharum* may indicate a more ancient origin for these species than *S. spontaneum*. This would agree with the conclusions of Wilson *et al.* (1999). An alternative explanation for preferential pairing, however, could be the presence of genome segments from other taxa (e.g. *Sclerotachya* or *Miscanthus*) present on certain chromosomes. In the cultivar R570, chromosomes of *S. spontaneum* origin or recombinant origin show preferential pairing within the largely *S. officinarum* derived genome (Grivet *et al.*, 1996; Hoarau *et al.,* 2001), whereas crosses within *S. spontaneum* in general do not show preferential pairing (Al-Janabi *et al.*, 1993; Ming *et al*., 1998). Thus, in *S. officinarum*, similar preferential pairing behaviour could be expected if a few chromosomes retain recombinant segments from an ancient hybridisation event.

Genome duplication within the base chromosome set is likely

Although it is well known that the base chromosome number is highly replicated (8-ploid in *S. officinarum*, and 5 to 16-ploid in *S. spontaneum*), little is known about duplication of genome segments within the monoploid chromosomes of *Saccharum*. Sequence information from *Arabidopsis thaliana* (2n=10, x=5) has shown that up to 60% of the genome is duplicated, and that duplicated sections have undergone further rearrangement. In addition, many genes are arranged in tandem arrays of up to 23 repeats (The *Arabidopsis* Genome Initiative, 2000). Large scale duplications detected within the maize genome (2n=20, x=10), provide evidence for a polyploid event in the evolution of maize. In polyploids, reliance on simplex markers for mapping prejudices the identification of duplicated regions. Ming *et al.* (1998) however, reported 47 proximal duplications within the same linkage group, and another 59 duplications across linkage groups, out of a total of 439 loci. It thus appears that duplication within the monoploid chromosome set of both *S. spontaneum* and *S. officinarum* could be fairly extensive. Multiple bands, many monomorphic, obtained from RFLP analysis provide further evidence that probed sequences are duplicated in the monoploid genome.

Hybrids may show an effective increase in monoploid number and genome duplication

The differences in chromosome structure between the two progenitor species and pairing behaviour reviewed above suggest that in *Saccharum* spp. hybrids, the 'hybrid monoploid number' is likely to be greater than ten. This would result in an increase in duplication of sections of the ancestral genomes that are rearranged with respect to each other, and are no longer homologous. In a polyploid where homologous regions are already highly replicated these duplicated regions may not have significant phenotypic effects, but they will influence genome maps. Homologous groups interpolated from common markers in different maps might contain sections of composite linkage groups assembled from duplicated non-homologous regions. In a study of linkage disequilibrium, Jannoo *et al.* (1999) observed 8 out of 59 cases of significant association that mapped

to different linkage groups in R570. These may represent duplications within the genome that have not been mapped in R570. Possible chromosomal rearrangements observed within *S. officinarum* and *S. spontaneum* genotypes by Ming *et al.* (1998), might also be explained by duplicated regions remaining undetected in relatively low density maps.

Aneuploidy in hybrids may result in erosion of chromosome numbers over generations

The complex genome, aneuploidy and chromosome mosaicism in sugarcane hybrids contribute to the formation of unbalanced gametes (Burner and Legendre, 1993), which can lead to loss of chromosomes. For example, a clone obtained from the cross US86-8 (2n=111) x CP77-1776 (2n=113) had a chromosome number of 2n=103, suggesting the loss of nine parental chromosomes. They also demonstrated that the frequency of chromosomes pairing as trivalents increased linearly with generations from the initial I_1 hybrids. In R570, Glaszmann et al. (2001) found that more than two chromosomes may be left unpaired during each meiosis, illustrating another mechanism for the formation of aneuploid gametes. Chromosome loss due to aneuploid gametes implies that continued recurrent breeding might result in the gradual erosion of chromosome number in modern *Saccharum* spp. hybrids.

Single genes, and interaction between genes are involved in the control of phenotype

The ultimate aim of many genome studies in plants is to enable manipulation of important phenotypic traits such as pest and disease resistance, and yield and quality characteristics. Dominant and recessive resistance genes have been found to control resistance, and are involved in broad-spectrum and race-specific resistances. Although good conservation of RFLP marker order in grasses has been demonstrated over large chromosomal segments (Devos and Gale, 1997), several disease resistance genes are not well conserved and may prove difficult to identify through comparative analysis (Keller and Feuillet, 2000). In polyploids, study of single genes may be complicated by potential interallelic interactions at one locus, and recessive genes are likely to be masked.

For yield traits, studies in rice have shown that epistasis (interactions between genes) is an important contributor to phenotype, and that yield components are associated more with multiple loci, than with specific alleles at individual loci (Li *et al.,* 2001). Epistatic effects have also been observed in tillering in barley. (Babb and Muehlbauer, 2001). Heterosis in wheat was correlated with differentially expressed sequences, suggesting that regulatory genes play an important factor in heterosis (Wu *et al*., 2001). Epistasis may be more important in determining phenotype than has been previously assumed, but only with the advent of molecular techniques has epistasis been studied in any detail. In polyploids, epistasis can be inherited, and its effects are likely to be confounded with additive gene effects.

Lessons for breeding and biotechnology

The value of information lies in its potential to guide changes in strategy and methods. Our understanding of the sugarcane genome has increased substantially over the past few years, although many questions still remain. New information allows the re-evaluation of the conceptual basis for research and development involving genetic improvement of sugarcane.

Germplasm introgression

The formation of aneuploid gametes in modern *Sacccharum* spp. hybrids suggests continued recurrent breeding could lead to a reduction in chromosome number over time. The effect of chromosome loss on long term breeding programmes is unknown. If unpaired chromosomes of *S. spontaneum* or recombinant origin have a higher probability of being lost, this, coupled with a high selection intensity for sucrose content, could lead to the reduction in proportion of the *S. spontaneum* genome in advanced generation clones. Although this may not be serious under tropical growing conditions, loss of desirable *S. spontaneum* traits for hardiness, tillering and ratooning ability could affect the long-term success of breeding programmes in subtropical areas such as South Africa. Selection may to some extent mitigate the effects of chromosome loss, but efficiency will be affected.

This question is being addressed in a new research programme at SASEX. A long-term germplasm introgression programme is being initiated to provide a range of selected I_1 hybrid material for use in the breeding programme, and alternative breeding strategies involving a combination of recurrent selection and germplasm introgression are being developed. Chromosome numbers within the elite breeding population will be estimated using flow cytometry, and the relative proportion of *S. spontaneum* and *S. officinarum* genome in selected individuals will be investigated using genomic *in situ* hybridisation (GISH). In addition to counteracting chromosome loss in advanced generation hybrids, the introgression programme also will bring new genes from previously unutilised basic germplasm into the breeding population.

Epistasis, breeding and markers

Breeding programmes generally have relied on models exploiting general combining ability (GCA), or additive genotypic effect in determining breeding decisions. Although GCA models have been successful, evidence for the importance of epistasis should not be ignored. Raghavan and Govindaswamy (1956a) presented data on a *S. spontaneum* (2n=64) x *Sclerostachya* (2n=30) hybrid. The I_1 progeny had 2n=79, indicating functioning 2n gametes in the female parent. Although the parents had Brix in juice of 8º and 3º respectively, 29% of I_1 progeny had Brix greater than 14.5º, and in the F2, 47% of progeny had Brix greater than 14.5º, with five individuals having Brix greater than 20º. Grassl (1980), also remarked on exceptionally high sucrose progeny obtained from a cross between 'sugarcane' and *Sclerostachya*. This provocative data suggests strong epistatic effects for sucrose content between genes from *Saccharum* and *Sclerostachya*. Crosses showing heterosis for certain traits, such as that described above, would be useful for mapping and marker studies for epistatic genes. As epistatic effects can be inherited in polyploids (unlike diploids), family selection can exploit positive epistasis, but will be confounded with additive genotypic effects.

Pest and disease resistance are controlled by both dominant and recessive genes in many crops. In a polyploid such as sugarcane, recessive genes are likely to be masked by other alleles at homologous loci, and thus may not be useful in breeding. In addition, the concept of dominance does not translate from diploids to polyploids where interaction between multiple alleles at a locus occurs - a phenomenon more similar to epistasis. Interactions between genes could complicate the search for molecular markers associated with phenotype. Methods of data analysis that include interactions between markers (e.g. 2-way ANOVA) will provide more information on the effect of the markers than traditional linear regression models, and should be included in analysis procedures.

Mapping

Because of the high ploidy level of *Saccharum* spp. hybrids, mapping is restricted to the use of simplex and duplex markers (Wu *et al.*, 1992), using the pseudo-testcross approach. In progeny of bi-parental crosses, segregation in the progeny depends on the marker genotype of both parents. Marker loci with identical alleles present in both parents will not be available for mapping, but this is dependent on the allele frequency in the population. For example, in a decaploid (approximation of some genome regions in modern hybrids), only 3% of loci with an allelic frequency (p) of 0.2 in the breeding population will be mapped in an out-cross. In a selfed population, however, all simplex markers present in the selfed parent will be able to be mapped (equal to 27% of loci in the base population with p = 0.2). Mapping selfed populations would thus result in higher density maps than out-crossed populations.

Mapping in *Saccharum* spp. hybrids detects a higher relative proportion of *S. spontaneum* loci, due to the lower ploidy level of this species in the hybrid genome (for example, see Grivet *et al.,* 1996). This is because mapping efficiency depends on gene frequency. Important sections of the *S. officinarum* genome are thus likely to remain poorly covered. Crossing an n+n I_l hybrid to *S. spontaneum* should give progeny with only 20 *S. officinarum* chromosomes. Mapping a selfed population from such an individual could give a higher density map of the *S. officinarum* genome, yielding new information on specific traits such as sucrose accumulation.

The level of duplication within the base genome will not be revealed by mapping, due to the confounding effect of ploidy, whereby multiple loci are difficult to distinguish from multiple alleles at one locus. For example, different alleles at one locus will appear to be unlinked loci. In low density maps, homologous groups formed from co-segregation groups through comparative mapping or common markers will assign these alleles to duplicated loci in the same linkage group. This is one possible interpretation of the data of Ming *et al.* (1998), where 47 proximal duplications within the same linkage group were detected. Analysis of the complete segregation patterns of individual RFLP probes could, however, provide lower and upper estimates of the number of loci. For example, assume that in a 'hybrid monoploid value' of x=12, an RFLP probe gives four monomorphic bands and two polymorphic bands in out-crossed progeny of *Saccharum* spp. hybrids. This could arise from a minimum of 26 (4x6+2) or a maximum of 50 (4x12+2) alleles. This would mean a lower limit of three separate loci, or an upper limit of five loci could be responsible for the observed banding pattern. Although subjective, this data could start to provide some picture of gene duplication within sugarcane, and aid in interpreting segregation data. The use of fluorescence in situ hybridisation of BAC clones, ESTs or other specific genic sequences to metaphase or interphase chromosomes could provide more information on the level of gene duplication.

Challenges for the future

Obviously, much is still unknown about the sugarcane genome, and its complex polyploid nature presents difficulties in unravelling its mysteries. One of the main challenges in exploiting the genome for cultivar development is the understanding of gene regulation and expression, in relation to gene sequence and structure. Silencing and differential expression of high copy number genes in polyploids may result in poor correlation between the presence of genes or specific alleles, and the expression of phenotype. Sun *et al.* (2001) demonstrated that heterosis in wheat was correlated with the differential expression of genes between parents and progeny, with 98% of multigene-family-specific cDNAs being differentially expressed. Differential expression of genes could account for as much variation in phenotype as allelic or DNA sequence differences in the coding regions of genes. This introduces new levels of complexity into an already complex system. Despite these difficulties, progress is being made in gene mapping, molecular marker studies, molecular cytology, and genetic transformation. These advances must be incorporated into useful tools that can be used to breed and select improved cultivars. To this end, breeders and biotechnologists need to combine their skills and start using the knowledge gained from the past decade of genome exploration.

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