*Proceedings of the Australian Society of Sugar Cane Technologists*, volume 42, 573–584, 2021

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Australian Society of Sugar Cane Technologists

## Peer-reviewed paper

# Genomic organization of sugarcane cultivars revealed by chromosome-specific oligonucleotide probes

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**Abstract** Sugarcane (*Saccharum* spp.) is probably the crop with the most complex genome. Modern cultivars (2n=100-120) are derived from interspecific hybridization between the noble cane *S. officinarum* (2n=80) and the wild cane *S. spontaneum* (2n=40-128). We investigated the genome organization of important sugarcane cultivars and their parental species using chromosome-specific probes combined with genomic *in situ* hybridization (GISH). This allowed the genomic and genetic characterisation of Australian sugarcane cultivars and one of the major contributing parental clones, Mandalay. The *S. spontaneum* clone Mandalay follows the classical organization of *S. spontaneum* clones with x=8 with a major discrepancy related to an extra six chromosomes compared to the previously reported 2n=96 for Mandalay's clone. Our previous results reported the rearrangements between the *S. officinarum* (x=10) and *S. spontaneum* (x=8) chromosomes, with a most likely scenario of a two-step process leading to x= 9 and then x=8, where each step involved three chromosomes that were rearranged into two. Further polyploidization led to the wide geographical dispersion of *S. spontaneum* clones with x= 8. In modern cultivars, the 13-20% of the *S. spontaneum* contribution originated from cytotypes with x=8. Modern cultivars have mainly 12 copies of each of the first four basic chromosomes and a more variable number for those basic chromosomes whose structure differs between the two parental species. These new insights and cytogenetic tools substantially improve our understanding of the extreme level of complexity of modern sugarcane cultivar genomes and could lead to guiding breeding strategies in the development of new improved varieties for the Australian industry.

**Key words** *Saccharum*, chromosome-specific oligo, FISH, GISH, polyploid, interspecific hybrid, chromosome rearrangements

## INTRODUCTION

Modern sugarcane cultivars (*Saccharum* spp., 2n=100-120) are highly polyploid, aneuploid and of interspecific origin with a genome complexity that exceeds that of all other crops. Interspecific hybrids were first produced in Java and India and mainly involved two *Saccharum* species, *S. officinarum* and *S. spontaneum* (Roach 1972). *S. officinarum* (2n=80), the sugar-producing species or 'noble cane', is believed to have been domesticated from the wild species *S. robustum* (2n=60 or 80 and up to 200) in New Guinea, possibly as far back as 8,000 years ago (Arceneaux 1965; Price 1965). *S. spontaneum* (2n=40 to 128), the wild species, has a very wide distribution from the Mediterranean to the Pacific. It has thin stalks, no or very low sugar, high vigour and resistance to biotic and abiotic stresses.

The concept of interspecific hybridisation in sugarcane is attributed to Soltwedel in 1887 for his first unsuccessful attempt at crossing the two species in search of sugarcane clones resistant to major diseases (Bremer 1961b). Jewiest and Van Harrenveld persisted into systematic nobilisation of *S. officinarum* and *S. spontaneum* progenies and in 1921 the creation of POJ2878 represented the pinnacle of their perseverance. POJ2878 became one of the most proficient clones in sugarcane nobilisation history. Interspecific hybridization provided a major breakthrough in sugarcane breeding, solving some of the disease problems but also delivering unexpectedly large benefits in increasing yield and improving ratooning ability and adaptability under different stress conditions (Roach

1972). Modern sugarcane cultivars are derived essentially from intercrossing of these first 'nobilised' hybrids and involved only a few parental clones (Arceneaux 1965; Price 1965).

Despite the limited number of clones involved in the nobilisation process, the genetic makeup of sugarcane cultivars is particularly complicated due to the high number of chromosomes per clone and the number of backcrosses involved in the nobilisation process. Given this genomic complexity, sugarcane genetics lags behind other major crops and sugarcane breeding remains largely conventional.

Past classical cytogenetic studies have shown that all *Saccharum* species are polyploid and that aneuploidy is frequent in this genus (reviewed in Sreenivasan *et al.* 1987). The lowest ploidy level recorded is 4x and is found only in *S. spontaneum*. However, because all *Saccharum* species are highly polyploid and have small chromosomes of similar size both within and among species, information such as basic chromosome number and the precise behaviour of wild chromosomes during introgression processes, including their exact contribution to present cultivars, could not be established precisely.

The development of molecular cytogenetics in sugarcane in the 1990s initiated an analysis of its complex genome structure. D'Hont *et al.* (1998) showed a basic chromosome number of x=10 in *S. officinarum* and x=8 in *S. spontaneum* using fluorescent in situ hybridization (FISH), and she and her team also characterized the interspecific genomic constitution of modern cultivars (D'Hont *et al.* 1995, 1996). Total genomic DNA of *S. officinarum* and *S. spontaneum* was used to perform genomic in situ hybridisation (GISH) on chromosome preparations of several modern cultivars and showed 10-23% of entire *S. spontaneum* chromosomes and 8-13% of chromosomes resulting from exchanges between *S. officinarum* and *S. spontaneum* chromosomes (D'Hont *et al.* 1996; Cuadrado *et al.* 2004; Piperidis and D'Hont 2001; Piperidis *et al.* 2010).

Recently, Garsmeur *et al.* (2018) proposed, based on a dense SNP genetic map of a cultivar, that this variation in basic chromosome number in *S. spontaneum* with x=8, compared to *S. officinarum* with x=10 resulted from two pairs of three chromosomes that were rearranged into two pairs of two chromosomes.

Several genetic maps have been developed in modern sugarcane cultivars (reviewed by Zhang *et al.* 2014), but none of the current sugarcane maps are saturated and thus it has been impossible to precisely outline the genome architecture of modern cultivars. In particular, the number of copies of each basic chromosome has not been determined.

Molecular cytogenetics has been very important in the last 20 years in interpreting complex genomes such as sugarcane (D'Hont *et al.* 1996, Piperidis *et al.* 2010). Prior to the release of the sugarcane sequence assembly (Garsmeur *et al.* 2018), bacterial artificial chromosomes (BAC) were used to investigate the genome architecture of cultivars. However, this method proved difficult in sugarcane because of the high content of repeated sequence, mainly transposable elements, in the sugarcane genome. Chromosome-specific oligo probes have recently been used to establish karyotypes and to identify chromosome rearrangements in several crops, including rice, maize and potatoes (Braz *et al.* 2018; Albert *et al.* 2019; Xi *et al.* 2020). With the release of the sugarcane reference sequence, we designed chromosome-specific oligo probes and used them to analyse the genome architecture of some modern cultivars and their parental species.

## MATERIAL AND METHODS

### Plant material

Accessions analysed in this study included *S. officinarum* clone Badila, three *S. spontaneum* clones (Coimbatore, Mandalay and SES 196) all from the Sugar Research Australia (SRA) collection based in Meringa, Australia, and four cultivars, R570, Q241$^{\Phi}$, SRA10$^{\Phi}$, SRA27, sourced from SRA Meringa or Mackay (Australia). Sugarcane is propagated vegetatively, so accessions consisted of clones.

### Chromosome preparation

Root tips were harvested from sugarcane plants grown in a glasshouse in 20 L pots containing a mixture of 50% vermiculite/perlite at SRA Mackay in central Queensland. Protoplast preparation, chromosome spreads and GISH experiments followed protocols previously described in Piperidis *et al.* (2013) and Piperidis (2014).

### Chromosome oligo probe design

We used the sugarcane reference sequence assembly of Garsmeur *et al.* (2018) that was assembled in 10 chromosomes to design one oligo probe per chromosome (Probes 1 to 10). Probes were designed by Arbor Biosciences (Ann Arbor, USA) using their proprietary software. The design was based on targeting interspaced regions spread over each chromosome. A combination of sequence similarity and thermodynamic parameters was used to reject any candidates with significant potential cross-hybridization. Overlapping qualified probes were discarded to obtain a final set of unique non-overlapping target-specific probes. Probes were labelled with three different fluorochromes, ATTO 488 (green fluorescence), ATTO 550 (red fluorescence), ATTO 633 (far-red fluorescence).

### FISH/GISH preparation

FISH with chromosome-specific oligo probes and GISH was performed as described previously (Piperidis *et al.* 2013; Piperidis 2014) without DNA blocking, as the chromosome probe designs provided sufficient probe specificity.

Chromosome-specific oligo probes were used separately or in combination depending on the experiment. Not all combinations of probes were used, so in the few cases where we observed inter-chromosome translocation, it was often not possible to identify one of the two chromosomes involved.

For GISH, we used genomic DNA from *S. officinarum* (Badila or BNS 3066) and *S. spontaneum* (Coimbatore or Mandalay). GISH experiments were performed on de-hybridized slides used for FISH after removing the cover slips in 4x SSC/Tween20 at 42°C for 10 minutes followed by a 2xSSC at 42°C for another 10 minutes. Slides then followed the same hybridization protocol used for the chromosome-specific oligo probes.

Finally, slides were counterstained with 4'-6-diamidino-2-phenylindole (DAPI, Vectashield Mounting Media with DAPI). Images were digitally captured with a CCD camera attached to a BX53 Olympus microscope and final contrast of images was processed with the Olympus Cellsens software. Chromosomes for each clone were analyzed from at least four cells and at least two root tips.

## RESULTS

### Chromosome-specific oligo-based FISH probes in *Saccharum*

The probes mainly painted the distal parts of the chromosomes as expected since they were designed on the chromosome arms of a monoploid mosaic chromosome. To simplify the reading of the results, we report either painting on a chromosome (both arms) or half a chromosome (one arm). Some variation in the intensity of painting of individual chromosomes might occur at times and could correspond to technical artifacts or to variation in the degree of homology between the various chromosome copies.

Some of the following results for Badila, Coimbatore, SES 196 and R570 have already been described (Piperidis and D'Hont 2020) and are briefly summarized here for a better understanding of our new results.

### Genome architecture of *S. officinarum* Badila

Each of the 10 oligo probes painted eight (in one case nine) chromosome copies in *S. officinarum* Badila and, thus, painted one basic chromosome set in this clone. These results validated the chromosome specificity of these probes in *S. officinarum* that will be further referred to as probes P1 to P10, corresponding to chromosome 1 to 10 in *S. officinarum.*

In Badila, we found 81 chromosomes instead of the 2n=80 previously reported for this clone (Bremer 1924; Jagathesan *et al.* 1970, cited in Sreenivasan *et al.* 1987) and each of the oligo probes painted eight chromosomes, with the exception of probe P9 that painted an extra ninth chromosomes (Figures 1 and 2a,e). This clone is thus aneuploid, with eight copies of each basic chromosome and one additional copy of chromosome 9 (Figure 1).

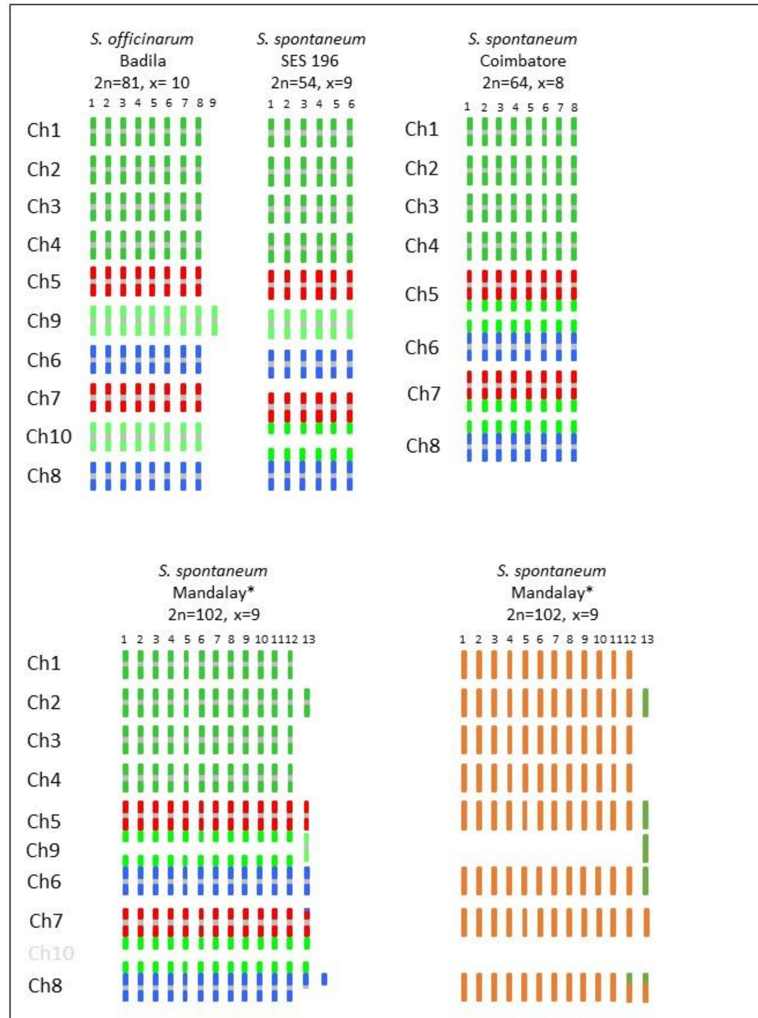


***Figure 1.*** Karyotype schematic representations of the analysed *S. officinarum* and *S. spontaneum* clones. Each bar represents a chromosome, with colours corresponding to oligo probes used in Figure 2. Translocated chromosome segments are represented on the right. On the right side for the Mandalay* representation, *S. officinarum* chromosomes are represented in green and *S. spontaneum* chromosomes in orange, based on estimation from GISH results. Interspecific recombined chromosomes and translocated chromosome arms are represented arbitrarily.

### Genome architecture of *S. spontaneum*

The 10 oligo-probes were used individually or in combination on chromosome preparations of three *S. spontaneum* accessions with different chromosome numbers.

Coimbatore is a *S. spontaneum* accession originating from India (Chennai region) with 2n=64 chromosomes (Panje and Babu 1960). The probes P1 to P4 each painted eight chromosomes, probes P5 and P6 each painted eight chromosomes and probe P9 painted an extra distal part for each of the 16 chromosomes (Figure 1). Probes P7 and P8 each painted eight chromosomes and probe P10 painted an extra distal part for the 16 chromosomes (Figure 2b,f). These results showed a basic chromosome number of x=8 in *S. spontaneum* Coimbatore and the hexaploid level of this clone with 2n=8x=64 (Figure 1).

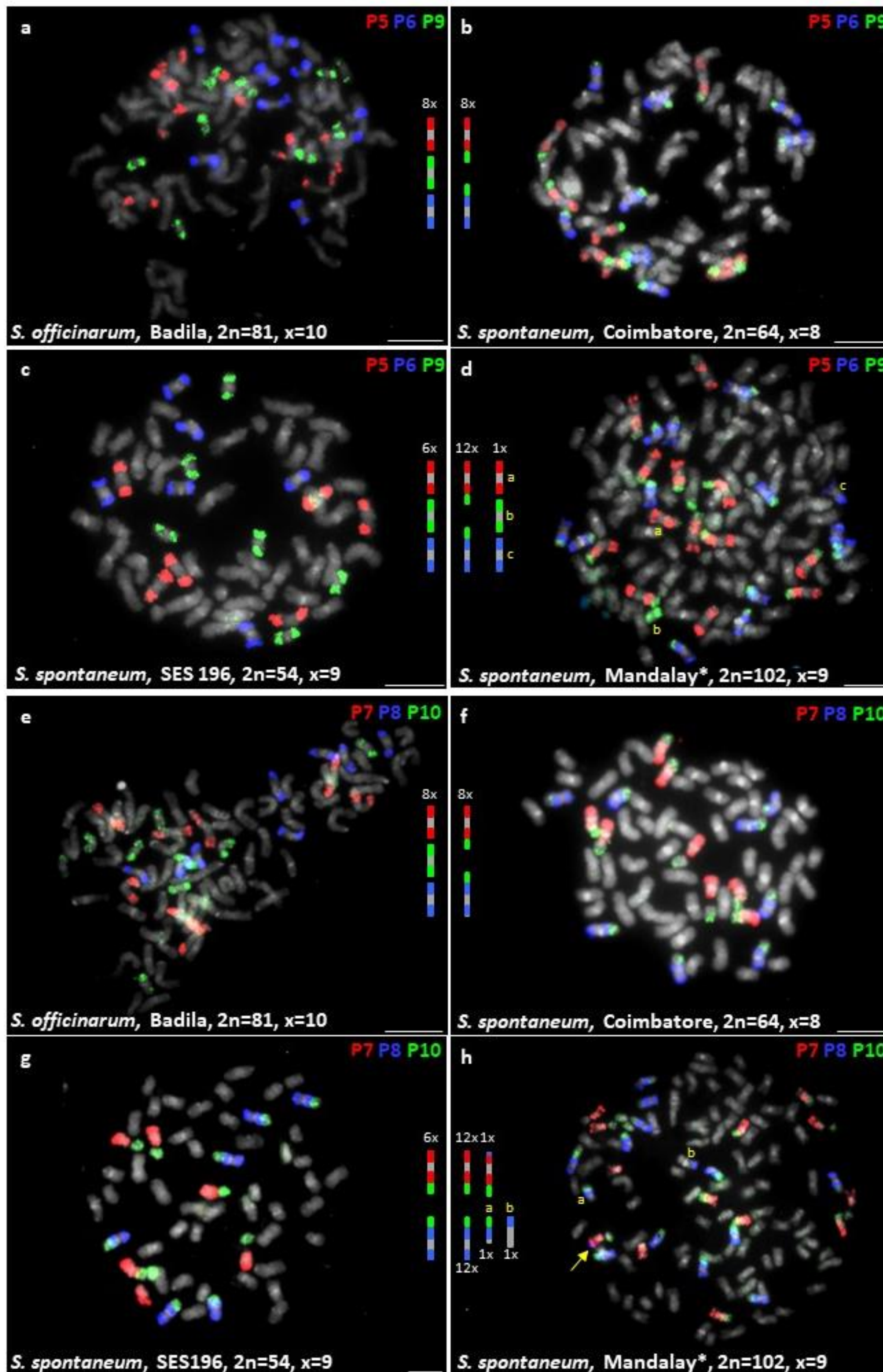


***Figure 2.*** FISH on metaphase mitotic chromosomes of Badila, Coimbatore, SES196 and Mandalay* with chromosome-specific oligo probes combination P5, 6, 9 and P7, 8, 10 indicated on each picture. Chromosomes were counterstained in DAPI (displayed in grey). Schematic representations of the observed chromosome painting patterns are included, and letters (a, b, c) shows refer to the chromosomes on the picture. Arrow on picture (h) points to a translocated insertion of a fragment of chromosome 8 in chromosome 7/10. Scale bar, 5 µm.

Mandalay is a *S. spontaneum* clone originating from Myanmar with a reported chromosome number of 2n=96 (Price 1957; Panje and Babu 1960). However, the clone we used, although labelled as Mandalay (here called

Mandalay* to avoid confusion and enable differentiation), has 102 chromosomes organized in 12 copies of the eight basic chromosomes of *S. spontaneum* with x=8 and an additional six chromosomes (Figure 1). The probes P2, P5, P6 and P9 each painted one of these additional chromosomes (Figures 1 and 2d (a: additional P5, b: additional P9, c: additional P6)). The remaining two chromosomes have rearranged structures with: i) a chromosome labelled by probe P7/P10 which also has a small, translocated fragment painted by P8 at one extremity (Figures 1 and 2h, yellow arrow); ii) a chromosome half painted by probe P8 and P10 (Figures 1 and 2h (a)), while the other half of the chromosome is unknown. In addition, probe P8 also labelled a chromosome segment translocated to a yet unknow chromosome (Figures 1 and 2h (b)). These results showed that this clone had chromosomes with distinct basic chromosome structures.

A GISH experiment using *S. officinarum* and *S. spontaneum* total DNA as probes on chromosome preparations showed that 96 chromosomes were from *S. spontaneum*, three chromosomes were from *S. officinarum,* and three were recombined chromosomes between *S. officinarum* and *S. spontaneum*. Mandalay* is thus not a pure *S. spontaneum* and it cannot be classified as a cultivar either, and it raises some questions about its genome structure. In Figure 3, we have circled in yellow two chromosomes 8 (labelled with P8 and 10) that are of interspecific origin.

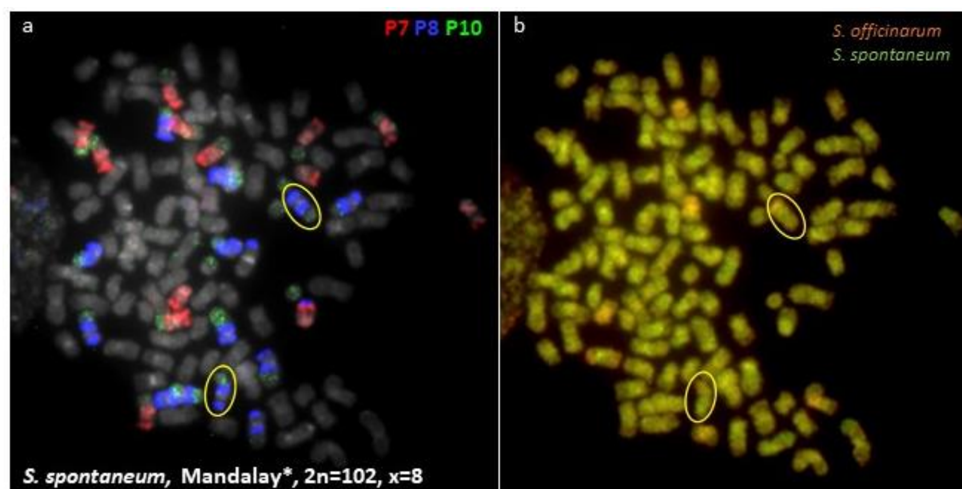


***Figure 3.*** ***(a)*** FISH on metaphase mitotic chromosomes of Mandalay* with chromosome-specific oligo probes P7, 8, 10. Probe combinations are indicated on each picture. Chromosomes were counterstained in DAPI (displayed in grey). ***(b)*** GISH with *S. officinarum* and *S. spontaneum* total DNA detected in orange and green, respectively, and performed as a second hybridization on the metaphase cell. Two yellow circles show two chromosomes 8 of an interspecific origin. Scale bar, 5 µm.

SES196 is a *S. spontaneum* clone collected from northern India (West Bengal region) with a reported chromosome number of 56 (Panje and Babu 1960). We found 54 chromosomes painted as follows: probes P1 to P6 and P9 each painted six chromosomes; probes P7 and P8 each painted six chromosomes; probe P10 painted the remaining distal part of these six chromosomes (Figures 1 and 2c,g).

These results showed a basic chromosome number of x=9 in *S. spontaneum* clone SES 196, with the same basic chromosome organization as the one seen in *S. officinarum* for chromosomes 1 to 6 and 9 and the same basic chromosome organization as in *S. spontaneum* with x=8 for chromosomes 7 and 8. Therefore, SES196 is a hexaploid with 2n=6x=54.

### Genome architecture of modern cultivars

The 10 oligo-probes were used individually or in combination on chromosome preparations of four modern sugarcane cultivars, R570, Q241$^{\phi}$, SRA10$^{\phi}$ and SRA27. GISH was performed as a second hybridization on the same slide after the FISH experiment with genomic DNA from *S. officinarum* and *S. spontaneum* to identify the specific origin of the chromosomes.

In R570, we counted 114 chromosomes, of which 10 (9%) correspond to *S. spontaneum* chromosomes and 10 (9%) to interspecific recombinant chromosomes (Figures 3 and 4). There were 12 copies for chromosomes 1 to 4, with one to four copies corresponding to *S. spontaneum* or interspecific recombinant chromosomes. There were nine copies of *S. officinarum* chromosomes 5, 6, and 9; two copies of *S. spontaneum* chromosome 6, and three copies of *S. spontaneum* chromosome 5, of which two copies corresponded to interspecific recombinants. We found 10 copies of *S. officinarum* chromosomes 7, 8, and 10, and two copies of *S. spontaneum* chromosomes 7 and 8, one of them corresponding to an interspecific recombinant. In addition, we detected a translocated segment of chromosome 5 to one copy of *S. officinarum* chromosome 8 (Figure 4).

***Figure 4.*** Karyotype schematic representation of the analysed cultivars. Each bar represents a chromosome. Colours correspond to oligo probes used in Figure 5. Translocated chromosome segments (T) are represented on the right, some between two-headed black-arrows linking the chromosome segments involved, when known. Interspecific recombined chromosomes and translocated chromosome arms are represented arbitrarily. For R570, the second schematic represents the results from GISH with *S. officinarum* chromosomes represented in green and *S. spontaneum* chromosomes in orange.

In Q241$^{\Phi}$, we counted 112 chromosomes (Figures 4 and 5a,b). There were 10 copies for chromosome 1, and 11 copies for chromosomes 2 to 4. For chromosomes 1 to 3 we also found two translocated copies onto two undetermined chromosomes . We identified seven to nine copies of chromosomes 7 to 10 that we attribute to *S. officinarum* given their structures and three and four copies that we attribute to *S. spontaneum* given their structures. One segment of chromosome 10 appeared translocated to one copy of chromosome 1.

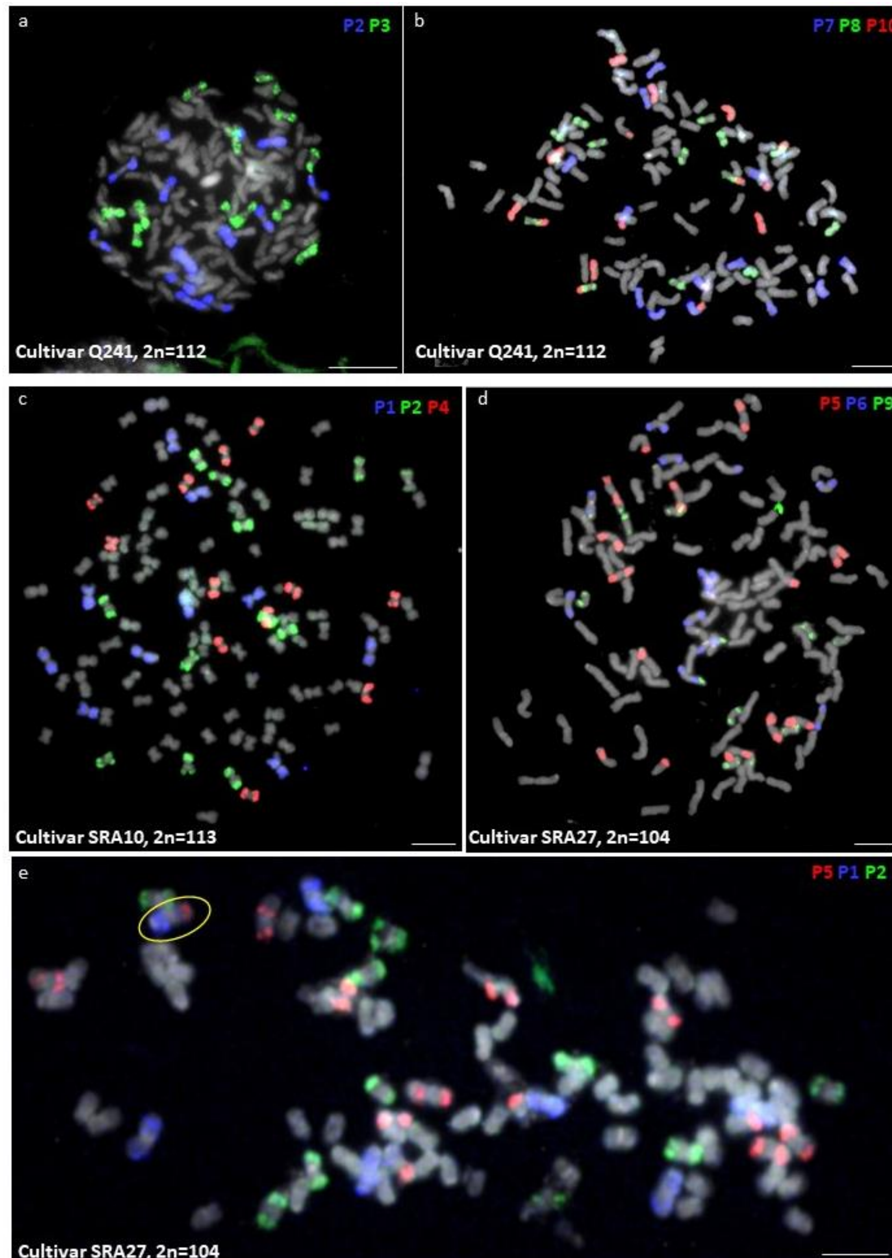


***Figure 5.*** FISH on cultivars Q241$^{\Phi}$, SRA10$^{\Phi}$ and SRA27 with chromosome-specific oligo probes on metaphase mitotic chromosomes. Probe combinations and accession names are indicated on the pictures. (e) The yellow circle shows the translocated chromosome 1/5 in cultivar SRA27. Chromosomes were counterstained in DAPI (displayed in grey). Scale bar, 5 µm.

In SRA10$^{\Phi}$, we counted 113 chromosomes (Figures 4 and 5a,b). We found 12 copies for chromosomes 1 to 4. We identified seven to nine copies of chromosomes 6 to 10 that we attributed to *S. officinarum*, and two to four copies that we attributed to *S. spontaneum* given their structures. Twelve translocated chromosomes have been revealed for chromosomes 5 to 10, but we have yet to determine the other chromosomes involved in these translocations.

In SRA27, we counted 104 chromosomes (Figures 4 and 5d). We found 11 copies for chromosome 1, a translocated chromosome between a chromosome 1 and a chromosome 5 (Figures 4 and 5e), and 12 copies for chromosomes 2 to 4. We identified seven to eight copies of chromosomes 6 to 10 that we attribute to *S. officinarum*, and two and three copies that we attribute to *S. spontaneum* given their structures. Another six translocated segments found from chromosome 5, 6 and 7 have yet to be attributed.

## DISCUSSION

We analysed the genome of *S. officinarum* Badila and three *S. spontaneum* clones (Coimbatore, SES196, Mandalay*),as well as the architecture of four modern sugarcane cultivars using chromosome-specific oligo probes. The probes were designed from the reference sequence of the clone R570, assembled in 10 *S. officinarum* basic chromosomes in Garsmeur *et al.* (2018). One probe was designed for each of the 10 assembled chromosomes.

In *S. officinarum* Badila, we showed that each of the 10 oligo probes painted eight chromosome copies in *S. officinarum* (with an extra chromosome painted with probe 9), showing that each of the 10 probes corresponded to the 10 basic chromosomes in this species.

The sugar-rich group of *S. officinarum*, also called the noble canes, is believed to have been domesticated in New Guinea from the wild species *S. robustum* (2n= 60, 80 and up to 200) (Brandes 1956; review in Sreenivasan *et al.* 1987). A basic chromosome number of x=10 has been proposed, based on being the most common number in the Andropogoneae tribe (Bremer 1961a) which is also supported by the physical mapping of two types of rRNA genes (5S and 45S) using FISH on a few *S. officinarum* clones (D'Hont *et al.* 1998). *S. officinarum* Badila has eight (nine in one case) copies of 10 chromosomes, confirming a basic number of x=10 and a global octoploid level despite the extra chromosome 9 that we found.

Although clones from the species *S. officinarum* were reported to mainly have 2n=80, atypical aneuploids with some exceptions in the range of 78 to 120 have been reported (reviewed in Sreenivasan *et al.* 1987). Larger deviations were also reported in some accessions (Bremer 1924; Price 1960; Jagathesan *et al.* 1970, cited by Sreenivasan *et al.* 1987) that were later shown by GISH to be interspecific hybrids with *S. spontaneum* (Piperidis *et al.* 2010). We found no trace of interspecific hybridization in the Badila clone we analyzed; it thus appears to be a pure *S. officinarum* with an aneuploidy chromosome number of 2n=81.

Garsmeur *et al.* (2018) proposed, based on a high-density SNP genetic map of a modern cultivar, that the rearrangements between the basic chromosome of x=10 in *S. officinarum* and the basic chromosome of x= 8 in *S. spontaneum* consisted of two cases where three chromosomes were rearranged into two chromosomes. This scenario was then supported by the genome sequence assembly of a haploid *S. spontaneum* clone SES 208 that was assembled in 32 chromosomes corresponding to four copies of eight basic chromosomes (Zhang *et al.* 2018).

The *S. spontaneum* clone Coimbatore (originating from southern India, Chennai region) showed a basic chromosome number of x=8, with a chromosome organization supporting the genome structure organization proposed by Garsmeur *et al.* (2018).

The second *S. spontaneum* clone that we studied, SES 196, showed a basic chromosome number of x= 9. In this clone, we found that seven chromosomes (1 to 6 and 9) had an identical structure as the seven *S. officinarum* chromosomes, while the two chromosomes 7 and 8 share the same structure as the one found in *S. spontaneum* clones with a basic chromosome number of x=8. Thus, *S. spontaneum* SES196 with x=9 has one rearrangement in common with *S. spontaneum* with x=8 and could represent an intermediate step between x=10 and x=8.

Recently, Meng *et al.* (2019) used oligo-FISH-barcode probes to identify one *S. spontaneum* clone (Nepal-X) with a basic chromosome number of x=10 with 2n=4x=40 chromosomes. This clone showed a global chromosome architecture similar to *S. officinarum*.

The combination of these results suggests that, after its divergence from the lineage of *S. officinarum*, rearrangements occurred in the *S. spontaneum* lineage involving cytotypes with x=10 that through an intermediate step of x=9 led to cytotypes with x=8. Identifying the mechanisms involved will require further investigation, but could imply two successive reciprocal translocations, as reported in Brassicaceae and other Poaceae (Mandkov *et al.* 2010; Wang and Bennetzen 2012).

Clones with x=10 and x=9 are also much less frequent than those with x=8. The chromosome rearrangements that led to x=8 may have been associated with selective advantages and/or the rearranged chromosomes may be preferentially transmitted to the progeny and therefore colonized the species (Martin *et al.* 2017). A recent study of *S. spontaneum* clones from India showed different levels of ploidy in 12 cytotypes, with five euploids 2n=40 (5x), 48 (6x), 56 (7x), 64 (8x), 72 (9x) and seven aneuploids 2n=50, 52, 54, 60, 70, 74 and 76. This suggests different pathways for the evolution of *S. spontaneum* chromosomes with a preferential mesh-type relationship hypothesis providing better adaption to different ecological environment (Sobhakumari 2020).

The third *S. spontaneum* clone in our study is Mandalay. Mandalay has been a "star clone" in Australia as it was widely utilized in nobilisation studies between 1976-1980 (Symington 1989). The high percentage of progenies

derived from Mandalay in selection trials classify Mandalay as the most successful clone used in nobilisation. The clone Mandalay, collected in 1929 by Mangelsdorf, was imported to Australia through the CSR quarantine facility (N. Berding, pers. comm.). In the early 1960s, a panicle of POJ2878 was shipped to the David North Facility at Indooroopilly and was pollinated by Mandalay. After two backcrosses with commercial hybrids, the clone QN66-2008 was produced. This made the initial cross one of the most successful nobilisation crosses in Australia, as QN66-2008 is the parent and/or the grandparent of at least 40 Q cultivars and two SRA cultivars. Cytological studies by Price (1957) reported that Mandalay had 2n=96. The clone used in the cytological experiments was imported from Myanmar in 1929 and kept at the Hawaiian Sugar Planters' Association, while the clone Mandalay* used in our study was sourced from the Australian clone collection kept at SRA in Meringa. We found that Mandalay* had 102 chromosomes and was not pure *S. spontaneum* because six extra chromosomes appeared to derive from *S. officinarum*. Three chromosomes (5, 6 and 9) are individually painted with the probe 5, 6 and 9, precisely as the *S. officinarum* structural organization in Badila. Bielig *et al.* (2003) reported between 46 and 51 bivalents for Mandalay, which suggests that the Mandalay they studied has between 92-102 chromosomes. This shows that their clone Mandalay could have been the same as the one we studied. We still do not know if the clone Mandalay used in the original cross in the 1960s was the same one that was studied by Price in 1957, or if it had already been compromised or mislabelled. Nevertheless, it is important to understand the genomic/chromosomic status of Mandalay as in the last 20 years Mandalay has been used as a *S. spontaneum* clone in cytological/cytogenetical studies, but its interspecific nature could have biased results (Piperidis *et al.* 2000, 2010; Reffay *et al.* 2005). Recently, Mandalay* has also been used by SRA's breeding program in crossing strategies based on its previous performances as a parent, therefore the expected outcomes might differ greatly if the clones are different.

Nonetheless, results obtained on Coimbatore and Mandalay* validated that the variation in basic chromosome number in *S. spontaneum* with x=8 compared to *S. officinarum* with x=10 resulted from two pairs of three chromosomes that were rearranged in two chromosomes as proposed by Garsmeur *et al.* (2018). The results from clone SES196 showed an intermediate step in the evolution of the genome structure of *S. spontaneum*.

The four cultivars that we analysed had between 105 and 114 chromosomes, which sits in the normal range of chromosomes reported for cultivars between 100-120 chromosomes (Piperidis *et al.* 2000, 2010; D'Hont 2005).

In the four modern cultivars we analysed, the *S. spontaneum* chromosomes all came from *S. spontaneum* cytotypes with x=8 ; these are the most frequent types. In the four basic chromosomes (1-4) where the chromosome structure between *S. officinarum* and *S. spontaneum* with x=8 is identical, we found mainly 12 chromosome copies with random translocated chromosomes. For the other chromosomes (5-10) with a distinct structure between the two parental species, we observed a more variable number of chromosome copies (between 8 and 13). Large chromosome structural variations such as the one observed between *S. officinarum* and *S. spontaneum* are known to generate unbalanced gametes, which could explain the more variable chromosome copy numbers for basic chromosomes.

Variation of ploidy between chromosomes or chromosome segments, such as aneuploidy or segmental aneuploidy due to the coexistence of distinct basic chromosome structure from the parental species or due to inter-chromosome translocations are probably tolerated in sugarcane cultivars and their parental species thanks to the high polyploid context.

Our study provides new insights regarding the architecture and evolution of the genomes of the two species, *S. officinarum* and *S. spontaneum*, at the origin of modern cultivars. The complexity level in cultivars results from a combination of at least three main components: 1) the two different species involved that have distinct basic chromosome number; 2) different copy number for each set of homoeologous chromosome; and 3) the number of interspecific chromosomes as well as the number of translocated chromosomes.

Chromosome composition of additional cultivars has been initiated to get a better understanding of the relationships as well as the mechanisms involving the different factors implicated in the making of cultivars. Some clones, such as Mandalay*, POJ2878 and their progenies, have also been integrated in our experimental design in order to better understand what makes one sugarcane a better parent than others, and what makes a cultivar better than the next one in order to guide breeding strategies to boost the breeding of better sugarcanes for the Australian industry.

## ACKNOWLEDGEMENT

We acknowledge financial support from Sugar Research Australia Limited.

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