Meiotic abnormalities in sugarcane (Saccharum spp.) and parental species: Evidence for peri- and paracentric inversions

Article *in* Annals of Applied Biology · August 2023

DOI: 10.1111/aab.12855

CITATIONS
2

READS
40

10 authors, including:

Nina Soares
Escola Superior de Agricultura "Luiz de Queiroz"
7 PUBLICATIONS **26** CITATIONS
SEE PROFILE

Zirlane Portugal
Escola Superior de Agricultura "Luiz de Queiroz"
19 PUBLICATIONS **171** CITATIONS
SEE PROFILE

Raquel Moura Machado
University of Campinas
20 PUBLICATIONS **70** CITATIONS
SEE PROFILE

Amanda Teixeira Mesquita
University of Campinas
9 PUBLICATIONS **45** CITATIONS
SEE PROFILE

Received: 23 April 2023 | Revised: 25 June 2023 | Accepted: 5 July 2023

DOI: 10.1111/aab.12855

Annals of Applied Biology
An international journal of the aab

WILEY

ORIGINAL ARTICLE

Meiotic abnormalities in sugarcane (*Saccharum* spp.) and parental species: Evidence for peri- and paracentric inversions

Gleicy Kelly Oliveira[1] | Nina Reis Soares[1] | Zirlane Portugal Costa[1] | Carmelice Boff Almeida[1] | Raquel Moura Machado[2] | Amanda Teixeira Mesquita[2] | Monalisa Sampaio Carneiro[3] | Eliana R. Forni-Martins[2] | Mateus Mondin[1] | Maria Lucia Carneiro Vieira[1]

[1]Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, Brazil

[2]Instituto de Biologia, Universidade Estadual de Campinas, Campinas, Brazil

[3]Universidade Federal de São Carlos, Araras, Brazil

Correspondence
Maria Lucia Carneiro Vieira, Escola Superior de Agricultura "Luiz de Queiroz," Universidade de São Paulo, 13418-900, Piracicaba, São Paulo, Brazil.
Email: mlcvieir@usp.br

Funding information
Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)

Abstract

The modern cultivars of sugarcane (*Saccharum* spp.) are highly polyploid and accumulate aneuploidies due to their history of domestication, genetic improvement and interspecific hybrid origin involving the domesticated sweet species *Saccharum officinarum* ('noble cane') and the wild *Saccharum spontaneum*, both with an evolutionary history of polyploidy. The first hybrids were backcrossed with *S. officinarum*, and selection from progenies in subsequent generations established the genetic basis of modern cultivars. *Saccharum* genome complexity has inspired several molecular studies that have elucidated aspects of sugarcane genome constitution, architecture and cytogenetics. Herein, we conducted a comparative analysis of the meiotic behaviour of representatives of the parentals *S. officinarum* and *S. spontaneum*, and the commercial variety, SP80-3280. *S. officinarum*, an octoploid species, exhibited regular meiotic behaviour. In contrast, *S. spontaneum* and SP80-3280 exhibited several abnormalities from metaphase I to the end of division. We reported and typified, for the first time, the occurrence of peri- and paracentric inversions. Using in-situ hybridisation techniques, we were able to determine how pairing association occurred at diakinesis, the origin of lagging chromosomes and, in particular, the mitotic chromosome composition of SP80-3280. Interestingly, *S. spontaneum* and recombinant chromosomes showed the most marked tendency to produce laggards in both divisions. Future attempts to advance knowledge on sugarcane genetics and genomics should take meiotic chromosome behaviour information into account.

KEYWORDS
chromosomal inversions, chromosome pairing, in situ hybridisation, meiotic behaviour, *Saccharum*

1 | INTRODUCTION

The sugarcane (*Saccharum* spp.) crop is of considerable industrial importance, accounting for nearly 80% of global sugar production. It is grown in more than 120 countries situated along the tropics and subtropic regions of the world (see Masoabi et al., 2023). Sugarcane is generally regarded as the most sustainable source of biomass for producing biofuels, with high potential for mitigating the effects of climate change without affecting food security (Kline et al., 2017; Long et al., 2015). Crops and by-products can be developed for producing bioelectricity, bioplastics and fertilisers, in addition to cellulosic ethanol.

Saccharum species originated in New Guinea, where sugar canes have been grown for millennia. The earliest record of domestication dates back to around 8000 BCE, and cultivation gradually spread across human migration routes to Southeast Asia and India. This long history of cultivation has facilitated the generation of a diversified germplasm which includes species of the *Saccharum* complex (two wild, *Saccharum spontaneum* and *Saccharum robustum*, and four cultivated species, *Saccharum officinarum*, *Saccharum sinense*, *Saccharum barberi* and *Saccharum edule*) and four interbreeding genera (*Erianthus*, *Miscanthus*, *Narenga* and *Sclerostachya*). Collections currently include interspecific hybrids, commercial cultivars and elite clones (see Barreto et al., 2021; Cursi et al., 2022).

Selection practices in former times resulted in *S. officinarum* clones with a higher sugar content and fewer fibres. These are known as 'noble canes' (Simmonds, 1975). Subsequently, in the late 19th century, new varieties emerged from interspecific hybridisation of the formerly cultivated species (*S. barberi* and *S. officinarum*) and wild *S. spontaneum*. These hybrids were then successively crossed with *S. officinarum* in order to recover the sucrose content. *S. spontaneum* was chosen due to its peculiar attributes, especially hardiness, resistance to diseases, tillering and ratooning ability, which are of remarkable value in the profitability of the crop everywhere (see Barreto et al., 2021; Cheavegatti-Gianotto et al., 2011; Grivet et al., 2004; Matsuoka & Stolf, 2012). Importantly, due to a mechanism known as meiotic restitution, unreduced gametes were transmitted by *S. officinarum* (i.e., 2*n*, its somatic chromosome number) to its progenies (Bremer, 1961a, 1961b; Price, 1963a, 1963b), which accounts for the overrepresentation of the *S. officinarum* genome in subsequent generations. Overall, the origin of modern cultivars is well documented (see Pompidor et al., 2021).

The genus *Saccharum* includes diverse forms of polyploids and exclusively higher order polyploid species (>4*x*), such as *S. officinarum*, a typical octoploid ($2n = 8\times = 80$, $x = 10$), *S. robustum* ($2n = 60$, 80 to 200), and its presumed natural mutant clone, *S. edule* ($2n = 60$ to 122; Grivet et al., 2006). *S. spontaneum* is an autopolyploid with variable chromosome number and aneuploid accessions (Panje & Babu, 1960). It is considered as a mixed ploidy species, with chromosome numbers higher than expected for species in multiples of 8 ($2n = 40$ to 128). The basic number of *S. spontaneum* ($x = 8$) was supposedly reached in two steps by rearrangements from $x = 10$ leading to $x = 9$ and then $x = 8$ (Piperidis & D'Hont, 2020). Current sequencing results for a typical contemporary cultivar (12 homoeologous haplotypes of the R570 cultivar) suggest the existence of three founding genomes in modern sugarcanes, two contributed by *S. officinarum* and also found in its presumed ancestor, *S. robustum*, and one contributed by *S. spontaneum* (Pompidor et al., 2021).

According to pioneering molecular cytogenetic studies, *S. officinarum* and *S. spontaneum* account respectively for 75%–85% and 15%–25% of sugarcane chromosomes. The remaining chromosomes are recombinant from both origins (Cuadrado et al., 2004; D'Hont et al., 1996; Piperidis et al., 2010; Piperidis & D'Hont, 2020), due to pairing and recombination between homoeologous chromosomes. In addition, the incorporation of other germplasm into cultivated backgrounds has so far stymied attempts to decipher the genetic architecture and genomic organisation of modern sugarcane cultivars. Importantly, due to the geographical locations of experimental stations (US, India, Brazil, Australia, etc.) and agricultural requirements, each sugarcane pedigree has particular features. There are differences in the contributions of each ancestral species and hybrid genotypes within the pedigrees. According to Wang, Zhang,, et al. (2022), the genetic complexity of *Saccharum* species has slowed attempts to understand their genomic structures and has hindered efforts in molecular breeding.

As a result of all these processes, sugarcane has an 'artificial' genome of interspecific constitution (polyploid and aneuploid), produced by human intervention, and a complexity that exceeds that of most crops (Gouy et al., 2013). Despite its redundant origin (all modern varieties have primarily the same origin) and genome complexity, including a variable number of chromosomes ($2n = 110$ to 130), from a meiotic point of view, several classic studies have suggested that both parental species and interspecific hybrids predominantly form bivalents at meiosis, as well as the contemporary cultivars (Bielig et al., 2003; Burner, 1991; Nair, 1975; Price, 1963a, 1963b; Suzuki, 1941). Recently, our group has confirmed a bivalent association in the IACSP93-3046 variety ($2n = 112$). This was done using fluorescent in situ hybridisation (FISH) with labelled probes targeting the centromeric regions at diakinesis. These probes allowed us to enumerate the number of centromeres (i.e., 56 bivalents), although in some cells one or two univalents were also found (Vieira et al., 2018).

Herein, our aim was to investigate the meiotic behaviour of representatives of the parental species (*S. officinarum* and *S. spontaneum*) and the SP80-3280 commercial variety. We examined in detail the frequency and types of meiotic irregularities, such as the incidence of both peri- and paracentric inversions in SP80-3280 cells. Using in situ hybridisation techniques, we were able to determine how pairing association occurs in diakinesis and its mitotic chromosome composition. We also analysed the origin of lagging chromosomes (if species-specific or recombinants) during the first and second meiotic divisions. Our findings have implications for sugarcane genetic mapping, genomics and molecular cytogenetics.

2 | MATERIALS AND METHODS

2.1 | Plant material

The following plant material was investigated: (i) clone Caiana Fita ($2n = 80$), representative of *S. officinarum*; (ii) accession SES205 ($2n = 64$), representative of *S. spontaneum* and (iii) the commercial variety, SP80-3280, for which genetic and genomic data are available (Garcia et al., 2013; Balsalobre et al., 2017; Souza et al., 2019). The pedigree of SP80-3280 is shown in Figure S1.

Plant material was kindly provided by the Universidade Federal de São Carlos (UFSCar; http://pmgca.dbv.cca.ufscar.br), a member of the Inter-university Network for the Development of the Sugar and Ethanol Industry (RIDESA); and the IAC Sugarcane Center (https://www.iac.sp.gov.br/areasdepesquisa/cana/index.php?lang=en) run by

the Agronomic Institute of Campinas (IAC), both Brazilian public institutions located in Southeastern Brazil.

Immature panicles (pre-emerged inflorescences still wrapped in the flag leaf sheath) were collected from Caiana Fita and SES205 at the Sugarcane Hybridization Station (IAC) in Uruçuca (14°35′34″ S, 39°17′2″ W, Bahia State), and from the SP80-3280 variety at UFSCar in Araras (22°21′25″ S, 47°23′03″ W, São Paulo State).

2.2 | Meiotic chromosome behaviour

Immature inflorescences of Caiana Fita, SES205 and SP80-3280 were collected and fixed in Carnoy (three acetic acid:one ethanol) solution at room temperature (24 ± 2°C). After 24 h, the fixative solution was replaced with 70% absolute ethanol and flasks stored at 4°C.

Following conventional protocols (Sharma & Sharma, 1980), flower buds were carefully dissected, and anthers placed on a slide in a drop of 2% acetic carmine. After cross-sectioning with a scalpel blade, anthers were lightly crushed to expel the microsporocytes on the slide covered with a 20 × 20 coverslip. Slides were visualised under the microscope and selected for examination.

Meiotic cells from metaphase I to telophase II (including tetrads) were analysed and images captured using an OPTIKAM B3 camera (Optika) and Adobe Photoshop CS5 (Adobe Systems). The percentage of cells with chromosome irregularities was estimated at each stage for a total of 200 cells each of species' representatives (Caiana Fita and SES205), and 850 cells of commercial variety (SP80-3280) due to its interspecific hybrid origin and breeding history.

2.3 | Pairing investigation using FISH with centromeric and telomeric probes

Anther cells at diakinesis were previously selected to prepare a cell suspension according to Murata and Motoyoshi (1995) and Vieira et al. (2018), with modifications. First, the anthers were washed in distilled water and placed in a microcentrifuge tube containing an enzyme mixture consisting of 2% cellulase (Onozuka), 20% pectinase (Sigma) and 1% macerozyme (Sigma) and then kept at 37°C for 2–3 h. Microsporocytes were carefully separated using a micropipette in order to obtain a cellular suspension that was then centrifuged (13,000 rpm for 5 min). The pellet was washed in distilled water (50 µL), centrifuged as above and fixed in Carnoy solution (50 µL) for 5 min. Cells were then resuspended in a new fixative solution (30 µL) and 10 µL of the suspension was dropped on a clean slide and dried at room temperature. High-contrast images were examined under a microscope (Nikon E200) and selected for hybridisation.

To investigate chromosome pairing, we used in situ hybridisation with fluorescent probes to detect centromere sequences. First, genomic DNA was extracted from Caiana Fita, SES205 and SP80-3280 using the CTAB method as described in Vieira et al. (2018). Next, a primer pair previously designed to amplify sugarcane CENT repeats was used (Nagaki et al., 1998; Vieira et al., 2018). The oligonucleotide sequences were CENT-F (5′-GGGTGCGTCCAAAATTATTTC-3′) and CENT-R (5′-GTACCATAGGCTCAACAATC-3′).

The amplification reaction consisted of 1 × buffer solution, 1.5 mM $MgCl_2$, 0.2 mM of dNTP, 0.3 µM of each primer, 1 U of GoTaq Flexi DNA Polymerase (Promega), 40 ng of genomic DNA and ultrapure water for a final volume of 20 µL. The amplifications were performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems) with an initial denaturation step (95°C, 5 min), followed by 35 amplification cycles (95°C for 40 s, 60°C for 50 s, 72°C for 1 min 30 s) and a final extension at 72°C for 10 min.

A standard gel electrophoresis was run to check the size of the PCR products, which were then purified using a Wizard SV Gel and PCR Clean-Up System kit (Promega). Purified DNAs were labelled using the DIG-nick translation labeling kit (Roche) with digoxigenin-11-dUTP, following the manufacturer's instructions.

FISH procedures were carried out according to Schwarzacher and Heslop-Harrison (2000) and Vieira et al. (2018), with modifications. Slides were treated with RNase (100 µg/mL for 1 h, 37°C), fixed in paraformaldehyde (4%, w/v) for 10 min and dehydrated in an ethanol series (70%, 90% and 100%, 5 min each). The hybridisation mixture consisted of formamide (50%, v/v), dextran sulphate (10%, w/v), saline sodium citrate (2 × SSC), sodium dodecyl sulphate (0.13%, w/v SDS) and 3 ng/µL of DNA probe. The hybridisation mixture was previously denatured (10 min, 90°C) and applied to chromosomal preparations. Slides were denatured and hybridised for 10 min at 90°C and 37°C, respectively, in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems) and then incubated in a humidity chamber overnight at 37°C.

The CENT probe was detected with anti-digoxigenin conjugated to rhodamine (Roche). Slides were mounted in DAPI-Vectashield (Vector Labs). Images at diakinesis were captured using a DFC365 FX digital camera (Leica) coupled to a DM 4000B fluorescence microscope (Leica). The selected images were processed using Adobe Photoshop CS5 (Adobe Systems). The hybridisation sites of the top 10 cells were analysed to determine chromosome pairing.

Chromosome associations were also investigated in early prophase I cells, specifically at pachytene using DAPI, a blue fluorescing DNA-specific stain, to reveal chromosome details (see Ahmad et al., 2021) and FISH using telomeric probes. At this stage, the telomeres were labelled using rhodamine-labelled synthetic oligonucleotides (5′ TELO1F—FLUORO—CCC TAA ACC CCT AAA CCC TAA ACC CTA AAC CCT AAA 3′ and 3′ TELO1R—RHOD—CCC TAA ACC CTA CCT AAA CCC TAA ACC CTA AAC CCT AAA 5′, Life Technologies).

2.4 | Origin of lagging chromosomes by genomic in situ hybridisation

Meiotic cells were prepared as mentioned above. Total genomic DNA probes were digoxigenin- and biotin-labelled using the respective DIG-Nick translation kit (Roche) and BioNick DNA Labeling System (Invitrogen). Slides were treated with RNAse (100 µg/mL;

Sigma-Aldrich) at 37°C for 45 min, fixed in formaldehyde (4%, w/v), denatured in 70% formamide in 2 × SSC at 80°C for 1 h 45 min and then dehydrated in an ethanol series (70%, 90% and 100%, 5 min each) at −20°C. The hybridisation mixture (50 µL per slide) consisted of 50% formamide, 10% dextran sulphate, 2 × SSC, 1.5 µL Salmon sperm DNA and 100 ng of each probe, that is, digoxigenin-labelled *S. officinarum* (digoxigenin-11-dUTP kit, Roche Biochemicals) and biotin-labelled *S. spontaneum* (biotin-14-dATP kit, Invitrogen). The hybridisation mixture was denatured at 97°C for 10 min and stored on ice for 15 min. Hybridisation was performed in a humid chamber for 48 h at 37°C.

S. officinarum digoxigenin-labelled probes were detected with sheep anti-digoxigenin FITC (Roche Biochemicals) and Alexa-Fluor 488 (Life Technologies), and *S. spontaneum* biotin-labelled probes detected with anti-mouse biotin (Dako Corporation) and rabbit anti-mouse TRITC (Dako Corporation). Slides were mounted using Vectashield with 4′,6-diamidino-2-phenylindole (DAPI) (Vector Labs). Meiotic chromosome images were captured by a DFC365 FX digital camera (Leica) coupled to a DM 4000B fluorescence microscope (Leica).

2.5 | Counting and mitotic chromosome constitution by GISH

Sugarcane stalks were collected from SP80-3280 field plants and cut into pieces ∼8 cm in length. In the laboratory, the cuttings were placed on trays containing sphagnum moss watered daily and kept at 28±3°C to induce bud rooting. Roots ∼2 cm long were excised and pre-treated with a blocking solution of 8-hydroxyquinoline (0.03% w/v; Sigma) and cyclohexamide (25 ppm; Cayaman Chemical Company) for 4 h 30 min at room temperature. The roots were then fixed in Carnoy solution (3:1 v/v ethanol: acetic acid) for 24 h, transferred to a 70% ethanol solution and stored at 4°C.

For slide preparation, the roots were washed twice in distilled water, hydrolysed in 1 N HCl at 60°C for 8 min, washed again and stained as usual using the Schiff's reagent for 45 min in the dark. Digestion was performed using an enzymatic solution of 2% cellulase (Onozuka), 20% pectinase (Sigma) and 1% macerozyme (Sigma) at 37°C for approximately 90 min. Then the roots were washed twice in distilled water, immersed in 45% acetic acid for 2 min, and the root tips squashed in a drop of 1% acetic carmine. Slides were mounted in Entellan embedding agent (Merck) and examined under an Olympus BX50 microscope. Metaphase images were captured using an OPTIKAM B3 camera (Optika) and Adobe Photoshop CS5 (Adobe Systems). Twenty three intact cells showing well-spread chromosomes were selected for chromosome counting.

For determining the mitotic chromosome constitution, roots were collected, pretreated, fixed and digested as described above. Root tips were immersed in Carnoy solution to prepare slides by the flame-drying technique (Dong et al., 2000). For genomic in situ hybridisation (GISH) analysis, slightly modified previous protocols optimised for sugarcane (D'Hont et al., 1996; Piperidis et al., 2010) were used. Genomic DNA from Caiana Fita and SES205 was extracted as described above and accurately quantified with a Qubit 4 Fluorometer (Invitrogen). DNA integrity was checked by agarose gel (1.2% w/v) standard electrophoresis. To do this, DNA bands were stained with SYBR Safe 0.5× (Invitrogen) and visualised under a UV transilluminator. Genomic DNA probes were labelled by nick translation with digoxigenin-11-dUTP (Roche Biochemicals) for Caiana Fita and the biotin14-dATP kit (Invitrogen) for SES205.

Slides were treated with RNAse, fixed, denatured and then dehydrated as described above. Chromosome hybridisation, detection of *S. officinarum* and *S. spontaneum* labelled probes, and image capturing were performed as described for meiotic chromosomes.

3 | RESULTS

3.1 | Meiotic chromosome behaviour in *S. officinarum* and *S. spontaneum* representatives

As expected, the octoploid *S. officinarum* exhibited regular microsporogenesis: for both divisions, abnormalities were visualised in only ∼6% of the cells (12/209), some at metaphase I (2/34) (e.g., chromosomes not lined up at the equatorial plate) and some at anaphase I (4/23) (e.g., lagging chromosomes). In the second division, the percentage of cells with chromosomes not lined up at the equatorial plate was ∼13% (5/39, metaphase II) and those with laggards, 11% (1/9, anaphase II), both acceptable percentages in autopolyploids (Table 1).

In contrast, ∼52% (111/214) of *S. spontaneum* cells exhibited irregularities, 42% (35/84) in the first and 58% (76/130) in the second division (Table 1). Thirty seven percent (11/30) of metaphase I cells exhibited chromosomes not lined up at the equatorial plate and ∼ 43% (17/40) of anaphase I cells exhibited lagging chromosomes (up to nine chromosomes, although predominantly one or two). Only two cells were found to exhibit chromosome bridges. Lagging chromosomes were observed in up to 36% (5/14) of telophase I cells, although usually only one or two.

During meiosis II, no chromosomal abnormality was found in 42% (54/130) of the cells. At prophase II, ∼54% (28/52) of the cells exhibited up to two chromosomes not incorporated into the nucleus. At metaphase II, up to six chromosomes were found not lined up at the equatorial plate (more frequently two chromosomes). Lagging chromosomes were observed in only one cell (1/4) at anaphase II. Meiosis II is known to be a faster division, and this may account for the low number of cells observed at anaphase II. Nevertheless, a high number of cells was found at telophase II, 67% (16/24) of them exhibiting lagging chromosomes not incorporated into the nuclei.

Remarkably, we visualised asynchronous cells in *S. spontaneum* but not in *S. officinarum*. *S. spontaneum* is a mixed-ploidy species, which may explain the incidence of asynchronous cells. Furthermore, approximately 40% of tetrad cells exhibited four nuclei with no micronuclei, but in the remaining cells (20/33) up to five micronuclei were observed. This leads to the conclusion that a very low number of irregularities occurs in *S. officinarum*, in contrast to *S. spontaneum*, in which 52% of meiotic cells exhibited abnormalities from metaphase I up to the subsequent phases.

TABLE 1 Meiotic abnormalities in pollen mother cells of *Saccharum officinarum* (Caiana Fita, 2*n* = 80) and *Saccharum spontaneum* (SES205, 2*n* = 64). Numbers in brackets are percentages.

	S. officinarum		*S. spontaneum*		
Meiotic phase	No. of cells examined	No. of cells with abnormalities	No. of cells examined	No. of cells with abnormalities	Abnormality
Metaphase I	34	2 (5.9)	30	11 (36.7)	Chromosomes not lined up at the equatorial plate
Anaphase I	23	4 (17)	40	17 (42.5)	Lagging chromosomes
		0		2 (5)	Chromosome bridges
Telophase I	35	0	14	5 (35.7)	Lagging chromosomes
Subtotal	92	6 (6.6)	84	35 (41.7)	
Prophase II	23	0	52	28 (53.8)	Chromosomes outside the nucleus
Metaphase II	39	5 (12.8)	14	8 (57.1)	Chromosomes not lined up at the equatorial plate
Anaphase II	9	1 (11.1)	4	1 (25)	Lagging chromosomes
Telophase II	15	0	24	16 (66.7)	Lagging chromosomes
Metaphase/Anaphase	0	0	1	1	Asynchrony
Anaphase/Telophase	0	0	2	2	Asynchrony
Tetrad	31	0	33	20 (60.6)	Micronucleus
Subtotal	117	6 (5.1)	130	76 (58.4)	
Total	209	12 (5.7)	214	111 (51.8)	

TABLE 2 Meiotic abnormalities in pollen mother cells of the SP80-3280 commercial variety (2*n* = 112). Numbers in brackets are percentages.

Meiotic phase	No. of cells examined	No. of cells with abnormalities	Abnormality
Metaphase I	160	89 (55.6)	Chromosomes not lined up at the equatorial plate
Anaphase I	76	65 (85.5)	Lagging chromosomes
		5 (6.6)	Chromosome bridges
Telophase I	145	132 (91.0)	Lagging chromosomes
		4 (2.7)	Chromosome bridges
Subtotal	381	295 (77.4)	
Prophase II	120	115 (95.8)	Chromosomes outside the nucleus
Metaphase II	100	49 (49)	Chromosomes migrating precociously to poles
		38 (38)	Chromosomes not lined up at the equatorial plate
Anaphase II	12	8 (66.6)	Lagging chromosomes
		4 (33.3)	Chromatid bridges
Telophase II	59	50 (84.7)	Lagging chromosomes
		2 (3.4)	Chromatid bridges
Metaphase/Anaphase	15	15	Asynchrony
Metaphase/Telophase	6	6	Asynchrony
Anaphase/Telophase	10	10	Asynchrony
Tetrad	147	67 (43.5)	Micronucleus
Subtotal	469	361 (76.9)	
Total	850	656 (77.1)	

3.2 | Meiotic chromosome behaviour in the SP80-3280 commercial variety

Of a total of 850 pollen mother cells assessed, only 194 (~23%) exhibited regular behaviour in both meiosis I (excepting prophase I, not analysed herein) and II. The remaining cells (656/850, ~77%) exhibited irregularities that varied in type and frequency depending on the meiotic phase (Table 2). A similar rate of irregularities was found in cells undergoing the first and second division 77.4% (295/381) and 76.9% (361/469), respectively.

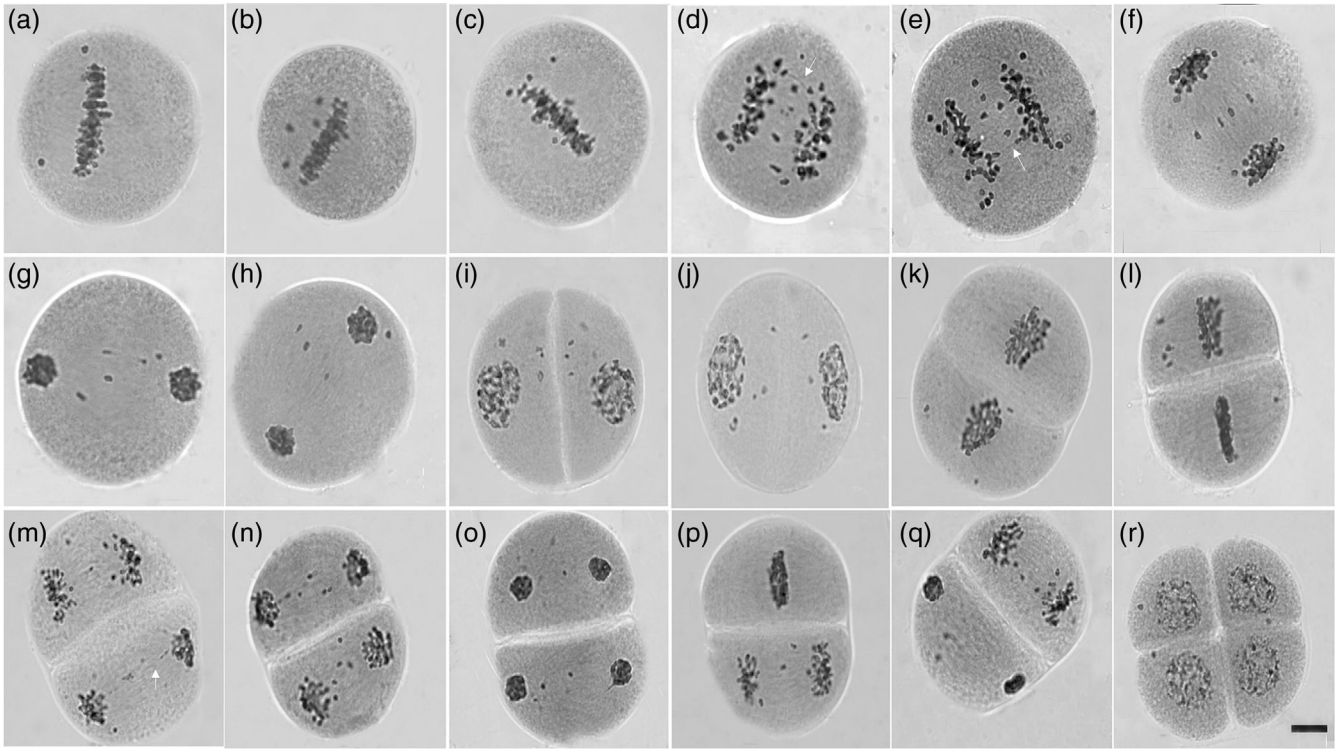

FIGURE 1 Microsporocytes of the SP80-3280 variety ($2n = 112$) during meiosis I (a–h) and II (i–r) exhibiting chromosomes not lined up at the equatorial plate (a–c). Lagging chromosomes at anaphase I (d–f) and telophase I (g–h). Bridges at anaphase I support the existence of paracentric inversions (d–e, white arrow). Prophase II cells exhibiting 2–8 chromosomes not incorporated into the nuclei, randomly distributed throughout the cytoplasm (i–j). Metaphase II cells (k–l), some chromosomes exhibiting early segregation. A tiny chromatid bridge in the bottom cell (m, white arrow) and several lagging chromosomes at late anaphase II (n). Telophase II cells (o) and asynchronously dividing cells with lagging chromosomes (p–q). Tetrad cell exhibiting typical micronuclei, two of them closer to or inside the cell wall, indicating non-inclusion in the final microspore (r). Bars, 10 μm.

Approximately half of metaphase I cells exhibited irregular behaviour (89/160), with chromosomes (predominantly 1 or 2) not lined up at the equatorial plate (Figure 1a–c). In both anaphase I (Figure 1d–f) and telophase I (Figure 1g,h), the percentage of cells with irregularities was notably high, with respective values of ∼92% (70/76) and ∼ 94% (136/145). In addition, up to eight non-aligned chromosomes were frequently visualised. Bivalents were visualised as rod-shaped chromosomes (∼27%, predominantly one or two; Figure 1f). Chromosome bridges were found at percentages of 6.6% (5/76) and 2.7% (4/145) respectively at anaphase I and telophase I.

Almost all prophase II cells (∼96%) exhibited up to eight chromosomes not incorporated into the nuclei (Figure 1i,j). Of the total of metaphase II cells (=100), chromosomes non-aligned on the equatorial plate were visualised in 38% (Figure 1k,l). In the remaining cells (49%), chromosomes seemed to migrate early to the poles (Figure 1l). A tiny chromatid bridge is shown in the bottom cell of Figure 1m. Several lagging chromosomes were identified in anaphase II (Figure 1m,n) and may be remnants not incorporated into telophase I nuclei (Figure 1o); the most frequent values were from two to five chromosomes. Only 11% (7/59) of telophase II cells exhibited complete nuclei with no micronuclei.

We also visualised ∼8% of cells with asynchronous behaviour, (including metaphase/anaphase and anaphase/telophase; Figure 1p,q).

TABLE 3 Chromosomal associations at diakinesis in cells of *Saccharum officinarum* (Caiana Fita; $2n = 80$), *Saccharum spontaneum* (SES205; $2n = 64$) and SP80-3280 ($2n = 112$).

Cell	*Saccharum officinarum*	*Saccharum spontaneum*	SP80-3280
1	38II + 2I	30II + 2I	53II + 3I
2	40II	30II + 1I	53II + 3I
3	40II	32II	54II + 4I
4	40II	31II	55II + 2I
5	40II	31II + 1I	56II
6	38II + 2I	21II + 1I	54II + 3I
7	39II + 2I	32II	55II
8	40II	31II + 2I	52II + 4I
9	40II	32II	52II + 2I
10	40II	32II	55II + 2I

At the end of meiosis, approximately half of the resulting daughter cells (80/147) exhibited four normal nuclei, but in the remaining cells (67/147) there were up to four chromosomes (or fragments) entrapped in micronuclei (Figure 1r).

3.3 | Chromosome association analysis using FISH and centromeric sequence validation

With the aim of identifying chromosome associations at prophase I, we used FISH with centromeric probes. Intense fluorescent signals were observed in centromeric regions at diakinesis in *S. officinarum* (Caiana Fita), *S. spontaneum* (SES205) and SP80-3280 (Table 3).

Ten cells were analysed for each genotype. Most of the *S. officinarum* cells exhibited 40 bivalents (Figure 2a–c). Although

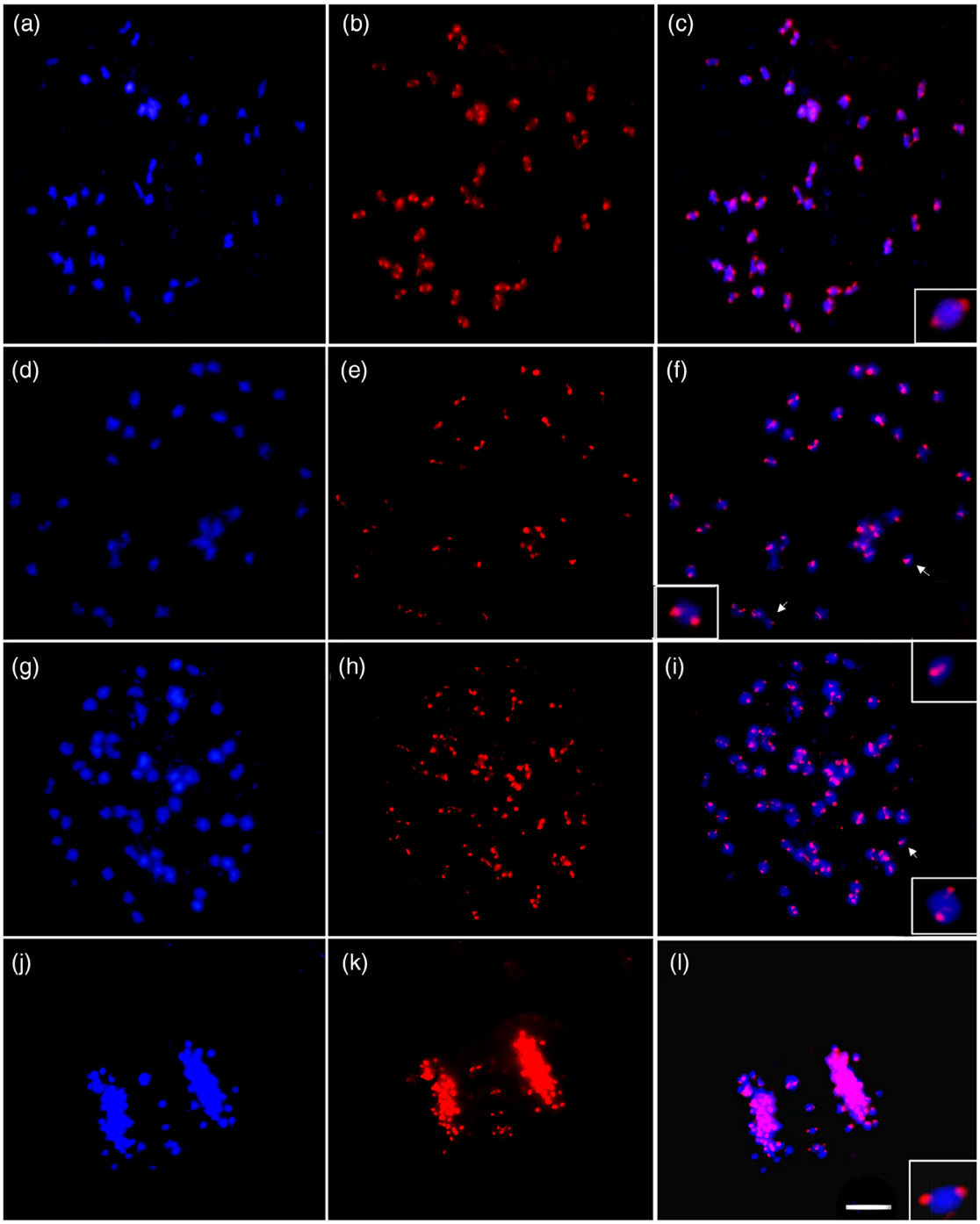

FIGURE 2 Fluorescent in situ hybridisation of centromeric probes hybridised at diakinesis: Chromosomes of Caiana Fita stained with DAPI (blue) (a); Centromeric sites hybridised with the CENT probe detected with anti-DIG-rhodamine (red) (b); Merged images (a/b) showing 40 bivalents; the inset shows a typical bivalent (c); Chromosomes of SES205 stained with DAPI (blue) (d); Centromeric sites hybridised with the CENT probe detected with anti-DIG-rhodamine (red) (e); Merged images (d/e) showing 31II + 2I (arrowed); (f) Chromosomes of SP80-3280 stained with DAPI (blue) (g); Centromeric sites hybridised with the CENT probe detected with anti-DIG-rhodamine (red) (h); Merged images (g/h) showing 56II + 1I (arrowed); the insets show a typical bivalent and univalent (i); Chromosomes of SP80-3280 at anaphase I stained with DAPI (blue) (j); Centromeric sites hybridised with the CENT probe detected with anti-DIG-rhodamine (red) (k); Merged images (j/k) showing lagging chromosomes at anaphase I (l). Bar, 10 μm.

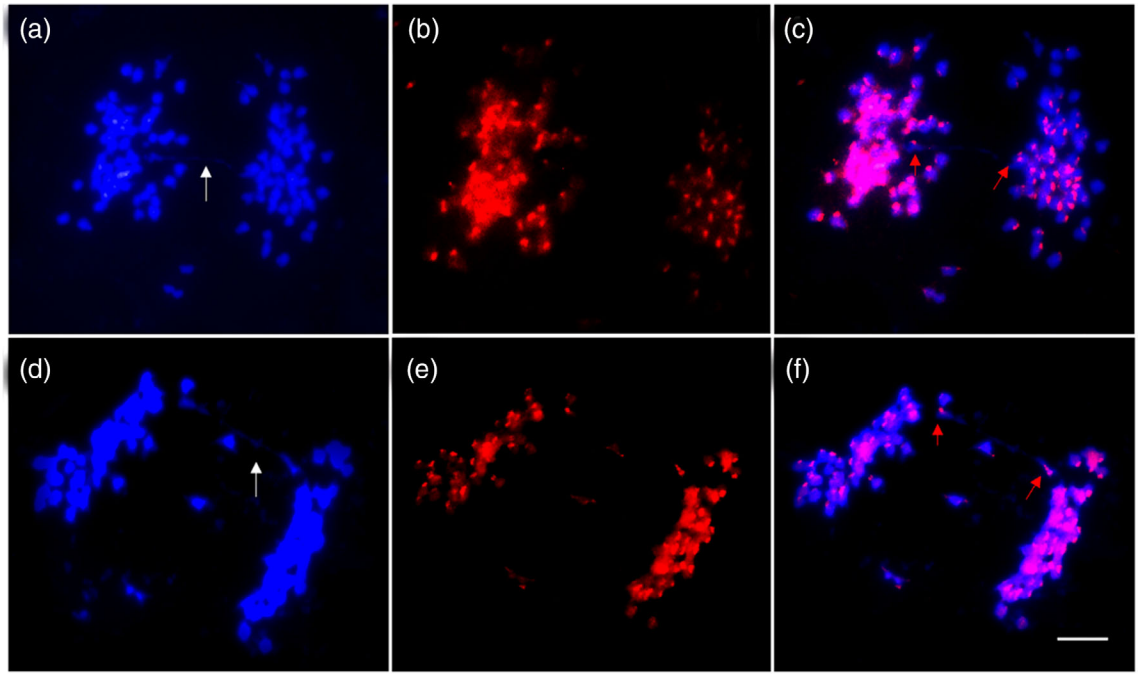

FIGURE 3 Chromosome bridges (arrowed) in meiotic cells of the SP80-3280 variety: Anaphase I-cells stained with DAPI (a, d); Centromeric sites hybridised with the CENT probe detected with anti-DIG-rhodamine (red) (b, e); Merged images (a/b and d/e) exhibiting chromosome bridges; the arrows (in red) show dicentric chromosomes (c, f). Bar, 10 μm.

bivalents (II) were also prevalent in *S. spontaneum*, one or two univalents (I) were observed in half of the cells (Figure 2d–f). With regard to SP80-3280, 56 bivalents were observed in just one cell, with bivalent chromosome associations prevalent at diakinesis, together with two to four univalents (Figure 2g–i), and even lagging chromosomes associated as bivalents (Figure 2j–l).

Centromeric-specific probes were also used for in situ hybridisation of anaphase I cells. For the first time in sugarcane, dicentric bridges were identified (Figure 3a–f).

3.4 | Chromosome analysis in early prophase I cells of SP80-3280

In order to enrich our analysis of chromosomal association, pachytene cells were also examined (Figure 4a–i). Due to the high number of chromosomes, it was not possible to trace with any certainty the individual chromosomes along their length. Sites of possible pairing partner switching were observed in pachytene cells, in which a chromosome may synapse with more than one partner simultaneously (Figure 4b,e,f). However, it was possible to identify some unpaired chromosomal segments (Figure 4c) suggesting a lack of homology. Additionally, centromeric and telomeric probes were used to hybridise pachytene chromosomes, allowing us to observe fluorescent signals consistently with $n = 56$ (Figure 4g–i).

We created a diagram to explain the inversion loop visualised in pachytene cells (Figure 5a–c). To induce a pericentric inversion, two breaks occur on opposite arms or sides of the centromere. The region between the breaks is inverted, and the ends are rejoined to the rest of the chromosome. The presence of a heterozygous inversion involves forming a loop to pair during meiosis (Figure 5d), so that the homologues can line up along their lengths (Figure 5e).

If just one crossover occurs within the inverted region, one chromatid will end up with the inverted region and the other will be normal. The two others will be unbalanced products. Thus, the larger the inverted region, the greater the chance of producing aneuploid gametes, which may not be seen in offspring.

We also visualised chromosome dicentric bridges in some anaphase I and II cells and in *S. spontaneum*. We created schemes to explain their origin (Figures S2 and S3). Undoubtedly, the presence of paracentric inversions is supported by the results. When two breaks in one chromosome arm rejoin after the excised piece has inverted, not including the centromere, this results in a paracentric inversion, and the incidence of two crossovers, one within and another outside the inverted segment, results in a dicentric chromosome and an acentric fragment, which are not transmitted normally. Ultimately, the dicentric bridge will fragment somewhere along its length. The existence of bridges (with or without chromosome fragments) in the first division is a consequence of this type of rearrangement.

Bridges at anaphase II also confirm that a paracentric inversion did occur. As mentioned above, a bridge in only one cell of the dyad indicates that two crossovers have happened, one inside and another outside the inverted segment adjacent (Figure S2). Bridges in both cells of the dyad indicate that three crossovers occurred at pachytene. All the four chromatids are involved, and two crossovers occur inside

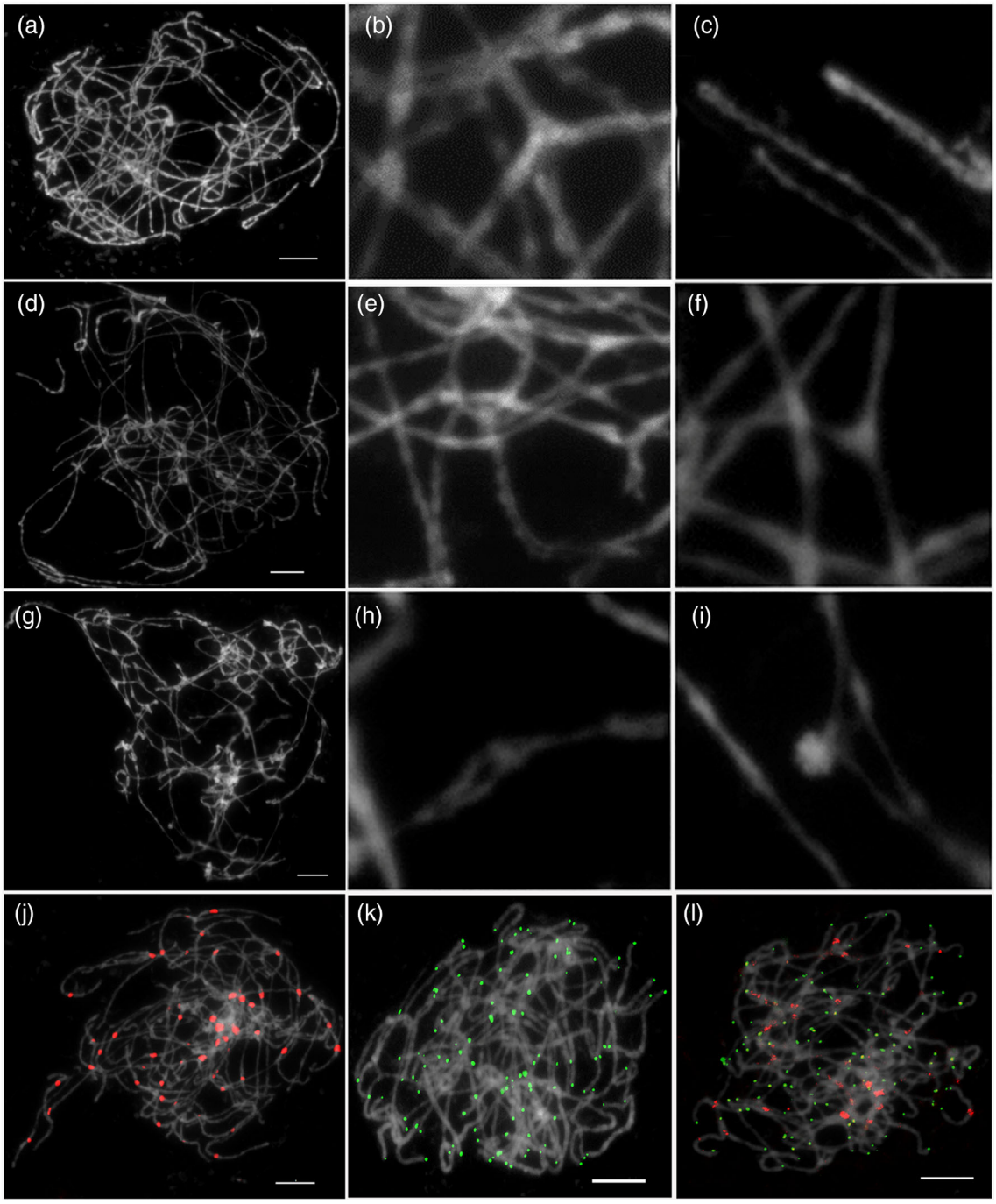

FIGURE 4 SP80-3280 typical pachytene cells (a, d, g–i). Possible sites of pairing partner switching (b, e, f). Unpaired chromosomal regions (c). Fluorescent in situ hybridisation with centromeric probes detected with anti-DIG-rhodamine (red), indicating 56 centromere signals (g). Fluorescent in situ hybridisations with telomeric probes (green) (h). Centromere and telomere signals (i). Bar, 10 μm.

the inversion loop and one outside adjacent. Consequently, two dicentric chromatids are formed. The presence of two acentric fragments can be observed, but is not obligatorily (Figure S2). During anaphase II both dicentric chromatids should be resolved, and bridges become evident in both dyad cells.

In both cases, the acentric fragments are not necessarily present. The fragment size depends on the length of the segment involved in the inversion and the position of the crossover inside the loop.

Alternatively, the presence of inverted duplicated segments and the formation of one crossover inside the loops could explain the excess of bridges at anaphase I. This type of rearrangement results in dicentrics that are resolved during anaphase I. In this case, no acentric fragments are formed (Figure S4).

In both peri- and paracentric inversions, crossovers within the inversion loop generate duplicated/deficient gametes that may result in zygotic lethality. The presence of duplicated/deficient gametes is

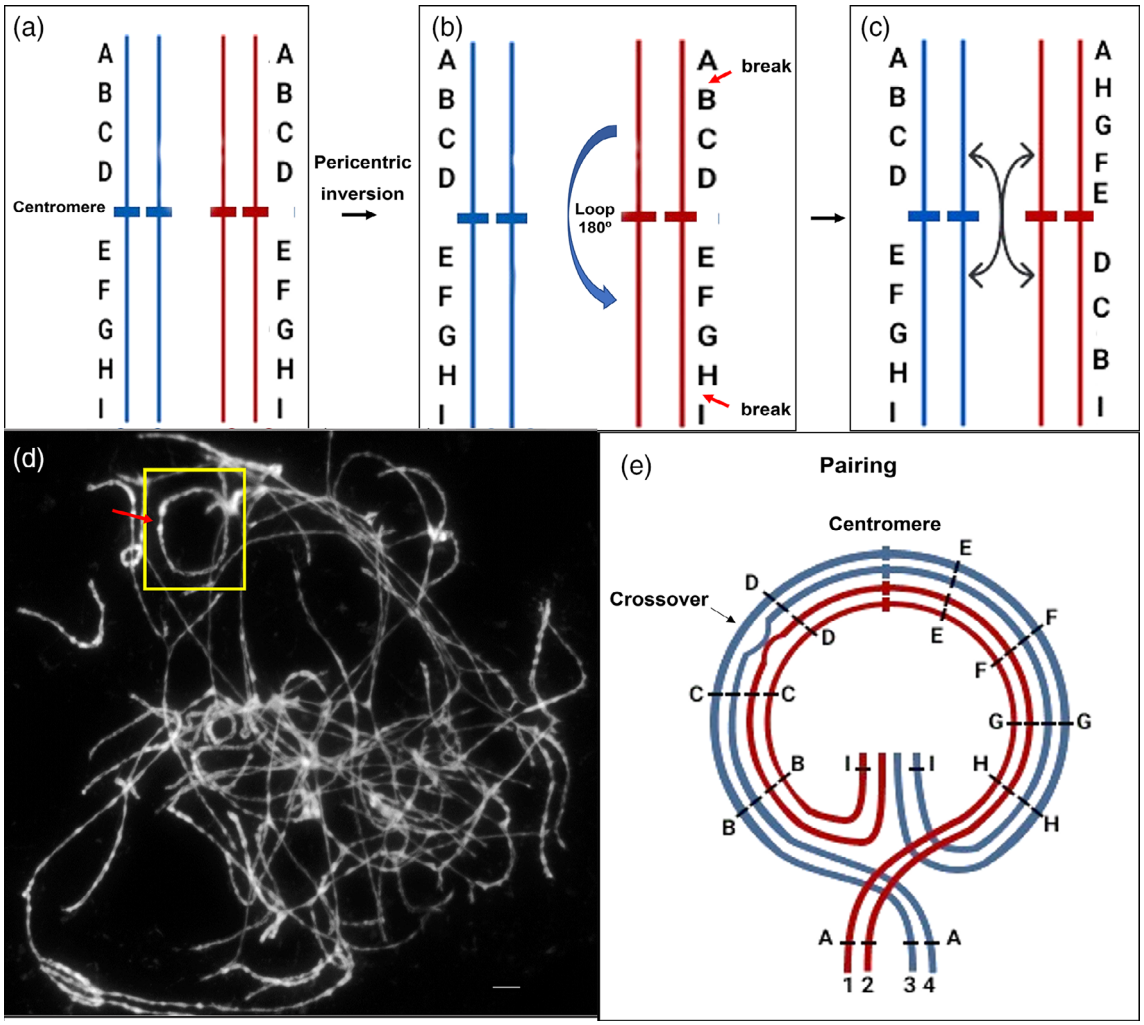

FIGURE 5 Schematic representation of a hypothetical pair of homologues (a): Two breaks occur on opposite sides of the centromere; the region between the breaks is inverted and the ends rejoined to the rest of the chromosome (b–c); An inversion loop in a SP80-3280 pachytene cell (yellow box); the red arrow indicates the centromere (d) (Bar 10 μm); Pairing during meiosis and loop formation so that homologues (or homeologs) can line up along their lengths (e).

expected to translate into a reduction in fertility in inversion heterozygotes. This assumes that the inversion is sufficiently large to induce a crossover probability close to unity.

3.5 | The origin of laggard chromosomes

Laggard chromosomes (one or two in each cell) were visualised as chromosomes not aligned on the equatorial plate. In metaphase I cells half of the laggards were from *S. officinarum* (3) and half from *S. spontaneum* (3). At this stage, no laggards originating from recombinant chromosomes were found. Some 37 laggards (up to six in each cell) were visualised in anaphase I cells. Approximately 19% (7/37) were from *S. officinarum*, ~33% (12/37) from *S. spontaneum*, and ~51% (19/37) from recombinant chromosomes (Figure 6a,b). At the end of the first division, 11 laggards were visualised. Forty-five percent (5/11) were recombinant chromosomes, ~25% (3/11) were from *S. officinarum* and ~25% from *S. spontaneum* (Figure 6c,d; Table 4).

Prophase II cells exhibited up to eight chromosomes not incorporated into the nucleus, totalising 27 laggards. Approximately 18% (5/27) were from *S. officinarum*, ~50% (14/27) from *S. spontaneum* and ~30% (8/27) were recombinant chromosomes (Figure 7a). In metaphase II cells, 13 not aligned on the equatorial plate were visualised. Some 25% (3/12) originated from *S. officinarum*, 33% (4/12) from *S. spontaneum* and ~42% (5/12) were recombinant chromosomes.

Laggards were identified as chromosomes not incorporated into the telophase I nuclei and are most likely remnants from anaphase II cells. Overall, anaphase II and telophase II cells (four in total) contained 19 laggards (up to eight in each cell). Only one chromosome (1/19) was of *S. officinarum* origin. Approximately half of the remaining laggards were of *S. spontaneum* origin (9/19), and half recombinants (9/19) (Figure 7b,c).

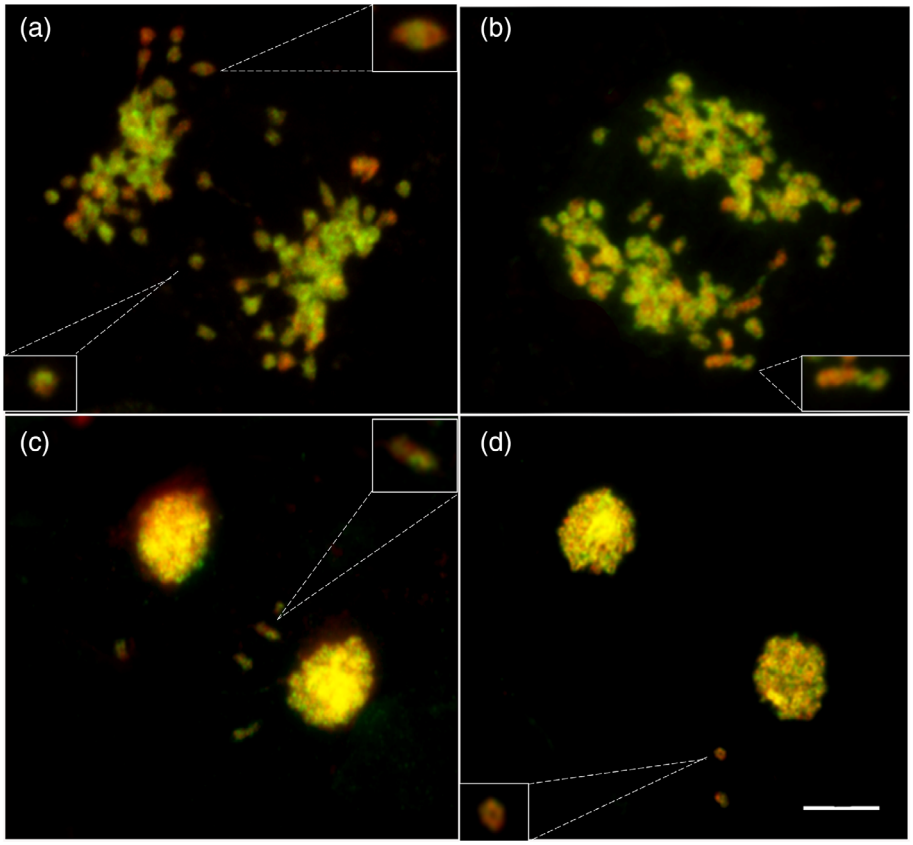

FIGURE 6 SP80-3280 cells at the first meiotic division labelled with genomic probes. The yellow–green (FITC) and red–orange (TRITC) fluorescence indicate hybridisation with *Saccharum officinarum* and *Saccharum spontaneum* DNAs, respectively. (a, b) Anaphase I and (c, d) telophase I. The insets show laggards. Bar, 10 μm.

TABLE 4 Origin of lagging chromosomes in meiotic cells of SP80-3280 using GISH.

Meiotic phase	No. of cells analysed	No. of laggards	*S. officinarum* origin	*S. spontaneum* origin	Recombinant origin
Metaphase I	3	6	3	3	0
Anaphase I	8	38	7	12	19
Telophase I	3	11	3	3	5
Prophase II	13	27	5	14	8
Metaphase II	3	12	3	4	5
Anaphase II	2	13	1	8	4
Telophase II	2	6	0	1	5
Tetrad	4	10	3	5	2
Total	38	123	25	50	48

We also visualised cells with asynchronous behaviour (including metaphase/anaphase and anaphase/telophase cells). At the end of the second division, 10 laggards remained (up to five in each cell) trapped in micronuclei (Figure 7d). These included ~30% from *S. officinarum* (3/10), 50% from *S. spontaneum* (5/10) and 20% recombinants (2/10) (Table 4).

In conclusion, we were able to detect 123 laggards in GISH-treated cells, from metaphase I up to tetrad, and determine their origin. Around 20% (25/123) were from *S. officinarum*, and approximately half of the remaining laggards (50/123) were from *S. spontaneum* and half (48/123) of interspecific origin, indicating the prevalence of laggards originating from the wild species (*S. spontaneum*) and from interspecific recombination events. This technique allowed cytological distinction of the three sugarcane chromosome types with a laggard tendency and capable of originating aneuploid gametes.

3.6 | Counting and mitotic chromosome constitution by GISH

We examined 23 mitotic cells of SP80-3280 to determine their chromosome numbers. The modal value was $2n = 112$ chromosomes (Figure S5). The combination of cycloheximide and 8-hydroxyquinoline resulted in

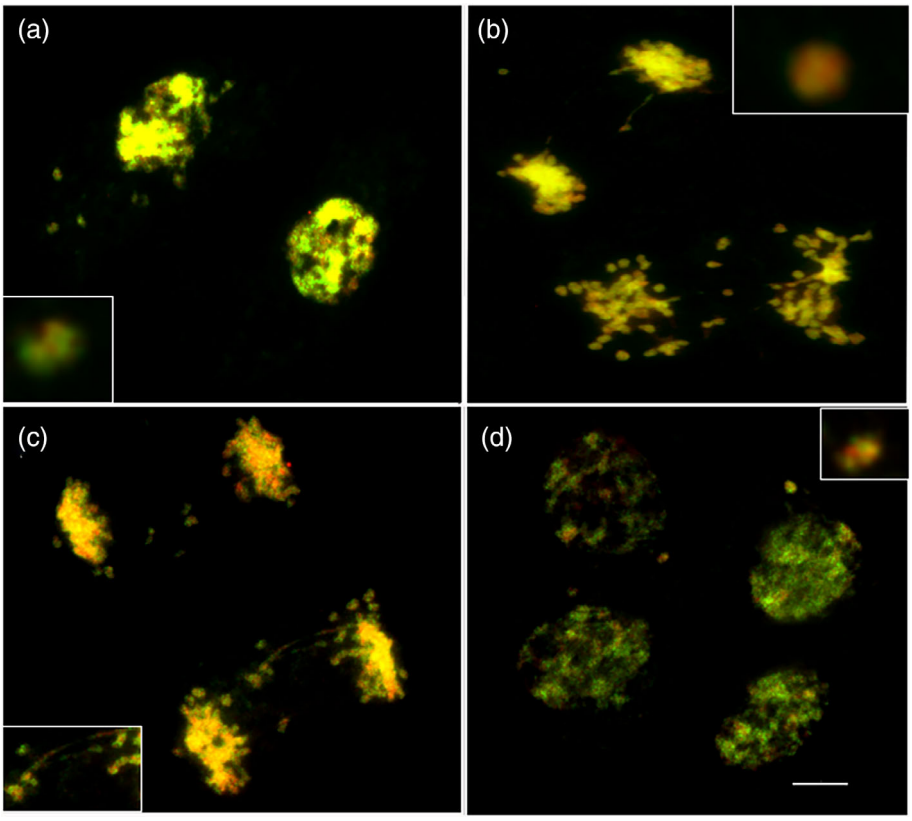

FIGURE 7 SP80-3280 cells at the second meiotic division labelled with genomic probes. The yellow–green (FITC) and red–orange (TRITC) fluorescences indicate hybridisation with *Saccharum officinarum* and *Saccharum spontaneum* DNAs, respectively. The insets show laggards. (a) Prophase II, up to four chromosomes were shown not incorporated into the nuclei; (b, c) Anaphase II; (d) A tetrad cell with micronuclei. Note the recombinant chromosome with laggard tendency. Bar, 10 μm.

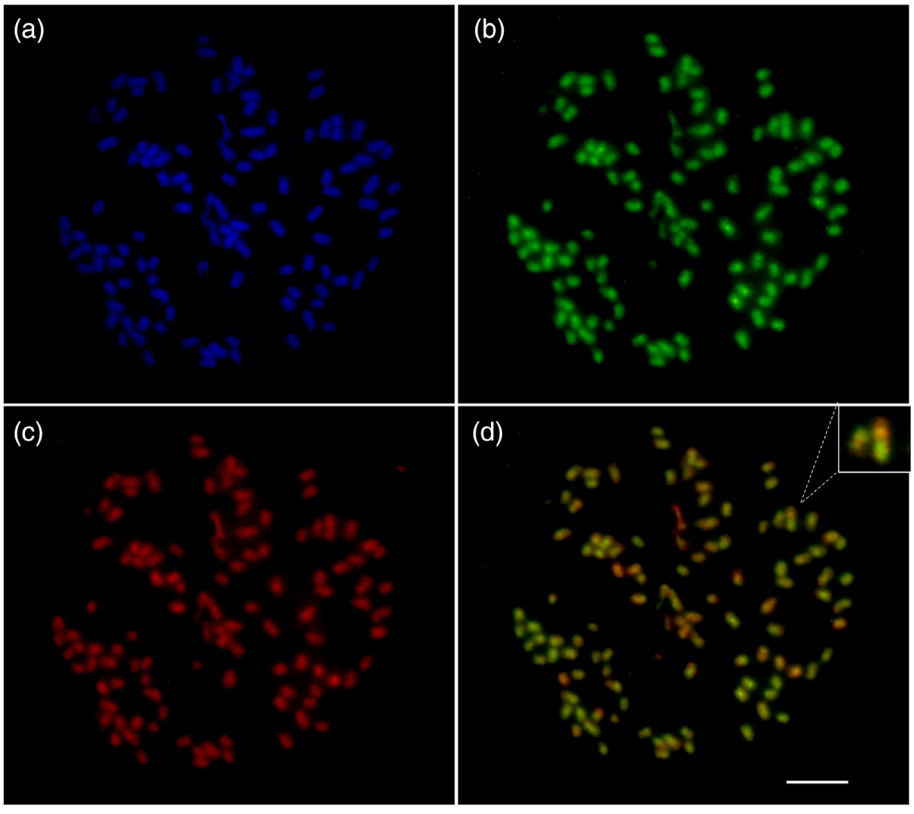

FIGURE 8 Genomic in situ hybridisation of chromosome preparations of the SP80-3280 variety using labelled genomic DNA of *Saccharum officinarum* (Cana Fita) and *Saccharum spontaneum* (SES205): Mitotic metaphase counterstained with DAPI (a); Yellow–green fluorescence (FITC) indicating hybridisation with *S. officinarum* DNA (b); Red fluorescence (TRITC) indicating hybridisation with *S. spontaneum* DNA (c); Merged images (b/c). The inset shows a typical recombinant chromosome (d). Bar, 10 μm.

good chromosome spreads and accumulation of prometaphase and metaphase cells, confirming the effectiveness of this method for sugarcane chromosome counting and allowing us to proceed with GISH.

Herein, we describe Caiana Fita and SES205 genomic probe hybridisation on SP80-3280 chromosomes. These *Saccharum* representatives were selected based on our current knowledge of the

pedigrees of some commercial sugarcane varieties. For instance, Caiana Fita, a noble cane, was one of the first accessions of *S. officinarum* introduced into and cultivated in Brazil ($x = 10$, $2n = 8x = 80$) (Figueiredo, 2008). However, SES205 is a very divergent accession introduced from India and a representative of the wild species, *S. spontaneum* ($x = 8$, $2n = 8x = 64$; see Medeiros et al., 2020).

We examined 10 cells, all with $2n = 112$ chromosomes. Eighty-nine (80%) chromosomes were entirely labelled in green and corresponded to *S. officinarum*; 13 (11%) were entirely labelled in orange corresponding to *S. spontaneum* and 10 (9%) were labelled in green/orange, revealing their interspecific origins due to chromosome exchanges or recombination (Figure 8). There were no non-hybridised chromosome regions.

4 | DISCUSSION

The genus *Saccharum* is well-known for its exclusively higher-order polyploid species, such as *S. officinarum*, *S. robustum* and *S. spontaneum*, the latter two with variable chromosome numbers or cytotypes. In this study, we confirmed that *S. officinarum* exhibits a relatively low number of meiotic abnormalities (~6%), that is, regular meiosis, similar to that of other polyploids in the grass family. In contrast to our findings, some studies have reported abnormalities higher than 30%. Several aspects can influence the rate of normal and abnormal meiocytes, in particular the evolutionary history of the polyploid species (Comai, 2005) and their mode of reproduction. In *Brachiaria brizantha*, a grass of African origin, high levels of meiotic abnormalities were found, and are normally present in odd polyploids, compromising pollen viability and inducing asexual reproduction, for example, apomixis (Risso-Pascotto et al., 2003). In the genus *Avena*, cytogenetic and geographical combined data of diploids and polyploids has shown that the frequencies of multivalent associations and laggards have been influenced by local environmental stress conditions within natural populations (Aissat et al., 2019). On the other hand, *S. officinarum* is a well-adapted, perennial species whose evolutionary trajectory of diploidisation forms the most prevalent bivalents.

Approximately 50% of the cells of SES205 (representing *S. spontaneum*) exhibit abnormalities, including lagging chromosomes from metaphase I to subsequent phases. Moreover, we visualised some asynchronous cells, confirming the classic findings (Sreenivasan & Jagathesan, 1975). The evolutionary history of the autopolyploid *S. spontaneum* may explain the meiotic chromosomal instability observed herein. To clarify, *S. spontaneum* is a mixoploid species (see Zhang et al., 2018), with chromosome numbers in multiples of 8 ($2n = 40$ to 128). According to the classic study by Panje and Babu (1960), the cytotypes should have a typical geographic distribution: in West Asia the numbers range from $2n = 112$ to 128, in the East, $2n = 80$ to 112 and in the Centre, $2n = 40$ to 80. Chromosome numbers of $2n = 64$ are common in India, the origin of SES205.

Subsequently, a novel tetraploid accession (Np-X) that belongs to the ancient Pan-Malaysia group was found to have $2n = 40$ and an unusual $x = 10$ (Meng et al., 2020). According to the authors, this finding suggests a parallel evolutionary pathway of genomes and polyploid series with different basic chromosome numbers. However, at the time, it was also proposed that rearrangements occurred from a basic chromosome of $x = 10$ (probably in the Northern part of India) in two steps, leading to $x = 9$ and then $x = 8$ (descending disploidy). Each step involved three chromosomes that were rearranged to form only two. Further polyploidisation led to wide geographical distribution of clones with $x = 8$ (Piperidis & D'Hont, 2020). Insertional dysploidy has been recorded in three grass subfamilies and appears to be the dominant mechanism of basic chromosome number reduction in grasses (Luo et al., 2009).

Despite the interspecific origin of modern varieties, bivalent pairing prevails, and this type of chromosome association has been documented in classic studies. Subsequently, using FISH on meiotic chromosomes of the IACSP93-3046 variety ($2n = 112$), Vieira et al. (2018) confirmed the predominance of the bivalent configuration. Chromosomal abnormalities were visualised in approximately 70% of IACSP93-3046 meiotic cells, for example, chromosomes not aligned on the equatorial plate, laggards and chromosomes not incorporated into telophase I nuclei, resulting in micronuclei at the end of division and explaining, at least in part, the origin of univalents.

Similarly, it was possible to identify a significant number of cells with irregularities (~77%) in the SP80-3280 variety, including asynchronous cells which were also present in SES205 but not in the representative of *S. officinarum*. Remarkably, the strategy of using centromeric probes proved to be critical for confirming the predominance of bivalents at diakinesis, including laggards at anaphase. It is not always possible to recognise lagging bivalents using conventional protocols.

In conclusion, our findings lend weight to the idea that stable chromosome segregation occurs in modern sugarcane varieties and this has implications, for instance, on linkage analysis. One may assume that sugarcane behaves as a diploid during meiosis. Our results suggest that a synapse regulatory mechanism exists in *Saccharum*, in which probable multivalent associations are resolved into bivalents towards the end of prophase I. This mechanism has been extensively researched and proven to exist in wheat and *Brassica* (Griffiths et al., 2006; Jenczewski et al., 2003; Nicolas et al., 2009; Rey et al., 2017, 2021; Riley & Chapman, 1958). *Brassica napus* is an established allopolyploid species with good meiotic control (see Quezada-Martinez et al., 2022). There are gene clusters responsible for regulating the progression of meiosis and the most promising candidate gene to play this role is thought to be *ZIP4* (reviewed in Soares et al., 2021). It has been suggested that *ZIP4* acts as a scaffold protein containing tetratricopeptide repeats, facilitating the assembly of protein complexes and promoting homologous crossovers (Chelysheva et al., 2007; Shen et al., 2012).

With this in mind, we decided to observe the pachytene cells in SP80-3280. The pachytene is one of the most informative meiotic phases from a chromosomal standpoint. We were able to visualise several possible sites of pairing partner switching, in which a chromosome can synapse with more than one partner simultaneously. It is a common phenomenon in polyploids due to the occurrence of complex interactions and the pairing of three or more chromosomes starting

simultaneously at different points along their length (see Choudhary et al., 2020). Our findings seem to show that this is corrected by the end of pachytene to produce bivalents.

In addition, inversion loops were observed as a result of pericentric inversions leading to unbalanced gametes, confirming a previous report. The Thai sugarcane KPS 01-01-25 cultivar exhibited pairing partner switches and a few small loops that point to inversions (though not typified), duplications or deletions (Thumjamras et al., 2016). No dicentric chromosomes derive from this type of inversion.

Some chromosomal bridges were visualised in anaphase I and telophase I cells of the SP80-3280 commercial variety and *S. spontaneum* (Figure S6), but not in the representative of *S. officinarum*. Finally, we also detected bridges and other irregularities in the final stages of division. These bridges originate due to the formation of dicentric chromosomes. Homologous pairing during meiosis in a paracentric inversion heterozygote is also maximised by the formation of an inversion loop. If a crossover happens within this loop, dicentric and acentric chromosomes are formed. The acentric fragment is lost during meiosis as it cannot be pulled to either pole due to the absence of the centromere. However, if the chromosome bridge is mechanically broken at a random place, this could lead to 100% unbalanced products. Bridges and fragments in the second meiotic division are not as common as they are in the first, but bridges without fragments occur more frequently at this stage (see Huang & Rieseberg, 2020). Finally, as first described by McClintock (1939), inverted duplications may also form a bridge configuration in anaphase I or II, depending on the position of the crossover.

Furthermore, we investigated the constitution of laggards and showed that most were of *S. spontaneum* origin or recombinants. The laggard tendency seems to be inherited from *S. spontaneum*. The duration of meiosis is characteristic of the species and can vary due to environmental factors (e.g., temperature), nuclear DNA content, ploidy level and genotype (Bennett, 1977; Wijnker & Schnittger, 2013). The last three factors are intrinsic differences between *S. officinarum* and *S. spontaneum*. Alternatively, we may speculate that if homoeologous synapsis occurs at late zygotene and pachytene, as previously reported in wheat (Martín et al., 2014), the delay could also explain the constitution of the laggards investigated herein. Furthermore, in wheat lacking the *Ph1* locus (a major factor in the control of meiotic processes), overall synapsis of the telomere bouquet is delayed, and further synapsis occurs after the bouquet stage, at which time homoeologous synapsis is also possible (Martín et al., 2017).

Pioneering studies on mitotic chromosomes have established that the constitution of modern varieties consists of approximately 75%–80% *S. officinarum*, 10%–25% *S. spontaneum* and 10%–15% recombinant chromosomes (D'Hont et al., 1996; Piperidis et al., 2010; Piperidis & D'Hont, 2020). However, using *S. spontaneum*-specific chromosome probes, Wang, Cheng, et al. (2022) reported unexpected proportions of interspecific recombinants (11.9%–40.9%) in some cultivars. The above figures are corroborated herein, that is, a respective 80%, 11% and 9% of *S. officinarum*, *S. spontaneum* and recombinant chromosomes. Importantly, as recently reported in *Brassica* allohexaploids (Quezada-Martinez et al., 2022), *Saccharum* hybrids and sugarcane modern varieties were able to tolerate multiple chromosome rearrangements over generations, despite the putative impact of these on meiosis.

It would be interesting, in the near future, to focus on immunolocalisation of the axis and synaptonemal complex proteins in the parental contributors of sugarcane to characterise their role in stabilising sugarcane meiosis. Future work could also focus on the pairing partner switches observed at pachytene that could be confirmed and visualised in greater detail, as was the case in *Arabidopsis arenosa* (Morgan & Wegel, 2020). Finally, for the first time ever, we found that both types of inversions occur in sugarcane, and progress on sugarcane genome architecture decrypting may elucidate their chromosome localisation.

ACKNOWLEDGEMENTS

This study was supported by the following Brazilian institutions: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Grant No. 2020/07741-0; 2019/07838-6), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Finance Code 001 and CAPES/PrInt, fellowship awarded to NRS) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

CONFLICT OF INTEREST STATEMENT

The authors have no competing interest to declare.

ORCID

Mateus Mondin https://orcid.org/0000-0002-7641-0546
Maria Lucia Carneiro Vieira https://orcid.org/0000-0003-0341-5714

REFERENCES

Ahmad, F., Poerba, Y. S., Kema, G. H. J., & Jong, H. (2021). Male meiosis and pollen morphology in diploid Indonesian wild bananas and cultivars. *The Nucleus*, *64*, 181–191. https://doi.org/10.1007/s13237-021-00350-7

Aissat, A., Amirouche, R., & Amirouche, N. (2019). Cytotaxonomic investigation and meiotic behaviour of natural populations of genus *Avena* in Algeria. *Euphytica*, *215*, 158. https://doi.org/10.1007/s10681-019-2490-6

Balsalobre, T. W., Silva Pereira, G., Margarido, G. R., Gazaffi, R., Barreto, F. Z., Anoni, C. O., Cardoso-Silva, C. B., Costa, E. A., Mancini, M. C., Hoffmann, H. P., de Souza, A. P., Garcia, A. A. F., & Carneiro, M. S. (2017). GBS based single dosage markers for linkage and QTL mapping allow gene mining for yield related traits in sugarcane. *BMC Genomics*, *18*, 72. https://doi.org/10.1186/s12864016-3383-x

Barreto, F. Z., Balsalobre, T. W. A., Chapola, R. G., Garcia, A. A. F., Souza, A. P., Hoffmann, H. P., Gazaffi, R., & Carneiro, M. S. (2021). Genetic variability, correlation among agronomic traits, and genetic progress in a sugarcane diversity panel. *Agriculture*, *11*(6), 533. https://doi.org/10.3390/agriculture11060533

Bennett, M. D. (1977). The time and duration of meiosis. *Philosophical Transactions of the Royal Society B*, *277*, 201–226. https://www.jstor.org/stable/2417709

Bielig, L. M., Mariani, A., & Berding, N. (2003). Cytological studies of 2n male gamete formation in sugarcane, *Saccharum* L. *Euphytica*, *133*(1), 117–124. https://doi.org/10.1023/A:1025628103101

Bremer, G. (1961a). Problems in breeding and cytology of sugar cane. II. The sugar cane breeding from a cytological viewpoint. *Euphytica*, *10*(2), 121–133. https://doi.org/10.1007/BF00037206

Bremer, G. (1961b). Problems in breeding and cytology of sugar cane. I. A short history of sugar cane breeding the original forms of *Saccharum*. *Euphytica*, *10*(1), 59–78. https://doi.org/10.1007/BF00037206

Burner, D. M. (1991). Cytogenetic analyses of sugarcane relatives (*Andropogoneae*: *Saccharinae*). *Euphytica*, *54*(1), 125–133. https://doi.org/10.1007/BF00145639

Cheavegatti-Gianotto, A., Abreu, H. M. C., Arruda, P., Bespalhok, J. C., Burnquist, W. L., Creste, S., Di Ciero, L., Ferro, J. A., Figueira, A. V. O., Filgueiras, T. S., de Fátima Grossi-de-Sá, M., Guzzo, E. C., Hoffmann, H. P., de Andrade Landell, M. G., Macedo, N., Matsuoka, S., de Castro Reinach, F., Romano, E., da Silva, W. J., … Ulian, E. C. (2011). Sugarcane (*Saccharum* × *officinarum*): A reference study for the regulation of genetically modified cultivars in Brazil. *Tropical Plant Biology*, *4*, 62–89. https://doi.org/10.1007/s12042-011-9068-3

Chelysheva, L., Gendrot, G., Vezon, D., Doutriaux, M. P., Mercier, R., & Grelon, M. (2007). Zip4/Spo22 is required for class I CO formation but not for synapsis completion in Arabidopsis thaliana. *PLoS Genetics*, *3*, e83. https://doi.org/10.1371/journal.pgen.0030083

Choudhary, A., Wright, L., Ponce, O., Chen, J., Prashar, A., Sanchez-Moran, E., Luo, Z., & Compton, L. (2020). Varietal variation and chromosome behavior during meiosis in *Solanum tuberosum*. *Heredity*, *125*, 212–226. https://doi.org/10.1038/s41437-020-0328-6

Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nature Reviews Genetics*, *6*, 836–846. https://doi.org/10.1038/nrg1711

Cuadrado, A., Acevedo, R., Moreno Díaz, de la Espina, S., Jouve, N., & de la Torre, C. (2004). Genome remodelling in three modern *S. officinarum* × *S. spontaneum* sugarcane cultivars. *Journal of Experimental Botany*, *55*(398), 847–854. https://doi.org/10.1093/jxb/erh093

Cursi, D. E., Castilho, R. O., Tarumoto, Y., Umeda, M., Tippayawat, A., Ponragdee, W., Racedo, J., Perera, M. F., Hoffmann, H. P., & Carneiro, M. S. (2022). Origin, genetic diversity, conservation, and traditional and molecular breeding approaches in sugarcane. In P. M. Priyadarshan & S. M. Jain (Eds.), *Cash Crops* (pp. 83–116). Springer.

D'Hont, A., Grivet, L., Feldmann, P., Rao, S., Berding, N., & Glaszmann, J. C. (1996). Characterization of the double genome structure of modern sugarcane cultivars (*Saccharum* spp.) by molecular cytogenetics. *Molecular and General Genetics*, *250*(4), 405–413. https://doi.org/10.1007/BF02174028

Dong, F., Song, J., Naess, S. K., Helgeson, J. P., Gebhardt, C., & Jiang, J. (2000). Development, and applications of a set of chromosome-specific cytogenetic DNA markers in potato. *Theoretical and Applied Genetics*, *101*(7), 1001–1007. https://doi.org/10.1007/s001220051573

Figueiredo, P. (2008). Breve história da cana-de-açúcar e do papel do Instituto Agronômico no seu estabelecimento no Brasil. 2008. In L. L. Dinardo-Miranda, A. C. M. de Vasconcelos, & M. G. A. Landell (Eds.), *Cana-de-Açúcar* (pp. 31–44). Camppinas, Instituto Agronômico.

Garcia, A. A. F., Mollinari, M., Marconi, T. G., Serang, O. R., Silva, R. R., Vieira, M. L. C., Vicentini, R., Costa, E. A., Mancini, M. C., Garcia, M. O. S., et al. (2013). SNP genotyping allows an in depth characterization of the genome of sugarcane and other complex autopolyploids. *Scientific Reports*, *3*(1), 3399. https://doi.org/10.1038/srep03399

Gouy, M., Nibouche, S., Hoarau, J. Y., & Costet, L. (2013). Improvement of yield per se in sugarcane. In R. K. Varshney & R. Tuberosa (Eds.), *Translational genomics for crop breeding. Abiotic stress, yield, and quality* (pp. 211–237). Wiley. https://doi.org/10.1002/9781118728482.ch13

Griffiths, S., Sharp, R., Foote, T. N., Bertin, I., Wanous, M., Reader, S., Colas, I., & Moore, G. (2006). Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat. *Nature*, *439*, 749–752. https://doi.org/10.1038/nature04434

Grivet, L., Daniels, C., Glaszmann, J. C., & D'Hont, A. (2004). A review of recent molecular genetics evidence for sugarcane evolution and domestication. *Ethnobotany Research and Applications*, *2*, 9–17. https://doi.org/10.17348/era.2.0.9-17

Grivet, L., Glaszmann, J.-C., & D'Hont, A. (2006). Molecular evidence of sugarcane evolution and domestication. In T. Motley (Ed.), *Darwin's harvest: New approaches to the origins, evolution, and conservation of crops* (pp. 49–66). Columbia University Press. https://doi.org/10.7312/motl13316-004

Huang, K., & Rieseberg, L. H. (2020). Frequency, origins, and evolutionary role of chromosomal inversions in plants. *Frontiers in Plant Science*, *11*, 296. https://doi.org/10.3389/fpls.2020.00296

Jenczewski, E., Eber, F., Grimaud, A., Huet, S., Lucas, M. O., Monod, H., & Chèvre, A. M. (2003). *PrBn*, a major gene controlling homeologous pairing in oilseed rape (*Brassica napus*) haploids. *Genetics*, *164*(2), 645–653. https://doi.org/10.1093/genetics/164.2.645

Kline, K. L., Msangi, S., Dale, V. H., Woods, J., Souza, G. M., Osseweijer, P., Clancy, J. S., Hilbert, J. A., Johnson, F. X., McDonnell, P. C., & Mugera, H. K. (2017). Reconciling food security and bioenergy: Priorities for action. *GCB Bioenergy*, *9*, 557–576. https://doi.org/10.1111/gcbb.12366

Long, S. P., Karp, A., Buckeridge, M. S., Davis, S. C., Jaiswal, D., Moore, P. H., Moose, S. P., Murphy, D. J., Onwona-Agyeman, S., & Vonshak, A. (2015). Feedstocks for biofuels and bioenergy. In G. M. Souza, R. L. Victoria, C. A. Joly, & L. M. Verdade (Eds.), *Bioenergy and sustainability: Bridging the gaps* (pp. 302–346). SCOPE.

Luo, M. C., Deal, K. R., Akhunov, E. D., Akhunova, A. R., Anderson, O. D., Anderson, J. A., Blake, N., Clegg, M. T., Coleman-Derr, D., Conley, E. E., Crossman, C. C., Dubcovsky, J., Gill, B. S., Gu, Y. Q., Hadam, J., Heo, H. Y., Huo, N., Lazo, G., Ma, Y., … Dvorak, J. (2009). Genome comparisons reveal a dominant mechanism of chromosome number reduction in grasses and accelerated genome evolution in Triticeae. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 15780–15785. https://doi.org/10.1073/pnas.0908195106

Martín, A. C., Rey, M. D., Shaw, P., & Moore, G. (2017). Dual effect of the wheat *Ph1* locus on chromosome synapsis and crossover. *Chromosoma*, *126*(6), 669–680. https://doi.org/10.1007/s00412-017-0630-0

Martín, A. C., Shaw, P., Phillips, D., Reader, S., & Moore, G. (2014). Licensing MLH1 sites for crossover during meiosis. *Nature Communications*, *5*, 4580. https://doi.org/10.1038/ncomms5580

Masoabi, M., Snyman, S., & van der Vyver, C. (2023). Characterisation of an ethyl methanesulfonate-derived drought-tolerant sugarcane mutant line. *Annals of Applied Biology*, *182*, 343–360. https://doi.org/10.1111/aab.12823

Matsuoka, S., & Stolf, R. (2012). Sugarcane tillering and ratooning: Key factors for a profitable cropping. In J. F. Gonçalves & K. D. Corrêa (Eds.), *Sugarcane: production, cultivation and uses* (pp. 137–157). Nova Science Publishers.

McClintock, B. (1939). The behavior in successive nuclear divisions of a chromosome broken at meiosis. *Proceedings of the National Academy of Sciences of the United States of America*, *25*, 405–416. https://doi.org/10.1073/pnas.25.8.405

Medeiros, C., Balsalobre, T. W. A., & Carneiro, M. S. (2020). Molecular diversity and genetic structure of *Saccharum* complex accessions. *PLoS One*, *15*(5), e0233211. https://doi.org/10.1371/journal.pone.0233211

Meng, Z., Han, J., Lin, Y., Zhao, Y., Lin, Q., Ma, X., Wang, J., Zhang, L., Yang, Q., & Wang, K. (2020). Characterization of a *Saccharum spontaneum* with a basic chromosome number of $x = 10$ provides new insights on genome evolution in genus *Saccharum*. *Theoretical and Applied Genetics*, *133*, 187–199. https://doi.org/10.1007/s00122-019-03450-w

Morgan, C., & Wegel, E. (2020). Cytological characterization of *Arabidopsis arenosa* polyploids by SIM. *Methods in Molecular Biology*, *2061*, 37–46. https://doi.org/10.1007/978-1-4939-9818-0_4

Murata, N. M., & Motoyoshi, F. (1995). Floral chromosomes of *Arabidopsis thaliana* for detecting low-copy DNA sequences by fluorescence in situ hybridization. *Chromosoma*, *104*, 39–43. https://doi.org/10.1007/BF00352224

Nagaki, K., Tsujimoto, H., & Sasakuma, T. (1998). A novel repetitive sequence of sugarcane, SCEN family, locating on centromeric regions. *Chromosome Research*, *6*(4), 295–302. https://doi.org/10.1023/A:1009270824142

Nair, M. K. (1975). Cytogenetics of *Saccharum officinarum* L. and *S. spontaneum* L. IV. Chromosome number and meiosis in *S. officinarum* × *S. spontaneum* hybrids. *Caryologia*, *28*(1), 1–14. https://doi.org/10.1080/00087114.1975.10796591

Nicolas, S. D., Leflon, M., Monod, H., Eber, F., Coriton, O., Huteau, V., Chèvre, A. M., & Jenczewski, E. (2009). Genetic regulation of meiotic crossovers between related genomes in *Brassica napus* haploids and hybrids. *The Plant Cell*, *21*(2), 373–385. https://doi.org/10.1105/tpc.108.062273

Panje, R. R., & Babu, C. N. (1960). Studies in *Saccharum spontaneum*. Distribution and geographical association of the chromosome number. *Cytologia*, *25*(2), 152–172. https://doi.org/10.1508/cytologia.25.152

Piperidis, G., Piperidis, N., & D'Hont, A. (2010). Molecular cytogenetic investigation of chromosome composition and transmission in sugarcane. *Molecular Genetics and Genomics*, *284*(1), 65–73. https://doi.org/10.1007/s00438-010-0546-3

Piperidis, N., & D'Hont, A. (2020). Sugarcane genome architecture decrypted with chromosome specific oligo probes. *The Plant Journal*, *103*, 2039–2051. https://doi.org/10.1111/tpj.14881

Pompidor, N., Charron, C., Hervouet, C., Bocs, S., Droc, G., Rivallan, R., Manez, A., Mitros, T., Swaminathan, K., Glaszmann, J. C., Garsmeur, O., & D'Hont, A. (2021). Three founding ancestral genomes involved in the origin of sugarcane. *Annals of Botany*, *127*(6), 827–840. https://doi.org/10.1093/aob/mcab008

Price, S. (1963a). Cytogenetics of modern sugar canes. *Economic Botany*, *17*, 97–106. https://doi.org/10.1007/BF02985359

Price, S. (1963b). Cytological studies in *Saccharum* and allied genera. VIII. F_2 and BC_1 progenies from 112- and 136 chromosome *S. officinarum* × *S. spontaneum* hybrids. *Botanical Gazette*, *124*(3), 186–190. https://doi.org/10.1086/336190

Quezada-Martinez, D., Zou, J., Meng, J., Batley, J., & Mason, A. S. (2022). Allele segregation analysis of F_1 hybrids between independent *Brassica* allohexaploid lineages. *Chromosoma*, *131*, 147–161. https://doi.org/10.1007/s00412-022-00774-3

Rey, M. D., Martín, A. C., Higgins, J., Swarbreck, D., Uauy, C., Shaw, P., & Moore, G. (2017). Exploiting the *ZIP4* homologue within the wheat *Ph1* locus has identified two lines exhibiting homoeologous crossover in wheat wild relative hybrids. *Molecular Breeding*, *37*, 95. https://doi.org/10.1007/s110320170700-2

Rey, M. D., Ramírez, C., & Martín, A. C. (2021). Wheat, rye, and barley genomes can associate during meiosis in newly synthesized trigeneric hybrids. *Plants*, *10*(1), 113. https://doi.org/10.3390/plants10010113

Riley, R., & Chapman, V. (1958). Genetic control of the cytologically diploid behaviour of hexaploid wheat. *Nature*, *182*, 713–715. https://doi.org/10.1038/182713a0

Risso-Pascotto, C., Pagliarini, M. S., & Valle, C. B. (2003). Chromosome number and microsporogenesis in a pentaploid accession of *Brachiaria brizantha* (Gramineae). *Plant Breeding*, *122*(2), 136–140. https://doi.org/10.1046/j.1439-0523.2003.00825.x

Schwarzacher, T., & Heslop-Harrison, P. (2000). *Practical in Situ Hybridization* (p. 203). BIOS Scientific.

Sharma, A. K., & Sharma, A. (1980). *Chromosome techniques: Theory and practice* (3rd ed., p. 711). Butterworths.

Shen, Y., Tang, D., Wang, K., Wang, M., Huang, J., Luo, W., Luo, Q., Hong, L., Li, M., & Cheng, Z. (2012). ZIP4 in homologous chromosome synapsis and crossover formation in rice meiosis. *Journal of Cell Science*, *125*, 2581–2591. https://doi.org/10.1242/jcs.090993

Simmonds, N. W. (Ed.) (1975). Sugar-canes. In *Evolution of crop plants* (pp. 104–108). Longman.

Soares, N. R., Mollinari, M., Oliveira, G. K., Pereira, G. S., & Vieira, M. L. C. (2021). Meiosis in polyploids and implications for genetic mapping: A review. *Genes*, *12*(10), 1517. https://doi.org/10.3390/genes12101517

Souza, G. M., Van Sluys, M. A., Lembke, C. G., Lee, H., Margarido, G. R. A., Hotta, C. T., Gaiarsa, J. W., Diniz, A. L., Oliveira, M. M., & Ferreira, S. S. (2019). Assembly of the 373k gene space of the polyploid sugarcane genome reveals reservoirs of functional diversity in the world's leading biomass crop. *Gigascience*, *8*(12), giz129. https://doi.org/10.1093/gigascience/giz129

Sreenivasan, T. V., & Jagathesan, D. (1975). Meiotic abnormalities in *Saccharum spontaneum*. *Euphytica*, *24*, 543–549. https://doi.org/10.1007/BF00028230

Suzuki, E. (1941). Cytological studies of sugar cane. I. Observations on some POJ varieties. *Cytologia*, *11*(4), 507–514.

Thumjamras, S., Iamtham, S., Prammanee, S., & Jong, H. (2016). Meiotic analysis and FISH with rDNA and rice BAC probes of the Thai KPS 01-01-25 sugarcane cultivar. *Plant Systematics and Evolution*, *302*, 305–317. https://doi.org/10.1007/s00606-015-1264-4

Vieira, M. L. C., Almeida, C. B., Oliveira, C. A., Tacuatiá, L. O., Munhoz, C. F., Cauz-Santos, L. A., Pinto, L. R., Monteiro-Vitorello, C. B., Xavier, M. A., & Forni-Martins, E. R. (2018). Revisiting meiosis in sugarcane: Chromosomal irregularities and the prevalence of bivalent configurations. *Frontiers in Genetics*, *9*, 213. https://doi.org/10.3389/fgene.2018.00213

Wang, K., Cheng, H., Han, J., Esh, A., Liu, J., Zhang, Y., & Wang, B. (2022). A comprehensive molecular cytogenetic analysis of the genome architecture in modern sugarcane cultivars. *Chromosome Research*, *30*, 29–41. https://doi.org/10.1007/s10577-021-09680-3

Wang, K., Zhang, H., Khurshid, H., Esh, A., Wu, C., Wang, Q., & Piperidis, N. (2022). Past and recent advances in sugarcane cytogenetics. *The Crop Journal*, *11*, 1–8. https://doi.org/10.1016/j.cj.2022.08.004

Wijnker, E., & Schnittger, A. (2013). Control of the meiotic cell division program in plants. *Plant Reproduction*, *26*, 143–158. https://doi.org/10.1007/s00497-013-0223-x

Zhang, J., Zhang, X., Tang, H., Zhang, Q., Hua, X., Ma, X., Zhu, F., Jones, T., Zhu, X., Bowers, J., Wai, C. M., Zheng, C., Shi, Y., Chen, S., Xu, X., Yue, J., Nelson, D. R., Huang, L., Li, Z., … Ming, R. (2018). Allele-defined genome of the autopolyploid sugarcane *Saccharum spontaneum* L. *Nature Genetics*, *50*, 1565–1573. https://doi.org/10.1038/s41588-018-0237-2

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Oliveira, G. K., Soares, N. R., Costa, Z. P., Almeida, C. B., Machado, R. M., Mesquita, A. T., Carneiro, M. S., Forni-Martins, E. R., Mondin, M., & Vieira, M. L. C. (2023). Meiotic abnormalities in sugarcane (*Saccharum* spp.) and parental species: Evidence for peri- and paracentric inversions. *Annals of Applied Biology*, *183*(3), 271–286. https://doi.org/10.1111/aab.12855