Meiotic chromosome behavior in *Saccharum* species, including the Brazilian variety SP80-3280

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Thesis presented to obtain the degree of Doctor in Science. Area: Genetics and Plant Breeding

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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 species S. officinarum ('noble cane') and the wild S. 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 behavior of representatives of the parentals S. officinarum and S. spontaneum, and the Brazilian variety, SP80-3280. S. officinarum, an octoploid species, exhibited regular meiotic behavior. In contrast, S. spontaneum and the Brazilian variety, SP80-3280, exhibited several abnormalities, including lagging chromosomes from metaphase I to the end of division. We reported and typified, for the first time, the presence of pericentric inversions and dicentric chromosomes. Using in-situ hybridization techniques, we were able to determine how pairing association occurred at diakinesis and, in particular, the chromosome composition of SP80-3280. Our findings have implications for sugarcane genetic mapping, genomics and molecular cytogenetics, and also for studies on resynthesized polyploids.

Keywords: Saccharum spp.; meiotic behavior; chromosome associations; in situ hybridization; chromosomal inversion; dicentric chromosomes

1.1. Introduction
The sugarcane (Saccharum spp.) crop is of considerable industrial importance, accounting for nearly 80% of global sugar production (see https://www.isosugar.org/sugarsector/sugar). 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 (Long et al. 2015; Kline et al. 2017). Crops and by-products can be developed for producing bioelectricity, bioplastics and fertilizers, in addition to cellulosic ethanol. Importantly, the energy contained in sugarcane-derived ethanol and in the electricity generated from burning sugarcane bagasse accounts for 17.5% of the Brazilian energy matrix (see http://www.mme.gov.br).
Saccharum species originated in New Guinea, where sugar canes have been grown for millennia. The earliest record of domestication dates back to around 8,000 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, S. spontaneum and S. robustum, and four cultivated species, S. officinarum, S. sinense, S. barberi, and S. 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. 2021).

Selection practices in former times resulted in Saccharum officinarum clones with a higher sugar content and fewer fibers. These are known as ‘noble canes’ (Simmonds 1975). Subsequently, in the late 19th century, new varieties emerged from interspecific hybridization of the formerly cultivated species (Saccharum barberi and S. officinarum) and wild Saccharum 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, including hardiness, ratooning capability and resistance to diseases (see Grivet et al. 2004; Cheavegatti-Gianotto et al. 2011; Barreto et al. 2021). Importantly, due to a mechanism known as meiotic restitution, unreduced gametes were transmitted by S. officinarum (i.e., 2n, 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 2021).

The genus Saccharum includes diverse forms of polyploids and exclusively higher order polyploid species (>4x), such as S. officinarum, a typical octoploid (2n = 8x = 80, x = 10), S. robustum (2n = 60, 80 to 200), and its presumed natural mutant clone, Saccharum edule (2n = 60 to 122) (Grivet et al. 2006). S. spontaneum is an autopolyplaid with variable chromosome number and aneuploid accessions (Panje and Babu 1960). It is considered 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 and D’Hont 2020). Current sequencing results for a typical contemporary cultivar (R570) suggest the existence of three founding genomes in modern sugarcane, 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 analysis, S. officinarum and S. spontaneum account respectively for 75 to 85% and 15 to 25% of sugarcane chromosomes. The remaining chromosomes are recombinant from both origins (D’Hont et al. 1996; Cuadrado et al. 2004; Piperidis et al. 2010; Piperidis and 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 organization of modern sugarcane cultivars. Due to the geographical locations of experimental stations (India, US, Australia, Brazil, 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. Overall, they are called as low-inbreeding genealogies (Amadeu et al. 2020).

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 analyses have suggested that both parental species and interspecific hybrids predominantly form bivalents, as well as the contemporary cultivars (Nair 1975; Price 1963a, 1963b; Suzuki 1941; Pagliarini et al. 1990; Burner 1991; Bielig et al. 2003).

Recently, our group has confirmed a bivalent association in the Brazilian variety, IACSP93-3046 ($2n = 112$). This was done using FISH (fluorescent in situ hybridization) with labeled 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 1 or 2 univalents were also found (Vieira et al. 2018).

Herein, our aim was to investigate the meiotic behavior of representatives of the parental species ($S. officinarum$ and $S. spontaneum$) and the Brazilian variety, SP80-3280. We examined the frequency and types of meiotic irregularities, such as those exhibiting the incidence of pericentric inversions. Using in situ hybridization techniques, we were able to determine chromosome composition and how pairing association occurs in early SP80-3280 prophase cells, and especially at diakinesis. Our findings have implications for sugarcane genetic mapping, genomics and molecular cytogenetics, and also for studies on resynthesized polyploids.

**1.2. Conclusion**

Herein, we conducted a comparative analysis of the meiotic behavior of representatives of the parentals $S. officinarum$ and $S. spontaneum$, and the Brazilian variety, SP80-3280. $S. officinarum$, an octoploid species, exhibited regular meiotic behavior. In contrast, $S. spontaneum$ and the Brazilian variety, SP80-3280, exhibited several abnormalities, including lagging chromosomes from metaphase I to the end of division. We reported and typified, for the first time, the presence of pericentric inversions and dicentric chromosomes. Using in-situ hybridization techniques, we were able to determine how pairing association occurred at diakinesis and, in particular, the chromosome composition of SP80-3280. Our findings have implications for sugarcane genetic mapping, genomics and molecular cytogenetics, and also for studies on resynthesized polyploids.
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Modern sugarcane varieties emerged through artificial interspecific hybridizations between the long-term cultivated species *Saccharum officinarum* and the wild *S. spontaneum*. A backcrossing program was then necessary for transferring the sucrose content from *S. officinarum* to the hybrids. Selection over generations and the incorporation of other germplasm into cultivated backgrounds established the genetic basis of modern cultivars, which are highly-polyploid and possess a complex and large genome (~10 Gb). In the present study, our group has introduced for the first time the genomic *in situ* hybridization approach to investigate the sugarcane meiotic chromosomes. As expected, our results show that most of bivalents were of single constitution; however paired chromosomes of interspecific origin were also observed. At diakinesis, we were only able to visualize univalents of *S. officinarum* origin. Interestingly, *S. spontaneum* and recombinant chromosomes were those to mostly show a laggard tendency in both the first and second divisions. Our studies contribute to the understanding how the two subgenomes behavior of during meiosis.

**Keywords** *Saccharum*; cytogenetics; meiotic behavior; GISH; interspecific hybridization, current variety

### 2.1. Introduction

The sugarcane crop (*Saccharum* spp.) is responsible for nearly 80% of the world's sugar production and is generally regarded as the most sustainable source of biomass for producing biofuels. Crops and by-products can be used for producing bioelectricity, bioplastics and fertilizers, in addition to cellulosic ethanol (Long et al. 2015; Kline et al. 2017).

The cultivated species *Saccharum officinarum* is believed to have been domesticated around 8000 BC from the wild species *S. robustum* in the New Guinea region. Subsequently, the clones were spread to Southeast Asia, India, and China. This long history of cultivation and empiric selection has facilitated the generation of a diversified germplasm which includes species of the *Saccharum* complex (two wild, *S. spontaneum* and *S. robustum*, and four cultivated species, *S. officinarum*, *S. sinense*, *S. barberi*, and *S. edule*) and four interbreeding genera (*Erianthus*, *Miscanthus*, *Narenga*, and *Sclerostachya*) (see Barreto et al. 2021; Cursi et al. 2021).

The emergence of modern sugarcane varieties occurred through interspecific hybridizations. The attributes of the so-called noble canes (*S. officinarum*) have sustained its cultivation for many
centuries; nevertheless, it has shown to be susceptible to diseases. Thus, breeders intended to promote resistance by crossing different clones of *S. officinarum* with *S. spontaneum*, a wild and vigorous species. Subsequently, successive backcrosses were made for a progressive improvement of the hardy and disease resistant but otherwise inferior hybrids (see Stevenson 1965). The peculiarity in this process is that the hybrid progenies receive $2n$ gametes from *S. officinarum* (when it was used as the female parent) and $n$ gametes from *S. spontaneum* (Bremer, 1961; Price 1961). This $2n + n$ transmission during ‘nobilization’ accelerated the elimination of most of the *S. spontaneum* undesirable traits while retaining the attractiveness and high sugar content of *S. officinarum* clones.

Later on, due to the geographical locations of experimental stations (US, India, Egypt, Réunion Island, Brazil, Australia, etc.) and agricultural requirements, each sugarcane pedigree has particular features. Intensive work has resulted in the development of varieties that have in their lineage different hybrids and selected species-specific clones.

As a consequence of all these practices, the current cultivars have 100 up to 130 chromosomes, most of them (~80%) deriving from *S. officinarum*, 10-20% from *S. spontaneum*, and ~10% from interspecific recombination, giving support for the occurrence of interspecific pairing and the formation of recombinant/translocated chromosomes (Piperidis 2010; Piperidis and D’Hont 2020).

Although there are homoeologues and recombinants, multivalent configurations are rarely evidenced in diakinesis and metaphase I, with bivalents predominating in accordance with conventional methods (Bremer 1961; Price 1963; Burner 1991) and fluorescent in situ hybridization (Vieira et al. 2018). It suggests that there is coordinated segregation of chromosomes, regardless of their origin, whether from *S. officinarum*, *S. spontaneum*, or recombinant.

Recently, our group performed a comparative analysis of the meiotic behavior in representatives of *S. officinarum* and *S. spontaneum* and the Brazilian variety SP80-3280. The two latter exhibited several abnormalities, including delayed chromosomes, from metaphase I to the end of the division, while the *S. officinarum* clone showed regular behavior. In this study, we visualized the presence of dicentric chromosomes, and a pericentric inversion that was particularly evidenced by a detailed pachytene analysis (Oliveira et al. 2022).

2.2. Conclusion

Herein, we describe, for the first time, the use of genomic in situ hybridization (GISH) for analyzing the sugarcane meiotic chromosomes. As expected, most of bivalents were of single constitution (*S. officinarum*-*S. officinarum* or *S. spontaneum*-*S. spontaneum*) but paired chromosomes of interspecific origin were also observed. At diakinesis, we were only able to visualize univalents of *S. officinarum* origin. Interestingly, *S. spontaneum* and recombinant chromosomes were those to mostly show a laggard tendency in both the first and second divisions. Our studies contribute to the understanding how the two subgenomes behavior of during meiosis.


