Characterization of Chromosome Inheritance of the Intergeneric BC$_2$ and BC$_3$ Progeny between Saccharum spp. and Erianthus arundinaceus

Yongji Huang$^{1*}$, Jiayun Wu$^2$, Ping Wang$^1$, Yanquan Lin$^1$, Cheng Fu$^2$, Zuhu Deng$^{1,3*}$, Qinnan Wang$^2$, Qiwei Li$^2$, Rukai Chen$^1$, Muqing Zhang$^3$

$^1$ Key Lab of Sugarcane Biology and Genetic Breeding, Ministry of Agriculture, Fujian Agriculture and Forestry University, Fuzhou, China, $^2$ Guangdong Key Laboratory of Sugarcane Improvement and Biorefinery, Guangzhou Sugarcane Industry Research Institute, Guangzhou, China, $^3$ Guangxi Collaborative Center for Sugarcane & Cane Sugar Industries, Guangxi, China

☯ These authors contributed equally to this work.

* dengzuhu@163.com

Abstract

Erianthus arundinaceus (E. arundinaceus) has many desirable agronomic traits for sugarcane improvement, such as high biomass, vigor, rationing ability, tolerance to drought, and water logging, as well as resistance to pests and disease. To investigate the introgression of the E. arundinaceus genome into sugarcane in the higher generations, intergeneric BC$_2$ and BC$_3$ progeny generated between Saccharum spp. and E. arundinaceus were studied using the genomic in situ hybridization (GISH) technique. The results showed that the BC$_2$ and BC$_3$ generations resulted from n + n chromosome transmission. Furthermore, chromosome translocation occurred at terminal fragments from the E. arundinaceus chromosome in some progeny of Saccharum spp. and E. arundinaceus. Notably, the translocated chromosomes could be stably transmitted to their progeny. This study illustrates the characterization of chromosome inheritance of the intergeneric BC$_2$ and BC$_3$ progeny between Saccharum spp. and E. arundinaceus. This work could provide more useful molecular cytogenetic information for the germplasm resources of E. arundinaceus, and may promote further understanding of the germplasm resources of E. arundinaceus for sugarcane breeders to accelerate its progress in sugarcane commercial breeding.

Introduction

Sugarcane (Saccharum spp.) is a large perennial grass that is indigenous to tropical and sub-tropical regions. As the most important sugar-producing crop worldwide, sugarcane has significant potential to contribute to the global sugar security and produces approximately 75% of the world’s raw sugar [1]. In addition, as a C$_4$ plant, sugarcane is an efficient crop in converting solar energy into chemical energy. Therefore, it has been heralded as an alternative source of...
fuel and petrochemical feedstock for the production of first-generation bioethanol to alleviate the current energy crisis [2].

The genus *Saccharum* is an important member of the *Poaceae* family that consists of six species, including *S. officinarum*, *S. spontaneum*, *S. robustum*, *S. barberi*, *S. sinense*, and *S. edule*. Modern sugarcane cultivars are highly complex aneuployploids, and most are primarily derived from interspecific hybridization between *S. officinarum* (2n = 80) and *S. spontaneum* (2n = 40–128) through nobilization [3]. This term was first coined by Dutch breeders Jesweit in Java during the early 1900s to denote the process of introgression of *S. spontaneum* into *S. officinarum* following hybridization and successive backcrossing. During the nobilization process, interspecific F1 hybrids were obtained from crosses between *S. officinarum* as the female parent and *S. spontaneum* as the male parent, and then were repeatedly backcrossed to *S. officinarum* as the female parent. Using this approach, progeny conserve the entire genome of *S. officinarum* in the first interspecific cross (F1) and the first backcross (BC1) [4]. Hence, the chromosome inheritance of progeny in F1 and BC1 exhibits 2n + n transmission. This not only allows for a quick recovery of the high sugar content from *S. officinarum*, but also integrates resistance genes to biotic and abiotic stresses from *S. spontaneum* [5]. Jesweit succeeded in the selective breeding of some new cultivars with high resistance to disease, and significantly contributed to the perseverance through the sugar crisis in Java at that time due to disease outbreaks [2]. POJ2878, hailed as the "wonder cane", is one of the most successful examples of the utilization of nobilization.

However, due to the frequent utilization of a limited number of progenitors in sugarcane breeding programs, modern sugarcane cultivars have given rise to a sharp decline in genetic diversity [6,7]. Genetic erosion renders sugarcane increasingly vulnerable to resistance against biotic and abiotic stresses. As a result, the genetic potential for yield and quality improvement has hardly allowed for any advancement in the past several decades. Therefore, options for remedying the growing concern of a dearth of genetic variation has become an urgent and necessary task for sugarcane breeders. One efficient approach for combating this issue is by tapping into wild relatives to introduce favorable genes for increased productivity and better adaptability to a wide large range of growing conditions as well as providing more robust disease resistance. The genus *Saccharum* together with the four related genera, namely *Erianthus*, *Miscanthus*, *Narenga*, and *Sclerostachya*, comprise the “Saccharum complex” [8]. These four related genera serve as a rich gene pool for sugarcane improvement with tolerance to abiotic stresses and resistance to biotic stresses. As one of the most important wild relatives of sugarcane, *Erianthus arundinaceus* (2n = 80) and *E. arundinaceus* (n = 21). POJ2878, hailed as the "wonder cane", is one of the most successful examples of the utilization of nobilization.

In sugarcane, there are various types of chromosome transmission such as n + n, 2n + n, n + 2n, and 2n + 2n [19,21–26]. Compared to the common type of chromosome transmission (n + n), the other three specific types of chromosome transmission (2n + n, n + 2n, and 2n + 2n) are derived from unilateral and bilateral sexual polyploidization. In the plant kingdom, sexual polyploidization, leading to unreduced gametes (2n gametes) with the somatic chromosome number rather than the gametophytic number (n gamete), is generally believed to be the predominant mechanism of polyploidization [27,28]. Based on the different gametogenesis, the
unreduced gametes are divided into 2n egg gametes and 2n male gametes. 2n egg gametes typically originate from the unreduced ovule during megasporogenesis, whereas 2n male gametes are the result of the unreduced pollen during microsporogenesis [29]. The consequence of unilateral sexual polyploidization is 2n + n (result from the fertilization of unreduced ovule by normal haploid pollen) or n + 2n (result from the fertilization of normal ovule by unreduced pollen), while the result of bilateral sexual polyploidization is 2n + 2n (result from the fertilization of unreduced ovule by unreduced pollen). Several mechanisms have been described that the types of meiotic abnormalities responsible for the production of 2n gametes. Due to the different parental heterozygosity rate that each mechanism transmits to the progeny, the genetic consequences of different types of 2n gametes formation are highly divergent [28]. Hence, the use of 2n gametes, resulting in the different types of chromosome transmission during the establishment of sexual polyploids, is of prime importance to develop and conduct breeding strategies for crop improvement [30]. Indeed, it has already been proven effective for improvement of crops such as lily, potato, banana, and citrus [31–38]. In nobilization of S. spontaneum, the utilization of 2n gametes transmission from S. officinarum in interspecific crosses with S. spontaneum is such a typical example of the cytological peculiarity of 2n gametes.

Genomic in situ hybridization (GISH) is a powerful molecular cytogenetic tool to unravel the chromosome composition for the detection of different chromosomes sets derived from two or more distinct species and even recombinant chromosome segments in allopolyploids. During allopolyploid speciation and evolutionary process, the occurrence of chromosomal rearrangement is common, such as translocation and inversion. So far, this molecular cytogenetic technology has been widely used in investigating the chromosome composition and chromosomal translocation in a wide range of natural allopolyploids or artificial polyploid progeny [39–46]. Thus, knowledge of inferring the chromosome transmission from the chromosome composition in allopolyploid will make it possible to implement a strategy for developing useful varieties through breeding. Using GISH, much insight has been gained into sugarcane chromosomal inheritance and genomic recombination over the past several decades [11,19–21,24]. A previous study indicated that modern sugarcane cultivars possess approximately 120 chromosomes, with 70–80% derived from S. officinarum, 10–20% from S. spontaneum, and a few chromosomes derived from interspecific recombination [24].

In this study, two generations, including nine BC2 progeny and eight BC3 progeny, were characterized by GISH. The objectives were as follows: (1) to determine the chromosome transmission in these two generations, which can provide a reference for breeding strategies for further deployment of genes and traits from E. arundinaceus; and (2) to determine the presence of various types of intergeneric chromosomal translocation and obtain information on whether they can be inherited, which can provide a basic understanding for efficient utilization in sugarcane breeding.

**Materials and Methods**

**Plant materials**

The plant materials used in this study consisted of 17 progeny derived from two generations (BC2 and BC3) of intergeneric hybrids between Saccharum spp. and E. arundinaceus (Table 1). In the F1 generation, F1 hybrids between S. officinarum and E. arundinaceus were derived from crosses between Badila (S. officinarum, 2n = 80) as the female parent and HN 92–77 (E. arundinaceus, 2n = 60) or HN 92–105 (E. arundinaceus, 2n = 60) as the male parent. In the BC1 generation, F1 hybrids between S. officinarum and E. arundinaceus were used as the female parent. CP 84–1198 (2n = 120), a commercial cultivar containing germplasms from S. officinarum, S. spontaneum, S. barberi and S. robustum without contribution from E. arundinaceus, was used
as the male parent \[21\]. In the BC2 and BC3 generation, all the female parents and the male parents are listed in detail in Table 1. Among them, ROC10, ROC20, ROC23, YT73-204, YT91-976, YT93-159, NJ57-416, and YC95-46 are the commercial cultivars containing germplasm from \textit{Saccharum} \textit{spp.} without contribution from \textit{E. arundinaceus}, while “YCE” series are the progeny of \textit{E. arundinaceus}. The progeny analyzed in this study were generated at the Hainan Sugarcane Breeding Station of Guangzhou Sugarcane Industry Research Institute. All plant materials were grown in the greenhouse at Fujian Agriculture and Forestry University.

### Genomic in situ hybridization (GISH) procedure

Chromosome preparation and the GISH experiment were carried out according to the method described by D’hont et al. [9]. Genomic DNA from Badila (\textit{S. officinarum}) and YN82-114 (\textit{S. spontaneum}) was labelled with Biotin, the Biotin-labeled probe was detected with Avidin D, Rhodamine 600 (XRITC) and a Biotinylated anti-avidin antibody (Vector Laboratories, Burlingame, CA), respectively. Genomic DNA from HN92-77 or HN92-105 (\textit{E. arundinaceus}) was labelled with Digoxigenin, and the Digoxigenin-labeled probe was detected with sheep-anti-Digoxin-FITC (Roche, Lewes, UK) and rabbit-anti-sheep-FITC secondary antibody (Roche, Lewes, UK). Chromosomes were then counterstained with 4', 6-diamidino-2-phenylindole (DAPI) in a Vectashield anti-fade solution (Vector Laboratories, Burlingame, CA). FISH signals were captured using an AxioScope A1 Imager fluorescent microscope (Carl Zeiss, Göttingen, Germany). In this study, results are presented as the modal number and the range of chromosomes counting four to 22 metaphases for each progeny (Table 2). The images were processed using an AxioCam MRc5 and AxioVision v.4.7 imaging software (Carl Zeiss, Göttingen, Germany).

| Generation | Progeny     | Female parent | Male parent     |
|------------|-------------|---------------|-----------------|
| BC2        | YCE03-01    | NJ57-416      | YCE01-116 (BC1) |
| BC2        | YCE03-06    | YCE01-116 (BC1) | NJ57-416      |
| BC2        | YCE03-16    | YCE01-91 (BC1) | ROC23          |
| BC2        | YCE03-168   | YCE01-123 (BC1) | ROC10          |
| BC2        | YCE03-218   | YT73-204      | YCE01-105 (BC1) |
| BC2        | YCE03-249   | YCE01-69 (BC1) | YT73-204      |
| BC2        | YCE03-378   | ROC20         | YCE01-92 (BC1)  |
| BC2        | YCE04-55    | YC95-46       | YCE01-102 (BC1) |
| BC2        | YCE05-179   | ROC20         | YCE01-134 (BC1) |
| BC3        | YCE05-64    | YT73-204      | YCE03-133 (BC2) |
| BC3        | YCE05-150   | YCE03-218 (BC2) | ROC10          |
| BC3        | YCE06-61    | ROC10         | YCE03-01 (BC2)  |
| BC3        | YCE06-63    | ROC10         | YCE03-01 (BC2)  |
| BC3        | YCE06-92    | YCE04-51 (BC2) | YT93-159       |
| BC3        | YCE06-111   | YCE03-168 (BC2) | YT93-159       |
| BC3        | YCE06-140   | YCE03-218 (BC2) | ROC10          |
| BC3        | YCE06-166   | YCE03-168 (BC2) | YT91-976       |

**Note:** “YCE” series are the progeny of \textit{E. arundinaceus}, the other plant materials are the commercial cultivars containing germplasm from \textit{Saccharum} \textit{spp.} without contribution from \textit{E. arundinaceus}.

doi:10.1371/journal.pone.0133722.t001
Table 2. Chromosome composition of the intergeneric BC<sub>2</sub> and BC<sub>3</sub> progeny between Saccharum spp. and E. arundinaceus.

| Generation | Progeny     | Chromosome composition                          | No. of chromosomes | No. of metaphases analyzed |
|------------|-------------|-------------------------------------------------|--------------------|----------------------------|
|            |             | Modal number Range                               | Saccharum spp.     | E. arundinaceus            | Type of recombinants |
|            |             | Total (2n cell)                                  |                    |                            |                           |
| BC<sub>2</sub> | YCE03-01   | 2n = 119 = 105S + 14E                            | 119                | 115–122 105               | 103–106 14 12–14 0       | — 10                      |
| BC<sub>2</sub> | YCE03-06   | 2n = 119 = 105S + 14E                            | 119                | 118–123 105               | 104–107 14 13–15 0       | — 16                      |
| BC<sub>2</sub> | YCE03-16   | 2n = 113 = 100S + 13E                            | 113                | 109–113 100               | 98–101 13 12–14 0        | — 15                      |
| BC<sub>2</sub> | YCE03-168  | 2n = 110 = 100S + 9E + E/S                       | 111                | 108–113 100               | 99–103 10 8–10 1          | E/S 10                    |
| BC<sub>2</sub> | YCE03-218  | 2n = 107 = 97S + 10E                            | 107                | 105–108 97                | 97–99 10 9–12 0          | — 14                      |
| BC<sub>2</sub> | YCE03-249  | 2n = 110 = 97S + 13E                            | 110                | 107–113 97                | 96–100 13 12–15 0        | — 22                      |
| BC<sub>2</sub> | YCE03-378  | 2n = 121 = 104S + 16E + S/E                     | 121                | 115–121 104               | 101–105 16 15–17 1       | S/E 8                     |
| BC<sub>2</sub> | YCE04-55   | 2n = 111 = 98S + 13E                            | 111                | 109–114 98                | 96–100 13 11–13 0        | — 18                      |
| BC<sub>2</sub> | YCE05-179  | 2n = 112 = 99S + 13E                            | 112                | 108–116 99                | 96–101 13 13–14 0        | — 12                      |
| BC<sub>3</sub> | YCE05-64   | 2n = 118 = 107S + 6E + 2(E/S) + 3(S/E)          | 118                | 113–118 107               | 103–113 6 6 5            | 2(E/S)+3(S/E) 4           |
| BC<sub>3</sub> | YCE05-150  | 2n = 116 = 108S + 8E                            | 116                | 114–119 108               | 102–111 8 8 0            | — 14                      |
| BC<sub>3</sub> | YCE06-61   | 2n = 114 = 107S + 7E                            | 114                | 109–115 107               | 102–108 7 7 0            | — 20                      |
| BC<sub>3</sub> | YCE06-63   | 2n = 105 = 98S + 7E                             | 105                | 101–106 98                | 94–99 7 5–8 0            | — 9                       |
| BC<sub>3</sub> | YCE06-92   | 2n = 118 = 109S + 7E + 2(E/S)                   | 118                | 115–119 109               | 102–112 7 7 2            | 2(E/S) 10                 |
| BC<sub>3</sub> | YCE06-111  | 2n = 108 = 103S + 4E + E/S                      | 108                | 104–110 103               | 100–106 4 4 1            | E/S 15                    |
| BC<sub>3</sub> | YCE06-140  | 2n = 112 = 106S + 5E + S/E                      | 112                | 108–112 106               | 100–109 5 5–6 1          | S/E 11                    |
| BC<sub>3</sub> | YCE06-166  | 2n = 110 = 105S + 5E                            | 110                | 106–111 105               | 101–106 5 5 0            | — 4                       |

Note: Since small variation of chromosome counts can occur due to the loss or the overlapping of a few chromosomes from the preparation, the modal number of chromosomes and the range of total numbers of chromosomes in 2n cell are presented for the sugarcane clones analyzed. S and E indicate Saccharum spp. chromosome and E. arundinaceus chromosome, respectively. S/E and E/S indicate Saccharum spp. centromere with E. arundinaceus chromosome segment and E. arundinaceus centromere with Saccharum spp. chromosome segment, respectively.

doi:10.1371/journal.pone.0133722.t002
Results and Discussion

n + n chromosome transmission in BC2 and BC3 progeny

GISH experiments of nine BC2 progeny revealed plants with a total chromosome complement ranging from 107 to 121 chromosomes, of which 97–105 were derived from *Saccharum* spp. and 9–16 from *E. arundinaceus*, respectively (Table 2, Fig 1A–1I; Figs A-I in S1 File). Since 22–35 chromosomes were derived from *E. arundinaceus* in BC1 as parents (YCE01-69, YCE01-102, YCE01-92, YCE01-134, YCE01-105 and YCE01-116) [21], these results indicate that the number of *E. arundinaceus* chromosomes in the BC2 progeny was reduced by approximately half of the *E. arundinaceus* chromosomes of the BC1 parents. For instance, YCE01-116 (BC1) was the male parent of YCE03-01; YCE01-116 and YCE03-01 bore 28 and 14 chromosomes derived from *E. arundinaceus*, respectively [21]. This indicates that the BC2 progeny were products of n + n transmission. GISH experiments of eight BC3 progeny revealed plants with a total chromosome complement ranging from 105 to 118 chromosomes, of which 98–109 were derived from *Saccharum* spp. and 4–8 from *E. arundinaceus*, respectively (Table 2 and Fig 2A–2H; Figs A-H in S2 File). Since 9–14 chromosomes were derived from *E. arundinaceus* in BC2 as parents (YCE03-168, YCE03-218 and YCE03-01), these results indicate that approximately half of the *E. arundinaceus* chromosomes of the BC2 parents was transmitted to the BC3 progeny. For example, YCE03-01 (BC2) was the male parent of YCE06-63; YCE03-01 and YCE06-63 bore 14 and 7 chromosomes derived from *E. arundinaceus*, respectively. Our results indicate that the eight BC3 progeny were products of n + n transmission. Piperidis et al. [19,20] reported that the similar transmission was in some different intergeneric BC2 and BC3 progeny between *Saccharum* spp. and *E. arundinaceus*.

In this study, YCE05-150 is a sibling line of YCE06-140 with five *E. arundinaceus* chromosomes. However, the number of *E. arundinaceus* chromosomes in YCE05-150 is eight. That is, more than half of the *E. arundinaceus* chromosomes in YCE03-218 was transmitted to YCE05-150. Given the results of chromosome count, the number of total chromosomes in YCE05-150, YCE03-218 (as the female parent) and ROC10 (as the male parent) were 116, 107 and 112, respectively. We can exclude the possibility that YCE05-150 was the product of 2n + n or n + 2n transmission. In fact, YCE05-150 was also the product of n + n transmission.

In noblization of *S. spontaneum*, the interspecific F1 hybrids and BC1 progeny result from 2n + n transmission. The speedy process of noblization is conducted to recover high biomass yield and sugar content from *S. officinarum* while retaining the stress tolerance characteristics from *S. spontaneum*. As a general rule, the improved varieties are obtained in the BC2 or BC3. However, the increasing times of noblization may negatively impact the recovery of vigor and resistance to biotic or abiotic stresses. Molecular cytogenetic studies of *E. arundinaceus* indicated that chromosome transmission was n + n in F1, BC2, and BC3 generations, but was 2n + n in the BC1 generation [19–21]. Compared to the progress of noblization for utilization of *S. spontaneum*, this slows down the progress of noblization in utilization of *E. arundinaceus* and may require the improved varieties from the BC3, BC4, or even higher generations. Interestingly, Wu et al. [21] recently reported that an unexpected inheritance pattern of *E. arundinaceus* chromosomes resulted from more than a 2n + n transmission in the BC1 generation. This may lead to the presence of a larger number of new, multilocus allelic combinations and potentially creates a massive opportunity for selection of desirable traits in newly synthesized germplasm.

Smut caused by *Sporisorium scitamineum* is a destructive and worldwide disease of sugarcane, resulting in severe yield reductions and considerable loss in sugar content. Wild relatives represent potentially important sources of desirable genes for sugarcane improvement. As one of the most important wild relatives of sugarcane, *E. arundinaceus* has the potential to improve...
Fig 1. GISH analysis of the intergeneric BC$_2$ progeny between *Saccharum* spp. and *E. arundinaceus*. *Saccharum* spp. chromosomes are visualized in red and *E. arundinaceus* chromosomes in green. (A) YCE03-01: 2n = 119 = 105S + 14E; (B) YCE03-06: 2n = 119 = 105S + 14E; (C) YCE03-16: 2n = 113 = 100S + 13E; (D) YCE03-168: 2n = 111 = 100S + 10E + E/S; (E) YCE03-218: 2n = 107 = 97S + 10E; (F) YCE03-249: 2n = 110 = 97S + 13E; (G) YCE03-378: 2n = 121 = 104S + 16E + S/E; (H) YCE04-55: 2n = 111 = 98S + 13E; (I) YCE05-179: 2n = 112 = 99S + 13E. The arrowheads in Fig 1D and Fig 1G show the translocated...
disease resistance in sugarcane. Introgressing from wild relatives into sugarcane is an effective approach to broadening the genetic basis of sugarcane germplasm. More strikingly, recent reports have proven that YCE05-179, a BC2 progeny, is resistant to sugarcane smut [47,48].

The rest of the other yet-to-be-identified progeny between Saccharum spp. and E. arundinaceus might provide superior lines resistant to abiotic and biotic stresses. It is necessary to screen the progeny between Saccharum spp. and E. arundinaceus for those most likely to improve commercial and agricultural traits.

**Chromosomal translocation in the intergeneric BC₂ and BC₃ progeny between Saccharum spp. and E. arundinaceus**

The current results obtained in this study together with those present in a previous study indicate that eight progeny harbored an intergeneric chromosomal translocation between Saccharum spp. and E. arundinaceus (Table 2, Fig 1D and 1G; Figs D and G in S1 File; and Fig 2A, 2E, 2F, 2G; Figs A, E, F, G in S2 File). In our previous study, out of 13 BC₁ progeny analyzed, two BC₁ progeny (YCE01-36 and YCE01-92) both carried an intergeneric translocated chromosome, and the chromosomal translocation occurred at a terminal fragment from the E. arundinaceus chromosome [21]. In this present study, out of nine BC₂ progeny analyzed, two BC₂ progeny (YCE03-168 and YCE03-378) both contained one translocated chromosome, and the chromosomal translocation occurred at a terminal fragment from the E. arundinaceus chromosome (Table 2, Fig 1D and 1G; Figs D and G in S1 File). Moreover, out of eight BC₃ progeny analyzed, there were four BC₃ progeny (YCE05-64, YCE06-92, YCE06-111, and YCE06-140) with chromosome translocations. Piperidis et al. [20] also reported the presence of intergeneric chromosomal translocations between Saccharum spp. and E. arundinaceus in BC₃. In our study, YCE06-111 and YCE06-140 possessed an intergeneric translocated chromosome, and YCE06-92 and YCE05-64 carried two and five translocated chromosomes, respectively. The chromosomal translocation occurred at the terminal fragment from E. arundinaceus chromosomes in all these cases (Table 2, Fig 2A, 2E, 2F and 2G; Figs A, E, F, G in S2 File). Notably, multiple chromosome translocations tend to occur in YCE06-92 and YCE05-64, indicating that these two progeny were more prone to translocation. These results revealed that chromosome breakpoint tended to occur at the terminal fragment from Saccharum spp. chromosome and/or E. arundinaceus chromosome. But in essence, there are basically two distinct types of recombinant chromosome in these chromosomal translocation events: (1) the recombinant chromosome of Saccharum spp. with terminally located fragment of E. arundinaceus (S/E); and (2) the recombinant chromosome of E. arundinaceus with terminally located fragment of Saccharum spp. (E/S). In both types, S/E and E/S indicate Saccharum spp. centromere with E. arundinaceus chromosome segment and E. arundinaceus centromere with Saccharum spp. chromosome segment, respectively. In the light of the GISH results in our previous and present study, the type of recombinant chromosome in YCE01-36, YCE01-92, YCE03-378 and YCE06-140 belongs to the former type, whereas that in YCE03-168, YCE06-92 and YCE06-111 belongs to the latter one. In addition, the type of recombinant chromosome in YCE05-64 is the admixture of S/E and E/S, including 2(E/S) and 3(S/E).
Fig 2. GISH analysis of the intergeneric BC$_3$ progeny between *Saccharum* spp. and *E. arundinaceus*. *Saccharum* spp. chromosomes are visualized in red and *E. arundinaceus* chromosomes in green. (A) YCE05-64: 2n = 118 = 107S + 8E + 2(E/S)+3(S/E); (B) YCE05-150: 2n = 116 = 108S + 8E; (C) YCE06-61: 2n = 114 = 107S + 7E; (D) YCE06-63: 2n = 105 = 98S + 7E; (E) YCE06-92: 2n = 118 = 109S + 7E + 2(E/S); (F) YCE06-111: 2n = 108 = 103S + 4E + E/S; (G) YCE06-140: 2n = 112 = 106S + 5E + S/E; (H) YCE06-166: 2n = 110 = 105S + 5E. The arrowheads in Fig 2A, Fig 2E, Fig 2F and Fig 2G show the translocated chromosome. S and E indicate *Saccharum* spp. chromosome and *E. arundinaceus* chromosome.
Translocated chromosomes are stably transmitted to the progeny

Based on the pedigree, YCE01-92 (BC1) is the female parent for YCE03-378 (BC2), and YCE03-168 (BC2) is the male parent for YCE06-111 (BC3), respectively. In YCE01-92 there is one recombinant chromosome which is transmitted to YCE03-378. Similarly, in YCE03-168 there is also one recombinant chromosome which is transmitted to YCE06-111. That is, according to the transgenerational inheritance of the translocated chromosome, we can conclude that these translocated chromosomes could be stably transmitted to the progeny in subsequent generations. We recently reported that chromosome translocations only occur in the terminal regions and not the centromeric regions. This finding demonstrated that the terminal regions of the *E. arundinaceus* and/or *Saccharum* spp. chromosomes are more actively involved in translocations than the centromeric regions. It is possible that this is because intercalary translocations require more chromosome breakage events than terminal translocations, and therefore rarely occur. In addition, recombination events occur in different generations and in different progeny, suggesting that translocation events are not restricted to an individual progeny.

Significance of intergeneric chromosome translocation for sugarcane improvement

Previous molecular cytogenetic studies have suggested that a few chromosomes were derived from interspecific recombination between *S. officinarum* and *S. spontaneum* in modern sugar-cane cultivars [24]. In our study, despite a large genetic distance between *Saccharum* spp. and *E. arundinaceus* [15,16,49], the occurrence of intergeneric chromosomal translocations occurred within the BC1, BC2, and BC3 generations. These results suggest that intergeneric chromosome translocations can occur during an early generation. A considerable number of the recombinant chromosomes confirmed that homologous recombination occurs in the resulting progeny from *Saccharum* spp. and *E. arundinaceus*. Undoubtedly, the intergeneric chromosome translocations between *Saccharum* spp. chromosomes and *E. arundinaceus* chromosomes in this study represent a new genetic variation between these two genomes. From the point-of-view of sugarcane, intergeneric chromosome translocations can import *E. arundinaceus* chromosome segments or useful genes of *E. arundinaceus* into sugarcane. A translocation event can lead to a break in the genetic linkage, which increases the opportunity to segregate genetic variation and opens up the possibility for generating genetic and phenotypic novelty. Importantly, this kind of genetic variation may have a positive impact on sugarcane improvement.

Supporting Information

S1 File. GISH analysis of the intergeneric BC2 progeny between *Saccharum* spp. and *E. arundinaceus*. (A) *Saccharum* spp. chromosomes are visualized in red; (B) *E. arundinaceus* chromosomes are visualized in green; (C) All chromosomes are counterstained in blue; (D) A merged image is generated from the red and green channels. S and E indicate *Saccharum* spp. chromosome and *E. arundinaceus* chromosome, respectively. S/E and E/S indicate *Saccharum* spp. centromere with *E. arundinaceus* chromosome segment and *E. arundinaceus* centromere.
with *Saccharum* spp. chromosome segment, respectively. Scale bars: 5 μm.

(S2 File) GISH analysis of the intergeneric BC\textsubscript{3} progeny between *Saccharum* spp. and *E. arundinaceus*. (A) *Saccharum* spp. chromosomes are visualized in red; (B) *E. arundinaceus* chromosomes are visualized in green; (C) All chromosomes are counterstained in blue; (D) A merged image is generated from the red and green channels. S and E indicate *Saccharum* spp. chromosome and *E. arundinaceus* chromosome, respectively. S/E and E/S indicate *Saccharum* spp. centromere with *E. arundinaceus* chromosome segment and *E. arundinaceus* centromere with *Saccharum* spp. chromosome segment, respectively. Scale bars: 5 μm.

(S2 File)

**Acknowledgments**

We thank Angélique D’hont, Nathalie Piperidis and George Piperidis for technical assistance. We thank Guangzhou Sugarcane Industry Research Institute for providing the plant materials used in this study.

**Author Contributions**

Conceived and designed the experiments: YJH JYW ZHD. Performed the experiments: YJH JYW. Analyzed the data: YJH JYW PW YQL RKC MQZ ZHD. Contributed reagents/materials/analysis tools: JYW YQL RKC MQZ ZHD. Wrote the paper: YJH JYW PW ZHD. Provided the plant materials: CF QNW QWL.

**References**

1. FAOSTAT (2013). Available: http://faostat.fao.org.
2. Lam E, Shine J, Da Silva J, Lawton M, Bonos S, Calvino M, et al. Improving sugarcane for biofuel: engineering for an even better feedstock. GCB Bioenergy. 2009; 1(3): 251–255.
3. Jannoo N, Grivet L, Chantret N, Garsmeur O, Glaszmann JC, Arruda P, et al. Orthologous comparison in a gene-rich region among grasses reveals stability in the sugarcane polyploid genome. Plant J. 2007; 50(4): 574–585. PMID: 17425713
4. Bremer G. Problems in breeding and cytology of sugar cane. Euphytica. 1961; 10(1): 59–78.
5. Roach B. Nobilisation of sugarcane. Proc Int Soc Sugar Cane Technol. 1972; 14: 206–216.
6. Lu Y, D’Hont A, Walker D, Rao P, Feldmann P, Glaszmann J. Relationships among ancestral species of sugarcane revealed with RFLP using single copy maize nuclear probes. Euphytica. 1994; 78(1–2): 7–18.
7. Irvine J. *Saccharum* species as horticultural classes. Theor Appl Genet. 1999; 98(2): 186–194.
8. Amalraj VA, Balasundaram N. On the taxonomy of the members of "*Saccharum* complex". Genet Resour Crop Ev. 2006; 53(1): 35–41.
9. D’Hont A, Rao PS, Feldmann P, Grivet L, Islam-Faridi N, Taylor P, et al. Identification and characterisation of sugarcane intergeneric hybrids, *Saccharum officinarum* × *Erianthus arundinaceus*, with molecular markers and DNA in situ hybridisation. Theor Appl Genet. 1995; 91(2): 320–326. doi: 10.1007/BF00220949 PMID: 24169780
10. Rott P, Mohamed I, Klett P, Soupa D, de Saint-Albin A, Feldmann P, et al. Resistance to leaf scald disease is associated with limited colonization of sugarcane and wild relatives by *Xanthomonas albilineans*. Phytopathology. 1997; 87(12): 1202–1213. doi: 10.1094/PHYTO.1997.87.12.1202 PMID: 18945019
11. Piperidis G, Christopher MJ, Carroll BJ, Berding N, D’Hont A. Molecular contribution to selection of intergeneric hybrids between sugarcane and the wild species *Erianthus arundinaceus*. Genome. 2000; 43(6): 1033–1037. PMID: 11955351
12. Cai Q, Atkens KS, Fan YH, Piperidis G, Jackson P, McIntyre CL. A preliminary assessment of the genetic relationship between *Erianthus rockii* and the "*Saccharum* complex" using microsatellite (SSR) and AFLP markers. Plant Sci. 2005; 169(5): 976–984.
13. Jackson P, Henry RJ. *Erianthus*. In: Kole C, editors. Wild Crop Relatives: Genomic and Breeding Resources. Berlin Heidelberg: Springer; 2011. pp. 97–107.

14. Burner DM. Cytogenetic analyses of sugarcane relatives (*Andropogoneae: Saccharinae*). Euphytica. 1991; 54(1): 125–133.

15. Nair NV, Nair S, Sreenivasan TV, Mohan M. Analysis of genetic diversity and phylogeny in *Saccharum* and related genera using RAPD markers. Genet Resour Crop Ev. 1999; 46(1): 73–79.

16. Alik X, Paulet F, Glaszmann J, D’Hont A. Inter-Alu-like species-specific sequences in the *Saccharum* complex. Theor Appl Genet. 1999; 99(6): 962–968.

17. Sobral BWS, Braga DPV, LaHood ES, Keim P. Phylogenetic analysis of chloroplast restriction enzyme site mutations in the *Saccharinae* Griseb. subtribe of the *Andropogoneae* Dumort. tribe. Theor Appl Genet. 1994; 87(7): 843–853. doi: 10.1007/BF00221137 PMID: 24190471

18. Ram B, Sreenivasan T, Sahi B, Singh N. Introggression of low temperature tolerance and red rot resistance from *Erianthus* in sugarcane. Euphytica. 2001; 122(1): 145–153.

19. Piperidis N, Chen J, Deng H, Wang L, Jackson P, Piperidis G. GISH characterization of *Erianthus arundinaceus* chromosomes in three generations of sugarcane intergeneric hybrids. Genome. 2010; 53(5): 331–336. doi: 10.1139/g10-010 PMID: 20616864

20. Piperidis N, Aitken K, Hermann S. Towards a reliable method to select potentially high value *Erianthus* hybrids. Int Sugar J. 2013: 1–9.

21. Wu J, Huang Y, Lin Y, Fu C, Liu S, Deng Z, et al. Unexpected inheritance pattern of *Erianthus arundinaceus* chromosomes in the intergeneric progeny between *Saccharum* spp. and *Erianthus arundinaceus*. PLoS One. 2014; 9(10): e110390. doi: 10.1371/journal.pone.0110390 PMID: 25310831

22. Burner DM, Legendre BL. Chromosome Transmission and Meiotic Stability of Sugarcane (*Saccharum* spp.) Hybrid Derivatives. Crop Sci. 1993; 33(3): 600–606.

23. Piperidis G, Piperidis N, D’Hont A. Molecular cytogenetic investigation of chromosome composition and transmission in sugarcane. Mol Genet Genomics. 2010; 284(1): 65–73. doi: 10.1007/s00438-010-0546-3 PMID: 20532965

24. D’Hont A, Grivet L, Feldmann P, Rao S, Berding N, Glaszmann JC. Characterisation of the double genome structure of modern sugarcane cultivars (*Saccharum* spp.) by molecular cytogenetics. Mol Gen Genet. 1996; 250(4): 405–413. PMID: 8602157

25. Hermann SR, Aitken KS, Jackson PA, George AW, Piperidis N, Wei X, et al. Evidence for second division restitution as the basis for 2n+n maternal chromosome transmission in a sugarcane cross. Euphytica. 2012; 187(3): 359–368.

26. Deng ZH, Zhang MQ, Lin WL, Cheng F, Zhang CM, Li YC, et al. Analysis of Disequilibrium Hybridization in Hybrid and Backcross Progenies of *Saccharum officinarum* x *Erianthus arundinaceus*. Agric Sci China. 2010; 9(9): 1271–1277.

27. Lavia GI, Ortiz AM, Robledo G, Fernandez A, Seijo G. Origin of triploid *Arachis pintoi* (Leguminosae) by autoploidy evidenced by FISH and meiotic behaviour. Ann Bot. 2011; 108(1): 103–111. doi: 10.1093/aob/mcr108 PMID: 21693666

28. De Storme N, Geelen D. Sexual polyploidization in plants cytological mechanisms and molecular regulation. New Phytol. 2013; 198(3): 670–684. doi: 10.1111/nph.12184 PMID: 23421646

29. Mason AS, Pires JC. Unreduced gametes: meiotic mishap or evolutionary mechanism? Trends Genet. 2015; 31(5): 5–10. doi: 10.1016/j.tig.2014.09.011 PMID: 25445549

30. Ramanna MS, Jacobsen E. Relevance of sexual polyploidization for crop improvement—A review. Euphytica. 2003; 133(1): 3–18.

31. Barba-Gonzalez R, Lokker AC, Lim KB, Ramanna MS, Van Tuyl JM. Use of 2n gametes for the production of sexual polyplids from sterile Oriental x Asiatic hybrids of lilies (*Lilium*). Theor Appl Genet. 2004; 109(6): 1125–1132. PMID: 15290047

32. Lim KB, Shen TM, Barba-Gonzalez R, Ramanna MS, Van Tuyl JM. Occurrence of SDR 2n gametes in *Lilium* hybrids. Breeding Sci. 2004; 54(1): 13–18.

33. Park TH, Kim JB, Hutton RCB, van Eck HJ, Jacobsen E, Visser RGF. Genetic positioning of centromeres using half-tetrad analysis in a 4x-2x cross population of potato. Genetics. 2007; 176(1): 85–94. PMID: 17392117

34. Jansky SH, Peloquin SJ. Advantages of wild diploid *Solanum* species over cultivated diploid relatives in potato breeding programs. Genet Resour Crop Ev. 2006; 53(4): 669–674.

35. Cuenca J, Aleza P, Navarro L, Ollitrault P. Assignment of SNP allelic configuration in polyplids using competitive allele-specific PCR: application to citrus triploid progeny. Ann Bot. 2013; 111(4): 731–742. doi: 10.1093/aob/mct032 PMID: 23422023
36. Cuenca J, Froelicher Y, Aleza P, Juarez J, Navarro L, Ollitrault P. Multilocus half-tetrad analysis and centromere mapping in citrus: evidence of SDR mechanism for 2n megagametophyte production and partial chiasma interference in mandarin cv ‘Fortune’. Heredity. 2011; 107(5): 462–470. doi:10.1038/hdy.2011.33 PMID: 21587302

37. Aleza P, Juarez J, Cuenca J, Ollitrault P, Navarro L. Recovery of citrus triploid hybrids by embryo rescue and flow cytometry from 2x x 2x sexual hybridisation and its application to extensive breeding programs. Plant Cell Rep. 2010; 29(9): 1023–1034. doi:10.1007/s00299-010-0888-7 PMID: 20607244

38. Ssebuliba RN, Tenkouano A, Pillay M. Male fertility and occurrence of 2n gametes in East African Highland bananas (Musa spp.). Euphytica. 2008; 164(1): 53–62.

39. Zhang HK, Bian Y, Gou XW, Dong YZ, Rustgi S, Zhang BJ, et al. Intrinsic karyotype stability and gene copy number variations may have laid the foundation for tetraploid wheat formation. P Natl Acad Sci USA 2013; 110(48): 19466–19471.

40. Harper J, Armstead I, Thomas A, James C, Gasior D, Bisaga M, et al. Alien introgression in the grasses Lolium perenne (perennial ryegrass) and Festuca pratensis (meadow fescue): the development of seven monosomic substitution lines and their molecular and cytological characterization. Ann Bot. 2011; 107(8): 1313–1321. doi:10.1093/aob/mcr083 PMID: 21486927

41. Manzanero S, Vega JM, Houben A, Puertas MJ. Characterization of the constriction with neocentric activity of 5RL chromosome in wheat. Chromosoma. 2002; 111(4): 228–235. PMID: 12424523

42. An DG, Zheng Q, Zhou YL, Ma PT, Lv ZL, Li LH, et al. Molecular cytogenetic characterization of a new wheat-rye 4R chromosome translocation line resistant to powdery mildew. Chromosome Res. 2013; 21(4): 419–432. doi:10.1007/s10577-013-9366-8 PMID: 23836161

43. Markova M, Michu E, Vyskot B, Janousek B, Zluvova J. An interspecific hybrid as a tool to study phylogenetic relationships in plants using the GISH technique. Chromosome Res. 2007; 15(8): 1051–1059. PMID: 18075777

44. Zhang P, Li WL, Frieb B, Gill BS. The origin of a “zebra” chromosome in wheat suggests nonhomologous recombination as a novel mechanism for new chromosome evolution and step changes in chromosome number. Genetics. 2008; 179(3): 1169–1177. doi:10.1534/genetics.108.089599 PMID: 18562667

45. Mestiri I., Chague V, Tanguy AM, Hunecu C, Huteau V, Belcram H, et al. Newly synthesized wheat allohexaploids display progenitor-dependent meiotic stability and aneuploidy but structural genomic additivity. New Phytol. 2010; 186(1): 86–101. doi:10.1111/j.1469-8137.2010.03186.x PMID: 20149116

46. Kynast RG, Riera-Lizarazu O, Vales MI, Okagaki RJ, Maquieira SB, Chen G, et al. A complete set of maize individual chromosome additions to the oat genome. Plant Physiol. 2001; 125(3): 1216–1227. PMID: 11244103

47. Su Y, Guo J, Ling H, Chen S, Wang S, Xu L, et al. Isolation of a Novel Peroxisomal Catalase Gene from Sugarcane, which Is Responsive to Biotic and Abiotic Stresses. PloS One. 2014; 9(1): e84426. doi: 10.1371/journal.pone.0084426 PMID: 24392135

48. Su YC, Xu LP, Xue BT, Wu QB, Guo JL, Wu LG, et al. Molecular cloning and characterization of two pathogenesis-related beta-1,3-glucanase genes ScGluA1 and ScGluD1 from sugarcane infected by Sporisorum scitamineum. Plant Cell Rep. 2013; 32(10): 1503–1519. doi: 10.1007/s00299-013-1463-9 PMID: 23842683

49. Alix K, Baurens FC, Paulet F, Glaszmann JC, D'Hont A. Isolation and characterization of a satellite DNA family in the Saccharum complex. Genome. 1998; 41(6): 854–864. PMID: 9924794