Genetic recombination in *Sorghum bicolor* × *S. macrospurum* interspecific hybrids


**Abstract:** Sorghum has been improved by public and private breeding programs utilizing germplasm mostly from within the species *Sorghum bicolor*. Until recently, cross-incompatibilities have prevented hybridization of *S. bicolor* with most other species within the genus *Sorghum*. Utilizing germplasm homozygous for the *iap* allele, hybrids were readily produced between *S. bicolor* (*2n = 20; AAB₁B₁*) and *S. macrospurum* (*2n = 40; WWXXXYYYZZ*). These hybrids were intermediate to the parents in chromosome number (*2n = 30*) and overall morphology. Meiosis in both parents was regular; *S. bicolor* had 10 bivalents per pollen mother cell (PMC) and *S. macrospurum* had an average of 19.96 bivalents per PMC. Six hybrids were studied cytologically and meiosis was irregular, with the chromosomes associating primarily as univalents and bivalents. There was an average of 3.54 bivalents per PMC, with a range of 0–8 bivalents, most of which were rods (98%). Using FISH (fluorescent in situ hybridization), moderate levels (2.6 II per PMC) of allosyndetic recombination were observed. Genomic relationships were sufficient to assign *S. macrospurum* the genomic formula AAB₁B₁YYZZ (*Y* and *Z* remain unknown). Allosyndetic recombination in the interspecific hybrids indicates that introgression through genetic recombination should be possible if viable backcrosses can be recovered.

**Key words:** *Sorghum bicolor*, interspecific hybrids, genetic recombination.

**Résumé :** Le sorgho a été amélioré dans le cadre de programmes de sélection publics et privés faisant appel à des ressources génétiques appartenant principalement à l’espèce *Sorghum bicolor*. Jusqu’à récemment, les incompatibilités avaient empêché les croisements avec la plupart des espèces du sein du genre *Sorghum*. En employant des accessions homozygotes pour l’allèle *iap*, des hybrides ont été obtenus aisément entre le *S. bicolor* (*2n = 20; AAB₁B₁*) et le *S. macrospurum* (*2n = 40; WWXXXYYYZZ*). Ces hybrides étaient intermédiaires entre les parents pour ce qui est du nombre de chromosomes (*2n = 30*) et de leur morphologie générale. La méiose chez les deux parents était normale avec 10 bivalents par microsporocyte chez le *S. bicolor* et une moyenne de 19.96 bivalents par microsporocyte chez le *S. macrospurum*. Six hybrides ont fait l’objet d’analyses cytogénétiques et la méiose y était irrégulière avec les chromosomes montrant principalement des univalents et des bivalents. Entre 0 et 8 bivalents, pour une moyenne de 3.54 bivalents par microsporocyte, ont été observés principalement sous forme de bâtonnets (98 %). Lors d’hybridations in situ en fluorescence (FISH), des niveaux modestes (2.6 bivalents par microsporocyte) de recombinaison allosyndétique ont été observés. Les relations génomiques étaient suffisantes pour assigner au *S. macrospurum* la formule génomique AAB₁B₁YYZZ, *Y* et *Z* demeurant inconnus. La recombinaison allosyndétique chez les hybrides interspécifiques indiquerait que l’introggression via la recombinaison génétique est possible si l’on peut obtenir des rétrocroisements viables.

**Mots-clés :** *Sorghum bicolor*, hybrides interspécifiques, recombinaison génétique.

[Traduit par la Rédaction]

**Introduction**

*Sorghum* (*Sorghum bicolor* (L.) Moench) is an important food, feed, and forage crop in the USA and throughout the world. The genus *Sorghum* contains 5 sections; *S. bicolor* is a member of the section *Eu-sorghum* (Kunth) Hitch. and *S. halepense* (L.) Pers. The remaining 4 sections contain 19 species native to Africa, Australia, and Asia (Garber 1950; de Wet 1978; Lazarides et al. 1991). Breeding efforts have mainly used the primary gene pool of diverse germplasm within the species *S. bicolor* (Duncan et al. 1991). Limited efforts have been made to utilize the secondary gene pool consisting of the remaining species within *Eu-sorghum*. Interspecific hybrids are easily made with...
S. propinquum and, with some effort, S. halepense; however, few breeding programs have utilized the germplasm (Wooten 2001; Dweikat 2005). The tertiary gene pool contains all the remaining species within the genus, and despite many efforts to produce interspecific hybrids, until recently crosses had not been successful (Karper and Chisholm 1936; Ayyanger and Ponnaiya 1941; Garber 1950; Endrizzi 1957; Tang and Liang 1988; Wu 1990; Sun et al. 1991; Huelgas et al. 1996).

Hodnett et al. (2005) determined that pollen–pistil incompatibilities were the cause of reproductive isolation between S. bicolor and species in the tertiary gene pool. Arrested pollen tube growth of the wild species in the stigma and style of S. bicolor prevented successful fertilization. However, Price et al. (2005b) produced a single interspecific hybrid between cytoplastic male-sterile (CMS) S. bicolor (2n = 20) and the Australian species S. macrospermum Garber (2n = 40), indicating that the barriers to hybridization were strong but not complete. Further research determined that interspecific hybridization efficiency could be improved by the use of pollens of S. bicolor homozygous for the ssp allele. The ssp gene locus (Inhibition of alien pollen tubes) controls incompatibilities between pistils of S. bicolor and pollen of alien species (Laurie and Bennett 1989). Using homozygotes for the ssp allele, Price et al. (2006) reported interspecific hybrids between S. bicolor and three tertiary gene pool species: S. macrospermum, S. nitidum (Vahl) Pers., and S. angustum S.T. Blake. Interspecific hybrids were verified morphologically and cytologically, but only hybrids with S. macrospermum survived to maturity.

The genus Sorghum is divided into two distinct lineages, x = 5 and x = 10 (Price et al. 2005a). The x = 5 lineage (2n = 10, 20, 30, and 40) consists of species with large genomes and large chromosomes, while the x = 10 lineage (2n = 20 and 40) is characterized by small genomes and small chromosomes. Both S. macropermum and S. bicolor belong to the x = 10 lineage. The base chromosome number in Sorghum spp. is generally regarded as x = 5, and research has shown that S. bicolor is likely an ancient tetraploid (2n = 4x = 20) with distinct but related subgenomes (Garber 1950; Hadley 1953; Endrizzi and Morgan 1955; Celarier 1958; Doggett 1988; Tang and Liang 1988; Gomez et al. 1998; Zwick et al. 2000). Tang and Liang (1988) reviewed the data regarding genomic relationships between S. halepense and S. bicolor, and they designated the former as having the genomic formula AAAAB1B1B1B2 and the latter as having AAB1B2. They concluded that subgenomes B1 and B2 share enough homology that homoeologous chromosome pairing can occur. Subgenome A shares much less homology with subgenomes B1 and B2, but multivalents involving members of all three genomes are possible. Utilising FISH, the most recent cytogenetic research assigned S. bicolor the genomic formula A1A2B1B2B2, the subscript “b” representing bicolor, based on differential probe hybridization (Zwick et al. 2000). In this manuscript, S. bicolor will be designated AAB1B1. Little is known about the genomic relationships between S. bicolor and S. macropermum. Wu (1990) studied the karyotype of S. macropermum and suggested it was a polyploid with high amounts of similarity to the S. bicolor genome. That research showed that the chromosome sizes of both species were similar and overlapped, and that S. macropermum appeared to have two chromosomes for each individually identifiable S. bicolor chromosome. During meiosis, S. macropermum behaved as a diploid, forming 20 bivalents, although quadrivalents were observed. This suggests that S. macropermum is likely 2n = 8x = 40, with an unknown genomic formula, WWXXYYZZ.

Sorghum macropermum is the only member of the section Chaetosorghum and is native to the Katherine area in the Northern Territory of Australia (Lazarides et al. 1991). It is a nonhost or has ovipositional non-preference resistance to sorghum midge (Stenodiplosis sorgicola Coquillett) (Franzmann and Hardy 1996; Sharma and Franzmann 2001). It also is immune to sorghum downy mildew (Peronosclerospora sorghicola Weston and Uppal (Shaw)) (Kamala et al. 2002) and is highly tolerant to shoot fly (Atherigona soccata Rond.) (Sharma et al. 2005). There is interest in using this newly compatible species in an interspecific breeding program for the improvement of S. bicolor. The goal of such an interspecific breeding program is to transfer useful S. macropermum genetic variation to the S. bicolor genome. The most direct way for such introgression to occur is through allozyndetic recombination during meiosis, which is recombination between chromosomes of different parental genomes (Burnham 1962; Jauhar and Chibbar 1999). The level of allozyndetic recombination is largely a function of the amount of genetic similarity between the different genomes (Singh 2003). Homoeologous chromosomes, genetically related through ancestry, will pair if they retain sufficient homology with one another. Backcross progeny containing such recombinant chromosomes would possess S. macropermum genetic variation. Autosyndetic recombination, which is recombination between chromosomes from the same parental genome, does not result in introgression within the S. bicolor genome because no exchange occurs between the chromosomes of the different genomes. Therefore, determining whether allozyndetic recombination occurs in S. bicolor × S. macropermum hybrids will provide insight as to the possibility of introgression occurring.

The objectives of this research were to determine the genomic relationship between S. bicolor and S. macropermum, measure the frequency of allozyndetic recombination during meiosis, and recover backcrosses to the S. bicolor parent.

Materials and methods

Plant materials

Interspecific hybrids were produced using S. bicolor accession Nr481 (iap iap) as the hand-emasculated female parent and the wild species S. macropermum (AustTRC accession No. 302367) as the male parent. Nr481 is the genotype in which the iap gene was first identified (Laurie and Bennett 1989). Pollinated florets set approximately 25% seed with a shrunken endosperm. Approximately 60% of the seeds germinated on agar media and the seedlings were subsequently transplanted into soil. The hybrid seedlings were identified by the pubescence on their leaves, a trait absent in S. bicolor but present in both S. macropermum and the interspecific hybrids. The putative hybrids were confirmed by determining their chromosome number (2n = 30) from somatic root-tip spreads (Jewell and Islam-Faridi 1994).
Morphology of the interspecific hybrids did not differ from previously published descriptions (Price et al. 2005b). Six hybrids, as well as control plants of the two parents, were grown in a greenhouse in the summer of 2004 in College Station, Texas, for cytological studies. Backcrosses were attempted by collecting anthers (non-dehiscent) from the interspecific hybrids, disrupting the anthers in a glass Petri dish, and dusting pollen onto CMS *S. bicolor* (**iap iap**) stigmas with a fine-bristle paintbrush.

**Traditional cytogenetic analysis**

Immature panicles, with the flag leaf collar extended 3–4 inches above the last leaf collar, were harvested and fixed whole in Carnoy’s solution (ethanol–chloroform–glacial acetic acid, 6:3:1) for a minimum of 24 h and then transferred to 70% ethanol for storage. The anthers were dissected from the florets and macerated on a glass slide in a drop of aceticarmine stain. A cover slip was placed over the stain and the slide was heated over an alcohol flame before being squashed on a hard surface between filter paper. Pollen mother cells (PMCs) were examined by phase-contrast microscopy using a Zeiss Axioshot microscope (Carl Zeiss Inc., Gottingen, Germany) at 1000–2000× magnification. Meiotic analysis of both parents and the interspecific hybrids consisted of counting the numbers of univalents, bivalents (both rod and ring conformations), trivalents, and quadrivalents in each PMC. Note of laggard chromosomes at anaphase was also taken. Images were taken using a Nikon COOLPIX 4500 digital camera with a 57 mm to 38 mm stepdown adapter through the microscope eyepiece. Data were analyzed using GLM in SPSS version 11.5 (SPSS Inc., Chicago).

**Fluorescent in situ hybridization**

Slides to be used for FISH (fluorescent in situ hybridization) were prepared in the same manner as described above except the anthers were macerated in 20% acetic acid, and after squashing were immediately frozen at −80 °C. A cloned (pCEN38) centromere-associated sequence, present on all *S. bicolor* chromosomes and visually absent from *S. macrospermum* chromosomes, was used as a probe to visually differentiate the genomes (Zwick et al. 2000; Anderson 2005). Detection of the FISH probe followed a modified protocol of Jewell and Islam-Faridi (1994), as described by Hanson et al. (1995) and Kim et al. (2002). Purified CEN38 DNA was nick-translated with digoxigenin-11-dUTP (Roche Diagnostics, Indianapolis, Indiana). Meiotic chromosomes on glass slides were denatured in 70% formamide in 2× SSC for 1 min at 70 °C, then dehydrated in 70% (−20 °C), 85% (RT), 95% (RT), and 100% (RT) ethanol, for 2 min each. The hybridization mixture (25 µL per slide) contained 50 ng of labeled probe DNA, 50% formamide, and 10% dextran sulfate in 2× SSC. The hybridization mixture was denatured for 10 min at 95 °C, chilled on ice, and then added to the slide. Slides were sealed with rubber cement around a glass cover slip and incubated overnight at 37 °C. Following incubation, the slides were washed at 40 °C in 2× SSC and at room temperature in 4× SSC plus 0.2% Tween-20 for 5 min each. Slides were blocked with 5% (w/v) BSA in 4× SSC plus 0.2% Tween-20 at room temperature. The digoxigenin-labeled probe was detected with Cy3-conjugated anti-digoxigenin antibody. Slides were then washed in 4× SSC plus 0.2% Tween-20 at 37 °C and chromosomes were counterstained with 25 µL of DAPI with Vectashield. Slides were viewed through an Olympus AX-70 epifluorescence microscope and images were captured with a Macprobe version 4.2.3 imaging system (Applied Imaging Corp., Santa Clara, California). Meiotic analysis of the interspecific hybrids consisted of counting the numbers of bivalents (rods or rings) and multivalents in each PMC as well as associations with CEN38.

**Molecular markers**

DNA was extracted from fresh leaf tissue of 11 BC1F1 plants, their parents, and the parents (BTx623 and IS3620C) of a recombinant inbred line genetic mapping population (Menz et al. 2002) using FastDNA SPIN Kits (MP Biomedicals, Solon, Ohio). AFLP templates, using both *EcoRI*/*MseI* and *PstI*/*MseI* restriction enzyme combinations, were created using a procedure modified from Vos et al. (1995). The AFLP template, preamplification, and selective amplification reactions of the *EcoRI*/*MseI* and *PstI*/*MseI* fragments were as described by Klein et al. (2000) and Menz et al. (2002), respectively. Thirty *PstI*/*MseI* and 15 *EcoRI*/*MseI* primer combinations were used to amplify fragments in the DNA samples. Amplification products were analyzed on a LI-COR model 4200 dual-dye automated DNA sequencing system (LI-COR, Inc., Lincoln, Nebraska). Electrophoresis conditions were as described by Klein et al. (2000). Gels were analyzed visually and bands were scored as unique to *S. macrospermum* if they were not present in the CMS female parents; unique bands shared between the putative backcrosses and *S. macrospermum* were identified as introgression bands. Percent introgression was calculated as the total number of introgression bands found for a particular plant divided by the total number of unique *S. macrospermum* bands found. This number is an estimate of the amount of *S. macrospermum* genome that is present in each backcross plant.

**Results and discussion**

Meiotic analysis of the parents revealed mostly normal meiosis. *Sorghum bicolor* (acc. Nr481) had a mean chromosome pairing behavior of 10 bivalents per PMC, and no multivalents or univalents were observed (Table 1; Fig. 1A). The *S. macrospermum* parent had an average of 19.96 bivalents per PMC with only one quadrivalent in 48 cells observed (Table 1; Fig. 1B). Some level of quadrivalent formation was expected in the *S. macrospermum* parent, although the level observed was lower than previously reported (Wu 1990). Meiosis in the interspecific hybrids was abnormal: PMCs averaged 22.89 univalents and 3.54 bivalents, with 98% of the bivalents forming rods (Table 1; Fig. 1C). The number of bivalents ranged from 0 to 8, with the most common configuration being 24 I + 3 II (26% of PMCs), and multivalents were rare. Two interspecific hybrids had significantly lower bivalent formation than the others, indicating that recombination may be influenced by factors such as environment or genetic background (Table 1). The range of bivalents observed in the interspecific hybrids rules out exclusive *S. bicolor* autosynzygic pairing, as only 5
such bivalents are possible. Exclusive *S. macrospermum* autosynthetic pairing cannot be ruled out, since some homology exists within the genome and 10 such bivalents are possible. Preferential formation of rod-shaped bivalents is associated with lowered homology between genomes (Singh 2003), which would likely be the case if such pairing were allosyndetic. With such a low frequency of quadrivalent formation in the *S. macrospermum* parent, it is unlikely that all the bivalent formation in the hybrids can be attributed to autosyndesis within this parental genome. Traditional cytogenetic analysis cannot confirm allosyndetic recombination, as discrimination of these parental genomes is not possible, but these data do confirm that some recombination is occurring within the interspecific hybrids.

The FISH probe CEN38 does not visibly hybridize to *S. macrospermum* chromosomes but does differentially hybridize to 10 of the 20 somatic *S. bicolor* chromosomes (Zwick et al. 2000; Anderson 2005). Ten homologous *S. bicolor* chromosomes (SBI) show a strong CEN38 signal (SBI-01, -02, -03, -05, and -06) and 10 show a weak signal (SBI-04, -07, -08, -09, and -10) (Kim et al. 2005; J.S. Kim, personal communication). Zwick et al. (2000) suggested the differential hybridization corresponds to the subgenomes that make up *S. bicolor*: strong signal identifies subgenome A and weak signal identifies subgenome B1. The designation of CEN38 signal strength to subgenome was arbitrary, since it was not based on genome homology to *S. halepense*, which was used to assign the A and B1 subgenomes (Hadley 1953; Tang and Liang 1988), but these designations will be used in this discussion.

Meiotic FISH analysis of the interspecific hybrids revealed 4.3 bivalents per PMC, an estimate not significantly different from that obtained by traditional cytogenetic analysis. More importantly, it confirmed the presence of allosyndetic recombination between *S. bicolor* and *S. macrospermum* chromosomes, showing one chromosome with and one without CEN38 signal (Fig. 1D, 1). In fact, all three types of recombination were detected: allosyndetic, autosynthetic *S. macrospermum*, and autosyndetic *S. bicolor* (Table 2; Fig. 1D, 1–3). Allosyndetic recombination occurred in 61% of bivalents (2.6 II per PMC), which indicates that significant homology exists between some of the *S. bicolor* and *S. macrospermum* chromosomes. This agrees with Wu (1990), who suggested that *S. bicolor* may be one of the two ancestors of *S. macrospermum*.

Since the strong and weak CEN38 FISH signals differentiate *S. bicolor* subgenomes A and B1, the behavior of these subgenomes can be compared. In two different cells (Figs. 1E, 1F), four A and four B1 allosyndetic bivalents were separately detected. Together these represent 8 different *S. bicolor* chromosomes which are participating in recombination with members of the *S. macrospermum* genome. *Sorghum macrospermum* chromosomes cannot be differentiated in the present analysis; therefore, no definitive estimate can be given regarding the percentage of this genome that is subject to recombination with *S. bicolor*. It is likely that the 8 *S. bicolor* chromosomes are recombining with 8 different *S. macrospermum* homoeologous chromosomes. If so, then approximately 80% of the *S. bicolor* genome and possibly 40% of the *S. macrospermum* genome are subject to allosyndetic recombination. Clearly, the moderate level of allosyndetic recombination makes recovering introgression in backcross progeny very likely.

A and B1 *S. bicolor* chromosomes do not form allosynthetic bivalents with the *S. macrospermum* chromosomes at the same frequency. Chromosomes in subgenome A account for 71% of the allosyndetic bivalents in the interspecific hybrids (Table 3). So, while a majority of the genome has the capacity to recombine with *S. macrospermum* chromosomes, subgenome A chromosomes are more likely to be involved in recombination that results in introgression into an *S. bicolor* background.

Autosyndetic recombination occurred at a relatively high frequency within the *S. macrospermum* genome (36% of bivalents or 1.6 II per PMC) and at a very low frequency in the *S. bicolor* genome (2% of bivalents or 0.1 II per PMC) (Table 2). This indicates there is significant homology within the *S. macrospermum* haploid genome, and relatively little within the *S. bicolor* haploid genome. The relative differences in autosyndesis are likely a result of multiple factors. First, *S. bicolor* may have undergone significantly more diploidization, and thus have reduced homology within its subgenomes, than has *S. macrospermum* (Liu and Wendel 2002). Second, the differences may be due to an older evolutionary age for the tetraploid nature of *S. bicolor* and a more recent polyploidization event in *S. macrospermum*. Fi-

### Table 1. Chromosome pairing in *Sorghum bicolour, S. macrospermum*, and their interspecific hybrids.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>2n</th>
<th>No. of PMCs examined</th>
<th>Bivalents</th>
<th>Multivalent totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean*</td>
<td>Range % Rod*</td>
</tr>
<tr>
<td><em>S. bicolour</em></td>
<td>20</td>
<td>48</td>
<td>10.00B</td>
<td>NA</td>
</tr>
<tr>
<td><em>S. macrospermum</em></td>
<td>40</td>
<td>48</td>
<td>19.96A</td>
<td>18–20 NA</td>
</tr>
<tr>
<td><em>S. bicolour × S. macrospermum</em></td>
<td>30</td>
<td>312</td>
<td>3.54C</td>
<td>0–8 98A</td>
</tr>
<tr>
<td>Hybrid 1</td>
<td>30</td>
<td>54</td>
<td>3.59C</td>
<td>0–7 99A</td>
</tr>
<tr>
<td>Hybrid 2</td>
<td>30</td>
<td>53</td>
<td>3.96C</td>
<td>1–8 100A</td>
</tr>
<tr>
<td>Hybrid 3</td>
<td>30</td>
<td>28</td>
<td>1.68D</td>
<td>0–3 96A</td>
</tr>
<tr>
<td>Hybrid 4</td>
<td>30</td>
<td>48</td>
<td>3.58C</td>
<td>1–7 98A</td>
</tr>
<tr>
<td>Hybrid 5</td>
<td>30</td>
<td>115</td>
<td>3.95C</td>
<td>0–7 98A</td>
</tr>
<tr>
<td>Hybrid 6</td>
<td>30</td>
<td>14</td>
<td>2.07D</td>
<td>1–4 98A</td>
</tr>
</tbody>
</table>

LSD$_{0.05}$ 0.82 6
CV 26% 1%

*Values followed by different superscript letters are significantly different (p < 0.05).
Finally, it could be that polyploidization of *S. bicolor* involved two separate genomes with very little homology, while the nascent genomes of *S. macrospermum* were more closely related. Most likely all three describe the evolutionary history of these species. *Sorghum macrospermum* has been hypothesized to be an allooctaploid, originating from a chromosome doubling of two related *Eu-sorghum* species (Wu 1990), while *S. bicolor* is regarded as an allotetraploid, originating from doubling the chromosomes of the two more divergent genomes (Tang and Liang 1988; Gomez et al. 1998; Zwick et al. 2000).

Significant homology exists between the genomes of *S. bicolor* (2*n* = 4*x* = 20; AAB1B1) and *S. macrospermum* (2*n* = 8*x* = 40; WWXXYYZZ). Meiosis in the interspecific hybrids (2*n* = 6*x* = 30; AWXB1YZ) shows recombination, and FISH analysis reveals allozygotic recombination between as many as 8 *S. macrospermum* chromosomes and a minimum of 4 chromosomes from each of the *S. bicolor* subgenomes. Recombination between the *S. bicolor* subgenomes and their *S. macrospermum* homoeologs is not regular in that subgenomes A and B1 averaged 2.0 and 0.8 allozygotic bivalents per PMC, respectively (Table 3). Mul-
tivalents in the interspecific hybrids were very rare, indicating that only the homoeologous chromosomes from the related subgenomes of the two species have the potential to pair during meiosis. These findings indicate that the genomic formula of \( S. \) macrospermum is \( AAB_1B_1YYZZ \). Subgenomes \( A_b \) and \( A_m \) (\( b \) and \( m \) denoting \( S. \) bicolor and \( S. \) macrospermum, respectively) share more homology than subgenomes \( B_{1b} \) and \( B_{1m} \) as indicated by differences in pairing. Pairing between and within \( S. \) bicolor subgenomes was rare; one \( A_{B_b}B_{1b} \) bivalent, no \( A_{B_b}A_{B_b} \) bivalents, and two \( B_{1b}B_{1b} \) bivalents were observed in 46 cells examined. Only a moderate level of \( S. \) macrospermum autosyndetic pairing occurred in the hybrids (1.6 II per PMC). If the meiotic chromosome pairing between members of the \( A_b \) and \( B_{1b} \) subgenomes is a guide, it is unlikely that the \( S. \) macrospermum autosyndetic pairing in the hybrids can be explained as \( A_mB_{1m} \), \( A_mA_m \), and \( B_{1m}B_{1m} \) chromosome associations. Therefore, it is likely occurring from the association of the unknown subgenomes \( Y \) and \( Z \). Autosyndetic pairing may be between the two subgenomes \( Y_m \) and \( Z_m \) or between one of these subgenomes and either \( A_m \) or \( B_{1m} \). Much like in \( S. \) halepense, in which Tang and Liang (1988) hypothesized moderate levels of \( B_1B_2 \) pairing, subgenome \( Y \) or \( Z \) may be analogous to subgenome \( B_2 \). Thus, \( B_{1m}B_{2m} \) associations could explain the observed autosyndetic \( S. \) macrospermum pairing. However, these conclusions regarding genomic relationships of subgenomes \( Y \) and \( Z \) are hypothetical. Research addressing the genomic relations between \( S. \) macrospermum and \( S. \) halepense should clarify this issue.

Male fertility of the interspecific hybrids was estimated by observing I\(_2\)-KI pollen stainability, which revealed that normal pollen formation was rare. Only 5 fully stained pollen grains were observed among approximately 20000 examined. Approximately 3050 CMS \( S. \) bicolor florets were pollinated with pollen from the interspecific hybrids over a 4 month period and only 13 putative BC\(_1\)F\(_1\) seeds developed. All of the putative backcross progeny developed on 3 heads that were pollinated during a 2 week period that coincided with low light intensity, an environmental condition known to affect the stability of the male sterility. Thus, it is possible that “BC\(_1\)F\(_1\)” plants resulted from self-pollination or pollination by stray \( S. \) bicolor pollen and not interspecific pollination. The putative BC\(_1\)F\(_1\) progeny were grown to maturity and morphologically they did not differ from their maternal parent. Molecular markers were used in hopes of proving their interspecific paternity. DNA samples from 11 putative BC\(_1\)F\(_1\) plants, their respective female CMS parents, \( S. \) macrospermum, and mapping parents BTx623 and IS3620C (Menz et al. 2002) were used to evaluate the presence or absence of \( S. \) macrospermum introgression. A total of 825 unique \( S. \) macrospermum bands were scored and some were found in the putative BC\(_1\)F\(_1\) plants. Introggression estimates were between 0% and 1%, and none of the introgression bands was shared by the mapping parents. Thus, the genomic location of the potential introgression is unknown (data not presented).

The presence of introgression bands in these male-produced backcrosses is not irrefutable evidence of their backcross status. All introgression bands were AFLP markers, thus nothing is known regarding their sequence similarity, only that the fragments had similar mobility through the gel. Co-migration of non sequence-related bands can be a problem with AFLP markers when comparing different species (Mechanda et al. 2004). It is possible that stray \( S. \) bicolor pollen from a genotype that produces non sequence-related AFLP bands of the same size as those in \( S. \) macrospermum was the male parent of the putative BC\(_1\)F\(_1\)s, thus making “introgression bands” not indicative of introgression. It is also possible that these plants are actually backcrosses with only 0%–1% \( S. \) macrospermum introgression. Regardless, “BC\(_1\)F\(_1\)” plants were male-sterile like their CMS parent, and restoring fertility would have required another cross and selfing generation. It was decided that producing introgression in this manner was not ideal and another approach would be pursued. It may be necessary to use the interspecific hybrids as the female parents in combination with embryo rescue, to avoid questions of pedigree in recovered backcrosses.

**Conclusion**

Allosyndetic recombination was detected between \( S. \) bicolor and \( S. \) macrospermum chromosomes at a moderate frequency (2.6 II per PMC) in the interspecific hybrids. There was sufficient chromosome pairing to suggest that \( S. \) macrospermum has two genomes that share homology with the genomes of \( S. \) bicolor and that the wild species has the genomic formula \( AAB_1B_1YYZZ \), with \( Y \) and \( Z \) being unknown genomes. Interspecific backcrosses were sought for crossing and mapping.

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**Table 2.** Allosyndetic (\( B–M \)) and autosyndetic (\( B–B \) and \( M–M \)) chromosome pairing in \( S. \) bicolor \( \times \) \( S. \) macrospermum hybrids as revealed using FISH probe CEN38.

<table>
<thead>
<tr>
<th>Bivalents</th>
<th>No. observed in 46 PMCs</th>
<th>Mean no. per PMC*</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>( B–M )</td>
<td>121</td>
<td>2.63(^A)</td>
<td>61.4</td>
</tr>
<tr>
<td>( B–B )</td>
<td>4</td>
<td>0.09(^C)</td>
<td>2.0</td>
</tr>
<tr>
<td>( M–M )</td>
<td>72</td>
<td>1.57(^B)</td>
<td>36.5</td>
</tr>
<tr>
<td>Total</td>
<td>197</td>
<td>4.28</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.** Allosyndetic chromosome pairing involving subgenomes \( A \) and \( B_1 \) in \( S. \) bicolor \( \times \) \( S. \) macrospermum hybrids as revealed using FISH probe CEN38.

<table>
<thead>
<tr>
<th></th>
<th>No. of bivalents observed in 19 PMCs</th>
<th>Mean no. of bivalents per PMC*</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A–M )</td>
<td>39</td>
<td>2.05(^A)</td>
<td>70.9</td>
</tr>
<tr>
<td>( B_1–M )</td>
<td>16</td>
<td>0.84(^B)</td>
<td>29.1</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>2.89</td>
<td></td>
</tr>
</tbody>
</table>

*Note: \( M \) represents \( S. \) macrospermum chromosomes, \( A \) and \( B_1 \) represent chromosomes within subgenomes of \( S. \) bicolor. *Means with different superscript letters are significantly different \((p < 0.05)\).
among putative BC1F1 plants after pollinating *S. bicolor* with the interspecific hybrids, but irrefutable evidence of their hybridization remains elusive. Only extremely low levels (1%) of putatively alien AFLP bands were observed, i.e., too low to confirm that the recovered progeny were different from self fertilizations. Future research should focus on producing introgression in backcross progeny, as the current research predicts that *S. macrospermum* introgression is probable.

References


Sharma, H.C., and Franzmann, B.A. 2001. Host-plant preference and oviposition responses of the sorghum midge, *Stenodiplosis sorghicola* (Coquillett) (Dipt., Cecidomyiidae) towards wild...