A Molecular Cytogenetic Map of Sorghum Chromosome *1*: Fluorescence *in Situ* Hybridization Analysis With Mapped Bacterial Artificial Chromosomes

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ABSTRACT

We used structural genomic resources for *Sorghum bicolor* (L.) Moench to target and develop multiple molecular cytogenetic probes that would provide extensive coverage for a specific chromosome of sorghum. Bacterial artificial chromosome (BAC) clones containing molecular markers mapped across sorghum linkage group A were labeled as probes for fluorescence *in situ* hybridization (FISH). Signals from single-, dual-, and multiprobe BAC-FISH to spreads of mitotic chromosomes and pachytene bivalents were associated with the largest sorghum chromosome, which bears the nucleolus organizing region (NOR). The order of individual BAC-FISH loci along the chromosome was fully concordant to that of marker loci along the linkage map. In addition, the order of several tightly linked molecular markers was clarified by FISH analysis. The FISH results indicate that markers from the linkage map positions 0.0–81.8 cM reside in the short arm of chromosome *1* whereas markers from 81.8–242.9 cM are located in the long arm of chromosome *1*. The centromere and NOR were located in a large heterochromatic region that spans ~60% of chromosome *1*. In contrast, this region represents only 0.7% of the total genetic map distance of this chromosome. Variation in recombination frequency among euchromatic chromosomal regions also was apparent. The integrated data underscore the value of cytological data, because minor errors and uncertainties in linkage maps can involve huge physical regions. The successful development of multiprobe FISH cocktails suggests that it is feasible to develop chromosome-specific "paints" from genomic resources rather than flow sorting or microdissection and that when applied to pachytene chromatin, such cocktails provide an especially powerful framework for mapping. Such a molecular cytogenetic infrastructure would be inherently cross-linked with other genomic tools and thereby establish a cytogenomics system with extensive utility in development and application of genomic resources, cloning, transgene localization, development of plant "chromonomics," germplasm introgression, and marker-assisted breeding. In combination with previously reported work, the results indicate that a sorghum cytogenomics system would be partially applicable to other gramineous genera.

SORGHUM bicolor ($2n = 20$) is the world's fifth most important cereal crop. This grain is grown in arid and semiarid regions of the world due to its unusual tolerance to adverse environments (Doggett 1988). The diverse sorghum germplasm collection of >40,000 accessions has been used to generate populations for mapping important trait loci. These studies have identified sorghum loci regulating plant morphology, flowering time, disease resistance, environmental stress tolerance, and other traits (*e.g.*, reviewed by Doggett 1988; Lin *et al.* 1995; Paterson *et al.* 1995a,b; Childs *et al.* 1997; Rami *et al.* 1998; Fernandes and Coutinho 1999; Klein *et al.* 2001). As a first step toward cloning genes linked to marker loci and for comparative genome analysis, detailed sorghum genetic maps have been constructed and cross-referenced to other grass species (Whitkus *et al.* 1992; Berhan *et al.* 1993; Chittenden *et al.* 1994; Pereira *et al.* 1994; Xu *et al.* 1994; Dufour *et al.* 1997; Peng *et al.* 1999; Bhattramakki *et al.* 2000; Kong *et al.* 2000; Menz *et al.* 2002). Recently, construction of an integrated genetic and physical map of the sorghum genome has been initiated (Klein *et al.* 2000; Childs *et al.* 2001). A combination of techniques including restriction endonuclease fingerprinting of sorghum bacterial artificial chromosome (BAC) clone libraries and genetic marker content mapping of BAC clones has been used to construct an integrated physical and genetic map of sorghum. The development of a high-throughput PCR-based method for expanding BAC contigs and integrating markers in BAC contigs was paramount for the success of this effort (Klein *et al.* 2000).

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Cytogenetic analyses of the 10 sorghum chromosomes would complement and enhance the genetic and physical information being compiled for sorghum. Cytological determination of chromosomal infrastructure such as centromeres, NORs, and heterochromatin and determination of the variation in recombination frequency across chromosomes would facilitate the use of these maps for comparative genomics and map-based gene isolation. The utility of BACs as molecular cytogenetic probes in plants became evident through early studies using marker-selected BACs for fluorescence *in situ* hybridization (FISH; Hanson *et al.* 1995; Jiang *et al.* 1995). Utilizing marker-selected BACs from euchromatin in the distal regions of sorghum chromosomes, Gomez *et al.* (1997) used FISH to show that a 205-kb sorghum BAC containing a sequence complementary to the maize *sh2* cDNA produced strong signals at the distal end of one arm of a pair of midsized metacentric sorghum chromosomes. Zwick *et al.* (1998) used six *liguleless*-associated rice restriction fragment length polymorphism (RFLP) markers to select related sorghum BACs, which in turn were used to physically map a homologous region in the sorghum genome. FISH of the marker-selected BACs yielded signals on the distal region of a single chromosome arm. With one exception, their relative positions indicated that the order of the loci in sorghum was the same as in the rice genetic map. The BACs were end-cloned for RFLP mapping, and the relative linkage order in linkage group I (Xu *et al.* 1994) was fully concordant with the observed order of FISH markers.

In this article, we detail the key cytogenetic features of sorghum chromosome *1*. This characterization is based on FISH of BACs derived from contigs mapped along the entire length of the recombination-based genetic map of sorghum chromosome *1*. In addition, an 18S-28S ribosomal DNA clone and a cloned centromere-associated DNA sequence were hybridized to provide insight into the molecular architecture of sorghum chromosome *1*.

MATERIALS AND METHODS

Plant material: Root tips of sorghum inbred line BTx623 were collected from seedlings and/or plants growing in pots in a greenhouse. Root tips were pretreated in saturated α-monobromonaphthalene (*aq*) and/or 2.5 mm hydroxyquinoline for 1.5 and 4 hr, respectively, in darkness to accumulate metaphase cells. A protoplast technique (Jewell and Islam-Faridi 1994) was used to prepare chromosome spreads, where the duration of enzymatic digestion to remove cell walls ranged from 25 to 60 min, depending on the size of the root tip. Microsporocytes were removed from anthers and prepared similarly.

Probes: Most of the BACs used in this study came from two BAC libraries that contain a total of 26,016 clones (Woo *et al.* 1994; Tao and Zhang 1998). The BACs from these libraries are referred to numerically with the prefix "sbb" (***S**orghum **b**icolor* **B**AC). The BACs from the Woo *et al.* (1994) library are numbered sbb1 to sbb13440, and the BACs from the Tao and Zhang (1998) library are numbered sbb13441 to sbb26016. Nineteen of the BAC clones used in this study contained molecular markers previously used to develop the high-density genetic map (Table 1; Menz *et al.* 2002). Also used in this study was a BAC (110K5) that contains the sorghum *Adh* gene (Tikhonov *et al.* 1999) and that had previously been localized to chromosome *1* (Gomez 1997). Most of the markers were chosen by position, to collectively provide coverage across the entire linkage map. The short arm of sorghum chromosome *1* contains the nucleolus organizer region, and thus, 18S-28S rDNA (plasmid pGmr3, kindly provided by Dr. E. Zimmer) was used as an additional FISH marker. A cloned (pCEN38) centromere-associated sequence (Zwick *et al.* 2000) was also used.

TABLE 1

List of BACs used for FISH, their associated markers, and locations on linkage group A

BAC	Marker	Map position (cM)
sbb20978	*Xtxa2621*	5.2
sbb6585	*Xtxa224*	18.4
sbb3766	*PHYA*	28.4
sbb11993	*Xtxa6367*	40.2
sbb1763	*Xtxa2615*	67.6
sbb18958	*Xtxa523*	69.6
sbb9703	*Xtxa215*	80.2
sbb3890	*Xtxa2654*	80.2
sbb18256	*Xtxa325*	81.8
sbb19112	*Xtxa270*	93.8
sbb17193	*Xtxp43*	104.2
sbb16685	*Xtxp32*	119.2
sbb25572	*Xtxp58*	160.8
sbb10541	*Xtxs1765*	190.4
sbb9346	*Xtxa2304*	191.5
sbb11061	*Xtxa6057*	201.8
sbb15290	*Xtxp319*	221.4
sbb13961	*Xtxs1176.1*	236.8
sbb18861	*Xtxa2344*	236.8
110K5[a]		

[a] Randomly selected BAC.

Probe labeling and FISH: BAC DNA was isolated by alkaline lysis, digested with *Hin*dIII, and then further purified using Plant DNeasy spin columns (QIAGEN, Valencia, CA) using a modified protocol (Childs *et al.* 2001). BAC DNA was labeled with biotin-14-dATP (Bionick labeling system; Invitrogen, San Diego) and/or Dig-11-dUTP (F. Hoffmann-La Roche). For 18S-28S rDNA and CEN38, the whole plasmids were used for labeling. Samples of labeled DNA were electrophoresed through agarose gels to ensure that the lengths were between 300 and 500 bp. Labeled DNA was dot-blotted to verify incorporation of labeled nucleotides.

The hybridization mixture consisted of 50% deionized formamide, 10% dextran sulfate, 2× SSC, labeled BAC DNA (50 ng/slide), carrier DNA (salmon sperm DNA, 5 μg/slide), and blocking Cot-*1* DNA (10- to 50-fold excess of labeled BAC DNA). The Cot-*1* DNA fraction was prepared according to Zwick *et al.* (1997). The hybridization mixture was denatured in boiling water for 10 min, immediately placed on ice for 5 min, and incubated in a 37° water bath for 30 min to allow the Cot-*1* DNA to hybridize with the repetitive sequences of BAC DNA. Chromosomal DNA was denatured in 70% deionized formamide at 70° for 1.5 min. The slides were immediately

dehydrated through an ethanol series (70, 85, 95, and 100%) at −20° for 3 min each. Each slide was loaded with 20 μl of hybridization mixture and covered with a glass coverslip. The edges of the coverslip were sealed with rubber cement and placed in a humidity chamber at 37° for ~16 hr. The hybridization sites were detected with FITC, Cy3, or both, depending on labeled DNA used in the hybridization mixture. Digital images were recorded from an Olympus AX-70 epifluorescence microscope with suitable filter sets (Chroma Technology, Brattleboro, VT), using a Sensyx (Roper Scientific) camera and the MacProbe v4.2.3 digital image system (Applied Imaging, Santa Clara, CA). Images were processed with Adobe Photoshop 6.0.

Four sets of FISH experiments were conducted. First, each BAC was used individually in FISH on somatic metaphase chromosomes to identify the respective arm position relative to 18S-28S rDNA (short arm) and CEN38 (centromeric region). Second, adjacent BACs were used as probes (dual-color FISH) to assign their relative orientation (physical position). Third, we delimited the location of the centromere by FISH with flanking probes, initially using probes associated with opposite ends of the linkage map and then progressing toward the center of the chromosome. Fourth, we used 14 digoxygenin- and biotin-labeled BACs in a hybridization mixture to allow simultaneous detection of the corresponding 14 loci. FISH of this cocktail to pachytene chromosomes was used to provide additional linear resolution, to determine the physical position of each BAC along the pachytene bivalent, and to assess the feasibility of large-scale development of multiprobe cocktails for FISH to an individual sorghum chromosome.

RESULTS

Mitotic FISH of individual BAC-derived probes allowed for the evaluation of the FISH characteristics of each probe and for gross cytomolecular mapping of respective hybridization site(s). Chromosome *1* of sorghum was readily distinguishable in that it is much longer than the other chromosomes and bears a large secondary constriction due to the nucleolus organizing region (NOR) at a submetacentric position in the short arm. At mitotic metaphase, euchromatin constitutes ~40% of the sorghum chromosome *1*. It is the predominant form in distal regions, which are weakly 4′,6-diamidino-2-phenylindole (DAPI) fluorescent, whereas the pericentromeric region that accounts for ~60% of chromosome *1* is heterochromatic and strongly DAPI fluorescent.

All 20 sorghum BAC-derived probes yielded strong FISH signals on chromosome *1*. Probes derived from seven BACs (sbb1763, sbb18958, sbb9703, sbb3890, sbb18256, sbb19112, and sbb17193) also yielded scattered minor signals on all of the other chromosomes. The latter result indicates that each of these seven BACs contains one or more dispersed repetitive elements. The position of the primary mitotic FISH site on chromosome *1* for each probe revealed whether the respective BAC originated from the long or short arm of chromosome *1* (Figure 1). Eleven BACs hybridized to the long arm of chromosome *1* and nine BACs (plus the 18S-28S rDNA tandem repeat) were assigned to the short arm. The resolution offered by single-probe FISH made it clear that most of the BAC-FISH sites were located in euchromatin.

The relative order of tightly linked BACs within each arm on the cytomolecular map was determined by dual-color FISH of pairs of adjacent BACs to late prophase chromosomes (Figure 1, 9a–12d). DNA markers *Xtxs-1176.1* and *Xtxa2344* were mapped at 236.8 in linkage group A (LG-A), but unresolved in order (Menz *et al.* 2002; and see http://sorghumgenome.tamu.edu/). We determined that these marker loci are contained by BACs sbb13961 and sbb18861, respectively, and then used BAC-FISH to determine whether their positions on mitotic chromosomes were distinct and, if so, their relative positions. We observed BAC sbb13961 (*Xtxs-1176.1*) proximal to sbb18861 (*Xtxa2344*), where both were located near the end of the long arm of chromosome *1* (Figure 1, 9c, 13a, and 13c).

We used progressive sampling of distal to proximal loci in LG-A (Menz *et al.* 2002) to place the chromosome *1* centromere on the linkage map. At one stage of this process, three markers (*Xtxa215*, *Xtxa325*, and *Xtxa2654*) of a 24-marker bin in LG-A were utilized. FISH of the respective marker-selected BACs (sbb9703, sbb18256, and sbb3890) revealed the order of the markers to be as follows: short arm telomere → *Xtxa215* → *Xtxa2654* → centromere → *Xtxa325* → long arm telomere (Figure 3). Most importantly, the FISH signals of these BACs flanked the centromere. The BAC FISH signals corresponding to *Xtxa215* and *Xtxa2654* occurred in the short arm near the heterochromatin-euchromatin junction, whereas BAC-FISH signal for *Xtxa325* occurred in the long arm near the heterochromatin-euchromatin junction. Although the interval defined by markers *Xtxa325* and *Xtxa2654* spans only 0.7% of the chromosome *1* linkage group (1.7 of 242.9 map units), the corresponding physical segment defined by BACs sbb18256 and sbb3890 accounts for ~60% of the physical length of the somatic metaphase chromosome. The NOR is located in the short arm, near the centromere, embedded within the large pericentromeric heterochromatic block. Thus, the centromere, the heterochromatic block, and the NOR are located between markers *Xtxa325* and *Xtxa2654* (Figure 2, 13b).

A comparison of the physical spacing between BAC-FISH signals on chromosome *1* and spacing of these BACs on the sorghum linkage map indicates that the frequency of recombination varies widely in different regions of chromosome *1* (Figure 2, 13a and 13c, and Figure 3). Recombination in the pericentromeric heterochromatin of chromosome *1* was much lower than would be predicted on the basis of the physical size of this region. Recombination frequency varied within euchromatic regions of chromosome *1* as well. For example, the linkage map distances between markers *Xtxa224* and *PHYA* and between *PHYA* and *Xtxa2615* were 10.0 and 39.2 cM, respectively, whereas the physical

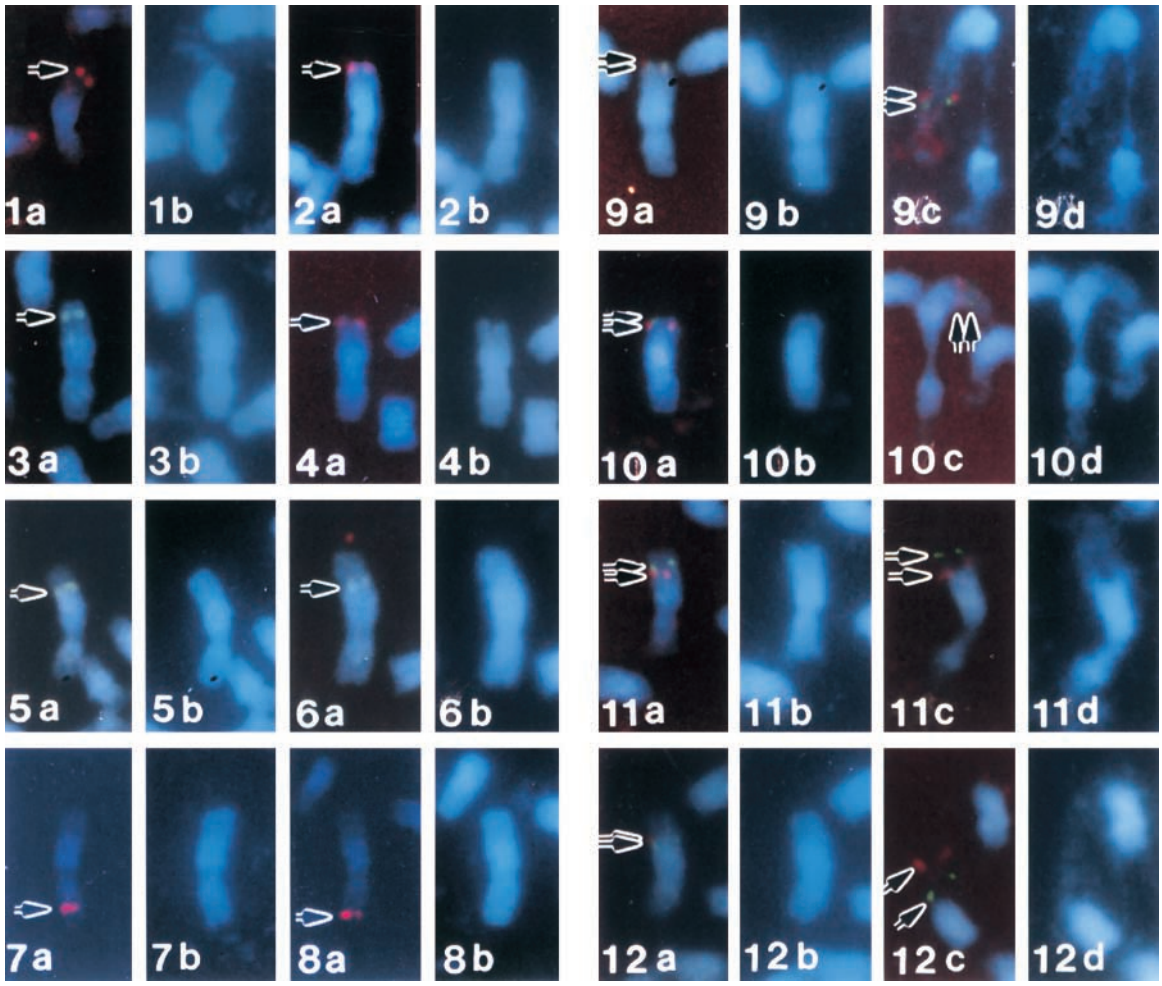


Figure 1.—Cytogenetic mapping of sorghum chromosome *1* using genetically marked BACs and FISH. Single (1a–8b) and dual (9a–12d) BAC-FISH signals on sorghum chromosome *1* are shown. BAC hybridization sites were detected with either FITC-conjugated anti-digoxygenin (for digoxygenin-labeled probe) or Cy3-conjugated streptavidin (for biotin-labeled probe). Examples of single-color BAC-FISH signals (arrows) are shown in 1a (BAC sbb18861), 2a (BAC sbb13961), 3a (BAC sbb9346), 4a (BAC sbb11061), 5a (BAC sbb16685), 6a (BAC sbb17193), 7a (BAC sbb3766), and 8a (BAC sbb6585). (1b, 2b, 3b, 4b, 5b, 6b, 7b, and 8b) The corresponding DAPI-stained chromosomes. (9a, 10a, 11a, and 12a) A set of four dual-color BAC-FISH signals on metaphase chromosome *1*. (9c, 10c, 11c, and 12c) FISH on late prophase chromosomes clearly demonstrates the order and physical position of the BACs. (9a) BACs sbb18861 and sbb13961 are located toward the end of the long arm of chromosome *1*. (9c) BAC sbb18861 is located distally (green FISH signals, arrowed) and BAC sbb13961 is located interstitially (red FISH signal, arrowed). (10a) BACs sbb11061 and sbb9346 are located toward the end of the long arm. (10a and 10c) BAC sbb11061 is located distal (green FISH signal) to BAC sbb9346 (red FISH signal). (11a and 11c) BAC sbb25572 (green FISH signal) is located distal to BAC sbb16685 (red FISH signal). BACs sbb16685 and sbb17193 are located on the long arm of chromosome *1*. (12a and 12c) BAC sbb17193 (green FISH signal) is proximal to BAC sbb16685 (red FISH signal). (9b, 9d, 10b, 10d, 11b, 11d, 12b, and 12d) DAPI-stained metaphase and prophase chromosomes.

size of these regions, as defined by the FISH with BACs that contain these markers, was similar, suggesting greater recombination in the region delineated by markers *Xtxa2615* and *PHYA* (Figure 3).

To permit the simultaneous identification of numerous sites of a single chromosome, somatic chromosome spreads were hybridized to a two-hapten FISH probe cocktail composed of 14 BACs distributed along chromosome *1*. The two-hapten FISH yielded strong primary signals on chromosome *1* and exhibited low levels of dispersed signals. Of the 14 BACs, 5 were from the short arm and 9 were from the long arm. Hybridization was successful, but signals of some nearby loci were confluent. To achieve spatial resolution among adjacent loci, the 14-BAC probe cocktail was hybridized to pachytene chromosomes, which are severalfold longer than somatic metaphase chromosomes (McClintock 1929; Ramanna and Prakken 1967; Stack 1984; Peterson *et al.* 1999) and are highly amenable to mapping markers using FISH (Gallagher *et al.* 1998; Fransz *et al.* 1998; Zhong *et al.* 1999; Cheng *et al.* 2001). All the BAC-FISH signals were clearly associated with the chromosome *1*

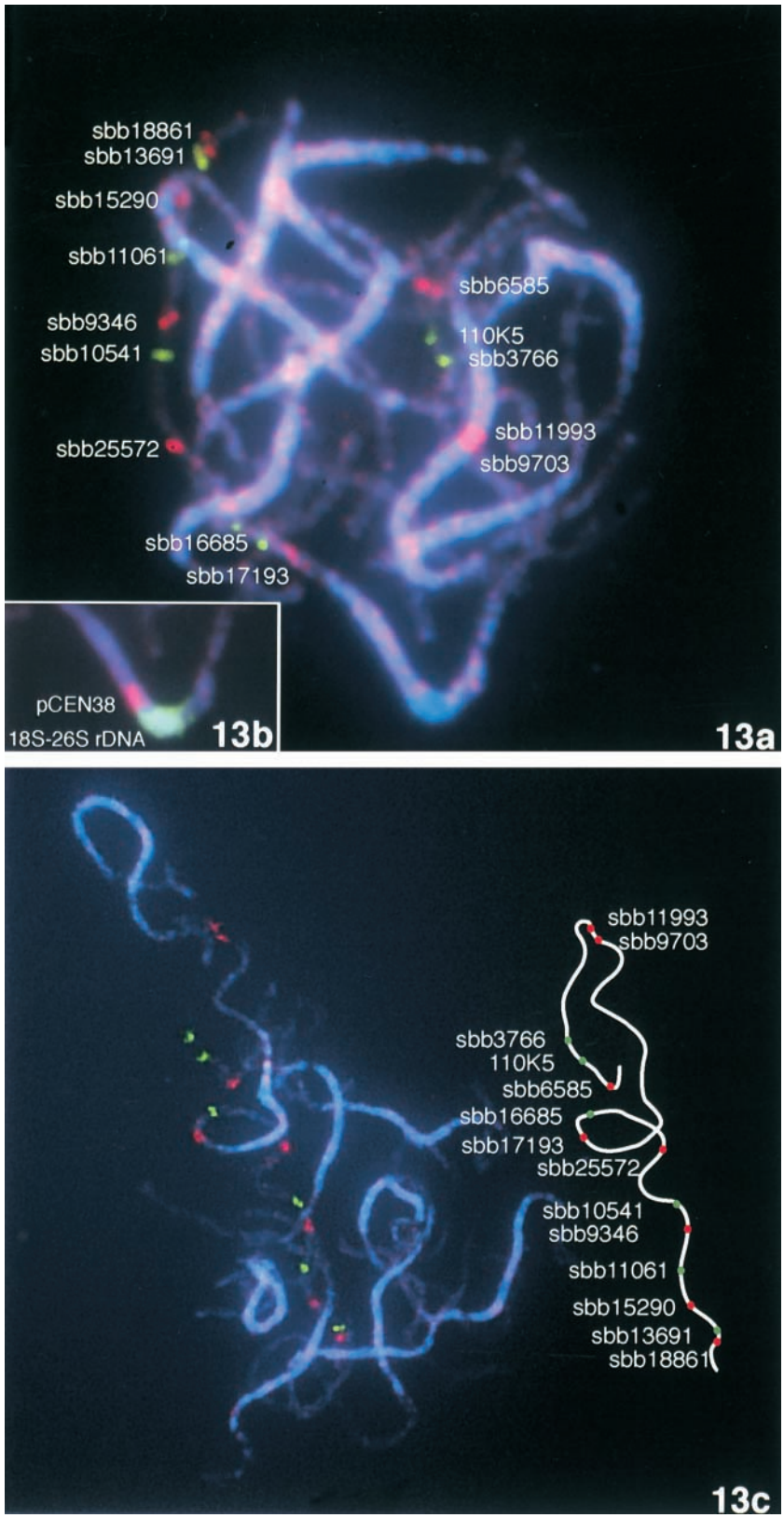
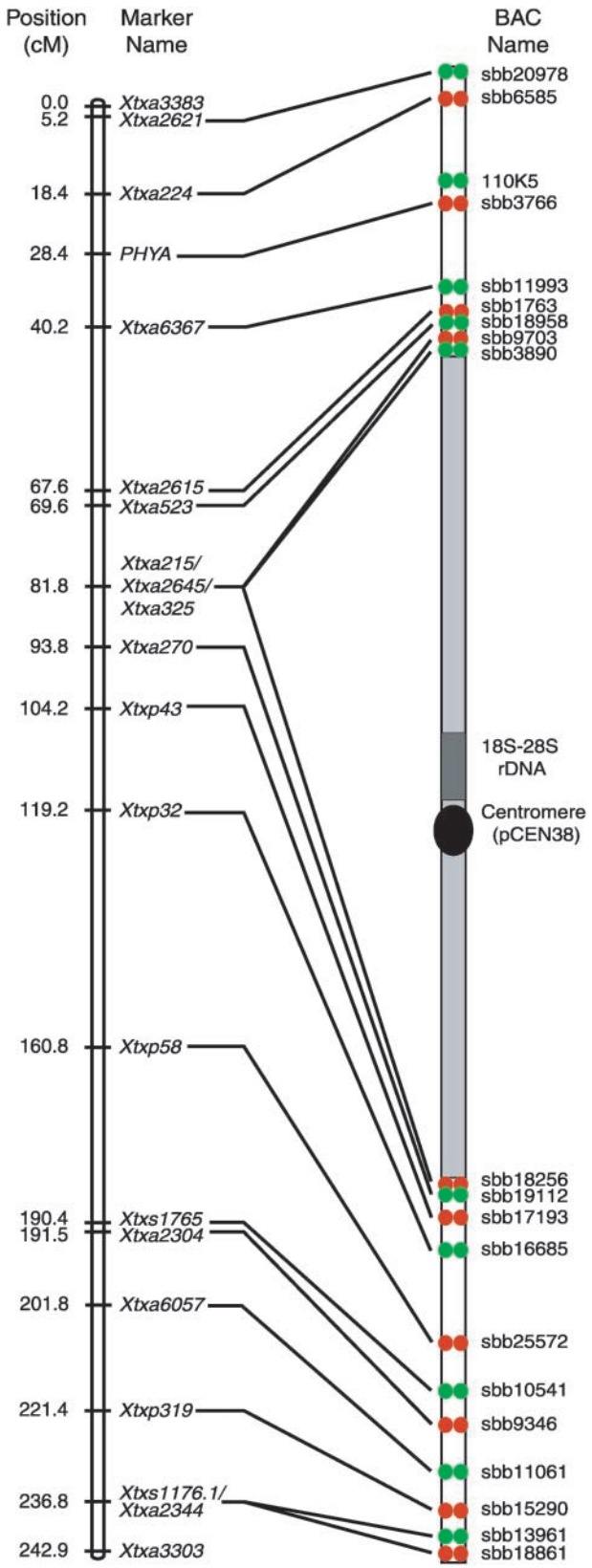


FIGURE 2.—FISH signals on sorghum chromosome *1* using a 14-BAC probe cocktail to pachytene chromosomes. The identity of each individual BAC used in the probe cocktail is given next to its representative FISH signal. (13b) The region around the centromere of 13a reprobed with CEN38 (centromere-associated probe) and 18S-28S ribosomal DNA. (13c) A pachytene spread with the same BAC probe cocktail as 13a with an additional line drawing showing the outline of chromosome *1*.

bivalent (Figure 2, 13a and 13c). The distribution of FISH signals on short *vs.* long arms of the pachytene bivalents was identical to that observed previously on mitotic chromosomes, but the pachytene preparations provided much greater within-arm resolution among the BAC-FISH sites. None of the BAC-FISH sites was found to overlay the pericentric heterochromatin, not even sbb9703, sbb3890, or sbb18256. In addition, re-

probing of these pachytene spreads with CEN38 and 18S-28S rDNA revealed more clearly the physical proximity of the nucleolus-organizing and centromere regions, as well as their respective positions relative to the pachytene heterochromatin pattern (Figure 2, 13b).



DISCUSSION

The sorghum genome has been mapped extensively through segregation analysis using molecular markers (*e.g.*, Peng *et al.* 1999; Bhattramakki *et al.* 2000; Kong *et al.* 2000; Menz *et al.* 2002). Unless integrated with cytomolecular maps and other genomic resources, linkage maps reveal little about the physical spacing, dispersion, and clustering of genes and genetic markers. Molecular cytogenetics can contribute significantly to a genome map through the assignment of linkage groups to chromosomes and markers to chromosome arms, by localizing the positions of centromeres on linkage groups, by resolving the order of closely linked markers (and associated BAC clones), and by confirming the physical positions of markers at the ends of linkage groups. In many instances, these tasks are readily accomplished by FISH of BACs associated with linkage map loci.

In this study, 20 BACs and two plasmids containing 18S-28S rDNA and centromere-associated CEN38 sequences were hybridized to metaphase chromosomes to assign their cytological location on chromosome *1*. FISH signals from all of these BACs occurred on chromosome *1*, and their physical order was fully concordant with the recombination-based map. Therefore, FISH analysis confirmed the relative order of the DNA markers that had been established through segregation analysis. In addition, FISH analysis was able to resolve the relative orders of two sets of DNA markers that had previously not been resolved by segregation analysis. The physical resolution of linkage maps is subject to variation in the level of recombination. In physical regions that are low in recombination, the exact order of DNA markers and hence associated BAC clones cannot be easily resolved without complementary information. This study indicates that cytological analysis of somatic and/or pachytene sorghum chromosomes can eliminate ambiguity in at least certain regions of the sorghum linkage map.

FISH analysis revealed several important organizational and structural features of chromosome *1* of sorghum. First, the position of the centromere was delimited to the linkage map segment between DNA markers *Xtxa325* and *Xtxa2654*. The location of a centromere on the sorghum linkage map had not been pre-

Figure 3.—A diagrammatic representation of the cytogenetic locations of 20 sorghum BACs, 18S-28S rDNA, and CEN38 (a centromere-associated clone) on sorghum chromosome *1* (right). The corresponding linkage marker positions (Peng *et al.* 1999; Menz *et al.* 2002) are shown on the left. The *Adh* locus (BAC 110K5) is yet to be placed on the genetic map.

viously reported for any of the 10 sorghum chromosomes. Second, the major 18S-28S rDNA coding region on sorghum chromosome *1* was located interstitially close to the centromere. In contrast, nucleolus organizing regions are located toward the end of a chromosome(s) in many plant species (Mukai *et al.* 1991; Leitch and Heslop-Harrison 1992; Hanson *et al.* 1996; Liu *et al.* 1997). The interstitial location of the 18S-28S rDNA coding region in sorghum chromosome *1* may be the result of an inversion and/or translocation event(s). Finally, DNA markers from map positions 0.0 to 81.8 cM were found to reside in the short arm and DNA markers from map positions 81.8 to 242.9 cM were located in the long arm of chromosome *1*.

FISH analysis of sorghum chromosome *1* showed that the frequency of genetic recombination varied widely across this chromosome. For example, DNA markers located in BACs ssb18256 and ssb3890 span only ~1.7 of the 242.9 map units that comprise chromosome *1*. However, FISH revealed that genomic DNA located between BACs ssb18256 and ssb3890 span ~60% of the physical length of the somatic metaphase chromosome *1*. This pericentromeric region of chromosome *1* corresponds to a large block of heterochromatin. FISH showed that this region of chromosome *1* has an unusually low rate of recombination. Suppressed recombination in the pericentromeric regions has been reported in wheat, barley, and tomato (Tanksley *et al.* 1992; Delaney *et al.* 1995a,b; Sherman and Stack 1995; Künzel *et al.* 2000). Recombination frequency also varied in euchromatic regions located in the distal portion of chromosome *1*. For example, the linkage map distances between *Xtxa244* and *PHYA* and *PHYA* and *Xtxa2615* were 10.0 and 39.2 cM, respectively. However, the physical distances between these markers were similar. Variation in relative rates of recombination across chromosomes has been observed for other crop plants (Xie *et al.* 1993; Gill *et al.* 1996; Faris *et al.* 2000; Künzel *et al.* 2000; Cheng *et al.* 2001). Faris *et al.* (2000) studied a gene-rich region of chromosome *5B* of wheat that undergoes frequent recombination. Although this region accounts for just ~4% of wheat chromosome *5B*, it is very highly recombinant (~50 cM).

Determining the distribution of heterochromatin and euchromatin and the frequency of genetic recombination within these regions is important to establish a guide to future genome sequencing activities and map-based gene cloning. As such, ~60% of the length of the sorghum genome at mitotic metaphase is pericentromeric heterochromatin that is enriched in repetitive DNA sequences and more condensed than euchromatin (Zwick *et al.* 1997). If sorghum heterochromatin contains fewer genes than euchromatin, as seems likely, it would be advantageous to focus early gene discovery efforts in regions of euchromatin. In this study, all of the BACs used for FISH analysis hybridized to euchromatin. The presence of genes in the BACs used for FISH analysis was assessed by obtaining 16 random sequences from each clone. This analysis showed that all of the BACs used in this study contain one or more genes (J. Mullet, unpublished data). Moreover, one of the BACs used for FISH has been completely sequenced and contains *PHYA* and at least 17 other genes (Childs *et al.* 2001). These results are consistent with the idea that gene density in the euchromatic regions of chromosome *1* is higher than that in the large pericentromeric heterochromatic region. However, further analysis of BACs located in the heterochromatic region of sorghum chromosome *1* will be required to confirm this observation. The results obtained here are consistent with results obtained by Gomez *et al.* (1997) and Zwick *et al.* (1998) that DNA markers and genes are located primarily in the distal euchromatic regions of sorghum chromosomes. This idea is also consistent with the work of Peng *et al.* (1999) who mapped 28 cDNAs on sorghum chromosome *1* and these 28 cDNAs all reside in the euchromatin regions. Similar results have been reported for other crop plants (Zhong *et al.* 1999; Cheng *et al.* 2001). A few markers reside near the border of the euchromatin and heterochromatin regions in both the short and long arms of chromosome *1*. This may aid analyses aimed at identifying factors that differentiate these two forms of chromatin. The molecular definition of such sequences, associated proteins, borders, and insulators would be important in any species. In gramineous species, they may have special importance given their reported propensity to utilize gene space with a narrow buoyancy (GC content) range (Barakat *et al.* 1997), *i.e.*, to be distributed very nonrandomly.

The integration of linkage, physical, and cytomolecular maps of sorghum will be useful to researchers working in various fields but particularly important to genomic investigations of grass species. In this study, we have demonstrated the feasibility of developing and simultaneously applying large numbers of FISH markers to a single sorghum chromosome. In previous studies, we have demonstrated the feasibility of simultaneously tagging all of the sorghum chromosomes with analogous multiprobe cocktails (Kim *et al.* 2002), as well as *trans*-generic FISH, *e.g.*, of sorghum BACs to rice and maize genomes (Zwick *et al.* 1998). Collectively, these studies strongly indicate the feasibility of developing a cytomolecular map that is of use not only for sorghum genomics, but comparatively for other Sorghum species and other grass genera, at least within the Poaceae and perhaps more broadly. A universal grass molecular karyotyping system would facilitate alignment of related chromosomal regions among grasses and facilitate direct genetic and cytogenetic studies of chromosome organization and evolution.

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