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Note

Chromosome Identification and Nomenclature of *Sorghum bicolor*

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ABSTRACT

Linkage group identities and homologies were determined for metaphase chromosomes of *Sorghum bicolor* ($2n = 20$) by FISH of landed BACs. Relative lengths of chromosomes in FISH-karyotyped metaphase spreads of the elite inbred BTx623 were used to estimate the molecular size of each chromosome and to establish a size-based nomenclature for sorghum chromosomes (SBI-01–SBI-10) and linkage groups (LG-01 to LG-10). Lengths of arms were determined to orient linkage groups relative to a standard karyotypic layout (short arms at top). The size-based nomenclature for BTx623 represents a reasonable choice as the standard for a unified chromosome nomenclature for use by the sorghum research community.

LINKAGE mapping of Sorghum has progressed quickly, using diverse mapping populations and markers (Whitkus *et al.* 1992; Chittenden *et al.* 1994; Pereira *et al.* 1994; Xu *et al.* 1994; Dufour *et al.* 1997; Ming *et al.* 1998; Tao *et al.* 1998, 2000; Boivin *et al.* 1999; Crasta *et al.* 1999; Peng *et al.* 1999; Bhattramakki *et al.* 2000; Kong *et al.* 2000; Haussmann *et al.* 2002; Menz *et al.* 2002; Bowers *et al.* 2003). The lack of a common nomenclature system for sorghum linkage groups, however, has made it difficult and cumbersome to compare and use results obtained by different groups. For most well-studied genomes, linkage group nomenclature and chromosomal designations are integrated and are usually based on biological parameters, *e.g.*, chromosome size, arm length, and arm orientation (Werner *et al.* 1992; Fransz *et al.* 1998; Künzel *et al.* 2000; Cheng *et al.* 2001; Kulikova *et al.* 2001; Howell *et al.* 2002; Anderson *et al.* 2003). Conventional and C-band karyotypes of Sorghum species were reported by Gu *et al.* (1984) and Yu *et al.* (1991), respectively, but means of evaluation were lacking and their relationship to molecular markers and genomic resources remains unknown. In contrast, identification of sorghum chromosomes by simultaneous fluorescence *in situ* hybridization (FISH) of a landed BAC cocktail was devised to establish a FISH-based karyotypic system for sorghum (Kim *et al.* 2002). It provides a cyto-genomic approach in which linkage group markers and cytological markers are integrated.

Here, we used FISH-based karyotyping in concert with analysis of chromosome lengths, arm lengths, and arm ratios to establish a size-based nomenclature for sorghum chromosomes. The ability to reliably identify contracted chromosomes facilitated development of a standardized karyotype (ideogram) for *Sorghum bicolor* (L.) Moench. The results enabled us to align and orientate the linkage maps relative to the 10 chromosome pairs, and to develop nomenclatures for chromosomes and linkage groups that are based on sorghum chromosome size.

MATERIALS AND METHODS

BACs used for FISH were derived from libraries prepared by Woo *et al.* (1994) and Tao and Zhang (1998). The BACs were located on the sorghum linkage map as described by Klein *et al.* (2000), and BAC DNA used for FISH was isolated as previously described (Islam-Faridi *et al.* 2002). Molecular cytogenetic methods were as described by Kim *et al.* (2002), except as follows. Root tips from glasshouse-grown sorghum [*S. bicolor* (L.) Moench] plants of the elite line BTx623 were treated with saturated aqueous α-monobromonaphthalene for 2 hr and then fixed and processed for slide making as described previously (Kim *et al.* 2002). Prior to FISH, chromosomal DNA on slides was denatured at 70° in 100 μl of 70% formamide in 2× SSC on a hot block for 1.5 min followed by dehydration in 70% ethanol at −20° and 85, 95, and 100% ethanol at room temperature, respectively. For single-probe

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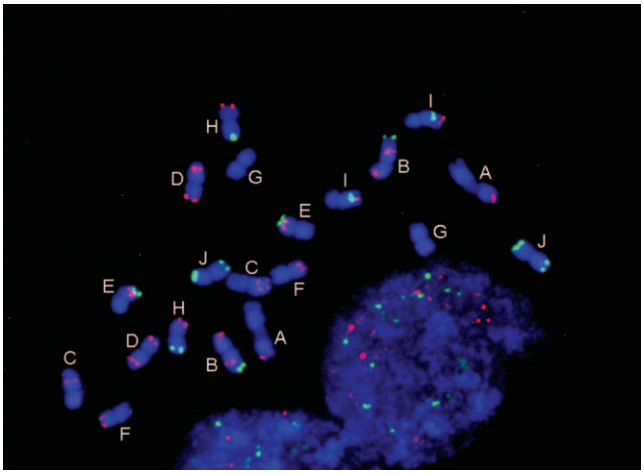


Figure 1.—Simultaneous FISH of a 17-BAC cocktail probe to sorghum mitotic metaphase chromosome spread. The patterns of signals enable FISH-based recognition of each chromosome pair and associate specific linkage groups with specific chromosomes. Each letter corresponds to a linkage group (Menz *et al.* 2002).

FISH, the hybridization mixture (25 μl) contained 10 ng of labeled BAC probe DNA, 50% formamide, 10% dextran sulfate, and 2× SSC. The mixture was denatured at 90° for 10 min, chilled on ice, and added to the slide. For FISH of the multi-probe cocktail from 17 BAC clones, 50× *Cot-1* DNA was added to the probe mixture, which was denatured at 90° for 10 min, chilled on ice, and then annealed for 30 min before application to the slide. Following overnight incubation at 37°, slides were rinsed at 40° for 5 min in a series of washes consisting of 2× SSC, 50% formamide in 2× SSC, 2× SSC, and 4× SSC plus 0.2% Tween 20, respectively.

Images were taken from an Olympus AX-70 epifluorescence microscope (Olympus America, Melville, NY) equipped with standard filter cubes, a Peltier-cooled monochrome 1.3 megapixel Sensys camera (Photometrics, Tucson, AZ), and MacProbe v.4.2.3 digital imaging system (Applied Imaging, San Jose, CA). Homologous chromosome pairs were identified with the aid of MacProbe v.4.2.3, according to the pattern of signal on each chromosome. For karyotyped images, DAPI-stained chromosomes were measured using Optimas v6.0 (Media Cybernetics, Silver Spring, MD). The centromere for each chromatid was identified by the primary constriction and also by FISH of the centromere-associated sequence pCEN38 (Zwick *et al.* 2000). FISH-identified chromatid arms were measured and averaged to determine the length for each arm of the genome. The arm ratio (average long arm/short arm ratio), total chromosome length (short arm + long arm), and relative chromosome length (length of the individual chromosome/total length of all chromosomes in the genome) were calculated for each chromosome in the complement. Data were exported to a spreadsheet (Microsoft Excel) and analyzed.

RESULTS AND DISCUSSION

FISH markers enabled identification of all 20 mitotic metaphase chromosomes with respect to homology (within cells) and common identity (across cells) and relative to linkage groups (Figure 1, Table 1). Although C-banding can be used for identification of sorghum chromosomes that are not fully condensed (Yu *et al.* 1991), for the purpose of molecular size estimation, it is important to target metaphase, *i.e.*, when molecular density is most uniform along the chromosome long axis and relative lengths most accurately reflect relative molecular size. Without FISH, reliable identification of all metaphase chromosomes would have been very difficult if not impossible, because distinctive features tend to vanish as chromatin becomes highly contracted.

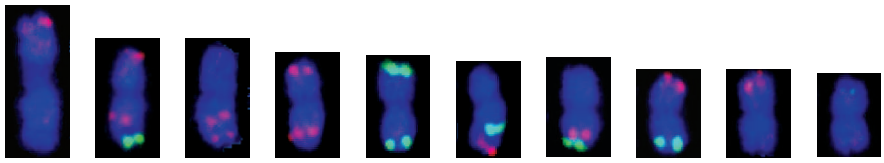
Metaphase chromosome arms were measured and tabulated and later sorted by total chromosome length (Table 1). A FISH-based karyotype of *S. bicolor* inbred line BTx623 was developed, in which chromosomes were ordered and designated according to total length at metaphase, namely SBI-01 (longest) to SBI-10 (shortest). The three-letter acronym SBI designates the genus and species, and the two-digit numeric code denotes the chromosome number. The consistent use of two digits will facilitate data sorting by computers. For linkage groups that relate well to the structure of the BTx623 genome, we suggest that they be referred to analogously, as LG-01 to LG-10 and that arms be oriented as is customary in karyotypes: p (short) arm at the top and q (long) arm at the bottom (Figure 2). The relationship between sorghum chromosomes and many of the published sorghum linkage maps is also shown in Table 1. Adoption of a common nomenclature for sorghum linkage groups will facilitate the integration of data and genomic resources developed by independent research laboratories.

The karyotype of BTx623 is grossly similar to those of other sorghum accessions and cultivars (Magoon and Shambulingappa 1960; Magoon and Ramana 1961; Magoon *et al.* 1964; Bennett and Laurie 1995; Sang and Liang 2000). BTx623 contained an exceptionally long pair of chromosomes, SBI-01, eight pairs of metacentric chromosomes closely graded in size, SBI-02, -03, -04, -05, -07, -08, -09, and -10, and one pair of midsized submetacentric chromosomes, SBI-06. SBI-01 is morphologically the most distinct chromosome of the sorghum haploid complement. In addition to its distinctive length (5.11 μm), SBI-01 is one of only two submetacentric pairs and is the only "satellite" chromosome. Lengths of the remaining chromosomes followed a somewhat bimodal distribution, with SBI-02, -03, -04, and -05 constituting the group of longer chromosomes (3.87–3.44 μm) and SBI-06, -07, -08, -09, and -10 constituting the group of shorter ones (3.15–2.97 μm).

The only secondary constriction and nucleolus organizing region (NOR) observed in BTx623 was located near the centromere in the short arm of chromosome 1, SBI-01p. It should be noted, however, that the relative length of the two SBI-01 arms shifts during the mitotic chromosome contraction. Because NORs contract differentially late in the cell cycle and are otherwise very

TABLE 1

Relationship of the FISH-based karyotype of sorghum and the linkage groups composing the various linkage maps of the sorghum genome

Chrom. no.[a]:	SBI-01	SBI-02	SBI-03	SBI-04	SBI-05	SBI-06	SBI-07	SBI-08	SBI-09	SBI-10
Linkage group (LG):	LG-01	LG-02	LG-03	LG-04	LG-05	LG-06	LG-07	LG-08	LG-09	LG-10
LG in Menz *et al.* (2002)[b]	A	B	C	D	J	I	E	H	F	G
LG in Pereira *et al.* (1994)	C	F	G	D	J	B	A	I	E	H
LG in Bowers *et al.* (2003)[c]	C	B	A	F	H	D	J	E	G	I
LG in Crastra *et al.* (1999)	G, K	D	A	C	J	F	E	H	I	B
LG in Boivin *et al.* (1999)[d]	C, K	F	G	D, L	J	B	A	I	E	H
LG in Whitkus *et al.* (1992)	B, C	D	F, M	H	G	E	A	K, L	I	J
FISH karyotype[e]										
Total length (μm)	5.11	3.87	3.85	3.5	3.44	3.15	3.13	3.07	2.98	2.94
Standard error[f]	0.047	0.035	0.038	0.032	0.037	0.029	0.028	0.026	0.029	0.023
Relative length[g]	14.59	11.06	10.98	9.99	9.82	9.00	8.92	8.75	8.51	8.39
Estimated DNA content[h]	119.3	90.5	89.8	81.7	80.3	73.6	73.0	71.6	69.6	68.6
Arm ratio[i]	1.32	1.16	1.13	1.14	1.02	1.42	1.06	1.10	1.02	1.04

[a] Chromosomes were ordered and numbered according to their rank of the total length at metaphase (full contraction).
[b] Linkage group designations are identical to those described in Peng *et al.* (1999), Kong *et al.* (2000), Bhattramakki *et al.* (2000), and Haussmann *et al.* (2002).
[c] Linkage group designations are identical to those described in Chittenden *et al.* (1994) and Tao *et al.* (2000).
[d] Linkage group designations are identical to those described in Dufour *et al.* (1997).
[e] The chromosomes are displayed according to cytogenetic convention with the short arm at the top of the vertical chromosome. The 17 BACs used for the karyotype are denoted in Figure 2 by an asterisk.
[f] The sample size for measurements was 40.
[g] Relative length = 100(chromosome length/genome length).
[h] Estimated DNA content = relative length × estimated genome size, *i.e.*, 818 Mbp (Price *et al.* 2005).
[i] Arm ratio = length of the long arm/the length of the short arm.

long, overall length of the NOR-bearing arm, SBI-01p, actually exceeds that of the long arm (SBI-01q) until the chromatin contraction process is nearly complete, *i.e.*, at metaphase. Thus, the designation of relative arm sizes at metaphase should connote relative molecular size as well.

In most higher eukaryotes, NORs are situated in short arms of subacrocentric or submetacentric chromosomes. The medial position seen in BTx623 is of interest, but not unique. NORs in most *S. bicolor* genotypes (and a number of other Sorghum species) occur in medial locations of the largest chromosome of the genome (Magoon and Shambulingappa 1960; Magoon and Ramana 1961; Magoon *et al.* 1964; Bennett and Laurie 1995; Sang and Liang 2000). However, a temporary constriction occurs in the fifth largest chromosome of a variety of *S. bicolor* cultivated for silage, in addition to the major constriction in its largest chromosome (Gu *et al.* 1984). The NOR of *S. bicolor* Combine Kafir 60 is located in the middle of the fifth longest chromosome (Yu *et al.* 1991). The NOR of its close rhizomatous relative, *S. propinquum*, is located in the short arm of the smallest chromosome (Magoon and Shambulingappa 1961). Such structural differences between parents can complicate linkage analysis (*e.g.*, see Bowers *et al.* 2003) and undermine the applicability of each linkage map beyond the respective parental combination.

We developed an integrated "cyto-genomic" map from FISH data on 24 BACs containing linkage markers from across the sorghum genome (Figure 2, Table 2). The centromere position of each chromosome was identified using the centromere-specific probe pCEN38, as previously described by Islam-Faridi *et al.* (2002; data not shown). Relative to the karyotyping convention (shorter arms at top), the orientations of linkage groups were concordant for SBI-01, -02, -04, -05, -06, -07, and -10, but inverted for SBI-03, -08, and -09 (Figure 2).

The adoption of a common reference for nomenclature of sorghum chromosomes and a related nomenclature for linkage groups would facilitate development of

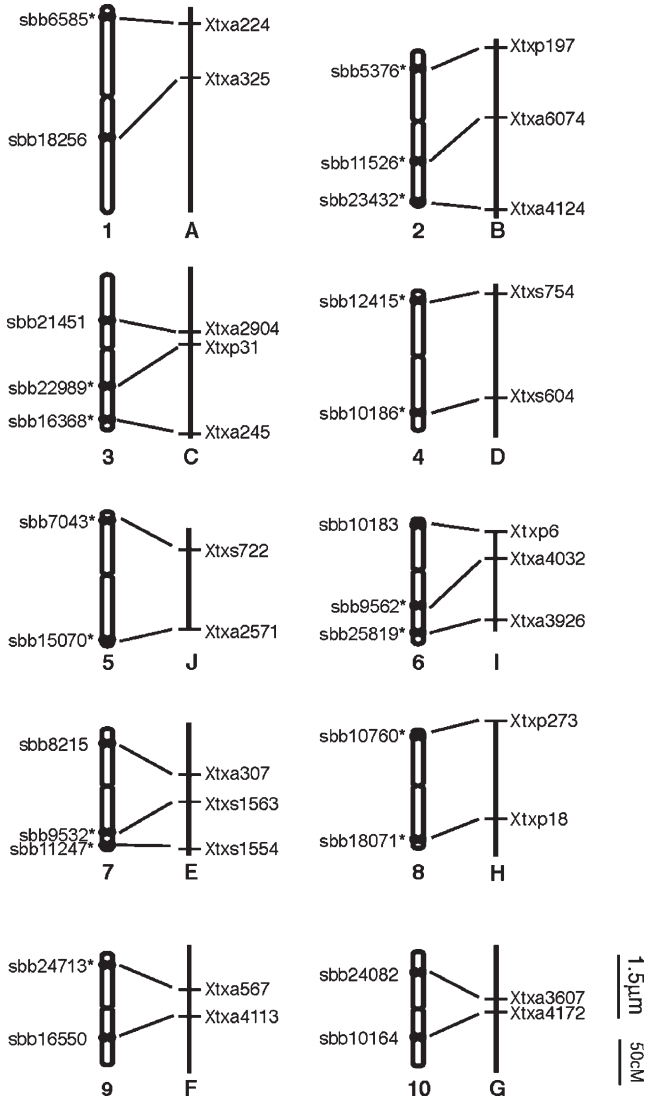


FIGURE 2.—Correlation between mitotic metaphase chromosomes and linkage groups of sorghum using the map of MENZ *et al.* (2002). Asterisks denote signals from the 17 BACs shown in Figure 1. Chromosomes are numbered according to size and linkage groups are labeled alphabetically. Chromosomes are depicted with the shorter arm in the top position. BAC clones are positioned on the ideogram according to their positions relative to the centromeres. Bar indicates 1.5 μm for metaphase chromosomes and 50 cM for linkage maps.

gramineous genomics, *e.g.*, by enhancing communication between research groups and data usage across genome maps. The unified nomenclature system for chromosomes and linkage groups of line BTx623 provide a reasonable basis for a genomic nomenclature for *S. bicolor* in that this line is readily available, highly inbred, and extensively used for genetic, breeding, and genomics research. However, caution must be exercised in applying the nomenclature to other mapping endeavors because the incidence of structural rearrangements in sorghum is inadequately studied, so it remains reasonably likely that genomes of mapping parents differ structurally. FISH-karyotypic analysis of parents and meiotic analysis of their F_1 hybrids might alert researchers to perturbations that could otherwise cryptically distort linkage maps and predictions derived from them or preclude expected genetic gains.

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TABLE 2

List of genetic markers and their anchored BACs used for integrating linkage and cytogenetic maps

Chromosome map			Linkage map			
Chromosome no.	BAC	Arm location of signal	Linkage group	Marker	Map distance	Total map distance (cM)
SBI-01	sbb6585	Short	LG-01	*Xtxa224*	16.2–19.1	232.2
	sbb18256	Long		*Xtxa325*	75.6–80.5	
SBI-02	sbb5376	Short	LG-02	*Xtxp197*	7.9–11.1	205.2
	sbb11526	Long		*Xtxa6074*	89.7	
	sbb23432	Long		*Xtxa4124*	193.4–196.9	
SBI-03	sbb21451	Short	LG-03	*Xtxa2904*	76.5–79.9	196.5
	sbb22989	Long		*Xtxp31*	91.0–94.4	
	sbb16368	Long		*Xtxa245*	190.3–196.5	
SBI-04	sbb12415	Short	LG-04	*Xtxs754*	9.7	174.6
	sbb10186	Long		*Xtxs604*	130.1	
SBI-05	sbb7043	Short	LG-05	*Xtxs722*	23	118
	sbb15070	Long		*Xtxa2571*	Off-end (118)	
SBI-06	sbb10183	Short	LG-06	*Xtxp6*	0	115.8
	sbb9562	Long		*Xtxa4032*	26.0–29.3	
	sbb25819	Long		*Xtxa3926*	102.3	
SBI-07	sbb8215	Short	LG-07	*Xtxa307*	73.9	155.9
	sbb9532	Long		*Xtxs1563*	92.2-97.2	
	sbb11247	Long		*Xtxs1554*	151.2	
SBI-08	sbb10760	Short	LG-08	*Xtxp273*	0	152.3
	sbb18071	Long		*Xtxp18*	109.5-111.2	
SBI-09	sbb24713	Short	LG-09	*Xtxa567*	54.4	153
	sbb16550	Long		*Xtxa4113*	85	
SBI-10	sbb24082	Short	LG-10	*Xtxa3607*	64.3	148
	sbb10164	Long		*Xtxa4172*	77.9–80.3	

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