FISH of a maize *sh2*-selected sorghum BAC to chromosomes of *Sorghum bicolor*

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Abstract: Fluorescence in situ hybridization (FISH) of a 205 kb *Sorghum bicolor* bacterial artificial chromosome (BAC) containing a sequence complementary to maize *sh2* cDNA produced a large pair of FISH signals at one end of a midsize metacentric chromosome of *S. bicolor*. Three pairs of signals were observed in metaphase spreads of chromosomes of a sorghum plant containing an extra copy of one arm of the sorghum chromosome arbitrarily designated with the letter D. Therefore, the sequence cloned in this BAC must reside in the arm of chromosome D represented by this monotelosome. This demonstrates a novel procedure for physically mapping cloned genes or other single-copy sequences by FISH, *sh2* in this case, by using BACs containing their complementary sequences. The results reported herein suggest homology, at least in part, between one arm of chromosome D in sorghum and the long arm of chromosome 3 in maize.

Key words: sorghum, maize, *shrunken* locus, physical mapping, fluorescence in situ hybridization, bacterial artificial chromosomes.

Résumé : Une hydration in situ à fluorescence (FISH) a été réalisée à l'aide d'un chromosome bactérien artificiel (BAC) contenant 205 kb d'ADN du *Sorghum bicolor*. Ce clone contient une séquence complémentaire à l'ADNc du gène *sh2* du maïs et il a produit une paire de forts signaux FISH à une extrémité d'un chromosome métacentrique de taille moyenne chez le *S. bicolor*. Trois paires de signaux ont été observées sur des étalements de chromosomes en métaphase chez un plant de sorgho contenant une copie supplémentaire d'un bras du chromosome du sorgho qui a été arbitrairement désigné à l'aide de la lettre D. Ainsi, la séquence clonée dans ce BAC doit provenir du bras de chromosome qui est présent chez cette lignée monotélosomique. Il s'agit donc d'une nouvelle approche permettant de réaliser la cartographie physique, par la technique FISH, de gènes clonés ou d'autres séquences en simple copie, *sh2* dans ce cas-ci, au moyen de BAC contenant les séquences complémentaires. Les résultats rapportés ici suggèrent qu'il existe de l'homologie, à tout le moins partielle, entre un bras du chromosome D du sorgho et le bras long du chromosome 3 du maïs.

Mots clés : sorgho, maïs, locus *shrunken*, cartographie physique, hybridation in situ à fluorescence, chromosomes bacteriens artificiels.

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Introduction

Sorghum is among the most widely cultivated cereals in the world (Doggett 1988). Owing to high drought tolerance, it is especially important in arid and semiarid regions. The genetic characterization of sorghum lags behind several other economically important cereals, e.g., maize, rice, and wheat. Nevertheless, well-saturated maps of sorghum based on RFLPs (restriction fragment length polymorphisms) have been developed in recent years (Xu et al. 1994; Chittenden et al. 1994; Pereira et al. 1994). However, the RFLP linkage groups have not been conclusively assigned to specific chromosomes.

The similarity in structure and size of most sorghum chromosomes has hindered reliable karyotypic identification of chromosomes and subchromosomal regions, except for chromosome I, which is the largest chromosome and houses the NOR, and chromosome IV, which has a characteristic arm ratio (Gu et al. 1984; Doggett 1988). There are no reports in which the sorghum chromosomes have been characterized satisfactorily solely on the basis of banding patterns. However, Yu et al. (1991) were successful in identifying every chromosome of *Sorghum bicolor* cv. Combine Kafir 60, using a Giemsa C-banding technique in conjunction with chromosome length and arm ratio measurements.

The integration of physical and genetic maps is a goal for sorghum, as well as for other crop plants. Fluorescence in situ hybridization (FISH) is a rapid and reliable method for physically mapping DNA sequences (Price et al. 1990; Crane et al.

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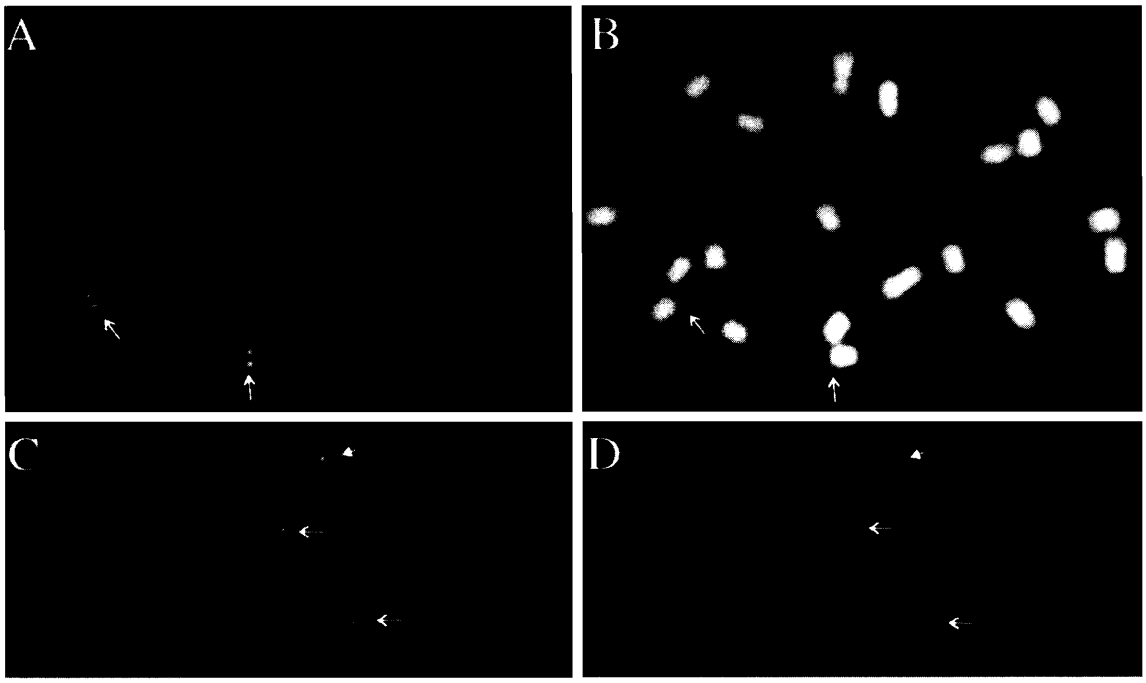
Fig. 1. Fluorescent images of FISH signals on somatic chromosomes of disomic and monotelotrisomic *Sorghum bicolor*. Grayscale images at the left (A and C) show Cy3-detected hybridization sites and their paired images at the right (B and D) show DAPI-revealed chromatin distribution. (A and B) BAC 86B6 signals (arrows) on a pair of metacentric chromosomes of disomic sorghum. (C and D) BAC 86B6 FISH signals on chromosomes of a sorghum plant monotelotrisomic for chromosome D. Note signals on the two normal homologs (large arrows) and on the monotelosomic chromosome (small arrow). Since this monotelosome is derived from chromosome D, the BAC 86B6 carrying the sequence complementary to the maize *sh2* gene must reside distally on the arm of chromosome D represented by this monotelosome.



1993; Lehfer et al. 1993; Leitch and Heslop-Harrison 1993). To date, in situ hybridization (ISH) of single-copy plant DNA sequences that are less than several thousand nucleotides in length has been difficult, and there are few reports where successful ISH of such sequences resulted in high quality cytological images (Leitch and Heslop-Harrison 1993; Lehfer et al. 1993). However, Hanson et al. (1995) demonstrated that large single-copy DNA sequences (>100 kb) cloned in bacterial artificial chromosome (BAC) vectors are ideal FISH probes in that they can yield superb visual and photographic images, with high rates of signal detection at somatic interphase or metaphase, without the need for image processing.

Conservation of gene order and nucleotide sequences across many regions of cereal genomes has permitted the use of the same set of probes for gene mapping in different cereals (Whitkus et al. 1992; Ogihara et al. 1994; Pereira et al. 1994; Dunford et al. 1995). Pereira et al. (1994) reported similarity between the genomes of sorghum and maize in that locus order is generally conserved, genetic distances are similar, and about two-thirds of all maize genomic probes strongly hybridize with sorghum DNA.

Woo et al. (1994) utilized the similarity of single-copy genes in maize and sorghum to screen a sorghum BAC library for individual BACs containing sequences homologous to maize *shrunken2* (*sh2*) cDNA. We report herein the physical mapping of a BAC containing the maize *sh2* locus to the sorghum chromosome arbitrarily designated "D" by Schertz (1974).

Materials and methods

Plant materials

Sorghum bicolor primary trisomics ($2n + 1 = 21$) were from the cytogenetic stocks of Dr. K. Schertz (Schertz 1966, 1974). The available *S. bicolor* trisomics (designated by K.F. Schertz with the letters D, E, G, H, and I) were grown from seed. The trisome for chromosome D (a midsize metacentric chromosome) possesses two normal D chromosomes and a monotelosome consisting of only one arm of chromosome D.

Plants were grown in pots in a greenhouse or in a growth chamber with controlled temperature (24–25°C, day; 18–21°C, night). Root tips were collected from these plants and used for chromosome preparation. Trisomics were identified by chromosome counts of at least 20 intact cells from each plant using phase contrast microscopy.

Description of the BACs containing the *sh2* gene

The BAC clone used in this study was isolated from a *S. bicolor* BAC library of 13 440 clones constructed at Texas A&M University (Woo et al. 1994). The BAC library was screened with a plasmid DNA (pcSh2, obtained from J. Bennetzen) insert as probe having sequences of *Zea mays sh2* cDNA (Woo et al. 1994). Three sorghum BAC clones were detected that hybridized to pcSh2: BAC 11C12 (50 kb), BAC 71A1 (80 kb), and BAC 86B6 (205 kb) (Woo et al. 1994). BAC 86B6 was selected as a probe for FISH experiments because of its large insert size.

Preparation of chromosome spreads

Root tips (ca. 4 mm long) were treated with a 0.4% 8-hydroxyquinoline solution for 5 h at room temperature, fixed in 95% ethanol –

glacial acetic acid (4:1, v/v), rinsed several times with distilled water, hydrolyzed for 5 min in 0.1 N HCl, rinsed 5 min with distilled water, and washed in citrate buffer (pH 4.5) for 5 min. To digest the cell walls, root tips were treated for 15–50 min at 37°C with pH 4.5 aqueous 5% cellulase (Onozuka R-10; Yakult Honsha Co. Ltd., Tokyo, Japan) and 1.0% pectolyase Y-23 (Seishin Corporation, Tokyo, Japan), and rinsed 3 times with distilled water. Chromosome spreads were prepared using the procedures of Jewell and Islam-Faridi (1994).

Preparation of the DNA probes

BAC 86B6 DNA was isolated by alkaline lysis plasmid maxiprep as described by Silhavy et al. (1984). The whole plasmid DNA and insert were labeled with biotin-14-dATP by nick translation, according to the standard protocol provided by the manufacturer (BioNick, Gibco BRL).

FISH

FISH techniques were as described by Islam-Faridi and Mujeeb-Kazi (1995), with the following modifications. For hybridization, each slide was loaded with 25 μL of denatured probe solution containing 50% deionized formamide, 10% dextran sulfate, 20× SSC (1× SSC: 0.15 M NaCl plus 0.015 M sodium citrate), 20 ng BAC 86B6 probe, 12.5 μg *Escherichia coli* carrier DNA, and 400 ng C_0t-1 DNA. C_0t-1 DNA was prepared as described in Zwick et al. (1997). The probe solution was denatured for 10 min at 82°C and allowed to reanneal with C_0t-1 DNA in a ratio of 1:20 (probe : C_0t-1 DNA) for 45 min at 37°C to block the BAC repetitive DNA sequences (Hanson et al. 1995; Zwick et al. 1997). A cover slip was placed over the probe solution and sealed with rubber cement. The slides were placed in a humidity chamber at 37°C for overnight hybridization.

The fluorescent hybridization signal was detected following treatment with monoclonal mouse antibiotin (Jackson ImmunoResearch) amplified with Cy3™ conjugated anti-mouse (Jackson ImmunoResearch) (5 μg/mL) in 5% NGS (normal goat serum; Vector Laboratories) at 37°C for 20 min. All slides were stained with DAPI (4′,6-diamidino-2-phenylindole), as described by Hanson et al. (1995). Two drops of antifade solution (Vectashield™, Vector Laboratories) were placed on each slide and covered with a cover slip.

Metaphase observation and photography

Images were photographed directly on Fuji HG ASA-400 professional film through a camera mounted on an Olympus AX-70 epifluorescence microscope using an Olympus triple bandpass filter. Color prints made commercially from photographic negatives were digitized as 8-bit grayscale on a conventional flatbed scanner, assembled into a plate, labeled, and printed with a dye-sublimation printer.

Results and discussion

FISH to mitotic metaphase chromosomes of *S. bicolor* disomics and trisomics was conducted using as a probe the BAC clone designated 86B6 (205 kb) containing sequences of the maize *sh2* gene. In disomic metaphase spreads of sorghum chromosomes subjected to FISH with BAC 86B6, four signals were consistently observed (one per chromatid) at the distal end of one arm of a pair of midsize metacentric chromosomes (Figs. 1A and 1B). FISH of BAC 86B6 to chromosome spreads of the four trisomic plants, E, G, H, and I, also resulted in four signals, one per chromatid, on two homologous chromosomes (not shown). Association was noted with monotelotrisomic chromosome D (Figs. 1C and 1D). The results were consistent across multiple cells, slides, and root tips, and indicate that the sequences cloned in BAC 86B6 that included the *sh2* gene must reside in the arm of chromosome D represented by monotelotrisomic chromosome D.

A *shrunken* mutant is not in the list of sorghum mutants summarized in Doggett (1988). In maize, the *sh2* locus is located at map position 149.2 of chromosome 3 (Coe et al. 1988). Therefore, it can be concluded that homology, at least in part, occurs between one arm of chromosome D in sorghum and the long arm of chromosome 3 in maize.

The results further support the concept that large single-copy DNA sequences, cloned in vectors such as BACs, can readily be used as FISH probes, yielding strong signals that are easily viewed and recorded without the need of digital image enhancing procedures (Hanson et al. 1995). The results also demonstrate a novel procedure to physically map cloned genes or other single-copy sequences by using BACs containing their complementary sequences. Polymorphism is not required. This, coupled with the use of cytogenetically aberrant tester plants, provides a powerful set of tools for assigning cloned single-copy DNA sequences to physical positions on specific chromosomes.

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