A RFLP linkage map of *Sorghum bicolor* (L.) Moench

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**Abstract** A RFLP linkage map of sorghum composed principally of markers detected with sorghum low-copy-number nuclear DNA clones has been constructed. The map spans 1789 cMs and consists of 190 loci grouped into 14 linkage groups. The 10 largest linkage groups consist of from 10 to 24 markers and from 103 to 237 cMs, and the other 4 linkage groups consist of from 2 to 5 markers and from 7 to 62 cMs. The map was derived in *Sorghum bicolor* ssp. *bicolor* by analysis of a F$_2$ population composed of 50 plants derived from a cross of IS 3620C, a guinea line, and BTx 623, an agronomically important inbred line derived from a cross between a zera zera (a caudatum-like sorghum) and an established kafir line. The restriction fragment length polymorphism (RFLP) frequency detected in this population using polymerase chain reaction (PCR)-amplifiable low-copy-number sorghum clones and five restriction enzymes was 51%. A minimal estimate of the number of clones that detect duplicate sequences is 11%. Null alleles occurred at 13% of the mapped RFLP loci.

**Key words** Sorghum • RFLPs • Linkage map

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**Introduction**

*Sorghum* (*Sorghum bicolor* L. Moench) is one of world's most important cereal crops. It is well-adapted to growth under semi-arid conditions, and both plants and grain have numerous valuable uses. Yield and quality are constrained by many factors including disease and insects, however, and the species is poorly characterized genetically, hindering agronomic improvement.

The value of using DNA restriction fragment length polymorphisms (RFLPs) to obtain linkage maps of crop plant species and then of using the maps to analyze economic-trait loci (ETLs) has been amply demonstrated (Nienhuis et al. 1987; Osborn et al. 1987; Paterson et al. 1988, 1990, 1991; Young et al. 1988; Keim et al. 1990; Klein-Lankhorst et al. 1991; Yu et al. 1991). Two RFLP-based linkage maps of sorghum have been produced using low-copy-number maize genomic DNA clones; one is composed of 92 markers (Whitkus et al. 1992) and the other of 96 markers (Berhan et al. 1993; see also Hulbert et al. 1990). Most low-copy-number maize genomic clones will hybridize to genomic blots of sorghum DNA, but they do not do so at a uniform level. Almost 50% of the maize clones tested by Hulbert et al. (1990) hybridized more strongly (and about 15–20% hybridized approximately 10 times more strongly) to maize DNA than to sorghum DNA. We found (see below) that low-copy-number sorghum genomic DNA clones hybridize to sorghum genomic DNA blots at a higher level and much more uniformly than maize DNA clones. Also, it is possible that maize genomic DNA clones will not readily identify markers located throughout the sorghum genome. To enable efficient usage of sorghum RFLP markers and to insure broad map coverage of the sorghum genome, it is desirable to develop a sorghum linkage map composed principally of loci detected with low-copy-number sorghum DNA clones. In this paper we report the development of such a map.

**Materials and methods**

Plant material

Six diverse sorghum lines from which four F$_1$ hybrids had been derived were evaluated as possible parents of a F$_2$ population. F$_1$
plants obtained from the parents that were chosen (see below) were self-pollinated to generate the F2 population consisting of 50 individuals that was used in this study.

Young leaves were collected every 3 weeks from plants of the parental lines and the F2 population. After F3 seeds were collected, new illers arising from the F3 plants provided an additional source of DNA. Leaf samples were either freeze-dried and powdered in a Wiley mill for storage or stored frozen at -80°C.

DNA isolation, digestion and Southern hybridization

Total genomic DNA was extracted and purified from either frozen or freeze-dried leaf tissue as described by Murray and Thompson (1980) and Saghai-Maroof et al. (1984) except that tissue samples were extracted in CTAB solution at twice the described concentration for 3-4 h at 65°C with occasional gentle inversion. DNA present in the supernatant was precipitated according to the described protocol, redissolved in Tris-EDTA (TE) buffer (10 mM Tris, 1 mM EDTA, pH 8.0) and quantified by fluorometry (TKO 100, Hoefer).

Genomic DNAs (7.5-10 μg per lane) digested with BamHI, EcoRI, EcoRV, HindIII or XbaI were routinely used for the detection of polymorphism between the two parental sorghum lines. Electrophoresis (Maniatis et al. 1982), blotting to Zeta Probe membranes (Reed and Mann 1985) and hybridization (Helenjas et al. 1986) followed established protocols. Overnight hybridization was at 65°C, except for maize probes, for which the temperature was 60°C. When high stringency washes were needed, the temperature was raised to 72°C, and blots were washed at least once more for 30 min.

Sorghum genomic DNA library construction and initial screening

Total sorghum genomic DNA was digested to completion with PsiI. The DNA fragments were size-selected on a sucrose gradient (15-30% in TE buffer) prepared by the freeze-thaw method (Baxter-Gabbard 1972; Davis and Pearson 1978). Fragments 0.4-2.5 kb in size were ligated into pUC18 and transformed into Escherichia coli strain DH5α. Bacterial cells containing recombinant plasmids were selected based on ampicillin resistance and the 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal) and isopropylthio-β-D-galactoside (IPTG) screening procedure (Maniatis et al. 1982).

Colony hybridization was performed using total sorghum genomic DNA as a probe to screen for clones that contained single or low-copy-number DNA sequences. Colonies that showed a strong signal on the autoradiograph were considered to be clones containing recombinant plasmids with repetitive sequences and were eliminated.

Probe sources, nomenclature and preparation

Sorghum genomic DNA clones were generated from PsiI fragments of genomic DNA as described above. Maize genomic clones previously used to map RFLP loci in maize chromosomes were obtained from the University of Missouri. Pd1, a vector clone, is essentially a primer-dimer sequence from the multiple-cloning site of pUC18 (Xu et al. 1993). The sorghum clones were assigned the prefix pSbTXS, where p = plasmid, Sb = Sorghum bicolor and TXS = Texas A&M University, and consecutive numbers as they were isolated.

Insert DNA fragments were generated from either PsiI digestion of recombinant plasmids or polymeerase chain reaction (PCR) amplification of the inserts directly from Escherichia coli cells containing recombinant plasmids as described previously (Birnboim and Doly 1979; Xu et al. 1993). PCR-amplified DNA products and PsiI-generated insert fragments were resolved by electrophoresis (5 V/cm for 3 h) in 1% Nusieve GTG (FMC) low-melting-point (LMP) agarose and sliced from the gel. DNA fragments (25 ng for survey blots or 100 ng for F2 mapping blots) were labeled with [32P]-dATP using the method of Feinberg and Vogelstein (1983). Labeled DNA fragments were purified through a spin column (Sephadex G-50 in STE), denatured and then added to the hybridization solution (Maniatis et al. 1982).

Detection of polymorphisms and segregation analysis

Clones that revealed polymorphisms in survey blots containing DNA from the parental lines digested with five restriction enzymes (see above) were used for segregational analysis of the population. Loci were scored either for the presence versus absence of codominant alleles or the presence versus absence of dominant and recessive (= null) alleles. F2 data for those loci that were scored for null alleles were analyzed assuming dominance. Linkage analysis was performed on F2 segregation data with the computer program Mapmaker Linkage 3.0 obtained from S. V. Tinge of the Dupont Company. The entire set of markers was first processed using 2-point analysis with LOD = 3.0 and maximum theta = 0.30 (i.e., maximum recombination = 0.30) to infer possible linkage group assignments. The suspected groups were then processed using multipoint analysis (LOD = 3.5) with a maximum of 6 markers by using the "compare" command to determine an acceptable order for these markers, which served as a framework for the linkage map. Thereafter, the remaining markers were placed into the framework by using the "try" command, and the orders of markers were confirmed by using the "ripple" command (Lander et al., 1987). CentiMorgan (cM) values were calculated using the Kosambi function (Kosambi 1944). One interval in which the likelihood of linkage between the markers falls between a LOD of 3 and 3.5 is indicated on the map.

Genetic nomenclature

Loci detected by anonymous RFLP clones were designated with the symbol "X" followed by the same laboratory designator and number as was assigned to the clone. An additional number in parenthesis was used to designate 2 or more loci detected by a single probe. All characters in the locus designation are italicized. In this manuscript, the X is omitted from locus designations in some contexts.

Results

Selection of the F2 mapping population

Genomic DNAs of six parents of four potential F2 mapping populations were digested with three restriction enzymes (EcoRI, EcoRV and HindIII) and probed with 20 maize and 11 sorghum DNA clones. The maximum RFLP frequency detected, 54.8%, was between IS 3620C, a guinea line, and BTx 623, an agronomically important inbred sorghum line derived from a cross between a zera zera (a caudatum-like sorghum) and an established kafir line. The other sets of parents had from 32.3% (between SA 7078 and QL3) to 38.7% (Chinese Natl acc ‘422’ and BTx 623; RTx 430 and QL3) RFLP. On this basis, a F2 population derived from the cross of IS 3620C and BTx 623 was selected as a mapping population.

Hybridization of maize DNA to sorghum DNA

Radiolabeled sorghum genomic DNA was hybridized to 248 maize genomic DNA clones and two sorghum cDNA clones on dot blots. Under conditions that gave a good signal level for the sorghum cDNA clones, a