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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

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 uniformaly 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.

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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 F_2 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 F_2 population. After F_3 seeds were collected, new tillers arising from the F_2 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 m*M* TRIS, 1 m*M* EDTA, pH 8.0) and quantified by fluorometry (TKO 100, Hoefer).

Genomic DNAs (7.5–10 µg per lane) digested with *Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII or *Xba*I 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 (Helentjaris 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 *Pst*I. 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 DH5-α. 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 *Pst*I 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 *Pst*I digestion of recombinant plasmids or polymerase 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 *Pst*I-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 F_2 mapping blots) were labeled with [^{32}P]-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. F_2 data for those loci that were scored for null alleles were analyzed assuming dominance. Linkage analysis was performed on F_2 segregation data with the computer program Mapmaker Macintosh V 1.0 obtained from S. V. Tingey 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 F_2 mapping population

Genomic DNAs of six parents of four potential F_2 mapping populations were digested with three restriction enzymes (*Eco*RI, *Eco*RV and *Hin*dIII) 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 QL_3) to 38.7% (Chinese Natl acc '422' and BTx 623; RTx 430 and QL_3) RFLP. On this basis, a F_2 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

detectable signal level was observed for 186 (76%) of the maize clones. The 186 maize clones were then used as probes on sorghum genomic DNA on Southern blots under the hybridization and post-hybridization wash stringency conditions normally used for sorghum genomic DNA probes. Under these conditions, the signal level detected for about two-thirds of the maize clones was no more that 20% of the level typical for sorghum low-copy-number genomic DNA clones, and only about 10% of the maize clones produced a signal level equal to that of typical sorghum low-copy-number genomic DNA clones.

Sorghum genomic DNA library characterization

A partial clone library of genomic DNA was constructed. The library consists of approximately 2500 clones ranging from 300 to 2500 bp in length. Of the 96 clones assayed using the colony hybridization procedure, 92 (95.8%) gave weak signals on autoradiograms, presumably because the clones contain single- or low-copy-number DNA sequences. Sixty-five of these clones were further tested by being used as probes on DNA from IS 3620C and BTx 623. Only 3 of the clones (4.6%) hybridized to repetitive DNA.

Cloned DNA inserts that were amplifiable by PCR were used for mapping. Successful amplification was obtained with 651 of the 922 clones tested (70.6%), and typical yields were 0.5–2 μg of product in a 25 μl reaction. Radiolabeled DNA inserts were hybridized to digested genomic DNA of IS 3620C and BTx 623 to identify clones that contained low-copy-number sequences and reveal RFLPs. Of 499 sorghum clones tested, 25 (5.6%) were found to contain highly repetitive DNA sequences, 5 (1.1%) to contain multiple-copy-number sequences that hybridized to an average of about 5 fragments/parental line, 6 (1.3%) to contain organellar DNA and 413 (92.0%) to contain low-copy-number sequences.

Restriction fragment length polymorphism

Initially, eight restriction enzymes (*Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII, *Hin*fI, *Msp*I, *Pst*I and *Xba*I) were used to test for RFLPs in the mapping population. Five of the enzymes, *Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII and *Xba*I, were subsequently selected for further use on the basis of their being the most efficient in revealing polymorphisms and the least expensive. Of 413 sorghum clones containing single or low-copy-number sequences that were tested, 210 (50.8%) revealed a RFLP between the two parental lines with at least one of the five restriction enzymes. The RFLP frequency detected with each restriction enzyme is shown in Table 1. Ninty-four probes (23%) detected only a single fragment per parent, whereas 37 probes (9%) detected 2 or more fragments per parent with each of the five restriction enzymes. Overall, the average number of fragments detected/per probe was 1.55, and the range of the averages among the five restriction enzymes was from 1.51 to 1.61 (Table 1).

Segregation analysis and RFLP linkage map

One-hundred and seventy-three single and low-copy-number sorghum clones, ten maize clones and one primer-dimer probe were used for mapping. Among these clones, 63% hybridized to a single fragment from one parent and 1 or 2 from the other parent, 28% hybridized to 2 fragments from one parent and 2 or 3 fragments from the other parent (1 or 2 of which were polymorphic), and 9% hybridized to 3 or more fragments/parent (2 or more of which were polymorphic). Segregation data were analyzed for 195 markers, including 184 detected by sorghum probes, 10 by maize probes, and 1 by the primer-dimer sequence. Distorted segregation of alleles ($P < 0.05$) occurred at 26 loci and, among these, the BTx 623 allele was favored at 21 loci (80.8%) and the IS 3620C allele was favored at 5 loci (19.2%). An excess of heterozygotes was observed at 5 loci.

Fourteen linkage groups were established by consegregational analysis (Fig. 1). The map covers a total of 1789.3 cMs and consists of 190 loci. The 10 largest linkage groups consist of from 10 to 24 loci and from 103.3 cMs to 237.2 cMs. The average map distance between pairs of loci in these linkage groups is 9.3 cMs. Approximately 60% of the intervals between adjacent loci are smaller than or equal to 10 cMs, and 37% are from 10 to 30 cMs. Four intervals between 2 loci are larger than 30 cMs. However, the LOD score of these intervals is at least 3.5. Each of the 4 other linkage groups consists of 2–5 loci and 6.6–62.3 cMs. Five markers remain unlinked. The 26 loci that gave distorted segregation ratios are designated with asterisks in Fig. 1.

Co-dominant alleles were scored at 168 loci (86.2%), and dominant alleles (characterized by the presence of a fragment in one parent and the absence of a homologous fragment in the other) were scored at 27 loci (13.8%). Eight clones detected duplicated loci and 2 clones (pSbTXS361 and pSbTXS1163) detected triplicated loci. The distribution of these duplicated and triplicated loci is shown in Table 2.

Table 1 Polymorphism levels between BTx 623 and IS 3620C detected by sorghum low-copy-number genomic clones using five restriction enzymes[a]

Restriction enzyme	% polymorphism revealed	Cumulative RFLP (%)	Number of fragments detected/probe
*Xba*I	29.8	29.8	1.61
*Eco*RI	29.3	41.2	1.53
*Eco*RV	25.9	46.0	1.51
*Hin*dIII	25.1	49.6	1.56
*Bam*HI	18.1	50.8	1.52

[a] Based on analysis of 413 DNA clones

Discussion

The signal-level intensities produced by maize genomic DNA clones or Southern blots containing sorghum genomic DNA were both much lower and much more variable than those produced by sorghum genomic DNA clones. For this reason, we chose to principally use sorghum clones for mapping and to use only those maize clones that gave a satisfactory signal level under hybridization and post-hybridization wash stringency conditions similar to those suitable for sorghum clones.

Distorted segregation was observed at a total of 26 of 190 mapped loci, including 1 or more loci in 7 of the 10 largest linkage groups (see Fig. 1). The alleles showing distorted segregation generally fall into clusters (see linkage groups D, E and H). Neighboring loci showed similar though not statistically significant segregation patterns (locus *txs1745* in the center of a cluster on linkage group F involves a null allele, so homozygosity for one parent cannot be distinguised from heterozygosity, making distortion difficult to detect). Most isolated loci showing distorted segregation ratios are either well separated from neighboring loci (e.g. *txs 558* in linkage group B) or differ very little from neighboring loci in regard to the parental origin of alleles. Given the small size of the F_2 mapping population, chance is probably responsible for most of the distortion. However, a few closely linked clusters of loci showing distorted segregation exist (e.g. see linkage group F), and one or more of these may well contain a gene that is subject to selection in gametes or in early development. Similar or higher frequencies of distorted segregation to that observed in this study have been observed in mapping studies conducted in several other species, including maize (Helentjaris et al. 1986), lettuce (Kesseli et al. 1990), barley (Heun et al. 1991), common bean (Nodari et al. 1993), rice (18%, McCouch et al. 1988), potato (25%, Gebhardt et al. 1988) and *Brassica napus* (21%, Landry et al. 1991). The clustering of loci with distorted segregation has been reported in potato (Bonierbale et al. 1988) and barley (Heun et al. 1991).

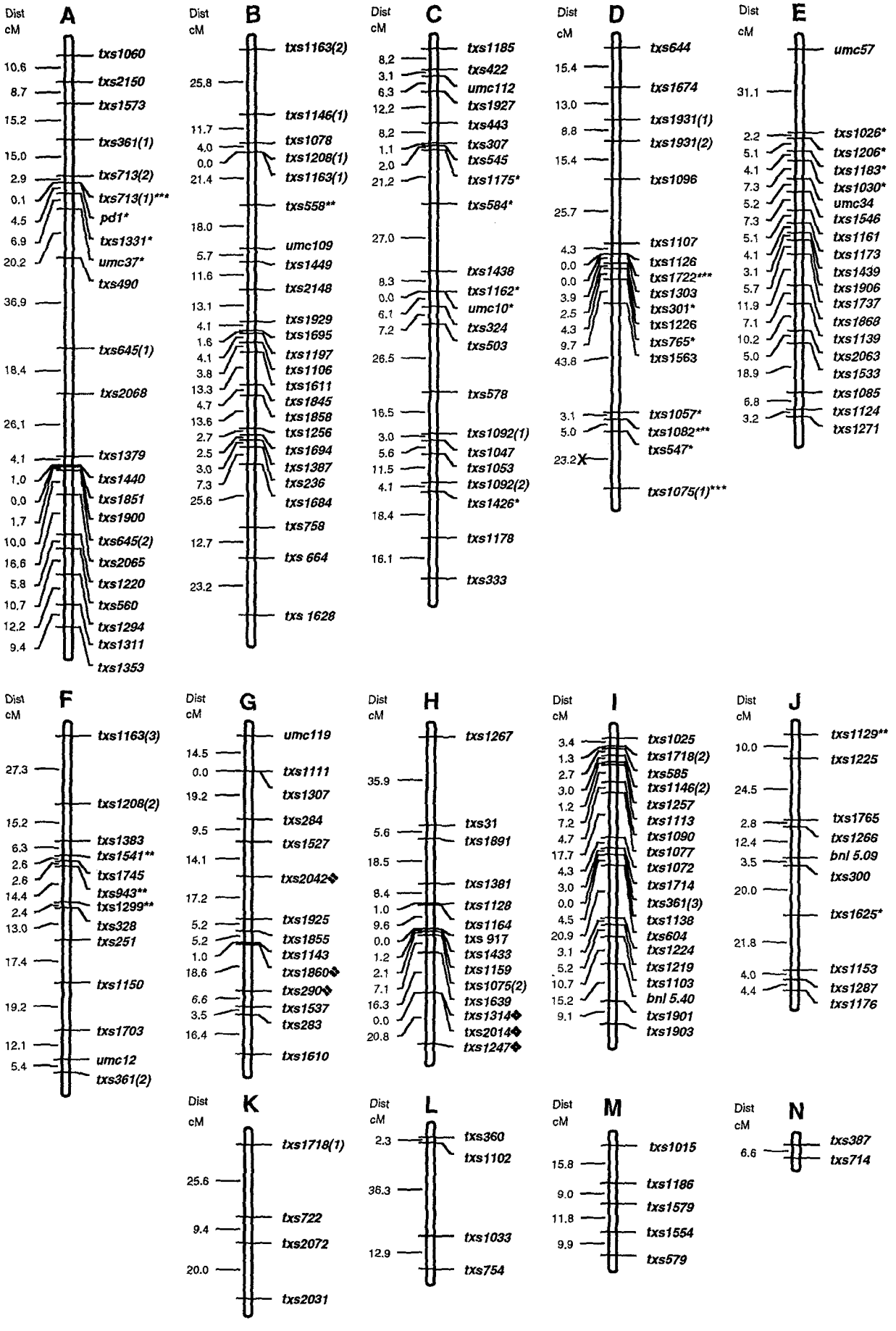
Our linkage map consists of 14 linkage groups, 4 more than number of sorghum chromosomes, and 5 loci remain unlinked. Given the large differences in size between the 10 largest linkage groups (linkage groups A–J) and the 4 smallest groups (K–N), it is likely that each sorghum chromosome contains 1 of the 10 largest linkage groups and that up to four chromosomes contain 1 of the other groups.

The 14 linkage groups span 1789 cMs. At a minimum, the linkage of the 4 smallest groups and the 5 unlinked markers to the 10 largest groups will enlarge the linkage map by 270 cMs to about 2060 cMs. The size of this map contrasts markedly with the 949 cMs and 709 cMs that comprise the sorghum maps of Whitkus et al. (1992) and of Berhan et al. (1993), respectively. It is similar, however, to that of maps constructed for maize [the review of Coe et al. (1990) reports a RFLP map for maize composed of about 1800 cMs, while Beavis and Grant (1991),

Fig. 1 Sorghum RFLP linkage maps. Loci with co-dominant alleles at which distorted segregation of alleles was detected at $P = 0.05$ are identified with one *one or two asterisks*, with *one asterisk* (*) indicating segregation favoring the BTx 623 allele and *two asterisks* indicating segregation favoring the IS 3620C allele. Loci with dominant and recessive alleles at which distorted segregation favored the BTx 623 allele are indicated with *three asterisks*. (Distorted segregation favoring the IS 3620C alleles at loci with dominant and recessive alleles was not observed.) Loci at which an excess of heterozygotes were observed are indicated by ◆. The maps were constructed using multipoint analysis wiith a LOD of 3.5 except for the interval designated by *X* in linkage group D, in which the likelihood of linkage between *txs547* and *txs1075(1)* falls between a LOD of 3 and 3.5

Table 2 Number of loci, centiMorgans (cMs), average distance in cMs between loci and distribution of duplicated loci in the linkage groups

Linkage group	Number of loci	CentiMorgans	Average cMs between loci	Duplicated loci
A	23	237.2	10.3	*Xtxs361(1); Xtxs645(1), (2); Xtxs713(1), (2)*
B	24	233.3	9.7	*Xtxs1146(1); Xtxs1163(1), (2); Xtxs1208(1)*
C	22	214.6	9.8	*Xtxs1092(1), (2)*
D	17	178.1	10.5	*Xtxs1931(1), (2); Xtxs1075(1)*
E	19	143.4	7.6	
F	13	137.9	10.6	*Xtxs361(2); Xtxs1208(2); Xtxs1163 (3)*
G	14	131.0	9.4	
H	14	126.5	9.0	*Xtxs1075(2)*
I	19	117.1	6.2	*Xtxs361(3); Xtxs1146(2); Xtxs1718(2)*
J	10	103.3	10.3	
K	4	62.3	15.6	*Xtxs1718(1)*
L	4	51.5	12.9	
M	5	46.5	11.6	
N	2	6.6	3.3	
Total	190	1789.3	9.4	


A
Dist cM
txs1060
10.6
txs2150
8.7
txs1573
15.2
txs361(1)
15.0
txs713(2)
2.9
txs713(1)***
0.1
pd1*
4.5
txs1331*
6.9
umc37*
20.2
txs490
36.9
txs645(1)
18.4
txs2068
26.1
txs1379
4.1
txs1440
1.0
txs1851
0.0
txs1900
1.7
txs645(2)
10.0
txs2065
16.6
txs1220
5.8
txs560
10.7
txs1294
12.2
txs1311
9.4
txs1353
B
Dist cM
txs1163(2)
25.8
txs1146(1)
11.7
txs1078
4.0
txs1208(1)
0.0
txs1163(1)
21.4
txs558**
18.0
umc109
5.7
txs1449
11.6
txs2148
13.1
txs1929
4.1
txs1695
1.6
txs1197
4.1
txs1106
3.8
txs1611
13.3
txs1845
4.7
txs1858
13.6
txs1256
2.7
txs1694
2.5
txs1387
3.0
txs236
7.3
txs1684
25.6
txs758
12.7
txs 664
23.2
txs 1628
C
Dist cM
txs1185
8.2
txs422
3.1
umc112
6.3
txs1927
12.2
txs443
8.2
txs307
1.1
txs545
2.0
txs1175*
21.2
txs584*
27.0
txs1438
8.3
txs1162*
0.0
umc10*
6.1
txs324
7.2
txs503
26.5
txs578
16.5
txs1092(1)
3.0
txs1047
5.6
txs1053
11.5
txs1092(2)
4.1
txs1426*
18.4
txs1178
16.1
txs333
D
Dist cM
txs644
15.4
txs1674
13.0
txs1931(1)
8.8
txs1931(2)
15.4
txs1096
25.7
txs1107
4.3
txs1126
0.0
txs1722***
0.0
txs1303
3.9
txs301*
2.5
txs1226
4.3
txs765*
9.7
txs1563
43.8
txs1057*
3.1
txs1082***
5.0
txs547*
23.2
txs1075(1)***
E
Dist cM
umc57
31.1
txs1026*
2.2
txs1206*
5.1
txs1183*
4.1
txs1030*
7.3
umc34
5.2
txs1546
7.3
txs1161
5.1
txs1173
4.1
txs1439
3.1
txs1906
5.7
txs1737
11.9
txs1868
7.1
txs1139
10.2
txs2063
5.0
txs1533
18.9
txs1085
6.8
txs1124
3.2
txs1271
F
Dist cM
txs1163(3)
27.3
txs1208(2)
15.2
txs1383
6.3
txs1541**
2.6
txs1745
2.6
txs943**
14.4
txs1299**
2.4
txs328
13.0
txs251
17.4
txs1150
19.2
txs1703
12.1
umc12
5.4
txs361(2)
G
Dist cM
umc119
14.5
txs1111
0.0
txs1307
19.2
txs284
9.5
txs1527
14.1
txs2042◆
17.2
txs1925
5.2
txs1855
5.2
txs1143
1.0
txs1860◆
18.6
txs290◆
6.6
txs1537
3.5
txs283
16.4
txs1610
H
Dist cM
txs1267
35.9
txs31
5.6
txs1891
18.5
txs1381
8.4
txs1128
1.0
txs1164
9.6
txs 917
0.0
txs1433
1.2
txs1159
2.1
txs1075(2)
7.1
txs1639
16.3
txs1314◆
0.0
txs2014◆
20.8
txs1247◆
I
Dist cM
txs1025
3.4
txs1718(2)
1.3
txs585
2.7
txs1146(2)
3.0
txs1257
1.2
txs1113
7.2
txs1090
4.7
txs1077
17.7
txs1072
4.3
txs1714
3.0
txs361(3)
0.0
txs1138
4.5
txs604
20.9
txs1224
3.1
txs1219
5.2
txs1103
10.7
bnl 5.40
15.2
txs1901
9.1
txs1903
J
Dist cM
txs1129**
10.0
txs1225
24.5
txs1765
2.8
txs1266
12.4
bnl 5.09
3.5
txs300
20.0
txs1625*
21.8
txs1153
4.0
txs1287
4.4
txs1176
K
Dist cM
txs1718(1)
25.6
txs722
9.4
txs2072
20.0
txs2031
L
Dist cM
txs360
2.3
txs1102
36.3
txs1033
12.9
txs754
M
Dist cM
txs1015
15.8
txs1186
9.0
txs1579
11.8
txs1554
9.9
txs579
N
Dist cM
txs387
6.6
txs714

based on data from four F_2 populations for which the individual maps ranged from about 1550 to 2200 cMs, reported a composite map consisting of about 1700 cMs].

Among the likely causes for the above-mentioned size differences among sorghum linkage maps are differences in the amount of the sorghum genome covered by the maps and differences in recombination frequencies in the F_1 hybrids from which the F_2 populations analyzed were derived. Berhan et al. (1993) concluded that the 709 map units that comprise the linkage groups that they identified cover less than one-half of the sorghum genome. Findings indicating incomplete coverage of the genome included the identification of 15 rather than 10 linkage groups, the failure to link 15 markers to any other marker, and the failure to detect any sorghum loci that are orthologous to loci located in 8 of 20 maize chromosome arms. The map of Whitkus et al. (1992) consists of 949 cMs and 13 linkage groups, but no unlinked markers were reported and sorghum loci orthologous to loci in all 20 maize chromosome arms were identified. The authors suggested a minimum length for the sorghum linkage map of 1099 cMs.

The Whitkus et al. (1992) map was derived from a study of a F_2 population obtained from a cross of two *S. bicolor* subspecies, while our map and that of Berhan et al. (1993) were derived from studies of F_2 populations obtained from crossing *S. bicolor* ssp. *bicolor* races. Less recombination in the former cross than in the latter two is a possibility. However, another recently developed sorghum RFLP map based on a F_2 population obtained from a cross of two *S. bicolor* subspecies consists of about 1750 cMs (M. Lee, personal communication), making it unlikely that differences in recombination frequencies in the F_1 hybrids from which the F_2 populations were derived is the major cause of the large difference in size between our map and that of Whitkus et al. (1992).

Whitkus et al. (1992) obtained information about the frequency of duplicate loci in sorghum by probing restriction enzyme-digested DNAs of seven inbred lines with maize genomic DNA clones. A minimum of 2 loci was assumed to be detected with a probe when the probe hybridized to 2 or more fragments across all restriction enzymes and lines. On this basis, they estimated that 38% of the 146 maize clones that they tested hybridized to 2 or more loci in sorghum. We hybridized 413 sorghum clones to genomic DNAs obtained from IS 3620C and BTx 623 (the parents of the F_2 population that we used for mapping) that had been digested with five restriction enzymes. Forty-five (11%) of the clones hybridized to 2 or more fragments per line with each of the restriction enzymes. Consequently, a minimum estimate of the percentage of sorghum clones that hybridized to duplicate loci in the lines studied is 11%. It should be noted that our procedure for clone isolation was designed to isolate a high frequency of clones that hybridize to single or very-low-copy-number sequences, a factor which could contribute to underestimating the frequency of duplicate loci. On the other hand, it is likely that our estimate would have been even lower if we had probed more than two lines, as did Whitkus et al. (1992). It is unlikely that the frequency of duplicated RFLP loci can be as low as 11%. Further exploration of this matter using additional sorghum clones is needed.

Of the 184 clones used to obtain segregation data 8 detected polymorphism at 2 loci and 2 detected polymorphism at 3 loci (Table 2). These data provide little information about possible evolutionary relationships among sorghum chromosomes, although 2 clones detected duplicate loci in the same pair of linkage groups (pSbTXS1163 and pSbTXS1208 in linkage groups B and F).

Only a few common clones have been used in the construction of the four sorghum linkage maps that have been produced to date. We have initiated a project to produce an integrated sorghum RFLP map in a recombinant inbred line population developed from IS 3620C and BTx 623 using selected clones that were previously used to construct the four maps.

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References

Baxter-Gabbard KL (1972) A simple method for the large-scale preparation of sucrose gradients. FEBS Lett 20:117–119

Beavis WD, Grant B (1991) A linkage map based on information from four F_2 populations of maize (*Zea mays* L.). Theor Appl Genet 82:636–644

Berhan AM, Hulbert SH, Bulter LG, Bennetzen JL (1993) Structure and evolution of the genomes of *Sorghum bicolor* and *Zea mays*. Theor Appl Genet 86:598–604

Birnboim HC, Doly J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 7:1513—1523

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Coe EH Jr, Hoisington DA, Neuffer MG (1990) Linkage map of corn (*Zea mays* L.). In: O'Brien SJ (ed) Genetic maps. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp 6.39–6.67

Davis PB, Pearson CK (1978) Characterization of density gradients prepared by freezing and thawing a sucrose solution. Anal Biochem 91:343–349

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Gebhardt C, Ritter E, Debener T, Schachtschabel U, Walkemeier B, Uhrig H, Salamini F (1989) RFLP analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet 78:65–75

Helentjaris T, Slocum M, Wright S, Schaefer A, Nienhuis J (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet 72:761–769

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrells ME, Tanksley SD (1991) Construction of a restriction fragment length polymorphism map for barley (*Hordeum vulgare*). Genome 34:437–447

Hulbert SH, Richter TE, Axtell JD, Bennetzen JL (1990) Genetic mapping and characterization of sorghum and related crops by means of maize DNA probes. Proc Natl Acad Sci USA 87:4251–4255

Keim P, Diers BW, Shoemaker RC (1990) Genetic analysis of soybean hard seededness with molecular markers. Theor Appl Genet 79:465–469

Kesseli RV, Paran I, Michelmore RW (1990) Genetic linkage map of lettuce (*Lactuca sativa*, 2n = 18). In: O'Brien SJ (ed) Genetic maps. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., pp. 6.100–6.102

Klein-Lankhorst R, Rietveld P, Machiels B, Verkerk R, Weide R, Gebhardt D, Koornneef M, Zabel P (1991) RFLP markers linked to the root knot nematode resistance gene *Mi* in tomato. Theor Appl Genet 81:661–667

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Landry BS, Hubert N, Etoh T, Harada JJ, Lincoln S (1991) A genetic map for *Brassica napus* based on restriction fragment length polymorphisms detected with expressed DNA sequences. Genome 34:543–552

Maniatis F, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Murray MG, Thompson WF (1980) Rapid isolation of high-molecular-weight plant DNA. Nucleic Acids Res 8:4321–4325

Nienhuis J, Helentjaris T, Slocum M, Ruggero B, Schaefer A (1987) Restriction fragment length polymorphism analysis of loci associated with resistance in tomato. Crop Sci 27:797–803

Nodari RO, Tsai SM, Gilbertson RL, Gepts P (1993) Towards an integrated linkage map of common bean 2. Development of an RFLP-based linkage map. Theor Appl Genet 85:513–520

Osborn TC, Alexander DC, Fobes JF (1987) Identification of restriction fragment length polymorphisms linked to genes controlling soluble solids content in tomato fruit. Theor Appl Genet 73:350–356

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335:721–726

Paterson AH, DeVerna JW, Lanini B, Tanksley SD (1990) Fine mapping of quantitative trait loci using selected overlapping recombinant chromosomes in an interspecies cross of tomato. Genetics 124:735–742

Paterson AH, Damon S, Hewitt JD, Zamir D, Rabinowitch HD, Lincoln SE, Lander ES, Tanksley SD (1991) Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics 127:181–197

Reed KC, Mann DA (1985) Rapid transfer of DNA agarose gels to nylon membranes. Nucleic Acids Res 13:7207–7221

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Whitkus R, Doebley J, Lee M (1992) Comparative genome mapping of sorghum and maize. Genetics 132:1119–1130

Xu GW, Magill CW, Hart GE (1993) Primers that amplify inserts in a multi-cloning site also hybridize to *Sorghum bicolor* (L.) Moench DNA. Genome 36:198–201

Young ND, Zamir D, Ganal MW, Tanksley SD (1988) Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *Tm2a* gene in tomato. Genetics 120:579–585

Yu ZH, Mackill DJ, Bonman JM, Tanksley SD (1991) Tagging genes for blast resistance in rice via linkage to RFLP markers. Theor Appl Genet 81:471–476