Theor Appl Genet (1994) 87:925–933

THEORETICAL AND APPLIED GENETICS

© Springer-Verlag 1994

A detailed RFLP map of *Sorghum bicolor* × *S. propinquum*, suitable for high-density mapping, suggests ancestral duplication of *Sorghum* chromosomes or chromosomal segments

L. M. Chittenden[1], K. F. Schertz[2], Y.-R. Lin[1], R. A. Wing[1], A. H. Paterson[1]

[1] Department of Soil and Crop Sciences, Texas A & M University, TX 77843-2474, USA
[2] USDA-ARS, College Station, TX 77843-2474, USA

Received: 15 April 1993 / Accepted: 23 July 1993

Abstract. The first "complete" genetic linkage map of *Sorghum* section *Sorghum* is described, comprised of ten linkage groups putatively corresponding to the ten gametic chromosomes of *S. bicolor* and *S. propinquum.* The map includes 276 RFLP loci, predominately detected by PstI-digested *S. bicolor* genomic probes, segregating in 56 F_2 progeny of a cross between *S. bicolor* and *S. propinquum.* Although prior cytological evidence suggests that the genomes of these species are largely homosequential, a high level of molecular divergence is evidenced by the abundant RFLP and RAPD polymorphisms, the marked deviations from Mendelian segregation in many regions of the genome, and several species-specific DNA probes. The remarkable level of DNA polymorphism between these species will facilitate development of a high-density genetic map. Further, the high level of DNA polymorphism permitted mapping of multiple loci for 21 (8.2%) DNA probes. Linkage relationships among eight (38%) of these probes suggest ancestral duplication of three genomic regions. Mapping of 13 maize genomic clones in this cross was consistent with prior results. Mapping of heterologous cDNAs from rice and oat suggests that it may be feasible to extend comparative mapping to these distantly-related species, and to ultimately generate a detailed description of chromosome rearrangements among cultivated *Gramineae.* Limited investigation of a small number of RFLPs showed several alleles common to *S. bicolor* and *S. halepense* ("johnson-grass"), but few alleles common to *S. propinquum* and *S. halepense*, raising questions about the origin of *S. halepense.*

Communicated by F. Salamini
Correspondence to: A. H. Paterson

Key words: *S. halepense* – Comparative genetic mapping – Maize – RAPD – Marker-assisted selection

Introduction

Sorghum bicolor (L.) Moench. (sorghum), ranks fifth in importance among the world's grain crops, being well-adapted to areas with too-little rainfall for growing other food and feed grains. Sorghum is a staple food crop in parts of Africa and Asia, and a significant feed crop in the U.S., where it covers approximately 14 million acres annually, producing a crop valued at $ 1.2 billion in 1990 (Agricultural Statistics Board 1991). Increasing demand for limited fresh water supplies, coupled with global warming trends, indicate that dryland crops such as sorghum will become increasingly important to agriculture in both arid regions and more moderate climates.

The genus *Sorghum* includes five sections, with chromosome numbers ranging from 2n = 10 to 2n = 40 (Doggett 1988). *Sorghum* section *Sorghum* harbors genetic variation in fundamental attributes of grain crops as well as grasses, making it valuable for the study of a wide range of agriculturally-important traits in both. *S. bicolor* encompasses five cultivated "races" and a larger number of wild varieties with a center of diversity in eastern central Africa (Doggett 1976). While *S. bicolor* is cultivated primarily as a grain crop (sorghum), the Asian species *S. propinquum* exemplifies many fundamental attributes of both wild and cultivated grasses, notably, small seeds, abundant tillering, narrow leaf shape, and well-developed-rhizomes. This "rhizomatous" trait is largely responsible for the tenacity of another, related sorghum,

S. halepense (johnson-grass), which is considered to be one of the most noxious weeds found in U.S. and world agriculture, (Holm et al. 1977; McWhorter 1989).

Linkage maps based upon DNA markers are widely recognized as essential tools for genetic research in many species. Application of DNA markers to crop-improvement objectives has been described in detail by numerous investigators (see Paterson et al. 1992 for a recent review). Likewise, genetic maps have been utilized to document comparative chromosome arrangement in several closely-related crops (Bonierbale et al. 1988; Tanksley et al. 1988, 1992; Hulbert et al. 1990; Gebhardt et al. 1991; Binelli et al. 1992; Whitkus et al. 1992).

The long-range objective of our research is to develop a high-density map of sorghum, using DNA markers. Constructing such a map can be expedited by the use of the interspecific cross of *S. bicolor* × *S. propinquum* which yields viable, fertile F_2 offspring, shows normal chromosome pairing (Doggett 1988), and harbors a high level of DNA polymorphism. As the gene pools of many domestic crop species are quite narrow, intraspecific crosses often do not exhibit sufficient DNA polymorphism to construct a high-density map. While crosses among conspecific races or subspecies show more polymorphism, this polymorphism may not be uniformly distributed across the chromosomes, due to the introgression that has occurred as a result of natural processes or designed breeding programs (e.g., Stephens et al. 1967; Rosenow and Clark 1987).

The present results describe a detailed RFLP map of an *S. bicolor* × *S. propinquum* F_2 population, linked to an estimated 93% of the genome, with markers at average spacing of 5.2 cM. This map provides evidence supporting ancestral duplication of chromosomes or chromosome segments in the evolution of modern-day "diploid" (n = 10) sorghums, and casts some doubt on the putative origin of one modern-day polyploid, *S. halepense* (n = 20), or "johnsongrass". The mapping population utilized in this study is uniquely predisposed to the rapid accumulation of markers required to ultimately provide plant breeders and molecular geneticists with a high-density map of sorghum.

Materials and methods

Genetic stocks

A cross was made of *S. bicolor* cultivar BTx623 × *S. propinquum* (unnamed accession). The F_1 was made by pollinating a single BTx623 plant with pollen from a single *S. propinquum* plant. A single F_1 was verified using two RFLPs, and selfed in the greenhouse to produce the F_2 progeny used in this experiment.

DNA extraction, digestion, and blotting

Young leaves were harvested from both parent plants and each of 56 greenhouse-grown F_2 offspring. The material was lyophilized and stored at −80 °C until grinding. For each DNA extraction, 0.5 g of lyophilized tissue was frozen in liquid nitrogen and ground by mortar and pestle. The ground material was either extracted immediately or stored at −80 °C until needed. The DNA extraction procedure was modified from a protocol developed by Saghai-Maroof et al. (1984). For each sample, 9 ml of extraction buffer, [100 mM Tris, pH 7.5; 1% hexadecyltrimethyl-ammonium bromide (CTAB); 0.7 M NaCl; 10 mM EDTA, pH 8.0; 1% fresh 2-mercaptoethanol], were added to 0.5 g of ground tissue, incubated for 30 min at 65 °C, and centrifuged at 2,700 g for 10 min. The supernatant was extracted twice with chloroform/octanol (24:1), and DNA was precipitated by adding 0.6 vol ice-cold isopropanol. The DNA was then washed in 70% ethanol, air-dried to evaporate the ethanol, dissolved in 200 µl of TE, and stored at 4 °C until use. This technique yielded about 50 – 100 µg DNA per extraction.

Approximately 5 µg of DNA per lane was digested with 15 units of either *Eco*R1, *Hin*d3, or *Xba*1, (Promega[1]), electrophoresed for 16 h at 22V through an 0.8% agarose gel immersed in 1 × NEB (neutral electrophoresis buffer: 0.1 M Tris, 12.5 mM sodium acetate, 1 mM EDTA, pH 8.1), and stained in ethidium bromide (Sigma) to verify complete digestion. Gels were blotted onto Hybond N+ (Amersham), using 0.4 N NaOH. (Reed and Mann 1985). After overnight transfer, blots were rinsed in 2 × SSC and stored at 4 °C until use.

Probe preparation, hybridization, and autoradiography

*Pst*I-digested *S. bicolor* (cv BTx623) genomic fragments of 0.5–1.0 kb in length were isolated from a 1.5% agarose preparative gel, using the BioRad "Prepagene" kit. Following *Pst*I digestion and dephosphorylation of the vector, pT7/T3 (Gibco BRL), the genomic fragments were ligated to the vector at room temperature overnight. The ligation mix was ethanol-precipitated, resuspended in double-distilled H_2O, and aliquots electroporated into *E. coli* (DH5α). White recombinant clones were selected from ampicillin plates containing X-gal (Sambrook et al. 1989).

Genomic inserts were amplified by PCR from bacterial cell lystate (McCabe 1990), using M13 forward and reverse primers flanking the multiple cloning site in pT7/T3. An aliquot of the PCR products was electrophoresed in 1% agarose to verify amplification of a single product. Reactions with multiple products were discarded. PCR products were separated from excess reaction components in Sephadex G50 (Sigma) spun-columns, (Sambrook et al. 1989). Most candidate probes were screened for repetitive elements by hybridizing labelled genomic DNA to dot-blots of 20 ng of DNA from each probe. Probes emitting a detectable signal after a 48-h exposure were eliminated. About 20 ng of PCR-amplified fragment was labelled with 10 µCi of ^{32}P-dCTP, by the random hexamer technique (Feinberg and Vogelstein 1983). Hybridization was in 35 × 150 mm glass bottles containing 5 ml of hybridization buffer, [6 × SSC, 5% Denhardts, 2.5% SDS, (Sigma)], incubated at 65 °C overnight in a hybridization rotisserie (Hybaid). Blots were washed three times at 65 °C in 0.1 × SSC, 0.1% SDS, and autoradiographed on Kodak XAR5 film with Spectra L-Plus (Picker International) intensifying screens.

Linkage analysis

Southern blots of genomic DNA from 56 F_2 individuals, digested with *Eco*RI, *Hin*dIII, or *Xba*I, were analyzed with each DNA probe that revealed polymorphism on survey blots, Linkage was

[1] The naming of suppliers for reagents used in this research in no way implies endorsement of these products by the United States Department of Agriculture

assessed using MAPMAKER version 1.0 (Lander et al. 1987) for the Macintosh computer (provided by S. Tingey, DuPont Co.), run on the Apple Quadra 700. Initial linkage associations ("group") employed a LOD threshold of 4.0, unusually conservative for a genome of ten chromosomes and approximately 1500 cM, but necessary to avoid spurious associations with genomic regions showing severely-distorted segregation (see Results). Local ordering of markers ("ripple", see Fig. 1) was based on the customary LOD 2.0 difference from alternate orders.

Results

Level of DNA polymorphism between S. bicolor and S. propinquum

The *S. bicolor* and *S. propinquum* parents showed polymorphism for 95% of the low-copy probes surveyed, with at least one of three restriction enzymes (*Eco*RI, *Hin*dIII, *Xba*I). Overall, 76% of the probes surveyed were polymorphic with *Eco*RI, 77% with *Hin*dIII, and 83% with *Xba*I.

Probes which were polymorphic with none or all three enzymes occurred more frequently than expected, while probes polymorphic with one or two enzymes occurred less frequently than expected (Table 1). In the extreme, if all RFLPs were due mostly to localized genomic rearrangements (insertions/deletions), one would expect that a given DNA probe would detect polymorphism either with no restriction enzymes, or with all three – whereas if RFLPs are largely due to base substitutions in restriction sites, polymorphism with one enzyme would be independent of polymorphism with others, and "two-enzyme" or "three-enzyme" probes would occur at a frequency predictable from rates of polymorphism detected for each single enzyme. Significant deviation from this frequency in our data (Table 1) suggests that localized genomic rearrangements (insertions/deletions) contribute to DNA polymorphism between *S. bicolor* and *S. propinquum.*

Table 1. The number of restriction enzymes revealing polymorphism between *S. bicolor* and *S. propinquum* for low-copy DNA probes[a]

	None	Any one restriction enzyme	Any two restriction enzymes	All three restriction enzymes
Observed	17	23	58	145
Expected	2	26	96.5	118.5

$\chi^2 = 134.07$, 3 df; deviation is significant ($P \leq 0.001$)

[a] Only low-copy DNA probes which yielded unambiguous results for all three restriction enzymes were included in this analysis. Consequently, the numbers presented in this table do not add up to the number of probes analyzed (presented in text), because of occasional artifacts on autoradiograms

Among the 375 probes surveyed, 337 were low copy, and the remaining 38 were repetitive. Among these repetitive probes, only four distinct band patterns were observed. An additional 46 uncharacterized repetitive elements were eliminated prior to the survey (as described in Materials and Methods).

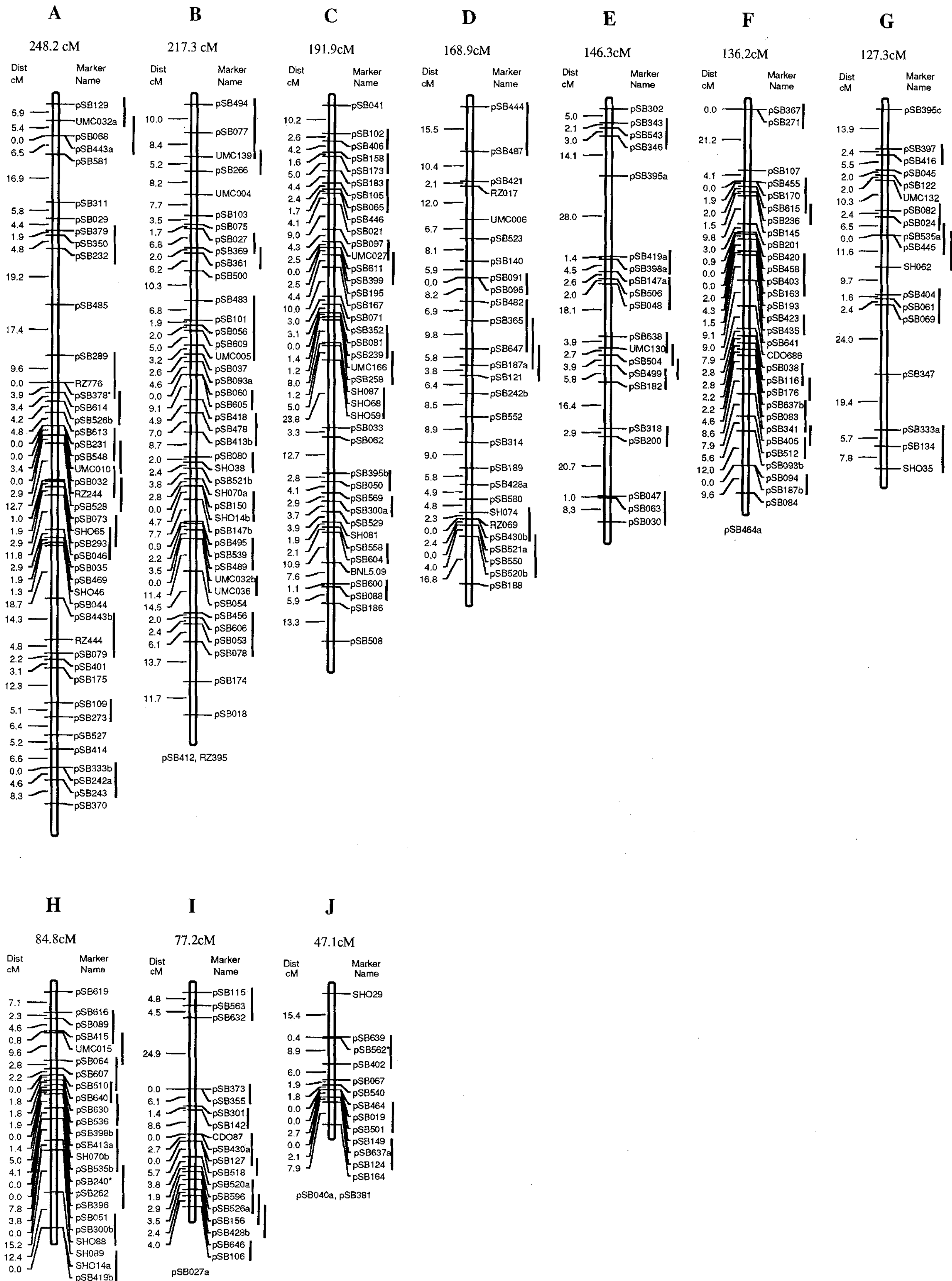
Of the 337 low-copy probes, 320 (95%) detected polymorphism between *S. bicolor* and *S. propinquum.* However, 85 were deemed unsuitable for mapping, due to the presence of too many bands, or bands too close in molecular weight to be reliably discerned.

A few heterologous probes from maize (UMC, BNL), rice (RZ), and oat (CDO), were also studied. Of 29 maize probes successfully surveyed, 25 were polymorphic, and 13 considered suitable for mapping. Of the limited surveys done with rice and oat cDNAs, five rice and two oat markers were also mapped.

The high level of DNA polymorphism observed with RFLPs is paralleled by a high level of random amplified polymorphic DNA (RAPD)-PCR polymorphisms. As part of a related study to be published separately, 242 decamers were applied to template DNA from BTx406 (an *S. bicolor* accession which is similar to BTx623 for DNA markers), and the *S. propinquum* mapping parent. Among 1513 bands produced, 1041 (68.8%) were unique to BTx406 or *S. propinquum.* Assuming that the PCR products derived from each RAPD primer represent different loci (Williams et al. 1990), and that our map of 1445 cM represents a 93% coverage of the sorghum genome (see below), these bands should provide RAPD-PCR polymorphisms at an average spacing of 1.5 cM (i.e., 1445 cM/1041 bands × 93% coverage) throughout the genome.

Segregation and recombination in F_2 progeny

The 255 polymorphic probes (235 sorghum and 20 heterologous) deemed suitable were mapped in 56 F_2 progeny of the *S. bicolor* × *S. propinquum* cross. While most loci showed Mendelian segregation ratios, some deviated significantly. Across the entire genome, individuals were homozygous for the *S. bicolor* allele over an average of 28.6($\pm$12.5)% of the genome, homozygous for the *S. propinquum* allele over 21.2($\pm$12.5)% of the genome, and heterozygous over 50.2($\pm$8.3)% of the genome. Thirteen unlinked genomic regions, distributed over seven of the ten chromosomes, showed significant deviations from Mendelian segregation, with only linkage groups D, E, and H free from segregation distortion. Six of the thirteen regions showed excess heterozygotes (near RZ244 and pSB370 on gp. A; pSB077 on gp. B; pSB041 on gp. C; pSB641 on gp. F; pSB301 on gp. I; pSB077 on gp. B), four showed deficiency of the *S. propinquum*


A
248.2 cM
Dist cM
Marker Name
pSB129
UMC032a
pSB068
pSB443a
pSB581
pSB311
pSB029
pSB379
pSB350
pSB232
pSB485
pSB289
RZ776
pSB378*
pSB614
pSB526b
pSB613
pSB231
pSB548
UMC010
pSB032
RZ244
pSB528
pSB073
SHO65
pSB293
pSB046
pSB035
pSB469
SHO46
pSB044
pSB443b
RZ444
pSB079
pSB401
pSB175
pSB109
pSB273
pSB527
pSB414
pSB333b
pSB242a
pSB243
pSB370
B
217.3 cM
pSB494
pSB077
UMC139
pSB266
UMC004
pSB103
pSB075
pSB027
pSB369
pSB361
pSB500
pSB483
pSB101
pSB056
pSB609
UMC005
pSB037
pSB093a
pSB060
pSB605
pSB418
pSB478
pSB413b
pSB080
SHO38
pSB521b
SH070a
pSB150
SHO14b
pSB147b
pSB495
pSB539
pSB489
UMC032b
UMC036
pSB054
pSB456
pSB606
pSB053
pSB078
pSB174
pSB018
pSB412, RZ395
C
191.9cM
pSB041
pSB102
pSB406
pSB158
pSB173
pSB183
pSB105
pSB065
pSB446
pSB021
pSB097
UMC027
pSB611
pSB399
pSB195
pSB167
pSB071
pSB352
pSB081
pSB239
UMC166
pSB258
SH087
SHO68
SHO59
pSB033
pSB062
pSB395b
pSB050
pSB569
pSB300a
pSB529
SH081
pSB558
pSB604
BNL5.09
pSB600
pSB088
pSB186
pSB508
D
168.9cM
pSB444
pSB487
pSB421
RZ017
UMC006
pSB523
pSB140
pSB091
pSB095
pSB482
pSB365
pSB647
pSB187a
pSB121
pSB242b
pSB552
pSB314
pSB189
pSB428a
pSB580
SH074
RZ069
pSB430b
pSB521a
pSB550
pSB520b
pSB188
E
146.3cM
pSB302
pSB343
pSB543
pSB346
pSB395a
pSB419a
pSB398a
pSB147a
pSB506
pSB048
pSB638
UMC130
pSB504
pSB499
pSB182
pSB318
pSB200
pSB047
pSB063
pSB030
F
136.2cM
pSB367
pSB271
pSB107
pSB455
pSB170
pSB615
pSB236
pSB145
pSB201
pSB420
pSB458
pSB403
pSB163
pSB193
pSB423
pSB435
pSB641
CDO686
pSB038
pSB116
pSB176
pSB637b
pSB083
pSB341
pSB405
pSB512
pSB093b
pSB094
pSB187b
pSB084
pSB464a
G
127.3cM
pSB395c
pSB397
pSB416
pSB045
pSB122
UMC132
pSB082
pSB024
pSB535a
pSB445
SH062
pSB404
pSB061
pSB069
pSB347
pSB333a
pSB134
SHO35
H
84.8cM
pSB619
pSB616
pSB089
pSB415
UMC015
pSB064
pSB607
pSB510
pSB640
pSB630
pSB536
pSB398b
pSB413a
SH070b
pSB535b
pSB240*
pSB262
pSB396
pSB051
pSB300b
SHO88
SH089
SHO14a
pSB419b
I
77.2cM
pSB115
pSB563
pSB632
pSB373
pSB355
pSB301
pSB142
CDO87
pSB430a
pSB127
pSB518
pSB520a
pSB596
pSB526a
pSB156
pSB428b
pSB646
pSB106
pSB027a
J
47.1cM
SHO29
pSB639
pSB562*
pSB402
pSB067
pSB540
pSB464
pSB019
pSB501
pSB149
pSB637a
pSB124
pSB164
pSB040a, pSB381

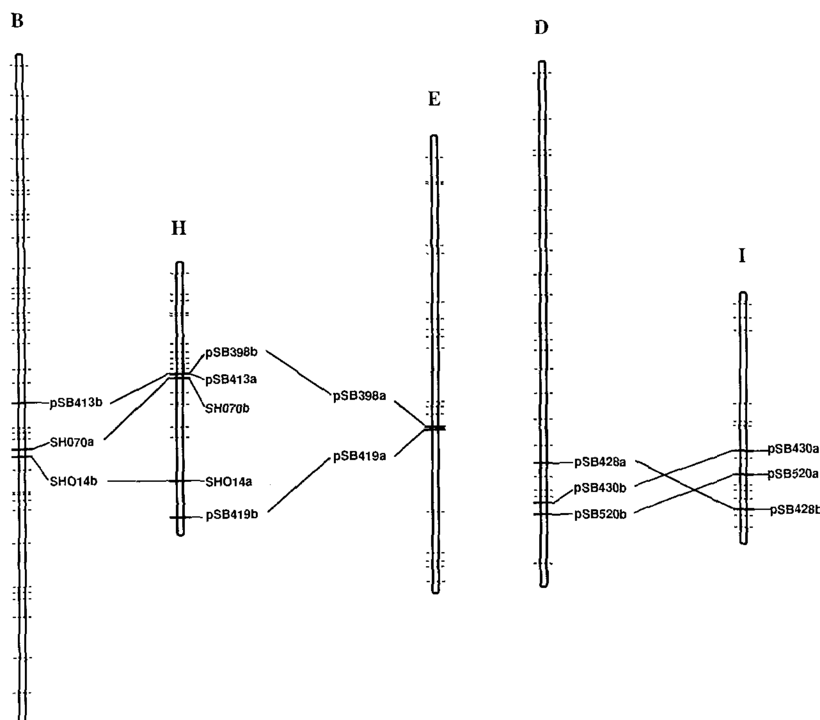


Fig. 2. Map orders of duplicated loci which fall in common regions along linkage groups B and H, H and E, and D and I. These parallelisms suggest polyploidization events in the evolution of the *Sorghum* genome (see Discussion: genome evolution)

homozygote (near pSB464 on gp. J; pSB352 and pSB600 on gp. C; pSB115 on gp. I), two showed deficiency of the *S. bicolor* homozygote (near pSB232 and pSB289 on gp. A), and one showed heterozygote deficiency (near pSB122 on gp. G). The most extreme case of segregation distortion was pSB352 (gp. C): of the 51 individuals which could be scored with certainty, 41 were homozygous for the *S. bicolor* allele, ten were heterozygous, and none were homozygous for the *S. propinquum* allele.

A total of 276 segregating loci fell into ten linkage groups, putatively corresponding to the ten chromosomes of *S. bicolor* and *S. propinquum*. These ten linkage groups span a genetic distance of 1445 cM with an average of 5.2 cM between markers. Six loci showed tenative linkage with particular groups but could not be reliably placed within those groups (see Fig. 1). Another 12 loci did not show association with any group, although eight of these markers did fall into three small groups among themselves. Four loci showed linkage to no other loci. Assuming that markers are distributed randomly across the recombinational length of the genome (an albeit tenuous assumption), the map covers about 93% (258/276) of the genome.

Duplication of RFLP loci

Of the 255 markers mapped, 21 (8.2%) demonstrated multiple bands segregating for two (or three, for pSB395) separate loci. While many mechanisms of DNA sequence duplication are known, linkage relationships suggest that some of these duplicated loci may be the result of duplicated chromosomes or chromosomal segments. Among these 21 markers, eight (38%) detected loci which fell into three linked sets,

Fig. 1. RFLP map of *S. bicolor* × *S. propinquum*. The map includes 276 loci, comprising ten linkage groups ranging in size from 47.1 to 248.2 cM, for a total length of 1445 cM. The average distance between markers is 5.2 cM. *Pst*I sorghum genomic clones are denoted by *pSB-* or *SHO-*; maize genomic clones are labeled *UMC-* or *BNL-*; and rice and oat cDNAs are labeled *RZ-* and *CDO-*, respectively. Markers with names followed by "*" are specific to the *S. bicolor* genome, (see Discussion: genome evolution). Markers with names followed by *a*, *b*, or *c* correspond to DNA probes which detect polymorphism at more than one locus on the map. The most likely order of all markers is presented. *Lines to the right* of closely-linked markers indicate that alternate marker orders cannot be ruled out at LOD 2.0, ("ripple" command, MAPMAKER; Lander et al. 1987). The six markers placed immediately *underneath* linkage groups B, F, I, and J are tenatively associated to that linkage group, but could not be mapped to an accurate location within the group. Another eight loci fell into three small groups which did not link to the map (pSB057-UMC019; pSB108-pSB541-pSB491-pSB204; and pSB517-pSB526b). Finally, four loci (pSB074, pSB305, pSB607b, and UMC134) showed no associations to any other markers

duplicated onto common linkage groups (Fig. 2). Three linked loci were duplicated homosequentially on groups B and H; two linked loci were duplicated on groups H and E; and three linked loci were duplicated on groups D and I, with one inversion.

Rapidly-evolving DNA sequences

Three DNA probes isolated from *S. bicolor* (BTx623) detected no homologous sequences in *S. propinquum*, based on genomic Southern-blots including digests with three different restriction enzymes (*Eco*RI, *Hin*dIII, *Xba*I). These DNA probes mapped as dominant/recessive markers to different linkage groups (designated with * in Fig. 1; pSB378 – gp. A, pSB240 – gp. H, pSB562 – gp. J). These apparent *S. bicolor*-specific elements have been examined in *Eco*RI, *Hin*dIII, and *Xba*I digests from a single accession each of the *S. bicolor* races bicolor, caudatum, durra, guinea, and kafir, and from each of the *S. bicolor* subspecies, aethopicum, verticilliflorum, arundinaceum, feterita, and virgatum. Two elements (pSB240, pSB562) were present in all races and subspecies of *S. bicolor*, while the third (pSB378) was present in all except the race guinea accession IS3620C.

Discussion

The interspecific cross, *S. bicolor* × *S. propinquum*, is uniquely well-suited to creating a high-density map of sorghum. The 95% rate of RFLPs among single-copy DNA probes, and the estimated 93% coverage of the genome by the 276 loci, facilitated the assembly of the first "complete" genetic map of sorghum, with ten linkage groups putatively corresponding to the ten gametic chromosomes. This is in contrast to reports of lower levels of polymorphism within *S. bicolor* (Binelli et al. 1992). The marker orders established in this interspecific cross should be common to crosses within *S. bicolor*, as cytological evidence indicates homosequentiality among the chromosomes of *S. bicolor* and *S. propinquum* (Doggett 1988). The remarkable level of DNA polymorphism between *S. bicolor* and *S. propinquum* will expedite construction of a high-density map, as well as comparative mapping of sorghum and other species, since approximately 95% of low-copy DNA clones can be mapped.

The relatively small genome of sorghum (n = 10, C = 0.8 pg and approximately 750 Mbp; Arumuganathan and Earle 1991), is better suited to physical mapping and map-based cloning than the genomes of many other cultivated grains or grasses. A high density map, in conjunction with a detailed yeast artificial chromosome (YAC) library, would permit cloning of genes with agricultural or developmental importance in sorghum. RFLPs at 5.2-cM intervals, and RAPD-PCR polymorphisms at 1.5-cM intervals throughout the genome will facilitate high-resolution analysis of targeted genomic regions, using near-isogenic lines (Martin et al. 1991), or synthetic DNA pools (Giovannoni et al. 1991; Michelmore et al. 1991). Preliminary success with the construction of YACs (R.A.W., A.H.P., K.F.S., unpublished data) bodies well for the prospect of map-based gene cloning in sorghum.

Duplication of Sorghum chromosomes or chromosome segments?

A high-density map of *S. bicolor* × *S. propinquum* will be useful for the study of chromosome evolution. For example, 16 duplicated RFLP loci map to common regions on three pairs of linkage groups (Fig. 2), suggesting ancient duplication(s) of sorghum chromosomes or chromosome segments. This supports a model of sorghum genome evolution which involves some degree of chromosome duplication (Whitkus et al. 1992, Model B), and essentially rules out an alternative model (Whitkus et al. 1992, Fig. 4, Model A) which involves no chromosome (or segment) duplication.

The existence of n = 5 species within the genus *Sorghum* (Doggett 1988), together with cytotaxonomic evidence for polyploidy of sorghum (Brown 1943; Garber 1944; Kidd 1952; Hadley 1953; Endrizzi and Morgan 1955; Sharma and Bhattacharjee 1957; Celarier 1958; Bennett and Merwine 1966; Tang and Liang 1988), make it tantalizing, but premature, to suggest that sorghum may be derived from a primitive ancestor with only five gametic chromosomes. A distinction between the hypothesis of a genome-wide duplication event, and the alternative hypothesis of one or more segmental duplication events, awaits additional evidence. Further, a satisfactory model should account for duplicated probes (of which there are presently 13), which cannot be explained by chromosome (or segment) duplication. Duplicated loci are widely-distributed across the genome, being found on all ten linkage groups (Fig. 1). Although there do appear to be "clusters" of duplicated loci in some chromosomal regions (e.g., gp. D, lower end; Fig. 1), an insufficient number have been mapped to determine whether or not such "clusters" might simply occur by chance. Some duplicated loci may be accounted for by pseudogenes or retrotransposon-like duplications, such as one case of proximal duplication (pSB443a, b on gp. A; Fig. 1). Others may fall in genomic regions which have undergone substantial rearrangement since divergence of "homoeologs" though the density of duplicated loci on the map is as yet too low to reveal homoeology. Substantial rearrangement between putatively "homoeologous" regions is strongly indicated, since one of the putative duplications involves an inversion (gps. D and I; Fig. 2), and a second includes an insertion, comprised of the third, on one group (H; Fig. 2).

The high level of polymorphism between *S. bicolor* and *S. propinquum* facilitates genetic analysis of ancestral duplications – since the mapping of regions involved in such duplications requires that both "homoeologous" loci detected by a DNA probe exhibit RFLPs. Specifically, if the likelihood that a single genomic restriction fragment exhibits polymorphism is x, then the likelihood that a DNA probe would detect polymorphism at two "homoeologous" loci is x^2. For a high level of polymorphism per restriction enzyme, x^2 is close to x. For progressively lower levels of polymorphism, x^2 becomes exponentially smaller than x, so that it becomes exponentially more difficult to map duplicated loci in crosses exhibiting lower levels of DNA polymorphism. Thus, for intraspecific crosses such as have been used in other sorghum maps (Hulbert et al. 1990; Binelli et al. 1992; Pereira et al. 1992; Whitkus et al. 1992; G. Hart, personal communication), it would be relatively more difficult to identify chromosomal or segmental duplications.

Comparative mapping of Sorghum and other Gramineae

Comparisons of chromosome organization between members of the genus *Sorghum* and other genera can be made more informative with the availability of a detailed genetic map of *Sorghum*. The comparative organization of maize and sorghum chromosomes has been studied by several investigators (Hulbert et al. 1990; Binelli et al. 1992; Whitkus et al. 1992). We mapped only 13 maize clones but, based on information from the large number of sorghum DNA clones mapped, we suggest that linkage groups B and C of Whitkus et al. (1992) are part of the same linkage group (our group C, Fig. 1). Similarly, we have mapped several rice and oat cDNAs (provided by S. Tanksley and M. Sorrells), indicating the feasibility of extending comparative genome mapping beyond n = 10 Gramineae such as maize, sorghum, and sugarcane, to n = 7 Gramineae such as wheat, oat, barley, and rye, and n = 12 Gramineae such as rice.

Relationships among species within Sorghum section Sorghum

The occurrence of *S. bicolor*-specific low-copy probes (see Results), reinforces the observed high level of polymorphism between *S. bicolor* and *S. propinquum*, in suggesting that these species have diverged considerably from their most-recent common ancestor. This suggestion is supported by the non-overlapping contemporary geographical distributions of *S. bicolor* and *S. propinquum* (Doggett 1976), although overlap of each species with different parts of the distribution of *S. halepense* might offer a possible means of gene flow.

We briefly examined the relationship among *S. bicolor*, *S. propinquum*, and *S. halepense*, or johnsongrass (2n = 40). It has been suggested (Doggett 1976) that *S. halepense* may be a polyploid descendant of a cross between *S. bicolor* and *S. propinquum*. This hypothesis is reinforced by (1) the natural geographic distribution of *S. halepense*, which lies between and overlaps the distributions of *S. bicolor* and *S. propinquum*, and (2) the observation that *S. propinquum*, *S. halepense*, and *S. halepense*-derived *S. almum* ("Columbus Grass") are the only members of the genus known to be rhizomatous (Doggett 1976). Seven DNA probes mapping to three different linkage groups were applied to *Eco*R1 digests of *S. bicolor* (BTx623), *S. propinquum*, and *S. halepense* (locally-collected johnsongrass). These probes revealed 13 restriction fragments in *S. bicolor*, 16 fragments in *S. propinquum*, and 25 fragments in *S. halepense*. A total of 4 (30%) of the 13 *S. bicolor* restriction fragments, each detected by a different DNA probe, were shared with *S. halepense*, and two (15%) of the *S. bicolor* restriction fragments were shared with *S. propinquum*. However, only one very faint fragment was shared between *S. propinquum* and *S. halepense*. While we emphasize that the small number of loci and accessions examined make these results tenative, this may suggest that *S. propinquum* is a highly-polymorphic species, that the contribution of *S. propinquum* to *S. halepense* is ancient and the RFLPs have undergone much divergence, or that *S. propinquum* may have contributed less to *S. halepense* than was previously thought. A more definitive study is in progress.

Applications of a high-density sorghum map

A high-density genetic map of sorghum is essential for many investigations in transmission genetics. Comprehensive mapping of quantitative trait loci (QTLs) requires informative markers for all regions of the genome (Paterson et al. 1988; Lander and Botstein 1989). Likewise, a high-density map facilitates marker-assisted selection, especially between closely-related types, by increasing the probability of finding an informative polymorphic marker in any particular genomic region. High-resolution genetic mapping (Paterson et al. 1990), physical mapping, and map-based cloning (cf. Tanksley et al. 1992; Paterson and Wing 1993) all require DNA markers at short intervals along the chromosomes.

Integration of existing maps of sorghum has been facilitated by the mapping of partly-overlapping sets of maize clones in different populations (Hulbert et al. 1990; Binelli et al. 1992; Pereira et al. 1992; Whitkus et al. 1992; G. Hart, personal communication). Exchange of a small number of DNA probes can provide a "coarse-level" integration sufficient for many purposes. In this regard, we have recently exchanged two clones from each of our linkage groups for two from each linkage group of an *S. bicolor* "BTx623" × *S.*

bicolor "IS3620C" map (being made by G. Hart and colleagues) to facilitate integration of interspecific and intraspecific sorghum maps.

The *S. bicolor* × *S. propinquum* RFLP map provides valuable information regarding both transmission genetics and evolutionary genetics, and will aid in mapping QTLs associated with the divergence of grain sorghums from grasslike relatives. Moreover, a detailed map of DNA markers is a valuable link between the disciplines of classical plant breeding and molecular biology, with many potential applications for the improvement of sorghum and related species, ranging from marker-assisted selection to map-based cloning.

Acknowledgements. The authors thank M. Dahmer, S. Elmore, M. Chase, and J. Garza for technical assistance, J. Doebley, G. Hart, J. Dahlberg, and R. Duncan for valuable comments, S. Tanksley, M. Sorrells (Cornell Univ.), and J. Gardiner (Univ. of Missouri, Columbia) for DNA clones. Aspects of this work were supported by the Texas Agricultural Experiment Station (A.H.P., R.A.W.), USDA-ARS (K.F.S.), and USDA-NRICGP (92-37300-7654, to A.H.P., K.F.S., and R.A.W.).

References

Agricultural Statistics Board (1990) Annual crop summary. NASS, USDA

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Bennett HW, Merwine NC (1966) Meiotic behavior of a Hodo Sorgo × johnsongrass hybrid. Crop Sci 6:127–131

Binelli G, Gianfranceschi L, Pe ME, Taramino G, Busso C, Stenhouse J, Ottaviano E (1992) Similarity of maize and sorghum genomes as revealed by maize RFLP probes. Theor Appl Genet 84:10–16

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

Brown MS (1943) Haploid plants in sorghum. J Hered 34: 163–166

Celarier RP (1958) Cytotaxonomic notes on the subsection *Halepense* of the genus *Sorghum*. Bull Torrey Bot Club 85:49–62

Doggett H (1976) Sorghum. In: Simmonds NW (ed) Evolution of crop plants. Longman Scientific and Technicial, Essex, UK, pp 112–117

Doggett H (1988) Sorghum, 2nd edn. John Wiley and Sons, New York, pp 150–197

Endrizzi JE, Morgan DT (1955) Chromosomal interchanges and evidence for duplication in haploid *Sorghum vulgare*. J Hered 46:201–208

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

Garber ED (1944) A cytological study of the genus *Sorghum:* subsections *Parasorghum* and *Eusorghum*. Am Nat 78:89–94

Gebhardt C, Ritter E, Barone A, Debener T, Walkemeier B, Schactschabel U, Kaufmann H, Thompson RD, Bonierbale MW, Ganal MW, Tanksley SD, Salamini F, (1991) RFLP maps of potato and their alignment with the homoeologous tomato genome. Theor Appl Genet 83:49–57

Giovannoni JJ, Wing RA, Ganal MW, Tanksley SD (1991) Isolation of molecular markers from specific chromosomal intervals using DNA pools from existing mapping populations. Nucleic Acids Res 19:6553–6558

Hadley HH (1953) Cytological relationships between *Sorghum vulgare* and *S. halepense*. Agron J 45:139–143

Holm LG, Plucknett DL, Pancho JV, Herberger JP (1977) *Sorghum halepense* (L.) Pers. In: The world's worst weeds: distribution and biology. University Press of Hawaii, Honolulu, Hawaii, pp 54–61

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

Innis MA, Gelfand DH, Sninsky JJ, White TJ (1990) PCR Protocols: a guide to methods and applications. Academic Press, San Diego, California

Kidd HJ (1952) Haploid and triploid sorghum. J Hered 43:204–225

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

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Martin GB, Williams JGK, Tanksley SD (1991) Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato using random primers and near isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

McCabe PC (1990) Production of single-stranded DNA by asymmetric PCR In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic Press, San Diego, California, pp 76–83

McWhorter CG (1989) History, biology, and control of johnsongrass. Rev Weed Sci 4:85–121

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked-segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828–9832

Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors, 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, Tanksley SD, Sorrells ME (1992) DNA markers in crop improvement. In: Sparks DL (ed) Advances in agronomy. Academic Press, New York, pp 39–90

Paterson AH, Wing RA (1993) Genome mapping in plants. Curr Opin Biotechnol (in press)

Pereira MG, Lee M, Bramel-Cox P (1992) RFLP genetic linkage mapping in sorghum. Agron Abstr 1992:110

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

Rosenow DT, Clark LE (1987) Utilization of exotic germplasm in breeding for yield stability. In: Proc 15th Biennial Grain Sorghum Research and Utilization Conference, Lubbock, Texas, pp 49–56

Saghai-Maroof MA, Soliman KM, Jorgenson R, 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

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Sharma AK, Bhattacharjee D (1957) Chromosome studies in *Sorghum.* I. Cytologica 22:287–311

Stephens JC, Miller FR, Rosenow DT (1967) Conversion of alien sorghums to early combine genotypes. Crop Sci 7:396

Tang H, Liang GH (1988) The genomic relationship between cultivated sorghum [*Sorghum bicolor* (L.) Moench] and johnsongrass [*S. halepense* (L.) Pers.]: a re-evaluation. Theor Appl Genet 76:277–2e84

Tanksley SD, Bernatzky RB, Lapitan NL, Prince JP (1988) Conservation of gene repertoire but not gene order in pepper and tomato. Proc Natl Acad Sci USA 85:6419–6423

Tanksley SD, Ganal MW, Prince JP, deVicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Yound ND (1992) High-density molecular maps of the tomato and potato genomes. Genetics 132:1141–1160

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

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) Oligonucleotide primers of arbitrary sequence amplify DNA polymorphisms which are useful as genetic markers. Nucleic Acids Res 18:6531–6535