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# Construction of a combined sorghum linkage map from two recombinant inbred populations using AFLP, SSR, RFLP, and RAPD markers, and comparison with other sorghum maps

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**Abstract** Sorghum [*Sorghum bicolor* (L.) Moench] is an important crop in the semi-arid tropics that also receives growing attention in genetic research. A comprehensive reference map of the sorghum genome would be an essential research tool. Here, a combined sorghum linkage map from two recombinant inbred populations was constructed using AFLP, SSR, RFLP and RAPD markers. The map was aligned with other published sorghum maps which are briefly reviewed. The two recombinant inbred populations (RIPs) analyzed in this study consisted of 225 (RIP 1) and 226 (RIP 2) $F_{3:5}$ lines, developed from the crosses IS 9830 × E 36-1 (RIP 1) and N 13 × E 36-1 (RIP 2), respectively. The genetic map of RIP 1 had a total length of 1,265 cM (Haldane), with 187 markers (125 AFLPs, 45 SSRs, 14 RFLPs, 3 RAPDs) distributed over ten linkage groups. The map of RIP 2 spanned 1,410 cM and contained 228 markers (158 AFLPs, 54 SSRs, 16 RFLPs) in 12 linkage groups. The combined map of the two RIPs contained 339 markers (249 AFLPs, 63 SSRs, 24 RFLPs, 3 RAPDs) on 11 linkage groups and had a length of 1,424 cM. It was in good agreement with other sorghum linkage maps, from which it deviated by a few apparent inversions, deletions, and additional distal regions.

**Keywords** *Sorghum bicolor* · Genetic linkage map · AFLP · RFLP · SSR · RAPD

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## Introduction

Sorghum [*Sorghum bicolor* (L.) Moench], an important crop in the diet of millions of people in the semi-arid tropics, is receiving growing attention in genetic research. A number of genetic linkage maps of sorghum have been published in the last decade (see Table 1). Several are purely based on RFLP (restriction fragment length polymorphism) markers (Hulbert et al. 1990; Binelli et al. 1992; Whitkus et al. 1992; Berhan et al. 1993; Chittenden et al. 1994; Pereira et al. 1994; Ragab et al. 1994; Xu et al. 1994; Dufour et al. 1997; Ming et al. 1998; Crasta et al. 1999; Peng et al. 1999; Bowers et al. 2000; Xu et al. 2000); others include also AFLP (amplified fragment length polymorphism; Boivin et al. 1999; Klein et al. 2001), SSR (simple-sequence repeats; Taramino et al. 1997; Tao et al. 1998, 2000; Bhattramakki et al. 2000; Kong et al. 2000; Bennetzen et al. 2001; Klein et al. 2001), RAPD (randomly amplified polymorphic DNA; Tuinstra et al. 1996, 1997), or morphological markers (Bennetzen et al. 2001). But all of these genetic maps still have deficiencies, including the absence of mapped telomeres and centromeres, and a lack of connections to the physical chromosomal structures. Moreover, these maps have relatively few markers in common which would allow a comparison across sorghum populations or between sorghum and other plant species (Bennetzen et al. 2001). Ideally, all genetic and physical maps of sorghum should be integrated to form a comprehensive reference map. This would facilitate the choice of markers that are either evenly distributed across the genome, or specific to certain genomic regions. It would also ease the comparison of sorghum maps with maps of other *Gramineae* species and several aspects of gene discovery and isolation. Our objective here was to construct a combined sorghum linkage map from two recombinant inbred populations using AFLP, SSR, RFLP, and RAPD markers. Employing previously mapped markers as anchors, we aligned it with other recently published sorghum maps.

## Materials and methods

### Plant material

Two recombinant inbred populations (RIPs) of sorghum, consisting of 225 (RIP 1) and 226 (RIP 2) $F_3$-derived $F_5$ lines ($F_{3:5}$ lines), were developed from the crosses IS 9830 × E 36-1 (RIP 1) and N 13 × E 36-1 (RIP 2). Line IS 9830 is a tall Sudanese feterita belonging to the caudatum race. Line N 13 from India is a durra sorghum. Line E 36-1 is assigned to the guinea/caudatum hybrid race with Ethiopian origin. The crosses were selfed and 226 $F_2$ plants per population advanced by single-seed descent to the $F_4$ generation. The $F_4$ lines were multiplied by selfing 40 panicles per line, and the resulting $F_5$ seed was bulked. These $F_3$-plant-derived bulks in $F_5$ are called $F_{3:5}$ lines here. Each $F_{3:5}$ line represents the gene content of one $F_3$ plant. One $F_{3:5}$ line in RIP 1 proved to be an off-type and was therefore removed from the data set.

### Marker analyses

Marker analyses were performed with bulked DNA from 20 seedlings per $F_{3:5}$ line. The genomic DNA was extracted following a modified CTAB (cetyl trimethyl ammonium bromide) extraction protocol (Saghai Maroof et al. 1984). RIP 1 was genotyped at 225 marker loci (131 codominantly and 20 dominantly scored AFLPs, 51 SSRs, 17 RFLPs, 6 RAPDs) and RIP 2 at 292 marker loci (122 codominantly and 75 dominantly scored AFLPs, 58 SSRs, 20 RFLPs, 17 RAPDs). The lower number of markers in RIP 1 was due to a lower genetic distance between IS 9830 and E 36-1 as compared to N 13 and E 36-1.

The AFLP markers were scored by Keygene Inc. (The Netherlands), following the procedures described by Vos et al. (1995). The totals of 151 and 197 AFLP markers in RIPs 1 and 2, respectively, were obtained from ten *Eco*RI/*Mse* primer combinations. Seven of the ten primer combinations were common to both RIPs: E11/M60, E12/M47, E13/M61, E14/M48, E14/M50, E14/M60 and E33/M50; three were applied to RIP 1 only: E11/M55, E12/M61 and E13/M59; and three to RIP 2 only: E11/M48, E11/M49 and E14/M61. Percentages of codominantly scored AFLP markers were 87% and 62% in RIPs 1 and 2, respectively. The seven primer combinations common for both RIPs yielded 34 common markers.

RFLP analyses were run by Biogenetic Services (Brookings, S.D., USA) according to standard procedures. The BNL (Burr and Burr 1991), CSU (Gardiner et al. 1993), PHP (Beavis and Grant 1991), UMC (Coe et al. 1990) and ISU (cDNA) maize probes were selected from sorghum genetic maps published by Pereira et al. (1994), Dufour et al. (1997), and Boivin et al. (1999). Using three restriction enzymes (*Eco*RI, *Eco*RV and *Hin*dIII), 201 probes were tested for polymorphism among the parent lines. The RIPs were then genotyped with one to three anchor RFLP markers per linkage group.

SSR analyses were done by Celera AgGen (Davis, Calif., USA) using microsatellites developed by Brown et al. (1996), Taramino et al. (1997), Kong et al. (2000) and Bhattramakki et al. (2000). PCR conditions were standard, following the recommendations of the aforementioned authors. The data was generated on an ABI Prism 377 DNA sequencer (Perkin Elmer Biosystems), and presented as alleles scored as estimated fragment sizes in base pairs compared to size standards or controls. The accuracy of scoring was approximately 0.67 base pairs (G. Hookstra, personal communication), but only markers with at least a two base pair difference between the parent lines were considered polymorphic. The base pair data were converted to letters corresponding to the respective parents, or 'H' when both bands were detected. A total of 241 SSRs was tested for polymorphism among the parent lines. RIPs 1 and 2 were genotyped with 51 and 58 selected SSRs, respectively. Most of these SSRs had been selected from the map of Bhattramakkki et al. (2000), either because of their telomeric position or because of their uniform distribution across the genome.

The RAPD analyses were performed by ICRISAT-India. A total of 221 primers (decamers) were used. Of these, 125 were from Operon technologies, Incorporated, and 96 from Genosys, USA. A total of 40 cycles were used for amplification with the PE 9600 GeneAmp system. PCR conditions applied for the first 39 cycles were; denaturation: 93 °C for 1 min; annealing: 40 °C for 1 min; extension: 72 °C for 2 min. In the final cycle the extension period was 10 min. Useful primers were identified using a three-phase screening. In primary screening (stage I), the three parents were screened with all the primers. In secondary screening (stage II), along with each pair of mapping parents, a subset of progeny ($F_{3:5}$ lines) were screened with only those primers showing polymorphism between parents at stage I. Finally, in the third phase of screening (stage III), primers selected in stage II were used and the whole set of mapping populations was screened along with the parents. Presence or absence of bands was scored for mapping at this stage.

### Statistical analysis

The chi-square test was used to test the markers for a 3:2:3 segregation ratio expected in the $F_3$ with codominant markers, or for a 3:5 segregation ratio expected with dominantly scored markers.

The computer software Joinmap 2.0 (Stam and Van Ooijen 1995) was employed for map construction. Maps were first computed separately for each RIP, then combined. Initially, markers were assigned to linkage groups, based on modified LOD scores of marker pairs. These modified LOD scores are based on a chi-square test for independence of segregation. The test of independence assures that the LOD scores are not affected by a contingent distortion of segregation, i.e., spurious linkages of markers with segregation distortion are avoided (Stam and van Ooijen 1995). The LOD grouping threshold was set to 5.0 in both RIPs. However, in RIP 2, linkage groups B and J stuck together and could only be separated at a LOD grouping threshold of 11.0. In a second step, recombination frequencies were computed for all marker pairs within each linkage group. Third, the maps for each linkage group were constructed using the "JMMAP" module. Data points with a LOD score below 0.1 or a recombination fraction above 0.49 were neglected in the final computations. These non-stringent thresholds were used to retain rather distantly linked markers in the data set. Most linkage groups reacted to the parameter settings ("LOD" and "REC" values) with changes in marker order. More stringent parameter settings resulted in erroneous marker orders as judged from the "list of top-linked markers" in the Joinmap output (see Stam and van Ooijen 1995) and compared to the map of Bhattramakki et al. (2000). Recombination frequencies were converted to centiMorgans (cM) with Haldane's mapping function (Haldane 1919). A "ripple" was performed after three marker additions/insertions. The "jump" and "triplet" thresholds were set to 4 and 9, respectively. The linkage groups were named according to common SSR markers selected from the map of Bhattramakki et al. (2000).

The goodness-of-fit of constructed maps, expressed as the chi-square value (Stam and van Ooijen 1995), was calculated as:

$$\chi^2 = -2.0 \ln \{\text{likelihood}[(\text{r\_joint})]/\text{likelihood}[(\text{r\_direct})]\},$$

where (r_joint) is the set of pairwise recombination rates corresponding to the calculated map, and (r_direct) are the estimates read from the input file. The calculated likelihood covers all pairwise distances in the map for which direct estimates are available.

The two maps had 67 loci in common (30 AFLPs, 33 SSRs and 4 RFLPs). Heterogeneity of recombination frequencies between common marker pairs was tested using the chi-square test in the "JMHET" module. The final combined map was constructed employing the same parameter settings described above.

## Results

### Polymorphism among parent lines

The proportion of polymorphic RFLP probes (polymorphic with at least one of the three restriction enzymes used) was 39.3% for the parent lines of RIP 1 (IS 9830 and E 36-1), and 48.8% for the parent lines of RIP 2 (N 13 and E 36-1). The degree of polymorphism for the 241 tested SSRs was 53.2% and 65.2%, respectively. The average number of polymorphic AFLP markers per primer combination amounted to 15.1 in RIP 1 and 19.7 in RIP 2. Only 2.7% and 7.2% of the tested RAPD markers were finally selected for genotyping the two mapping populations.

### Map of RIP 1 (IS 9830 × E 36-1)

Out of the 225 marker loci used for genotyping RIP 1, 84 (37%) showed significantly ($P \leq 0.01$) distorted segregation. The genetic map of RIP 1 had a total length of 1,264.6 cM, with 187 markers (125 AFLPs, 45 SSRs, 14 RFLPs and 3 RAPDs) distributed over ten linkage groups. Thirty eight marker loci were unlinked or largely disturbed the map construction, thereby decreasing the goodness-of-fit of the map. These markers (mostly RAPDs, dominantly scored AFLPs, or markers with highly distorted segregation) were removed from the data set. The average and maximal distances between two individual markers were 6.8 and 38.8 cM. There was some clustering of (mainly AFLP) markers, i.e., 17 positions in the genome were marked two-fold, and three positions three-fold. Large gaps (>25 cM) occurred in linkage groups B (two gaps), D (one), E (two), F (one) and G (two). A high goodness-of-fit (mean $\chi^2$ value <2.0 in the map construction of all linkage groups) indicated reliability of the linkage map. Loci with distorted segregation were found in all linkage groups, and mostly appeared in clusters. Linkage group D was exceptional in that 14 out of its 16 loci, covering 64.7 cM, showed significantly distorted segregation in favor of parent line IS 9830.

### Map of RIP 2 (N 13 × E 36-1)

Out of the 292 marker loci used for genotyping RIP 2, 110 (38%) showed significantly ($P \leq 0.01$) distorted segregation. The map of RIP 2 spanned 1,409.5 cM and contained 228 markers (158 AFLPs, 54 SSRs and 16 RFLPs) in 12 linkage groups. Anchor markers or AFLP markers common with RIP 1 indicated that linkage groups B and D were still split into two parts. Sixty three markers were unlinked or largely decreased the goodness-of-fit of the map. Markers which severely disturbed the mapping process were mostly RAPDs, dominantly scored AFLPs, or markers with highly distorted segregation. They were removed from the data set. The average and maximal distances between two individual markers were 6.2 and 42.7 cM. Some clustering of AFLP markers was observed, and 16 positions in the genome were marked two-fold, one four-fold, and one six-fold. Large gaps (>25 cM) occurred on linkage groups A (1 gap), C (2), D (1), E (4), G (2) and I (1). In addition, there are two missing links in the linkage groups B and D. As with RIP 1, a high goodness-of-fit (mean $\chi^2$ value <2.0 in the map construction of all linkage groups) indicated reliability of the linkage map for RIP 2. Loci with distorted segregation were found in all linkage groups, and mostly appeared in clusters. Linkage group G harbored the largest cluster of eight markers, spanning 52.4 cM, with significantly distorted segregation in favor of parent line N 13.

### Combined map of RIPs 1 and 2

The 67 common loci of the two individual maps were distributed over the linkage groups as follows: linkage group A: 10; B1: 0; B2: 8; C: 13; D: 4; E: 5; F: 6; G: 4; H: 4; I: 9; J: 4. The two individual maps generally showed conservation of loci order with four exceptions (on linkage groups A, C, E and I) where the position of tightly linked loci was exchanged. Heterogeneity of recombination frequencies was non-significant for the majority of common marker pairs. The numbers of marker pairs with significant ($P \leq 0.01$) heterogeneity of recombination frequencies were distributed as follows over the individual linkage groups: A: 2; B2: 9; C: 2; D: 1; E: 0; F: 2; G: 0; H: 0; I: 0; J: 0. The combined map contained 339 markers (249 AFLPs, 63 SSRs, 24 RFLPs and 3 RAPDs) distributed over 11 linkage groups and spanning 1,423.9 cM (Fig. 1). Linkage group B was still split into two parts. The average and maximal distances between two individual markers were 4.2 and 44.9 cM. Marker clusters contained mainly AFLP loci. Twenty seven positions in the genome were marked two-fold, seven three-fold, two four-fold, and one position five-fold. Large gaps (>25 cM) occurred on linkage groups B (the missing link), C (one gap), E (3) and G (1). A high goodness-of-fit (mean $\chi^2$ value <2.0 in the map construction of all linkage groups) indicated the reliability of the combined map.

## Discussion

### General features of the computed maps

The total lengths of the maps are within the range reported for sorghum in the literature (Table 1). The shorter map for RIP 1 (1,265 cM) compared to RIP 2 (1,410 cM) is probably due to lower genetic polymorphism between IS 9830 and E 36-1 (the parent lines of RIP 1) as compared to N 13 and E 36-1 (the parent lines of RIP 2). In the combined map, the average distance between markers (4.2 cM) is comparatively low. But there are still sev-

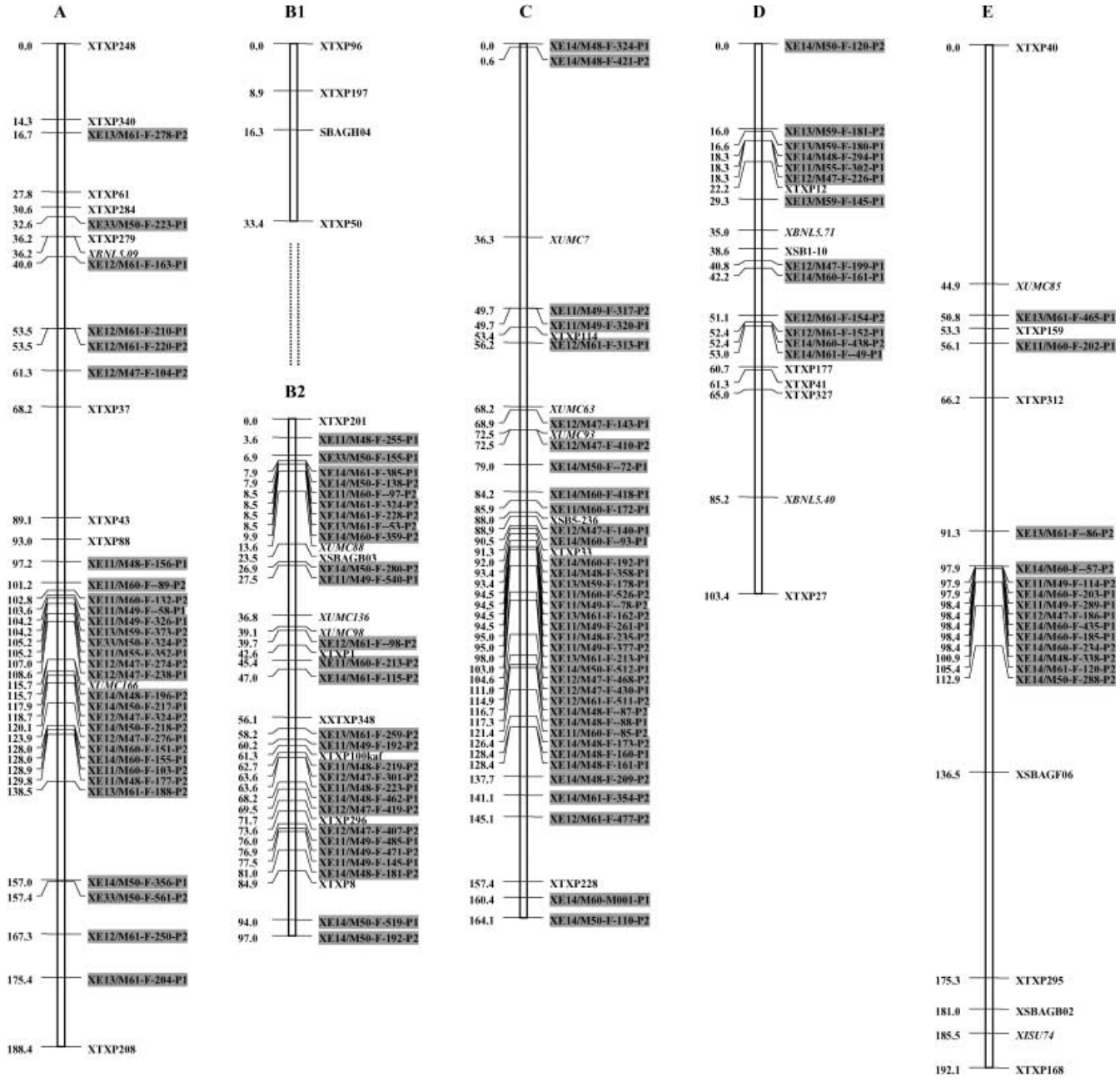


**Fig. 1** Combined map from two recombinant inbred populations of sorghum. *Marker names* are indicated to the right of each linkage group. *Numbers* to the left of the linkage groups indicate cumulative map distances in cM (Haldane). SSR markers are written in *normal font*; AFLP loci are marked with a *grey frame*; RFLP marker names are written in *italics*, and RAPD marker names are *underlined*. Linkage groups are named according to Bhattramakki et al. (2000) via common SSR markers

eral gaps that need to be filled. Besides the missing link in linkage group B, the largest gaps (about 40 cM) appeared on linkage groups C, E and G. On the other hand, there are regions in the combined map with very high marker density. Obviously, the distribution of the AFLP markers is not uniform across the genome. The upper part of linkage groups B and H, and the entire E linkage group are poorly covered with AFLPs. In the remaining linkage groups, the majority of AFLP markers tend to cluster in central regions. However, a few AFLPs are remarkable in that they mapped to unique, sometimes telomeric, positions. In the sorghum "AFLP RIL 379 genetic linkage map" of Boivin et al. (1999), AFLP markers also exhibited clustering and interspersion in the core RFLP map. Clusters of *Eco*RI AFLP markers have also been reported in other crops like maize (*Zea mays* L; Castiglioni et al. 1999; Vuylsteke et al. 1999), ryegrass (*Lolium perenne* L.; Bert et al. 1999), rice (*Oryza sativa* L.; Maheswaran et al. 1997), soybean (*Glycine max* (L.) Merr.; Keim et al. 1997), sugar beet (*Beta vulgaris* L.; Schondelmaier et al. 1996) and barley (*Hordeum vulgare*

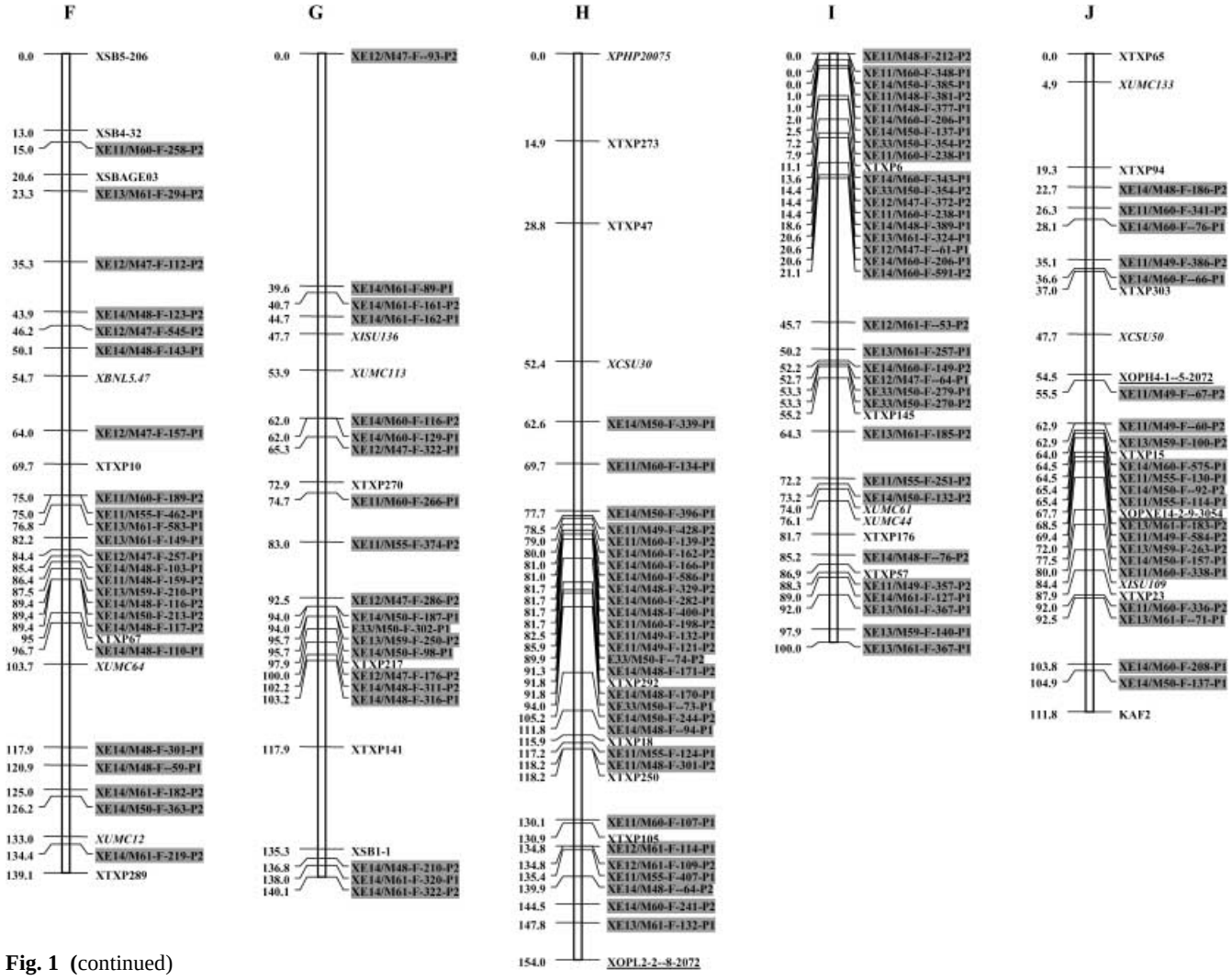


**Fig. 1** (continued)

L.; Becker et al. 1995). Vuylsteke et al. (1999) showed that the *Eco*RI-AFLP clusters colocalized well with the putative centromeric regions of the maize chromosomes. In centromeric regions, crossing-over during meiosis is greatly reduced (Luckaszewski and Curtis 1993), causing the markers to cluster. The high frequency of *Eco*RI-AFLP markers in centromeres may possibly be explained by an AT-richness in the pericentromeric heterochromatin (as shown for *Arabidopsis* by Ross et al. 1996) and the AT-rich target sequences of the restriction enzymes *Eco*RI and *Mse*I (Vuylsteke et al. 1999). According to Boivin et al. (1999), heterochromatic regions around centromeres and at the chromosome tips could also be well-accessible to *Eco*RI-based AFLP markers because these markers are non-sensitive to CG methylation. Since, in our study, SSR and RFLP markers were primarily selected for particular map positions, their distribution in the maps is "artificial" and will not be further discussed. Their linear order was mostly as expected and is described in more detail below.

## Alignment of the composite map with other sorghum genetic linkage maps

Peng et al. (1999) summarized the relationships among the linkage groups in their own and seven other published RFLP linkage maps of sorghum. Our combined map has markers in common with other recently published sorghum genetic linkage maps (Table 2). Our observations confirmed the relationships as summarized by Peng et al. (1999). The linkage group names in the maps of Bennetzen et al. (2001), Bhattramakki et al. (2000) and Kong et al. (2000), as well as those presented in this paper, correspond to those assigned initially by Peng et al. (1999).

Our combined map has 29 markers in common with the framework linkage map of sorghum proposed by Bennetzen et al. (2001). The order of common markers (where there are three or more in a linkage group) is fully consistent between the two maps. The largest difference between marker distances in the two maps appears on linkage group A: the distance between XTXP43 and XTXP 208 is 99.3 cM in our map but only about 55 cM in that of Bennetzen et al.

**Table 1** Overview of published sorghum genetic linkage maps

| Reference | Size and type of mapping pop. | Parent lines | Mapped markers | Linkage groups | Genome length (cM)[a] |
|---|---|---|---|---|---|
| Hulbert et al. 1990 | 55 $F_2$ plants | Shanqui Red (koaliang from China)<br>M91051 (zera zera from East Africa) | 37 RFLP maize probes or cloned genes | 8 | 283 R |
| Binelli et al. 1992 | 149 $F_2$ plants | IS 18729 (caudatum-bicolor from Texas)<br>IS 24756 (durra-caudatum from Nigeria) | 21 RFLP maize probes | 5 | 440 U |
| Whitkus et al. 1992 | 81 $F_2$ plants | IS2482C (*S. bicolor* ssp. *bicolor*)<br>IS18809 (*S. bicolor* ssp. *arundinaceum*) | 85 RFLP maize probes<br>7 Isoenzymes | 13 | 949 H |
| Berhan et al. 1993 | 55 $F_2$ plants | Same parents as Hulbert et al. 1990 | 96 RFLP maize probes or cloned genes | 15 | 709 R |
| Chittenden et al. 1994 | 56 $F_2$ plants | BTx 623 (*S. bicolor*)<br>Unnamed accession of *S. propinquum* | 256 RFLP sorghum probes<br>20 RFLP probes of maize, rice or oat | 10 | 1,445 U |
| Pereira et al. 1994 | 78 $F_2$ plants | CK 60 (*S. bicolor* ssp. *bicolor*)<br>PI229828 (*S. bicolor* ssp. *drummondii*) | 191 RFLP maize probes<br>10 RFLP sorghum probes | 10 | 1,530 U |
| Xu et al. 1994 | 50 $F_2$ plants | BTx 623 (zera zera × kafir)<br>IS 3620C (guinea line) | 179 RFLP sorghum probes<br>11 RFLP maize probes | 14 | 1,789 K |
| Ragab et al. 1994 | 93 $F_{2:3}$ families | BSC 35<br>BTx 631 | 38 RFLP sorghum probes<br>33 RFLP maize probes | 15 | 633 H |
| Lin et al. 1995 | 370 $F_2$ plants | Same parents as Chittenden et al. 1994 | 78 RFLP sorghum probes<br>124 RFLP loci mapped by Chittenden et al. 1994 | 11 | 935 K |
| Tuinstra et al. 1996, 1997 | 98 RIL ($F_{5:7–8}$) | TX 7078<br>B35 (durra sorghum from Ethiopia) | 150 RAPD<br>20 RFLP probes from maize or sorghum | 17 | Ca 1,580 R |
| Dufour et al. 1997 | 110 RIL ($F_5$) | IS 2807 (caudatum from Zimbabwe)<br>379 (guinea from South Africa) | 126 RFLP maize probes<br>19 RFLP sugarcane probes<br>4 cloned genes,<br>2 morphological markers | 13 | 977 H |
| | 91 RIL ($F_5$) | IS 2807 (caudatum from Zimbabwe)<br>249 (guinea from Burkina Faso) | 115 RFLP maize probes<br>8 RFLP sugarcane probes<br>4 cloned genes,<br>1 morphological marker | 12 | 878 H |
| | Composite map of the two populations | | 164 RFLP maize probes<br>19 RFLP sugarcane probes<br>3 cloned genes,<br>2 morphological markers | 13 | 1,095 H |
| Taramino et al. 1997 | 68 $F_2$ plants | Same parents as Pereira et al. 1994 | 7 SSR added to map of Pereira et al. 1994 | 10 | 1,575 U |
| Ming et al. 1998 | 56 $F_2$ plants | Same parents as Chittenden et al. 1994 | 328 RFLP probes of sorghum and other cereals | 10 | 1,750 K |
| Tao et al. 1998 | 120 RIL ($F_5$) | QL39<br>QL41 (both elite lines from Australia) | 68 RFLP sorghum probes<br>87 RFLP of maize, sugarcane, rice, oat, barley<br>8 SSR, 3 morphological traits | 21 | 1,400 U |
| Boivin et al. 1999 | Composite map with populations as Dufour et al. 1997 | | 343 RFLP and morphological markers | 11 | 1,352 H |
| | 110 RIL ($F_5$) | First population of Dufour et al. 1997 | 128 RFLP sorghum, rice, oat or barley probes<br>176 AFLP | 11 | 1,899 H |

**Table 1** (continued)

| Reference | Size and type of mapping pop. | Parent lines | Mapped markers | Linkage groups | Genome length (cM)[a] |
|---|---|---|---|---|---|
| Boivin et al. 1999 | | | 151 RFLP loci mapped by Dufour et al. 1997 | | |
| Crasta et al. 1999 | 96 RIL ($F_{6:7}$) | B35 (durra from Ethiopia) | 142 RFLP sorghum, maize, rice or wheat clones | 14 | 1,602 K |
| | | Tx 430 | | | |
| Peng et al. 1999 | 137 RIL ($F_{6-8}$) | Same parents as Xu et al. 1994 | 323 RFLP from sorghum, maize, rice, oat, barley | 10 | 1,347 K |
| Bowers et al. 2000 | 65 $F_2$ plants | Same parents as Chittenden et al. 1994 | 2,399 Loci based on 1925 RFLP probes | 10 | 1,200 U |
| Kong et al. 2000 | 137 RIL ($F_{6-8}$) | Same parents as Xu et al. 1994 | 33 SSR | 10 | 1,287 K |
| | | | 111 RFLP probes from Peng et al. 1999 | | |
| Tao et al. 2000 | 152 RIL ($F_5$) | Same parents as Tao et al. 1998 | 101 RFLP | 14 | 1,871 U |
| | | | 17 SSR<br>166 markers mapped by Tao et al. 1998 | | |
| Xu et al. 2000 | 98 RIL ($F_7$) | B 35 (durra from Ethiopia) | 162 RFLP sorghum and maize probes, cloned genes or sequenced DNA probes | 10 | 837 H |
| | | Tx 7000 (elite public line used in USA) | | | |
| Bhattramakki et al. 2000 | 137 RIL ($F_{6-8}$) | Same parents as Xu et al. 1994 | 116 SSR | 10 | 1,406 K |
| | | | 354 RFLP or SSR markers mapped by Peng et al. 1999 or Kong et al. 2000 | | |
| Bennetzen et al. 2001 | Framework map derived from comparison of the maps of Kong et al. 2000, Peng et al. 1999, Pereira et al. 1994, and Berhan et al. 1993. | | 154 RFLP | 10 | 1,450 U |
| | | | 34 SSR, 10 morphological markers | | |
| Klein et al. 2001 | 125 RIL ($F_5$) | RTx 430 (elite line from USA) Sureño | 44 SSR<br>85 AFLP, 1 morphological trait | 10 | 970 K |
| Haussmann et al., present publication | 225 RIL ($F_{3:5}$) | IS 9830 (caudatum line from Sudan) | 125 AFLP | 10 | 1,265 H |
| | | E 36-1 (Ethiopian guinea-caudatum) | 45 SSR, 14 RFLP, 3 RAPD | | |
| | 226 RIL ($F_{3:5}$) | N 13 (Indian durra) | 158 AFLP | 12 | 1,410 H |
| | | E 36-1 (Ethiopian guinea-caudatum) | 54 SSR, 16 RFLP | | |
| | Composite map of the two populations | | 339 AFLP, SSR, RFLP and RAPD markers | 11 | 1,424 H |

[a] H, K = Map distances estimated using the mapping functions of Haldane (1919) and Kosambi (1944), respectively; U = mapping function not specified; R = Recombination frequency (%)

Sixty six markers are shared by our combined map and the map of Bhattramakki et al. (2000). The order of common markers is generally conserved, except for three marker pairs (XTXP97 and XTXP96 on linkage group B, XTXP289 and UMC12 on F, and XTXP217 and XTXP270 on G). These markers are closely linked in both maps but their order is reversed in our map. On our linkage group G, there is an additional reversal in the order of the markers XTXP141 and SB1-1 which are 17.4 distant in our map, and 29.3 cM apart in the map of Bhattramakki et al. (2000). The largest missing regions in our combined map relative to the map of Bhattramakki et al. are: approximately 27 cM on linkage group A at the XTXP208 end, about 35 cM on linkage group B between XTXP50 and XTXP201, and about 46 cM on linkage group D at the XTXP12 end. Additional regions in our map (compared to the same map) were detected in linkage groups H (15 cM at the XTXP273 end) and I (11 cM at the XTXP6 end).

The number of common markers between the map of Klein et al. (2000) and our combined map is 20. In linkage group A, the order of the markers XTXP43 and

**Table 2** Linkage groups in our combined map *versus* corresponding linkage groups in selected, recently published sorghum maps. Numbers of markers shared with our map given in parentheses.

| Reference | Linkage group in our combined map | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B1+2 | C | D | E | F | G | H | I | J |
| Bennetzen et al. 2001 | A (4) | B (3) | C (3) | D (2) | E (2) | F (4) | G (2) | H (3) | I (4) | J (2) |
| Bhattramakki et al. 2000 | A (9) | B(11) | C (7) | D (5) | E (6) | F (5) | G (6) | H (6) | I (5) | J (6) |
| Klein et al. 2000 | A (3) | B (3) | C (1) | D (2) | E (2) | F (1) | G (0) | H (2) | I (3) | J (3) |
| Kong et al. 2000 | A (2) | B (2) | C (1) | D (1) | E (1) | F (2) | G (1) | H (2) | I (1) | J (3) |
| Tao et al. 2000 | C (1) | B (1) | – | F (3) | – | G (2) | I (2) | – | – | H (1) |
| Boivin et al. 1999 | C (2) | F (3) | G (3) | D (2) | A (1) | E (3) | H (1) | I (2) | B (2) | J (2) |
| Peng et al. 1999 | A (2) | B (2) | C (3) | D (0) | E (1) | F (2) | G (2) | H (0) | I (1) | J (0) |

XTXP88, which are closely linked in both maps, is reversed in our map. In the other linkage groups with three common markers (B, I and J), marker order is completely conserved between the two maps. Distances between common markers are similar, except for linkage group J where our estimated distance between XTXP23 and XTXP303 is about 20-cM shorter.

Sixteen markers are common between our combined map and that of Kong et al. (2000). The distance between the common marker pair XTXP 37 and XTXP 43 on linkage group A is 12-cM shorter in our combined map than in that of Kong and his colleagues. On the other hand, the distances between common marker pairs on the linkage groups F (XTXP 10, UMC 12), H (XTXP 47, XTXP 18) and I (XTXP 65 and XTXP 15) are, respectively, 11-, 33-, and 26-cM longer in our map.

The map of Tao et al. (2000) shares ten markers with our combined map. A major difference is the order of common markers on our linkage group D (XTXP12–SB1-10–XTXP41) versus (SB1-10–XTXP12–XTXP41) in their linkage group F.

Twenty one markers are common with the composite map of Boivin et al. (1999). Distances between common marker pairs are in good agreement, with two exceptions. The distance between UMC 64 and BNL 5.47 is 49.0 cM in our map (linkage group F) but 89.6 cM in the linkage group E of Boivin et al.; and the distance between CSU 30 and PHP 20075 on linkage group J (in both maps) is 52.4 cM in our map but only 28.8 cM in the map of Boivin et al. (1999).

The map of Peng et al. (1999) contains 13 markers common to our combined map. The only major difference in distance between common markers occurred in linkage group A, between the markers BML 5.09 and UMC 166 (distance of 79.4 cM in our map versus about 100 cM in the map of Peng et al.).

In conclusion, the agreement of our combined map with other recently published sorghum maps is generally good, with a few inversions, deletions, and additional regions that we detected.

## Outlook

Efforts are needed to enhance the number of common markers in different sorghum maps, to allow for a more comprehensive comparison and integration. The search should continue for new, highly polymorphic SSRs that would map to regions in the sorghum genome which are currently poorly covered with SSRs. There is also an urgent need to relate the available genetic maps to a physical map. This would facilitate the map-based cloning of genes, comparative genome analysis, and provide sequence-ready clones for genome sequencing projects (Klein et al. 2000). Physical maps of sorghum are currently under construction at Texas A&M University in the group of P.E. Klein, R.R. Klein and J.E. Mullet (Klein et al. 2000), and at the University of Georgia/Texas A&M University in the group of A.H. Paterson (Bowers et al. 2000).

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