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K. Boivin · M. Deu · J-F. Rami
G. Trouche · P. Hamon

Towards a saturated sorghum map using RFLP and AFLP markers

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Abstract A near-saturated sorghum genetic linkage map was produced using RFLP, AFLP and morphological markers. First a composite, essentially RFLP-based genetic linkage map was obtained from analyses of two recombinant inbred populations. This map includes 343 loci for 11 linkage groups spanning 1352 cM. Since this map was constructed with many previously mapped heterologous probes, it offers a good basis for synteny studies. Separately, an AFLP map was obtained from the analysis of 168 bands revealed from 12 primer pair combinations. It includes 137 loci for 11 linkage groups spanning 849 cM. Taking into account the different data sets, we constructed a combined genetic linkage map including 443 loci spanning 1899 cM. Two main features are to be noted: (1) the distribution of AFLPs along the genome is not uniform; (2) an important stretching of the former core map is induced after adding the AFLPs.

Key words Sorghum · RFLP · AFLP · Genetic linkage map

Introduction

Since the appearance of first works on maize and tomato (Helentjaris et al. 1986), restriction fragment length polymorphism (RFLP) markers have proved to be valuable in establishing linkage maps of many crop species. More recently, the amplified fragment length polymorphism (AFLP) technique developed by Zabeau and Vos (1993) has been used as an alternative to RFLPs. This newer technique, based on the selective amplification of restricted fragments, provides dominant markers in most cases. It enables the estimation of genetic distances among inbred lines (Smith et al. 1993, 1994), DNA fingerprinting (Vos et al. 1995) and mapping (Becker et al. 1995; Meksem et al. 1995; Thomas et al. 1995; Schondelmaier et al. 1996; Maheswaran et al. 1997). In this last case, the authors drew attention to the localisation of AFLP markers within the genome. Their results showed that AFLPs can integrate a core RFLP map in two ways : clustered or interspersed. Furthermore, in some cases, they can induce a stretching of the former RFLP map. Recently, Rouppe van der Voort et al. (1997) applied AFLP technology to comparative mapping by using the allele specificity of comigrating AFLPs.

During the last few years, several good or high-density sorghum RFLP maps have been produced from analyses of inter-specific crosses (Chittenden et al. 1994; Lin et al. 1995), an inter-subspecific cross (Pereira et al. 1994) and intra-specific crosses (Xu et al. 1994; Tao et al. 1996; Dufour et al. 1996, 1997). In some cases, they permit quantitative trait loci (QTLs) identification (Lin et al. 1995; Dufour 1996; Rami et al. 1998) and in other cases comparative genome mapping beyond poaceae (Pereira et al. 1994; Lin et al. 1995; Dufour et al. 1996, 1997). However, the critical point remains that the RFLP technique needs large amounts of DNA and that the realisation of higher density maps is a long, time-consuming process.

In the investigation presented in this paper, we used Dufour's maps (Dufour 1996; Dufour et al. 1996, 1997) as a starting point to obtain a more saturated composite genetic linkage map which further could permit us to extend comparative genome mapping with rice.

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K. Boivin · M. Deu
CIRAD-CA, BP 5035, F-34032 Montpellier cedex, France

J.-F. Rami
RUSTICA PROGRAIN GENETIQUE, 7 rue hermès,
Parc technologique du canal, F-31520 Ramonville St-Agne, France

G. Trouche
CIRAD/INERA, 01 BP596, Ougadougou, Burkina Faso

P. Hamon (✉)
Université P Valery, route de Mende, F-34199 Montpellier cedex 5,
France/CIRAD, BP 5035, Montpellier cedex, France

Then, we studied the distribution of AFLP markers within the sorghum genome and their possible use in sorghum breeding.

Materials and methods

Plant material

Two populations of 110 and 91 recombinant inbred lines (RILs) were developed from two intra-specific crosses within *S. bicolor ssp bicolor* (IS2807 × 379 and IS2807 × 249, respectively) at the Saria experimental station, Burkina Faso. IS2807 from ICRISAT collection, used as the female parent, belongs to the race caudatum; the male parents 379 and 249 (CIRAD collection) belong to the race guinea. The two populations are named RIL379 and RIL249.

Probes

One hundred and forty probes (SbRPG prefix) were obtained from a sorghum cDNA library produced in collaboration with RUSTICA PROGRAIN GENETIQUE and CIRAD. Sixty-four rice probes (RZ prefix), 59 oat probes (CDO prefix) and 27 barley probes (BCD prefix) were provided by Cornell University (Causse et al. 1994; Heun et al. 1991). Twenty-six barley probes (cMWG and MWG prefix) were provided by Graner et al. (1994). Forty-two pearl millet probes (Xpsm prefix) were provided by Dr. M.D. Gale, John Innes Centre, Norwich and 12 maize probes (CSU prefix) by Gardiner et al. (1993). Three wheat cloned genes (*psR 170*, *fba 137* and *KsuD30*) were also mapped.

Morphological markers

Three simple morphological traits were mapped. The awnless trait (a*A* gene; Sieglinger et al. 1934) was recorded on both populations, the IS2807 genotype was *AA* while both the 379 and 249 genotypes were *aa*. Coleoptile colour (*rs2Rs2* gene, Woodworth 1936) was noted on the RIL249 population. The IS2807 genotype was *Rs2Rs2*, while the 249 genotype was *rs2rs2*. Finally, the pearly trait (*zZ* gene; Ayyangar et al. 1934) was evaluated on the population RIL379. The IS2807genotype was *zz* while the 379genotype was *ZZ*.

RFLP protocol

RFLP protocol was as described in Dufour et al. (1996). Seven restriction enzymes (*Eco*RI, *Eco*RV, *Hin*dIII, *Dra*I, *Sst*I, *Bam*HI and *Bgl*II) were screened for polymorphism. Analyses were performed only on the RIL379 population.

AFLP protocol

The AFLP protocol is as described by Zabeau and Vos (1993) and Vos et al. (1995) and following Gibco BRL instructions. These analyses were also only performed only on the RIL379 population.

Data analyses

The new RFLP and morphological data set was included into that obtained by Dufour et al. (1997) for generating a more saturated RIL379 linkage map and then a composite map was constructed by combining the RIL379 and RIL249 data sets.

AFLP marker nomenclature includes the letter E for the *Eco*RI primer and the letter M for the *Mse*I primer, each followed by a number representing the three selective nucleotides and then a number corresponding to the polymorphic band analysed. The polymorphic bands were numbered serially in decreasing order of molecular weight. For AFLP analysis, cosegregating bands were considered as codominant marker pairs and thus were noted as alleles.

All markers were tested for the expected 1 : 1 segregation by the chi-square test at the $P = 5\%$ level.

The AFLP marker data set was added to the RFLP and morphological data set to produce the combined map. Linkage analyses were performed using MAPMAKER version 2.0 software (Lander et al. 1987). The multipoint analyses were carried out using a minimum LOD score of 4 with a maximum recombination value of 0.30; three-point and n-point analyses were performed to determine the most likely order of the markers. The "Try" command was used to determine the most likely marker's position on a linkage group and then the "Ripple" command was used to verify the order of the markers on each linkage group. Genetic distances were estimated with the Haldane mapping function.

Results

RIL379 genetic linkage map

Among the 373 newly tested probes for polymorphism between the two parents, 187 were polymorphic and 128 of these were subsequently used for mapping. Among them, 72 were unique copies, 40 were low-copy probes (2 or 3 bands revealed for all restriction enzymes tested) and 16 were multi-copy probes. In total, 142 segregating loci were added to the 156 previously studied by Dufour et al. (1997). Using the complete RFLP and morphological data set, we constructed a new map. It included 298 loci spanning a genetic distance of 1370 cM distributed into 12 linkage groups (2 clusters of 2 and 3 markers remained independent).

Linkage groups D and L in Dufour's map were merged but L.G. I split into L.Gs.I1 and I2. Linkage groups C and K, two parts of the same linkage group (Dufour 1996), noted here C2 and C1, respectively, remained unlinked.

In total, 40 significant deviations from the expected 1 : 1 Mendelian segregation ratio were observed. The corresponding loci were essentially distributed into L.G. F (20 loci). The others were placed on L.Gs. G (7 loci), D (5 loci), B, H, J (2 loci), C and E (1 locus). In the main cases (31 out of 40 loci), the distortion was in favour of the male allele.

RIL249 genetic linkage map

The data were those obtained by Dufour et al. (1997), completed by three morphological traits. The map includes 131 loci distributed on 12 linkage groups (plus 2 small clusters).

Composite genetic linkage map

The two maps have 88 loci in common. The composite map (Fig. 1) includes 343 loci distributed on 11 linkage groups (plus 2 small clusters) for a total genetic distance of 1352 cM. Despite the mapping of 26 new loci on L.Gs. C1 and C2, their merging was not obtained as well as the 2 small clusters remained independent.

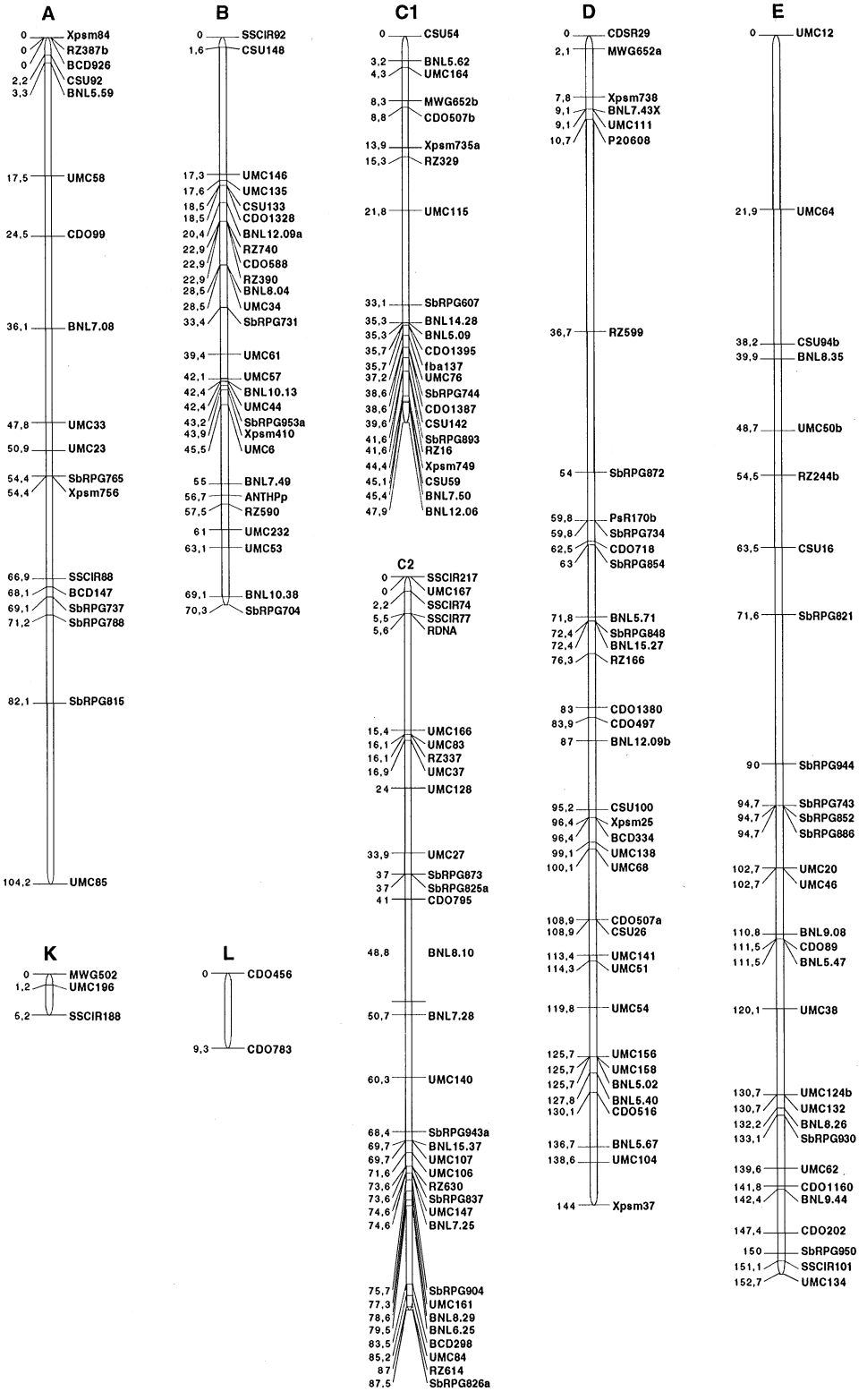

Fig. 1 Composite linkage map of the sorghum genome obtained from analyses of two RIL populations derived from IS2807 × 249 and IS2807 × 379 crosses

Fig. 1 Continued

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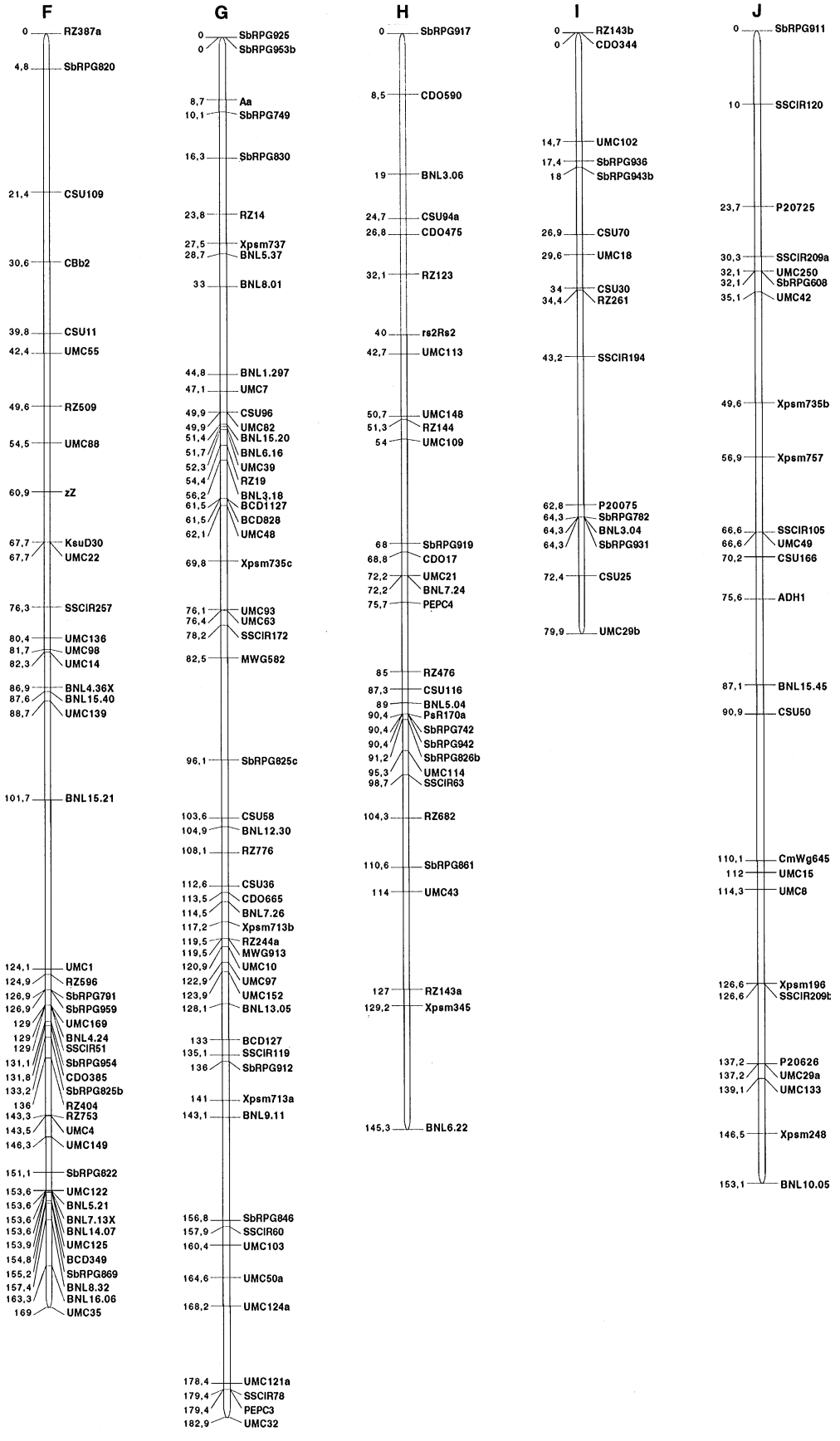


When these clusters are excluded, the average distance between markers is 4 cM. Two linkage groups (L.Gs. F and G) were mainly implied in Mendelian segregation distortions and these were in favour of the male allele.

AFLP RIL379 genetic linkage map

The 64 available primer pairs combinations were tested for the polymorphism revealed between the two parents. Only 1 (E-ACA × M-CTC) was monomorphic, while 7 revealed a low polymorphism. Among the remaining 56 primer pairs, 12 showed a high level of polymorphism and were used in this AFLP mapping (Table 1).

For each primer pair combination, the number of visible bands ranged from 79 to 148, while the number of polymorphic bands ranged from 8 to 26. When we took into account the 12 couples of primers, 197 out of 1328 bands (14.8%) were polymorphic, and these were used for mapping. Out of the 197 polymorphic bands, 16 were visually assigned to codominant marker pairs since cosegregant bands were close in molecular weight. A first run analysis on the remaining 181 bands was performed, and an additional 13 bands were identified as codominant markers. In total, 168 bands were recorded, of which 29 were coded as codominant marker pairs.

The AFLP map ultimately included 137 loci (31 remained independent) for 11 linkage groups spanning a genetic distance of 849 cM. Eight linkage groups consist of 11–20 loci spanning 55.3 to 148.8 cM. The average distance between markers is 6.2 cM. In total, 16 loci showed Mendelian segregation distortion. For 7 of these the distortion was in favour of the female allele; 5 were mapped to the linkage group homologous to L.G. J.

Combined RFLP-AFLP RIL379 genetic linkage map

Using the complete RIL379 data set (RFLP, morphological and AFLP markers), we constructed a combined map (Fig. 2). It includes 443 loci for 1899 cM distributed into 11 linkage groups (plus 1 cluster and 21 independent AFLP markers). The coverage of various linkage groups with AFLP markers is not uniform. For example, linkage groups B, J and G are well-covered, but AFLP markers are unequally distributed along L.Gs. D, F, H and I. Linkage group E is very poorly marked. AFLP markers are incorporated into the core map in two ways: interspersed between RFLP markers (for example on L.G. G) or mapped in clusters, as is observed for L.G. I or L.G. H. Ten independent AFLP markers were mapped and distributed on 7 linkage groups. No great disturbance was observed with respect to the order of the RFLP markers, but when one was noted, it involved markers very close to each other.

Table 1 Extract of the 8 × 8 available primer pair combinations indicating the 12 polymorphic combinations used for AFLP mapping

*Eco*RI *Mse*I	E-AAG	E-ACC	E-ACG	E-AGC	E-AGG
M-CAA		E5/M1	E6/M1	E7/M1	
M-CAC			E6/M2		
M-CAG	E2/M3		E6/M3		
M-CAT			E6/M4	E7/M4	
M-CTA			E6/M5	E7/M5	E8/M5
M-CTC			E6/M6		

Discussion

The composite sorghum mainly RFLP-based linkage map presented here covers 1352 cM for 343 loci distributed over 11 linkage groups plus 2 clusters of 2 and 3 independent loci. The average distance between markers (4 cM) is lower than those previously obtained by Chittenden et al. (1994, 5.2 cM), Xu et al. (1994, 9.4 cM), Pereira et al. (1995, 8 cM) and Dufour et al. (1997, 5.5 cM).

Most of the probes used in this study are heterologous, largely distributed upon request and mapped on several genomes. Despite the absence of markers between L.Gs. C1 and C2 and the impossibility of assigning 1 of the clusters to a linkage group, this map would be very useful in an extensive comparative mapping programme between sorghum, sugarcane, maize and rice genomes.

As was previously noted by Becker et al. (1995) on barley, the polymorphism rate of AFLPs is generally not too high (11.3% for barley versus 14.8% for sorghum), but the number of polymorphic bands generated per gel is sufficient to provide a good efficiency in AFLP sorghum mapping within a short time.

Although AFLP distribution on the genome is almost random their distribution along most linkage groups is not even. AFLP clusters have also been reported on barley (Becker et al. 1995), sugar beet (Schondelmaier et al. 1996), soybean (Keim et al. 1997) and rice (Maheswaran et al. 1997). However, non-uniform distribution also can be noted with RFLP markers. For Tanksley et al. (1992), the recombination rate is not uniform along the genome; cold regions are represented by clusters. Given such distributions, one question arises: do RFLP and AFLP clusters mark the same or different chromosomal regions? In this study, we show that in the mixed map, RFLP and AFLP clusters do not intermingle. In the same manner, Becker et al. (1995) and Schondelmaier et al. (1996) reported that AFLP clusters tend to flank RFLP

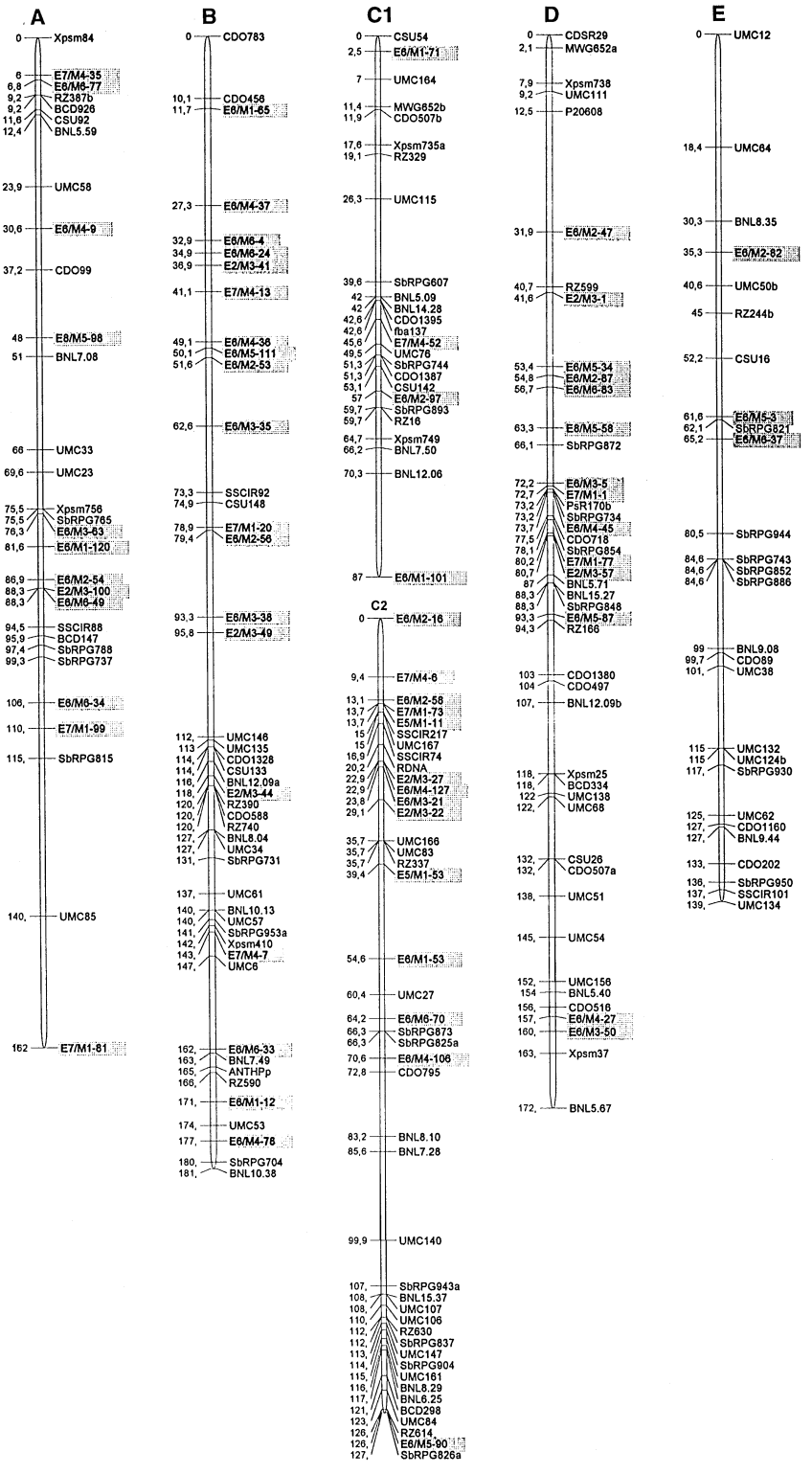
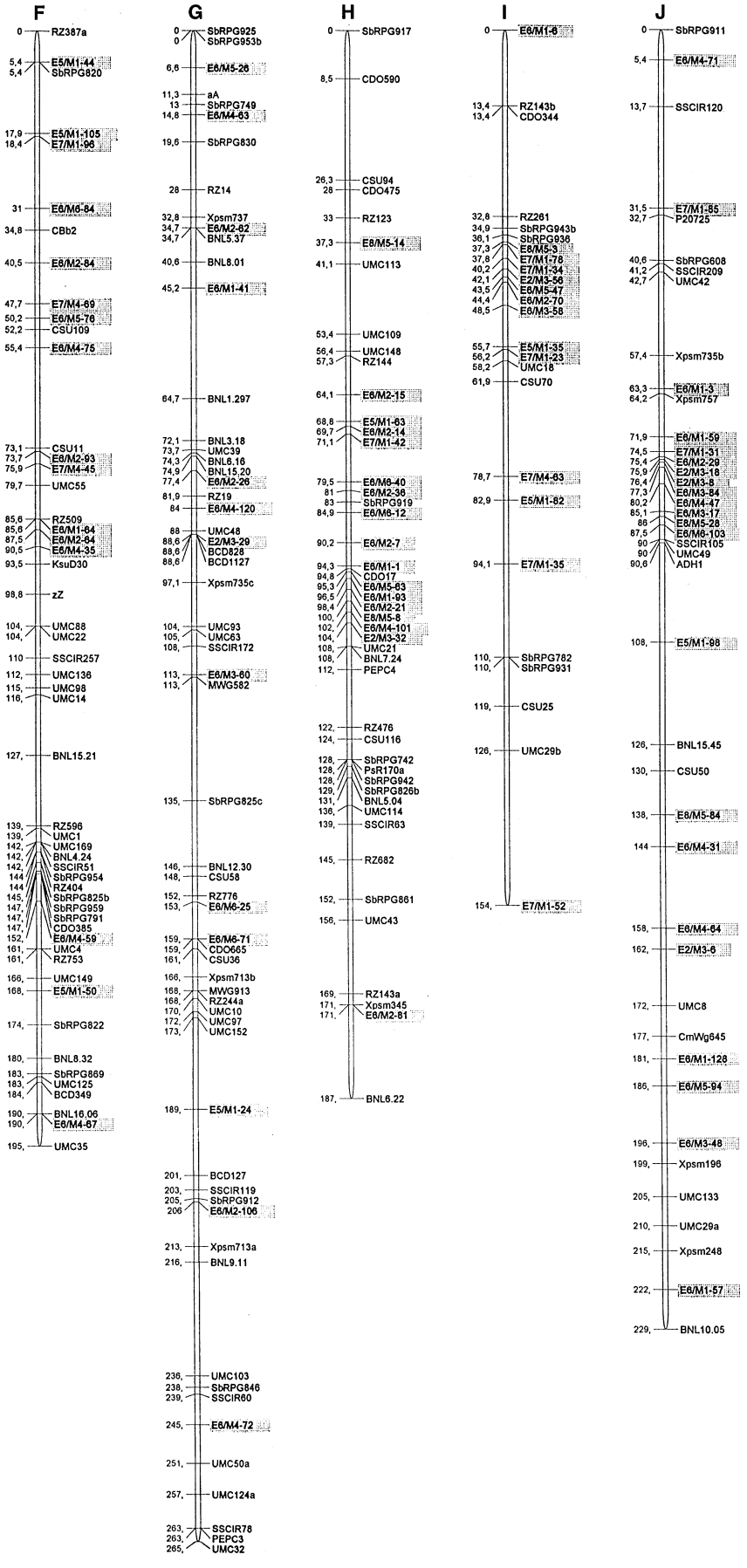


Fig. 2 Distribution of AFLP markers on the sorghum genetic linkage map. AFLP markers are denoted in *grey boxes*

Fig. 2 Continued on p 326

clusters. Using RAPDs, Giese et al. (1994) also observed that although these loci are distributed throughout the barley genome, they tend to cluster when mixed with RFLP markers. This observation could have some basis in the specificity of each type of marker. In plant genomes, it is generally admitted that hypo-methylated sequences are associated with actively expressed genes, while hyper-methylated sequences show a reduction in gene expression. As RFLP markers are mainly generated from cDNA or *Pst*I (sensitive to CG methylation)

Fig. 2 Continued



genomic probes, a good accessibility to expressed regions is expected. In this study, the AFLP markers were generated using a methylation restriction enzyme (*Eco*RI) non sensitive to CG. Thus, it is to be expected that some particular regions, such as heterochromatin, regions around centromeres and chromosome tips, would be accessible to *Eco*RI-based AFLP markers. In such regions, crossing-over during meiosis is greatly reduced (Luckaszewski and Curtis 1993) and markers tend to cluster. As well, more analogy is expected between the distribution of *Pst*I-based AFLPs and RFLPs than between *Pst*I- and EcoRI-based AFLPs. The difference in distribution of AFLPs according to the six-base-cutting restriction enzyme used has been documented in soybean (Young et al. 1998). These authors compared the distribution of *Eco*RI- and *Pst*I-based AFLP markers on the genome. Approximately, one-quarter of the *Eco*RI-based AFLPs mapped to clusters, while the *Pst*I-based AFLPs were underrepresented in the clusters. However, our results are not in concordance with those of van Eck et al. (1995). These authors reported a similar distribution of *Eco*RI/*Mse*I AFLPs and RFLPs on the potato genome. This discrepancy could be associated with different genomic structures, i.e. the importance of repeated sequences in the genome and their distribution.

Another main feature observed in some mixed maps is the stretching of the map. On the basis of the few studies reported to date, this stretching seems to be independent of the restriction enzyme used and the distribution of AFLPs along the genome. For example, despite a near-random distribution of AFLPs, an increase in the length of the barley map (1096 to 1873 cM) was reported by Becker et al. (1995). In the same manner, Maheswaran et al. (1997) using *Pst*I-based AFLPs observed an important stretching of the rice map (1811 to 3058 cM). On the contrary, although the AFLP clusters were present, no stretching of the sugar beet map was obtained by Schondelmaier et al. (1996).

In our study, a stretching of 547 cM was observed. Of this, 63.2 cM corresponds to the filling of one of the predictable gaps of the map, i.e. the merging L.G. B and a cluster of 2 markers, CDO 783 and CDO 456. AFLPs also permit the stretching (plus 94.4 cM) on one end of several linkage groups : L.G. A (plus 21.1 cM), L.G. C1 (plus 16.7 cM), L.G. C2 (plus 15 cM), L.G. I (plus 41.6 cM). According to Pereira et al. (1994), Lin et al. (1995) and Paterson et al. (1995), L.Gs. C1 and C2 have to be linked between BNL12.06 and SSCIR217. However, although the stretching of 16.7 and 15 cM at these two ends, no merging is obtained. Two hypotheses could be formulated: (1) unfortunately, interesting probes have not yet been tested and thus the gap remains; (2) this gap is constitutive in the two mapping populations and reliable to the genomic structure of the three genitors used. To test the first hypothesis, we can find appropriate probes in our sorghum library quickly only by chance or after testing a great number of probes. But, using synteny, we can increase this chance if probes are available. In particular, this region could be homoeologous to rice chromosome 10, and enough markers of this chromosome have not yet been tested and mapped on our mapping populations.

Finally, given the time required to generate AFLP markers, the ones described here show great potential for accelerating the search for QTLs (in adequate populations) and could have good applications in marker-assisted selection using advanced back-cross schemes (Tanksley and Nelson 1996). However, studies are required to improve the efficiency of different primer pair combinations in order to determine a minimal set which would permit a good coverage of the genome. Furthermore, it would be interesting to test *Pst*I instead of *Eco*RI and then, depending on the objective (help to saturate a core RFLP map or a particular region, generate a new map for QTL analyses, …), to choose the better process by which to generate AFLPs.

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