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Similarity of maize and sorghum genomes as revealed by maize RFLP probes**

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Summary. Densely saturated genetic maps of neutral genetic markers are a prerequisite either for plant breeding programs to improve quantitative traits in crops or for evolutionary studies. cDNA and genomic clones from maize were utilized to initiate the construction of a RFLP linkage map in *Sorghum bicolor*. To this purpose, an F_2 population was produced from starting parental lines IS 18729 (USA) and IS 24756 (Nigeria) that were differentiated with regard to many morphological and agronomical traits. A total of 159 maize clones were hybridized to the genomic DNA of the two parents in order to detect polymorphism: 154 probes hybridized to sorghum and 58 out of these were polymorphic. In almost all of the cases hybridization patterns were similar between maize and sorghum. The analysis of the segregation of 35 polymorphic clones in an F_2 population of 149 individuals yielded five linkage groups. The three principal ones recall regions of maize chromosomes 1, 3 and 5: in general, colinearity was maintained. A possible inversion, involving a long region of maize chromosome 3, was detected. Simulations were also performed to empirically obtain a value for the lowest number of individuals of the F_2 population needed to obtain the same linkage data.

Key words: Sorghum – Restriction Fragment Length Polymorphism – Genetic maps – Genomic structure – Maize

Introduction

Molecular genetic markers which describe differences at the level of the DNA sequence provide the investigator with powerful tools for the analysis of the inheritance of quantitative traits (Beckmann and Soller 1988; Paterson et al. 1988, 1991; Ottaviano et al. 1991) and evolutionary phenomena (Debener et al. 1990; Miller and Tanksley 1990), and for varietal and species identification (Soller and Beckmann 1983).

In plant breeding programs, molecular markers such as RFLPs and their recently developed offspring, the RAPDs, (Welsh and McClelland 1990; Williams et al. 1990; Martin et al. 1991) can satisfy the need for a kind of marker that describes the genotype of the individual directly and is not affected by environmental bias. The possibility exists of improving quantitative traits in crops by applying a selection based on molecular markers together with classical selection methods. Recently, Paterson et al. (1991) have mapped QTL for characters of agronomical interest in tomato at a resolution as high as 3 cM.

As to the evolutionary aspect, RFLPs and RAPDs could be useful in resolving the ambiguities and uncertainties of plant taxonomy: almost all of our molecular data (evolutionary trees) on plants derive from studies on plastidic genomes (Wolfe et al. 1989; Soreng et al. 1990) or very conserved genes such as *cytc* (Syvanen et al. 1989). The analysis of nuclear sequences other than genes for rRNA (Zurawski and Clegg 1987) and RuBisCo (Pichersky et al. 1986) could give higher accuracy to phylogenetic trees of plants.

However, the true basis for the development of effective strategies in each of these areas is the availability of a "classical" linkage map. Combined genetic maps based on conventional and molecular markers have reached a high degree of saturation in many crop plants, especially maize (Helentjaris et al. 1986; Burr et al. 1988), tomato (Bernatzky and Tanksley 1986), potato (Gebhardt et al. 1989) and rice (McCouch et al. 1988). *Sorghum bicolor*

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belongs to the same Andropogonaeae tribe as *Zea mays*, but lies far behind in terms of classical genetics, cytogenetics, mutant availability and accuracy of genetic maps. The agronomical importance of sorghum in developing countries of tropical climates is no less than that of maize in technologically advanced nations: breeding programs serving to improve resistance to biotic stress or to ameliorate hybrid production are therefore greatly needed.

First attempts at producing linkage maps in sorghum have already been made: Hulbert et al. (1990) used maize RFLP probes to analyze the cosegregation patterns in a F_2 population of 55 individuals and subsequently described eight linkage groups for 37 markers; these linkage groups were fairly colinear with corresponding regions of the maize genome.

In this paper we wish to contribute to the development of a linkage map in sorghum by the use of a partially different set of maize clones tested on a large F_2 population of 149 individuals. The relationship between maize and sorghum and some evolutionary aspects are discussed.

Materials and methods

Plant material

Sorghum lines IS 18729, a caudatum bicolor from Texas, and IS 24756, a durra-caudatum Nigeria landrace, from the ICRISAT (International Crop Research Institute for Semi-Arid Tropics) collection of inbred lines were crossed to produce the F_1 and F_2 populations.

Vegetative tissue from young leaves or tillers was collected from each of 149 individuals of the F_2 population, frozen in liquid nitrogen and stored at $-80\,^{\circ}C$ until used. Etiolated seedlings of the two sorghum parental lines, of the corresponding F_1 and of the maize inbred line Mo17 were also collected and stored.

DNA extraction, digestion and Southern analysis

DNA was isolated from each of the 149 plants of the F_2 and from seedlings of the two parental lines, the F_1s and the maize inbred line Mo17. One gram of frozen tissue was employed for each DNA extraction, which was carried out according to a modification of Dellaporta's procedure (1985).

For enzymatic digestion, restriction endonucleases *Pst*I, *Hin*dIII, *Eco*RI and *Bam*HI were purchased from New England Biolabs and Promega and used under the conditions specified by the suppliers in about ten fold excess. Genomic DNA (about 5–6 µg per lane) was digested and run overnight on a 0.6% agarose gel. The DNA was transferred onto nylon membranes (Hybond N^+, Amersham) using the alkali technique and the vacuum blotting procedure in a Vacublot (Pharmacia) chamber according to the manufacturer's instructions.

Filters were hybridized at 42 °C for 36 h in 0.6–0.7 ml/cm^2 of Klessig's solution (Klessig and Berry 1983) with formamide, washed at 50 °C at a final stringency of 0.5 × SSC, 0.1% SDS and exposed for autoradiography (Kodak X-omat films).

Both genomic and cDNA maize fragments were used as probes for RFLP analysis. The clones were obtained from D. Grant (Pioneer Hi-Bred Int), B. Burr (Brookhaven National Laboratory) and D. Hoisington (University of Missouri, Columbia). All DNA fragments, cloned in pUC19, were recovered after *Pst*I digestion, purified by low-melting-point agarose gel electrophoresis and labelled by random primer extension using the kit from Boehringer.

Detection of polymorphism

Three restriction endonucleases (*Bam*HI, *Eco*RI and *Hin*dIII) were used to digest DNA from sorghum parental lines IS 18729 and IS 24756. Maize DNA probes were then hybridized to the genomic blots, and their ability to detect polymorphism recorded.

The linkage analysis was performed on a F_2 population of 149 individuals, whose genomic DNA was tested with those clones that detect polymorphism in the two parentals. The segregation patterns of all of the clones analyzed were not significantly different from the expected 1:2:1 ratio. The estimate of recombination frequencies between loci and the determination of the linkage groups were performed by multipoint analysis of the data using the MAPMAKER program, version 1.0 (Lander et al. 1987), run on a HP/UNIX mainframe.

Results

Maize clones and their ability to detect polymorphism in sorghum

The ability of maize clones to hybridize with sorghum genomic DNA was tested on parental lines IS 18729 and IS 24756 using a total of 159 RFLP clones, 82 from Pioneer Hi-Bred International (PIO), 42 from Brookhaven National Laboratory (BNL) and 35 from the University of Missouri Columbia (UMC). Five clones were unable to hybridize, the others yielded the hybridization patterns shown in Table 1. The patterns were fairly coincident in maize and sorghum: in particular, almost all of the clones showing a single-copy pattern in maize were also single copy in sorghum. The same holds true for two- or three-copy clones. Some of the clones showing a multiband pattern in maize detected low-copy sequences in sorghum and were therefore utilized in further analyses.

This comparison is based on data from simultaneous hybridizations, with conditions being exactly the same for the two species: the only difference in the resulting hybridization was that sometimes the signal for sorghum was fainter than that for maize.

Sorghum DNA was digested with *Bam*HI, *Eco*RI and *Hin*dIII in order to detect polymorphism: 58 maize

Table 1. Comparison of the hybridization patterns in maize and sorghum of 154 maize RFLP clones

Hybridization pattern	Number of clones
Low copy number in both	144
Low copy in maize, multicopy in sorghum	4
Low copy in sorghum, multicopy in maize	3
Multicopy in both	3

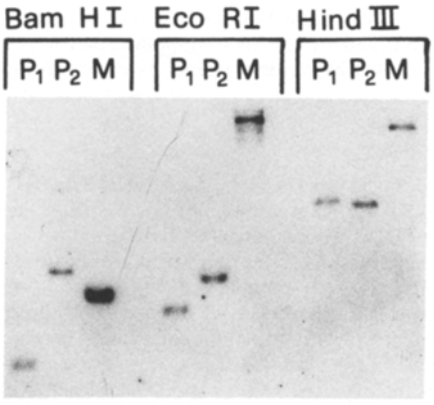

Fig. 1. Detection of polymorphism in two sorghum lines using a maize RFLP clone. Genomic DNA was fully digested with *Bam*HI, *Eco*RI or *Hin*dIII and hybridized with maize probe PIO 10-0080. P_1 Sorghum line IS 18729, P_2 sorghum line IS 24756, *M* maize inbred Mo17

clones (36.5% of the total) were found to be polymorphic, either in the form of an allelic difference or of the presence of band versus the absence of band(s); the former case being the more common. Each of the three enzymes detected about the same number of polymorphic clones, many of which were simultaneously polymorphic for all of the enzymes (Fig. 1). This could indicate that our two lines differ more because of chromosomal rearrangements than point mutation.

F_2 analysis

The segregation patterns of 35 polymorphic loci chosen among those mapping on chromosomes 1, 3, 4 and 5 of maize were analyzed on 149 individuals of an F_2 population. The genotype of each plant was scored, and the matrix of data analyzed by MAPMAKER. An example of this F_2 analysis is shown in Fig. 2. All but one of the polymorphic clones showed allelic differences between the two lines, thus giving more precision to our map (Ritter et al. 1990).

Linkage group detection

Twenty-one clones out of the 35 analyzed fall into five linkage groups. Parameters used to define linkage were as follows: (1) frequency of recombination, upper limit = 40%; (2) log-likelihood = 3.0, meaning that the ratio of the probability of two loci being associated to the probability that they are not is higher than 1000. In this way we obtained, by multipoint analysis, the most probable linkage maps for the markers considered: in no case was the "second best" map better than 100 times less probable. By using the above parameters, it was not possible to detect any association between the other 14 polymorphic clones.

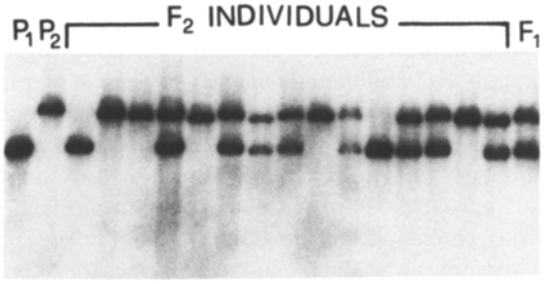

Fig. 2. Segregation analysis of the F_2 population derived from the cross IS 18729 × IS 24756. Genomic DNA was digested with *Bam*HI and hybridized with maize probe PIO 20-0817. P_1 and P_2 Parental lines, F_1 sample from a F_1 plant

Comparison of linkage in sorghum and maize

In Fig. 3 the five linkage groups are shown together with the position of corresponding markers on the currently available RFLP maps of maize (Pioneer Hi-Bred, December 1990, kindly provided by D. Grant). A good degree of colinearity is present. The most interesting features are:

1) Four RFLP markers mapping on chromosome 1 in maize are present in the same linkage group in sorghum, with the exception of clone PIO 20-0644. Assuming that the four loci represent hortologous genes, differences in linear order can be interpreted as a rearrangement produced by an inversion. This group includes the clone PIO 20-0817, whose multiband pattern in maize prevented its attribution to any linkage group.

2) Six clones, all of which map on maize chromosome 3, represent a linkage group in sorghum also. The inversion of a chromosomal region comprising the clones BNL 5.37, PIO 20-0521, PIO 10-0080 and PIO 20-0726 might account for the different relative positions these clones occupy in the two species.

3) Other possible chromosomal rearrangements during the divergent evolution of sorghum and maize might have involved regions of maize chromosomes 4 and 5. The linear order obtained could be due to an inversion and a translocation between chromosome 4 and chromosome 5. It is interesting to note that the second longest linkage group in sorghum contains four clones mapping on chromosome 5 in maize plus clone PIO 20-0608 from maize chromosome 4. On the other hand, the three-marker linkage group contains two clones (UMC 27 and PIO 20-0872) mapping on maize chromosome 5 and the clone PIO 10-0025, located on maize chromosome 4. Furthermore, we find clones BNL 15.07 and PIO 20-0689 associated in sorghum, mapping, respectively, on maize chromosomes 4 and 5.

The overall genetic distance covered by our linkage groups is 439.6 cM. Due to the rearrangements observed between the two species, the regions of the genome de-

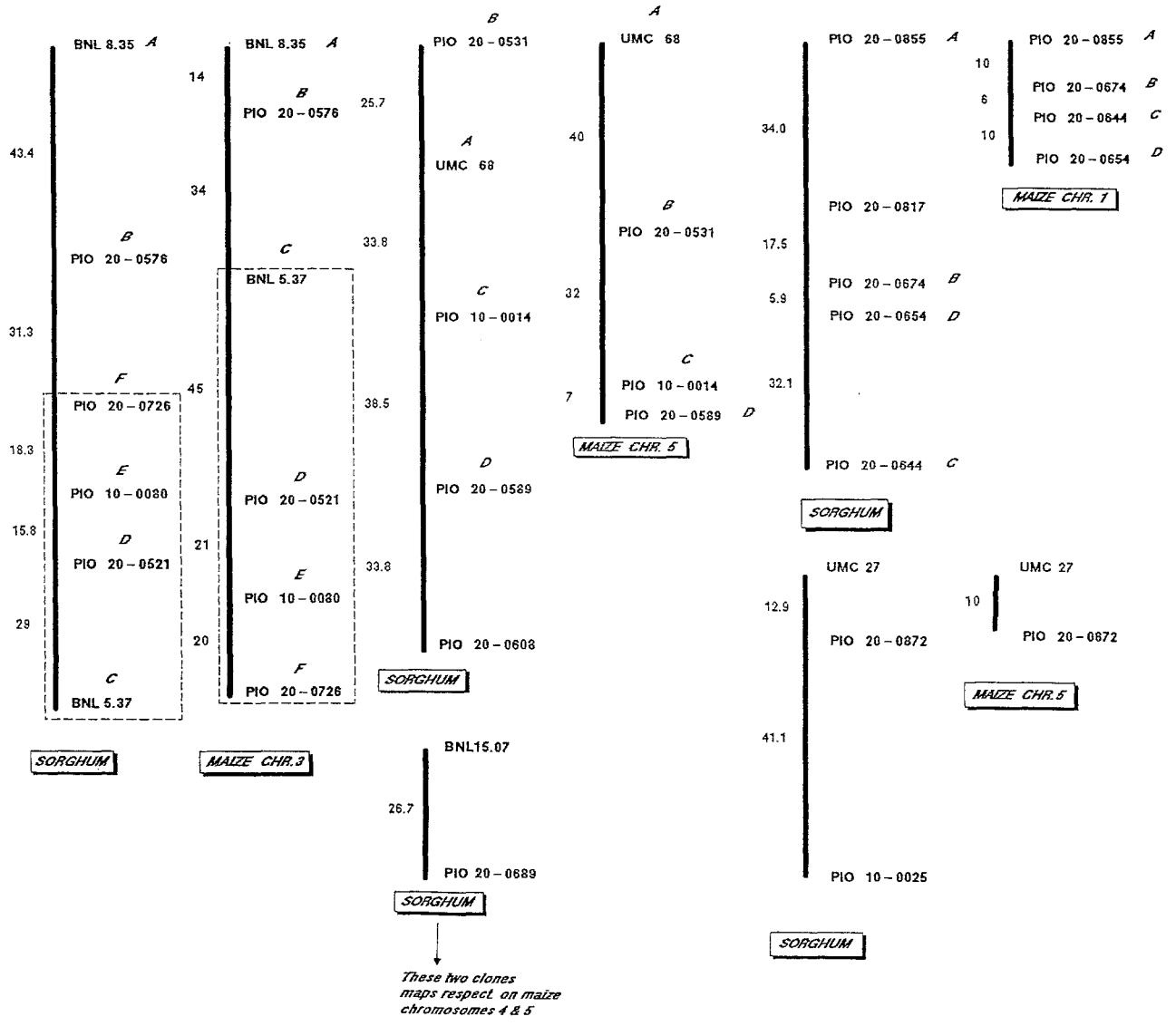

Fig. 3. Colinearity of the RFLP linkage map in maize and the RFLP linkage groups in sorghum. Each of the latter is represented to the *left* of the corresponding region of the maize genome. *Capital letters* indicate the same clone for easier reference. The association of clones BNL 15.07 and PIO 20-0689 is not present in maize (see text). *Numbers* indicate genetic distances in centiMorgans (cM)

fined by the same markers span 338.0 cM in sorghum and 249.0 cM in maize. It has to be pointed out that precise comparisons cannot be made because of the different origin of the data and the different progeny number in the two studies (map distances' estimates suffer from a large sampling error when samples are obtained from populations of a small size).

Computer simulations of dimensions of F_2 population

In an attempt to evaluate empirically the lowest number of individuals necessary to obtain the same data as obtained from our population of 149 plants, we analyzed the partial data sets relating to the linkage group composed of clones mapping on maize chromosome 3, of 40, 80 and 110 randomly chosen individuals from our population. The results of the simulations are presented in Fig. 4. Six clones are linked in our map: the analysis based on 40 individuals yields a linkage group of only three; the analysis on 80 individuals increases the number of linked loci to five; 110 individuals yield a five-loci linkage group, but not the same one as the "80 individual" simulation. Data similar to those of the full set are obtained only when the F_2 population dimension is 113 plants.

However, the order of the markers is always maintained, and distances between linked loci are already very close to those of the full data set even in the case of a very small population of 40 individuals.

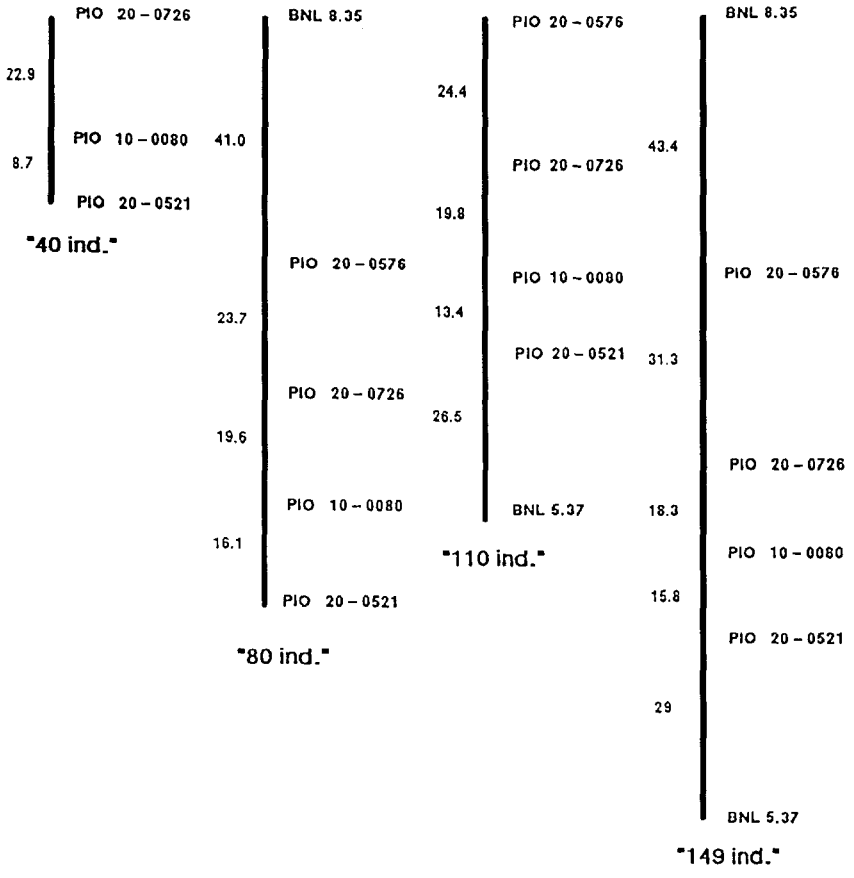

Fig. 4. Effects of the dimensions of the F_2 population on the detection of linkage between markers. Partial data sets have been analyzed, each relative to the number of individuals indicated. The result obtained from the analysis of the full data set is shown as "*149 ind*"

Discussion

One hundred and fifty-nine maize RFLP clones were tested for their ability to hybridize and detect polymorphism in *Sorghum bicolor*. The use of heterologous probes has been demonstrated to be effective for each of these purposes (Bonierbale et al. 1988). In our experiments, the close relationship between maize and sorghum was evident: only 5 clones out of 159 could not find homologous sequences in sorghum, allowing a divergence of 3% between the two genomes to be estimated. Fifty-eight maize clones were shown to hybridize and to reveal polymorphism between two inbred lines of sorghum. Under many aspects, the hybridization patterns in the two species did not differ: in particular, single-copy clones in maize were single-copy genes in sorghum. Many indications are available concerning both the genetic structure and the origin of traits shared by both maize and sorghum. Sorghum is considered a true diploid with $n=10$ (Doggett 1988), but a tetraploid origin cannot be ruled out. In the Sorghastrae, n is always a multiple of 5; on the other hand, both an allotetraploid origin of maize and its allopolyploid behavior at the cytological level have been proposed, as discussed in Helentjaris et al. (1988).

The degree of polymorphism observed in sorghum was, however, very low, 58 out of 159 clones = 36.5%; the same probes tested on two maize inbreds using the same restriction enzymes yielded a polymorphism of about 80% (our laboratory, unpublished data). In particular, we tested all of the available probes (42) of maize chromosome 5: of these, only 8 showed polymorphism in our two inbreds. This was not expected because the sorghum lines used in our work, IS 18729 and IS 24756, were chosen from a set of 16 lines previously tested for the presence of polymorphism. IS 18729 and IS 24756 are clearly differentiated with regard to: (1) geographic origin: IS 18729 is a bicolor from Texas and IS 24756, a Fara-Fara landrace from Nigeria; (2) morphological traits (height, tassel and ear morphology, colour); (3) agronomical traits (photoperiod, tiller production, seed weight). The greater polymorphism observed by Hulbert et al. (1990) may be explained by the different genomes studied and by the greater number of restriction enzymes used: 7 against our 3. However, polymorphism in sorghum appears to be lower than in maize. The use of mainly genomic maize probes could be responsible for this; in fact they may represent duplicated sequences, of which the maize genome appears to have in abundance (Helentjaris et al. 1988). The DNA content of sorghum,

as determined by microdensitometry (Laurie and Bennett 1985), is one-third of that of maize: presumably, sorghum possesses a more "condensed" genome. A more precise map will be obtained when sorghum clones are used for this purpose. Genomic clones have already been produced (our laboratory; G. Hart personal communication; M. Lee personal communication), and they appear to detect higher degrees of polymorphism (M. Lee, personal communication).

The segregation pattern of 35 polymorphic loci mapping on maize chromosomes 1, 3, 4 and 5 was analyzed in 149 individuals of the F_2 population: all of the loci showed the expected pattern 1:2:1. For 14 clones it was not possible to detect any linkage at the level of 40% recombination frequency. Twenty-one clones fell into five linkage groups, the three principal ones strongly recalling regions of maize chromosomes 1, 3 and 5. In general, colinearity was maintained, as previously demonstrated (Hulbert et al. 1990). However, it is possible to infer the presence of inversions (between clones PIO 20-0576 and BNL 5.37, PIO 20-0531 and UMC 68, PIO 20-0644 and PIO 20-0654) and the possibility that portions of maize chromosome 4 and 5 have undergone a reciprocal translocation in sorghum (or vice versa!). In particular, of interest is what appears to be a large inversion in sorghum of chromosome 3 in maize: although maize RFLP maps do not allow the unquestionable determination of the centromere position, it is likely that one end of the inversion (between PIO 20-0576 and BNL 5.37) approximately coincides with the centromere. Paracentric inversions between potato and tomato genomes have been detected, and their possible importance in plant evolution has been pointed out (Bonierbale et al. 1988).

The total length of the genetic map obtained in sorghum is 406 cM, while regions corresponding to maize chromosomes span 305 cM. In terms of genomic dimensions sorghum is smaller than maize, but this seems not to be the case with respect to genetic distances, indicated by the fact that in our experiment 14 clones mapped in maize were not found to belong to any linkage group in sorghum, at the recombination frequency of 40%. Cytological studies indicate that autogamous behavior in plants seems to be associated with an increased frequency of crossing-overs at meiosis in order to maintain a high level of recombination potential (Lewis and John 1963). A higher frequency of recombination should lead to the estimation of greater distances between markers. Our results go in this direction, showing a longer map for sorghum, whose reproductive behavior is self-pollinating, while maize is mostly an outbreeder. Little data on the frequency of chiasmata in maize and sorghum are available: in maize, Beadle (1933) and Darlington (1934) found that at diplotene the average number of chiasmata per bivalent was between 2.7 and 3.7. If the frequency of chiasmata depends on the genetic background, as would appear, one should expect higher values for sorghum. In *Sorghum bicolor*, the same estimate is around 2.9 (Lewis and John 1963): however, bearing in mind that the amount of nuclear DNA in sorghum is one-third of that in maize, similar chiasmata frequencies indicate shorter units of recombination, and therefore apparently longer distances. In addition, it has been shown in *Petunia*, *Lathyrus* and *Lolium* that a decrease in chiasmata frequencies per picogram DNA is associated with an increase in the amount of genomic DNA (Rees and Durrant 1986).

Our results indicate a good degree of colinearity between the maps of the two species: this feature has been already pointed out by Hulbert et al. (1990), although in their work they detected smaller distances in sorghum than in maize. The different sets of clones utilized and the different sizes of the segregating populations analyzed could account for this discrepancy, which will probably be overcome when more data on the genetic analysis of sorghum is obtained.

The usefulness of a genetic map depends both on the degree of saturation and on the precision of estimated distances between markers. Our F_2 population, consisting of 149 individuals, satisfies the second requisite, since the sampling error is reduced and the estimate of recombination frequencies is consequently more precise. In our simulation performed on the six markers of the longer linkage group, the number of F_2 individuals was the discriminating factor for their arrangement in a maximum likelihood map: with 40 individuals analyzed, only 3 clones out of 6 were linked; each of the other 3 segregated independently. The final arrangement was obtained only when the number of individuals exceeded 110. Therefore, population dimensions should be taken into account when comparing different maps.

Given the good feasibility of working with maize probes and the future use of homologous clones, the combined efforts of several research groups could soon lead to the definition of a RFLP linkage map in sorghum. Some difficulties might derive from low levels of intraspecific polymorphisms that do not allow a good saturation of the map in some regions of the genome. However, the use of cDNA clones, whose ability to detect higher degrees of polymorphism than genomic clones has been observed (at least in tomato; Miller and Tanksley 1990), RAPD techniques and interspecific crosses should serve to overcome these problems.

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