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Genomic organization of the ribosomal DNA of sorghum and its close relatives

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Summary. The structure and organization of the ribosomal DNA (rDNA) of sorghum *(Sorghum bicolor)* and several closely related grasses were determined by gel blot hybridization to cloned maize rDNA. Monocots of the genus *Sorghum* (sorghum, shattercane, Sudangrass, and Johnsongrass) and the genus *Saccharum* (sugarcane species) were observed to organize their rDNA as direct tandem repeats of several thousand rDNA monomer units. For the eight restriction enzymes and 14 cleavage sites examined, no variations were seen within all of the *S. bicolor* races and other *Sorghum* species investigated. Sorghum, maize, and sugarcane were observed to have very similar rDNA monomer sizes and restriction maps, befitting their close common ancestry. The restriction site variability seen between these three genera demonstrated that sorghum and sugarcane are more closely related to each other than either is to maize. Variation in rDNA monomer lengths were observed frequently within the *Sorghum* genus. These size variations were localized to the intergenic spacer region of the rDNA monomer. Unlike many maize inbreds, all inbred *Sorghum* diploids were found to contain only one rDNA monomer size in an individual plant. These results are discussed in light of the comparative timing, rates, and modes of evolutionary events in *Sorghum* and other grasses. Spacer size variation was found to provide a highly sensitive assay for the genetic contribution of different *S. bicolor* races and other *Sorghum* species to a *Sorghum* population.

Key words: Evolution – Restriction fragment length polymorphism – Grasses – Maize – Sugarcane

Introduction

Ribosomal RNA genes (rDNA) in eukaryotes exist in tandemly repeated multiple copies (Long and Dawid 1980). The 17S, 5.8S, and 26S rRNAs are transcribed as a single RNA molecule, separated by transcribed spacer regions which are removed when the large precursor molecule is cleaved to yield the mature rRNAs (Perry 1976). The rRNA-encoding regions of the rDNA repeat are separated by intergenic spacer (IGS) regions (Miller and Beatty 1969).

The rDNAs of many plant species exhibit repeat length variability (reviewed by Rogers and Bendich 1987). The variability in the length of the rDNA repeat is usually the result of differing numbers of repeated sequences in the IGS (Appels and Dvorak 1982a; Dover et al. 1982; Treco et al. 1982; Saghai-Maroof et al. 1984; Yakura et al. 1984). The rRNA-encoding regions of the rDNA repeat tend to be highly conserved (Appels and Dvorak 1982b; Messing et al. 1984; Eckenrode et al. 1985), while IGS regions show extensive sequence divergence (reviewed by Fedoroff 1979). Examination of the structural variation in ribosomal RNA genes between different species provides useful information concerning the points of divergence of related species and the molecular mechanisms of the evolution of genome structure. Distinctive restriction site patterns or polymorphisms within an rDNA repeat can also be employed as a molecular marker to identify the source of the rDNA in a particular plant population (Burr et al. 1983).

In this study, we examined the structure of the rDNA repeats in several lines of *Sorghum bicolor* [(L.) Moench] and its near relatives and have compared their structure to that of *Zea mays* and *Saccharum*. The results indicated that the transcribed region of the sorghum rDNA repeat is highly conserved within the species, while spacer length

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variation allowed differentiation between some sorghum varieties, races, and closely related species. Although the rDNA restriction map of sorghum was quite similar to that of maize and sugarcane, several restriction site variations permitted these related species to be distinguished.

Materials and methods

Plant material

Most sorghum varieties and races, and Piper Sudangrass (*S. bicolor*, ssp. *drummondii*) were obtained from J. Axtell, Purdue University, W. Lafayette/IN (USA). Colby, a milo sorghum variety, was provided by L. Dunkle, Purdue University, W. Lafayette/IN (USA). Johnsongrass [*S. halepense* (L.) Pers.] and shattercane (*S. bicolor* ssp. *arundinaceum*) were obtained from M. Ross, Purdue University, W. Lafayette/IN, (USA). See Table 1 for races. Maize inbred lines Q66, Q67, and B79 were provided by D. S. Robertson, Iowa State University, Ames/IA (USA). Maize inbred line W23 was obtained from L. F. Bauman, Purdue University, W. Lafayette/IN (USA). Pooled leaves of sugarcane cultivar CP 70-321, derived from an interspecific hybrid of *Saccharum* species *S. officinarum*, *S. spontaneum*, and *S. barberi* (Fanguy et al. 1979), were obtained from C. P. Chao, Louisiana State University, Baton Rouge/LA (USA).

DNA isolation

DNA was prepared from the mature leaves of individual plants by the method of Rivin et al. (1982).

Restriction enzyme digestions

All restriction enzymes were purchased from Bethesda Research Labs, New England Biolabs, or Boehringer Mannheim Biochemicals. A two-fold enzyme excess was employed under conditions specified by the manufacturer.

Gel blot hybridization

Genomic DNA was digested with the appropriate restriction enzyme(s) and fractionated on 0.8% agarose gels by constant voltage electrophoresis. The DNA was transferred to nitrocellulose or nylon filters. The filters were hybridized and washed as described previously (Bennetzen 1984). The rDNA hybridizational probe used (pZMR1) was provided by I. Rubenstein (McMullen et al. 1986). The entire plasmid or a gel-purified restriction fragment was labelled by either nick translation or random primer extension (Feinberg and Vogelstein 1983). Unincorporated nucleotides were removed by Sephadex G-75 gel filtration chromatography. The molecular weights of hybridizing fragments were determined in reference to digested lambda phage DNA samples run on each gel.

Results

Structure of the ribosomal repeat

Genomic DNAs from maize and several sorghum isolates were digested with the restriction enzyme *Xba*I, which cuts once within the rDNA repeat, and hybridized to a maize rDNA probe (Fig. 1 A). The sorghum rDNA hybridized with about one-fourth of the intensity of the maize rDNA to the maize probe, indicating good homology and similarly high rDNA copy numbers between the two species. The single 9-kb maize fragment observed was larger than that seen in Kaoliang, the sorghum with the shortest rDNA repeat in our study. In addition, the largest *Bgl*II/*Xba*I rDNA fragment detected was larger in maize (5.2 kb) than in Kaoliang sorghum (5.05 kb), while the smaller *Bgl*II/*Xba*I fragment was of the same size (3.8 kb) in both species (Fig. 1 B). These data indicate that Kaoliang sorghum rDNA is arranged as multiple direct tandem repeats of an approximately 8.85-kb unit. However, when genomic DNA was digested with *Sst*I, an enzyme that generates several rDNA restriction fragments (Fig. 1 c), the fragments observed in maize added up to a total (8.75 kb) which was less than the total of the Kaoliang sorghum *Sst*I rDNA fragments (9.0 kb). The reason for this sizing inconsistency is not known, although it may involve either the anomalous migration of one or more fragments due to an unusual secondary structure or an inability to detect a small *Sst*I fragment generated in maize. The *Xba*I digested DNAs from maize lines Black Mexican Sweet, B79, Q66, Q67, and W23 were run as controls. These all had the same rDNA repeat size by our gel blot hybridization criteria.

The restriction map that was generated for the cloned rDNA from Black Mexican Sweet maize (pZMR1) had

Table 1. Nature and source of plant materials

Line designation	Common name	Species (race)	Stock No.	Source
Colby	Sorghum	*S. bicolor* (durra)	Cultivar	L. Dunkle
Durra	Sorghum	*S. bicolor* (durra)	IS11167	J. Axtell
Kaoliang	Sorghum	*S. bicolor* (bicolor)	IS4225	J. Axtell
Msumbite	Sorghum	*S. bicolor* (guinea)	–	J. Axtell
Shallu	Sorghum	*S. bicolor* (guinea)	IS0466	J. Axtell
Piper	Sudangrass	*S. bicolor* ssp. *drummondii*	Cultivar	J. Axtell
Shattercane	Shattercane	*S. bicolor* ssp. *arundinaceum*	Field population	M. Ross
Johnsongrass	Johnsongrass	*S. halepense*	Field population	M. Ross
Sugarcane	Sugarcane	*Saccharum* (interspecific hybrid)	Cultivar	C. P. Chao
Black Mexican sweet corn	Maize	*Z. mays*	Exotic inbred	Maize Stock Center
W23	Maize	*Z. mays*	Inbred	L. Bauman
B79, Q66, Q67	Maize	*Z. mays*	Inbreds	D. Robertson

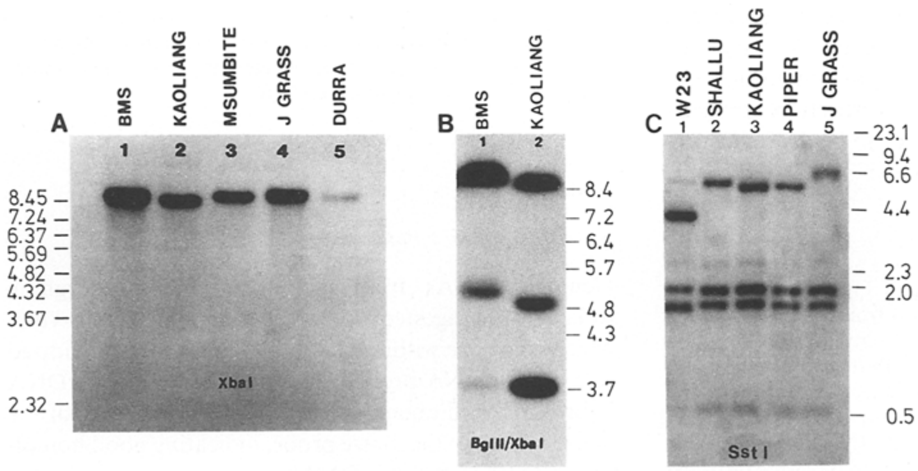

Fig. 1 A–C. Size variation of the rDNA repeat units of maize, sorghum, and related species. Restriction digests of genomic DNAs from individual plants. **A** *Xba*I digest. *Lane 1:* Black Mexican Sweet maize. *Lane 2:* Kaoliang. *Lane 3:* Msumbite. *Lane 4:* Johnsongrass. *Lane 5:* Durra. *Lane 1* contains 1 μg of DNA; *lanes 2–4* contain 2 μg of DNA; *lane 5* contains 0.5 μg of DNA. **B** *Bgl*II/*Xba*I digest. *Lane 1:* 1 μg of Black Mexican Sweet maize DNA. *Lane 2:* 2 μg of Kaoliang DNA. **C** *Sst*I digest. *Lane 1:* W23 maize. *Lane 2:* Shallu. *Lane 3:* Kaoliang. *Lane 4:* Piper. *Lane 5:* Johnsongrass. *Lane 1* contains 0.5 μg of DNA; *lanes 2–5* contain 2 μg of DNA. The hybridizational probe was hexamer-labelled plasmid pZMR1. Numbers refer to the molecular weights, in kbp, of *Bst*EII-digested (**A** and **B**) or *Hin*dIII (**C**) digested lambda DNA

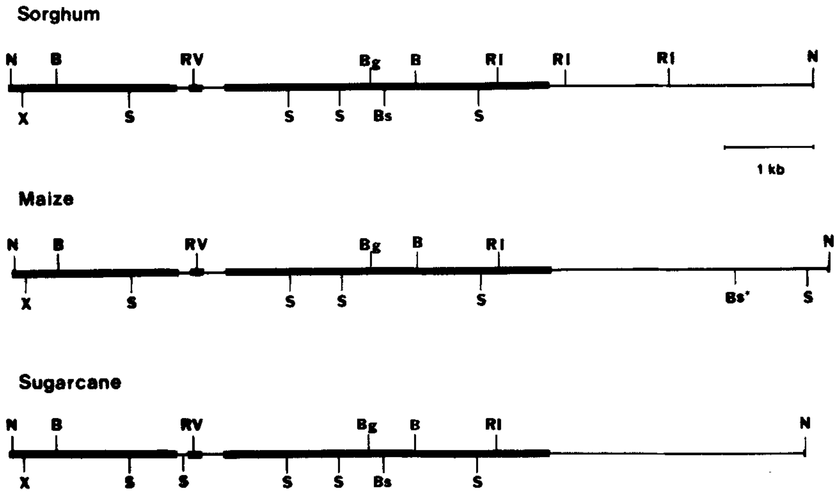

Fig. 2. Restriction map of the sorghum, maize, and sugarcane ribosomal DNA monomers. *B* – *Bam*HI, *Bg* – *Bgl*II, *Bs* – *Bst*EII, *N* – *Nde*I, *RI* – *Eco*RI, *RV* – *Eco*RV, *S* – *Sst*I, *X* – *Xba*I

two restriction size differences from the published sequence of the 17S rDNA coding region of the W22 maize line (Messing et al. 1984). An *Eco*RV site was observed which was not present in the sequence, and we were unable to detect an *Sst*I site which was in the published sequence. The *Sst*I site predicted by the published sequence (Messing et al. 1984) was not detected in any of the 21 maize lines (which did not include W22) investigated by Zimmer et al. (1988). The *Eco*RV site appeared to be a cloning artifact and was not included on the maize map, since it was not observed in genomic analyses of maize Black Mexican Sweet, B79, B37, Q66, Q67, and Wilbur's Knobless Flint DNA (data not shown and Zimmer et al. 1988). Partial digestion at a *Bst* EII site was detected near the 5′ end of the 17S coding region in the IGS region of the rDNA repeat in maize lines Q66, Q67, B79, and Black Mexican Sweet by gel blot hybridization analysis of total nuclear DNA (data not shown). Incomplete cleavage at this site was also observed in several of the maize inbreds investigated by Zimmer et al. (1988). Partial digestion of the rDNA monomer with *Bst*EII suggested that this site may only be present or accessible in some rDNA copies. We were unable to cut the pZMR1 clone of the maize rDNA repeat with *Bst*EII, indicating that the cloned Black Mexican Sweet rDNA did not contain this site. This *Bst*EII site is predicted by the DNA sequence of the IGS region of maize inbred A619 (Toloczyki and Feix 1986).

In general, the restriction maps of maize and sorghum rDNAs were found to be very similar (Fig. 2). Moreover, probes containing either the transcribed or intergenic regions of the maize rDNA repeat hybridized strongly to sorghum rDNA (data not shown). This indicated a very high degree of conservation of overall rDNA sequence between maize and sorghum. A few restriction site changes between maize and sorghum were observed, however (Fig. 2). These differences include a *Bst*EII site which was present in the IGS region of maize and absent in sorghum and a *Bst*EII size which was present in the 26S rRNA-encoding region of sorghum and absent in maize. In addition, there was a single *Sst*I site present in the IGS region of maize which was not found in sorghum. Several *Eco*RI sites were present in the IGS region of sorghum, but none were detected in maize. The general sorghum rDNA restriction map derived from these experiments was confirmed for Kaoliang sorghum, Shallu sorghum, Piper Sudangrass, and Johnsongrass.

A restriction map of the rDNA repeat of sugarcane was constructed using data derived from gel blot hybridization analyses with the same restriction enzymes and DNA probes employed for the sorghum and maize analyses (Figs. 2 and 3, and data not shown). The approximately 8.75-kb and 8.25-kb size classes of sugarcane rDNA repeats observed differed from sorghum only in their smaller size, the absence of the numerous *Eco*RI sites in the IGS region, and the addition of a unique *Sst*I site in the transcribed spacer (Figs. 2 and 3). This extra SstI site differentiated sugarcane from both sorghum and maize. The closer resemblance of sugarcane rDNA to sorghum rDNA than to maize rDNA was exemplified by the presence of a *Bst*EII site in the 25S rRNA-encoding region and the absence of *Bst*EII and *Sst*I sites in the intergenic spacer domain (Figs. 2 and 3).

Repeat size variation among Sorghum lines

Nine different sorghum lines were examined and were found to exhibit considerable variation in their rDNA monomer size (Figs. 1 and 4, and data not shown). The detected variation was localized to the IGS region, between the *Xba*I and *Eco*RI sites (Figs. 1 B, 2, and 4). A particular size class did not appear to differentiate between every sorghum isolate, however, as several lines had repeats which appear to be the same size within our level of sensitivity. The Kaoliang, Durra, and Colby sorghums had an rDNA monomer size of about 8.85 kb. Msumbite sorghum, Piper Sudangrass, and shattercane had an rDNA monomer size of approximately 9.05 kb. Shallu sorghum had a repeat size of about 9.15 kb, while Johnsongrass had rDNA monomer classes of approximately 9.85 kb, 10.05 kb, and 10.35 kb. Within the limited populations that we investigated, a particular inbred line contained only one rDNA size class. The

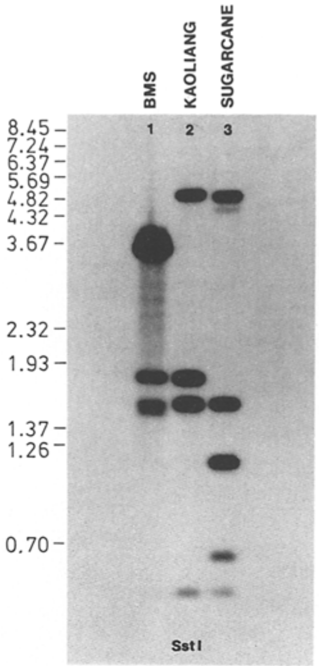

Fig. 3. *Sst*I digestion of genomic maize, sorghum, and sugarcane rDNAs. *Lane 1:* Black Mexican Sweet maize. *Lane 2:* Kaoliang. *Lane 3:* Sugarcane. *Lane 1* contains 0.5 µg of DNA; *lanes 2* and *3* contain 2 µg of DNA. The hybridizational probe was hexamer-labelled plasmid pZMR1. Numbers refer to molecular weights, in kbp, of *Bst*EII-digested lambda DNA

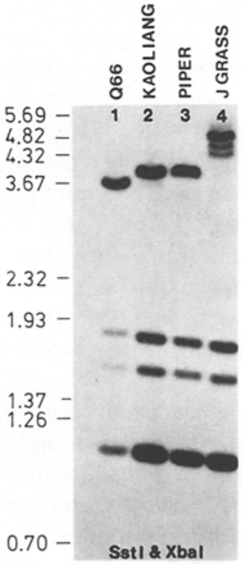

Fig. 4. *Sst*I/*Xba*I digestion of genomic rDNAs from individual plants. *Lane 1:* Q66 maize. *Lane 2.:* Kaoliang. *Lane 3:* Piper. *Lane 4:* Johnsongrass. *Lane 1* contains 0.5 µg of DNA; *lanes 2–4* contain 2 µg of DNA. The hybridizational probe was hexamer-labelled plasmid pZMR1. Numbers refer to molecular weights, in kbp, of *Bst*EII-digested lambda DNA

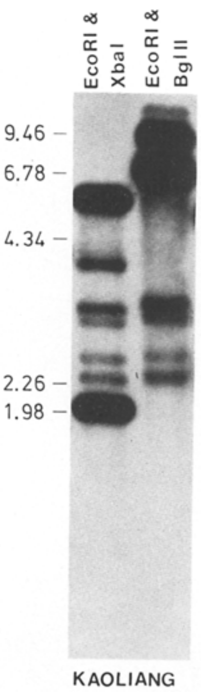

Fig. 5. *Eco*RI partial digestion combined with either complete *Xba*I or complete *Bgl*II digestion of genomic rDNA from a single Kaoliang sorghum plant. The hybridizational probe was a hexamer-labelled 2.5-kb *Sst*I spacer fragment of pZMR1. Numbers refer to molecular weights, in kbp, of *Hin*dIII-digested lambda DNA

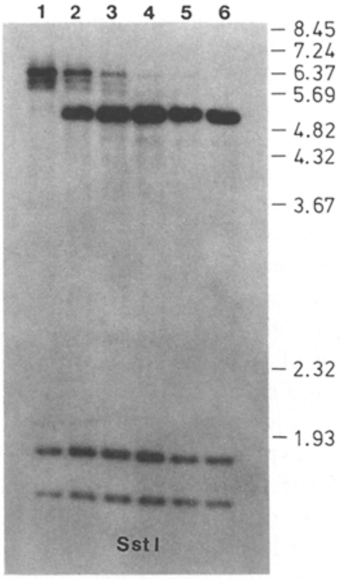

Fig. 6. Ribosomal DNA detection on gel blots containing varying concentrations of Johnsongrass and Piper sudangrass genomic rDNAs digested with *Sst*I. *Lane 1:* 100% Johnsongrass. *Lane 2.:* 50% Johnsongrass + 50% Piper. *Lane 3:* 20% Johnsongrass + 80% Piper. *Lane 4:* 5% Johnsongrass + 95% Piper. *Lane 5:* 2% Johnsongrass + 98% Piper. *Lane 6:* 100% Piper. The total amount of genomic DNA per lane is 2 μg. The hybridizational probe was hexamer-labelled plasmid pZMR1. Numbers refer to molecular weights, in kbp, of *Bst*EII-digested lambda DNA

exception to this rule was Johnsongrass, where an individual plant exhibited three distinct size classes. Two hybrid sorghum populations displayed the two rDNA size classes of their inbred parents (data not shown).

Multiple *Eco*RI sites were detected in the intergenic rDNA spacer in sorghum (Fig. 2). While only two of these sites could be definitively mapped, the multiple bands observed in an *Eco*RI partial digestion (Fig. 5) suggested that there were several other sites in the spacer. Many or all of these *Eco*RI sites appeared to be between the two spacer *Eco*RI sites that were mapped. These *Eco*RI sites may be encoded by the subgenomic repeats of the IGS region.

Since the rDNA monomer in sorghum exhibited some size variability, we decided to test whether this might serve as a restriction fragment length polymorphism (RFLP) marker for detecting the rDNA composition and origin of a sorghum population. Varying concentrations of Johnsongrass and Piper genomic DNA were mixed in order to determine what level of contamination could be detected with the maize rDNA probe. With gel-resolved DNA digested by the restriction enzyme *Sst*I, a 2% Johnsongrass DNA contamination of Piper DNA was detected (Fig. 6).

Discussion

The restriction maps of the sorghum, maize, and sugarcane rDNA monomers are, overall, very similar. As previously suggested by morphological and molecular (Hamby and Zimmer 1988) criteria, our data indicate that sorghum and sugarcane are more closely related to each other than either is to maize. Sorghum does exhibit some restriction site variability in the rDNA repeat compared to both maize and sugarcane. This variation is found both in the coding and IGS regions of the rDNA repeat unit. We did not find any restriction site polymorphisms in the rDNA repeat between three *Sorghum bicolor* lines (Kaoliang sorghum, Shallu sorghum, and Piper Sudangrass) and a closely related species (Johnsongrass), while maize exhibits extensive polymorphism between inbred lines (Zimmer et al. 1988). Repeat length variability is frequent among sorghums and closely related species, as it also is in maize and (from our very limited sampling) sugarcane. This length variation is localized to the IGS region in sorghum, as it is in other plant species.

The absence of rDNA map variation within a species and its close relatives can be simply explained by a relatively recent common origin of the related individuals. However, when variations in spacer length are common while restriction site polymorphisms are absent in the same comparison, a more elaborate evolutionary model is required. For instance, spacer length variability may be generated more rapidly than are restriction site polymorphisms, while these size variations may be constrained within specific limits. Hence, a more recently or narrowly derived species may show similar levels of spacer length variability but less restriction site polymorphism than a more ancient species. Unequal crossing over events between the rDNA monomers and the short tandem repeats in the IGS region are believed to account for spacer length variability (Rogers et al. 1986; Smith 1974; Szostak and Wu 1980; Tartof 1974), while restriction site polymorphisms are presumably derived from fixed mutational events. A higher relative frequency of unequal crossing over compared to mutation in sorghum than in maize might also explain the differences in modes of rDNA heterogeneity in these genera (Williams and Strobeck 1985).

Maize and all of the *Sorghums* described here have a haploid chromosome number of 10. The exception to this rule is Johnsongrass, which contains 40 chromosomes per nucleus. Although a presumed tetraploid version of sorghum, it is not clear whether Johnsongrass may have arisen through allopolyploidy or autopolyploidy events (Hoang-Tang and Liang 1988). The unusually large size of the rDNA spacer on both chromosome sets suggests that the presumed polyploidization which gave rise to Johnsongrass occurred within or between sorghum relatives that had an rDNA repeat size larger than any of the diploid *Sorghums* investigated in this study. Alternatively, parallel or concerted evolution may have led to an increase in the rDNA repeat size in both Johnsongrass rDNA arrays or a decrease in the rDNA monomer size in the other *Sorghums*. Cases where different rDNA repeat sizes have been tolerated (i.e., fixed) within a single rDNA tandem array are seen in maize inbreds (Zimmer et al. 1988) but were not observed in any diploid inbred *Sorghum*. Of the three rDNA monomer size classes that were observed in an individual Johnsongrass plant, the largest size class was about two-fold more frequent than either of the two smaller classes. This result could be explained by a size heterozygosity for the rDNA repeat on one pair of homologues and homozygosity on the other pair of chromosomes carrying an rDNA cluster in this tetraploid. Hence, there was no evidence for heterogeneity of rDNA spacer length within a single rDNA tandem cluster in any *Sorghum*. The heterogeneous rDNA repeat length detected in sugarcane could be due to the polyploid nature of this interspecific hybrid, the fact that this cultivar was derived from the genetic contributions of three inbreds, and/or our use of leaves pooled from several individual plants as a DNA source.

Most wheat and soybean lines (Appels and Dvorak 1982a; Doyle and Beachy 1985; Flavell et al. 1986), like sorghum, lack single rDNA tandem arrays with two or more monomer structures, while intra-cluster heterogeneity is common in maize (Zimmer et al. 1988). This striking difference may simply be due to the much more frequent inbreeding of sorghum, wheat, and soybean than maize. Since inbreeding would rapidly generate homozygosity for the two homologues which carry rDNA clusters, recombination between two structurally different rDNA clusters to generate a single heterogeneous tandem array (Krystal et al. 1981) would be relatively infrequent.

Theoretically, restriction site variability and repeat length heterogeneity in the rDNA repeat should allow determination of the evolutionary relatedness of various races of sorghum. One might, for instance, conclude that their common rDNA repeat size indicates that Kaoliang, Durra, and Colby sorghums are more closely related to each other than to any of the other sorghums in our study. These results agree with data indicating the early origin of durra race sorghums (Durra and Colby) from the bicolor race (Kaoliang). By this same rDNA monomer size criterion, Msumbite sorghum, Piper Sudangrass, and shattercane are more closely related to each other than to the other lines, while Shallu sorghum and Johnsongrass are less closely related to each other and to the other races. These data may not be significant given the apparent rate at which the rDNA repeat length is changing in sorghum and the potential for parallel events.

The current models of sorghum race and variety distribution (de Wet 1978; Harlan and de Wet 1972; Harlan and Stemler 1976; Mann et al. 1983) differentiate the main *Sorghum bicolor* races as bicolor, caudatum, durra, guinea, and kafir. All of these races are likely to have been derived and disseminated from an ancient bicolor race prior to three thousand years ago (Mann et al. 1983). Subsequent introgressions between these races are believed to have created the numerous intermediate types now observed. Due to these many ill-defined introgressions, the actual DNA contributions of any given sorghum population to what we now call sorghum races can only be crudely estimated. Our limited data on rDNA spacer length variation does place the Kaoliang, Colby, and Durra materials, derived from sorghums segregated to Asia over 3000 years ago, in a class separate from Msumbite sorghum, Piper Sudangrass, and shattercane, all of which are thought to have had an exclusively African history. Conversely, a spacer length difference was detected between two guinea race sorghums, Shallu and Msumbite.

We were able to use a maize rDNA probe to detect a 2% Johnsongrass contamination in a Piper DNA preparation. This result indicates that the rDNA repeat in sorghum can be useful as a restriction fragment length polymorphism (RFLP) marker for determining the percentages of parental genomic contributions in traditional breeding or population genetics programs. The high copy number of the rDNA repeat should make this a particularly sensitive probe for the detection of low percentage contributions.

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