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Short Communication

A physical map of the sorghum chloroplast genome

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Summary

The chloroplast genome of the IS1112C cytoplasm of sorghum was mapped by the construction of a *Bam*HI library in pUC8, and hybridization with *Bam*HI, *Sal*I, and *Pst*I digests of chloroplast DNA (ctDNA) of sorghum and maize. The molecules are extensively colinear, with only one of 13 *Sal*I fragments differing slightly from maize. Seven of 70 restriction sites differed in the two species. A total molecular size of ca. 138 kb was estimated for sorghum. The inverted repeat was not conserved between sorghum and maize, as revealed by a slightly larger *Bam*HI 16S rDNA fragment in sorghum. Homology of a sequence adjacent to the γbcl gene and one end of the inverted repeat was detected. These homologies were also observed in maize, and suggest that the ctDNA genomes of sorghum and maize share small reiterations of sequences of the inverted repeat.

Introduction

The chloroplast genome of angiosperms consists of a single circular molecule ranging in size from 120 kb to 217 kb (10). Within the *Gramineae,* the chloroplast genomes of maize, wheat, barley, and *Pennisetum* have been mapped (1, 5, 11, 14), and share several basic characteristics: an inverted repeat (IR) of ca. 21–22.5 kb, single copy regions totalling ca. 110 kb, and the same approximate positions of the psbA, rbcL, and rDNA regions. The chloroplast DNA (ctDNA) of *Sorghum bicolor* (L.) Moench. displays restriction endonuclease patterns very similar to that of maize (12). Within the species, three ctDNA patterns were apparent in male-sterile cytoplasms; the milo group, with 16 members, the IS1112C group, with five members, and the 9E group, with two members (2, 13). The cloning and mapping of IS1112C ctDNA was initiated to elucidate the structure of the genomes and the characterization of polymorphisms.

Materials and methods

Seed of IS1112C, a male-sterile cytoplasm, but male-fertile in the IS1112C background, was provided by K. F. Schertz, USDA-ARS, College Station, TX. Chloroplast DNA was prepared according to Pring *et al.* (12), with modifications. Leaf tissue was homogenized in 10 volumes grinding buffer; following SDS-proteinase K digestion, the preparations were processed according to Dellaporta *et al.* (4). *Bam*HI-digested chloroplast DNA was ligated into the *Bam*HI site of pUC8, transformed, and recombinant colonies were selected by standard procedures (6, 15). Restriction, electrophoresis, blotting, nick translation, hybridization, and molecular size determinations were as described (8, 9). Maize chloroplast clone pZls91, containing the rbcL coding sequence, was graciously provided by Dr L. McIntosh. Wf9(N) was utilized for maize ctDNA.

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Results and discussion

Restriction patterns

Chloroplast DNA restriction patterns of maize and sorghum are quite similar (Fig. 1). *Bam*HI generated 28 visible fragments from maize and sorghum, although mapping and computer simulation revealed at least 37 from maize (5). *Pst*I digestion showed 16 fragments in sorghum and 17 in maize, with apparent co-migration of 13 fragments less than 14 kb. *Sal*I digestion revealed 11 apparently co-migrating fragments from both species, although 0.4% agarose electrophoresis revealed that *Sal*I B of sorghum was perhaps 250 bp larger than *Sal*I B of maize. A summary of *Pst*I kb values for the sorghum and maize digests (Table 1) showed a total kb of ca. 138 for sorghum and ca. 137 for maize. The sorghum value is only slightly lower than the mapped 139 kb total for maize (5).

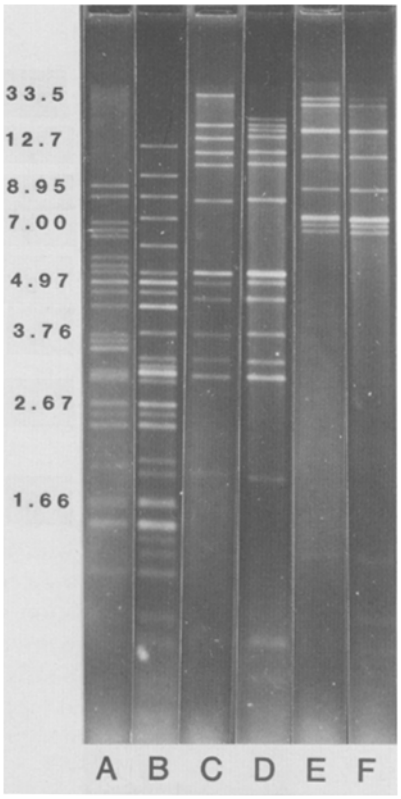

Fig. 1. *Bam*HI (A, B), *Pst*I (C, D) and *Sal*I (E, F) restriction endonuclease patterns of sorghum (A, C, E) and maize (B, D, F) chloroplast DNA electrophoresed 16 h at 2 V/CM in 0.8% agarose gels. Numbers are kb values of selected fragments.

Mapping of the sorghum ctDNA

Each of the 24 unique clones were individually hybridized to Southern bi-directional blots of *Bam*HI, *Pst*I, and *Sal*I digests of sorghum and maize ctDNA. By reference to the maize map, these clones were then placed on a linear map (Fig. 2). The zero coordinate of this map, the *Pst*I site between fragments 5 and 8, is probably within 320 bp of the zero coordinate of the maize map (5). Seven major alterations were found between maize and

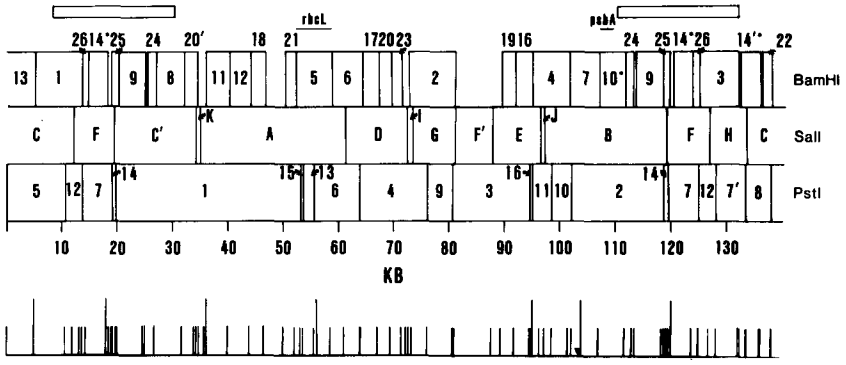

Fig. 2. The restriction endonuclease map of IS1112C sorghum chloroplast DNA. Numbering scheme is as developed for maize (5). Zero coordinate is the *Pst*I junction of *Pst*I fragments 5 and 8 of IS1112C. Bottom; short lines, sorghum and maize restriction sites identified in this study as conserved; tall lines, sites unique to sorghum or maize. Triangle at 104 kb is approximate location of an insertion in sorghum.

Table 1. Molecular sizes of *Bam*HI, *Pst*I, and *Sal*I fragments of sorghum and maize chloroplast DNA, and identification of the sorghum *Bam*HI pUC8 clones. Numbers or letters in enclosures are fragment designations based on decreasing kb. Fragments of apparent similar kb are listed on the same line. Designations are as the system utilized for maize (5), except that (′) designations are not used for fragments within the inverted repeat. Designation NC are fragments not represented in clone library.

Clone	*Bam*HI sorghum	Maize	*Sal*I sorghum	Maize	*Pst*I sorghum	Maize
		12.70 (1)	26.40	26.40 (A)	33.50 (1)	
		9.85 (2)	22.25	22.00 (B)		17.75 (1)
pLD1	8.95		15.00	15.00 (C)	17.00 (2)	
pLD2	8.25	8.25 (3)	15.00	15.00 (C′)		16.60 (2)
		7.20 (4)	11.40	11.40 (D)		15.30 (4)
pLD3	7.00		8.65	8.65 (E)	14.00 (3)	14.00 (4)
pLD4	6.65		7.20	7.20 (F) (2)	12.12 (4)	12.00 (5)
		6.05 (5)	7.20	7.20 (F′)	10.75 (5)	10.75 (6)
pLD5	6.40		6.80	6.80 (G)	8.20 (6)	8.20 (7)
pLD6	5.60		6.68	6.68 (H)	5.20 (7) (2)	5.20 (8) (2)
pLD7	5.35		1.22	1.22 (I)	5.20 (7′)	5.20 (8′)
pLD8	5.20	5.10 (6)	0.84	0.84 (J)	4.98 (8)	4.98 (9)
pLD9	4.97	4.97 (7)	0.66	0.66 (K)	4.55 (9)	4.55 (10)
NC (10)	4.65	4.65 (8)	136.50	136.25	3.78 (10)	3.78 (11)
		4.36 (9)			3.30 (11)	3.30 (12)
pLD11	4.36	4.36 (9′)			3.10 (12) (2)	3.10 (13) (2)
pLD12	3.76	3.78 (10)			1.95 (13)	1.90 (14)
pLD13	3.67				0.72 (14) (2)	0.72 (15) (2)
NC (14)	3.50				0.54 (15)	0.54 (16)
		3.35 (11)			0.42 (16)	0.435 (17)
		3.29 (12)			138.33	137.23
NC (15)	3.16					
pLD16	3.08					
		3.10 (13)				
		3.10 (13′)				
pLD17	3.00	3.00 (14)				
		2.67				
pLD18	2.67	2.67 (15′)				
pLD19	2.55	2.55 (16)				
pLD20	2.40	2.40 (17)				
pLD20′	2.40	2.40 (17′)				
		2.05 (18)				
pLD21	2.00	1.92 (19)				
pLD22	1.66	1.65 (20)				
		1.62 (21)				
pLD23	1.62	1.62 (21′)				
pLD24	1.49	1.49 (22)				
		1.49 (22′)				
pLD25	1.40	1.42 (22/23)				
		1.36 (24)				
		1.28 (25)				
pLD26	1.14	1.14 (26)				

sorghum of 70 mapped restriction sites; comparison with a bar graph (Fig. 2) shows random distribution of the polymorphisms. An additional *Bam*HI site is found in the small single copy region in sorghum, generating fragments 1 and 13. *Bam*HI 14 in sorghum is 400 bp larger than its counterpart in maize, *Bam*HI 13. The 33.5 kb *Pst*I fragment 1 is generated by the deletion of a *Pst*I site found in maize. Similarly, *Bam*HI 5 in sorghum is generated by deletion of a *Bam*HI site in maize. The *Bam*HI site separating fragments 4 and 16 is absent in maize. The loss of a *Bam*HI site in sorghum within fragment 7 is characterized by maize fragments 11 and 25; the latter has been positioned adjacent to

maize fragment 2 by cosmid clones of maize ctDNA (D. M. Lonsdale, personal communication). *Bam*HI fragment 7 is ca. 720 bp larger than maize fragments 11 and 25, indicating an insertion; this insertion is also reflected in *Sal*I and *Pst*I fragments. Ambiguities include the uncloned fragments 10, 14, 14′, 15, and numerous small fragments not cloned. Additions to the maize map can be inferred from sorghum data (Table 1); *Pst*I fragments 15, 16, and 17 were positioned by their counterparts in sorghum.

Conservation of coding sequences

The extensive colinearity of the sorghum and maize ctDNAs allows the tentative assignment of coding regions, based on the maize map (5). The beta (atpB) and epsilon (atpE) subunits map in sorghum *Pst*I fragments 13 and 6, sites conserved in both species. Cloned sequences of the maize S cytoplasm S1 molecule hybridized strongly to *Bam*HI fragment 10 (4.65 kb) in sorghum and fragment 8 in maize. Sequences of S1 include part of the coding region of the psbA gene (C. S. Levings, III, personal communication; P. Bedinger and V. Walbot, personal communication). The 23S rRNA gene is positioned on sorghum and maize *Bam*HI fragment 1, which differs in size in the two species. Coding sequences of the 23S rDNA gene include one *Pst*I and one *Sal*I site (5), which are conserved in sorghum. Maize *Bam*HI 13 (3.1 kb), which carries the 16S rRNA (5), was replaced in sorghum by *Bam*HI 14 (3.5 kb), indicating variation within the inverted repeat.

Comparison with other sorghum cytoplasms

The chloroplast genomes of male-sterile milo and male-fertile kafir were compared with IS1112C. Kafir did not have a *Pst*I site at the junction of fragments 5 and 8, in the small single copy region. Milo varied in the large single copy region at *Bam*HI fragments 2 and 15.

Homology of the IR junction fragments with a single copy region

Clone pLD5, which carries the rbcL sequence as detected by the maize clone pZls91 (7), hybridized to maize *Bam*HI fragments 9 and 18. Additional

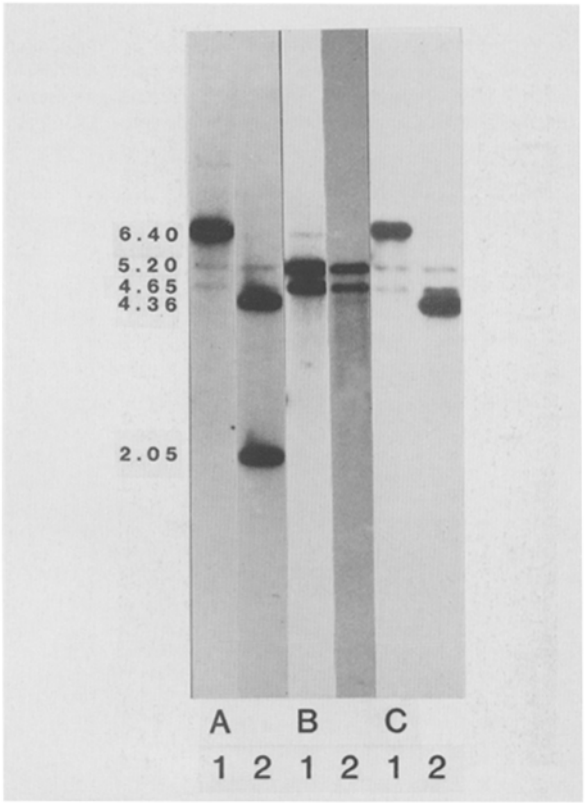

Fig. 3. Hybridization of pLD5 (A), pLD8 (B), and pZls91 (C) clones to *Bam*HI digest of sorghum (1) and maize (2) chloroplast DNA. Numbers are kb values of hybridizing fragments.

hybridization to pLD5, albeit lower than that of apparently homologous fragments, was detected in *Bam*HI fragments 8 and 10 in sorghum and 6 and 8 in maize (Fig. 3A). These fragments are the junction fragments of the IR and the large single copy region. Clone pLD8, a junction fragment opposite junction fragment 10, hybridized readily to fragment 10 in sorghum, fragments 6 and 8 in maize, and to *Bam*HI fragments 5 in sorghum and 9 in maize (Fig. 3B). Clone pZls91 also hybridized to the same fragments (Fig. 3C). Since pLD8 and pZls91 hybridized to *Bam*HI 5 but not 21, we can suggest that the homology observed occurs in a region of ca. 1.7 kb spanned by a *Bam*HI site and a *Pst*I site, the latter within the coding region of rbcL. Verification was obtained by restricting the insert of pLD5 with *Pst*I, and hybridization with pLD8. pLD8 hybridized with only a 1.75 kb fragment (Fig. 4A), which was assigned to the left edge of the pLD5 insert. Homology within the end of

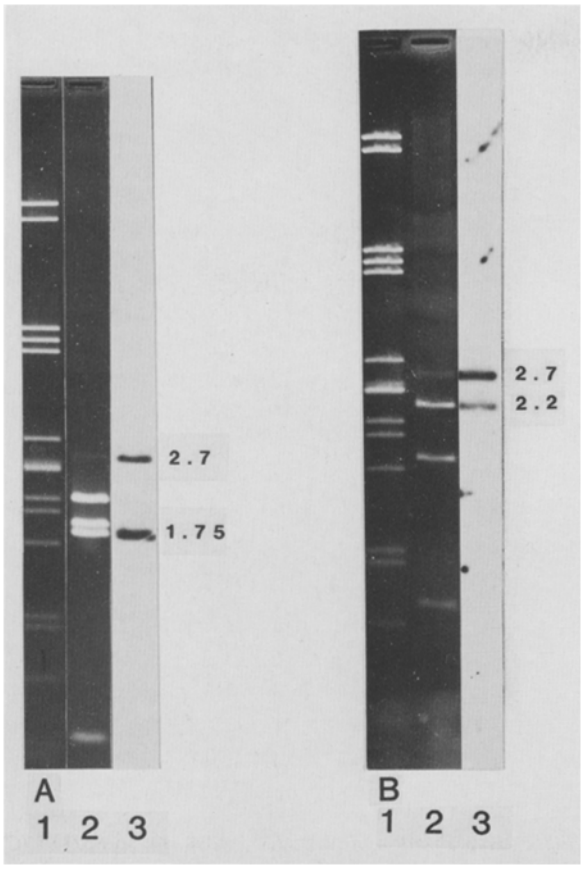

Fig. 4. Hybridization of sequences flanking the rbcL gene with sequences of the inverted repeat. A) ethidium-bromide stained gel of *Pst*I-digested bacteriophage lambda DNA (1), *Pst*I-digested insert of pLD5 (2), and hybridization with pLD8 (3). B) ethidium-bromide stained gel of *Pst*I-digested bacteriophage lambda DNA (1), *Eco*RI-digested insert of pLD8 (2), and hybridization with pLD5 (3). Hybridization at 2.7 kb is residual pUC8 DNA in the insert preparations.

the IR was established by *Eco*RI digestion of the pLD8 insert, and hybridization with pLD5 (Fig. 4B). Following the data of Larrinua *et al.* (5), *Eco*RI digestion of maize *Bam*HI 6 should generate fragments of 400 bp (part of *Eco*RI q), 2200 bp (*Eco*RI m), 1750 bp (*Eco*RI r), and 950 bp (part of *Eco*RI x); these kb values were apparently unchanged in sorghum. pLD5 hybridized to only *Eco*RI fragment m, 2200 bp, which includes the end of the inverted repeat in maize. Similar hybridization was noted in barley (3) and wheat (cited in 3). Thus these four species are characterized by reiterations of the IR near the rbcL gene.

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