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G.-W. Xu · Y.-X. Cui · K. F. Schertz · G. E. Hart

Isolation of mitochondrial DNA sequences that distinguish male-sterility-inducing cytoplasms in *Sorghum bicolor* (L.) Moench

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Abstract We have demonstrated that sorghum DNA sequences of mitochondrial origin can be used to distinguish different male-sterility-inducing cytoplasms. Six DNA clones containing single-copy mitochondrial sequences were hybridized on Southern blots to restriction enzyme-digested DNA of 28 sorghum lines representing sources of different cytoplasmic male-sterility (CMS) groups. Four cytoplasmic types were defined on the basis of the pattern of DNA fragments detected. Similar analyses of 50 additional diverse sorghum accessions suggested that three of the four cytoplasmic types may be diagnostic for CMS. Also, three other cytoplasmic types were discovered. These and other mitochondrial DNA clones may be useful molecular tools for "fingerprinting" sterility-inducing cytoplasms in breeding programs, determining cytoplasmic diversity among germ plasm accessions, and identifying new sources of cytoplasm that induce male sterility.

Key words Cytoplasmic male-sterility · *Sorghum bicolor* · Mitochondrial DNA clones

Introduction

Male-sterile female parents have been used to mass-produce F_1 hybrids in sorghum since the discovery of cytoplasmic-nuclear male sterility by Stephens and Holland (1954). At present, almost all commercial F_1 hybrid seed and their female parents depend on a single cytoplasmic source (3-Dwarf White Sooner Milo) for the induction of male sterility. Such cytoplasmic uniformity predisposes a crop to potential widespread damage, such as the 1970's southern corn leaf blight epidemic. Therefore, it is very important to develop and utilize diverse cytoplasms in the production of sorghum hybrids.

Other sorghum cytoplasms that induce male sterility, in addition to the initially discovered milo cytoplasm, have become available (Hussaini and Rao 1964; Mittal et al. 1958; Nagur and Menon 1974; Quinby 1980; Rao 1962; Ross and Hackerott 1972; Schertz 1977; Schertz and Ritchey 1978; Webster and Singh 1964; Worstell et al. 1984). They come from diverse races and species of *Sorghum* and from different geographic regions. Studies of cytoplasmic-nuclear male sterility in sorghum have been conducted principally in three groups of cytoplasmic-male-sterility (CMS) lines, Indian, Kansas, and Texas lines (Schertz and Pring 1982; Schertz et al. 1989). Some of the Indian CMS lines differ from milo in response to different fertility restorer lines (Tripathi et al. 1980). In Kansas, Ross (1965) and Ross and Hackerott (1972) developed a series of male-sterile lines with kafir nuclei and cytoplasms from wild sorghums, and designated them as KS34 through KS39. Three of the six KS lines have been confirmed to differ from milo in fertility restoration reactions and in mitochondrial (mt) DNA sequences (Conde et al. 1982). Schertz et al. (1989) divided 22 male-sterile lines (Rosenow et al. 1980; Schertz 1977; Schertz and Ritchey 1978; Quinby 1980; Webster and Singh 1964; Schertz, unpublished data) into seven cytoplasmic groups based on the fertility of F_1 progeny derived from crosses with different fertility restorer lines.

The 22 Texas isonuclear lines with diverse cytoplasms have been categorized into two groups based on anther morphology (Schertz et al. 1989). The first group contains CMS lines that have small, pointed anthers, similar to that found in ATx398, a line with milo (A1) cytoplasm, and the second group contains lines that have large, pointed anthers.

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G.-W. Xu
Department of Soil and Crop Sciences, Texas A & M University, College Station, TX 77843 USA, and Energy Biosystems Corporation, The Woodlands, TX 77381, USA

Y.-X. Cui
Department of Plant Pathology and Microbiology, Texas A & M University, College Station, TX 77843, USA

K. F. Schertz
USDR-ARS in Cooperation with Texas A & M University, Department of Soil and Crop Sciences, College Station, TX 77843, USA

G. E. Hart (✉)
Department of Soil and Crop Sciences, Texas A & M University, College Station, TX 77843, USA

Cytoplasm-specific DNA probes would be useful for "fingerprinting" sterility-inducing cytoplasms in breeding programs, determining cytoplasmic diversity among germ plasm accessions, and identifying new cytoplasms that induce male sterility. Previous molecular studies by Pring et al. (1982) and Schertz and Pring (1982) of 18 lines, 6 of which were described by Schertz et al. (1989), established three chloroplast groups and ten mitochondrial groups based on restriction fragment analyses of organellar DNA. More recently, DNA clones derived from 'known-function' genes have been used to distinguish among sorghum lines that contain different cytoplasms. Bailey-Serres et al. (1986) differentiated lines IS17218 (9E) and IS 7920C (A4) by the detection of a 1.9-kb *Hin*dIII fragment in IS7920C with a *coxI* (cytochrome c oxidase subunit) clone. Pring et al. (1988) distinguished lines IS17218 (9E) and IS1112C (A3) from one another using an *atp6* (ATPase subunit 6) probe, and IS17218 and IS1112C from milo, KS37, and KS39 using *rrn18* and *rrn26* (18 s and 26 s rDNA) probes. Also, Mullen et al. (1992), using an *atp6* probe, showed that Tx398 (fertile cytoplasm) and IS1112C (A3) differ from one another. In addition, Chase and Pring (1986) differentiated the cytoplasm of IS12565C (A3) from that of IS1112C (A3) by the occurrence of N1 and N2 plasmid-like DNAs and 1.7 and 2.3-kb minicircle plasmids in the latter.

Among the clones isolated from a sorghum DNA library during the development of a restriction fragment length polymorphism (RFLP) map of sorghum (Xu et al. 1994) were six clones that appeared to be of organellar origin and revealed unique polymorphic patterns. The purpose of the present study was to determine if these organellar clones could be used to distinguish among male-sterility-inducing sorghum cytoplasms.

Table 1 Sorghum lines containing male-sterility-inducing cytoplasms

Cytoplasm source			Cytoplasmic sterility group[c]
Line[a]	Race or species[b]	Origin	
3-Dwarf Milo[d]	D	Unknown	A1*
IS1116C[f]	G	India	A1
IS2266C	G	Sudan	A1
IS3579C	C	Sudan	A1
IS6705C	G	U. Volta	A1
IS6771C	G-C	India	A1
IS7007C	G	Sudan	A1
IS7502C	G	Nigeria	A1
IS8232C	(K-C)-C	India	A1
IS2573C	C	Sudan	A2
IS2816C	C	S. Rhodesia	A2
IS12662C	G	Nigeria	A2*
IS1112C	D-(DB)	India	A3*
IS6882C	K-C	Unknown	A3
IS12565C	C	Sudan	A3
IS7920C	G	Nigeria	A4*
IS17218	N/A	Nigeria	9E*
IS12603C	G	Nigeria	9E
IS7506C	B	Nigeria	A5*
IS1056C	D	India	A6*
IS2801C	D	S. Rhodesia	A6
IS3063C	D	Ethiopia	A6
SA1741	A		KS34
PI258806	A		KS35
PI208190	V		KS36
PI247722	*S. sudanense*		KS37
PI155140	*S. conspicuum*		KS38
Kenya 53262	*S. niloticum*		KS39

[a] The first 22 cytoplasms were in Tx 398 nuclear backgrounds and the last 6 were in Tx 3197 nuclear backgrounds
[b] B=bicolor, C=caudatum, D=durra, G=guinea, and K=kafir races of *S. bicolor* spp. *bicolor*. A=arundinaceum and V=verticilliflorum races of *S. bicolor* spp. *verticilliflorum*
[c] Based on fertility restoration response
[d] 3-Dwarf Milo=3-Dwarf White Sooner Milo
[e] IS/C number indicates a converted International Sorghum line
[f] Type member for each fertility group

Materials and methods

Plant materials

Twenty-eight sorghum CMS lines, including 22 Texas and 6 KS lines (Table 1), and 50 other sorghum accessions representing nine races and three subspecies of *S. bicolor* of different geographical origins (Table 2) were studied. The 22 Texas isonuclear CMS lines had been classified into seven groups based on observations of the fertility of F_1 progeny obtained by crossing the lines to a tester set (Schertz et al. 1989 and unpublished).

Young leaves were collected from 3-week-old greenhouse-grown seedlings of each sorghum line. Leaf samples were processed and stored as described previously (Xu et al. 1994).

Probe source and preparation

Six *Pst*I sorghum clones, pSbTXS349, pSbTXS1027, pSbTXS1058, pSbTXS1168, pSbTXS1177, and pSbTXS1278, which contain inserts ranging in size from 145 to 3260 bp, were used in this study (clones designations are abbreviated hereafter as TXS plus a number). The plasmids were isolated and purified according to the protocol of Birnboim and Doly (1977). DNA fragment inserts were released from the plasmids by *Pst*I digestion, purified by electrophoresis in low-melting-point agarose gels, and then labeled with [^{32}P]-dATP as described by Feinberg and Vogelstein (1983). The labeled DNA fragments were purified through a spin column, denatured, and added to the hybridization solution (Maniatis et al. 1982).

DNA isolation, digestion and Southern hybridization

Total DNA was extracted and purified from either fresh or freeze-dried leaf tissue by a modification (Saghai-Maroof et al. 1984) of the method of Murray and Thompson (1980). Plant samples, 0.6 g of dried powder or 6 g of fresh leaf that had been powdered by grinding in liquid nitrogen, were incubated in DNA extraction buffer (200 m*M* TRIS, pH 8.0, 10 m*M* EDTA, 25 m*M* β-mercaptoethanol, and 2% hexadecyltri-methylammonium bromide [CTAB]) for 3–4 h at 65°C with occasional gentle inversion. The DNA present in the supernatant was first precipitated according to the described protocol, then redissolved in TE buffer and quantified by fluorometry (TKO 100, Hoefer). DNA digestion, electrophoresis (5.0–7.5 µg/lane), blotting, hybridization, and post-hybridization washes were performed as described previously (Xu et al. 1994).

Isolation of the mitochondria from leaf tissue, and preparation and purification of mtDNA were performed according to the method of Pring et al. (1982).

Table 2 Sorghum lines used to determine cytoplasmic diversity and to identify new cytoplasm types

Line	Race	Origin	Cytoplasm type[a]
S. bicolor spp. *bicolor*			
Chinese Amber, FC8728	bicolor	China	B
Chinese Nat. Acc. No. 422	bicolor	China	B
CI171	bicolor	China	B
EBA-9	bicolor	Sudan	B
Shanqui Red	bicolor	China	E
Standard Broomcorn, CI556	bicolor	USA	B
Sweet Sudangrass, SA372	bicolor	Sudan	A
IS6964	bicolor	Sudan	B
IS1598C	bicolor	India	B
IS7542C	bicolor	Nigeria	B
BTx623	caudatum/kafir	USA	B
M91051	caudatum	USA	B
IS6710C	caudatum	Senegal	B
IS12568C	caudatum	Sudan	B
IS12608C	caudatum	Ethiopia	B
IS1022C	durra	India	B
IS12570C	durra	Sudan	B
IS7333C	durra	Nigeria	B
SA7078	durra	Unknown	B
Tx403	durra	USA	A
IS3477C	guinea	Sudan	B
IS3614C	guinea	Nigeria	B
IS3620C	guinea	Nigeria	A
IS3955C	guinea	Nepal	A
IS5332C	guinea	India	B
IS7173C	guinea	Tanzania	G
IS7419C	guinea	Nigeria	B
IS7920	guinea	Nigeria	B
BTx378	kafir	USA	B
BTx398	kafir	USA	B
BTx3197	kafir	USA	B
87-4325	kafir	S. Africa	B
87-4326	kafir	S. Africa	B
87-4327	kafir	S. Africa	B
87-4328	kafir	S. Africa	B
87-4329	kafir	S. Africa	B
S. bicolor spp. *verticilliflorum*			
IS14564	aethiopicum	Sudan	B
IS18820	aethiopicum	Ivory Coast	B
PI302105	aethiopicum	Ethiopia	B
PI156549	arundinaceum	Rhodesia	A
IS18826	arundinaceum	Ivory Coast	F
PI186570	arundinaceum	Nigeria	A
IS14505	verticilliflorum	India	B
IS18798	verticilliflorum	S. Africa	B
PI267331	verticilliflorum	India	B
IS18803	virgatum	Unknown	B
IS18809	virgatum	Egypt	B
B35	Unknown	USA	B
RTx430	Unknown	Unknown	B
QL3-India	Unknown	India	B

[a] Cytoplasm types were defined as A, B, C, E, F, and G on the basis of the pattern of DNA fragments detected using probe/enzyme combinations

Results

Demonstration that plasmids contain mtDNA

An organellar origin for the sorghum DNA sequences contained in the six clones that were used (TXS349, TXS1027, TXS1058, TXS1168, TXS1177, and TXS1278) was suggested by the fact that each of the clones hybridized to DNA fragments of different sizes in the parents of the F_2 mapping population used by Xu et al. (1994) but only fragments from the maternal parent were detected in the F_2 population. Evidence that the clones are of mitochondrial origin was obtained by the demonstration that radiolabeled mtDNA, but not nuclear DNA, hybridized to the clones on Southern blots. A mitochondrial origin of the sequences

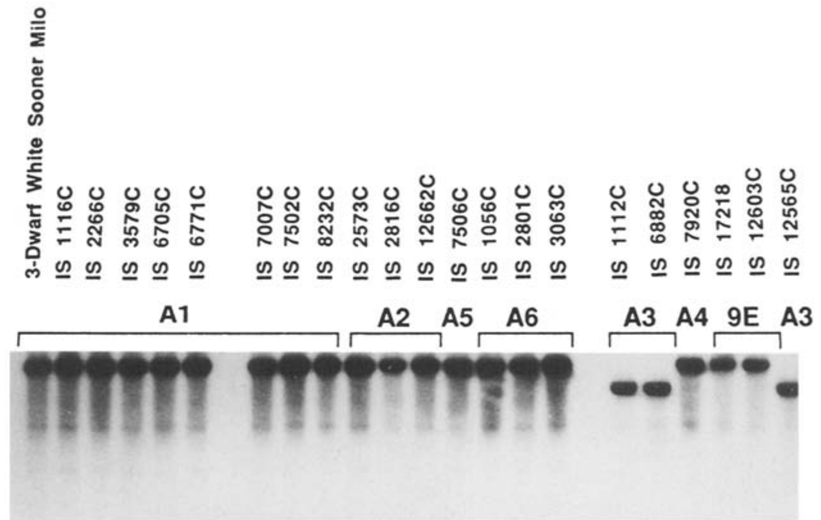

Fig. 1 Southern blot hybridized with TXS1058 as a probe. The blot contains *Xba*I-digested genomic DNAs from sorghum lines of the indicated A1, A2, A3, A4, A5, A6, and 9E CMS groups. The 5.5-kb fragment is found only in lines with A3 cytoplasm

was further confirmed using DNAs from accession IS1112C (a source of a sterility-inducing cytoplasm) and KS36, which were digested with *Bam*HI, *Hin*dIII, and *Pst*I and probed with sorghum total mtDNA and each of the six mtDNA clones, respectively, in an attempt to compare the DNA fragments detected. All of the DNA fragments detected using the mtDNA clones as probes corresponded to DNA fragments detected when total sorghum mtDNA was hybridized to the same Southern blot.

Distinguishing among male-sterility-inducing cytoplasms with DNA probes

Total genomic DNA purified from 28 sorghum lines (Table 1) possessing male-sterility-inducing cytoplasms was digested with five restriction enzymes (*Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I) and hybridized with the six TXS probes that contain mtDNA sequences.

All of the probes hybridized to single or low-copy-number DNA fragments. Within each cytoplasmic group, each probe detected the same pattern of DNA fragments when hybridized to Southern blots containing DNAs cut with the same restriction enzyme. No variation was found between the lines within any cytoplasmic sterility group. Variation in the pattern of DNA fragments was found, however, between some of the cytoplasmic sterility groups (Fig. 1).

On Southern blots prepared with each of the five restriction enzymes, clone TXS349 hybridized strongly to a single DNA fragment from all lines in the A3, A4, and 9E fertility groups, but it did not hybridize to any DNA fragment from lines in the A1, A2, A5, and A6 groups. When hybridized to DNA cut with *Eco*RV, TXS349 hybridized to a 9.2-kb A3 fragment, a 15.8-kb A4 fragment, and a 16.8-kb 9E fragment (Fig. 2A). With the other four restriction enzymes, the fragment to which TXS349 hybridized was the same in lines from all three (A3, A4, and 9E) groups (Table 3).

TXS1058, a 1630-bp sequence, hybridized to either one or two fragments per line in the DNAs from all the 28 male-sterile lines digested with each of the five restriction enzymes. When hybridized to DNA digested with *Eco*RV, two fragments were detected. One, 3.4 kb in size, had a high signal level but was invariant across all lines tested (Fig. 2B). The second fragment, with a low signal level, was 9.7 kb in size in A3 lines, 8.5 kb in 9E lines, and 8.1 kb in size in the other lines. TXS1058 also detected a 5.5-kb fragment that is specific to A3 lines when hybridized to *Xba*I-digested DNAs (results not shown).

TXS1168, a 1040-bp sequence, detected one fragment in each of the 28 lines. The fragment to which TXS1168 hybridized was invariant in size across all lines tested when the DNA of the lines was digested with *Eco*RI, but when it was digested with *Bam*HI, *Eco*RV, *Hin*dIII, or *Xba*I, A3, A4, and 9E lines had in common a fragment of one size and A1, A2, A5, A6, and KS lines had in common a fragment of a different size. With *Eco*RV, the A3-, A4-, and 9E-specific fragment was 8.1 kb in size and the other fragment was 5.8 kb in size (Fig. 2C).

TXS1177, a 1120-bp sequence, hybridized to one fragment per line on Southern blots containing DNA digested with *Eco*RV or *Xba*I. Results obtained with *Xba*I are shown in Fig. 2D. A 5.2-kb fragment was specific to A3 lines and a 7.8-kb fragment was detected in all other lines tested.

No polymorphism was observed among the 28 male-sterile lines listed in Table 1 with TXS1027 or TXS1278 with any of the five restriction enzymes.

The clone-restriction enzyme combination of TXS349 and *Eco*RV divided the 28 CMS lines studied into four groups, one consisting of A1, A2, A5, A6, and KS lines, a second of A3 lines only, a third of the A4 line and a fourth of 9E lines. The same grouping of lines was obtained using two clone/enzyme combinations, i. e., either TXS349 or TXS1168 with any one of four restriction enzymes (*Bam*HI, *Eco*RI, *Hin*dIII and *Xba*I) along with either TXS1058 or TXS1177 with either *Eco*RV or *Xba*I. The four

Table 3 RFLP patterns observed and cytoplasm types identified using mtDNA clones as probes on genomic DNA from 28 sorghum male-sterile lines digested with restriction enzymes

Cytoplasm sources	Cytoplasmic sterility group	pSbTXS probe Restriction enzyme(s)					Cytoplasm type[b]
		349 RV[a]	349 B, RI, H, X	1058 RV, X	1168 B, RV, H, X	1177 RV, X	
3-Dwarf Milo[c]	A1[d]	I	I	I	I	I	A
IS1116C	A1	I	I	I	I	I	A
IS2266C	A1	I	I	I	I	I	A
IS3579C	A1	I	I	I	I	I	A
IS6705C	A1	I	I	I	I	I	A
IS6771C	A1	I	I	I	I	I	A
IS7007C	A1	I	I	I	I	I	A
IS7502C	A1	I	I	I	I	I	A
IS8232C	A1	I	I	I	I	I	A
IS2573C	A2	I	I	I	I	I	A
IS2816C	A2	I	I	I	I	I	A
IS12662C	A2[d]	I	I	I	I	I	A
IS1112C	A3[d]	II	II	II	II	II	B
IS6882C	A3	II	II	II	II	II	B
IS12565C	A3	II	II	II	II	II	B
IS7920C	A4[d]	III	II	I	II	I	C
IS17218	9E[d]	IV	II	III	II	I	D
IS12603C	9E	IV	II	III	II	I	D
IS7506C	A5[d]	I	I	I	I	I	A
IS1056C	A6[d]	I	I	I	I	I	A
IS2801C	A6	I	I	I	I	I	A
IS3063C	A6	I	I	I	I	I	A
SA1741	KS34	I	I	I	I	I	A[f]
PI258806	KS35	I	I	I	I	I	A
PI208190	KS36	I	I	I	I	I	A[f]
PI247722	KS37	I	I	I	I	I	A
PI155140	KS38	I	I	I	I	I	A
Kenya 53262	KS39	I	I	I	I	I	A

* Type member for each fertility group
[a] B=*Bam*HI, RI=*Eco*RI, RV=*Eco*RV, H=*Hin*dIII, and X=*Xba*I
[b] As defined on the basis of the patterns of DNA fragments detected using probe/enzyme combinations
[c] 3-Dwarf Milo=3-Dwarf White Sooner Milo
[d] Type member for each fertily group
[e] RFLP patterns observed (see text)
[f] Cytoplasm type A with probe/restriction enzyme combinations listed in the table, but cytoplasm type F with TXS1027/*Sac*II; see text

cytoplasmic types defined on the basis of these findings are designated A (A1, A2, A5, A6, and KS lines), B (A3 lines), C (A4 line), and D (9E lines) (Table 3).

Cytoplasmic diversity among sorghum accessions

To evaluate the possibility that mtDNA clones can be used to identify diversity among cytoplasms of lines not known to differ in cytoplasm, DNA from 50 diverse sorghum accessions was purified, digested with restriction enzymes, and hybridized on Southern blots to radiolabeled mtDNA clones. Two of the cytoplasmic types defined above, A and B, were detected in 1 or more lines and three new cytoplasmic types, designated E, F, and G, were identified.

TXS349 is one of three clones that distinguishes A3 CMS lines from all other CMS lines assayed (Table 3). It detected an A3-specific DNA fragment (9.2 kb) when hybridized to *Eco*RV-digested total DNA from the lines. TXS1058 and TXS1177 did likewise when hybridized to either *Eco*RV- or *Xba*I-digested total DNA. On the basis of these findings, cytoplasm type B was defined. It is clear, however, that cytoplasm type B is found not only in CMS lines. As shown in Fig. 3, the DNA fragment which TXS349 detects in *Eco*RV-digested DNA is present in 43 of the 50 lines, including many lines that are not known to be CMS-inducing. The same results were obtained with TXS1058 and TXS1177 (results not shown).

In contrast to cytoplasm type B, the results obtained indicate that cytoplasm type A, found for all of the A1, A2, A5, A6, and KS CMS lines studied, cytoplasm type C, found for the 1 A4 CMS line studied (Table 3), and cytoplasm D, found for the 2 9E CMS lines studied, may well be specific to these CMS lines. Cytoplasm type A is defined by clones TXS349 and TXS1168. The former clone does not hybridize to a DNA fragment in any cytoplasm type A CMS line (Fig. 2A), while the latter detects a common DNA fragment (5.8 kb) among CMS lines of cytoplasm type A (Fig. 2C). Of the 50 diverse lines studied 6 (Sweet Sudangrass SA372, IS3620C, Tx403, IS3955C,

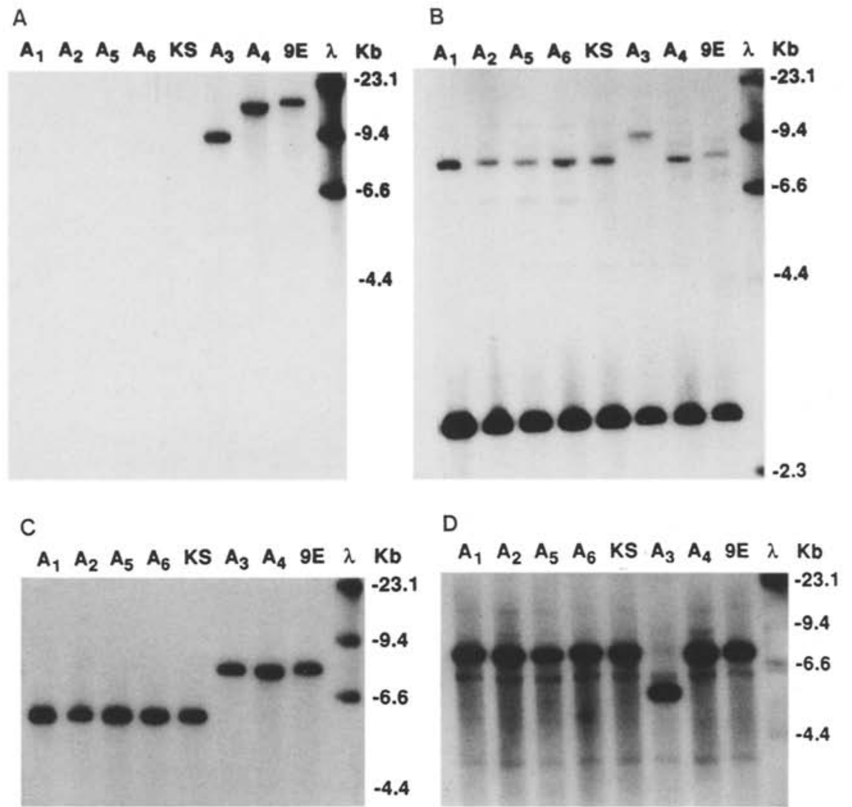

Fig. 2A–D Southern blots hybridized with TXS349 (**A**), TXS1058(**B**), TXS1168(**C**), and TXS1177(**D**) as probes, respectively. The blots contain *Eco*RV- (blots A, B, and C) or *Xba*I-digested (blot D) genomic DNAs from sorghum lines with the indicated types of male sterility-inducing cytoplasm. Molecular size standards are shown on the *right*

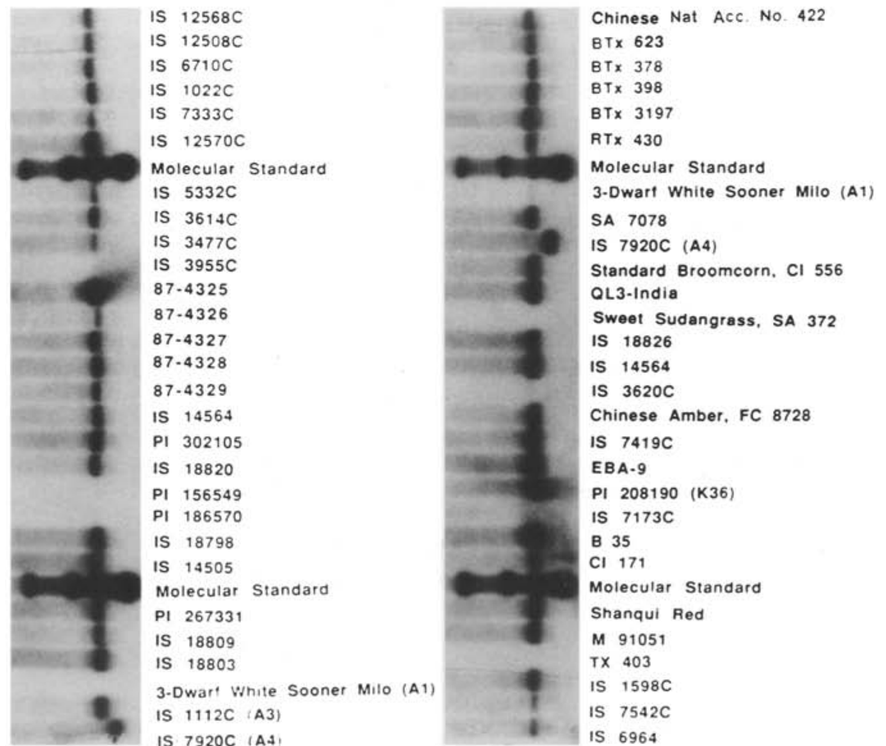

Fig. 3 Southern blot hybridized with TXS349. The blot contains *Eco*RV-digested genomic DNAs from 50 sorghum lines of a germ plasm collection and 6 lines known to have either an A1, A3, A4, or KS cytoplasm. Molecular size standards are present in the *7th* and *24th* lanes of both blots

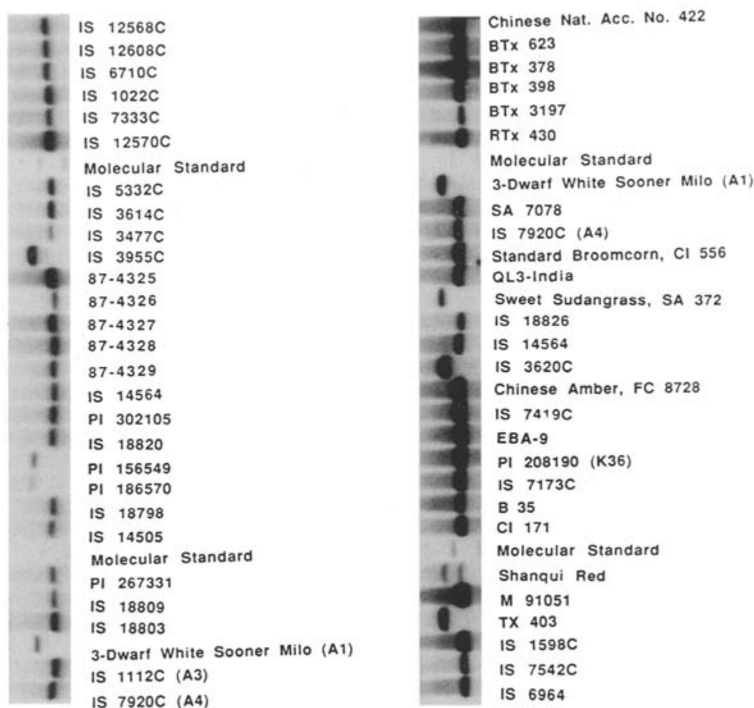

Fig. 4 Southern blot hybridized with TXS1168. The blot contains *Eco*RV-digested genomic DNAs from 50 sorghum lines of a germ plasm collection and 6 lines known to have either an A1, A3, A4, or KS36 cytoplasm. Molecular size standards are present in the *7th* and *24th* lanes of both blots

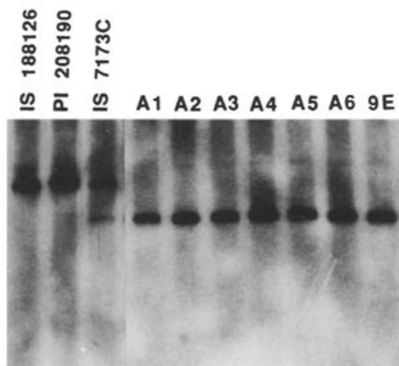

Fig. 5 Southern blot showing two newly-identified cytoplasmic types. The blot contains *Sac*II-digested genomic DNAs from sorghum lines with A1 through A6 and 9E cytoplasms and from accessions IS188126, PI208190, and IS7173 hybridized with TXS1027

PI156549, and PI186570) displayed the type A fragment pattern (Figs. 3 and 4). Cytoplasm type C is defined by the hybridization of clone TXS349 to a 15.8-kb *Eco*RV fragment and cytoplasm type D by the hybridization of TXS349 to a 16.8-kb *Eco*RV fragment. Neither of these cytotypes were detected among the 50 diverse lines studied.

TXS1168 hybridized to two Shanqui Red *Eco*RV fragments, one of 5.8 kb and one of 9.2 kb, identifying a new cytoplasm type, designated E. TXS1027 did not detect any differences among the 28 CMS lines, but it did identify two new cytoplasm types among the 50 diverse additional accessions studied. When hybridized to total DNAs digested with *Sac*II, TXS1027 hybridized to a 5.8-kb fragment found in lines IS188126 and PI208190 only (cytoplasm type F) and to both a 5.8-kb fragment and a 4.7-kb fragment in line IS7173C only (cytoplasm type G) (Fig. 5). When hybridized to *Eco*RI-digested DNA, TXS1027 hybridized to a 9.5-kb fragment found only in these same 3 lines among the 50 diverse lines studied (results not shown).

Discussion

Pring et al. (1982), Conde et al. (1982), and Schertz and Pring (1982) differentiated sorghum lines into groups based on the RFLP pattern produced by ethidium bromide staining of restriction enzyme-digested mitochondrial and chloroplast DNA. Three chloroplast and ten mitochondrial groups were identified among 19 CMS and 5 fertile lines tested. Our cytoplasmic types are based on different probe/enzyme combinations, and the fertility restoration groups are now better understood than at the time of those earlier reports. The associations described, therefore, do not completely correspond to the earlier reports.

It is interesting to note that the clone and restriction-enzyme combinations of TXS349 with *Bam*HI, *Eco*RI, *Hin*dIII, and *Xba*I and TXS1168 with *Bam*HI, *Eco*RV, *Hin*dIII, and *Xba*I distinguish the small-anther from the large-anther CMS groups as described by Schertz et al. (1989). They also reported that, based on fertility tests, A4 and 9E cytoplasms are similar but not identical. In our study, as in that of Bailey-Serres et al. (1986), A4 and 9E lines were

found to have different cytoplasmic types, consistent with the fertility results.

Five cytoplasmic types, four of which are potentially diagnostic for CMS lines, were identified among the 50 diverse accessions that we studied. Among the 6 accessions in this group with cytoplasm type A, 1 accession, IS3620C, has been reported to have a cytoplasm that induces male sterility (Kaul 1988). Cytoplasm types E and G, each found in one line, were not found in any of the 28 known CMS lines studied. Cytoplasm F was found in 1 known CMS line, PI208190, and 1 additional line, IS18826. We have initiated studies to determine the CMS capability of the cytoplasm type E, F, and G lines and of the 5 cytoplasm type A lines whose CMS capability is unknown.

We have demonstrated that RFLPs detected among mtDNAs using mtDNA clones as probes revealed correlations between CMS groups and RFLP groups. These and other mtDNA clones may be useful molecular tools for "fingerprinting" sterility-inducing cytoplasms in breeding programs, determining cytoplasmic diversity among germ plasm accessions, and identifying new sources of cytoplasm with the potential to induce male sterility.

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