Variations in mitochondrial DNA organisation between normal and male-sterile cytoplasms of maize

Richard D.Thompson, Roger, J.Kemble and Richard B.Flavell

Plant Breeding Institute, Maris Lane, Trumpington, Cambridge, CB2 2LQ, UK

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ABSTRACT

Mitochondrial DNA from male-sterile lines of maize carrying S cytoplasm contains two small DNA species which are absent from N (fertile) and other male-sterile cytoplasms. Portions of these species have been purified and amplified by constructing recombinant plasmids *in vitro*. Probes made with these plasmids have been used to demonstrate; i) a homologous region in the N mitochondrial genome, which may indicate the origin of the S specific DNA species. ii) two other DNA species present in low amounts in S cytoplasm only. iii) the absence of strong homology to the S specific DNA species in mitochondria from C and T male-sterile cytoplasms.

INTRODUCTION

Cytoplasmically inherited male sterility in maize (*Zea mays* L.) has been correlated with alterations in the organisation and expression of the mitochondrial genome. Four genetically different cytoplasms (1-3) are known in maize; N cytoplasm, which allows normal fertile pollen development, and T, C and S cytoplasms which cause pollen sterility. Mitochondria from each of these cytoplasmic types may be distinguished by agarose gel electrophoresis of their DNA species, either undigested (4-6) or digested with restriction endonucleases (7, 8), or by their proteins translated *in vitro* (9-11).

Mitochondrial DNA (mt DNA) from S lines contains two low molecular weight DNA species, S1 and S2, of 6.2 kb and 5.2 kb respectively. They are of unknown origin and are not seen in mitochondria from N, T or C cytoplasms (6). In S lines which have reverted to male-fertility due to cytoplasmically inherited changes (12) these DNA species are absent (13). This suggests that the organisation of these DNA sequences may be related to cytoplasmic male-sterility. Sequences of S1 and S2 have been cloned in the plasmid pBR322 and used as probes to study their organisation in

mt DNAs isolated from plants with N, T, C and S cytoplasms.

MATERIALS AND METHODS

Isolation of DNA

Mt DNA was isolated and purified from maize lines possessing C0192 x WJ nuclear background carrying N, T, C and S cytoplasms as previously described (5). The mt DNA isolated from the line with S cytoplasm was further purified for cloning by equilibrium centrifugation in CsCl-ethidium bromide gradients (14).

Plasmid DNAs were isolated from chloramphenicol-amplified cultures according to the method of Clewell (15).

Cloning of S1 and S2 DNAs

S1 and S2, the two low molecular weight mt DNA species found only in S cytoplasms (6) were separated from main-band mt DNA by velocity sedimentation on a linear 5-20 per cent sucrose gradient containing 0.1 M NaCl, 10 mM Tris HCl pH 8.0, 1 mM EDTA at 110,000 x g for 11 hours. Fractions containing S1 and S2 DNAs were detected by electrophoresis (6), pooled and reprecipitated with ethanol prior to partial digestion with Mbo I, which produces the same 5' cohesive projections (5'GATC-) as Bam HI. These fragments were reannealed with a large molar excess of pBR322 which had previously been digested with Bam HI. The mixture was incubated with T4 DNA ligase (16) and transformed (17) into E. coli HB101 (18).

Identification of clones

$\text{Amp}^R \text{ Tet}^S$ transformants were probed with cRNA (19) prepared from S1 and S2 enriched fractions of the sucrose gradients using the colony hybridisation technique of Grunstein and Hogness (20). Clones giving a positive signal were propagated, and plasmid DNA purified. To identify cloned sequences complementary to S1 and/or S2 DNA, a nick-translated (21) probe from each plasmid DNA was hybridised to a nitrocellulose filter (22) carrying S cytoplasm mt DNA.

DNA electrophoresis, transfer to nitrocellulose and hybridisations

Mt DNA samples were fractionated by electrophoresis on 1.0 per cent or 1.5 per cent agarose gels and transferred to nitrocellulose filters by the method of Southern (22). Filters were prehybridised at 65° in 2 x SSC containing 0.1 per cent SDS and 0.2 per cent each of Ficoll, BSA and PVP. Hybridisations were carried out by using fresh solution, adding 5×10^5 - 2.5×10^6 cpm freshly boiled nick-translated plasmid DNA, and

shaking rapidly at 65°C for 24 hours. Filters were washed in 2 changes of 500 ml 2 x SSC at 65°C with shaking for at least 3 hours prior to drying and autoradiography.

RESULTS

In order to study the distribution of sequences homologous to S1 and S2 in mt DNAs from plants carrying N, T, C and S cytoplasms it was necessary to obtain completely purified probes. This was achieved by cloning Mbo I fragments of S1 and S2 DNAs in the plasmid pBR322 as described in Materials and Methods. Three different kinds of clones were obtained; those which contained sequences complementary to S2 but not S1 (eg. pZmS4), those which contained sequences complementary to S1 but only slightly complementary to S2 (eg. pZmS42) and those which contained sequences complementary to S1 and S2 (eg. pZmS21). Properties of the inserted DNA in the three clones used in this study are summarised in Table 1.

DNAs extracted from mitochondria of plants carrying N, C, T and S cytoplasms were fractionated by electrophoresis on an agarose gel, with and without prior digestion by Bam HI restriction endonuclease (Fig. 1). The low molecular weight DNA species characteristic of undigested N, T, C and S mt DNAs are labelled S1, S2, t, oc, l, cl, c2 and ccc in Figure 1. They are described in detail elsewhere (4). Preparations of nuclear DNA (14) and high molecular weight mt DNA (purified free of S1 and S2) from a line carrying S cytoplasm were restricted with Bam HI and run in parallel tracks (Fig. 1). Figure 2 shows the autoradiograph obtained when nick-translated pZmS42 (homologous to S1 DNA) was hybridised to a nitro-

Table 1. Characterisation of clones from S1 and S2 mt DNAs.

Clone	Insert size (Kb)	Insert complementary to	
		S1	S2
pZmS4	2.1	-	+
pZmS21	4.0	+	+
pZmS42	1.5	+	faint

The inserts, which could not be excised with Bam HI, were sized by calculation from the HaeIII, EcoRI and HinIII single and double digest products.

Figure 1

Figure 2

cellulose filter (22) of this gel. The probe hybridised to S1 DNA of 6.2 Kb in both the undigested and Bam HI digested S mt DNA samples (tracks S and S') suggesting S1 does not have a Bam HI target. The faint hybridisation to 4.0 Kb fragments in the Bam HI digested S mt DNA coincides with the largest Bam HI digest product of S2 DNA (see below and Fig. 3) and reflects the slight complementarity of the probe to S2 (Table 1).

The S1 probe hybridised only very faintly to DNAs from lines carrying T and C cytoplasms, and to the S cytoplasm mt DNA preparation lacking S1 and S2 DNA (Fig. 2). Subsequent experiments show this more clearly (see below and Fig. 4). The probe hybridised strongly to high molecular weight mt DNA from lines with N cytoplasm, however. On digestion with Bam HI a single restriction fragment containing 7.2 Kb hybridised to the probe (track N').

Figure 3 shows the autoradiograph obtained when an S2-selective probe (pZmS4) was hybridised to a nitrocellulose filter of samples fractionated as in Figure 1. This probe hybridised strongly to S2 DNA (track S) and to two major Bam HI restriction fragments containing 4.0 and 0.95 Kb (track S'). The probe also hybridised to the 2.35 Kb DNA species found in undigested N, C, and S cytoplasm mt DNAs (labelled "t" in Fig. 1). In mt DNA from lines carrying T cytoplasm, the equivalent DNA species appears to be smaller (1.94 Kb), which suggests T cytoplasm carries a deletion in this DNA species. No other S2-specific hybridisation was seen in T or C cytoplasm mt DNA, but high molecular weight mt DNA from lines carrying N cytoplasm did show homology (track N), present in two Bam HI fragments (track N') containing 4.7 and 0.95 Kb. The smaller fragment has the

Figure 1. Electrophoresis on 1.5% agarose gel of mt DNA preparations from different maize cytoplasms in C0192 x WJ nuclear background. Tracks N, C, T and S are mt DNA extracts from the respective cytoplasms, not subjected to restriction endonuclease digestion, showing the characteristic banding patterns (labelled as DNA species) previously described (4). Tracks N', C', T' and S' are mt DNA extracts from the respective cytoplasms which have been digested with Bam HI. Track S^m is a Bam HI digest of an S cytoplasm mt DNA extract lacking S1 and S2 which were removed by fractionation on a linear sucrose gradient. Track nu^S is a Bam HI digest of nuclear DNA isolated from purified nuclei obtained from a line carrying S cytoplasm. Size markers were obtained from an Eco RI digest of lambda bacteriophage DNA and reference 4.

Figure 2. Autoradiograph showing hybridisation to a nitrocellulose filter (22) of the gel in Fig. 1 by pZmS42 which contains DNA sequences complementary to S1. Details of the gel tracks and markers are given in Fig.1.

Figure 4

Figure 3

same mobility in the gel as the fragment cleaved from S2 by Bam HI.

In order to study further the faint hybridisation observed in N, T and C cytoplasms, and that due to DNA species larger than S1 and S2 in S cytoplasm (Fig. 2) the mt DNA was electrophoresed for a longer time and on a 1 per cent, rather than 1.5 per cent, agarose gel. The amount of S cytoplasm mt DNA was also reduced by 10-fold to prevent adjacent tracks becoming obscured on autoradiography. After transfer to nitrocellulose filters (22) the DNAs were probed with pZmS21 which hybridises to both S1 and S2 DNA. The autoradiograph from this experiment is shown in Figure 4. In undigested mt DNA from lines carrying S cytoplasm, two species containing 13.2 and 12.1 Kb hybridised to the probe in addition to S1 and S2 (track S). Hybridisation occurred to fragments containing 16.5, 12.4 and 10.0 Kb in Bam HI-digested S mt DNA lacking S1 and S2 DNA species (Track S^m). Of these fragments only that containing 16.5 Kb is found in other mt DNAs. It is present in mt DNA from N and C but not T cytoplasms.

In Bam HI-digested high molecular weight mt DNA from S cytoplasm seven other fragments show faint hybridisation to the pZmS21 probe (Fig. 4, track S^m). The 6.2 and 4.0 Kb fragments probably represent residual S1 and S2. The 7.2 and 4.7 Kb fragments are similar in mobility to the two major hybridising Bam HI fragments in N mt DNA (track N') and to a 7.2 Kb fragment in T mt DNA (track T'). The 6.0 and 5.1 Kb Bam HI fragments are present in N, C, T and S mt DNAs. The 4.5 Kb fragment is also represented in Bam HI-digested mt DNA from C cytoplasm (track C'). Bam HI-digested mt DNA from T cytoplasm (track T') contains a hybridising fragment of 4.4 Kb. In nuclear DNA preparations (track nu^S), only fragments similar in mobility to S1 of Bam HI-digest products of S1 and S2 hybridised to the probe. This suggests a slight contamination of the nuclear DNA with mt DNA.

Figure 3. Autoradiograph showing hybridisation to a nitrocellulose filter (22) of the gel in Fig. 1 by pZmS4 which contains DNA sequences complementary to S2. Details of the gel tracks and markers are given in Fig. 1.

Figure 4. Autoradiograph showing hybridisation to a nitrocellulose filter (22) of a 1.0 per cent agarose gel by pZmS21 which contains DNA sequences complementary to S1 and S2. The samples electrophoresed on the gel were the same as those in Fig. 1 except track m which is a marker track obtained by single digests of pBR322 by Hin dIII and Taq I. The amount of sample in tracks S and S' is 10 times less than all the other tracks.

The hybridisation patterns in Figure 4 obtained from N, T, C and S cytoplasms in C0192 x WJ nuclear background have also been observed for N cytoplasm in B9A nuclear background, T cytoplasm in W33, Q cytoplasm (a member of the T cytoplasm group) (2,3,5,11) in C0192 x WJ, S cytoplasm in WF9, CA cytoplasm (a member of the S cytoplasm group) (2,3,5,11) in C0192 x WJ, and C cytoplasm in B37. This suggests that the hybridisation patterns in Figure 4 are representative of the four cytoplasmic types (5,11).

DISCUSSION

The hybridisation data presented here show a correlation between cytoplasmically inherited male sterility and the lack of strong hybridisation of S1 and S2 containing probes to high molecular weight mt DNA. In contrast, these probes hybridised strongly to high molecular weight mt DNA from plants with N, fertile, cytoplasm. In Bam HI digests of N mt DNA, the fragments hybridising to the S1 and S2 probes are longer than the sum of S1 and S2. This suggests that in N mitochondria fragments homologous to S1 and S2 are integrated into high molecular weight mt DNA. It is therefore possible that S1 and S2 have arisen by excision from mt DNA similar to that found in plants with N cytoplasm. In lines with S cytoplasm, S1 and S2 are produced in equimolar amounts and amplified to more than a five fold molar excess over the most abundant high molecular weight DNA species. Two other DNA species unique to S cytoplasm and smaller than high molecular weight mt DNA have been detected by hybridisation (Fig. 4). These DNA species are restricted by Bam HI to fragments containing approximately 12.4 and 10.0 Kb. These estimates suggest they may be dimer variants of S1 and S2.

The genetic stability of the male sterile cytoplasms are different. S cytoplasmic male sterility in some lines is relatively unstable and the majority of revertant plants transmit fertility cytoplasmically (12). Since high molecular weight mt DNAs in plants with N and S cytoplasm differ in the organisation of S1 and S2 sequences, it is possible that reversion to fertility in S lines involves the integration of S1 and S2 into mt DNA. This process would be expected to be rare in plants with T or C cytoplasms because sequences homologous to S1 and S2 are not maintained in high copy number. Although our data show that high molecular weight mt DNAs from T, C and S lines all lack high molecular weight S1 and S2 sequences, in contrast with mt DNA from N lines, other evidence,

eg. method of fertility restoration (12), the nuclear alleles involved, (1, 12) and the characteristic low molecular weight DNA species (4) suggests that the mechanism of cytoplasmic male sterility is not the same in the three cytoplasms.

In examining the faint hybridisation of S1 and S2 - containing probes to C, T and S high molecular weight mt DNAs, the presence of several Bam HI-digest fragments common to different cytoplasms have been observed. The extent of hybridisation may be low because of only short stretches of complete homology. Alternatively it may be low due to a small variant subpopulation of the total mt DNA molecules extracted. As some of the fragments are very similar in molecular weight to major hybridising fragments in N mt DNA, the latter possibility merits thorough investigation, particularly as, if validated, it would suggest differential replication of members of a heterogeneous population as an alternative route for amplification or diminution of mt DNA sequences.

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