Analysis of native mitochondrial DNA in male-fertile maize mutants resistant to *Helminthosporium maydis* race T obtained by mutagenic treatments of seeds with Texas cytoplasm.

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**RÉSUMÉ**

Mutagenic treatment of seeds with Texas male-sterile cytoplasm, with gamma rays or ethyl methyl sulfonate, allowed us to obtain male-fertile maize resistant to *Helminthosporium maydis* race T. These two traits were transmitted through the maternal parent. The study of the native mitochondrial DNA of 5 resistant male-fertile progenies showed that the 2.35 kb molecule of DNA which characterized the N, C and S cytoplasms was absent from our mutants just as it was from the T cytoplasm.

Like the revertants obtained by *in vitro* culture, the male-fertile resistant A types showed a native mitochondrial DNA which migrated in the same way as Texas type. The electrophoretic patterns of the mitochondrial DNA digested by various restriction enzymes belonged to the Normal type; however, the material obtained by *in vitro* culture showed electrophoretic patterns of Texas or intermediary type according to the enzymes used. Different hypotheses are discussed to explain these phenomena.

**Additional key words:** *Zea mays*, extranuclear inheritance, plasmid-like molecule.

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**SUMMARY**

Analyse du DNA mitochondrial natif de mutants de maïs mâle-fertiles résistants à *Helminthosporium maydis* race T, obtenus par traitements mutagènes de graines à cytoplasme Texas.

Le traitement mutagène par rayons gamma ou par méthyl sulfonate d'éthyle, de grains de maïs à cytoplasme mâle stérile Texas a permis d'obtenir des plantes mâle fertiles résistantes à *Helminthosporium maydis* race T. L'héritéité de ces deux caractères est cytoplasmique. L'étude du DNA mitochondrial natif de 5 descendance mâle fertiles résistantes montre qu'aucun de nos mutants, pas plus que le témoin à cytoplasme Texas, ne possède la molécule de DNA de 2.35 kb qui caractérise les cytoplasmes N, C et S.

Donc, comme les révertants obtenus par culture *in vitro*, les types A mâle-fertiles ont un DNA natif mitochondrial qui migre à la manière du type Texas.

Les diagrammes électrophorétiques du DNA mitochondrial digéré par diverses enzymes de restriction sont du type Normal au contraire du matériel obtenu par culture *in vitro* qui présente des diagrammes électrophorétiques du type Texas ou de type intermédiaire, selon les enzymes testées. Différentes hypothèses sont discutées.

**Mots clés additionnels:** *Zea mays*, hérédité extranucléaire, molécule de type plasmidial.

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**I. INTRODUCTION**

Four cytoplasmic types have been described for maize. Three of them are characterized by male sterility controlled by one or two nuclear genes specific to the respective cytoplasmic type (or group); they are the cytoplasms belonging to the Texas or « T », the U.S.D.A. or « S » and the Charrua or « C » groups. The fourth cytoplasmic type corresponds to the cytoplasmic group which does not show sterility, whatever nuclear genotype is combined with it; it is the Normal or « N » cytoplasmic group.

In 1970, the *Helminthosporium maydis* race T (HmT) outbreak in the U.S.A. forced the maize breeders to give up the use of Texas cytoplasmic male sterility and to proceed again to manual detasseling for obtaining hybrid varieties. Some research work was however undertaken in an attempt to obtain Texas male-sterile maize no longer susceptible to HmT. Two different approaches were applied: *in vitro* culture by GENGEBACH & GREEN (1975) and by BRETT-TELL et al. (1979, 1980) on the one hand, and mutagenesis on the other (CASSINI et al., 1977; CORNU et al., 1977). None of these methods gave Texas male-sterile plants with
heritable Hmt resistance although quite a lot of more or less male-sterile or more or less susceptible plants were obtained. Both methods also allowed one to get male-sterile plants resistant to Hmt quite similar to the phenotype of the Normal cytoplasmic group. Both Gengenbach and Brettell called these plants « variants » ; we called them « A type » (Cornu et al., 1981). In both cases this Normal phenotype (male-fertile, resistant) showed cytoplasmic inheritance: both characters were exclusively inherited through the maternal parent and of course the fertility was completely independent of the presence in the nucleus of restorer genes.

The most probable hereditary factor for male sterility and Hmt susceptibility in maize Texas cytoplasm has appeared to be the mitochondrial DNA (mtDNA). Gengenbach et al. (1981) and Kemble et al. (1982) have studied the mtDNA of variants obtained in vitro. According to the restriction enzymes used (BamH1 and Xho1) and according to the variants, the digestion patterns belonged to Texas type or differed both from Texas and Normal types; in all cases yet studied the migration of the native mtDNA belonged to Texas type. Contrary to these variants derived from in vitro cultures, the A type mutants resulting from the treatment with gamma rays or ethyl methyl sulfonate (EMS) of seeds treated 1.55 kb and 1.4 kb. Each maize cytoplasmic type can possess a linear DNA with about 2.35 kb. Each of the two cytoplasmic types S and C was characterized by the presence of « two unique DNA species » : for S, these two molecules called S1 and S2 had estimated sizes of 6.2 kb and 5.2 kb; for C, the two characteristic molecules were estimated 1.55 kb and 1.4 kb. Each maize cytoplasmic type can thus be defined by the migration of native mtDNA and this technique is recommended by Kemble et al. (1980) to determine to which of the different maize cytoplasmic groups a particular type belongs (Table 1).

In 1980, Kemble & Bedbrook proved that the migration of native mtDNA gave a band with a very high intensity for the main mtDNA and a variable number of DNA bands with low molecular weight and low intensity. The four cytoplasmic groups N, T, S and C had in common a DNA with 1.94 kilobases (kb) which appeared on the gels under different forms; these forms were, in increasing migration rate order, open circle (Oc), linear (L) and supercoiled (Ccc). All the cytoplasmic types, except T type, possessed a linear DNA with about 2.35 kb. Each of the two cytoplasmic types S and C was characterized by the absence of which is typical for T cytoplasm as well as for our five mutant families (lanes b, d, e, f, g, h). For the T control as for the mutants, the Oc form of the 1.94 kb DNA is easy to see whereas this is not the case for N and C controls. For all the mtDNA studied, the L form of this same molecule can be made out. The two typical DNA of C cytoplasm do not appear on figure 1. lane c.

In 1981, a first test on native mtDNA migration was carried out for family 135 and for controls with N cytoplasm. We observed differences between N and the mutant similar to those we reported above, but we could not come to a definite conclusion because the T control was absent. The work was done with mtDNA prepared from 50 g of etiolated shoots and from 10 g of green leaves. The nuclear genotypes were F 186, F 101, W 325 A for the N control and for family 135 (it was the fourth backcross for 135).

These two series of experiments prove that the A types are not N revertants, as their mtDNA does not include the 2.35 kb DNA molecule. It must also be stressed that this conclusion could be drawn if this 2.35 kb DNA did appear and if pattern variations took place after digestion by restriction enzymes. As a matter of fact, a certain heteroge-
neity of the restriction patterns could be observed for the N (Levings & Pring, 1977) and C (Pring et al., 1979) cytoplasmic groups, although the group affinities of the cytoplasm showing the differences could not be questioned.

IV. DISCUSSION

The studied mutants as well as the variants obtained in vitro had a native mtDNA of Texas type but not the same electrophoretic patterns after digestion by the restriction enzymes.

To explain that some Texas male-sterile plants susceptible to HmT revert to male fertile plants resistant to HmT after the mutagenic treatments, two hypotheses are advanced: i) transmission of Normal mitochondria by pollen; ii) initial association of mtDNA of Texas and Normal types. In both hypotheses Normal mtDNA would have been promoted instead of Texas mtDNA (Cassini et al., 1977; Cornu et al., 1977; Berville & Paillard, 1982). The hypothesis of a point mutation is not sufficient to explain the fact that the restriction patterns obtained after digestion by EcoRI, BamHI and Sall enzymes belong to the N type.

However, as the mtDNA electrophoretic patterns after digestion by the restriction enzymes are neither of Normal nor of Texas type, the phenotypic reversion of Texas to Normal type after in vitro culture could be explained as follows: mtDNA heterogeneity and « chromosomal rearrangements » (Genetebach et al., 1981; Kemble et al., 1982). This hypothesis is based on the work of various authors: Quetier & Vedel (1977) proved that in plants no agreement existed between the molecular weight (MW) estimated from the size of the mtDNA molecules and the MW estimated from restriction patterns. Levings & Pring (1979) revealed that there were several sizes of mtDNA molecules in maize and that the size frequencies varied according to the cytoplasmic group. Protoplast fusions in Nicotiana (Belliard et al., 1979) and in Brassica (Pelletier et al., 1983) have made it possible to observe recombinations between mtDNA. This work leads thus to assume that there are several types of mtDNA molecules in plant mitochondria and that intra- and/or intermolecular changes could take place to explain the cytoplasmic variants obtained on in vitro culture.

The mutants belonging to A type present plasmid-like molecules of Texas type. The two hypotheses on mutagenic treatment then seem little adapted to describe the appearance of A type. As in the case of in vitro culture, mechanisms of intra- and/or interchromosomal recombinations could be responsible for the A type. However Kemble et al. (1983) and Newton (1983) obtained, for the A188 and KY21 lines on Normal cytoplasm, a Texas type migration of

**Table 1**

<table>
<thead>
<tr>
<th>Cytoplasmic type</th>
<th>N</th>
<th>T</th>
<th>C</th>
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<td>6.2 KB</td>
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<td>++</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td></td>
<td>CCC</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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</tbody>
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OC: open circle, L: linear, CCC: supercoiled forms of the 1.94 kb DNA.
OC: forme circulaire ouverte, L: forme linéaire, CCC: forme superenroulée du DNA de 1.94 kb.
+++: forte intensité, ++: moyenne intensité, +: faible intensité.

![Figure 1](image-url)

Figure 1

Agarose gel electrophoretic patterns of native mitochondrial DNA of maize. Lanes a, b, c: A 632 × Mo 17 on N, T, C cytoplasm. Lanes d, e, f, g, h: the five mutants families 135, 473, 511, 532 and 1754. Molecular weight markers were digests of λ DNA by EcoRI and HindIII (Boehringer, marker III). T: the 2.35 kb DNA characteristic of N, C, and S cytoplasms. O: open circle; L: linear forms of the common DNA of 1.94 kb. Diagrammes électrophorétiques sur gel d’agarose, du DNA mitochondrial natif de maïs. Puits a, b, c: A 632 × Mo 17 sur cytoplasmes N, T, C. Puits d, e, f, g, h: les cinq familles de mutants 135, 473, 511, 532 et 1754. Les marqueurs de masse moléculaire sont des fragments de DNA du phage λ digéré par EcoRI et HindIII (Boehringer, marker III). T: DNA de 2.35 kb caractéristique des cytoplasmes N, C et S : O: forme circulaire ouverte. L: forme linéaire de la molécule de DNA commun à tous les types cytoplasmatiques, de masse moléculaire 1.94 kb.
the native mtDNA. The hypothesis of an initial association of Texas and Normal mtDNA will be difficult to maintain if the F7 line on Normal cytoplasm has a native mtDNA of the Normal type. To check the hypothesis of mitochondrial transmission by pollen, further studies will be made on F71.

REFERENCES BIBLIOGRAPHIQUES


