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Transmission of paternal chloroplasts in tobacco (*Nicotiana tabacum*)

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ABSTRACT

Medgyesy et al. (1986, Mol. Gen. Genet. 204, 195-198) have described in *Nicotiana plumbaginifolia* and in an interspecific cross involving *N. plumbaginifolia* and *N. tabacum* a procedure for selecting cell lines derived from seedlings carrying paternal chloroplasts by taking advantage of a plastid-encoded mutation which confers resistance to streptomycin. We have extended their demonstration of occasional transmission of chloroplasts through pollen to the case of an intraspecific cross in *N. tabacum*. The line used as maternal parent, ITB19(sua), displayed a cytoplasmic male sterility due to the presence of a cytoplasm originating from *N. suaveolens*. The line used as paternal parent, SR1, was fertile and possessed mutant chloroplasts conferring resistance to streptomycin. From cell lines derived from 204 seedlings, three were regenerated into streptomycin-resistant buds. The plants derived from these three clones were male-sterile. Their progeny, after crossing with a wild type tobacco line, XHFD8, was resistant to streptomycin. Tests of resistance of the seedlings to tentoxin and restriction analyses of the chloroplast DNA indicated that two clones still had the maternal chloroplasts and were thus probably new streptomycin-resistant mutants, whereas the third one had acquired the chloroplasts of the paternal parent, but had retained the mitochondria of the maternal parent.

Abbreviations: cp-DNA: chloroplast DNA
mt-DNA: mitochondrial DNA
Np: *Nicotiana plumbaginifolia*
Nt: *Nicotiana tabacum*

INTRODUCTION

As most angiosperm genera (Tilney-Bassett 1978, Sears 1980), *Nicotiana* was considered to display maternal inheritance of chloroplasts. In vitro selection for chloroplastic streptomycin-resistance in cell lines derived from seedlings, however allowed Medgyesy et al. (1986) to demonstrate that occasional transmission of chloroplasts through the pollen could take place in *Nicotiana plumbaginifolia* and in an interspecific cross involving *Nicotiana plumbaginifolia* as maternal parent and *Nicotiana tabacum* as paternal parent. No information was given on the possibility of such a paternal transmission in the case of intraspecific crosses in *Nicotiana tabacum*, thus leaving open the possibility that the phenomenon observed could be restricted to crosses in which *Nicotiana plumbaginifolia* is used as the maternal parent. This is not the case, since using the same method as Medgyesy et al., we could demonstrate that occasional paternal inheritance of plastids can also take place in *Nicotiana tabacum*.

MATERIALS AND METHODS

Plant Material

The ITB19(sua) tobacco line, kindly given by Dr. R. Delon, Institut Expérimental du Tabac, BP 168, 24108 Bergerac, was used as maternal parent. This line possesses the cytoplasm of *N. suaveolens* that causes its cytoplasmic male sterility (Izard and Hitier 1955). As paternal parent we used the streptomycin-resistant tobacco line SR1 obtained by Maliga et al. (1975). The A1-10 tobacco line which has acquired the streptomycin-resistant chloroplasts of SR1 was used as control in restriction analysis of chloroplast DNA and the wild type XHFD8 line was used in crosses for progeny analysis (Bourgin et al. 1986).

Selection of streptomycin-resistant lines

Seedlings of the ITB19(sua) X SR1 cross were germinated on Murashige and Skoog's derived medium B (Bourgin et al. 1979). After one week, hypocotyls of the seedlings were cut and the fragments obtained were cultured on a modified RMOP medium (Cseplö and Maliga 1984), in which the sucrose concentration was reduced to 1% and to which streptomycin sulfate was added aseptically to a final concentration of 1mg.ml^{-1}. Greeening buds formed on

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the resulting calli were subcultured 10 weeks later on medium B supplemented with 1mg.ml^{-1} streptomycin sulfate.

Test of seedlings for resistance to streptomycin and to tentoxin

Lots of 100 sterilized seeds per cross studied were sown in Petri dishes on medium B supplemented either with 1mg.ml^{-1} streptomycin sulfate or with 20 µg.ml^{-1} tentoxin (Sigma) (Durbin and Uchytil 1977). Cotyledons of resistant seedlings are green whereas those of the sensitive seedlings are white.

Analysis of chloroplast DNA

Cp-DNA of plants from the control lines and from the crosses of the streptomycin-resistant clones by XHFD8 line was isolated according to the method described by Charbonnier et al. (1987). The purified cp-DNA was digested by Eco RI and Hind III endonucleases (Boehringer, Mannheim) and the fragments were separated by electrophoresis on 0.7% agarose gels.

Analysis of mitochondrial DNA

Fifteen to 30 g of leaves derived from the parental lines and from the SR25 clone were used to prepare the mt-DNAs according to the method previously described by San et al. (1990). 1 µg of each mt-DNA was digested by Bam HI and Sal I endonucleases (Boehringer, Mannheim). The restriction fragments were separated by electrophoresis in 0.7 % agarose gels.

RESULTS

1. Selection of streptomycin-resistant clones

From a total of 204 hypocotyl explants originating from seedlings of the ITB19(sua) X SR1 cross, three clones, labelled SR4, SR10, and SR25, produced buds which remained green after the subculture on the streptomycin-containing medium B. Buds of clones SR10 and SR25 developed normally to plants after transfer to the greenhouse, whereas original clone SR4 was rootless and developed normally only after grafting to wild type tobacco plants. All these plants displayed the male sterility characteristic of the maternal parent.

2. Characterization of the plastid genome of the streptomycin-resistant clones

Plants of the three clones were pollinated with wild type XHFD8 plants. The seedlings of the resulting progeny were 100% resistant to streptomycin.

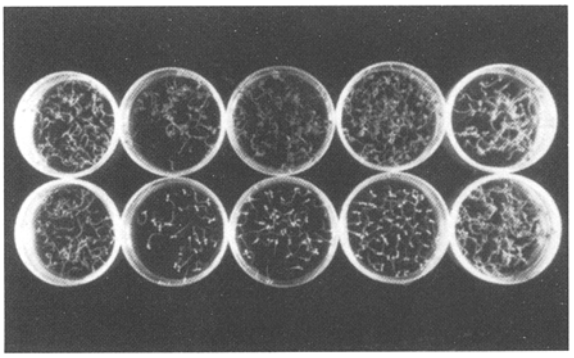

Figure 1. Test of sensitivity to tentoxin of seedlings from different lines. From left to right: clones A1-10, ITB19(sua), SR4, SR10, SR25. Upper line: control medium. Lower line: medium supplemented with tentoxin.

Since streptomycin-resistance in plant cell lines has always been attributed to cp-DNA encoded mutations (Maliga 1984, Fromm et al. 1989), this result was taken as an evidence of the maternal transmission of this character. In order to determine the origin of these chloroplasts confering streptomycin-resistance, resistance of the seedlings to tentoxin, a species dependent plastid trait (Durbin and Uchytil 1977), was also assessed. Seedlings of the crosses involving SR4 and SR10 were sensitive to tentoxin (Figure 1), as are seedlings of *N. suaveolens*, whereas seedlings from the SR25 clone were resistant to tentoxin, as are seedlings of *N. tabacum* (Burk and Durbin 1978).

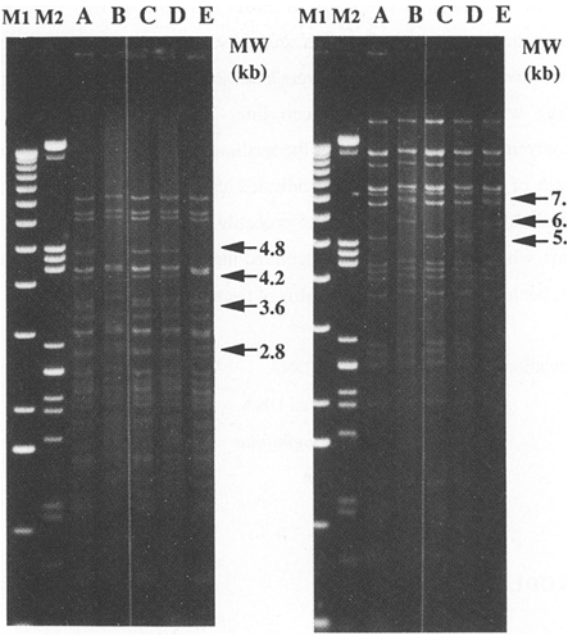

Figure 2. Restriction analysis of cp-DNA, respectively with Eco RI (A) and Hind III (B). Lanes: M1: 1kb DNA ladder; M2: lambda phage Hind III digest; A: ITB19(sua); B: A1-10: C: SR4; D: SR10; E: SR25. Arrows indicate fragments specific for either ITB19(sua) or A1-10

Differences in the restriction patterns between the cp-DNA of the maternal parent ITB19(sua) and that of line A1-10 representing the paternal parent are visible at 4.8, 4.2, 3.6, and 2.8 kb in the case of the digestion with Eco RI, and at 7.2, 6.0, and 5.4 kb in the case of the digestion with Hind III. Restriction patterns of cp-DNA of the three streptomycin-resistant clones confirmed the *N. suaveolens* origin of chloroplasts of clones SR4 and SR10, as well as the N. tabacum origin of those of the clone SR25 (Figure 2).

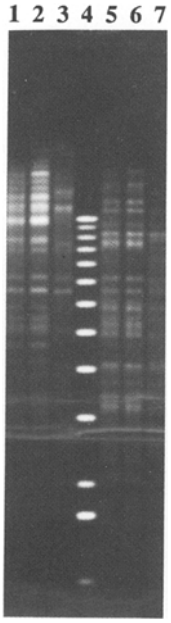

Figure 3. Restriction patterns of mt-DNA from parents and the SR25 clone. Lanes 1 to 3, Sal I digestions; lanes 4 to 7, Bam HI digestions. Lanes 1 and 5, ITB19(sua); lanes 2 and 5, SR25; lanes 3 and 7, XHFD8; lane 4, 1 kb DNA ladder.

3. Characterization of the mitochondrial genome of the SR25 clone

The mt-DNA restriction patterns of the maternal parent ITB19(sua) were very different from those of the paternal parent XHFD8 (Figure 3). We have compared the mt-DNA restriction patterns of the SR25 clone with those of the parents. The patterns of the SR25 were entirely identical to those of the maternal parent. The maternal mitochondrial inheritance was so confirmed.

DISCUSSION

Chloroplasts of clones SR4 and SR10, although associated with streptomycin-resistance, still displayed the restriction patterns and the tentoxin sensitivity characteristic of the line used as maternal line. We thus consider these clones to be spontaneous mutants, which can arise at rather high frequency, as already observed in the experiments of Medgyesy et al. (1986). Streptomycin-resistant clone SR25, on the contrary, possesses chloroplasts which display tentoxin-resistance and restriction patterns characteristics of tobacco chloroplasts. We therefore conclude that they are of paternal origin. The male sterility of this clone indicated that it had probably conserved the maternal *N. suaveolens* mitochondria, a situation similar to that observed in the cybrids obtained by Medgyesy et al. (1986). This was confirmed by comparison of the mt-DNA restriction patterns. Our experiment thus extends to intraspecific cross in *N. tabacum* the potential of the method devised by Medgyesy et al. (1986) for selecting the transfer of chloroplasts through pollen. In Medgyesy et al.'s experiment there was a great difference in the frequencies of paternal transmission between intraspecific cross (Np X Np: 2.5%) and the interspecific cross (Np X Nt: 0.07%). Although we obtained a single line in our experiment, the 0.5% frequency of paternal transmission of chloroplasts suggests that *N. tabacum* does not necessarily transmit plastids through the pollen at a much lower rate than *N. plumbaginifolia*. Efficient methods of production of plastid-encoded mutations conferring resistance to antibiotics (Fluhr et al. 1985, Mc Cabe et al. 1989) or to herbicides which affect photosystem II have been described in *Nicotiana* (Cseplö et al. 1985). Use of such biochemical markers should allow the extension of the spectrum of species for which occasional exceptions to the rule of maternal transmission of plastids may occur (*Solanum nigrum*: Gasquez et al. 1981, Mc Cabe et al. 1989; *Petunia hybrida*: Cornu and Dulieu 1988). Similarly, it would be interesting to test whether putative mitochondria-encoded oligomycin resistance selected in cell cultures of *Nicotiana sylvestris* (Aviv and Galun 1988, Durand and Harada 1989) could be used for selecting the transfer of mitochondria through pollen.

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