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Characterization of nuclear and cytoplasmic information in the progeny of a somatic hybrid between male sterile *Nicotiana tabacum* and *N. glutinosa*

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Summary. Several nuclear and cytoplasmic characters of the back-crossed progeny of a somatic hybrid between male sterile *Nicotiana tabacum* (*N. debneyi* cytoplasm) and *N. glutinosa* have been analysed. Progeny were obtained by repeated back-crossing of a somatic hybrid with pollen from either *N. tabacum* or *N. glutinosa*. Nuclear ribosomal RNA genes (rDNA) were found to be a reliable marker to determine the constitution of nuclear genomes in the progeny. The progeny obtained by back-crossing with *N. tabacum* pollen maintained uniformity in leaf morphology. On the other hand, variation in leaf morphology was observed in the second back-cross population obtained with *N. glutinosa* pollen. This may be due to a variable contribution of *N. tabacum* chromosomes. Segregation of rDNA was also found in individuals of the same back-crossed progeny, but was not related to the chromosome number. The stable inheritance of chloroplast DNA in the back-crossed generation was confirmed regardless of the type of pollen donor. Male sterility was consistently maintained throughout several generations, suggesting that the nuclear genome of either *N. tabacum* or *N. glutinosa* does not influence the expression of cytoplasmic male sterility.

Key words: *Nicotiana* somatic hybrid – Ribosomal RNA gene – Chromosome – Chloroplast DNA – Cytoplasmic male sterility

Introduction

Recent advances in the study of somatic hybridization of higher plants have made it possible to manipulate genetic information encoded by nuclear as well as cytoplasmic DNA (Schieder and Vasil 1980). The analysis of gene markers in somatic hybrid plants is crucial for the identification of their genome constitutions. Study of the inheritance of the characters of the hybrids to their progeny is necessary in the application of somatic hybridization to practical plant breeding.

In a previous communication, we reported on the inheritance of traits of a somatic hybrid between the male sterile *Nicotiana tabacum* and *N. glutinosa* in progeny obtained by back-crossing (Uchimiya et al. 1982). The markers investigated included Fraction I protein and cytoplasmic male sterility. In this communication, we report an analysis of ribosomal RNA genes (rDNA), chromosome number, chloroplast DNA and plant morphology in a somatic hybrid and progeny resulting from back-crosses.

Materials and methods

Back-crossing

Back-crossing was conducted in the manner reported earlier (Uchimiya et al. 1982). Briefly, stigmas of a hybrid plant obtained by protoplast fusion of male sterile *N. tabacum* containing *N. debneyi* cytoplasm (Uchimiya 1982) and *N. glutinosa* were pollinated with either *N. tabacum* or *N. glutinosa* pollen. Plants thus obtained were repeatedly crossed with the same pollen donors to yield extensively back-crossed progeny.

Ribosomal RNA genes (rDNA)

DNA was extracted from tobacco leaves and digested with restriction endonucleases (Uchimiya et al. 1983). DNA digests were subjected to 0.7% agarose gel electrophoresis, followed by a blot-hybridization with ^{32}P-labelled rRNA as probe according to the method described by Uchimiya and coworkers (1983).

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Chromosome number

Young leaves were treated with 0.05% colchicine for 3 h at 25 °C. The colchicine was removed and leaf tissues were washed three times in distilled water, followed by fixation in alcohol-acetic acid (3 parts of 95% ethanol to 1 part glacial acetic acid) for 12–24 h. The cells were transferred to 80% ethanol and stored at 4 °C. To prepare slides, the cells were rinsed twice with distilled water, hydrolysed in 1 N HCl at 60 °C for 8 min and rinsed twice in 0.1 M sodium acetate. The cells were stained in 1% aceto-carmine for 2–4 h and squashed.

Chloroplast DNA

Chloroplast DNA was prepared by the method of Saltz and Beckman (1981). Approximately 1–2 μg DNA was digested by EcoRI (2 U), and applied to an 0.8% agarose gel (Uchimiya et al. 1983). After electrophoresis for 16 h, DNA was stained with ethidium bromide, and photographed.

Results

Ribosomal DNA

In order to find the appropriate enzymes which discriminate between the rDNA of the two tobacco species, restriction fragment patterns generated with several restriction endonucleases were compared. Digestion of *N. tabacum* DNA with HindIII produced DNA fragments of 7.4 and 6.4 (minor band) $\times 10^6$ daltons hybridizing to labelled ribosomal RNA, whereas digestion of *N. glutinosa* DNA produced a 16×10^6 dalton rDNA. The DNA of the somatic hybrid contained all of these fragments (Fig. 1). The analysis of rDNA in successive back-cross generations with *N. tabacum* pollen showed that the *N. glutinosa* type rDNA was eliminated after the 2nd back-crossing (Fig. 2). In the case of back-crossing with *N. glutinosa*, some individuals of the population from the 2nd back-cross contained *N. tabacum* type rDNA (Fig. 3).

Leaf morphology and male sterility

In the progeny obtained by back-crossing with *N. tabacum*, all plants showed uniform leaf morphology (data not shown), while in the population after the second back-crossing with *N. glutinosa*, some variation was observed (Fig. 4). Previously, we demonstrated the inheritance of the male sterile character in the back-crossed progeny with *N. tabacum* (♂). Similarly, male sterility was also retained in the progenies of the 1st and 2nd back-crossing with *N. glutinosa* (♂) (Fig. 5).

Chromosome number

The result of the chromosome analysis is summarized in Table 1. The somatic hybrid plant had the amphiploid chromosome number (Fig. 6a, $2n = 72$), which is the summation of the two parental species, *N. tabacum*

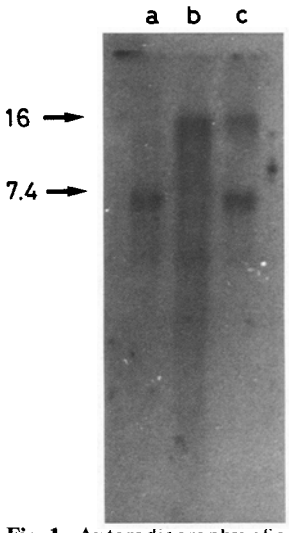

Fig. 1. Autoradiography of a blot-hybridization of ^{32}P-labelled −25S +17S rRNA to HindIII digests of nuclear DNA from leaves of (*a*) male sterile *N. tabacum*, (*b*) *N. glutinosa* and (*c*) their somatic hybrid. The reaction mixture contained 2 μg DNA, 5 U HindIII, 10 mM Tris-HCl (pH 7.5), 7 mM $MgCl_2$, and 60 mM NaCl. Numerals indicate molecular weight; $\times 10^6$ daltons

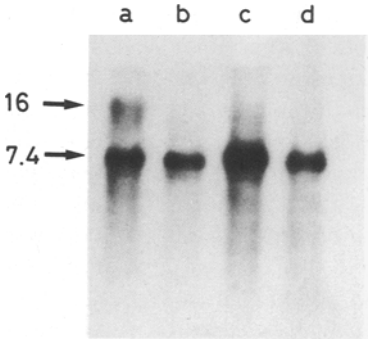

Fig. 2. Autoradiography of a blot-hybridization of ^{32}P-labelled −25S +17S rRNA to HindIII digests of nuclear DNA from leaves of progeny of a hybrid between male sterile *N. tabacum* and *N. glutinosa* crossed with *N. tabacum* (♂). (*a*) 1st, (*b*) 2nd, (*c*) 3rd and (*d*) 4th back-cross generation

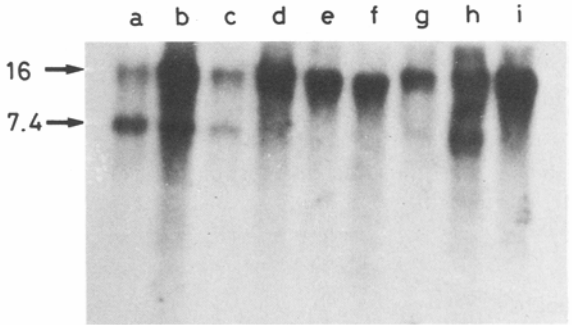

Fig. 3. Autoradiography of a blot-hybridization of ^{32}P-labelled −25S +17S rRNA to HindIII digests of nuclear DNA from leaves of progenies of a hybrid between male sterile *N. tabacum* and *N. glutinosa*, crossed with *N. glutinosa* (♂). (*a*) hybrid, (*b*) 1st back-cross generation, and (*c*) – (*i*) individuals in the population of the 2nd back-cross generation

Fig. 4 a – d. Morphological variation in the progeny of a hybrid between male sterile *N. tabacum* and *N. glutinosa*, back-crossed twice with *N. glutinosa* (♂). The chromosome numbers of the plants were (**a**) 36, (**b**) 34, (**c**) 38, (**d**) 35

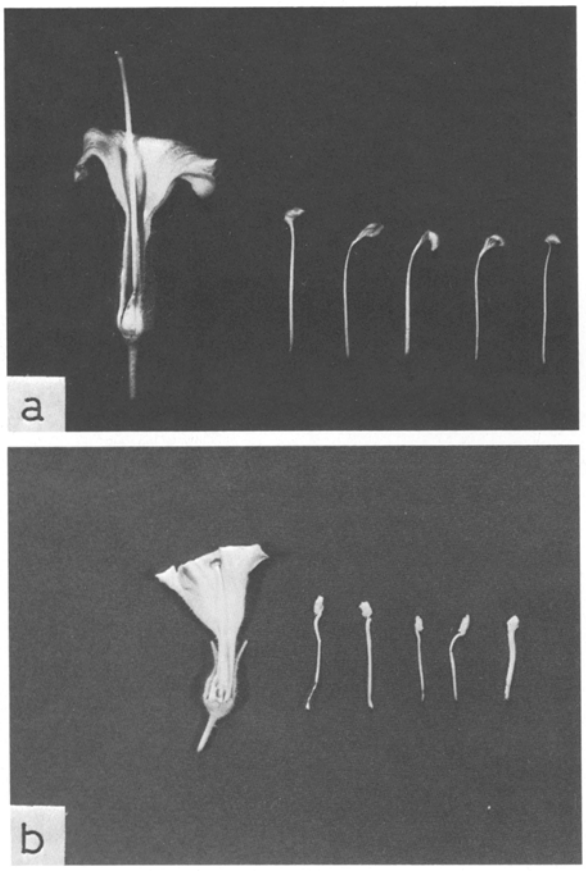

Fig. 5. Flower morphology of the progeny belonging to (**a**) the 1st and (**b**) 2nd back-cross generation with *N. glutinosa* (♂) of a hybrid between male sterile *N. tabacum* and *N. glutinosa*, Note the lack of anthers with pollen

(2n=48) and *N. glutinosa* (2n=24). The chromosome number of 48 in the plants (Fig. 6 b) resulting from pollination of the somatic hybrid with *N. glutinosa* indicates that the somatic hybrid is stable and produces normal gametes with 36 chromosomes (24 from *N. tabacum* and 12 from *N. glutinosa*). The meiotic behaviour of the hybrid was not studied since it lacked anthers and formed the stigmoid-like organ. Extensive chromosome analysis was made in the population originating from the 2nd back-crossing with *N. glutinosa,* where variation in leaf morphology was observed. As shown in Table 1 the individuals contained chromosome numbers ranging from 32 to 38. Typical examples of the segregants 4 and 5 are shown in Fig. 6 c and d, respectively. It is evident that between 8 and 14 chromosomes of the *N. tabacum* genome were transmitted by the female parent to give rise to plants with the observed chromosome constitution after pollination with *N. glutinosa.*

Chloroplast DNA

Figure 7 shows profiles of gel electrophoresis of EcoRI-digested chloroplast DNAs of male sterile *N. tabacum,*

Table 1. Summary of the nuclear and cytoplasmic markers in a *Nicotiana* somatic hybrid and backcrossed progeny. *T: N. tabacum* type, *G: N. glutinosa* type, *D: N. debneyi* type, *FIP:* Fraction I protein, *MS:* Male sterile, *MF:* Male fertile, *N.A.:* Not available

Plants	Nuclear markers			Cytoplasmic markers		
	FIP small[a]	rDNA	Chromosome no.	FIP large[a]	Chloro-plast DNA	Male sterility[a]
Parents:						
N. tabacum	T	T	48	D	D	MS
N. glutinosa	G	G	24	G	G	MF
Hybrid	T+G	T+G	72	G	G	MS
Back-crossed with *N. tabacum:*						
1 st	T+G	T+G	N.A.	G	G	MS
2 nd	T>T+G	T	N.A.	G	G	MS
3 rd	T	T	N.A.	G	G	MS
4 th	T	T	N.A.	N.A.	N.A.	MS
Back-crossed with *N. glutinosa:*						
1 st	T+G	T+G	48	G	G	MS
2 nd individuals: 1	N.A.	T+G	36	N.A.	G	N.A.
2	N.A.	G	32	N.A.	G	N.A.
3	N.A.	G	38	N.A.	G	MS
4	N.A.	G	34	N.A.	G	MS
5	N.A.	G	35	N.A.	N.A.	N.A.
6	N.A.	T+G	N.A.	N.A.	N.A.	N.A.
7	N.A.	G	38	N.A.	N.A.	N.A.

[a] Most of data are extracted from Uchimiya (1982) and Uchimiya et al. (1982)

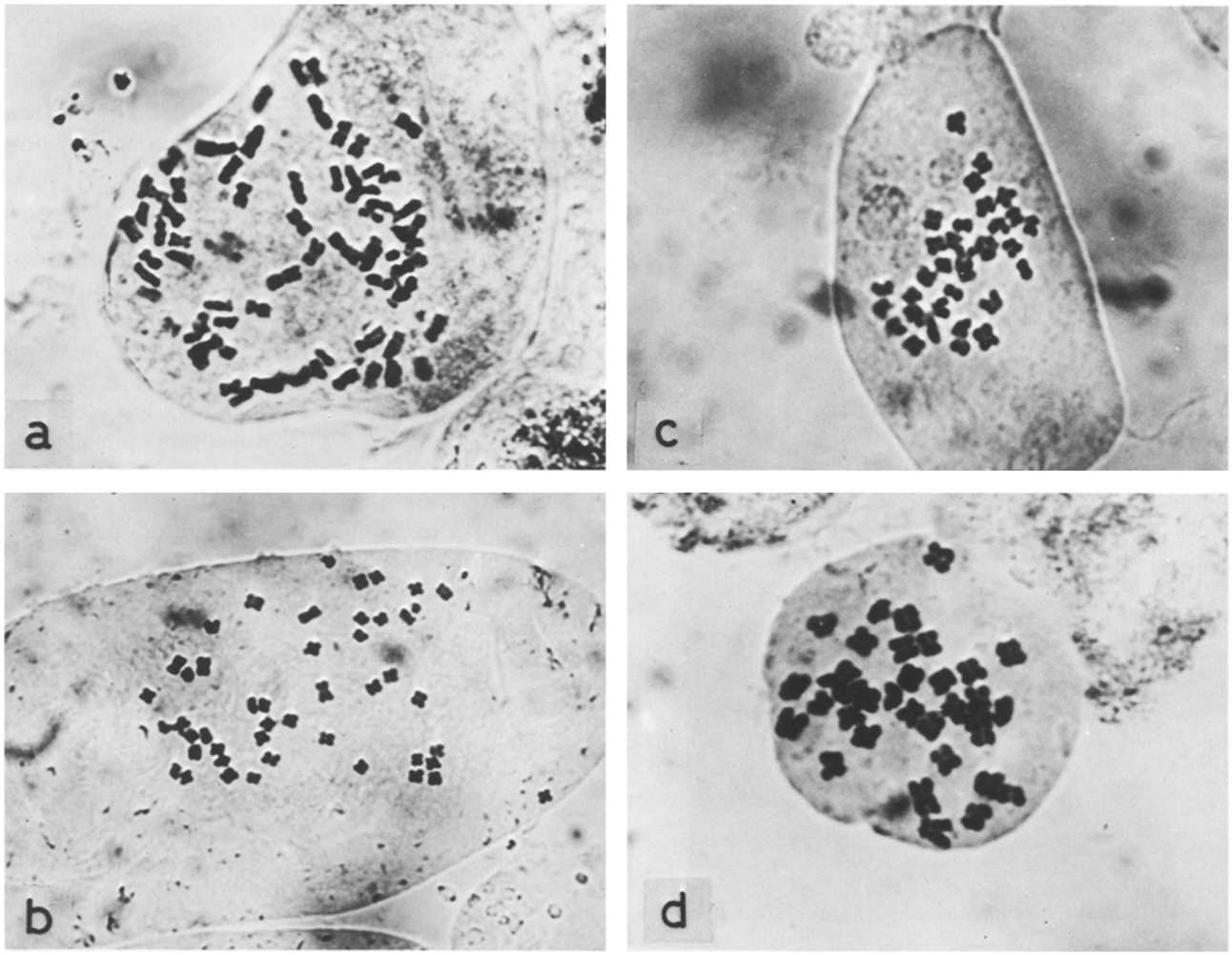

Fig. 6. Chromosomes observed in leaf tissues of (**a**) a somatic hybrid between male sterile *N. tabacum* (2n=48) and *N. glutinosa* (2n=24), and (**b**) the 1st and (**c, d**) 2nd back-cross progeny of the hybrid with *N. glutinosa* (♂)

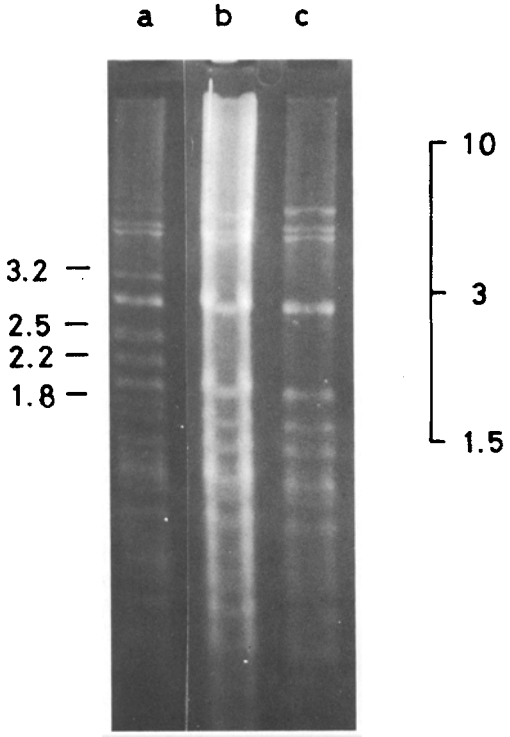

Fig. 7. A profile of agarose gel electrophoresis of EcoRI-digested chloroplast DNA from (*a*) male sterile *N. tabacum*, (*b*) *N. glutinosa* and (*c*) a somatic hybrid. Numerals indicate molecular weight; $\times 10^6$ daltons

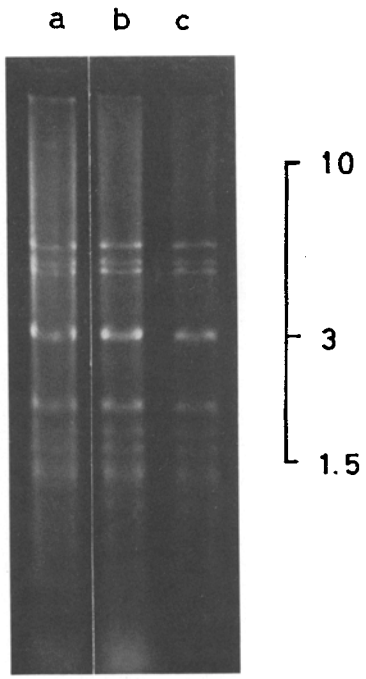

Fig. 8. A profile of agarose gel electrophoresis of EcoRI-digested chloroplast DNA from (*a*) a somatic hybrid between male sterile *N. tabacum* and *N. glutinosa* and individuals belonging to (*b*) the 2nd and (*c*) 3rd back-cross generation of a hybrid with *N. tabacum* (♂). Numerals indicate molecular weight; $\times 10^6$ daltons

N. glutinosa and a somatic hybrid. *Nicotiana glutinosa* chloroplast DNA lacks the 3.2, 2.5, 2.2 and 1.8×10^6 dalton fragments which were characteristic of male sterile *N. tabacum* (i.e. *N. debneyi*) chloroplast DNA. Furthermore, the chloroplast DNA of the somatic hybrid had the same fragment pattern as that of *N. glutinosa*. A similar analysis was made on back-cross progeny of the somatic hybrid with either *N. tabacum* or *N. glutinosa*. In the case of the progeny of the hybrid crossed with *N. tabacum*, the same EcoRI digestion pattern was observed (Fig. 8). Similar results were observed with progeny which had been obtained by back-crossing with *N. glutinosa* twice (data not shown). Consistency of the restriction pattern of chloroplast DNA in the somatic hybrid and the progeny was also shown when BamHI or HindIII were employed (our unpublished data).

Discussion

The results of an investigation of several characters in a somatic hybrid of male sterile *N. tabacum* and *N. glutinosa* and its progeny are summarized in Table 1, which also includes data reported elsewhere (Uchimiya 1982, Uchimiya et al. 1982). For the application of somatic hybridization into plant breeding, it is important to assess the inheritance of various characters in the progeny. In our case, the hybrid was male-sterile and self-pollination, therefore, was not possible. Progeny were obtained by cross-hybridization of a hybrid with other pollen donors. As previously reported (Uchimiya et al. 1983), elimination of the nuclear genome of *N. glutinosa* resulted in plants with normal morphology and such elimination was indicated by the type of the small subunit of Fraction I protein. However, when the hybrid was crossed with *N. glutinosa*, abnormal plant morphology was observed in the population after the 2nd back-crossing. Variations in plant morphology particularly in growth habit and leaf shape may be attributed to the presence of a variable number of *N. tabacum* chromosomes. Segregation of rDNA was also found in the individuals of the same back-cross, but was not related to the chromosome number.

Previously we reported that only one type of large subunit of Fraction I protein was apparent in the somatic hybrid and its progeny (Uchimiya et al. 1982). The outcome of chloroplast DNA analysis likewise reveals maternal inheritance of the chloroplast DNA. There have been several reports indicating the presence of a single type of chloroplast DNA prevailing in somatic hybrids (Belliard et al. 1979; Kumar et al. 1982; Schiller et al. 1982; Scowcraft and Larkin 1981).

Thus, present investigation provides further information on the inheritance of chloroplast DNA in the somatic hybrid and its progeny.

The transfer of cytoplasmic characters such as chloroplast DNA or male sterility would be one of the most advantageous application of somatic hybridization in higher plants. In this respect, the demonstrated stable inheritance of chloroplast DNA as well as cytoplasmic male sterility is encouraging.

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