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Reciprocal transfer of male sterile and normal plasmons in *Petunia* *

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Summary. The goal in this experiment was to achieve direct plasmon transfer via cell fusion. Two lines were used – a normal fertile line of *P. hybrida,* and a cytoplasmic male sterile (cms) line with the nuclear background of *P. parodii.* Two plants phenotypically similar to the original male sterile line were developed from protoplasts, but instead of being cms they were male fertile. On the other hand, two plants typical of the original normal line developed from protoplasts, but they were cms instead of fertile. Chromosome counts were done and in all cases the expected diploid number (= 14) was found. Genetic analysis showed that sorting out of cms and fertile segregants was evident in the first and second backcross of the cms cybrids. The fertile type cybrids were stable fertile for several generations of selfing and proper backcrossing. These results are discussed in the light of an earlier fusion experiment in which these two parental lines were involved.

Key words: *Petunia* – Plasmon transfer

Introduction

Recently, transfer of cytoplasmic male sterile (cms) plasmon by protoplast fusion has been demonstrated in *Nicotiana* (Belliard et al. 1977; Zelcer et al. 1978), in *Petunia* (Izhar and Power 1979; Izhar and Tabib 1980; Izhar et al. 1983; Lorz and Izhar 1983; Bergouniox-Bunissett and Perennes 1980) and in *Brassica* (Pelletier et al. 1983).

The genetic and molecular analysis of the protoplast fusion products showed that the plasmon transfer was not always achieved unilaterally and that a heteroplasmic state existed in the protoplast fusion products with respect to male sterility and fertility, and to the organelle populations (Belliard et al. 1979; Galun et al. 1982; Izhar et al. 1983; Boeshore et al. 1983).

Different results were obtained by Izhar and Tabib (1980) and Izhar et al. (1983). In the first report fertility was expressed by the heteroplasmon while in the second one male sterility was expressed by the heteroplasmic tissue of somatic hybrids. These data indicate possible nuclear control of the fate of the cytoplasmic sterility elements *ste* (Izhar et al. 1983) and fertility elements *fte* in the heteroplasmon of the protoplast fusion products, since different petunia lines were involved in these fusion experiments.

In the present study the data show instability of the cms plasmon transferred to the recipient normal line, but stability of the normal plasmon transferred to the originally cms line. These results are in agreement with above mentioned studies.

Materials and methods

Lines of two *Petunia* species were used for this experiment. (i) A cms line 3688, having *Petunia parodii* L.S.M. nuclear background and a sterile plasmon which had been introduced into *P. parodii* line 3699 by repeated backcrosses (Bc_{10}). Line 3688 has a morphology typical of *P. parodii:* erect growth type, narrow leaves, long corolla, a white flower and some anthocyanin seen in the corolla. (ii) Line 3704, a normal fertile line of *P. hybrida* (Hook) Vilm. This line has a compact growth type and red flowers with white sectors. Both lines were tested thoroughly (Izhar 1978) and found to have no male fertility restorer alleles.

Plants were grown in the greenhouse with supplemental artificial light to 16 h. The day temperatures were between 25° and 30 °C and night temperatures were 17 °C. Leaf mesophyll protoplasts were isolated and treated according to Izhar et al. (1983).

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Culture medium

The culture medium was based on that of Murashige and Skoog (1962) (MS) and consisted of organic and inorganic components (Flow Lab., Ltd.) to which were added sucrose (3%), mannitol (9%) and agar (0.6%) and growth regulators: NAA (1.0 mg/l), 2,4-D (1.0 mg/l) and BAP (0.5 mg/l) for the first 4 weeks. Later 2,4-D was omitted from the medium and NAA was increased to 2.0 mg/l. According to early experiments by Ettinger-Paltin (1981) leaf protoplasts of line 3704 did not divide on that medium. Protoplasts of line 3688 could grow to form small colonies but their growth slowed down when 2,4-D was removed.

Chromosome counts

These were made by the common anther-smearing technique, using acetocarmine (in fertile and some sterile hybrids); in addition, they were counted in the corolla tips according to Burns (1964).

Table 1. Crosses made with cybrid 1887 as male and as female parent with cms and normal lines. Cybrid 1917 was selfed only

Tester plants	Cybrid	No. of progeny	Type of progenies
562(4N;F) × 1887		12	all fertile[a]
1888(4N;S) × 1887		35	all fertile[a]
4556(2N;S) × 1887		35	all fertile
6697(4N;S) × 1887		25	all fertile[a]
3704(2N;F) × 1887		60	all fertile
3699(2N;F) × 1887		30	all fertile
3688(2N;S) × 1887		40	all fertile
1871(2N;F) × 1887		5	all fertile
Cybrid	Tester plants		
1887 × F–13	562 (4N;F)	2	all fertile[a]
1887 × 4556	(2N;S)	50	all fertile
1887 × 3704	(2N;F)	50	all fertile
1887 × 3699	(2N;F)	30	all fertile
Cybrid 1917 selfed		6	all fertile

2N; 4N = diploids and tetraploids, respectively
S; F = cms and normal fertile plasmon, respectively
[a] Triploid progeny

Table 2. Crosses made with cybrids no. 1917 and 1640 of the 3704 type, and a normal 3704 plant type regenerated from protoplasts (no. 1073 and 1740)

Male sterile cybrids × male parents	Progenies	Type
1716×3704	4	all sterile
1716×4501	12	all fertile
1640×3704	6	steriles
	2	fertiles (F3)
Plants of line 3704 fertile (from protoplasts)		
1740×3704	17	all fertile
1703 selfed	14	all fertile

Results

The selection conditions in the culture medium were in favour of the somatic hybrid between lines 3688 and 3704 (Ettinger-Paltin 1981). About 4,000 somatic hybrids regenerated from protoplasts (Izhar et al. 1983) and only a few parental-like plants escaped the selection system, including the cybrids.

About 100 plants of the 3688 parental line type developed from the protoplast fusion mixture. These plants were identical to line 3688.

Two plants which were identical morphologically to line 3688 were male fertile (cybrids no. 1887 and 1917). The number of chromosomes was 2N = 14 in the corolla tip and seven pairs in meiotic metaphase. The genetic analysis is shown in Table 1. Cybrid 1917 was a relatively weak plant and died after the first selfing was done. However, it was identified as male fertile and gave six fully fertile progenies after selfing. The genetic data presented in Table 1 for cybrid 1887 show that it is a male fertile cybrid with normal plasmon and no male fertility restorer alleles. The normal fertile progeny of the two cybrids showed consistent fertility in further generations and no sorting out of fertile and male sterile progenies.

The cms cybrids No. 1716 and 1640 of type 3704 shown in Table 2 were classified as male sterile plants (see 'Materials and methods'), but instability occurred in the progenies of the first generation: when cybrid 1716 was crossed to line 3704, only male sterile progeny were obtained. However, a cross to line 4501 (cv. 'Comanche') gave only male fertile progeny in the F_1. Cybrid 1640 yielded both male sterile and two F^3 progenies, thus showing sorting out of cms and fertility. The fertile 3704 plants which regenerated from protoplasts (Table 2) have only fertile progeny when crossed with 3704 or upon selfing.

Discussion

Our data (Tables 1 and 2) show that normal plasmon transfer yielded stable cybrids, while the transfer of cms plasmon yielded unstable cybrids which eventually lost the acquired sterility. The lack of stability in the cms plasmon described here (Table 2) is similar to the results obtained by Izhar et al. (1983) using the same parental lines for the protopast fusion. Most of the somatic hybrids between line 3704 and 3688 (97%) were fertile. Only 2% were stable sterile and less than 1% was sectorial somatic hybrid plants containing heterplasmon and showing fertility and sterility on the same plant. In Izhar et al. (1983, Fig. 1) instability in some somatic hybrids led to conversion of plant sectors from sterility to fertility. We see some similarity between these data and those in Table 2. It may well be that the nuclear

background of line 3704 favours the survival of the hypothetical *fte* elements over the *ste* elements in the heteroplasmon. The lack of stability of the cms cybrids is not always the case in cybrids of petunia. Izhar and Tabib (1980), using two different parental lines, obtained heteroplasmic cybrids which expressed fertility instead of sterility as in the present study and in Izhar et al. (1983).

The present data do not allow us to draw conclusions as to whether there is nuclear control over the survival of the *ste* or *fte* elements, but the present study and that of Izhar et al. (1983), as compared with Izhar and Tabib (1980) may suggest such a possibility.

So far, we do not have a molecular marker to indicate the presence of *ste* or *fte*. However, some molecular probes are available to indicate whether any change occurred in the mitochondrial DNA in the cybrids (Boeshore et al. 1983), as compared with the original parental lines. A study is under way in our laboratory, concerning the plant material in this report.

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