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Production and characterization of fertile somatic hybrids of eggplant (*Solanum melongena* L.) with *Solanum aethiopicum* L.

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Summary. In order to produce fertile somatic hybrids, mesophyll protoplasts from eggplant were electrofused with those from one of its close related species, *Solanum aethiopicum* L. Aculeatum group. On the basis of differences in the cultural behavior of the parental and hybrid protoplasts, 35 somatic hybrid plants were recovered from 85 selected calli. When taken to maturity either in the greenhouse or in the field, the hybrid plants were vigorous, all rapidly overtopping parental individuals. The putative hybrids were intermediate with respect to morphological traits, and all of their organs were larger, particularly the leaves and stems. DNA analysis of the hybrids using flow cytometry in combination with cytological analysis showed that 32 were tetraploids, 1 hexaploid and 2 mixoploids. The hybrid nature of the 35 selected plants was confirmed by a comparison of the isoenzyme patterns of isocitrate dehydrogenase (Idh), 6-phosphogluconate dehydrogenase (6-Pgd) and phosphoglucomutase (Pgm). Chloroplast DNA (ctDNA) restriction analysis using *Bam*HI revealed that among the 27 hybrid plants analyzed, 10 had *S. aethiopicum* patterns and the 17 remaining hybrids exhibited bands identical with those of eggplant without any changes. All of the somatic hybrid plants flowered. Both parental plants had 94% stainable pollen, while the hybrids varied widely in pollen viability ranging from 30% to 85%. The somatic hybrids showed high significant variation in fruit production. Nevertheless, there was a tendency for low fertility to be associated often with *S. aethiopicum* chloroplast type and/or with an abnormal ploidy level, while good fertility was mostly associated with the tetraploid level and eggplant chloroplasts. Interestingly, 2 tetraploid somatic hybrid clones were among the most productive, yielding up to 9 kg/plant. As far as the fertility of the F_1 sexual counterpart was concerned, only 2 fruits of 50 g were obtained. Hybrid fertility in relation to phylogenetic affinities of the fusion partners is discussed.

Key words: Electrofusion – Protoplasts – *S. melongena* – *S. aethiopicum* – Somatic hybrids – Field evaluation – Fertility

Introduction

Eggplant (*Solanum melongena* L.) is an economically important crop species. Tissue cultures are being employed in conjunction with classical breeding techniques for the improvement of this plant. Somatic hybridization by protoplast fusion provides the means by which to increase genetic variability through recombination of the nuclear and cytoplasmic genomes. In this way new combinations or organizations of cytoplasmic genomes can particularly be obtained (Belliard et al. 1979; Nagy et al. 1981; Galun et al. 1982; Robertson et al. 1987; reviewed by Medgyesy 1990). Somatic hybridization also offers a means of transferring desirable agronomic traits from wild to cultivated species (Primard 1984; Austin et al. 1985; Gibson et al. 1988; Serraf et al. 1991).

During the last 5 years interspecific somatic hybrids of eggplant have been produced from protoplast fusion with *S. sisymbriifolium* (Gleddie et al. 1986), *S. khasianum* (Sihachakr et al. 1988), *S. torvum* (Guri and Sink 1988a; Sihachakr et al. 1989) and *S. nigrum*

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(Guri and Sink 1988b). Preliminary evaluation of agronomic traits revealed that these somatic hybrids exhibit certain desirable properties, such as resistance to nematodes and spider mites inherited from *S. sisymbriifolium* (Gleddie et al. 1985), resistance to *Verticillium* wilt from *S. torvum* (Guri and Sink 1988a) and resistance to the herbicide atrazine from *S. nigrum* (Guri and Sink 1988b). However, the high sterility of the somatic hybrid plants produced so far has limited their use in the breeding program of eggplant. The question arises of whether this sterility may be due to the fact that the wild fusion partners used so far have been shown in morphological, serological and cross-ability studies to exhibit low phylogenetic affinities with eggplant. Following sexual crosses with eggplant, these wild fusion partners gave partially fertile hybrids or no hybrids at all (Daunay et al. 1991). If sexual and somatic hybridization seems to converge on hybrid fertility, depending on phylogenetic distance between the partners, protoplast fusion of two closely related species is expected to result in fertile hybrids.

In the investigation presented here our aim was to produce and characterize the fertile somatic hybrids that resulted following electrofusion between mesophyll protoplasts from eggplant and one of its closely related species, *S. aethiopicum* Aculeatum group. This wild species is used as a standard rootstock for Japanese commercial eggplant production because of its resistance to bacterial (*Pseudomonas solanacearum*) and fungus (*Fusarium oxysporum* f. sp. *melongenae*) wilts (Daunay et al. 1991). It can be crossed with eggplant, but the resulting seeds give rise to an F_1 hybrid with lowered fertility (Rao 1979). Nevertheless, promising eggplant lines carrying resistance to bacterial wilt have been recently obtained from this partially fertile interspecific hybrid (Ano et al. 1991). In order to evaluate the potential of somatic hybridization in the breeding program of eggplant, the somatic hybrid plants obtained in this study were also assessed in the field for morphology and pollen viability, and, in particular, for hybrid fruit production.

Materials and methods

Plant materials

The *Solanum melongena* cv 'Dourga' (an I.N.R.A. variety with white half-long fruit) and *Solanum aethiopicum* L. Aculeatum group (I.N.R.A. accession number = BOT2) were used. Seeds of these two lines were germinated on MS basal medium (Murashige and Skoog 1962) containing vitamins (Morel and Wetmore 1951) and 20 g/l sucrose and solidified with 7 g/l agar. Plants were then propagated by subculturing leafy node cuttings on the same medium at 4-week intervals. Environmental conditions were 12 h/day illumination (62 μE/m^2 per second), 27 °C and 60% humidity.

Protoplast isolation

The protoplast source was lamina taken from 3- to 4-week-old cuttings. Leaves of eggplant and *S. aethiopicum* were slightly scarified and then transferred into the filtered enzyme solution containing CPW salts (Frearson et al. 1973), 9.1% (w/v) mannitol, 1.5% (w/v) cellulase R-10, 0.5% (w/v) macerozyme R-10 and 0.05% (w/v) 2-(N-morpholino) ethanesulfonic acid (MES) buffer at pH 5.5–5.6. The leaves were placed face down on the solution and incubated overnight in the dark at 27 °C. At the end of the digestion period, protoplasts were separated from undigested materials by passage through metallic sieves (100-μm mesh), and the resulting suspension was washed once in a CPW solution (Frearson et al. 1973) containing 0.25 *M* mannitol and 0.125 *M* NaCl by centrifugation at 55 *g* for 5 min. The supernatant was removed, and the protoplasts were re-suspended in a CPW solution containing 21% (w/v) sucrose and then centrifuged at 120 *g* for 10 min. Floating protoplasts were washed twice in CPW (0.25 *M* mannitol + 0.125 *M* NaCl). Prior to fusion, the protoplasts were washed once in a 0.5 *M* mannitol solution supplemented with 0.2 m*M* $CaCl_2$ and then they suspended in this solution at a density of 3×10^5 protoplasts per milliter.

Electrofusion apparatus and fusion procedure

The electrical apparatus and fusion procedure described in Sihachakr et al. (1988) were used. The movable multi-electrodes were placed into a 15 × 50-mm petri dish containing 500- to 700-μl aliquots of a mixture (1:1) of protoplasts from both parents. In order to align the protoplasts we applied an A.C.-field at 125 V/cm and 1 Mhz for 15 s; subsequently, two square pulses developing 1.2 KV/cm for 20 μs each were applied to achieve protoplast fusion.

Protoplast culture and plant regeneration

Immediately after fusion, 6 ml culture medium was gradually added to the protoplast mixture. The culture medium was KM (Kao and Michayluk 1975) supplemented with 250 mg/l polyethyleneglycol 6000 (PEG), 0.2 mg/l 2,4-dichlorophenoxy-acetic acid (2,4-D), 0.5 mg/l zeatin, 1 mg/l α-naphthaleneacetic acid (NAA), 6.5% (w/v) glucose as osmoticum and 0.05% (w/v) MES buffer. Protoplasts were initially cultured in the dark at 27 °C for 7 days. Afterwards, they were exposed to 12 h/day illumination (62 μE/m^2 per second). On day 10, the cultures were diluted 8 times with the same medium, but growth regulators were replaced with 2 mg/l benzylaminopurine (BAP) and 0.1 mg/l 2,4-D. Fifteen days after the cultures were diluted, the calli showing increased vigor in their growth were selected and transferred onto the regeneration medium, which consisted of MS + vitamins (Morel and Wetmore 1951), 20 g/l sucrose, 7 g/l agar, 2 mg/l zeatin and 0.1 mg/l indol-3-acetic acid (IAA). Shoots were excised from the callus and rooted on hormone-free MS medium; the rooted plants were then transferred to the greenhouse (16 h/day illumination at 180 μE/m^2 per second, 20–30 °C and 70% humidity).

Identification of somatic hybrids

Identification of somatic hybrid plants was based on the vigor and morphological analysis of in vitro and greenhouse-grown plants, especially through the features of leaves, inflorescence, flowers and fruits. Hybridity was further confirmed by an examination of the patterns of three isoenzyme systems.

Isoenzyme analysis

Leaf extracts were prepared with leaves taken from in vitro plants according to the methods described in Sihachakr et al.

(1989). Isocitrate dehydrogenase (Idh) (E.C.1.1.1.42), 6-phosphogluconate dehydrogenase (6-Pgd) (E.C.1.1.1.44) and phosphoglucomutase (Pgm) (E.C.2.7.5.1.) were examined. They were separated by electrophoresis on 13% starch gels (Smithies 1955) and stained following the procedures described by Shields et al. (1983).

Determination of ploidy level and cytological analysis

Flow cytometry was used for the determination of ploidy level. One leaf taken from in vitro plants was chopped with a razor blade in 400 µl of a buffer solution consisting of CPW salts (Frearson et al. 1973), 9.1% (w/v) mannitol, 0.25% (w/v) PEG, 0.25% (v/v) 2-mercaptoethanol, 0.5% (v/v) triton, pH 6.5–7.0. Crude samples were passed through nylon nets with 40 µm meshes and stained with a DNA-specific dye, bisbenzimide Hoechst 33342 (2 µg/ml). Nuclei were analyzed in an Epics V flow cytometer (Spectra-Physics argon laser) using wavelengths of 351–364 nm for excitation. Each histogram was generated by the analysis of at least 10,000 nuclei. Cytological analysis was done on root tips taken from greenhouse-grown plants as described in Sihachakr et al. (1988). Briefly, root tips were pretreated with a saturated solution of α-chloronaphthalene for 2–3 h at room temperature, fixed in ethanol glacial acetic acid (3:1, v/v) for 24 h and hydrolyzed in 5 *N* HCl for 30 min. The preparation was then stained with acetocarmine (0.5% w/v acetocarmine in 45% acetic acid).

Analysis of chloroplast DNA (ctDNA)

The protocols for the isolation of chloroplast DNA- and restriction analysis using *Bam*HI enzyme are described in San et al. (1990).

Field trials

In order to study fertility and field assessment of somatic hybrids, the 35 selected hybrid clones and two parental lines were transplanted to an open field in Monfavet (I.N.R.A., France) during the summer of 1990 (Table 1). Plants were propagated by stem cuttings. The rooted cuttings, 25 cm tall, were then transplanted to soil within a plantation line: 2 × 2 plants of each of the check genotypes ('Dourga' and *S. aethiopicum* Aculeatum group) began and ended the line of the 35 somatic hybrid plants (1 plant per genotype for the hybrids). Cultural practices, weed control and fungicide treatments were similar to those used in commercial production. The cuttings were planted in May 1990. Individual plants were evaluated for several criteria: flower number per inflorescence and flower diameter (a mean value was calculated from 3–30 measurements, depending on the quantity of flowers available on each genotype in July and August), pollen stainability (determined by staining a minimum of 200 grains for each genotype) with 1% acetocarmine; a mean value for pollen stainability was estimated from sampling done in July and August), fruit production (plants were completely harvested in October, and both ripe and unripe fruits counted and weighed), weight of the seeds in the mature fruits (seeds from a sample of 12 fruit were extracted then weighed; the mean value of seed weight for one fruit was then estimated).

A reference control was provided by a F_1 sexual counterpart. The crosses between *S. melongena* cv 'Dourga' and *S. aethiopicum* BOT2 were carried out in the greenhouse in 1991. Seedlings of the resulting F_1 sexual hybrid were planted in the field in the summer of 1991 for evaluation.

Results

Electrofusion, protoplast culture and plant regeneration

Overall fusion frequencies of 25–30% (defined as the number of protoplasts relative to the number of aligned protoplasts) were obtained after the application of DC square pulses. Because of a great similarity in the shape and the presence of chloroplasts in protoplasts from both fusion partners, we were not able to visually identify heterokaryons. However, the frequency of binary fusions was estimated at being 30% of the fusion products. After 7 days of culture, the mixture of fused protoplasts underwent division at an estimated frequency of 15%, while 15% and 10% of eggplant and *S. aethiopicum* unfused protoplasts, respectively, underwent division in the control experiments.

Several thousands of calli were recovered from both the control and fusion experiments after the cultures were highly diluted with fresh medium supplemented with 2 mg/l BAP and 0.1 mg/l 2,4-D. At this time, three types of calli were distinguishable on the basis of their cultural behavior. Types-I and -II calli had the same rate of growth but could be distinguished from each other by their color. Type-I calli were faint green and type-II deep green; they were further identified as those derived from eggplant and *S. aethiopicum* protoplasts, respectively. The aspect of calli of type III was similar to that of type II except for the growth rate of the former being twice as rapid as that of the latter. When transferred onto regeneration medium in the control experiments, all of *S. aethiopicum* protoplasts, respectively. The aspect of shoots within 3 weeks, while eggplant calli required a regeneration time twice as long. Numerous small green shoots were scattered from *S. aethiopicum*-regenerating calli, but only 3–5 of them developed into plantlets bearing two green circular-shaped leaves. Very similar small green shoots, but fewer in number, were also observed over regenerating type-III calli. In this case, 1–2 shoots developed rapidly into plantlets that quite early exhibited a much stronger growth vigor than their parents. All of these shoots were carefully maintained and subjected to further detailed analysis since they were putative somatic hybrids. Finally, 28 of the 85 type-III calli selected gave rise to 35 putative hybrid shoots.

Cytometry and cytological analysis

Determination of the ploidy level of the 35 putative somatic hybrid plants was carried out by flow cytometry. The analysis of these plants showed that 32 were at the expected tetraploid level (4x), 1 was hexaploid (6x) and 2 were mixoploids (Table 1).

Table 1. Chromosome numbers, ctDNA type, number of flowers per inflorescence, flower diameter, pollen stainability, fruit yield and number, fruit mean weight and fruit seed content. The hybrids are designated by a number identifying the callus (DSA 1, 2 . . .) from which they are derived and a letter (a, b) when several plants are from the same cellus. The hybrid plants are classified according to their ctDNA type. Comparison of the means is made between DSA and SM and SA chloroplasts

Number of plants	Chromosome number	ctDNA type	Average number of flowers/ inflorescence	Average diameter of flowers (mm)	Pollen stainability (%)	Fruit yield (kg/plant)	Average number of fruits/plant	Fruit mean weight (g)	Average seed weight in a single fruit (g)
S. melongena	24	SM	1.0	39.2	94.3	2.55	15	170.0	2.50
S. aethiopicum	24	SA	10.0	19.3	94.1	1.71	66	25.8	2.10
F_1 sexual hybrid	24	–	–	–	10–30	0.05	2	0.02	–
DSA 1	48	SM	9.0	36.0	68.6	2.82	111	25.4	0.34
DSA 4a	48	SM	8.1	33.4	77.5	3.04	99	30.7	0.38
DSA 4b	48	SM	8.1	36.3	68.6	3.65	128	28.5	0.48
DSA 17a	48	SM	8.6	38.2	69.3	3.69	115	32.0	0.55
DSA 21	48	SM	6.9	34.5	78.8	9.14	214	42.7	0.77
DSA 26b	48	SM	8.2	31.8	85.0	5.06	179	28.2	0.95
DSA 27	48	SM	9.0	32.7	75.8	3.04	108	28.1	0.74
DSA 33	72 + 96	SM	–	–	–	–	–	–	–
DSA 34	48	SM	8.4	31.4	71.0	1.93	60	32.2	0.63
DSA 103a	48	SM	8.0	38.9	69.7	2.57	81	31.7	0.48
DSA 108	48	SM	7.8	31.6	65.2	2.24	78	28.7	0.50
DSA 110	48	SM	8.7	33.9	76.6	9.41	250	37.6	0.69
DSA 116	48	SM	7.1	32.1	69.2	0.58	30	19.2	0.30
DSA 120	48	SM	9.6	31.5	76.3	5.56	155	35.9	0.73
DSA 122	48	SM	8.4	32.0	80.8	5.89	179	32.9	0.54
DSA 127	48	SM	7.5	29.8	73.8	1.44	57	25.3	0.51
DSA 201a	48	SM	9.1	35.3	73.0	4.58	151	30.3	0.56
Means (DSA with SM chloroplasts)			8.2 NS	33.7 NS	69.4 NS	4.05*	125*	30.6 NS	0.57 NS
DSA 2	48	SA	7.5	36.3	73.6	3.02	114	26.5	0.74
DSA 3	48	SA	7.4	34.9	71.6	2.15	69	31.1	0.67
DSA 17b	48	SA	7.4	35.2	30.8	0.19	8	23.1	0.16
DSA 25a	48	SA	8.2	35.9	68.1	0.25	10	24.5	–
DSA 35	48	SA	7.6	36.4	65.6	2.39	103	23.2	0.61
DSA 104	48	SA	8.3	32.7	72.6	3.65	122	29.9	0.82
DSA 106b	48	SA	8.6	35.1	76.8	5.22	152	34.3	0.60
DSA 201b	48	SA	7.2	31.6	74.2	0.00	0	0.00	0.00
DSA 402	48	SA	7.3	33.9	80.8	5.85	167	35.0	0.56
DSA 501	48	SA	–	–	–	0.03	1	30.0	–
Means (DSA with SA chloroplasts)			7.9 NS	34.7 NS	68.2 NS	2.28*	75*	25.8 NS	0.52 NS
DSA 6	48 + 96	–	13.1	22.0	66.0	1.80	109	31.1	0.25
DSA 12	72	–	8.4	32.6	43.0	0.41	41	10.0	0.03
DSA 25b	48	–	7.5	35.1	74.9	3.34	94	35.5	0.63
DSA 26a	48	–	7.5	35.0	79.6	5.46	157	34.8	0.76
DSA 29	48	–	8.1	29.9	70.4	7.78	252	30.9	0.64
DSA 103b	48	–	8.0	32.5	71.0	5.72	186	30.8	0.56
DSA 106a	48	–	7.9	35.3	77.8	0.80	31	25.7	0.54
DSA 401	48	–	6.9	30.8	75.6	2.22	91	24.3	0.41
Means (ctDNA not analyzed)			8.4	31.7	69.8	3.44	120	27.9	0.48
DSA average	–	–	8.2	33.5	71.3	3.38	107	29.4	0.6
DSA min-max	–	–	2–26	18–48	30.8–85.0	0.00–9.41	0–252	10.0–42.7	0.03–1.00

* Significantly higher at $P = 0.05$; NS, non-significant at $P = 0.05$; SM, *S. melongena*; *S. aethiopicum*; DSA, somatic hybrids of *S. melongena* and *S. aethiopicum*

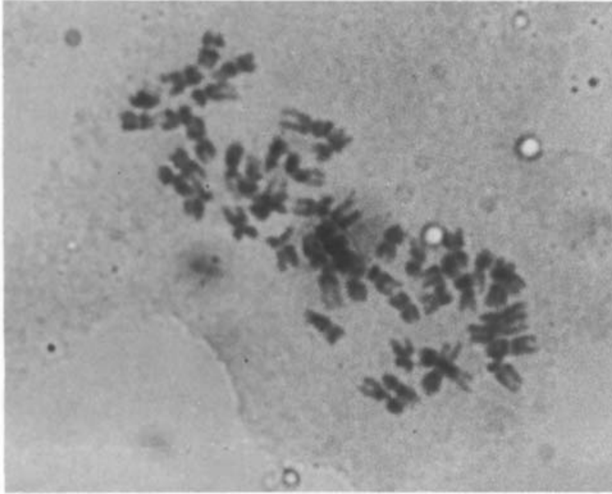

Fig. 1. A root-tip cell of a tetraploid somatic hybrid ($2n = 4x = 48$ chromosomes)

Chromosome counts made on a random sample of 20 plants taken among the hybrids, in particular those showing a ploidy level different from that of tetraploids, confirmed the results of flow cytometry. It was not possible to distinguish between the two chromosome sets in the somatic hybrids because of their great morphological similarity (Fig. 1).

Isoenzyme analysis

The hybrid nature of the 35 selected plants was confirmed by examining isoenzyme patterns for isocitrate dehydrogenase (Idh), 6-phosphogluconate dehydrogenase (6-Pgd) and phosphoglucomutase (Pgm). On the basis of the banding patterns of these three isoenzyme systems, the parents could be distinguished from each other as could somatic hybrids from the parents. For Pgm (Fig. 2A), the somatic hybrid patterns contained two specific bands that were identical with those found for the mixed extracts from both parents. For the banding patterns of 6-Pgd and Idh, in addition to the sum of the parental bands, the hybrids contained a heterodimer band not found in the mixed extracts of the parents (Fig. 2B, C).

Identification of chloroplasts

Chloroplasts were identified by means of ctDNA restriction analysis with *Bam*HI. Parental banding patterns were distinguishable from each other by the presence of specific bands. Two specific ctDNA bands

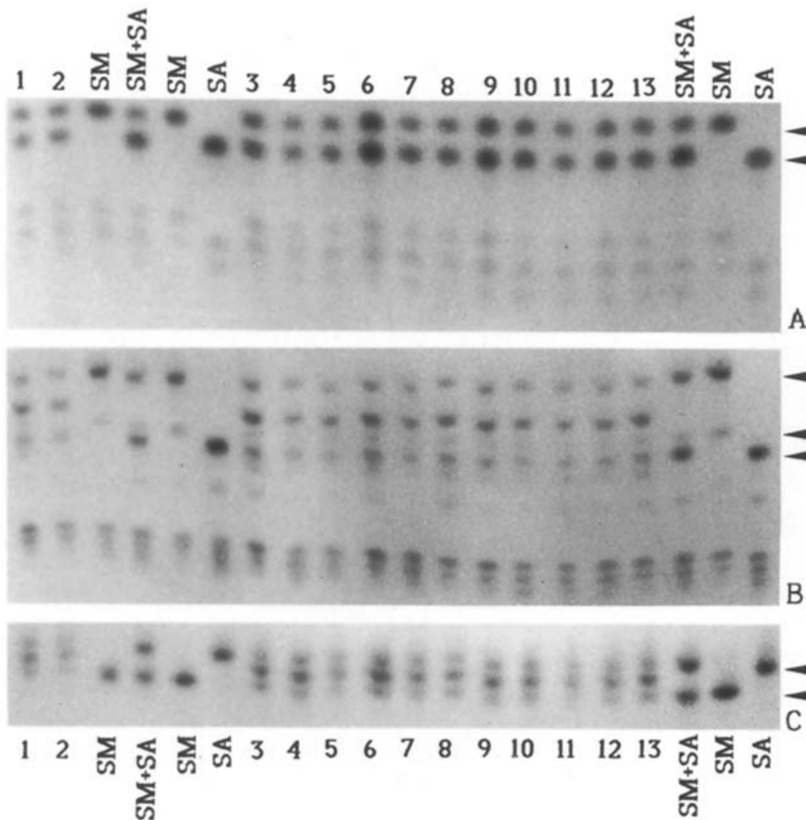

Fig. 2A–C. Electrophoresis banding patterns of **A** phosphoglucomutase (Pgm), **B** 6-phosphogluconate dehydrogenase (6-Pgd) and **C** isocitrate dehydrogenase (Idh) from a sample of somatic hybrids (*lanes 1–13*), eggplant (*SM*) *S. aethiopicum* (*SA*) and a mixture of both parents (*SM + SA*)

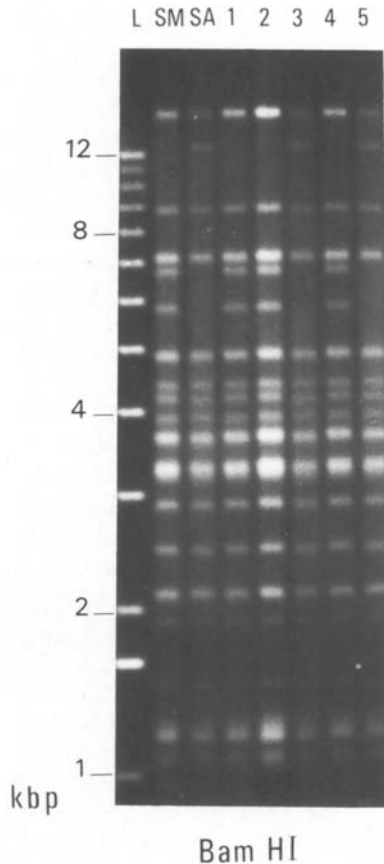

Fig. 3. *Bam*HI restriction patterns of *ctDNAs from* eggplant (*SM*), *S. aethiopicum* (*SA*) and 5 somatic hybrids (*lanes 1–5*). *L* 1-kbp ladder

of 5.8 and 6.7 kbp were found for eggplant and one of 13 kbp for *S. aethiopicum* (Fig. 3). Among the 27 hybrid plants analyzed, 10 had *S. aethiopicum* patterns without any changes (lanes 3 and 5; Fig. 3), and the 17 remaining hybrids had bands identical with those of eggplant (lanes 1, 2 and 4; Fig. 3) (Table 1). Neither a ct recombinant nor a mixture of parental ct types was found. Interestingly, the same callus may give rise to hybrid plants with either only one (DSA 4a and b with eggplant chloroplasts) or both chloroplast types (DSA 17 and DSA 201 with a: eggplant and b: *S. aethiopicum* chloroplasts; Table 1).

Analysis of morphology and fertility

When taken to maturity in the greenhouse, the putative somatic hybrid plants grew vigorously, all rapidly overtopping parental individuals (Fig. 4B). In addition to having a morphology intermediate to that of either parent, all of the organs of the hybrids were larger, in particular, the leaves and stems (Fig. 4A). The putative hybrid plants resembled *S. aethiopicum* with respect to presence of anthocyanin and few spins along stems and leaf veins, while the hybrid leaf blade was slightly lobed and its base united as in eggplant.

Transplanted to the open field, the hybrid plants again showed a strong vigor. They were morphologically intermediate between the two parents, except for 3 hybrid plants (DSA 6, DSA 12 and DSA 201b), which resembled the phenotype of *S. aethiopicum.*

Hybrids had mauve-edged petals with white sectors in the center; those from eggplant were uniformly mauve and those of *S. aethiopicum* were white (Fig. 4C). Taking this observation into account together with the isoenzyme banding patterns of the selected regenerants, we concluded that the 35 selected hybrid plants were indeed somatic hybrids of eggplant with *S. aethiopicum.*

Hybrid plants (except DSA 6) had an average flower number per inflorescence close to that of *S. aethiopicum* (high number), while their average flower diameter was close to that of eggplant (great diameter) at $P = 0.05$ (Table 1). Somatic hybrid DSA 6 had individually specific flower characteristics: more flowers per inflorescence with a similar flower diameter than *S. aethiopicum.* Analysis of pollen stainability revealed that both parental plants had a high percentage of stainable pollen, estimated to be 94%, while the somatic hybrids had lower and variable values that ranged from 30% to 85% (Table 1). The somatic hybrids showed high variation in fruit production. The fruit numbers ranged from 0 fruit/plant (full sterility) to 252 fruit/plant, and fruit yields from 0 kg/plant to 9.4 kg/plant, values that largely overlapped the parental values (Table 1). Interestingly, the somatic hybrids with eggplant chloroplasts showed nearly two-fold higher yields (4.05 kg/plant) than those with *S. aethiopicum* chloroplasts (2.28 kg/plant), while fruit mean weight was the same for all hybrids and very close to that of *S. aethiopicum* (Table 1). This resulted in a significantly higher number of fruit in the hybrids with eggplant-type chloroplasts (125 fruits/plant) than those with *S. aethiopicum* chloroplasts (75 fruits/plant) (Table 1). Fruit shape was half-long for 'Dourga', round and very fasciated for *S. aethiopicum* and oval and slightly fasciated for the hybrids except for 2 of them (DAS 6 and 12) whose fruit shape was very close to that of *S. aethiopicum.* Immature hybrid fruit were green; they turned orange when ripe, an intermediate color between the mature red fruits of *S. aethiopicum* and the ripe yellow ones of 'Dourga' (Fig. 4D). Lastly, hybrid fruit contained seeds but significantly fewer than those of the parental lines. The hybrid seeds resembled those of eggplant in their form, size and color, except those of hybrid DSA 6, which were slightly yellow like those of *S. aethiopicum.*

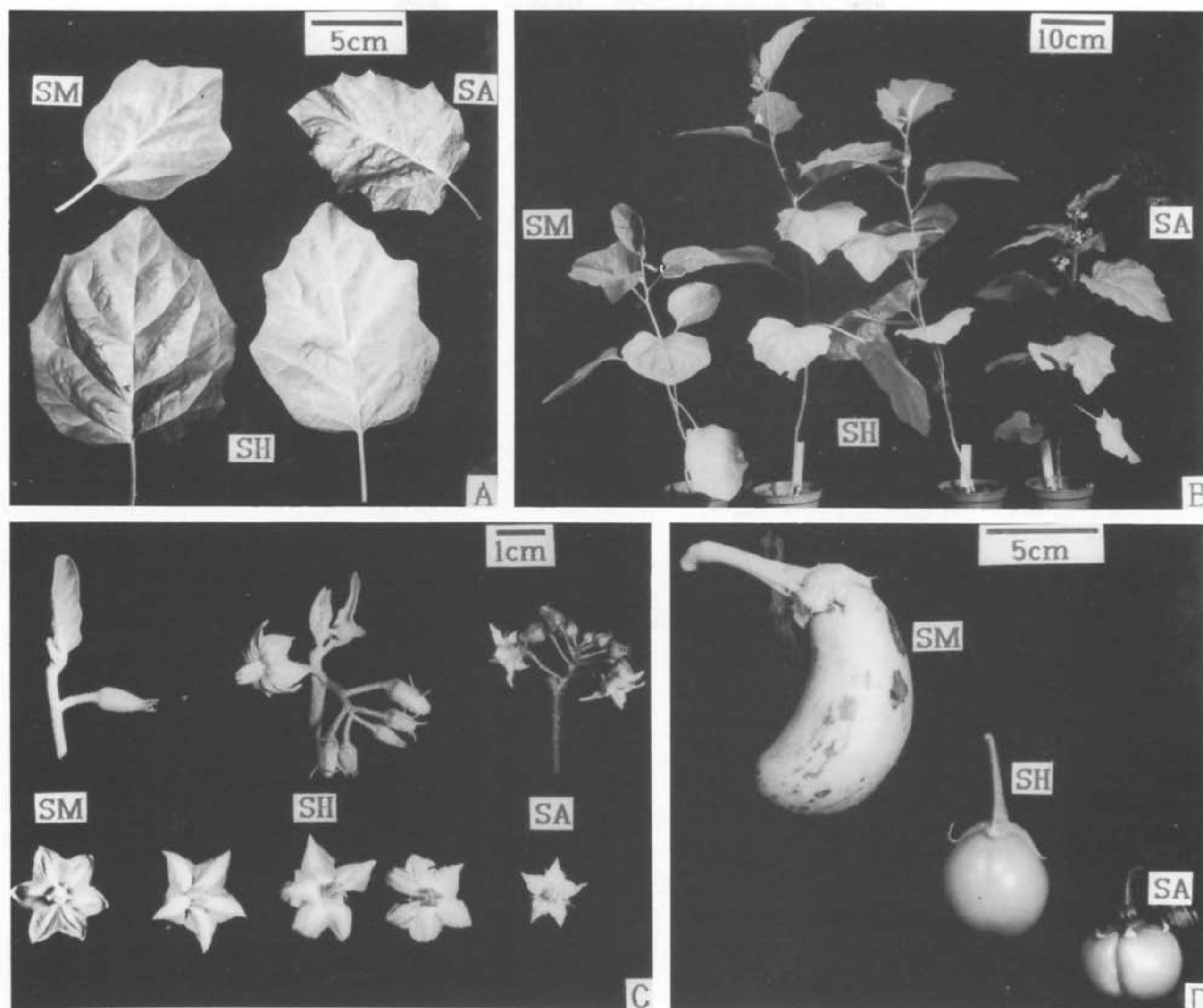

Fig. 4A–D. **A** Leaves, **B** plants, **C** inflorescence and flowers and **D** fruits of eggplant (*SM*), *Solanum aethiopicum* (*SA*) and their somatic hybrids (*SH*)

Very few seeds were obtained from the crosses between eggplant and *S. aethiopicum*, and those that were gave rise to vigorous individuals. Interestingly, the great resemblance between somatic and sexual hybrids, especially through morphology of the leaves, flowers and fruits, provided further evidence for the hybridity of the somatic hybrid plants. However, the F_1 sexual hybrids exhibited a very lower fertility, with only 2 fruit of 50 g/plant, than their somatic counterparts which yielded 107 fruit of 3.38 kg/plant on average (Table 1).

Discussion

In the investigation presented here many somatic hybrid plants were produced following electrofusion between mesophyll protoplasts from eggplant and one of its relatives species, *S. aethiopicum* Aculeatum group. Both the high rate of fusion induced by electrofusion and the particular culture behavior of hybrid calli certainly increased the effectiveness of hybrid selection. It has been reported that the technique of electrofusion induces a high rate of fusion (Tempelaar et al. 1987) with eggplant protoplasts in particular (Sihachakr et al. 1988, 1989), thus eliminating a requirement for elaborate heterokaryon selection schemes (Fish et al. 1988). Moreover, the early selection of hybrid calli of eggplant with *S. aethiopicum* was based upon the vigor in their growth. The inreased vigor of hybrid materials has also been used successfully for recovering hybrids after protoplast fusion, particularly those from potato (Debnath and Wenzel 1987; Serraf et al. 1991).

The ploidy level of the selected plants was determined by flow cytometry and confirmed by chromosome counts on a random sample of plants. The flow cytometric method has been reported to be efficient for the determination of ploidy level (Petit et al. 1986; De Laat et al. 1987) with a strong correlation between DNA content and chromosome numbers (Fahleson et al. 1988). The majority (91%) of somatic hybrid plants recovered in this study were at the expected tetraploid level. These results are in agreement with those from previous studies concerning the regeneration of mostly tetraploid hybrids of eggplants with *S. khasianum* (Sihachakr et al. 1988) and *S. torvum* (Guri and Sink 1988a; Sihachakr et al. 1989). The tetraploid state of the hybrids may be due to the use of differentiated tissues like leaves as the protoplast source; somatic hybrids regenerated from a cell suspension generally exhibit a higher variation in chromosome numbers (Schieder and Kohn 1986). This seemed to be the case in the recovery of aneuploid somatic hybrids of eggplant with *S. sisymbriifolium* (Gleddie et al. 1986) where protoplasts from a cell suspension were used as one of the fusion partners. The regeneration of primarily tetraploid hybrids of *S. melongena* with *S. aethiopicum* can also be attributed to the close phylogenetic affinities between these two species. When the fusion partners are distantly related, the final product is often an asymmetric combinaison of the two genomes (Pelletier et al. 1983; Gleba et al. 1984), resulting in the preferential elimination of the chromosomes of one parent (Pijnacker et al. 1989).

Protoplast fusion is a powerful means of overcoming maternal inheritance of cytoplasmic genomes. Somatic hybrids usually have chloroplasts from either one of the parents (Belliard et al. 1978; Fluhr et al. 1983) and sometimes a transitory mixture of both (Chen et al. 1977; Glimelius et al. 1981). In this study, 37% of the hybrids had *S. aethiopicum*-type chloroplasts, while the majority (63%) had ctDNA bands identical to those from eggplant. It has been reported that sorting-out follows rapidly after fusion (Morgan and Maliga 1987) and, therefore, hybrid plants with both parental types of chloroplasts were recovered from the same callus in this study. Interestingly, the great majority of somatic hybrids recovered so far from fusions between eggplant and three other related species *S. sisymbriifolium*, Gleddie et al. 1986; *S. khasianum*, Sihachakr et al. 1988; *S. torvum*, Guri and Sink 1988a; Sihachakr et al. 1989) had chloroplasts of the eggplant type. This information appears to be in favor of a biased organelle transmission. The latter case was described in somatic hybrids between tomato and *S. lycopersicoides*, where 68 out of 70 hybrids examined had tomato chloroplasts and only 1 had the wild-type plastids (Levi et al. 1988).

Up to the present time, although it is known that somatic hybridization can overcome sexual crosses by easily regenerating whole hybrid plants from fusions between eggplant and other wild species (*S. sisymbriifloium*, Gleddie et al. 1986; *S. khasianum*, Sihachakr et al. 1988; *S. torvum*, Guri and Sink 1988a; Sihachakr et al. 1989; *S. nigrum*, Guri and Sink 1988b), the usefulness of the hybrid plants in breeding programs has been limited by high sterility. As a matter of fact, no hybrids were obtained when eggplant was crossed with *S. sisymbriifolium* and *S. nigrum*, while the technique of embryo rescue was needed to produce the *S. melongena* × *S. torvum* (Daunay et al. 1991) and *S. melongena* × *S. khasianum* hybrids (Sharma et al. 1980). On the contrary, fusion products from the combinations of eggplant with these wild species proliferated actively at the callus stage, and finally regenerated plants. This observation seems to be coherent with the fact that somatic incompatibility may be bypassed mainly because of intrinsic characteristics of in vitro-cultured cells, as suggested by Negrutiu et al. (1989).

The relatively high fertility (pollen stainability, fruit production and fruit seed content) of the somatic hybrids of eggplant with *S. aethiopicum* can be attributed both to the relatively close phylogenetic relationship between the two fusion partners and to their polyploidy. As a matter of fact, if the interspecific F_1 hybrid resulting from the cross between *S. melongena* × *S. khasianum* hybrids (Sharma et al. be obtained in this study, as well as by others (Khan et al. 1978), its fertility in terms of pollen stainability is rather low (10–30% according to Daunay et al. 1991) and its exploitation for breeding is laborious because of difficulties in obtaining viable seeds on it and its progenies (Ano et al. 1991). But after colchicine treatment, the fertility of this 'sexual' hybrid (then allotetraploid) is increased (Ludilov 1974; Rao and Baksh 1979). It is well known that the allotetraploid status is able to restore the fertility of several other interspecific sexual *Solanum* hybrids (Rajasekaran 1970, 1971; Rao 1981).

Our measurements are unfortunately incomplete to estimate the respective incidence of the nuclear and cytoplasmic genomes on the variability of the somatic hybrids of eggplant with *S. aethiopicum* for morphology and fertility criteria, since only the ctDNA type has been determined (Table 1). Nevertheless, it is observed that hybrid fertility apparently varied with the chloroplast type and the ploidy level. As shown in Table 1 where the somatic hybrids are classified according to their chloroplast type, there is a tendency that low fertility, in terms of pollen stainability, fruit production and seed weight per fruit, is often associated with the *S. aethiopicum* chloroplast type and/or with an abnormal ploidy level (DSA 6, 12, 17b,

25a, 201b and 501), while good fertility is mostly associated with the tetraploid level and the eggplant chloroplast type (DSA 21, 26a, 26b, 110, 120 and 122). Interestingly, fruit yield and number of fruit per plant are significantly higher in the hybrids with eggplant chloroplasts than those with *S. aethiopicum* chloroplasts (Table 1). Lastly, somaclonal variation possibly induced by the callus phase during the regeneration process of the somatic hybrids could be involved in the variability observed, but this last hypothesis has to be verified on the progenies of the somatic hybrids (heritability of the variation observed among the hybrids).

Our principal interest in these interspecific somatic hybrids between *S. melongena* and *S. aethiopicum*, compared to their sexual F_1 counterpart, consists mainly in their distinct and variable cytoplasmic composition. Their ctDNA is either of the *S. aethiopicum* or eggplant type. The mtDNA patterns, not studied here, are probably variable from one to another hybrid as far as other interspecific somatic hybrids of eggplant are concerned (Bellamy 1989). Further, the potential usefulness of the somatic hybrids produced herein in eggplant breeding depends not only on their ability to transmit new traits of agronomic interest, but also on the possibility of bringing their tetraploid status back to the diploid level and then backcrossing them successfully with eggplant germplasm. Previous successful anther culture experiments carried out on allotetraploid 'sexual' progenies from the cross *S. melongena* × *S. torvum* (M. C. Daunay unpublished) have demonstrated that such a return to the diploid status was possible using an anther culture setup on eggplant (Dumas de Vaulx and Chambonnet 1982).

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