Theor Appl Genet (1990) 79: 632–640

THEORETICAL AND APPLIED GENETICS

© Springer-Verlag 1990

# Protoplast-fusion-derived *Solanum* cybrids: application and phylogenetic limitations

**A. Perl, D. Aviv and E. Galun** *

Department of Plant Genetics, Weizmann Institute of Science, Rehovot 76100, Israel

Received November 1, 1989; Accepted December 20, 1989
Communicated by R. Hagemann

**Summary.** We established interspecific *Solanum* cybrids in order to study the intrageneric nuclear-organelle compatibility and the introgression of advantageous plasmone-coded breeding traits into potato. Cybridization was performed by the donor-recipient protoplast-fusion procedure. We found that the plastomes of *S. chacoense*, *S. brevidens*, and *S. etuberosum* could be transferred into the cybrids having *S. tuberosum* nuclear genomes; chondriome components were likewise transferred from the former species into these cybrids. The combination with *S. chacoense* as organelle donor and potato as recipient resulted in green fertile plants with potato morphology. By using *S. etuberosum* as an organelle donor and potato as recipient, male-sterile cybrid plants, most of them having pigmentation abnormalities, were obtained. The combination of *S. brevidens* with potato resulted in pale-green (almost albino) regenerants. The latter albino plantlets had both the chloroplast DNA and the mitochondrial DNA of the donor (*S. brevidens*) and did not survive the transfer into the greenhouse. An immediately applicative result of this study is the de novo establishment of male-sterile plants in a potato cultivar. Such plants should be useful as seed parents in the production of hybrid, true-potato seeds.

**Key words:** Cybridization – *Solanum tuberosum* – Male sterility – Phylogenetic relationships

## Introduction

The ability to derive functional plants from isolated protoplasts paved the way for innovative cell genetics studies in angiosperms. One approach, started in our laboratory (Zelcer et al. 1978), was to establish plants having the nuclear genome of one species but plastomes and chondriome components of another species (see reviews: Galun and Aviv 1986; Galun et al. 1987, 1988). This approach, mediated by the donor-recipient protoplast fusion, was based on the utilization of X- or $\gamma$-irradiated protoplasts as organelle donors and recipient protoplasts, which contributed the nucleus of the derived cybrids. The procedure was improved by several means such as pre-fusion exposure of protoplasts to antimetabolites (iodoacetate – Sidorov et al. 1981; Rhodamine 6-G – Aviv et al. 1986), the utilization of donors or recipients with plastome (Belliard et al. 1978; Medgyesy et al. 1980; Menczel et al. 1983), and/or chondriome markers (Aviv and Galun 1988). These protoplast manipulation methods were supplemented with efficient protocols to identify plastome and chondriome compositions in the derived cybrids (Galun and Aviv 1986). Hence, a powerful tool was established to obtain cybrids containing novel plasmones. Consequently, cybrids were obtained in several angiosperm genera, in addition to *Nicotiana*, where the procedures were initially developed. Representatives of the former genera are *Citrus* (Vardi et al. 1987, 1989) *Brassica* (Barsby et al. 1987; Morgan and Maliga 1987; Chatterjee et al. 1988; Thomzik and Hain 1988), and *Daucus* (Ichikawa et al. 1987; Tanno-Suenaga et al. 1988). It is noteworthy that in some studies aimed to produce only somatic hybrid plants, cybrids, in addition to somatic hybrids, were derived from fusions where neither of the partners was X- or $\gamma$-irradiated (Gleba et al. 1984; Kushnir and Gleba 1987; Bottcher et al. 1989).

The evidence from the many reported interspecific cybrids of *Nicotiana* (loc cit) indicated that these cybrids were similar or identical in their morphological features to the recipient fusion partner. In none of these cybrids

* To whom correspondence should be addressed

could pigmentation deficiency or morphological defects be causally correlated to alien plasmones. The only reproducible manifestation of alien chondriome components in interspecific cybrids was de novo cytoplasmic male sterility (Aviv et al. 1984; Kumashiro et al. 1988 – in *Nicotiana*; Tanno-Suenaga et al. 1988 – in *Daucus*). *Solanum* is a vast genus containing about 1,600 species (D'arcy 1978). This genus therefore contributes attractive material for investigating alien plasmone/nuclear genome compatibility by the donor-recipient protoplast fusion.

Procedures to regenerate functional plants from isolated protoplasts of potato (*S. tuberosum*), a major food source, were reported by several authors (Gunn and Shepard 1981; Thomas et al. 1982; Bockelmann and Roest 1983; Haberlach et al. 1985; Tavazza and Ancora 1986; Masson et al. 1987). In the past, somatic hybridization but not cybridization was used, mainly in order to introduce advantageous breeding traits from wild *Solanum* species into potato (Butenko and Kuchko 1980, Binding et al. 1982; Barsby et al. 1984; Ehlenfeldt and Helgeson 1987; Austin et al. 1985a, b, 1986; Kemble et al. 1986; Sidorov et al. 1987; Fish et al. 1987, 1988). With the exception of one study (Sidorov et al. 1987), where an asymmetric hybridization was attempted to introduce advantageous genetic traits form *S. pinnatisectum* into *Solanum tuberosum* × *phureja* hybrids, no cybridization between potato and other *Solanum species* was recorded.

In the present work our aim was to study the compatibility between the nuclear genome of potato and organelles from alien *Solanum* species. Consequently, we employed the donor-recipient protoplast-fusion procedure and chose organelle donors that varied in their phylogenetic distance from potato. We employed Southern blot hybridization of chloroplast and mitochondrial DNAs to determine the organelle compositions of the fusion-derived cybrid plants, and we related these compositions to morphological abnormalities and floral-member malformations (i.e., male sterility) in the respective cybrids.

## Materials and methods

### *Plant material*

Shoot sections of *Solanum tuberosum* cv Desiree bearing one node were cultured axenically in Magenta GA7 boxes containing 50 ml of solidified (1% agar) Nitsch medium (Nitsch 1969) with only 1% sucrose. Silver thiosulfate (STS) was added (2 mg $l^{-1}$) to this medium to produce more leaf tissue and to increase the yield of protoplasts per unit tissue mass (Perl et al. 1988). The sections rooted, produced new shoots and were harvested after about 3 weeks of culture at 25 °C under 16-h cool-white inflorescent illumination providing 60–70 $\mu Em^{-2} s^{-1}$. The harvested shoots served for either reculture or for protoplast isolation.

Seeds of *Solanum chacoense* (PI 472816), *Solanum etuberosum* (PI 498311), *Solanum brevidens* (PI 245764), and *Solanum berthaultii* (PI 498107) were kindly supplied by Dr. J. Bamberg (Potato Introduction Station, Sturgeon Bay/WI, USA). The seeds were surface-sterilized with diluted commercial sodium hypochlorite (1% available chlorine), washed three times with sterile distilled water, and germinated. The resulting seedlings were further cultured as axenic shoots, as detailed for *S. tuberosum*.

### *Protoplast isolation and pre-fusion treatments*

One gram of leafy shoots harvested from axenic cultures was pricked, using a multineedle pricker, and incubated in 10 ml of maceration fluid as described previously (Perl et al. 1988). For protoplast isolation from the four wild *Solanum* species, the maceration fluid also contained 0.1% Driselase (Fluka). Donor protoplasts were $\gamma$-irradiated (10 krad). Recipient protoplasts were treated with 0.25 m*M* iodoacetate (recrystallized) for 30 min before fusion (Medgyesy et al. 1980).

### *Protoplast fusion and culture*

Protoplast fusion was performed with the addition of 100 mg $l^{-1}$ STS during the exposure of protoplasts to $Ca(NO_3)_2$ (following the polyethylene glycol treatment) and the fused protoplasts were cultured in liquid medium as described (Perl et al. 1988). After 3–4 weeks of culture in liquid medium with periodical reduction of the osmolarity, calli were transferred to solidified (0.8% agar) regeneration medium consisting of MS (Murashige and Skoog 1962) medium supplemented with 0.25 mg $l^{-1}$ zeatin-riboside and 0.1 mg $l^{-1}$ gibberellic acid. Regeneration commonly commenced after further 3–4 weeks. Shoots were rooted on a hormone-free MS medium and transferred to the greenhouse for further observation. In cases of sluggish regeneration the calli were transferred to regeneration medium with 4 mg $l^{-1}$ o-coumaric acid.

### *Characterization of organelle DNA*

DNA extraction and analysis were performed according to Mettler (1988). Thus, total DNA was extracted from 1 g of leaf tissue, digested with endonuclease run on agarose gel (0.8%), transferred to a GeneScreen Plus membrane, and hybridized with a nick-translated probe. For chloroplast DNA (ctDNA) analysis, the DNA was digested with BamHI and hybridized with a potato ctDNA clone, P153 (Heinhorst et al. 1988). For mitochondrial DNA (mtDNA) analysis, the total DNA was digested with one of several endonucleases and fragments from *Nicotiana* mt DNA (Aviv et al. 1984) or maize mitochondrial ATPase subunit 6 (Dewey et al. 1985) were used as radiolabelled probes.

### *Pollen germination*

Pollen was collected from several flowers immediately after anthesis and germinated in a hanging drop of a pollen medium described by Pallais et al. (1984).

## Results

In all the fusion combinations, we used protoplasts derived from axenic leafy shoots. Protoplasts from the potato cultivar Desiree served as recipients and were exposed to iodoacetate treatment. The latter protoplasts were fused with $\gamma$-irradiated protoplasts from one of the

**Table 1.** Organelle composition of protoplast-fusion-derived plants (calli) based on Southern blot hybridizations with organelle-specific DNA probes. Protoplasts of the potato cultivar Desiree served as recipients in all fusion combinations

| Donor | Fusion-derived plants (calli) | | Organelle analysis | | | | |
|---|---|---|---|---|---|---|---|
| | Total | Analyzed | Plastome | | Chondriome | | |
| | | | Donor | Recipient | Novel | Donor | Recipient |
| *S. chacoense* | 38 (10) | 17 (7) | 7 | 10 | 1 | 5 | 11 |
| *S. etuberosum* | 117 (36) | 88 (33) | 31 | 57 | 0 | 13 | 75 |
| *S. brevidens* | 10 (5) | 5 (5) | 5 | 0 | 0 | 5 | 0 |
| *S. berthaultii* | 78 (28) | 75 (22) | 0 | 75 | 0 | 8 | 67 |

four *Solanum* species serving as donors of organelles. Repeated control experiments indicated that when Desiree protoplasts were exposed to iodoacetate but not fused with donor protoplasts, the protoplasts succumbed in culture and no cell division occurred. Also, none of the four wild *Solanum* protoplasts produced cell colonies after $\gamma$-irradiation; neither did colonies form when the polyethylene glycol treatment was deleted from the fusion of donor to recipient protoplasts. We therefore assumed that the fusion-derived calli and plants resulted from heterologous fusion events.

*S. chacoense protoplasts as donors*

A total of 38 plants was regenerated from this fusion combination. All plants had the normal morphological (presumably nuclear-genome-coded) features of potato (i.e., Desiree). Root-tip squashes from five randomly collected plants indicated normal tetraploid mitotic karyotypes. The plants attained anthesis and had normal flowers. The pollen of these flowers germinated normally. The plastome composition of 17 plants was evaluated by ctDNA analysis as exemplified in Fig. 1. These analyses indicated that 10 plants contained the plastome of Desiree while 7 plants contained the plastome of *S. chacoense* (Table 1). No fusion-derived plant was heteroplastomic nor was plastome recombination indicated. The chondriomes were characterized in the same 17 plants by mtDNA analysis. The analysis of four cybrids is demonstrated in Fig. 2 and the total data are summarized in Table 1. Six of the 7 plants, found to contain the ctDNA of *S. chacoense*, contained also the mtDNA of the latter species; all 6 plants exept one (cyb G-23) had apparently pure mtDNA patterns of *S. chacoense.* Cybrid G-23 exhibited a novel mtDNA pattern that was similar (but not identical) to the pattern *S. chacoense* mtDNA. Eleven plants apparently retained the mtDNA pattern of the recipient fusion partner (Desiree). Our analysis does not reveal those *S. chacoense* or novel mtDNA fragments that do not hybridize with the radioactive probe. Hence it cannot be excluded that (some or even all of) these eleven plants contained components of the *S. chacoense* chondriome.

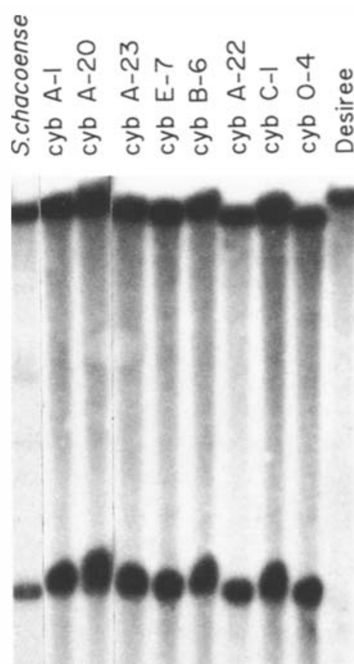


**Fig. 1.** Southern blot hybridization of ctDNA from *S. tuberosum* cv Desiree (recipient), *S. chacoense* (irradiated donor), and their cybrids. The ctDNA was digested with BamHI and probed with a *S. tuberosum* ctDNA BamHI fragment, P153

*S. etuberosum protoplasts as donor*

A total of 117 plants was regenerated from this fusion combination and all had the normal morphological features of potato (i.e., Desiree). Root-tip squashs from five randomly collected plants indicated normal tetraploid mitotic karyotypes. It should be noted that *S. etuberosum* is a tuberless wild species and is thus not closely related phylogenetically to *S. tuberosum*. Nevertheless, all fusion-derived plants produced normal potato tubers. Plastome composition was evaluated by Southern blot analysis of the ctDNA in 88 fusion-derived plants (Table 1). Of the latter, 31 contained the plastome of the donor (Fig. 3), while 57 fusion-derived plants retained the Desiree (i.e., *S. tuberosum*) plastome. As with *S. chacoense* as a donor, there was no indication of heteroplastomes or

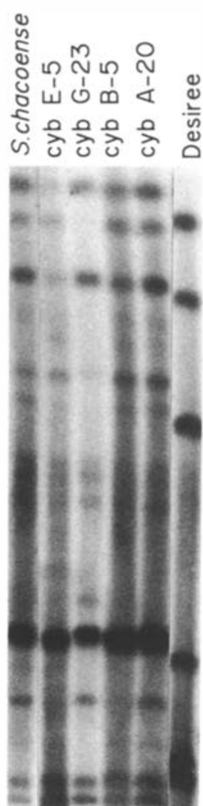


**Fig. 2.** Southern blot hybridization of mtDNA from *S. tuberosum* cv Desiree (recipient), *S. chacoense* (irradiated donor), and their cybrids. The mtDNA was digested with HindIII and probed with pSylSa8

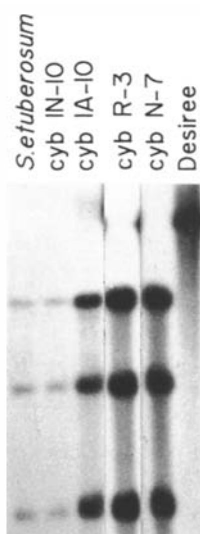


**Fig. 3.** Southern blot hybridization of ctDNA from *S. tuberosum* cv Desiree (recipient), *S. etuberosum* (irradiated donor), and their cybrids. The ctDNA was digested with BamHI and probed with a *S. tuberosum* ctDNA BamHI fragment, P153

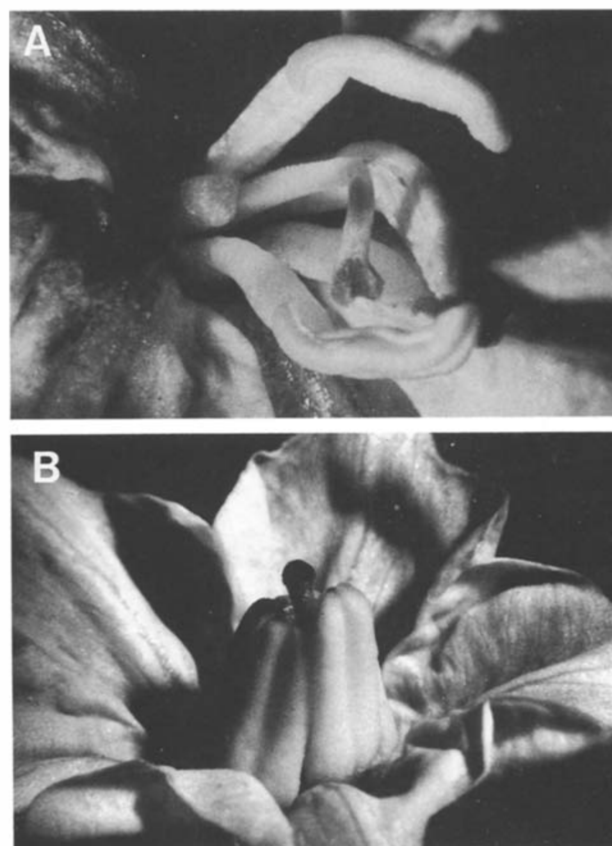


**Fig. 4 A and B.** Anthers of potato and cybrid flowers: **A** Malformed anthers of a CMS cybrid plant derived from a fusion between *S. etuberosum* (donor) and *S. tuberosum* (recipient). **B** Normal stamens of a *S. tuberosum* cv Desiree flower

recombination in the ctDNA. Putative cybrids containing chondriome components of *S. etuberosum* were detected by pollen germination tests. Eighteen plants were found to be male sterile. Among them, 5 plants had malformed anthers with almost no pollen (Fig. 4), while the other 13 sterile plants had structurally normal anthers but their pollen did not germinate. Apparently, pure mtDNA of *S. etuberosum* was detected in 13 cybrid plants (Table 1) regardless of the phenotypic expression of male sterility in those plants (Fig. 5). Most of the cybrids with *S. etuberosum* mtDNA contained also the ctDNA of *S. etuberosum*. Only two of the former cybrids retained the plastome of *S. tuberosum*. In 75 of the fusion-derived plants we detected the mtDNA pattern of *S. tuberosum*. The possibility cannot be excluded that some of these plants contained components of the *S. etuberosum* chondriome that were not revealed by the radiolabelled probe. Moreover, seven calli (among them calli N, IN, and S; Fig. 5) regenerated plants with *S. etuberosum* mtDNA as well as plants that retained the recipient (Desiree) mtDNA pattern. This fact indicates that these seven calli were derived from a fusion event

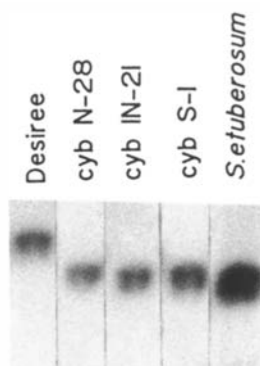


**Fig. 5.** Southern blot hybridization of mtDNA from *S. tuberosum* cv Desiree (recipient), *S. etuberosum* (irradiated donor), and their cybrids. The mtDNA was digested with *Eco*RI and probed with a mtDNA fragment of maize ATPase subunit 6

**Fig. 6.** Regenerated albino shoots derived of a fusion between *S. brevidens* (donor) and *S. tuberosum* cv Desiree (recipient)

and were not a result of recipient escapees. In contrast to the fusion with *S. chacoense* as donor of organelles where no abnormalities were revealed among the fusion-derived plants, in this combination with *S. etuberosum* two types of abnormalities were observed. One, male sterility (with or without floral-member malformation), was noted above. The second was an abnormal pigmentation presumably by anthocyanin. It was expressed in the leaves and the stems of part of the cybrids. This phenomenon, not expressed in the donor species, was correlated in the cybrids with the transfer of *S. etuberosum* plastomes. Cybrids that received only mtDNA components but not chloroplasts from the donor did not reveal any pigmentation abnormalities.

*S. brevidens protoplasts as donor*

Only five calli were derived from this fusion (Table 1) and they regenerated sluggishly. Regeneration did occur after 3 months, resulting in ten plantlets (two from each callus). All these plantlets were pale-green/albino (Fig. 6) and were propagated as axenic shoot cultures. Attempts to grow these plantlets autotrophically in the greenhouse were unsuccessful. These putative cybrid shoot cultures were morphologically similar to shoot cultures of *Solanum tuberosum* except for the deficient pigmentation. Root-tip squashes from five randomly collected plantlets indicated normal tetraploid mitotic karyotypes. The organelle composition of five plantlets (each representing one callus) was evaluated by Southern blot hybridization of their respective ctDNAs and mtDNAs. All five plantlets had the ctDNA pattern of *S. brevidens* (Fig. 7); likewise all these plantlets had the mtDNA pattern of *S. brevidens* (Fig. 8). These results indicate that cybrids of this combination contained both the chloroplasts and the mitochondria from *S. brevidens*.

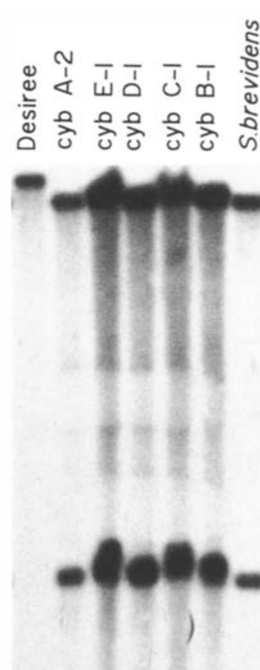


**Fig. 7.** Southern blot hybridization of ctDNA from *S. tuberosum* cv Desiree (recipient), *S. brevidens* (irradiated donor), and their cybrids. The ctDNA was digested with BamHI and probed with a *S. tuberosum* ctDNA BamHI fragment, P153

*S. berthaultii protoplasts as donor*

The calli derived from this fusion combination did not change their structure upon transfer to regeneration medium (i.e., lacking auxins). The calli maintained a friable growth pattern. In this respect they differed from the calli derived from the previously described fusion combinations. The latter calli became compact upon transfer to regeneration medium. We thus suspected that the calli

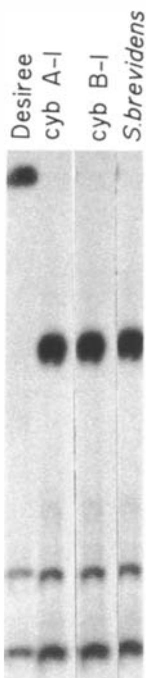


**Fig. 8.** Southern blot hybridization of mtDNA from *S. tuberosum* cv Desiree (recipient), *S. brevidens* (irradiated donor), and their cybrids. The mtDNA was digested with EcoRI and probed with pSylSa2

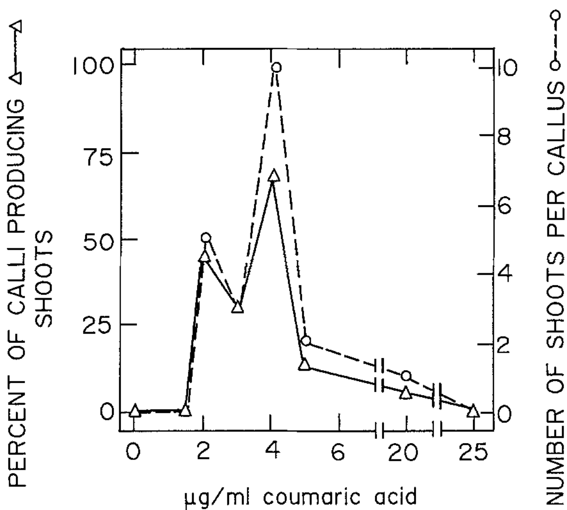


**Fig. 9.** Promotion of shoot regeneration from calli by o-coumaric acid. The calli were derived from the fusion of *S. tuberosum* protoplasts (recipient) with *S. berthaultii* (donor) protoplasts. Shoot regeneration was scored 21 days after transfer to regeneration medium; each point represents 10 calli

derived from the fusion with *S. berthaultii* were over-producing endogenous auxins and that this over-production retarded regeneration. Several compounds known to enhance auxin degradation were thus added to the regeneration medium. One of these compounds, o-coumaric acid, strongly promoted regeneration and shortened the time from transfer to regeneration medium to differentiation of plantlets by 3–4 weeks (Fig. 9). It should be noted that o-coumaric acid is considered a natural cofactor of IAA oxidase and is known to enhance IAA oxidation (Gortner and Sutherland 1958). This improvement in regeneration procedure yielded 78 plants. All these plants were morphologically similar to Desiree (i.e., *S. tuberosum*), suggesting they had *S. tuberosum* nuclei. Root-tip squashes from five randomly collected plants indicated normal tetraploid mitotic karyotypes. The organelle composition of 75 plants (from 22 calli) was analyzed by Southern blot hybridization (Table 1). All the plants retained *S. tuberosum* plastomes. Only 8 plants of 75 had apparently pure *S. berthaultii* chondriomes (data not shown). Four of these 8 plants attained flowering and were found to be male sterile. Our data cannot exclude the possibility that the majority of the plants regenerated from this fusion combination were escapees rather than cybrids. On the other hand, in the 8 verified cybrids the chondriomes were transferred from *S. berthaultii* without the co-transfer of plastomes.

## Discussion

The results of this study indicate that the donor-recipient protoplast-fusion technique is amenable for potato and may lead to the production of cybrid plants with the nuclear genome of potato and alien organelle and/or organelle-controlled features from different wild *Solanum* species. We have confirmed that the DNA-DNA hybridization analysis, using specific probes, can be used to identify cybrid plants. The morphology of the fusion-derived plants suggested that they were normal tetraploids, and this was verified by karyotype analyses. In a parallel study (unpublished data) we analyzed the meiotic and mitotic karyotypes of 21 *Solanum* cybrids derived from a different donor-recipient protoplast fusion; all but one of the cybrids had apparently normal chromosomes, identical to those of the recipient fusion partner (*S. tuberosum*). Hence, regeneration of potato plants from protoplasts, even after donor-recipient fusion, does not necessarily result in observed morphological or karyotype somaclonal variation, as indicated by some previous reports on protoplast-derived potato plants (Shepard et al. 1983). In spite of the morphological similarity, among the fusion-derived plants from a given combination (e.g., *S. etuberosum* as donor) as well as between these plants and the potato cultivar Desiree, the cybrid character of some of the fusion-derived plants could be determined even before organelle analysis, due to their male sterility.

In this research, our aim was to study primarily organelle/nuclear genome compatibility barriers among *Solanum* species. We used *S. tuberosum* as our "stan-

dard" nuclear genome "host" (recipient) and four other *Solanum* species as sources of alien organelles. To choose appropriate organelle-donor species having different phylogenetic relatedness to potato, we also considered the information reported by Hosaka and collaborators (Hosaka et al. 1984, 1988; Hosaka 1986; Hosaka and Hannemann 1988 b). These investigators used the endonuclease restriction profiles of ctDNA from *Solanum* species to evaluate plastome phylogeny in this genus. They found that the plastome of *S. chacoense* is very similar to that of *S. tuberosum* (the difference was revealed in only one restriction site). Our results showed that indeed *S. chacoense* chloroplasts (as well as chondriome components) could be transferred to potato without causing any morphological changes or male sterility in the respective cybrids. On the other hand, Hosaka and collaborators found a considerable phylogenetic distance between *S. tuberosum* and *S. etuberosum* plastomes: 29 differences in restriction sites were revealed. Our results showed that cybrids could be obtained from the fusion of *S. etuberosum* as organelle donor and *S. tuberosum* as recipient, and that the respective cybrids were morphologically similar to the recipient-fusion partner, but two abnormalities were revealed. Some had abnormal pigmentation and about one-fifth of the analyzed cybrid were male sterile (with or without floral-member deformation). All the abnormally pigmented cybrids had *S. etuberosum* chloroplasts and all the male-sterile had apparently pure *S. etuberosum* chondriomes. The rate of plastome transfer in this fusion combination was higher then the rate of mitochondrial transfer, but the two organelles were independently transferred, conforming to a similar independent transfer of organelle in *Nicotiana* cybrids (Aviv et al. 1984). Also, in agreement with the previously studied organelle transfer in *Nicotiana* (Aviv et al. 1984), organelle sorting-out was not completed at the callus stage: cybrids having different organelle composition may be derived from the same callus.

The species *S. brevidens* is considered even more far removed from *S. tuberosum* than *S. etuberosum*. The fusion combination of the former species with *S. tuberosum* resulted in pale-green/albino cybrids. Only a few plants could be regenerated, and in all of them there was a co-transfer of chondriome and plastome from *S. brevidens*. It should be noted that because of pathogen resistance traits, *S. brevidens* was a favorite species in somatic hybridizations with potato (Austin et al. 1985 b, 1986; Barsby et al. 1984; Ehlenfeldt and Helgerson 1987; Fish et al. 1987, 1988; Kemble et al. 1986). In contrast to our cybrids, the somatic hybrids derived from the fusion between protoplasts of *S. tuberosum* and *S. brevidens* did not show morphological abnormalities. A possible explanation for this difference is that the somatic hybrids contain the nuclear genome of both species, thus there is no manifestation of alloplasmonic incompatibility. An extreme case of intertribal nuclear/organelle combination was reported by Thanh et al. (1988) between *Nicotiana* and *Salpiglossis*. In that case, the transfer of *Salpiglossis* chloroplasts into *Nicotiana* nuclear background or vice versa resulted in normal green cybrids. On the other hand, interspecific alloplastomic incompatibilities are known from sexual crosses in *Oenothera* (Kutzelnigg and Stubbe 1974; Tilney-Bassett 1978). In the latter genus there exists a biparental transmission of chloroplasts in sexual hybrids, thus novel chloroplast/nuclear relations can be established by sexual crosses.

When *S. berthaultii* was used as donor and its irradiated protoplasts were fused with iodoacetate-treated potato protoplasts, none of the resulting plants had the donor chloroplasts. Furthermore, only 8 out of 75 analyzed, fusion-derived plants had the donor chondriome; thus, most of the fusion-derived plants could have been escapees. On the other hand, since unfused and iodacetated Desiree protoplasts never attained cell division, it is reasonable to assume that the initial fusion products were heteroplasmonic and that the *S. tuberosum* organelle composition was a result of later sorting-out. It is noteworthy that all cybrids with *S. berthaultii* chondriomes that attained flowering were found to be male sterile. This correlation between male sterility and chondriome/nuclear genome composition is in line with findings in other genera where alien chondriomes were considered to be causally related to male sterility (see reviews: Hanson and Conde 1985; Pring and Lonsdale 1985; Galun and Aviv 1986; Galun et al. 1988).

*Acknowledgements.* We are grateful to A. Dantes for her technical assistance during this work. This research was supported by an AID grant (No. DPE 5544-G-SS-7043) and by a grant (Disnat 449/142) from NCRD, Israel and GSF Munich, FRG. E.G. holds the Irene and David Schwartz Chair of Plant Genetics.

## References

Austin S, Baer MA, Ehlenfeldt M, Kazmeirczak PJ, Helgeson JP (1985 a) Intraspecific fusion in *Solanum tuberosum*. Theor Appl Genet 71:172–175

Austin S, Baer MA, Helgeson JP (1985 b) Transfer of resistance to potato leaf roll virus from *Solanum brevidens* into *Solanum tuberosum* by somatic fusion. Plant Sci 39:75–82

Austin S, Ehlenfeldt M, Baer MA, Helgeson JP (1986) Somatic hybrids produced by protoplast fusion between *S. tuberosum* and *S. brevidens*: phenotypic variation under field conditions. Theor Appl Genet 71:682–690

Aviv D, Galun E (1988) Transfer of cytoplasmic organelles from an oligomycin-resistant *Nicotiana* cell suspension into tobacco protoplasts yielding oligomycin resistant cybrid plants. Mol Gen Genet 215:128–133

Aviv D, Arzee-Gonen P, Bleichman S, Galun E (1984) Novel alloplasmic *Nicotiana* plants by donor recipient protoplast fusion: cybrids having *N. tabacum* or *N. sylvestris* nuclear genome and either or both plastomes and chondriomes from alien species. Mol Gen Genet 196:244–253

Aviv D, Chen R, Galun E (1986) Does pretreatment by Rhodamine 6-G affect the mitochondrial composition of fusion-derived *Nicotiana* cybrids? Plant Cell Rep 5:227–230

Barsby L, Shepard JF, Kemble RJ, Wong R (1984) Somatic hybridization in the genus *Solanum*: *S. tuberosum* and *S. brevidens*. Plant Cell Rep 3:165–167

Barbsy TL, Yarrow SA, Kemble RJ, Grant I (1987) The transfer of cytoplasmic male sterility to winter-type of oilseed rape (*Brassica napus* L.) by protoplast fusion. Plant Sci 53:243–248

Belliard G, Pelletier G, Vedel F, Quetier F (1978) Morphological characteristics and chloroplast DNA distribution in different cytoplasmic parasexual hybrids of *Nicotiana tabacum*. Mol Gen Genet 165:231–237

Binding H, Jain SM, Finger J, Mordhorst G, Nehls R, Gressel J (1982) Somatic hybridization of an atrazine-resistant biotype of *Solanum nigrum* with *Solanum tuberosum*. Theor Appl Genet 63:273–277

Bockelmann GS, Roest S (1983) Plant regeneration from protoplasts of *S. tuberosum* cv Bintje. Z Pflanzenphysiol 109:259–265

Bottcher UF, Aviv D, Galun E (1989) Complementation between protoplasts treated with either of two metabolic inhibitors results in somatic-hybrid plants. Plant Sci 63:67–77

Butenko RG, Kuchko AA (1980) Somatic hybridization of *Solanum tuberosum* and *Solanum chacoense* by protoplast fusion. In: Ferenczy L, Farkas GL (eds) Advances in protoplast research. Pergamon Press, Oxford, pp 293–300

Chatterjee G, Sikdar SR, Das S, Sen SK (1988) Intergeneric somatic hybrid production through protoplast fusion between *Brassica kuncea* and *Diplotaxis muralis*. Theor Appl Genet 76:915–922

D'arcy NG (1978) The classification of the Solanaceae. In: Hawkes JG, Lester RN, Skelding AD (eds) The biology and taxonomy of the Solanacae. Academic Press, New York, pp 3–47

Dewey RE, Levings CS, Timothy DH (1985) Nucleotide sequence of ATPase subunit 6 gene of maize mitochondria. Plant Physiol 79:914–919

Ehlenfeldt MK, Helgeson JP (1987) Fertility of somatic hybrids from protoplast fusion of *Solanum brevidens* and *S. tuberosum*. Theor Appl Genet 73:395–402

Fish N, Karp A, Jones MGK (1987) Improved isolation of dihaploid *S. tuberosum* protoplasts and the production of somatic hybrids between diploid *S. tuberosum* and *S. brevidens*. Theor Appl Genet 76:113–116

Fish N, Karp A, Jones MGK (1988) Production of somatic hybrids by electrofusion in *Solanum*. Theor Appl Genet 76:260–266

Galun E, Aviv D (1986) Organelle transfer. Meth Enzymol 118:595–611

Galun E, Aviv D, Breiman A, Fromm H, Perl A, Vardi A (1987) Cybrids in *Nicotiana*, *Solanum*, and *Citrus*: Isolation and characterization of plastome mutants, pre-fusion treatment, selection, and analysis of cybrids. In: Wettstein D von, Chua NH (eds) Plant molecular biology NATO Advanced Studies Institute, Ser A. Life Sci, pp 199–207

Galun E, Perl A, Aviv D (1988) Protoplast-fusion mediated transfer of male sterility and other plastome-controlled traits. Ciba Foundation Symp No. 137, Kyoto. In: Application of plant cell and tissue culture. Wiley, Chichester, pp 97–112

Gleba YY, Piven NM, Komarnitsky IK, Symik KM (1984) Transmission genetics of the somatic hybridization process in *Nicotiana*. 1. Hybrids and cybrids among the regenerants from cloned protoplast fusion products. Theor Appl Gent 69:121–128

Gortner AW, Sutherland GK (1958) Ferulic and coumaric acids in pineapple tissue as modifiers of pineapple indoleacetic acid oxidase. Nature 181:630–631

Gunn RE, Shepard JF (1981) Regeneration of plants from mesophyll-derived protoplasts of British potato (Solanum tuberosum L.) cultivars. Plant Sci Lett 22:97–101

Haberlach GT, Cohen BA, Riechert NA, Boer MA, Towill LE, Helgeson JP (1985) Isolation, culture, and regeneration of protoplasts from potato and several related *Solanum* species. Plant Sci Lett 39:67–74

Hanson MR, Conde MF (1985) Functioning and variation of cytoplasmic genomes: lessons from cytoplasmic-nuclear interactions affecting male fertility in plants. Int Rev Cytol 94:213–267

Heinhorst S, Cannon GC, Galun E, Kenschaft L, Weissbach A (1988) Clone bank and physical and genetic map of potato chloroplast DNA. Theor Appl Genet 75:244–251

Hosaka K (1986) Who is the mother of the potato; restriction endonuclease analyses of chloroplast DNA of cultivated potatoes. Theor Appl Genet 72:606–618

Hosaka K, Hanneman RE (1988) The origin of the cultivated tetraploid potato based on chloroplast DNA. Theor Appl Genet 76:172–176

Hosaka K, Ohihara Y, Matsubayashi M, Tsunewaki K (1984) Phylogenetic relationship between the tuberous *Solanum* species as revealed by restriction endonuclease analysis of chloroplast DNA. Jpn J Genet 59:349–369

Hosaka K, Zoeten GA de, Hanneman RE (1988) Cultivated potato chloroplast DNA differs from the wild type by one deletion-evidence and implication. Theor Appl Genet 75:741–745

Ichikawa H, Tanno-Suenaga L, Imamura J (1987) Selection of *Daucus* cybrids based on metabolic complementation between X-irradiated *D. capillifolius* and iodoacetamide-treated *D. carota* by somatic cell fusion. Theor Appl Genet 74:746–752

Kemble RJ, Barsby TL, Wong RSC, Shepard JF (1986) Mitochondrial DNA arrangements in somatic hybrids of *Solanum tuberosum* and *Solanum brevidens*. Theor Appl Genet 72:787–793

Kumashiro T, Asahi T, Komari T (1988) A new source of cytoplasmic male sterile tobacco obtained by fusion between *Nicotian tabacum* and X-irradiated *N. africana* protoplasts. Plant Sci 55:247–254

Kushnir SG, Gleba Y (1987) Functional cybrid plants possessing a *Nicotiana* genome and an *Atropa* plastome. Mol Gen Genet 209:159–163

Kutzelnigg H, Stubbe W (1974) Investigations on plastome mutants in *Oenothera*. 1. General considerations. Sub-Cell Biochem 3:73–79

Masson J, Lecerf M, Rousselle P, Perennec P, Pelletier G (1987) Plant regeneration from protoplasts of diploid potato derived from crosses between *Solanum tuberosum* and wild *Solanum* species. Plant Sci 53:167–176

Medgyesy P, Menczel L, Maliga P (1980) The use of cytoplasmic streptomycin resistance. Chloroplast transfer from *Nicotiana tabacum* to *Nicotiana sylvestris* and isolation of their somatic hybrids. Mol Gen Genet 179:693–698

Menczel L, Nagy F, Lazar G, Maliga P (1983) Transfer of cytoplasmic male sterility by selection for streptomycin resistance after protoplast fusion in *Nicotiana*. Mol Gen Genet 189:365–369

Mettler IJ (1988) A simple and rapid method for minipreparation of DNA from tissue cultured plant cells. Plant Mol Biol Rep 5:346–349

Morgan A, Maliga P (1987) Rapid chloroplast segregation and recombination of mitochondrial DNA in *B. napus* cybrids. Mol Gen Genet 209:240–246

Murashige T, Skoog F (1962) A revised medium for the rapid growth and bioassys with tobacco tissue cultures. Physiol Plant 15:473–497

Nitsch JP (1969) Experimental androgenesis in *Nicotiana*. Phytomorphology 19:389–404

Pallias N, Fony N, Berrios D (1984) Research on the physiology of potato sexual seed production. In: Innovative methods for propagating potatoes. Rep 28th Plan Conf, December 10, 1984. Int Potato Ctr, Lima, pp 149–168

Perl A, Aviv D, Galun E (1988) Ethylene and in vitro cultures of potato: suppression of ethylene vastly improves protoplast yield, plating efficiency, and transient expression of an alien gene. Plant Cell Rep 7:403–406

Pring DR, Lonsdale DM (1985) Molecular biology of higher plant mitochondrial DNA. Int Rev Cytol 97:1–46

Shepard JF, Bidney D, Barsby T, Kemble R (1983) Genetic transfer in plants through interspecific protoplasts fusion. Science 219:683–688

Sidorov VA, Menczel L, Nagy F, Maliga P (1981) Chloroplast transfer in *Nicotiana* based on metabolic complementation between irradiated and iodoacetate-treated protoplasts. Planta 152:341–345

Sidorov A, Zubko MK, Kuchko AA, Komarnitsky IK, Gleba YY (1987) Somatic hybridization in potato: use of $\gamma$-irradiated protoplasts of *Solanum pinnatisectum* in genetic reconstruction. Theor Appl Genet 74:364–368

Tanno-Suenaga L, Ichikawa H, Imamura J (1988) Transfer of the CMS trait in *Daucus carota* L. by donor-recipient protoplast fusion. Theor Appl Genet 76:855–860

Tavazza R, Ancora G (1986) Plant regeneration from mesophyll protoplasts in commercial potato cultivars (Prima, Kennebec, Spunta, Desiree). Plant Cell Rep 5:243–246

Thanh ND, Pa'y A, Smith MA, Medgyesy P, Marton L (1988) Intertribal chloroplast transfer by protoplast fusion between *Nicotiana tabacum* and *Salpiglossis sinuata*. Mol Gen Genet 213:186–190

Thomas E, Bright SW, Franklin J, Lancaster VA, Miflin BJ (1982) Variation among protoplast-derived potato plants. Theor Appl Genet 62:65–68

Thomzik JE, Hain R (1988) Transfer and segregation of triazine tolerant chloroplasts in *Brassica napus* L. Theor Appl Genet 76:165–171

Tilney-Bassett RAE (1978) The inheritance and genetic behaviour of plastids. In: Kirk JTO, Tilney-Bassett RAE (eds) The plastid. Elsevier, Amsterdam, pp 251–524

Vardi A, Brieman A, Galun E (1987) *Citrus* cybrids: production by the donor-recipient protoplast-fusion and verification by mitochondrial-DNA restriction profiles. Theor Appl Genet 75:51–58

Vardi A, Arzee-Gonen P, Frydman-Shani A, Bleichman S, Galun E (1989) Protoplast-fusion-mediated transfer of organelles from *Microcitrus* into *Citrus* and regeneration of novel alloplasmic trees. Theor Appl Genet 78:741–747

Zelcer A, Aviv D, Galun E (1978) Interspecific transfer of cytoplasmic male sterility by fusion between protoplasts of normal *Nicotiana sylvestris* and X-irradiated protoplasts of male-sterile *N. tabacum*. Z Pflanzenphysiol 90:397–407