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Mitochondrial DNA rearrangements in somatic hybrids of *Solanum tuberosum* and *Solanum brevidens*

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Summary. Thirty somatic hybrids between *Solanum tuberosum* and *Solanum brevidens* were analysed for mitochondrial and chloroplast genome rearrangements. In all cases, the chloroplast genomes were inherited from one of the parental protoplast populations. No chloroplast DNA alterations were evident but a range of mitochondrial DNA alterations, from zero to extensive intra- and inter-molecular recombinations, were found. Such recombinations involved specific 'recombination hot spots' in the mitochondrial genome. Not all hybrids regenerated from a common callus possessed identical mitochondrial genomes, suggesting that sorting out of mitochondrial populations in the callus may have been incomplete at the plant regeneration stage. Sorting out of organelles in planta was not observed.

Key words: Mitochondrial DNA – Mitochondrial recombination – Chloroplast DNA – *Solanum* – Somatic hybrids – Protoplast fusion

Introduction

The barrier of sexual incompatibility between the cultivated potato (*Solanum tuberosum* spp. *tuberosum*) and *Solanum brevidens,* a member of the non-tuberous diploid *Etuberosa* series, has recently been overcome through protoplast fusion. Some of the resulting somatic hybrids express beneficial agronomic traits from each parent, for example, tuber production (from *S. tuberosum*) and resistance to potato leaf roll virus (from *S. brevidens*). Phenotypic characters of these hybrids, which appear to have a realistic potential in potato breeding, have been described by Austin et al. (1985) and Barsby et al. (1984).

The work reported here was performed to determine if additional fusion-induced mtDNA rearrangements were present in the *S. tuberosum–S. brevidens* somatic hybrids because mitochondrial recombination has been implied in protoplast fusions involving tobacco (Aviv et al. 1984; Belliard et al. 1979; Galun et al. 1982; Nagy et al. 1981, 1983) petunia (Boeshore et al. 1983; Rothenberg et al. 1985) carrot (Matthews and Widholm 1985) and rapeseed (Chetrit et al. 1985), and mitochondrial DNA (mtDNA) rearrangements are common in plants regenerated from individual potato protoplasts (Kemble and Shepard 1984).

Materials and methods

Parental plant material

S. brevidens (2n = 2x = 24). Seed of *S. brevidens* (Accession no. 218288) were obtained from the USDA Potato Introduction Station, Sturgeon Bay, Wisconsin. Seed were soaked in 2,000 ppm gibberellic acid overnight and germinated in vermiculite. Plants were maintained as described previously (Barsby and Shepard 1983).

S. tuberosum (2n = 4x = 48). Chlorophyll-deficient protoplast-derived clones (protoclones) of *S. tuberosum* were obtained as described (Shepard 1980). A stable albino protoclone (designated 116) and a variegating protoclone (designated 774) were utilized in these experiments.

Construction and selection of hybrids

Mesophyll protoplasts were isolated and fused as described by Barsby et al. (1984). The selection of 116-*S. brevidens* somatic hybrids has been described previously (Barsby et al. 1984). In fusions between 774 and *S. brevidens,* the *S. brevidens* proto-

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plasts were treated with 4 mM sodium iodoacetate prior to fusion (after Medgyesy et al. 1980). Protoplasts derived from yellow tissue of 774 have altered culture requirements (Shepard 1980).

Regenerants were analysed for chromosome complement, leaf morphology, tuber production and the small subunit of ribulosebisphosphate carboxylase-oxygenase (RUBISCO) as previously described (Barsby et al. 1984).

Chloroplast and mitochondrial DNA analyses

Chloroplast DNA (cpDNA) and mtDNA were extracted according to the methods of Barsby et al. (1984) and Kemble and Shepard (1984), respectively. As an additional assurance against incomplete restriction enzyme digestion, all mtDNA preparations were repeated at least three times and further purified by cesium chloride gradient centrifugation. All DNAs were digested to completion with seven different restriction enzymes and electrophoresed on agarose gels (Kemble et al. 1980).

Results

Plants were regenerated from 25 calli arising from 116-*S. brevidens* fusions. Three of these gave rise only to albino plantlets which were axenically maintained. In addition, one callus produced a variegating plantlet. All four plants were morphologically different from the parental 116 and possessed the additive chromosome number of 72. The RUBISCO analysis was not performed on these four plantlets because their slow growth did not produce sufficient leaf material.

The remaining 21 calli all regenerated one or more green plants, 29 of which survived to maturity. Calli were numbered (1, 2, 3, etc.) and plants from a common callus origin were distinguished by letter (1A, 1B, 1C, etc.). All of these regenerants had the additive RUBISCO small subunit profiles of both parents. Only one plant (hybrid 30) had 72 chromosomes, the others being at, or close to, the octoploid condition (96 chromosomes). Possible explanations for these numbers have been presented previously (Barsby et al. 1984).

The one callus obtained from the 774-*S. brevidens* fusions produced a single green plant which had the additive RUBISCO small subunit profile of both parents and 66 chromosomes.

Restriction enzyme analyses of mtDNA and cpDNA were performed on the twenty nine 116-*S. brevidens* somatic hybrids, the single 774-*S. brevidens* hybrid, protoclone 116, protoclone 774 and wild-type *S. brevidens.* All cytoplasmic DNAs were subjected to incubation with each of the following restriction enzymes: *Eco* RI, *Bam* HI, *Xho* I, *Pst* I, *Xba* I, *Bgl* II and *Hin* dIII.

A representative array of *Eco* RI and *Bgl* II mtDNA restriction fragment patterns from individual 116-*S. brevidens* hybrids are shown in Fig. 1 and Fig. 2, respectively. Only two hybrids (12A and 12B) exhibited an *Eco* RI pattern which was identical to the *S. brevidens* parent and only hybrids 19 and 30 produced an

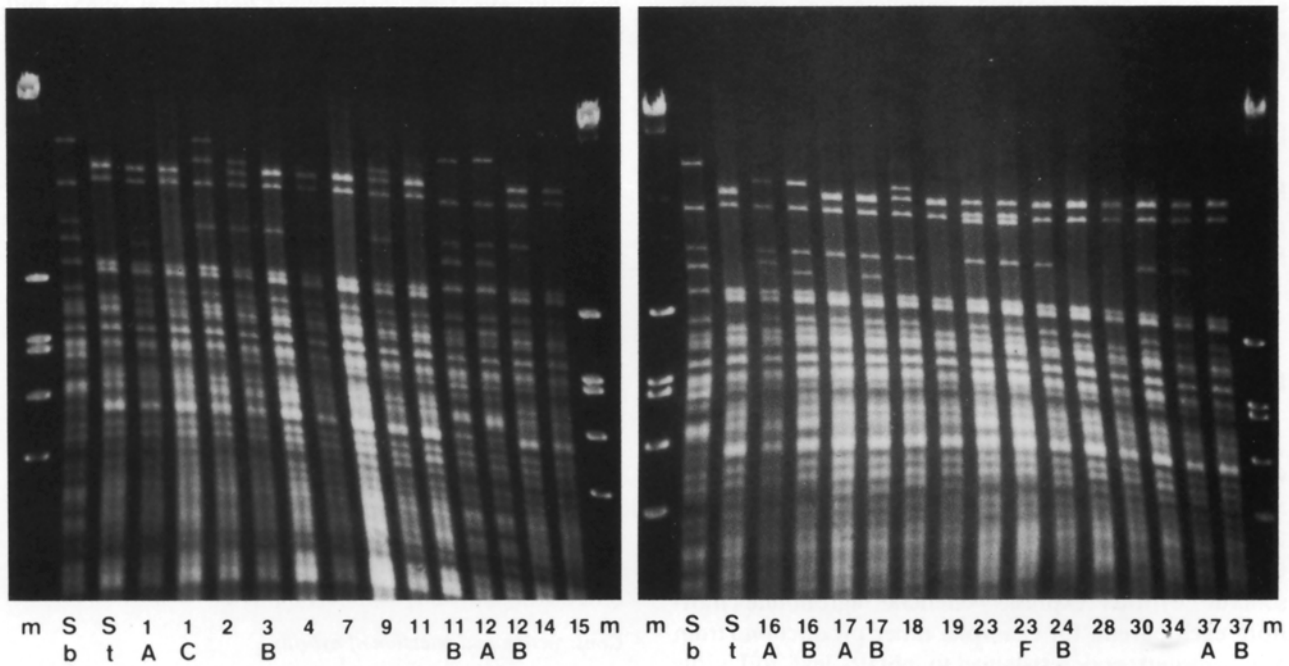

Fig. 1. Electrophoresis on 0.7% agarose gel of *Eco* RI fragmented mtDNAs from *S. brevidens* (Sb), *S. tuberosum* (St) and 27 different 116-*S. brevidens* hybrids (designated as 1A through 37B). *m* represents size marker fragments of 21.57, 7.54, 5.90, 5.68, 4.84 and 3.90 kb produced by independent digestions of lambda DNA with *Eco* RI and *Hae* III. DNA fragments smaller than approximately 2.7 kb are not shown

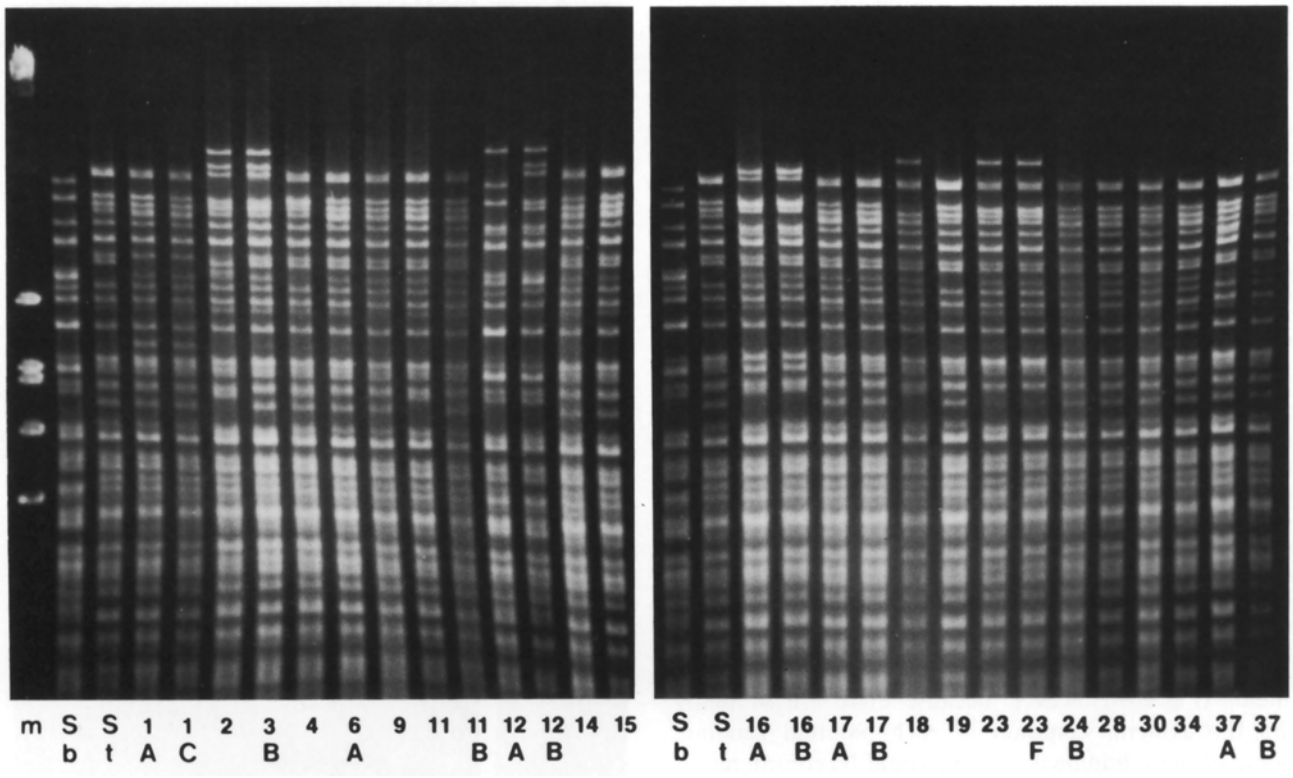

Fig. 2. Electrophoresis on 0.7% agarose gel of *Bgl* II fragmented mtDNAs. Labelling as in Fig. 1. DNA fragments smaller than approximately 2.5 kb are not shown

Eco RI pattern identical to the 116 parent (Fig. 1). However, eight other hybrids (1A, 1C, 7, 9, 11B, 15, 28 and 37B) exhibited a parental 116 pattern with an additional fragment equivalent in size to the sixth largest *S. brevidens Eco* RI fragment. Such a pattern was not observed in mtDNA isolated from many individual plants of 116, 774, two different accessions of the parental cultivar 'Russet Burbank' or other hexaploid potato cultivars analyzed. A further six hybrids (4, 14, 17A, 24B, 34 and 37A) possessed the 116 pattern plus two fragments equivalent in size to the third and sixth largest *S. brevidens Eco* RI fragments. Other hybrids which exhibited a 116 *Eco* RI pattern with additional fragments equivalent in size to those of *S. brevidens* are hybrid 17B (116 pattern plus third, fourth and sixth largest *S. brevidens* fragments) and hybrids 23 and 23F (116 pattern plus second, third and sixth largest *S. brevidens* fragments). Hybrids 3B, 11 and 18 demonstrated a 116 pattern plus the third and sixth largest *S. brevidens* fragments and, in addition, a fragment (the largest of the *Eco* RI pattern) not observed in either the 116 or *S. brevidens* patterns. Incubations involving different concentrations of *Eco* RI and DNA indicated that this large non-parental fragment was not caused by incomplete digestion. In complete digestions, this fragment and the majority of the other fragments were always present in equivalent stoichiometric amounts. Hybrids 2, 16A and 16B exhibited *Eco* RI restriction patterns similar to those described by others (Aviv et al. 1984; Belliard et al. 1979; Boeshore et al. 1983; Chetrit et al. 1985; Matthews and Widholm 1985; Nagy et al. 1983) as being indicative of mtDNA recombination. These patterns contain some, but not all, fragments of each parental pattern and, in addition, some non-parental fragments.

The *Eco* RI data (Fig. 1) also indicated that plants regenerated from the same callus do not necessarily contain the same mitochondrial genome organization. Hybrids 11 and 37A exhibit a 116 pattern plus the third and sixth largest *S. brevidens* fragments whereas their siblings, hybrids 11B and 37B respectively, exhibited a 116 pattern plus the sixth largest *S. brevidens* fragment only. Hybrids 17A and 17B similarly differed in the addition of one *S. brevidens* fragment. Both hybrids 16A and 16B exhibited patterns characteristic of mtDNA recombination but the patterns are slightly different to each other. However, hybrids 12A and 12B (which showed an unaltered parental *S. brevidens* pattern), hybrids 1A and 1C and hybrids 23 and 23F produced patterns indicating that plants regenerated

from the same callus can contain identical (as judged by restriction enzyme analyses) mitochondrial genomes.

The importance of using several restriction enzymes in any study of plant mtDNA reorganization is clearly demonstrated by the *Bgl* II results (Fig. 2). Again, only two hybrids (4 and 19) exhibited an unaltered 116 pattern, whereas twelve hybrids (6A, 9, 11B, 14, 15, 17A, 17B, 24B, 28, 30, 34 and 37B) produced a 116 pattern with an additional fragment equivalent in size to the fifth largest *S. brevidens* fragment. Consequently, data from just two restriction enzymes indicates that hybrid 19 is the only one that possibly possesses an unaltered parental 116 mitochondrial genome. It also indicates that several hybrids which exhibited an *Eco* RI pattern only slightly different to that of 116 exhibited larger differences with *Bgl* II, and vice versa. Hybrid 37A possessed a non-parental *Bgl* II fragment in addition to the full complement of 116 fragments whereas hybrids 1A, 1C, 3B and 11 exhibited an incomplete complement of 116 fragments but possessed various additional *S. brevidens* or non-parental fragments (Fig. 2). However, because there are so many *Bgl* II fragments common to each parental genome, some of these fragment patterns could be considered to represent mtDNA recombination. *Bgl* II did not identify any hybrids as having an unaltered parental *S. brevidens* pattern. Hybrid 12A possessed the complete *S. brevidens Bgl* II fragment pattern plus one 116 fragment and three non-parental fragments. The sibling hybrid, 12B, exhibited a pattern characteristic of mitochondrial recombination as did hybrids 2, 16A, 16B, 18, 23 and 23F. Thus only hybrids 2, 16A and 16B demonstrated characteristic mitochondrial recombination patterns with both *Eco* RI and *Bgl* II. Of the seven pairs of sibling hybrids analysed, only four pairs produced the same result with both enzymes; hybrids 1A and 1C and hybrids 23 and 23F possessed identical fragment patterns (within each pair) and hybrids 11 and 11B and hybrids 37A and 37B differed (within each pair).

Similar contrasting results were found between the other restriction enzymes used in this analysis of the 116-*S. brevidens* hybrids. For example, *Bam* HI and *Hin* dIII identified a *S. brevidens* fragment common to all hybrids except hybrids 4 and 19 (same result as *Bgl* II) whereas *Xho* I uncovered a *S. brevidens* fragment common to all hybrids except hybrids 19 and 30 (same result as *Eco* RI). In no case could these additional *S. brevidens* fragments be found in multiple mtDNA isolations from different plants of 116, 774, "Russet Burbank" accessions or other hexaploid potato cultivars. No such common *S. brevidens* fragments could be observed in the fragment patterns produced by *Pst* I or *Xba* I. Whereas six of the seven restriction enzymes employed produced some fragment patterns considered to be characteristic of mtDNA recombination, *Hin* dIII did not. This enzyme did, however, produce many fragment patterns in which incomplete complements of 116 fragments were augmented by *S. brevidens* fragments but no non-parental fragments were observed.

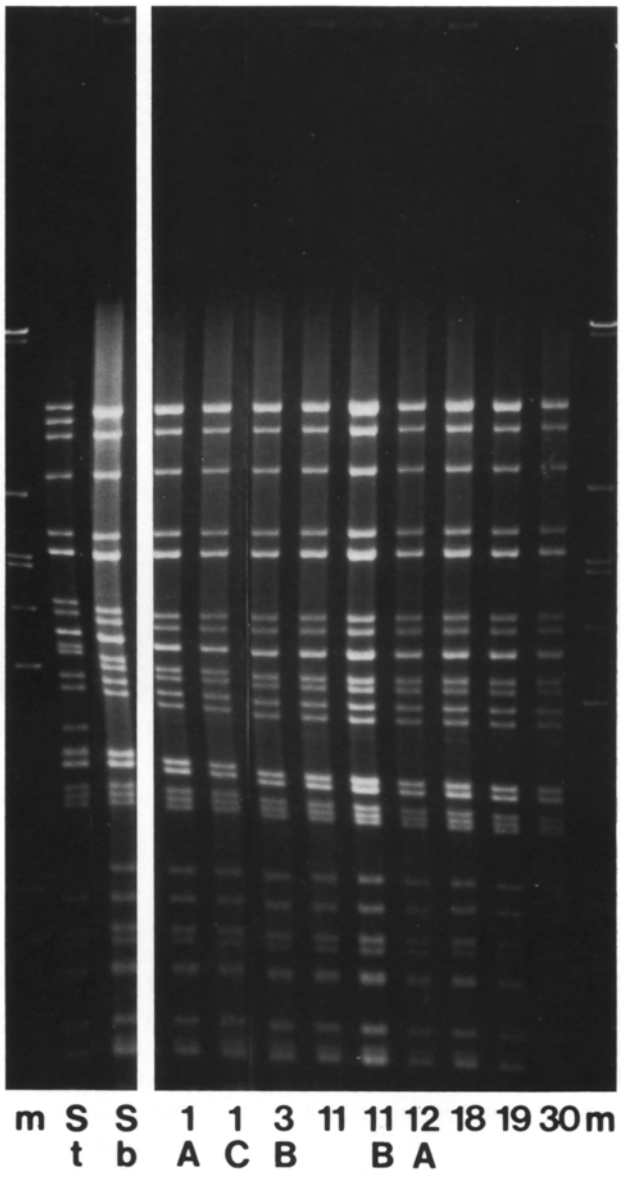

Fig. 3. Electrophoresis on 1% agarose gel of *Bgl* II fragmented cpDNAs. Labelling as in Fig. 1. DNA fragments smaller than approximately 1.5 kb are not shown

If the data generated by the seven restriction enzymes is averaged, then only hybrid 19 retained an unaltered parental 116 mitochondrial genome organization. No hybrid retained an unaltered *S. brevidens* mitochondrial genome. Thirteen other hybrids possessed a non-parental mitochondrial genome possibly

reorganized via recombination. However, it should be emphasized that several of the non-parental fragments and several of the fragments equivalent in size to those of *S. brevidens* exhibited by the hybrids were of identical size to some of those identified as new, non-parental fragments in a number of plants regenerated from individual potato protoplasts (Kemble and Shepard 1984). Consequently, several mtDNA rearrangements observed in the hybrids may be completely independent of the protoplast fusion process.

Most of the hybrids were vegetatively propagated by cuttings. MtDNA isolated from such cuttings always produced the same restriction fragment pattern as the original donor hybrid.

No DNA molecules except the high molecular weight mitochondrial chromosome were evident in any of the parental lines or hybrids when mtDNA preparations were electrophoresed on agarose gels without incubation with restriction enzymes.

The one 774-*S. brevidens* hybrid exhibited an unaltered parental 774 mtDNA pattern with each of the seven restriction enzymes (data not shown).

Figure 3 shows representative *Bgl* II digestions of cpDNA from some of the 116-*S. brevidens* hybrids. All 29 hybrids exhibited unaltered parental *S. brevidens* cpDNA patterns with all seven restriction enzymes. The 774-*S. brevidens* hybrid possessed an unaltered parental 774 cpDNA pattern with all enzymes.

Discussion

This study differs from other published work describing mtDNA recombination in somatic hybrids because plants regenerated from individual protoplasts of one of our parental lines (*S. tuberosum*) have previously been shown to undergo mtDNA rearrangements, presumably via intra-molecular recombination, independent of any fusion process (Kemble and Shepard 1984). In that case, approximately 15% of the 47 regenerants analysed possessed an altered mitochondrial genome. The present study indicates that exposure to the protoplast fusion system and the involvement of a second protoplast population increases the number of mtDNA arrangements observed in the regenerants. Approximately 52% of the somatic hybrids analysed exhibited mtDNA restriction patterns characteristic of mitochondrial recombination. Approximately 45% possessed a slightly rearranged, but nonetheless non-parental, *S. tuberosum* mitochondrial genome. Only one somatic hybrid did not reveal sequence deviations from the parental *S. tuberosum* mitochondrial pattern. There was no correlation between mitotic chromosome complement and degree of mtDNA rearrangements found in leaf tissue.

It is important to note, however, that several of the non-parental and *S. brevidens* mtDNA fragments found in the rearranged, but largely *S. tuberosum*-based, somatic hybrid mitochondrial genomes are equivalent in size to fragments observed in *S. tuberosum* plants regenerated from unfused protoplasts (Kemble and Shepard 1984). An explanation could be that since *S. tuberosum* and *S. brevidens* are taxonomically related, it is possible that their mtDNA evolved away from a common progenitor genome. It is likely that such mtDNA evolutionary changes would be via DNA recombination. If the stress of the protoplast tissue culture processes also causes a high degree of recombination it is, in effect, inducing similar changes although at a much faster rate. Consequently, one would expect to find fragments of similar size produced by recombinationally active 'hot spots' on each parental mitochondrial genome. Since these recombination events can occur in a single protoplast population (Kemble and Shepard 1984), several of the mtDNA recombination patterns and certainly the slightly rearranged *S. tuberosum* pattern could have been produced by intra-molecular recombination and be completely independent of the protoplast fusion process. However, some of the restriction patterns suggestive of mitochondrial recombination are sufficiently complex to indicate inter-molecular recombination involving both parental genomes.

Although point mutations in the genomes could explain some of the non-parental somatic hybrid fragments it is not considered the major mechanism. In addition to the evidence outlined above, the sizes of the non-parental fragments observed are the same in several hybrids. Again, this is suggestive of intra- and/or inter-molecular recombination via precise 'hot spots'. Such recombinationally active 'hot spots' have been reported in *Brassica* (Palmer and Shields 1984), maize (Lonsdale et al. 1984) and in *Cruciferae* somatic hybrids (P. Chetrit and F. Vedel, personal communication). Probing somatic hybrid mtDNA southern blots with cloned regions of mtDNA unique to either *S. tuberosum* or *S. brevidens,* if any exist, would clarify this hypothesis.

The present study has emphasized the importance of employing a large number of restriction enzymes and analysing a large number of somatic hybrids in establishing the extent of possible mtDNA rearrangements. *Hin* dIII, for example, did not produce any restriction pattern profiles which could be considered characteristic of mtDNA recombination. In addition, some enzymes did not differentiate the mitochondrial genomes of hybrids 12A and 12B from the parental *S. brevidens* pattern. Such anomalies are unavoidable when restriction fragments from large genomes (the *S. tuberosum* mitochondrial genome is estimated to be

in excess of 400 kb; Kemble and Shepard 1984) are displayed on agarose gels. Multiple fragments possess essentially equivalent electrophoretic mobility and, in attempts to better separate such fragments, many small fragments migrate off the gel.

None of the 30 somatic hybrids exhibited a mtDNA restriction fragment profile characteristic of a mixture of both parental types. This is further evidence that the plants were true hybrids and not chimeras.

Somatic hybrids regenerated from the same callus did not, in all cases, possess the same mitochondrial genomes indicating that sorting out of the genomes was incomplete at the plant regeneration stage. It also suggests that mitochondrial genome rearrangement may have been an ongoing phenomenon in callus. The argument that such plants could have regenerated from two different hybrid protoplasts adjacent to each other is not considered because the probability of such an event is extremely low among the relatively small number of fusion products present in the post-fusion cell population. In addition, the tissue culture procedure employed selects against development of clusters of different cell lineages (Barsby et al. 1984).

Multiple mtDNA extractions from the same hybrid plant or cuttings thereof, over a period of several months, always produced the same restriction pattern regardless of the enzyme employed. Therefore, the parental or non-parental mitochondrial genomes, or perhaps, the stoichiometric mixture of non-parental genomes did not continue to rearrange or sort out in planta.

Whereas mixed chloroplast populations have been reported in *Medicago* (Rose et al. 1986) and *Nicotiana* (Aviv et al. 1984; Fluhr et al. 1983, 1984; Gleba et al. 1984, 1985; Glimelius et al. 1981; Iwai et al. 1981; Sidorov et al. 1981) plants regenerated from protoplasts, all of our 116-*S. brevidens* hybrids analysed possessed unaltered *S. brevidens* chloroplast genomes. This cellular dominance may have occurred because the *S. brevidens* chloroplasts were photosynthetically superior to those of the albino 116 parent. However, it should be noted that three albino plantlets (all possessing the additive parental chromosome number of 72) were regenerated from green hybrid calli. Unfortunately, these plantlets did not produce sufficient leaf material for DNA analysis but the possibility exists that they may have contained unaltered 116 chloroplast genomes. In the one 774-*S. brevidens* hybrid analysed, the chlorophyll-deficient chloroplasts from the variegated 774 parent (protoplasts were isolated from non-green regions of leaves) reverted to green and were selected in preference to those of *S. brevidens*. In the latter fusion, the *S. brevidens* protoplast population was treated with the metabolic inhibitor, iodoacetic acid. Rearranged chloroplast genomes were not observed in any of the 30 hybrids analysed. This is in accord with previous evidence indicating that chloroplast genomes possessing inverted repeat regions are stable (Fluhr and Edelman 1981; Palmer and Thompson 1982) and that no cpDNA rearrangements were found in plants regenerated from individual *S. tuberosum* protoplasts (Kemble and Shepard 1984).

S. tuberosum–S. brevidens somatic hybrids appear to have potential in potato breeding. Previous studies have shown that they possess agronomically useful traits (Austin et al. 1985; Barsby et al. 1984). The present study shows that approximately half of the hybrids analysed possessed substantially rearranged or recombined mitochondrial genomes indicating that the protoplast fusion system offers a potential mechanism of incorporating mtDNA diversity or specific mtDNA-encoded traits. The other half of the hybrids exhibited unchanged or only marginally altered mitochondrial genomes indicating that protoplast fusion is also useful when the inheritance and maintenance of a particular parental mitochondrial genome is important.

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