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Analysis of the mitochondrial DNA of the somatic hybrids of *Solanum brevidens* and *S. tuberosum* using non-radioactive digoxigenin-labelled DNA probes

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Summary. Mitochondrial (mt) DNAs of somatic hybrids obtained by electrical and chemical fusion of mesophyll protoplasts of *S. brevidens* and a dihaploid line of *S. tuberosum* PDH 40 were analysed by Southern hybridization using the digoxigenin-labelled mtDNA sequences *nad5* or orf25. In the Southern analysis of the hybrid mtDNA probed with *nad5*, most of the 19 hybrids analyzed had an RFLP pattern similar, but not identical, to one of the parents, *S. tuberosum*, PDH40. Nineteen percent of the hybrids had most of the *S. brevidens* fragments. Five of the hybrids had an identical RFLP pattern to either one of the parents while another two hybrids had novel RFLP patterns. Similar results were obtained by Southern analysis with orf25. These results clearly show that mtDNA rearrangements had occurred at a high frequency in the somatic hybrids. There were no differences in the frequencies of rearrangements observed between the hybrids regenerated from chemical and electrical fusions.

Key words: Potato – *S. brevidens* – Mitchondrial DNA – Somatic hybrids – Non-radioactive labelling

Introduction

The production of somatic hybrid plants by protoplast fusion provides a useful approach for the combination of genetic material. It can be applied both to the formation of novel hybrids between sexually incompatible species and to the reconstruction of hybrids that can also be produced by sexual hybridization (Austin et al. 1985, 1986; Jones 1988; Chaput et al. 1990; Pehu et al. 1990). In protoplast fusion both nuclear and cytoplasmic genomes are combined, and this can lead to production of new genetic variability by the recombination of nuclear and cytoplasmic genomes. The production of somatic hybrids in the *Solanaceae* has been achieved in several laboratories (e.g., Puite et al. 1986; Austin et al. 1985, 1986; Fish et al. 1987, 1988a). The aim in most of these cases has been to transfer potentially useful agronomic traits from wild species into cultivated potato (*Solanum tuberosum*).

To fully understand the consequences and potential of protoplast fusion in the production of novel somatic hybrids, a detailed characterisation of such hybrids is necessary. Analysis of chloroplasts in fusion products indicates that they sort out during hybrid regeneration so that hybrid plants may contain chloroplasts either from one or other of the protoplast parents, or from one parent alone. The pattern found may depend on the phylogenetic distance between the species, the presence of selection pressure for one organelle type, or the relative number of plastids in each parental protoplast (Kumar and Cocking 1987; O'Connell and Hanson 1987; Gleba et al. 1988). Recombination of choroplast DNA (cpDNA) in fusion products may occur as a very rare event (Maliga et al. 1987; Medgyesy et al. 1990). In contrast to the situation with chloroplasts, recombination and/or rearrangements of mitochondrial DNA (mtDNA) in fusion products has been reported in somatic hybrids between various species, such as *Lycopersicon esculentum* and *L. peruvianum* (San et al. 1990; Derks et al. 1991), *L. esculentum* and *L. pennellii* (O'Connell and Hanson 1987; Wachocki et al. 1991), and

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Pennisetum americanum and *Panicum maximum* (Ozias-Akins et al. 1987). These rearrangements result in the production of restriction fragment patterns which differ from those of the parental mtDNA genomes. In most experiments it appears that chloroplasts and mitochondria segregate independently (Aviv and Galun 1980; Levi et al. 1988).

Our research has focussed on the South American diploid wild Potato species *S. brevidens* (Jones et al. 1990), which is resistant to Potato Leaf Roll Virus (Jones 1979) and to Potato Virus Y and X (Gibson et al. 1988, 1990). It would be useful to transfer these viral resistance traits to cultivated potato germplasm. Because direct sexual crosses cannot be made, the genome of *S. brevidens* was combined with that of cultivated potato by protoplast fusion using both chemical and electrofusion procedures (Fish et al. 1987, 1988a). The hybrids have been characterized previously by isoenzymes, morphology, chromosome number, nuclear hybridity and cpDNA (Fish et al. 1987, 1988b; Pehu et al. 1989), and for field growth under government guidelines (Fish et al. 1988b). Here we report the RFLP analysis of the mtDNA of the hybrids using a non-radioactive labelling method.

Materials and methods

Plant materials

The somatic hybrids selected for this study between dihaploid *S. tuberosum* (PDH 40, 24 chromosomes) and *S. brevidens* CPC 2451 (dihaploid, 24 chromosomes) were produced by Fish et al. (1987, 1988a) using both chemical and electrical fusion techniques. Nuclear hybridity has been confirmed by molecular, cytological and morphological methods (Fish et al. 1987, 1988a; Pehu et al. 1989). The hybrids used in this study were selected to represent two ploidy levels (tetraploid and hexaploid) and both parental cpDNA types as determined by Pehu et al. (1989). The hybrids included eight tetraploids, four hexaploids, five aneuploids at the tetraploid level, and seven aneuploids at the hexaploid level. Of the hybrids, 13 had *S. tuberosum* chloroplasts, and 11 had *S. brevidens* chloroplasts (see Table 1).

DNA isolation, restriction and Southern transfer

Total cellular DNA was isolated from in-vitro cultured material using the method of Draper et al. (1988). Four micrograms of this DNA was restricted for 4–5 h at 37 °C in 30 µl according to the supplier's instructions (New England Biolabs) and separated by agarose-gel (0.8%) electrophoresis in Tris-Borate EDTA (TBE) buffer at 2 v/cm for 18 h. After electrophoresis, the gel was depurinated for 10 min in 0.25 N HCl, denatured for 30 min in 0.5 M NaOH, 1.5 M NaCl solution and neutralized for 30 min in 1 M Tris, 1.5 M NaCl, pH 7.5 solution. The DNA was transferred overnight, onto a DNA-binding filter, Hybond-N (Amersham) in 20 × SSC by capillary transfer.

The mtDNA probes

The mtDNA probes used in the study were cytochrome oxidase I [coxI (Isaac et al. 1985)], cytochrome oxidase II; [cox II (Fox and Leaver 1981)], maize cytochrome b (Dawson et al. 1984), open reading frame (orf25) from *Petunia* (Stamper et al. 1987; the petunia orf25 gene is 100% homologous to tobacco orf25.) and *nad5* from wheat (Bonen et al., unpublished). They were kindly supplied by Prof. C. S. Levings and Dr. M. Hanson.

Southern analysis

The probes were non-radioactive digoxigenin-labelled according to the instructions of the supplier (Boehringer Mannheim Co.). Hybridization and immunological detection were also carried out following the procedures given by the supplier. The filter was washed for 15 min twice at 68 °C with 0.3 × SSC, SDS 0.1% (w/v). The filter was then incubated with about 10 ml of colour solution and sealed in a plastic bag in the dark. The colour precipitate started to form within a few minutes and the reaction was usually complete after 1 day.

Results

Mitochondrial DNA analysis of the fusion parents S. brevidens and S. tuberosum

To identify different RFLP patterns between the mtDNAs of *S. brevidens* and *S. tuberosum*, the patterns produced by different mtDNA probes in combination with four restriction endonucleases (*Bam*HI, *Eco*RI, *Hin*dIII and *Pst*I, New England Biolabs) were analyzed. Clear differences were found in the mtDNA pattern of both parents when *Eco*RI digests were probed with the *Bam*HI-*Hin*dIII fragment from the *nad5* gene of wheat (Fig. 1) and the *Bam*HI fragment from the orf25 of *Petunia* (see Fig. 3). There were no common bands in the parents.

Mitochondrial DNA analysis of the somatic hybrids

In the Southern analysis of the 19 hybrids digested with *Eco*RI and probed by *nad5*, there were three groups of RFLP patterns (Fig. 1, Table 1): (1) patterns similar, but not identical, to the parental species, (2) Unique RFLP patterns, and (3) patterns identical to the parents. Twelve of the hybrids had an RLFP pattern similar, but not identical, to either the *S. brevidens* or *S. tuberosum* parent. Among them, ten of the hybrids had an RFLP pattern similar, but not

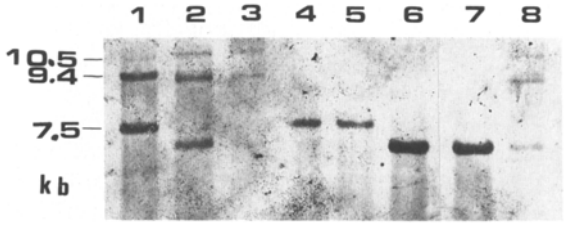

Fig. 1. Hybridization of the digoxigenin-labelled mitochondrial *nad5* to *Eco*RI digests of total genomic DNA. *Lane 1, S. brevidens. Lane 2, S. tuberosum* PDH40. *Lanes 3–8*, somatic hybrids. The sized bands indicates *S. brevidens*-specific fragments

Table 1. Summary of hybridization patterns of the somatic hybrids obtained with the mitochondrial gene probes. The endonuclease used to identify the RFLPs was *Eco*RI. E represents electrofusion, C chemical fusion. PDH and SB indicate hybridization patterns that are identical to either *S. tuberosum* PDH40 or *S. brevidens*. PD and B indicate hybridization patterns that have some bands of either *S. tuberosum* PDH40 or *S. brevidens*, but not all of them. R indicates a hybridization pattern that has novel bands, different from both of the parents

Plant no.	Fusion type	Chromosome no.	Nuclear genome constitution		Chloroplast type	*nad5*	orf25
			s.b.	s.t.			
81043	E	72	4x	2x	SB	PDH	
81081	E	71	2x	4x	PDH	PDH	PDH
70023	C	48	2x	2x	PDH	PD	PD
70067	C	47	2x	2x	PDH	PD	
81013	E	89			SB	PD	
81068	E	69			PDH	PD	PD + R
81115	E	72	2x	4x	SB	PD	
81130	E	69			PDH	PD	PD
81136	E	72	4x	2x	PDH	PD	PD + R + B
84042	E	48	2x	2x	PDH	PD	PD
84152	E	45	2x	2x	PDH	PD	PD
84155	E	72	2x	4x	SB	PD	B
65006	C	48	2x	2x	SB	SB	
65013	C	48	2x	2x	SB	SB	SB
84054	E	48	2x	2x	PDH		SB
65003	C	47	2x	2x	Sb	B	B
65009	C	71	4x	2x	SB		B
81005	E	47	2x	2x	SB	B	B
81011	E	47	2x	2x	SB		B
84054	E	48	2x	2x	PDH		B
84091	E	71	2x	4x	PDH		B + R
84153	E	71	2x	4x	SB	B	PD + R + B
81078	E	48	2x	2x	PDH	PD + R	PD + R
84140	E	48	2x	2x	PDH	R	PD + R + B

identical, to the *S. tuberosum* parent. Half of them shared only one band (6.0 kb) with *S. tuberosum*, the other half had two of *S. tuberosum* bands (Fig. 1, Table 1), a 9.0 kb band and in addition either a 6.0 kb or an 11 kb band from PDH40. Two of the hybrids had two bands from *S. brevidens*; however, they did not have the 10.5 kb band of *S. brevidens*. Two of the hybrids belonged to the second group, i.e., they had a novel RFLP pattern. One of these hybrids had a unique mtDNA RFLP (lane 1 in Fig. 2). The other one had one band (6.0 kb) from *S. tuberosum*, one band (9.4 kb) from *S. brevidens* and in addition four unique bands (1.8, 3.0, 4, 12 kb) not present in the parental lines. Lastly, five of the hybrids had exactly the same RFLP pattern as one of the parents. Three of these hybrids had an mtDNA RFLP pattern identical to *S. brevidens* while the remaining one had the same RLFP pattern as *S. tuberosum* PDH40. In conclusion, among the 19 analyzed, 74% of the mtDNA RFLP profiles differed from those of the parents.

Similarly three groups of RFLP patterns were observed in Southern analysis of the hybrid DNA digested with *Eco*RI and probed with orf25 (Fig. 3, Table 1), although the banding patterns were more complex than in the *nad5*/*Eco*RI hybridization (Fig. 3). Some of the hybrids had novel bands (2–4) in addition to the bands from the parents (Fig. 3, Table 1). From the 19 hybrids analyzed, only three had a completely identical RFLP pattern to one of the parents, 84% of the hybrids having different mtDNA RLFP patterns than the parental lines.

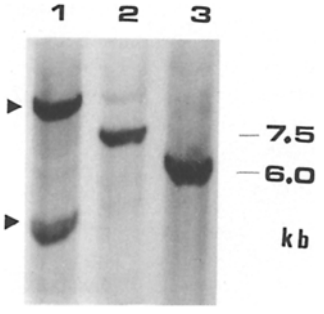

Fig. 2. Hybridization of the digoxigenin-labelled mitochondrial *nad5* to *Eco*RI digests of total genomic DNA. *Lane 1*, somatic hybrid having a unique mtDNA pattern (indicated by *arrows*). *Lanes 2–3*, somatic hybrids having *S. brevidens*-based and *S. tuberosum*-based mtDNA patterns, respectively

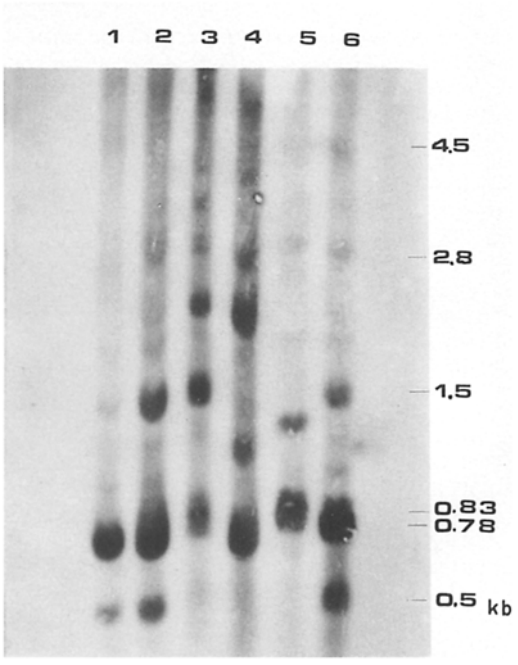

Fig. 3. Hybridization of the digoxigenin-labelled mitochondrial orf25 to *Eco*RI digests of total genomic DNA. *Lanes 1–4*, somatic hybrids. *Lane 5*, *S. tuberosum* PDH40. *Lane 6*, *S. brevidens*. The sized bands indicate species-specific fragments

Of the hexaploid hybrids containing two *S. brevidens* and one *S. tuberosum* genome, two had *S. tuberosum*-based mtDNA patterns, the remaining one being *S. brevidens*-based (Table 1). In the five hexaploid hybrids having one *S. brevidens* and two *S. tuberosum* genomes, two had *S. brevidens*-based mtDNA patterns. One of these hybrids had a *S. brevidens*-based mtDNA pattern if hybrid DNA was digested with *Eco*RI and probed by orf25, whereas it had a *S. tuberosum*-based mtDNA pattern if it was digested by *Eco*RI and probed by *nad5*. The remaining hybrids had *S. tuberosum*-based mtDNA patterns (Table 1).

Discussion

The study revealed a high frequency of mtDNA sequence rearrangements in somatic hybrids between *S. tuberosum* and *S. brevidens*, which is in agreement with the previous findings of Kemble et al. (1986) for the same species combination. In the present study mtDNA rearrangements were detected in 74–84% of the somatic hybrids, depending on the probe/restriction enzyme combination used. The report of Kemble et al. (1986) showed a higher number of the 29 hybrids analysed possessing an altered mitochondrial genome. in the latter study, most of the hybrids (28 out of 29) exhibited mtDNA restriction patterns characteristic of mitochondrial recombination or else a slightly rearranged mitochondrial genome. The fact that the frequency of mtDNA rearrangements detected in our experiments was lower could be due to the limited number of hybrids analysed in our study or the limited mtDNA probes used.

As in the study of Kemble et al. (1986) we also observed non-parental mtDNA fragments in the somatic hybrids which might have arisen through mitochondrial recombination. However, Kemble and Shepard (1984) found that 15% of plants regenerated from individual protoplasts of *S. tuberosum* had undergone mtDNA rearrangements, presumably via intra-molecular recombination, independent of any fusion process. Since mtDNA undergoes such changes it is probable that protoplast fusion leads to an increase in the number of mtDNA rearrangements observed in the fusion products.

In the present study, eight hexaploid hybrids with different parental nuclear genome composition were included. Based on the mtDNA patterns of these hexaploid hybrids, there was no association between the ploidy of the hybrids and the mtDNA type of the hybrids (Table 1). The previous cpDNA analysis of these hybrids indicated that each of the 58 somatic hybrids analysed contained either one parental chloroplast genome or the other (Pehu et al. 1989). As in the case of the nuclear genome composition, no association was observed between the cpDNA type and the mtDNA type among the hybrids (Table 1).

Among the 19 analysed hybrids, five derived from chemical fusion were included. Unlike the study of San et al. (1990) on the somatic hybrids between *Lycopersicon esculentum* Mill. and *L. peruvianum* Mill., we did not detect a difference in the degree of sequence rearrangements between the hybrids produced by chemical or electrical fusion (Table 1).

The RFLP patterns from several probe/enzyme combinations had to be surveyed to identify differences between the parental lines, and this indicates that the organellar genomes of *S. tuberosum* and *S. brevidens* are similar in composition. Similar results have been obtained by Derks et al. (1991). *S. tuberosum* and *S. brevidens* are taxonomically related, belonging to the same genus, and probably evolved from a common progenitor genome.

Southern-blot hybridization analysis of digested total DNA probed with cloned mtDNA fragments, has been shown to be useful for the characterization of the mitochondrial genomes of somatic hybrids/cybrids (Galun and Aviv 1986; Wachocki et al. 1991). Southern analysis in the present study using total DNA, rather than restriction analysis of extracted mtDNA, was chosen because the method required only 0.5–1 g of leaf tissue, and such a small amount of tissue is readily available from in-vitro shoot cultures

of the hybrids. This reduced the need to grow larger plants. A further improvement in technique was the non-radioactive labelling and hybridization procedure. Signal detection was quick and reproducible, and cost less than the use of radioactive labelling. In optimizing the digoxigenin labelling and hybridization procedure, the amount of labelled DNA used for the hybridization was found to be important for reducing the background staining. The optimum quantity was 20 ng of labelled DNA per one milliliter of hybridization buffer. Moreover, at the onset of colour precipitation it was important not to agitate the mix in order to ensure sharpness of the hybridization bands. In our experiments, the sensitivity of the non-radioactive technique was almost equal to that of radioactive labelling. The possibility of long-term storage of the labelled DNA (up to 1 year) and the possibility of reusing the hybridization filter and the probes are added advantages of digoxigenin dUTP labelling over the already established radioactive method.

Based on our results, it can be concluded that extensive sequence rearrangements were present in the mtDNA of hybrids, whereas the cpDNA in hybrids was unchanged from the parental lines (Pehu et al. 1989). Furthermore, segregation of chloroplasts and mitochondria following fusion was independent of each other, as well as of the nuclear genome composition, in the hexaploid hybrids. This provides a complete picture of the molecular characteristics of the cytoplasmic genomes of the somatic hybrids between *S. tuberosum* PDH40 and *S. brevidens* produced by Fish et al. (1987, 1988a) and Jones et al. (1990).

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References

Austin S, Baer MA, Helgeson JP (1985) 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 (1980) Restoration of fertility in cytoplasmic male-sterile (CMS) *Nicotiana sylvestris* by fusion with X-irradiated *N. tubacum* protoplasts. Theor Appl Genet 58:121–127

Chaput MH, Sihachakr D, Ducreux G, Marie D, Barghi N (1990) Somatic hybrid plants produced by electrofusion between dihaploid potatoes: BF15 (H1), Aminca (H6) and Cardinal (H3). Plant Cell Rep 9:411–414

Dawson AJ, Jones VP, Leaver CJ (1984) The apocytochrome b gene in maize mitochondria does not contain introns and is preceded by a potential ribosome binding site. EMBO J 3:2107–2113

Derks FHM, Wijbrandi J, Koornneef M, Colijn-Hooymans CM (1991) Organelle analysis of symmetric and asymmetric hybrids between *Lycopersicon peruvianum* and *Lycopersicon esculentum*. Theor Appl Genet 81:199–204

Draper J, Scott R, Armitage P, Walden R (1988) Plant genetic transformation and gene expression. A laboratory manual. Blackewell Scientific Publications, London, pp 212–214

Fish N, Karp A, Jones MGK (1987) Improved isolation of dihaploid *Solanum tuberosum* protoplasts and the production of somatic hybrids between dihaploid *S. tuberosum* and *S. brevidens*. In Vitro 23:575–580

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

Fish N, Steele SH, Jones MGK (1988b) Field assessment of dihaploid *S. tuberosum* and *S. brevidens* somatic hybrids. Theor Appl Genet 76:880–886

Fox TD, Leaver CJ (1981) The *Zea mays* mitochondrial gene coding cytochrome oxidase subunit II has an intervening sequence and does not contain TCA codons. Cell 26:315–323

Galun E, Aviv D (1986) Organelle transfer. In: Weissbach A, Weiss bach H (eds) Plant molecular biology. Methods Enzymol 118:595–611

Gibson RW, Jones MGK, Fish N (1988) Resistance to potato leaf roll virus and potato virus Y in somatic hybrids between dihaploid *Solanum tuberosum* and *S. brevidens*. Theor Appl Genet 76:113–117

Gibson RW, Pehu E, Woods RD, Jones MGK (1990) Resistance to potato virus Y and potato virus X in *Solanum brevidens*. Ann Appl Biol 116:151–156

Gleba YY, Hinnisdaels S, Sidorov VA, Kaleda VA, Parokonny AS, Boryshuk NV Cherep NN, Negrutiu I, Jacobs M (1988) Intergeneric asymmetric hybrids between *Nicotiana plumbaginifolia* and *Atropa belladonna* obtained by 'gamma-fusion'. Theor Appl Genet 76:760–766

Isaac PG, Jones VP, Leaver CJ (1985) The maize cytochrome c oxidase subunit I gene: sequence, expression and rearrangement in cytoplasmic male-sterile plants, EMBO J 4:1617–1623

Jones MGK (1988) Fusing plant protoplasts. Biotechnology 6:153–158

Jones MGK, Dunckley R, Steele S, Karp A, Gibson R, Fish N, Valkonen J, Poutala T, Pehu E (1990) Transfer of resistance to PLRV, PVX and PVY from *S. brevidens* to potato by somatic hybridization: characterisation and field evaluation. In: Nijkamp HJJ, Vanderplas LHW and Vanaartrijk J (eds) Progress in plant cellular and molecular biology. Kluwer Academic Publishers, Dordrecht Boston London, pp 286–292

Jones RAC (1979) Resistance of potato leaf roll virus in *Solanum brevidens*. Potato Res 22:137–144

Kemble RJ, Shepard JF (1984) Cytoplasmic DNA variation in a potato protoclonal population. Theor Appl Genet 69:211–216

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

Kumar A, Cocking EC (1987) Protoplast fusion: a novel approach to organelle genetics in higher plants. Am J Bot 74:1289–1303

Levi A, Ridley BL, Sink KC (1988) Biased organelle transmission in somatic hybrids of *Lycopersicon esculentum* and *Solanum lycopersicoides*. Curr Genet 14:177–182

Maliga P, Fejes E, Svab Z (1987) Recombination of chloroplasts in cultured plant cells. In: Green CE, Somers DA, Hackett

WP, Bies Boer DD (eds) Plant tissue and cell culture. Alan R. Liss, New York, pp 265–274

Medgyesy P, Thanh ND, Hopvath GV, Buzas B (1990) Chloroplast or mitochondrial DNA recombination after protoplast fusion forms an accommodated new organelle genome in incompatible nucleus-organelle combinations. Abstr 7th Int Congr Plant Tissue and Cell Culture, p 204

O'Connell MA, Hanson MR (1987) Examination of genome stability in cultured *Lycopersicon*. Theor Appl Genet 75:83–89

Ozias-Akins P, Pring DR, Vasil IK (1987) Rearrangements in the mitochondrial genome of somatic hybrid cell line *Pennisetum americanum* (L.) K. Schum. + *Panicum maximum* Jacq. Theor Appl Genet 74:15–20

Pehu E, Karp A, Moore K, Steele S, Dunckley R, Jones MGK (1989) Molecular, cytogenetic and morphological characterization of somatic hybrids of dihaploid *Solanum tuberosum* and diploid *S. brevidens*. Theor Appl Genet 78:696–704

Pehu E, Gibson RW, Jones MGK, Karp A (1990) Studies of the genetic basis of resistance to potato leaf roll virus, potato virus Y and potato virus X in *Solanum brevidens* using somatic hybrids of *Solanum brevidens* and *Solanum tuberosum*. Plant Sci 69:95–101

Puite KJ, Roest S, Pijnacker LP (1986) Somatic hybrid potato plants after electrofusion of diploid *Solanum tuberosum* and *Solanum phureja*. Plant Cell Rep 5:262–265

San HL, Vedel F, Sihachakr D, Remy R (1990) Morphological and molecular characterization of fertile tetraploid somatic hybrids by protoplast electrofusion and PEG-induced fusion between *Lycopersicon esculentum* Mill. and *Lycopersicon peruvianum* Mill. Mol Gen Genet 221:17–26

Stamper SE, Deway RE, Bland MM, CS Leving, III (1987) Characterization of the gene *urf13-T* and an unidentified reading frame, ORF 25, in maize and tobacco mitochondria. Curr Genet 12:457–463

Wachocki SE, Bonnema AB, O'Connell MA (1991) Comparison of the organization of the mitochondrial genome in tomato somatic hybrids and cybrids. Theor Appl Genet 81 : 420–427