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Organelle DNA analysis of *Solanum* and *Brassica* somatic hybrids by PCR with 'universal primers'

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Abstract In order to set up a quick and easy procedure for determining the cytoplasmic composition of somatic hybrids, we tested a set of 'universal primers' for plastidial and mitochondrial DNA on 13 genotypes belonging to the following species: *Nicotiana tabacum*, *Solanum commersonii*, *Solanum tuberosum*, *Solanum etuberosum*, *Solanum phureja*, *Brassica oleracea*, *Brassica rapa*, 'Anand' CMS *B. rapa*, 'Chiang' CMS *B. oleracea*, and 'Ogura' CMS *B. oleracea*. Such primers are homologous to conserved coding sequences and amplify polymorphic intergenic or intronic regions. cpDNA polymorphism within *Solanum* and *Brassica* spp. was found with two and four primer pairs, respectively. The primers for the intergenic region between the *trnF* and *trnV* genes gave polymorphism among several tested species and were used in *S. commersonii* (+) *S. tuberosum* somatic hybrids, and *B. oleracea* (+) 'Anand' CMS *B. rapa* cybrids. Two primer pairs for mtDNA revealed polymorphism between *S. commersonii* and *S. tuberosum*, and one showed intraspecific polymorphism in *S. tuberosum*. The primer pair for the intergenic region between the *rps14* and *cob* genes (pumD) showed a fragment of about 1.5 kb in *S. tuberosum* and *S. phureja*. A shorter fragment and no amplification were found in *S. etuberosum* and *S. commersonii*, respectively, suggesting frequent intrageneric rearrangements in this genome region. All Brassicaceae evidenced a fragment about 150-bp longer than in *S. tuberosum*. The same primers were also used with interspecific *Solanum* spp. somatic hybrids. Both PCR with pumD primers and hybridization with *rpl5/rps14* genes indicated lack of linkage between *rpl5/rps14* and *cob* genes in *S. commersonii*. Compared to direct visualization of restricted organellar DNA or Southern analysis with labelled probes, amplification of cpDNA and mtDNA with universal primers, followed by electrophoresis of either entire or restricted amplified fragments, is a simpler, more rapid and less expensive method to determine the organelle genome composition of interspecific *Solanum* and *Brassica* somatic hybrids.

Keywords *Solanum* · *Brassica* · Somatic hybrids · Organelle DNA · Universal primers

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Introduction

Solanum and *Brassica* genera include many cultivated and wild related species amenable to exploitation in breeding through biotechnological approaches. In both genera, somatic hybridization has been extensively used for interspecific gene transfer across sexual barriers (Cardi et al. 1993; Waara and Glimelius 1995; Bastia et al. 2000). Further, in contrast to sexual crosses, somatic hybridization can also induce a large variability in cytoplasmic genomes and generate novel nuclear-cytoplasmic interactions. The production of cytoplasmic hybrids (cybrids) through the donor-recipient method has been largely used to transfer cytoplasmic genes into a particular nuclear background (Earle 1995; Cardi and Earle 1997). Plastidial and mitochondrial genes, or their interaction with nuclear ones, can control several important traits related to plant and flower developement, (male) fertility, and stress resistance (Earle 1995; Cardi et al. 1999).

The cytoplasmic composition of somatic hybrids has been mostly investigated through the Southern analysis of organellar or total DNA with mitochondrial and plastidial probes, or through mt and cpDNA restriction-profile analysis (Earle 1995; Cardi et al. 1999; Bastia et al. 2000). These methods are usually expensive and/or time consuming. Moreover, a considerable amount of DNA is required. More recently, new types of PCR-based molecular markers for cp and mtDNA have been described. Several 'universal primers' homologous to highly conserved coding sequences of cp and mtDNA, and amplifying non-coding and highly variable regions, have been designed and tested on many land plants (Taberlet et al. 1991; Demesure et al. 1995; Dumolin-Lapègue et al. 1997b; Petit et al. 1998).

Direct sequencing or restriction analysis of fragments amplified with universal primers for organellar DNA (mainly cpDNA) has been used in phylogenetic studies in different plant species (Gielly and Taberlet 1994; Demesure et al. 1996; El Mousadik and Petit 1996; Tsumura et al. 1996) and in studies for determining the inheritance of chloroplasts and mitochondria in pedunculate oak (Dumolin et al. 1995). In this paper we tested the usefulness of universal primers for an early screening of the cytoplasmic composition of interspecific somatic hybrids and their parental genotypes. To this end we used a set of universal primers for cp and mtDNA with total DNA isolated from *Solanum* and *Brassica* species, interspecific somatic hy(cy)brids, and *Nicotiana tabacum*.

Materials and methods

Plant material

Various genotypes belonging to the Solanaceae and Brassicaceae families were used in this study. The former included: *Solanum commersonii* PI243503, PI472833, PI472834 (coded Cmm1, Cmm2, Cmm3, respectively); *Solanum tuberosum* dihaploid clones SVP11 and Atl9 (derived from clone W72-22-492 and cv Atlantic, respectively); *Solanum etuberosum* PI558054 (etb); *Solanum phureja* PI584955 clone IVP35 (phu); *N. tabacum* cv Samsun NN (Nt); Cmm1 (+) SVP11 and Cmm3 (+) Atl9 somatic hybrids (SH and FPN4, respectively) (Bamberg et al. 1996; Cardi et al. 1999; Bastia et al. 2000). The *Brassica* genotypes included: rapid cycling (RC) *Brassica oleracea* (fertile *oleracea* cytoplasm) CrGC (Crucifer Genetics Cooperatve) # 3-1 (coded Bo); RC *Brassica rapa* (fertile *rapa* cytoplasm) CrGC # 1-1 (Br); RC *B. rapa* (sterile 'Anand' cytoplasm) CrGC # 1-31 (M2); RC *B. oleracea* (sterile 'Chiang' cytoplasm) CrGC # 3-10 (M4); *B. oleracea* cabbage (sterile 'Ogura' cytoplasm) 90WC65 (M1C); Bo (+) M2 cybrids (Williams 1985; Cardi and Earle 1997; E.D. Earle, personal communication).

PCR analyses

Total DNA was extracted from fresh or frozen leaves following the procedure of Doyle and Doyle (1990) modified as reported in Cardi and Earle (1997). Ten to twenty five nanograms of genomic DNA were used in PCR amplifications with a set of universal primers for cpDNA and mtDNA (Table 1). In addition, other primer pairs for cpDNA and mtDNA as described by Dumolin-Lapegue et al. (1996) and Petit et al. (1998) were tested but not further used because, in our hands, they gave inconsistent results. Amplification reactions were generally performed in volumes of 25 µl containing 0.4 µM of each primer, 200 µM each of dATP, dCTP, dGTP and dTTP, 0.2 units of *Taq* DNA polymerase and 1 × reaction buffer supplied by the manufacturer (Perkin Elmer Cetus) (E. Tribulato, personal communication). Amplifications were performed in a DNA Thermal Cycler, programmed as follows: (1) program 1 = 2 min at 92°C, 10 cycles of 5 s at 92°C / 15 s at the annealing temperature (Table 1) / 1 min at 72°C, 20 cycles as before with a 10-s increase of the extension step per each cycle, a final extension of 10 min at 72°C (E. Tribulato, personal communication); (2) program 2 = 4 min at 94°C, 30 cycles of 45 s at 92°C / 45 s at the annealing temperature / 4 min at 72°C, a final extension of 10 min at 72°C (Demesure et al. 1995). Amplification products were analyzed by electrophoresis in 0.8–1% agarose gels stained with ethidium bromide, directly or after digestion with some of the following restriction enzymes: *Afa*I, *Hae*III, *Hha*I, *Hinf*I, *Hpa*II and *Taq*I. Their sizes were estimated (average CV = 2.5%) by comparison with a 1-kb DNA-ladder size marker. Lengths of fragments obtained by amplification with universal primers for cpDNA were also compared with those obtained after amplification of *N. tabacum* whose complete cpDNA sequence is known (Shinozaki et al. 1986).

Table 1 Primer pairs used for amplification of specific cpDNA or mtDNA regions. The annealing temperatures and amplification cycles employed are also indicated

Code	Primers		Program [a]	Annealing temperature	Reference for origin and sequence [b]
	Forward	Reverse			
cpDNA					
pucA	*trnH*	*trnK*	1	55	1
pucB	*trnD*	*trnT*	1	55	1
pucC	*psbC*	*trnS*	1	55	1
pucD	*trnS*	*trnfM*	1	55	1
pucE	*trnS*	*trnT*	2	57.5	1
pucF	*trnM*	*rbcL*	1	55	1
pucG	*trnK*	*trnK*	2	55	1
pucH	*trnC*	*trnD*	2	55	1
pucI	*psaA*	*trnS*	1	55	1
pucJ	*trnF*	*trnV*	2	57.5	2
pucK	*trnV*	*rbcL*	1	57.5	2
pucL	*trnQ*	*trnR*	1	57.5	2
pucM	*trnT*	*psbC*	1	55	2
pucN	*rbcL*	*aacD*	1	55	3
pucO	*trnT*	*trnF*	1	55	3
pucP	*rbcL*	*psaI*	1	55	3
pucQ	*rpoC2*	*rpoC1*	2	55	3
pucR	*trnK*	*trnQ*	1	47.5	2
pucS	*rpoC1*	*trnC*	1	47.5	2
pucT	*trnfM*	*psaA*	1	47.5	2
pucX	*psaA*	*psaA*	1	55	4
mtDNA					
pumA	*nad1* ex B	*nad1* ex C	1, 2	55	1
pumB	*nad4*- ex 1	*nad4*- ex 2	2	55	1
pumC	*nad4*- ex 2	*nad4*- ex 3	1, 2	55	3
pumD	*rps14*	*cob*	1	57.5	1
pumE	*rrn18*	*rrn5*	1, 2	55	3

[a] See Materials and methods
[b] 1 = Demesure et al. 1995; 2 = Dumolin-Lapegue et al. 1996; 3 = Petit et al. 1998; 4 = Tsumura et al. 1996

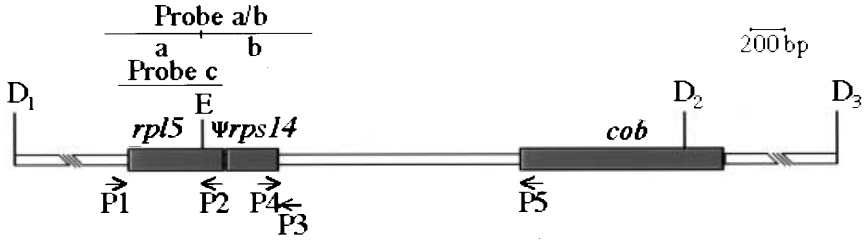

Fig. 1 Localization of probes and primers used for the analysis of the mitochondrial *rpl5-rps14-cob* locus in *Solanum* spp. The genomic organization of *S. tuberosum* mtDNA is reported according to Zanlungo et al. (1991) and Quiñones et al. (1996): *E* = *Eco*RI restriction site within the *rpl5* gene; D_2 = *Dra*I restriction site within the *cob* gene. D_1 and D_3 *Dra*I restriction sites are hypothesized on the basis of hybridization results with the *cob* gene (Cardi et al. 1999): D_1-D_2 = 6.5 kb; D_2-D_3 = 2.8 or 3.0 kb. P4 and P5 = pumD primer pair (see Table 1). For probe and P1 - P3 primer description see Materials and methods. *Arrows* are not drawn to scale and indicate the approximate position of the primers

Table 2 Amplification patterns of cpDNA in different *Solanum* and *Brassica* spp. genotypes.

Primer pairs[a]	Genotypes									
	Nt	*Solanum* spp.				*Brassica* spp.				
		cmm[b]	tbr	etb	phu	Bo	Br	M2	M4	M1C
pucA	1	2[c]	2	2	2	3	-[d]	3	2	2
pucG	1	1	1	1	1	1	1	1	1	1
pucH	1	1	1	1	1	2	2	2	2	2
pucB	1	2	2	1	2	1	1	3	1	3
pucM	1	1	1	1	1	2	2	2	2	2
pucC	1	1	1	1	1	2	2	2	2	2
pucD	1	2	2	2	2	2	–	–	–	–
pucX	1	1	1	1	1	1	1	1	1	1
pucI	1	1	1	1	1	2	3	2	3	3
pucE	1	2	2	2	2	2	2	2	2	2
pucO	1	1	1	1	1	2	2	2	2	2
pucJ	1	1	2	1	1	3	4	2	4	4
pucK	1	1	1	1	1	2	2	2	2	2
pucF	1	1	1	1	1	2	2	2	2	2
pucN	1	2	2	2	2	3	3	–	3	–
pucP	1	2	2	2	2	–	–	–	3	–

[a] Listed according to the position on the *N. tabacum* plastidial genome (Shinozaki et al. 1986). Primer codes as in Table 1

[b] Cmm = *S. commersonii* PI243503 (Cmm1), PI472833 (Cmm2) and PI472834 (Cmm3); tbr = *S. tuberosum* dihaploid clones SVP11 and Atl9; etb = *S. etuberosum* PI558054; phu = *S. phureja* PI584955; Bo = male-fertile *Brassica oleracea* CrGC#3–1; Br = male fertile *B. rapa* CrGC#1–1; M2 = 'Anand' CMS *B. rapa* CrGC#1–31; M4 = 'Chiang' CMS *B. oleracea* CrGC#3–10 ; M1 C = 'Ogura' CMS *B. oleracea* 90WC65

[c] For each primer pair, the same number indicates that the molecular weight was the same in the tested genotypes. The MWt found in *N. tabacum* is indicated by 1

[d] – = result not available

For the amplification of the *rpl5*/*rps14* genome region, P1 (5′-TAGGAGTTGGCGGTCTTC-3′) and P3 (5′-ACTTTTCGGTC CGGAGCC-3′) primers complementary to the upstream and downstream sequences of the *rpl5* and *rps14* genes, respectively, were used (Fig. 1). One hundred nanograms of total DNA from two SH somatic hybrids and their parental genotypes were amplified in 50-µl volumes of the same reaction buffer described for PCR with universal primers. Amplifications were carried out for 3 min at 94°C, 29 cycles of 45 s at 94°C / 45 s at 50°C / 1 min at 72°C, a final extension of 3 min at 72°C. The amplified DNAs were analyzed by agarose/EtBr gel-electrophoresis.

Southern analyses

Southern analyses were carried out with the two mitochondrial probes "a/b" or "c" (Fig. 1). The former, which was kindly provided by Dr. X. Jordana, P. Univ. Catolica de Chile, Santiago, Chile, included a 0.55-kb *Eco*RI fragment consisting of 140 nt 5′ region and most of the *rpl5* coding region, and a 0.65 kb *Eco*RI fragment consisting of 150 nt of the *rpl5* 3′ end, ψ*rps14*, and 200 nt of the 3′ region. The latter was obtained by amplification of the *rpl5* gene with primers P1 and P2 (5′-ACTGAGTTTCCCCCTCATCT-3′). Total DNA (2.5–3 µg) from a sample of SH somatic hybrids and parental species was digested with *Dra*I, electrophoresed for about 20 h in 1 × TAE agarose gel (0.8% w/v) at 1.2–1.4 V/cm, blotted onto a positively charged membrane under alkaline conditions (0.4 N NaOH), hybridized under high stringency conditions, and analyzed by a radioactive method after washing one to three times at 42°C (10 min each) with 0.1 × SSC, 0.1% SDS.

Results and Discussion

cpDNA

Out of the 21 cpDNA primer pairs tested, 16 gave clear and repeatable amplifications on different *Solanum* and *Brassica* spp. genotypes (Table 2). In *N. tabacum*, the

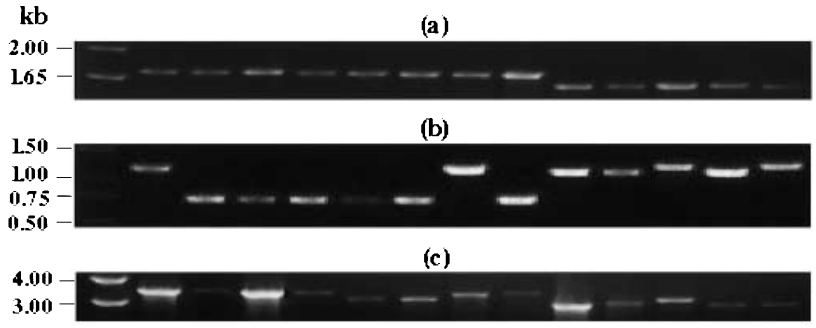

Fig. 2 Amplification of total DNA from different genotypes of the Solanaceae and Brassicaceae using the cpDNA primer pairs pucO (*a*), pucB (*b*) and pucJ (*c*). In all photos, from left to right: DNA size marker, Nt, cmm (1, 2, 3), tbr (SVP11, Atl9), etb, phu, Bo, Br, M2, M4, M1C. For primer pair and genotype codes see Tables 1 and 2, respectively

size of amplified fragments estimated by gel electrophoresis was generally close (±2%) to that determined by its complete sequence (Shinozaki et al. 1986). After amplification, the primer pairs pucG and pucX failed to detect any polymorphism among the tested genotypes. This is probably related to the fact that, in contrast with the other primers, they amplify intronic regions within the *trnK* and *psaA* genes, respectively (Shinozaki et al. 1986; Demesure et al. 1995; Tsumura et al. 1996). Six primer pairs (pucH, M, C, O, K and F) discriminated between the Solanaceae from Brassicaceae amplifying regions that were conserved within each family (Fig. 2a). Within the Solanaceae we could distinguish *Solanum* spp. from *N. tabacum* with five primer pairs (pucA, D, E, N and P). Only two primer pairs (pucB and J) revealed polymorphism within the *Solanum* genus. In particular, pucJ differentiated *S. tuberosum* (approximate fragment length 3.00 kb) from *S. commersonii*, *S. etuberosum* and *S. phureja* (3.30 kb); and pucB differentiated *S. etuberosum* (1.15 kb) from the other *Solanum* species (0.72 kb) (Fig. 2b and c). No differences were observed among *S. commersonii* accessions (Cmm1, Cmm2 and Cmm3) and between the two *S. tuberosum* genotypes SVP11 and Atl9 with any of the primer pairs tested.

Four primer pairs (pucA, B, I and J) revealed differences within the Brassicaceae and showed polymorphism between *B. oleracea* (Bo) and the other *Brassica* genotypes (Table 2). In particular, pucA differentiated Bo (approximate length 1.55 kb) from M4 and M1 C (1.80 kb); pucB revealed polymorphism between Bo (1.15 kb) and M2 and M1 C (1.21 kb); pucI discriminated Bo (3.00 kb) from Br, M4 and M1 C (3.05 kb); pucJ differentiated Bo (2.84 kb) from Br, M4 and M1 C (2.92 kb) and from M2 (3.00 kb) (Fig. 2b and c).

Based on amplification only, the primer pair pucJ for the intergenic region between the *trnF* and *trnV* genes showed polymorphism among several tested genotypes. Previous results based on Southern analysis with the probe pStB153 revealed a high degree of variability in the same region of the plastidial genome in *Solanum* (Perl et al. 1991; Cardi et al. 1999; Bastia et al. 2000) as well as *Brassica* spp. (Cardi and Earle 1997).

Restriction analysis of fragments amplified from different *Solanum* and *Brassica* spp. genotypes with six primer pairs for cpDNA revealed various polymorphisms, some of which were not detected by amplification alone (Table 3). The combinations pucX/*Hae*III, pucO/*Hha*I and *Hae*III, pucF/*Hae*III, and pucC/*Hha*I differentiated the Solanaceae from the Brassicaceae, whereas pucM/*Hinf*I and *Hae*III, pucI/*Hha*I, and pucC/*Hae*III detected differences between the *N. tabacum* and *Solanum* spp. genotypes. Within *Solanum* spp., pucM/*Hae*III, pucF/*Hha*I, and pucC/*Hae*III revealed polymorphisms between etb and the other genotypes tested (Fig. 3a). The combination pucX/*Hha*I could differentiate M2 from the other *Brassica* spp. genotypes (Fig. 3b).

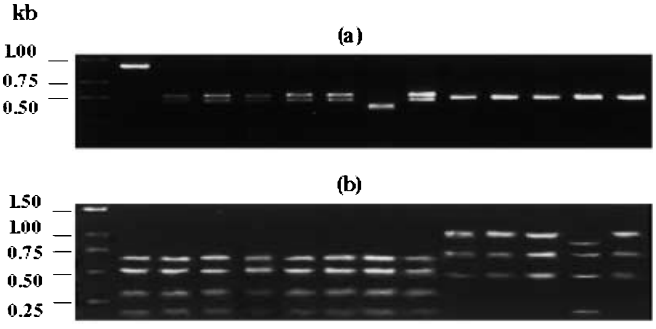

Fig. 3 Digestion patterns of fragments amplified from different genotypes. (*a*) Universal primer pair pucC with the restriction enzyme *Hae*III, from left to right: DNA size marker, Nt, cmm (1, 2, 3), tbr (SVP11, Atl9), etb, phu, Bo, Br, M2, M4, M1C; (*b*) pucX/*Hha*I, from left to right: DNA size marker, Nt, cmm (1, 2, 3), tbr (SVP11, Atl9), etb, phu, Bo, Br, M4, M2, M1C. For primer pair and genotype codes see Tables 1 and 2, respectively

Within the Solanaceae, we could more easily differentiate *S. tuberosum* from *S. etuberosum* than from *S. commersonii* and *S. phureja*. *S. phureja* is a cultivated diploid species in South America. Both *S. commersonii* and *S. etuberosum* are wild diploid species also originating from South America (Hawkes 1990). However, while it is known that the tuber-bearing species *S. commersonii* is slightly differentiated from *S. tuberosum* in both cytoplasmic and nuclear genomes, *S. etuberosum* is cytoplasmically very far from the tuber-bearing Solanums (Hosaka et al. 1984; Matsubayashi 1991; Perl et al. 1991; Bryan et al. 1999).

In comparison with the Solanaceae, cpDNA polymorphism was more commonly found within the Brassicaceae. With respect to *B. oleracea*, the most polymorphic genotypes were M1C ('Ogura' cytoplasm derived from *Raphanus sativus*), followed by M2 ('Anand' cytoplasm

Table 3 Analysis of restriction patterns of fragments amplified from different *Solanum* and *Brassica* spp. genotypes with six primer pairs for cpDNA[a]

Primer pairs	Enzyme	Genotypes									
		Nt	*Solanum* spp.				*Brassica* spp.				
			cmm	tbr	etb	phu	Bo	Br	M2	M4	M1C
pucM	*Hinf* I	1 [b]	2	2	2	2	3	3	3	3	3
	Hae III	1	2	2	3	2	4	4	4	4	4
pucX	*Hha* I	1	1	1	1	1	2	2	3	2	2
	Hae III	1	1	1	1	1	2	2	2	2	2
pucO[c]	*Hha* I	1	1	1	1	1	2	2	2	2	2
	Hae III	1	1	1	1	1	2	2	2	2	2
pucF	*Hha* I	1	1	1	2	1	3	3	3	3	3
	Hae III	1	1	1	1	1	2	2	2	2	2
pucI	*Hha* I	1	2	2	2	2	3	4	3	4	4
	Hae III	1	1	1	1	1	– [d]	–	–	–	–
pucC	*Hha* I	1	1	1	1	1	2	2	2	2	2
	Hae III	1 [c]	2	2	3	2	4	4	4	4	2

[a] For primer pair codes see Table 1. For genotype codes see Table 2
[b] For each primer pair / restriction enzyme combination, the same number indicates that the digestion patterns were the same in the tested genotypes. The restriction pattern found in *N. tabacum* is indicated by 1
[c] No restriction sites
[d] – = result not available

derived from *Brassica tournefortii*) and M4 ('Chiang' cytoplasm derived from *Brassica napus*). While it is known that the chloroplast DNAs of 'Ogura' and normal *R. sativus* are virtually identical (Makaroff and Palmer 1988), phylogenetic studies based on cpDNA RFLP analysis indicate that *B. tournefortii* is very distant from *B. oleracea* and is included in the 'Nigra' lineage, whereas *B. oleracea*, *R. sativus*, *B. napus* and *B. rapa* are included in the 'Rapa/Oleracea' lineage (Warwick and Black 1991).

Although restriction analysis of fragments amplified with cpDNA universal primers revealed intraspecific polymorphism in other species, such as common beech (Demesure et al. 1996) and white oaks (Dumolin-Lapègue et al. 1997a), based on our results the use of such primers seems to be more useful for determining the cytoplasmic genome composition of interspecific *Solanum* and *Brassica* spp. somatic hybrids. Accordingly, intraspecific polymorphism was not detectable in coffee and mangrovias (Orozco-Castillo et al. 1996; Parani et al. 2000). In order to analyze the intraspecific variability in organellar genomes, or to determine the cytoplasmic composition of intraspecific somatic hybrids, it would probably be useful to test other molecular markers, such as cpDNA simple sequence repeats (cpSSRs), which showed a high degree of intraspecific polymorphism in *S. tuberosum* (Bryan et al. 1999), or PCR-SSCPs (To et al. 1993; Dumolin-Lapègue et al. 1996).

The distribution of parental chloroplasts among SH and FPN4 *S. commersonii* (+) *S. tuberosum* somatic hybrids and BCy *B. oleracea* (+) 'Anand' CMS *B. rapa* cybrids was analyzed by amplification with the primer pair pucJ (Fig. 4). Most of the SH somatic hybrids showed the *S. commersonii* amplification pattern, where-

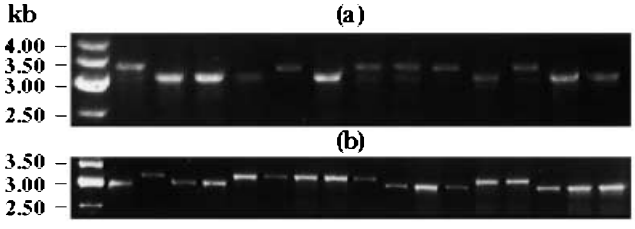

Fig. 4 Amplification of total DNA from SH *Solanum* spp. somatic hybrids (*a*) and from BCy *Brassica* spp. cybrids (*b*) with the universal primer pair pucJ. From left to right: (*a*) DNA size marker, Cmm1, SVP11, SH somatic hybrids; (*b*) DNA size marker, Bo, M2, BCy cybrids. For primer pair and genotype codes see Tables 1 and 2, respectively

as the FPN4 somatic hybrids and the *Brassica* cybrids showed a random segregation of parental chloroplasts (Table 4). These results are consistent with those obtained after Southern analysis of the same genotypes with the plastidial probe pStB153 (Cardi and Earle 1997; Cardi et al. 1999; Bastia et al. 2000).

mtDNA

As far as the mitochondrial genome is concerned, the primer pairs pumA, pumB, pumC and pumE (see Table 1) were tested on fusion parents of *Solanum* somatic hybrids and *Brassica* cybrids; however, we obtained clear and repeatable amplification results only on *Solanum* genotypes. The primer pair pumC revealed interspecific variation between *S. commersonii* and *S. tuberosum*, since two amplification fragments were evident in the former (about 1.06 kb and 1.22 kb) and one in

Table 4 *Solanum* and *Brassica* spp. somatic hybrids showing the indicated parental pattern after amplification with universal primers for cpDNA (primer pair pucJ) and mtDNA (primer pair pumD) [a]

Fusion combination	Code	Primer pair	Hybrids w/ parental pattern (no.)	
Solanum spp.			Cmm	Tbr
Cmm1 (+) SVP11	SH	pucJ	19	3
Cmm1 (+) SVP11	SH	pumD	11	21
Cmm3 (+) Atl9	FPN4	pucJ	5	6
Cmm3 (+) Atl9	FPN4	pumD	3	7
Brassica spp.			Bo	M2
Bo (+) M2	BCy	pucJ	8	7

[a] For primer pair codes see Table 1. For genotype codes see Table 2

the latter (1.06 kb) (data not shown). Amplification with pumA indicated intraspecific variation in *S. tuberosum*, since we found an amplification product of about 1.65 kb in Cmm1, Cmm3 and SVP11, and of about 1.52 kb in Atl9 (data not shown). No polymorphism among *Solanum* genotypes was found after amplification with pumB and pumE.

The primer pair pumD, which gave the most-reliable amplification results, was tested on different *Solanum* and *Brassica* spp. genotypes (Fig. 5a). It revealed polymorphism between the Solanaceae and the Brassicaceae as well as within the *Solanum* genus. An amplification product of about 1.46 kb was found in *S. tuberosum* genotypes (SVP11 and Atl9) and in *S. phureja*. A shorter fragment was found in *S. etuberosum* (1.0 kb), and no amplification was evident in *S. commersonii* accessions (Cmm1, Cmm2 and Cmm3), suggesting frequent rearrangements in the intergenic region between the *rps14* and *cob* genes. In particular, lack of amplification in *S. commersonii* suggests that the *rps14* and *cob* genes are not linked in this species. All *Brassica* genotypes showed a fragment of about 1.61 kb after amplification with the same primer pairs. We found polymorphism between M1C and the other *Brassica* genotypes only after *Hae*III-restriction of pumD amplification products. No polymorphisms were detected after digestion with five other restriction enzymes (*Afa*I, *Hha*I, *Hinf*I, *Hpa*II and *Taq*I).

After amplification with pumD, most of the SH (66%) and FPN4 (70%) *Solanum* somatic hybrids showed the *S. tuberosum* pattern; the remaining hybrids showed no amplification product, like the *S. commersonii* parent (Fig. 5b and Table 4). Segregation results largely confirmed those previously obtained by Southern analysis with the *cob* gene as a probe (Cardi et al. 1999). However, it is not possible to conclude whether the somatic hybrids not showing any amplification inherited the *S. commersonii* mitochondrial genome region or whether the lack of amplification in somatic hybrids was due to a new rearrangement of the *rps14-cob* locus. Out of 32 somatic hybrids analyzed, both by Southern analysis with the *cob* gene as a probe and by PCR with pumD, only two showed contrasting results in that they displayed the *S. tuberosum* pattern after Southern analysis (Cardi et al. 1999) and no amplification product after pumD amplification. This discrepancy could be due either to an artifact of PCR analysis or to a rearrangement in that region.

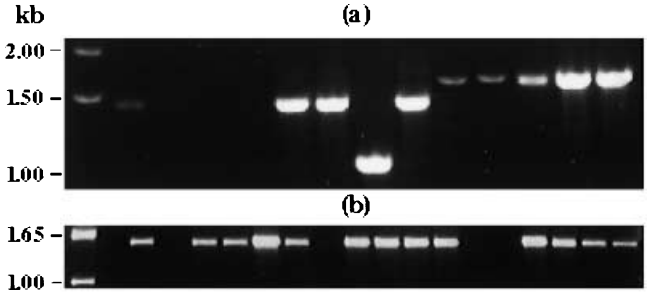

Fig. 5 Amplification of total DNA from different genotypes of the Solanaceae and Brassicaceae (*a*), and SH *Solanum* spp. somatic hybrids (*b*) with the primer pair pumD for mtDNA. From left to right: (*a*) DNA size marker Nt, cmm (1, 2, 3), tbr (SVP11, Atl9), etb, phu, Bo, Br, M2, M4, M1C; (*b*) DNA size marker, Cmm1, SVP11, SH somatic hybrids. For primer pair and genotype codes see Tables 1 and 2, respectively

Organization of *rpl5-rps14-cob* region in *S. commersonii*, *S. tuberosum* and interspecific somatic hybrids

The *rpl5-rps14-cob* gene cluster arrangement is conserved in several plant mitochondrial genomes, including those of pea, *B. napus*, *Arabidopsis thaliana* and *S. tuberosum* (Aubert et al. 1992; Brandt et al. 1993; Ye et al. 1993; Quiñones et al. 1996; Hoffmann et al. 1999). In the latter two species *rps14* is a pseudogene. In *Vicia faba* and *Oenothera*, the *rpl5* coding region upstream of *rps14* is either absent or only partially present (Wahleithner and Wolstenholme 1988; Schuster 1993). Our results from amplification with the pumD primer pair indicate that the linkage between *rps14* and *cob* is maintained in all the *Brassica*/*Raphanus* cytoplasms investigated as well as in the primitive cultivated species *S. phureja* and in the wild species *S. etuberosum*, although in the latter the shorter fragment obtained by PCR suggests a deletion in the intergenic region. On the other hand, no amplification in *S. commersonii* suggests a lack of linkage between the two genes in this species.

Southern analysis with either ''a/b'' or ''c'' probes detected a 6.5-kb *Dra*I fragment in *S. tuberosum*, and a 16.3-kb fragment in *S. commersonii* (a 12.2-kb weak signal, probably due to similar DNA region present in substoichiometric amounts, was also found in the wild

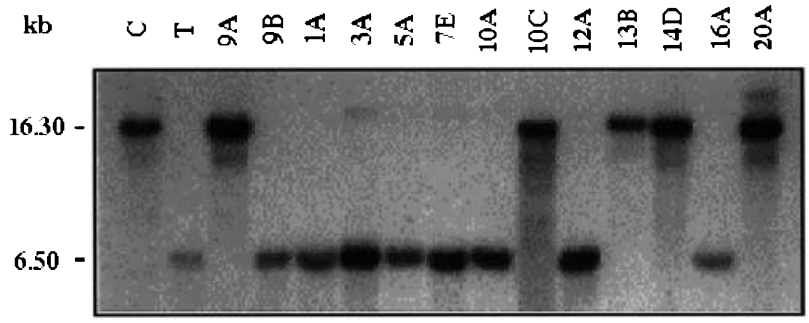

Fig. 6 Hybridization patterns in parental genotypes and a sample of SH *Solanum* spp. somatic hybrids with the mitochondrial probe ''c'' (= *S. tuberosum rpl5* gene). From left to right: *C* = Cmm1, *T* = SVP11, *9 A – 20 A* = somatic hybrids

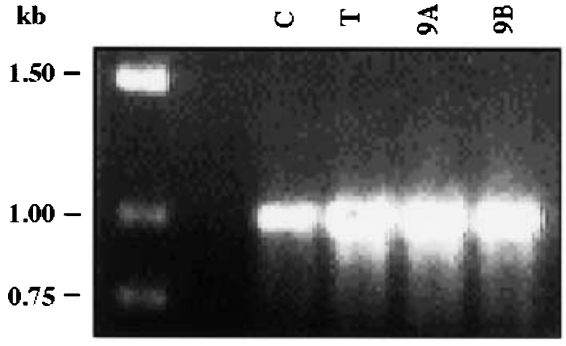

Fig. 7 Amplification products of the *rpl5-rps14* mitochondrial region with P1 and P3 primers (see Materials and methods) from Cmm1, SVP11, and two SH *Solanum* spp. somatic hybrids

species) (Fig. 6). Since in previous Southern experiments with the *cob* gene as a probe the same 6.5-kb fragment was found in tbr, but not in the wild species (Cardi et al. 1999), the hybridization results corroborate the hypothesis of lack of linkage between *rpl5/rps14* and *cob* in *S. commersonii*. On the other hand, results of PCR analysis with primers complementary to the 5′ *rpl5* and 3′ *rps14* coding regions gave an amplification product of about 1 kb in *S. tuberosum* as well as in *S. commersonii* and two somatic hybrids (Fig. 7), confirming that the two genes are linked in both species.

Only 2 out of 18 somatic hybrids analyzed both by Southern and PCR analyses did not indicate the same origin of the parental *rpl5*, *rps14* and *cob* genes. However, only in one of them (SH10C) the lack of amplification with the pumD primer pair probably depends on a rearrangement between the two genes, since the hybridization results made it clear that the hybrid inherited *rpl5* from tbr and *cob* from cmm. In the other hybrid (SH1 A), Southern analyses with *cob* and *rpl5* indicated that both genes were derived from tbr (Cardi et al. 1999). Hence, the null PCR result was probably due either to a minor rearrangement or mutation in the *rps14/cob* genes, or to an artifact of PCR. Rearrangements in this genome region were previously reported in intraspecific potato somatic hybrids (Lössl et al. 1999).

Our results showed that amplification of intergenic / intronic regions with universal primers for cpDNA and mtDNA, followed by electrophoresis of either entire or restricted amplified fragments, can be a useful method to determine the organelle genome composition of interspecific *Solanum* and *Brassica* somatic hybrids. It is simpler, more rapid and less expensive compared to the direct analysis of restricted organellar DNA as well as the use of radioactive or non-radioactive labelled probes. In addition, since total DNA can be used, it does not require any laborious cpDNA or mtDNA isolation. Finally, because of the small amount of total DNA required, it can be applied to in vitro plantlets allowing a very early screening of the cytoplasmic composition of somatic hybrids.

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