

Organelle Genome Analysis in Somatic Hybrids Between *Solanum tuberosum* and *S. pinnatisectum* Revealed Diverse Cytoplasm Type in Potato

Jagesh Kumar Tiwari¹ · Sapna Devi¹ · Poonam Chandel¹ · Nilofer Ali¹ ·
Vinay Bhardwaj¹ · Bir Pal Singh¹

Received: 5 June 2015 / Accepted: 28 November 2015 / Published online: 6 January 2016
© NAAS (National Academy of Agricultural Sciences) 2016

Abstract Organelle genome diversity was analysed in interspecific potato somatic hybrids using chloroplast (cp)- and mitochondrial (mt)-specific molecular markers. Out of total 25 markers (15 cpDNA and 10 mtDNA) tested in total 16 samples, only four mtDNA primers (rpS14/cob, Nsm2, ALM4/ALM5 and ALM6/ALM7) detected polymorphism, whereas other primers were monomorphic. Cluster analysis showed higher genetic diversity among the genotypes by mtDNA profiles than that by cpDNA. Ten haplotypes were grouped by cluster analysis comprised of maximum seven genotypes in haplotype no. 3. Monomorphic markers did not reveal variability in our samples and suggest highly conserved organelle genomic regions. New genomic arrangements were observed in the somatic hybrids for mt polymorphic loci. Our study suggests that somatic hybrids are comprised of diverse cytoplasm types consisting predominantly of T-, W-, and C-, with a few A- and S-type chloroplast, and α -, β - and γ -type mitochondrial genome, and have unique potential to widen the cultivated potato gene pool by breeding methods.

Keywords Cytoplasmic type · cpDNA · mtDNA · Potato · Somatic hybrids · Monomorphic markers · Polymorphism · Haplotypes

Introduction

Somatic hybridization has been used extensively for inter-specific gene transfer across the sexual barriers in potato [14, 17, 18]. In contrast to sexual crosses, somatic (protoplast) fusion can induce genetic variability within cytoplasmic organelle genomes [chloroplast (cp) and mitochondrial (mt) DNA] and novel cytoplasmic–nuclear interactions [10]. Considering the importance of organelle genomes, their analysis is immensely useful for introgression of desirable agronomic and/or stress (biotic and abiotic) response traits. In sexual crosses, organelle genomes are normally maternally inherited and exhibit very low mutation rates as compared to nuclear genome [4]. In potato, five basic cpDNA

types (A, C, S, T and W) and mtDNA types (α , β , γ , δ and ϵ) and thus five main cytoplasm types (cp/mt), i.e. W/ α , T/ β , W/ γ , W/ δ and S/ ϵ , have been identified [10]. Importantly, the T-type chloroplast coexists with the β -type mitochondrial genome, while mt types α , γ and δ were found in combination with W-type chloroplast in wild species; and mt type ϵ was found in A- and S-types in cultivated species [11]. In general, most cultivated Chilean potato (*Solanum tuberosum* ssp. *tuberosum*) has T-type chloroplast and β -type mitochondrial genomes (hence referred to T/ β cytoplasm), which is lacking in Andean potato (*S. tuberosum* ssp. *andigena*) and wild species. Moreover, the T/ β cytoplasm is predominant in the common potato, so sterility problem is unavoidable when T/ β cytoplasm is present [11].

The wild *Solanum* species represent a reservoir of genes for genetic improvement and have been used for both sexual and somatic hybridization. For example, *S. tuberosum* ssp. *andigena* has wide diversity, and *tuberosum* (female) \times *andigena* (male) cross results in increased heterotic vigour and yield but high male sterility, while reciprocal cross is male fertile

✉ Jagesh Kumar Tiwari
jageshtiwari@gmail.com

¹ Central Potato Research Institute, Shimla, Himachal Pradesh
171 001, India

indicating an important role of cytoplasm in potato breeding [6]. Previous researchers have investigated the recombinant cytoplasmic genome types in the cultivated gene pool by introgression from wild species through somatic hybridization and highlighted their importance for better characterization of genetic resources and utilization in potato breeding. Cytoplasm types have been analysed earlier by cpDNA and/or mtDNA markers in potato somatic hybrids, for example, *S. tuberosum* (+) *S. etuberosum* [17], *S. bulbocastanum* (+) *S. tuberosum* [2, 9] and *Solanum* spp. [1, 13, 15].

The aim of this study was to analyse organelle genome diversity using chloroplast and mitochondrial genome-specific primers in our previously developed interspecific potato somatic hybrids. These somatic hybrids were produced between the dihaploid of cultivated *Solanum tuberosum* ‘C-13’ and the diploid wild species *S.*

pinnatisectum via protoplast fusion for late blight resistance to widen the cultivated potato gene pool.

Materials and Methods

Plant Materials

In the present study, 14 interspecific potato somatic hybrids (P1, P2, P3, P4, P5, P6, P7, P8, P9, P10, P11, P12, P13 and P14) and two parents (*S. tuberosum* dihaploid parent ‘C-13’ and diploid wild species *S. pinnatisectum*) developed earlier [14] were used. The late blight-resistant potato somatic hybrids were developed at the Cell and Molecular Biology Laboratory, Division of Crop Improvement, Central Potato Research Institute, Shimla, India. In vitro

Table 1 Polymorphism detected by mt- and cpDNA markers in potato somatic hybrids

SN	Marker	No. of scorable fragments	No. of polymorphic fragments	Detected alleles (bp) and absolute frequencies (in brackets)	PIC ^a
<i>cpDNA marker</i>					
1	H1	1	0	455 (16)	0
2	H2	1	0	335 (16)	0
3	H3	1	0	900 (16)	0
4	NTCP3	2	0	199 (16), 200 (16)	0
5	NTCP4	1	0	162 (16)	0
6	NTCP6	3	0	173 (16), 174 (16), 175 (16)	0
7	NTCP7	2	0	173 (16), 174 (16)	0
8	NTCP8	2	0	252 (16), 253 (16)	0
9	NTCP9	3	0	279 (16), 288 (16), 289 (16)	0
10	NTCP10	2	0	120 (16), 121 (16)	0
11	NTCP12	2	0	124 (16), 125 (16)	0
12	NTCP14	2	0	150 (16), 151 (16)	0
13	NTCP18	2	0	186 (16), 188 (16)	0
14	NTCP39	2	0	155 (16), 156 (16)	0
15	ALC1/ALC3	2	0	622 (16)	0
	Total	28	0		
<i>mtDNA marker</i>					
16	nad1B/1C	1	0	1800 (16)	0
17	rpS14/cob	5	5	700 (13), 600 (3), 500 (3), 425 (2), 300 (3)	0.65
18	A14-1	2	0	146 (16), 150 (16)	0
19	T11-2	1	0	148 (16), 149 (16), 150 (16), 151 (16)	0
20	T12-3	1	0	188 (16), 195 (16), 198 (16), 200 (16)	0
21	T11-1	1	0	163 (16), 167 (16), 171 (16), 180 (16)	0
22	NSm2	4	4	3000 (15), 1530 (14), 1000 (15), 850 (13)	0.93
23	ALM1/ALM3	1	0	1200 (16)	0
24	ALM4/ALM5	3	3	2400 (15), 1600 (14), 480 (14)	0.67
25	ALM6/ALM7	5	4	2400 (16), 1200 (15), 480 (15), 400 (14), 280 (15)	0.80
	Total	24	16		

^a Polymorphic information content

propagated plants were used for plastome and chondriome genome analyses.

DNA Analysis

Leaf samples from in vitro propagated plants were used for genomic DNA isolation using DNeasy Plant DNA extraction kit (Qiagen). DNA quality and quantity were determined with NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). Genomic DNA was used in PCR amplification using cpDNA- and mtDNA-specific primer pairs, amplified genomic regions at annealing temperature (T_a) as detailed in Table 3. The polymerase chain reaction (PCR) was performed in a Mastercycler Gradient (Eppendorf, Hamburg, Germany) in a total volume of 25 μ l and consisted of 50 ng DNA templates in 1 \times PCR buffer, 2.5 mM $MgCl_2$, 200 μ M dNTP, 0.5 μ M of primer, 1 unit Taq polymerase (Qiagen). The PCR procedure included: 5 min at 94 °C followed by 33 cycles of 1 min at 94 °C, 1 min at T_a and 1 min at 72 °C, with a final extension of 7 min at 72 °C. Gel electrophoresis and documentation were followed as described in Tiwari et al. [17]. SSR type cp- and mtDNA primers were analysed on ‘3500 Genetic Analyzer’ (Applied Biosystems, California, USA). Fragment analysis of SSR data was performed using GeneMapper® Software version 4.1 (ABI). A 500-bp ‘GS 500 ROX’ standard was used to estimate the molecular size of the fragments.

Data Analysis

All reactions were repeated at least twice, and only distinct, reproducible and well-resolved bands across the run were considered for analysis. A data matrix was constructed on the basis of the presence (1) or absence (0) of bands of the amplified DNA fragments. Missing data were scored as ‘9’. In the SSR fragment analyses, higher peak intensity (≥ 1000) and band size (≥ 100 bp) were considered for analysis, which were scorable. Number of alleles, allele size, frequencies and polymorphic information content (PIC) of each marker were calculated for 16 samples. The PIC of each SSR marker was calculated according to the formula: $PIC = 1 - \sum(P_i^2)$, where P_i is the frequency of the i th allele of a marker detected in all accessions. Genetic diversity analysis was performed with the program NTSYS-PC 2.21 [12]. A similarity matrix was calculated by Jaccard’s similarity coefficient, and the dendrogram was generated using unweighted pair group method with arithmetic mean (UPGMA) clustering method.

Results

In the present study, organelle genome diversity was analysed in potato somatic hybrids using cpDNA and mtDNA-specific markers. Detected polymorphism, number of alleles, allelic absolute frequencies and PIC values of all 25 markers amplified in total 16 samples are presented in Table 1. Fifteen cpDNA markers resulted into one to two

Table 2 Amplification product (size in bp) for polymorphic mitochondrial DNA regions in potato somatic hybrids

Genotypes	rpS14/cob	NSm2	ALM4/ALM5	ALM6/ALM7	Cytoplasm type ^a
C-13	700	3000, 850	2400	2400, 400	W/ α , W/ γ
<i>S. pinnatisectum</i>	425, 700	3000, 1530, 1000	1600, 480	2400, 1200, 480, 280	W/ α , W/ γ , T/ β
P1	300, 500, 600	1530, 1000, 850	2400, 1600, 480	2400, 1200, 480, 400, 280	W/ α , W/ γ , T/ β
P2	700	3000, 1000, 850	2400, 1600, 480	2400, 1200, 480, 400, 280	W/ α , W/ γ , T/ β
P3	700	3000, 1530, 1000, 850	2400, 1600, 480	2400, 1200, 480, 400, 280	W/ α , W/ γ , T/ β
P4	300, 500, 600	3000, 1530, 1000, 850	2400, 1600, 480	2400, 1200, 480, 400, 280	W/ α , W/ γ , T/ β
P5	700	3000, 1530, 1000, 850	2400, 1600, 480	2400, 1200, 480, 400, 280	W/ α , W/ γ , T/ β
P6	700	3000, 1530, 1000, 850	2400, 1600, 480	2400, 1200, 480, 400, 280	W/ α , W/ γ , T/ β
P7	700	3000, 1530, 1000, 850	2400, 1600, 480	2400, 1200, 480, 400, 280	W/ α , W/ γ , T/ β
P8	700	3000, 1530, 1000, 850	2400, 1600, 480	2400, 1200, 480, 400, 280	W/ α , W/ γ , T/ β
P9	700	3000, 1530, 1000, 850	2400, 1600, 480	2400, 1200, 480, 400, 280	W/ α , W/ γ , T/ β
P10	700	3000, 1530, 1000, 850	2400	2400, 1200, 480, 400, 280	W/ α , W/ γ
P11	700	3000, 1530, 1000, 850	2400, 1600, 480	2400, 1200, 480, 400, 280	W/ α , W/ γ , T/ β
P12	300, 500, 600	3000, 1530, 1000, 850	2400, 1600, 480	2400, 1200, 480, 280	W/ α , W/ γ , T/ β
P13	700	3000, 1530, 1000, 850	2400, 1600, 480	2400, 1200, 480, 400, 280	W/ α , W/ γ , T/ β
P14	425, 700	3000, 1530, 1000, 850	2400, 1600, 480	2400, 1200, 480, 400	W/ α , W/ γ , T/ β

^a Cytoplasm types are based on the mt- and cpDNA markers primers, namely ALC1/ALC3 (622 bp), ALM1/ALM3 (1200 bp), ALM4/ALM5 (1600 and 2400 bp) and ALM6/ALM7 (2400 bp) which indicated W/ α -, W/ γ - and T/ β -type cytoplasm

Table 3 Primer pairs used to detect chloroplast (cp) and mitochondrial (mt) DNA polymorphisms in potato somatic hybrids

SN	Marker	Sequence (5' → 3')	T_a (°C)	Genomic region	Type	References
<i>cpDNA marker</i>						
1	H1	F: GGAGGGGTTTTCTTGGTTG R: AAGTTTACTCACGGCAATCG	55	<i>ndh1C/trn</i>	SCAR	Hosaka [7]
2	H2	F: GCATCGAGCGTGTGTTGGA R: AGTCCACCGCGAAGACATTC	55	<i>rbcL</i>	CAPS (<i>Hae</i> III)	Hosaka [7]
3	H3	F: CAGGGGTCCATTCCCTTGAC R: AGAAAGAAATCCACCAGGGC	60	<i>ycf4</i> and <i>ycf10</i>	CAPS (<i>Dra</i> I)	Hosaka [7]
4	NTCP3	F: AAGTCAAAAGAGCGATTAG R: TGATACATAGTGCATACAG	50	<i>trnK</i>	cpSSR	Bryan et al. [3]
5	NTCP4	F: TTGGATTAGATTTGTAGTTCCA R: ATCCACTTCATTTATCACAATG	55	<i>trnK/rps16</i>	cpSSR	Bryan et al. [3]
6	NTCP6	F: GGTTCGAATCCTTCCGTC R: GATTCTTTCGCATCTCGATTTC	60	<i>rps16/trnQ</i>	cpSSR	Bryan et al. [3]
7	NTCP7	F: TGATCCCGACGTAATCC R: CGAATCCCCTCTCTTCCG	60	<i>ORF98/trnS</i>	cpSSR	Bryan et al. [3]
8	NTCP8	F: ATATTGTTTTAGCTCGGTGG R: TCATTTCGGCTCCTTTATG	55	<i>trnG</i>	cpSSR	Bryan et al. [3]
9	NTCP9	F: CTTCCA AGCTAACGATGC R: CTGTCCTATCCATTAGACAATG	55	<i>trnG/trnR</i>	cpSSR	Bryan et al. [3]
10	NTCP10	F: TGCTGAATCGACGACCTA R: AATATTTCGGAGGACTCTTCTG	55	<i>atpF</i>	cpSSR	Bryan et al. [3]
11	NTCP12	F: CCTCCATCATCTCTTCCAA R: ATTTATTTTCAGTTCAGGGTTCC	60	<i>rps2/RF862</i>	cpSSR	Bryan et al. [3]
12	NTCP14	F: AATCCGTAGCCAGAAAAATAAA R: CCGATGCATGTAATGGAATC	60	<i>psbM/trnD</i>	cpSSR	Bryan et al. [3]
13	NTCP18	F: CTGTTCTTTCCATGACCCCTC R: CCACCTAGCCAAGCCAGA	60	<i>psbC/trnS</i>	cpSSR	Bryan et al. [3]
14	NTCP39	F: GTCACAATGGGGTTTTGAATA R: GACGATACTGTAGGGGAGGTC	60	<i>trnR/rnr5</i>	cpSSR	Bryan et al. [3]
15	ALC1/ALC3	F: TAGAATCAGGAGGTCTT R: TTAICTCACGGCAATC	44	<i>atpE</i>	W/ α + W/ γ type = 622 bp T/ β type = 381 bp	Lössl et al. [11]
<i>mtDNA markers</i>						
16	nad1B/1C	F: GCATTACGATCTGCAGCTCA R: GGAGCTCGATTAGTTTCTGC	55	<i>nad1B/1C</i>	SCAR	Hosaka and Sanetomo [8]
17	rpS14/cob	F: CACGGGTCGCCCTCGTTCCG R: GTGTGGAGGATATAGGTTGT	57	<i>rpS14-cob</i>	CAPS (<i>Mse</i> I)	Hosaka and Sanetomo [8]
18	A14-1	F: TGGGGGTCTTCATCACTCT R: GAAGGGCTTATCGCTTCTCC	55	90062–90214*	mtSSR	Hosaka and Sanetomo [8]
19	T11-2	F: TCTGCTTTTTCCTGGATGCT R: ATTTCCGACCTCTTGCAATG	55	268587–268737*	mtSSR	Hosaka and Sanetomo [8]
20	T12-3	F: TGGATTGAATCTTCCGCTTT R: CCCATCAATCATCCAACACA	55	140566–140760*	mtSSR	Hosaka and Sanetomo [8]
21	T11-1	F: GTGGGATCAACTGCTCGTTT R: GATTTATGAAAAGCCCGGTGA	55	179805–179954*	mtSSR	Hosaka and Sanetomo [8]
22	NSm2	F: CACGGGTCGCCCTCGTTCCG R: TTTTCCCTTATCCGAACGGGTCTT	55	<i>rps14-rps10</i> exonII	SCAR	Scotti et al. [15]
23	ALM1/ALM3	F: CACAAATCCATCTTTGTTTATGC R: GCGTTGGCTTACAGCGAACTAG	57	<i>atp6</i>	W/ α + W/ γ types = 1.2 kb	Lössl et al. [11]

Table 3 continued

SN	Marker	Sequence (5' → 3')	T_a (°C)	Genomic region	Type	References
24	ALM4/ALM5	F: AATAATCTTCCAAGCGGAGAG R: AAGACTCGTGATTCAGGCAAT	57	<i>cob</i> , <i>rps10</i>	W/ α type = 2.4 kb, T/ β type = 1.6 kb	Lössl et al. [11]
25	ALM6/ALM7	F: ATTTAGGCCCGGCTAGCAACA R: AACCCAGTCCCTATGGTATCTCTCT	57	<i>cob</i>	W/ γ type = 2.4 kb	Lössl et al. [11]

* Location in tobacco (EMBL sequence accession no. BA000042)

Table 4 Cytoplasm (chloroplast and mitochondrial) types based on the PCR products of markers

Marker	PCR product (size in bp)										References
	Chloroplast (cp) type					Mitochondrial (mt) type					
	W	C	A	S	T	α	β	γ	δ	ϵ	
NTCP6	172, 173, 175	173, 174, 175	174	127	173	–	–	–	–	–	Sukhotu et al. [16]
NTCP7	173, 174	173, 174	174	127	173	–	–	–	–	–	Sukhotu et al. [16]
NTCP8	252, 253	249, 250, 251	250	251	252	–	–	–	–	–	Sukhotu et al. [16]
NTCP9	247, 279	288, 289, 317	289	289	279	–	–	–	–	–	Sukhotu et al. [16]
NTCP12	234, 235, 236	236, 237	237	239	235	–	–	–	–	–	Sukhotu et al. [16]
NTCP14	149, 150, 151, 152, 153	150, 151, 152	151	150	149	–	–	–	–	–	Sukhotu et al. [16]
NTCP18	186, 187, 188	186, 196	186	186	188	–	–	–	–	–	Sukhotu et al. [16]
ALC1/ALC3	622	–	–	–	381	622	381	622	–	–	Lössl et al. [11]
ALM1/ ALM3	1200	–	–	–	–	1200	–	1200	–	–	Lössl et al. [11]
ALM4/ ALM5	2400	–	–	–	1600	2400	1600	–	–	–	Lössl et al. [11]
ALM6/ ALM7	2400	–	–	–	–	–	–	2400	–	–	Lössl et al. [11]

scorable bands per primer and generated total of 28 monomorphic bands ranging from 120 to 900 bp, whereas 10 mtDNA markers amplified one to five scorable bands per primer in the samples with total of 24 bands ranging from 146 to 3000 bp, from which 16 mtDNA bands were polymorphic (Tables 2, 3). The PIC value for markers *rpS14/cob*, *Nsm2*, *ALM4/ALM5* and *ALM6/ALM7* was 0.65, 0.93, 0.67 and 0.80, respectively, whereas other markers were monomorphic. Based on the classification of cytoplasm types as given in Table 4, somatic hybrids showed five chloroplast types (A, C, S, T and W) and three mitochondrial types (α , β and γ). In particular, amplification patterns of the *ALC1/ALC3*, *ALM1/ALM3*, *ALM4/ALM5* and *ALM6/ALM7* markers showed mixed cytoplasm types (W/ α , W/ γ and T/ β) in the somatic hybrids. Monomorphic loci indicated the presence of conserved organelle genomic regions in the hybrids. To illustrate, organelle genome profiles of the hybrids using selected polymorphic mtDNA markers *ALM4/ALM5* and *ALM6/ALM7* are shown in Fig. 1. Moreover, amplification product of SSR type mtDNA primers A14-1 and T11-2 in the

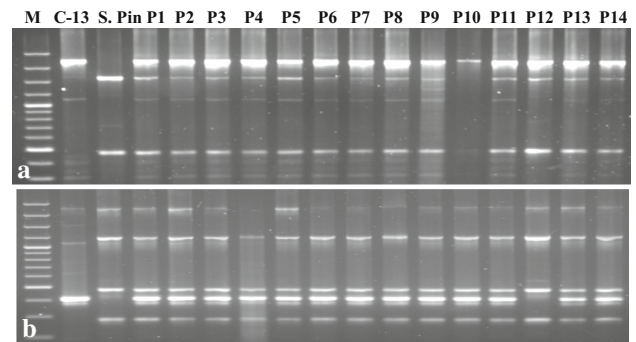


Fig. 1 Organelle genome amplification profiles of somatic hybrids using mtDNA markers **a** *ALM4/ALM5* and **b** *ALM6/ALM7*; *M* = 100-bp ladder; parents (C-13, and *S. pin* = *S. pinnatisectum*); and somatic hybrids (P1–P14)

hybrids, for example P1, analysed on the ‘3500 Genetic Analyser’ (ABI) is shown in Fig. 2.

Cluster analysis based on the Jaccard’s similarity coefficient values of cp- and mtDNA primers amplified in the samples could be grouped into 10 different haplotypes (Fig. 3). Each haplotype comprising genotype(s) was

Fig. 2 mtDNA fragments profile of somatic hybrids using SSR type mtDNA primers **a** T11-2 and **b** A14-1 analysed using '3500 Genetic Analyzer' (ABI)

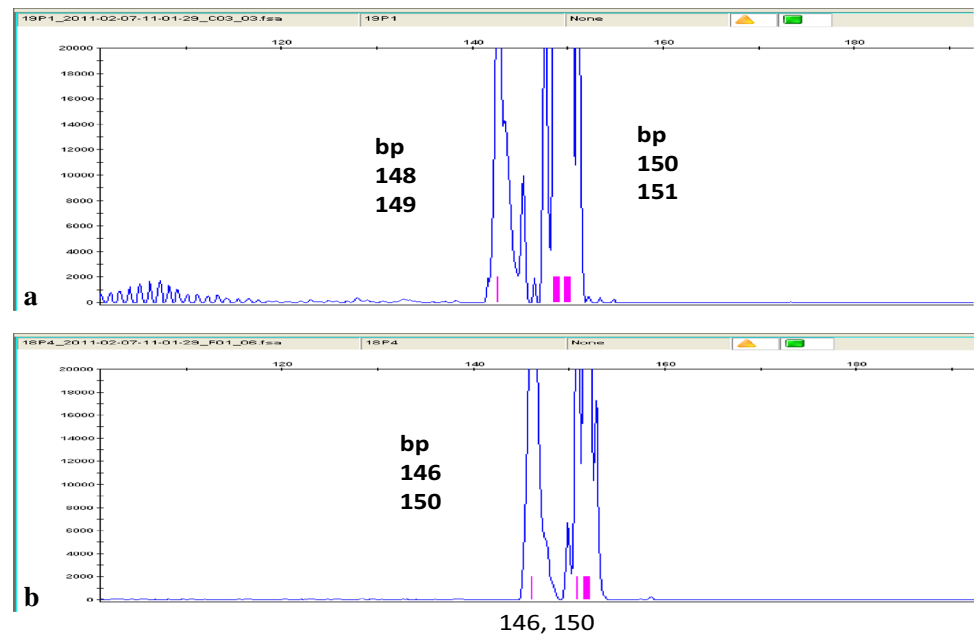
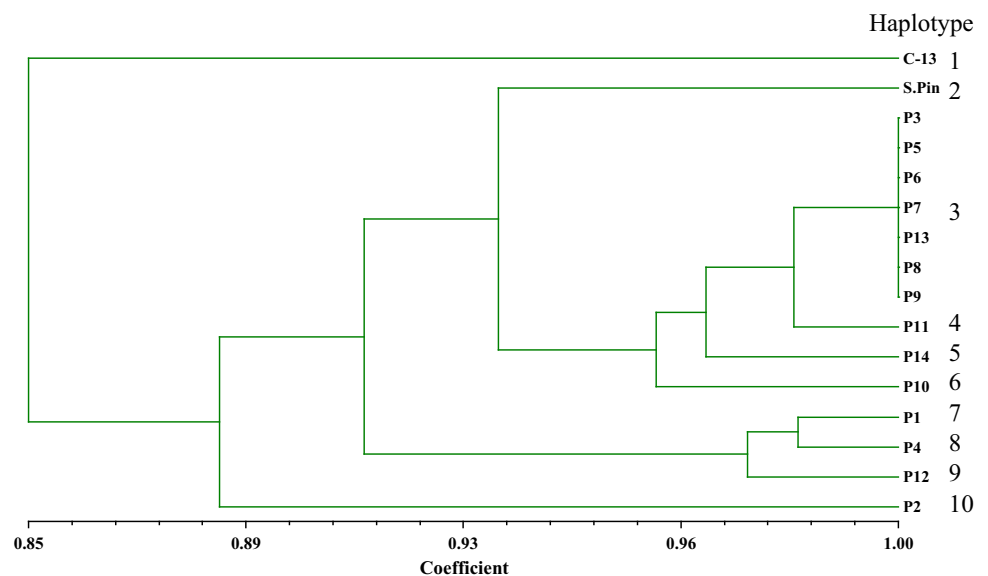


Fig. 3 Cluster analysis based on the Jaccard's similarity coefficient of cp- and mtDNA markers shows diversity among the somatic hybrids



characterized by similar amplification patterns in which only one haplotype no. 3 was comprised of seven genotypes (P3, P5, P6, P7, P13, P8 and P9) and other haplotypes had single genotype. Analysis revealed that among the haplotype no. 3 included genotypes comprised of common genomic regions, viz. *rps14/cob*, NSm2 (*rps14-rps10*), ALM4/ALM5 (*cob* and *rps10*) and ALM6/ALM7 (*cob*) (Table 2).

Discussion

Variations among the chloroplast and mitochondrial genomic regions using organelle-specific primers (cpDNA and mtDNA) were detected among the somatic hybrids.

Out of total 25 markers (15 cpDNA and 10 mtDNA), only mtDNA primers detected the polymorphism in the hybrids. Based on the classification of cytoplasm types using mitochondrial and chloroplast types, somatic hybrids had five chloroplast types (A, C, S, T and W), three mitochondrial types (α , β and γ) and overall mixed cytoplasm types (W/ α , W/ γ and T/ β). Our amplification patterns of cpDNA and mtDNA markers matched with the earlier reports [3, 7, 8, 15, 17]. In many plant species, a high genetic diversity in mtDNA profiles has been known to that in cpDNA, which is explained by slow evolution and low mutation rates of mtDNA [8]. The difference between cytoplasmic genomes variability depends on their various mechanisms of molecular evolution, coding and non-

coding regions, and patterns of transmission to progenies. The known mtDNA and cpDNA markers were characterized earlier in wild and cultivated potatoes [15, 17]. The genomic region analysed was previously found to be involved in the rearrangement of the potato plastome and chondriome in somatic hybrids [9, 10].

Past studies highlighted the narrow genetic base of modern Indian potato cultivars predominantly T/ β type with a few W/ α and A/ ϵ cytoplasm types [5], and also German varieties had mt types β , α and γ [11]. Breeding constraints imposed by the pollen sterility and the T/ β -type cytoplasm in potato may suggest the need for a change in breeding practices involving more diverse material, either from within the cultivated gene pool or from wild species [10]. Therefore, researchers have highlighted the importance of cytoplasmic diversity and stressed the need to increase levels of cytoplasmic variability found in the modern potato gene pool from wild sources. Present study shows that somatic hybrids possess diverse cytoplasm predominantly of T/ β , W/ α and W/ γ types. In another study, Hosaka and Sanetomo [8] showed phylogenetic relationship of the cultivated potatoes and closely related wild *Solanum* species using cpDNA and mtDNA markers. Further, Scotti et al. [15] studied evolutionary patterns by mitochondrial DNA variation in cultivated and wild potato species and highlighted importance of mtDNA for better characterization of potato genetic resources. Organelle genomes diversity analysis in our potato somatic hybrids revealed divergence among the genotypes. Somatic hybrids derived from wild and cultivated potato species have potential to widen the cultivated gene pool by using as parental lines in breeding. Germplasm analysis with more polymorphic markers, high-throughput fragment analysis and genome sequencing can allow a more efficient selection of cytoplasmic donors and the characterization of hybrids providing a novel useful tool to enlarge the gene pool of cultivated potato.

Acknowledgments The authors are grateful to the ICAR-Central Potato Research Institute, Shimla, and the DBT, Government of India (BT/PR4291/AGR/2/838/2011), for providing financial supports and necessary facilities. Heartly thanks are extended to Mr. Seeshram Thakur for in vitro maintenance of somatic hybrids plants.

Compliance with Ethical Standards

Conflict of interest None.

Authors contribution J.K. Tiwari, S. Devi, P. Chandel and N. Ali have performed the original research work. J.K. Tiwari has planned the work, analysed the data and prepared the manuscript. V. Bhardwaj and B.P. Singh have contributed in language correction of the manuscript.

References

- Ames M, Spooner DM (2008) DNA from herbarium specimens settles a controversy about origins of the European potato. *Am J Bot* 95:252–257
- Aversano R, Savarese S, De Nova JM, Frusciant L, Punzo M, Carputo D (2009) Genetic stability at nuclear and plastid DNA level in regenerated plants of *Solanum* species and hybrids. *Euphytica* 165:353–361
- Bryan GJ, Mcnicoll J, Ramsay G, Meyer RC, De Jong WS (1999) Polymorphic simple sequence repeat markers in chloroplast genomes of Solanaceous plants. *Theor Appl Genet* 99:859–867
- Cardi T, Bastia T, Monti L, Earle ED (1999) Organelle DNA and male fertility variation in *Solanum* spp. and interspecific somatic hybrids. *Theor Appl Genet* 99:819–828
- Chimote VP, Chakrabarti SK, Pattanayak D, Pandey SK, Naik PS (2008) Molecular analysis of cytoplasm type in Indian potato varieties. *Euphytica* 162:69–80
- Grun P (1990) The evolution of cultivated potatoes. *Econ Bot* 44(suppl):39–55
- Hosaka K (2003) T-type chloroplast DNA in *Solanum tuberosum* L. ssp. *tuberosum* was conferred from some populations of *S. tarijense* Hawkes. *Am J Potato Res* 80:21–32
- Hosaka K, Sanetomo R (2009) Comparative differentiation in mitochondrial and chloroplast DNA among cultivated potatoes and closely related wild species. *Genes Genet Syst* 84:371–378
- Iovene M, Savarese S, Cardi T, Frusciant L, Scotti N, Simon PW, Carputo D (2007) Nuclear and cytoplasmic genome composition of *Solanum bulbocastanum* (+) *S. tuberosum* somatic hybrids. *Genome* 50:443–450
- Lössl A, Adler N, Horn R, Frei U, Wenzel G (1999) Chondriome-type characterization of potato: mt α , β , γ , δ , ϵ and novel plastid-mitochondrial configurations in somatic hybrids. *Theor Appl Genet* 98:1–10
- Lössl A, Götz M, Braun A, Wenzel G (2000) Molecular markers for cytoplasm in potato: male sterility and contribution of different plastid-mitochondrial configurations to starch production. *Euphytica* 116:221–230
- Rohlf F (2006) NTSYSpc: numerical taxonomy system (ver. 2.2). Exeter Publishing, Ltd, Setauket
- Sanetomo R, Hosaka K (2011) A maternally inherited DNA marker, descended from *Solanum demissum* ($2n = 6x = 72$) to *S. tuberosum* ($2n = 4x = 48$). *Breed Sci* 61:426–434
- Sarkar D, Tiwari JK, Shruti Sharma, Poonam Sharma Sanjeev, Gopal J, Singh BP, Luthra SK, Pandey SK, Pattanayak D (2011) Production and characterization of somatic hybrids between *Solanum tuberosum* L. and *S. pinmatisectum* Dun. *Plant Cell Tissue Organ Cult* 107:427–440
- Scotti N, Cozzolino S, Cardi T (2007) Mitochondrial DNA variation in cultivated and wild potato species (*Solanum* spp.). *Genome* 50:706–713
- Sukhotu T, Hosaka K (2004) Origin and evolution of Andean potatoes revealed by chloroplast and nuclear DNA markers. *Genome* 49:636–647
- Tiwari JK, Chandel P, Singh BP, Bhardwaj V (2014) Analysis of plastome and chondriome genome types in potato somatic hybrids of *Solanum tuberosum* (+) *S. etuberosum*. *Genome* 57:1–7
- Tiwari JK, Poonam Sarkar D, Pandey SK, Gopal J, Kumar SR (2010) Molecular and morphological characterization of somatic hybrids between *Solanum tuberosum* L. and *S. etuberosum* Lindl. *Plant Cell Tissue Organ Cult* 103:175–187