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Molecular analysis of cytoplasm type in Indian potato varieties

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Abstract Thirty-eight Indian potato varieties and fifty-two advanced hybrid lines were analyzed for cytoplasm types using both plastid and mitochondrial genome specific markers. Indian genotypes thus analysed could be broadly grouped into 4 cytoplasm types i.e. T/β (69), W/α (18), W/γ (1) and A/ε (2). The T/β type cytoplasm, typical of common cultivated potato (ssp. *tuberosum*) was absent in six released varieties (Kufri Chipsona-1/-2/-3 series, Kufri Jawahar, Kufri Megha and Kufri Himalini) and fifteen out of fifty two hybrids analyzed. This information was further used to predict cytoplasm type on the basis of common shared maternal pedigree in thirty-eight other advanced hybrids, which revealed majority (25) had T/β type cytoplasm with W/α and A/ε cytoplasm observed in 12 and 1 genotype, respectively. T/β type cytoplasm was observed in all 28 early bulking hybrids studied along with all old genotypes. It was revealed that considerable broadening of maternal base was observed in recently developed genotypes. W/α type cytoplasm was present in most of processing (all 3 chipping varieties and 9 of 12 MP hybrids) and late blight resistant (11of 23 hybrids) genotypes.

Keywords Cytoplasmic divergence · Introgression · Mitochondrial genome · Plastid genome · Potato

Abbreviations

cp-type	Plastid genome type
T	*tuberosum* type
A	*andigena* type
GM	Maternal grandmother
cpDNA	Plastid DNA
mt-type	Mitochondrial genome type
W	Wild type
cpSSR	Chloroplast simple sequence repeats
GGM	Matrilineal great grandmother
mtDNA	Mitochondrial DNA

Introduction

Potatoes (*Solanum tuberosum* L. ssp. *andigena*) originated in the Andean mountains of South America and are adapted to tubering in short days. In

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contrast, Chilean potatoes (*S. tuberosum* ssp. *tuberosum*) are adapted to tubering in long days. The potato is a highly heterozygous, autotetraploid crop that suffers inbreeding depression on selfing (Mendoza and Haynes 1974). There are no homozygous breeding lines in potato for exploiting heterotic vigour. The exploitation of a few South American clones, which were successfully introduced to Europe and USA during the 17th century, and their use in most of the potato breeding programmes worldwide resulted in a narrow genetic base of cultivated potatoes. This was further aggrevated by cytoplasmic-genetic male sterility, which restricts use of many genotypes as male parents in breeding. As a consequence, substantial gains in the yield potential of newly developed varieties have not been obtained. All these factors call for broadening of genetic base of cultivated potato.

S. tuberosum ssp. *andigena* has wide diversity and, therefore, has been extensively used in neo-tuberosum programmes (Glendinning 1983) and in effecting *tuberosum* × *andigena* crosses, which results in enhanced heterosis and vigour. In *tuberosum* × *andigena* crosses, yield gains are accompanied by reduced tuber size and increased male sterility (Maris 1989), while reciprocal cross is male fertile indicating that cytoplasm plays an important role in potato breeding (Grun 1990). Wild species have also been successfully used for introgression of many genes bearing desirable traits in potato. However, their use is limited due to lengthy prebreeding that often involves co-segregation of linked undesirable traits.

Considering the importance of organelle genomes (plastid and mitochondrial genomes) in potato breeding, their analysis is of immense use in studying introgression of agronomic traits. Typically, organelle genomes are haploid, non-recombinant and are usually maternally inherited. The very low mutation rates of organelle genomes as compared to the nuclear genome make them a powerful tool for such studies. Most cultivated Chilean potatoes (ssp. *tuberosum*) have T-type plastid DNA and β type mitochondrial genome, which is lacking in Andean potato ssp. *andigena* as well as wild *Solanum* species. Five basic cpDNA types (T/C/W/S/A) have been identified in cultivated landraces of potato (Hosaka and Hanneman 1988). These organelle genome types are not species specific but their frequencies vary in different species, with frequencies varying with latitude in the Andes within the same species. T cp-type is linked to β mt-type (thereby defined as *tuberosum* mt genome), while mt-types α, γ and δ were found in combination with W cp-type in wild species; and mt-type ε was found in A- and S-type cultivated species (Lossl et al. 1999).

In a crop like potato, which is highly heterozygous and auto-tetraploid, organelle studies are expected to provide a better picture of available diversity. This study was therefore undertaken to analyse divergence revealed by plastid as well as mitochondrial genome type in thirty-eight Indian varieties and fifty-two advanced hybrids. The present study included three markers (H1, H2, H3) based on chloroplast genome deletion (Hosaka 2003) and four cpSSR markers (Provan et al. 1999), to investigate plastid genome type. Mitochondrial genome type was investigated using PCR analysis (ALM1 + 3, ALM4 + 5 and ALM6 + 7) specific for mitochondrial genome types (Lossl et al. 1999; 2000).

Materials and methods

Plant material and isolation of DNA

The present study included thirty-eight Indian varieties and fifty-two advanced hybrids (Table 1a, b). For DNA extraction, leaves were collected from 45 day old single plants grown in a glasshouse in the long summer days of Shimla (Latitude: 31.60 N, Longitude: 77.13 E, Altitude: 2202 msl). A modified CTAB method (Doyle and Doyle 1987) was used to isolate genomic DNA from 2 g of fresh leaves. The amount of DNA was quantified by spectrophotometer and quality was checked both by A260/280 ratio and by gel-electrophoresis.

Chloroplast and mitochondrial DNA markers

A total of ten PCR based markers consisting of three chloroplast deletions (H1, H2 and H3) (Hosaka 2003); four cpSSR (NTCP6, NTCP 8, NTCP 9 and NTCP 14) (Provan et al. 1999) and three mitochondrial PCR markers (ALM1 + ALM3; ALM4 + ALM5 and ALM6 + ALM7) (Lossl et al. 1999; 2000) were used in the present study (Table 2). H1 marker is known to amplify a 446 bp fragment in wild/*andigena* potato plastid genome region;

Table 1 (a) Details of Indian potato varieties used in present study

Variety	Female parent	Cp-type	Mt-type	Remarks including matrilineal pedigree	Year of release	Year of crossing
1. Atlantic	Wauseon	T	β	Atlantic = Wauseon × Lenape (with *S. chacoense* in pedigree)		
2. Kufri Alankar (A3649)	Kennebec	T	β	GM = Seedling 96-56; GGM = Earlane	1968	1957
3. Kufri Arun (MS/92-2105)	Kufri Lalima	T	β	GM = Kufri Red	2004	1991
4. Kufri Ashoka (PJ-376)	EM/C-1020	T	β	–	1996	N.A.
5. Kufri Badshah (JF4870)	Kufri Jyoti	T	β	GM = 3069 d(4)	1979	1963
6. Kufri Chamatkar (ON1202)	Ekishirazu	T	β	–	1968	1943
7. Kufri Chandramukhi (A2708)	Sd. 4485	T	β	–	1968	1957
8. Kufri Chipsona-1 (MP/90-83)	**MEX.750826**	**W**	α	**MEX.750826 (from Mexico)**	**1997**	**1989**
9. Kufri Chipsona-2 (MP/91-G)	**F-6**	**W**	α	**F-6 (from Peru)**	**1997**	**1990**
10. Kufri Chipsona-3 (MP/97-583)	**MP/91-86**	**W**	α	**GM = F6 (from Peru)**	**2005**	**1996**
11. Kufri Dewa	Craig's Defiance	T	β		1973	1959
12. Kufri Giriraj (SM/85-45)	SLB/J-132	T	β	GM = 3345d(1)	1997	1985
13. Kufri Himalini (SM/91-1515)	**I1062**	**W**	α	**DM = 22%**	**2005**	**1999**
14. Kufri Jawahar (JH222)	Kufri Neelmani	T	β	GM = Kufri Kundan	1996	N.A.
15. Kufri Jeevan (SLB/E427)	M109-3	T	β	M109-3 has complex pedigree (involving *andigena* var. Landiforme and *S. demissum* line S75)	1968	N.A.
16. Kufri Jyoti (SLB/Z 389)	3069 d(4)	T	β	GM: 2182EF(7)	1968	N.A.
17. Kufri Kanchan (SE/I-1307)	SLB/Z-405(a)	T	β	SLB/Z-405 is a *tuberosum* × *andigena* hybrid	2000	N.A.
18. Kufri Khasigaro	Taborky	T	β	–	1968	1958
19. Kufri Kumar (S1758)	Lumbri	T	β	–	1958	1943
20. Kufri Kundan (Hybrid 9)	Ekishirazu	T	β	–	1958	1939
21. Kufri Lalima (BS/C 1753)	Kufri Red	T	β	GM = Clonal selection of Darjeeling Red Round	1982	1964
22. Kufri Lauvkar (A7416)	Serkov	T	β	–	1972	1957
23. Kufri Megha	**SLB/K-37**	**W**	α	**GM = Z-443**	**1989**	**N.A.**
24. Kufri Muthu (SLB/Z 785)	3046 (1)	T	β	–	1971	N.A.
25. Kufri Naveen (SLB/e 402)	3070 d(4)	T	β	GM = 2182EF	1968	1962
26 Kufri Neela (A1528)	Katahdin	T	β	GM = USDA40568	1963	1957
27. Kufri Pukhraj (JEX/C166)	Craig's Defiance	T	β	Kufri Pukhraj = *tuberosum* × andigena hybrid	1997	1978

Table 1 continued

Variety	Female parent	Cp-type	Mt-type	Remarks including matrilineal pedigree	Year of release	Year of crossing
28. Kufri Pushkar (JW-160)	QB/A 9-120	T	β		2004	1994
29. Kufri Red	Darjeeling Red Round	T	β	Clonal selection of Darjeeling Red Round	1958	–
30. Kufri Shailaja (SM/87-185)	Kufri Jyoti	T	β	GM = 3069 d(4) Kufri Shailaja : *tuberosum* × andigena hybrid	2004	1987
31. Kufri Sheetman (C3745)	Craig's Defiance	T	β	–	1968	1959
32. Kufri Sherpa (F5242)	Ultimus	T	β	GM = Rhode Star, GGM = Prof. Wohltman	1983	1963
33. Kufri Sindhuri (C140)	Kufri Red	T	β	GM = Clonal selection of Darjeeling Red Round	1967	1959
34. Kufri Bahar (E3797)	Kufri Red	T	β	GM = Clonal selection of Darjeeling Red Round	1980	1961
35. Kufri Surya (HT/92-621)	Kufri Lauvkar	T	β	GM = Serkov	2004	1991
36. Kufri Sutlej (I5857)	Kufri Bahar	T	β	GM = Kufri Red	1996	N.A.
37. Kufri Swarna (O 5)	Kufri Jyoti	T	β	Male parent $(VTn)^2$62.33.3 has *S. vernei* in pedigree	1985	1972
38. Kufri Anand (MS/92-717)	Kufri Ashoka	T	β	GM = EM/C 1020	1999	1991

(b) Advanced hybrid lines -chloroplast andmitochondrial genome type

Name	Maternal parent	Cp-type	Mt-type	Remarks	Year of introduction to AICPIP trials	Year of crossing
1. 83-P-47	JI 5857	T	β	GM = Kufri Bahar	1994	1983
2. 94-P-31	86-P-40	T	β	GM = Kufri Chandramukhi	2001	1994
				94-P-31 = *tuberosum* × andigena		
3. 94-P-59	Cruza-27	T	β	Cruza-270 (Peruvian accession)	2001	1994
4. B-420(2)	387415.47	T	β	387415.47 (CIP)	2002	1995
5. Ex/A 680-16	**N0.507.14**	**A**	$\boldsymbol{\varepsilon}$	–	**2002**	**1993**
6. HB/83-39	**VB/A-64**	**W**	$\boldsymbol{\alpha}$	**GM = VB-8**	**1992**	**1982**
7. HT/93-707	Kufri Laukar	T	β	GM = Serkov	2001	1992
8. J/92-13	JN2207	T	β	GM = JF4920	2000	1991
9. J/92-164	JN2207	T	β	GM = JF4920	2000	1991
10. J/92-167	JN2207	T	β	GM = JF4920	2000	1991
11. J/93-139	Croft	T	β	GM: 2895F(G)	2000	1992
12. J/93-4	Kufri Jyoti	T	β	GM = 3069 d(4)	2001	1992

Table 1 continued

(b) Advanced hybrid lines -chloroplast andmitochondrial genome type

Name	Maternal parent	Cp-type	Mt-type	Remarks	Year of introduction to AICPIP trials	Year of crossing
13. J/93-58	Kufri Pukhraj	T	β	GM = Craig's Defiance	2002	1992
14. J/93-77	Croft	T	β	GM = 2895F(G)	2001	1992
15. J/93-81	Croft	T	β	GM = 2895F(G)	2000	1992
16. J/93-86	MS/82-638	T	β	GM = JN46	2001	1992
17. J/93-87	MS/82-638	T	β	GM = JN46	2000	1992
18. J/94-90	EB/C 899	T	β	–	2002	1993
19. JX/108	Krirrne	T	β	–	1992?	1985?
20. J/95-227	JY712	T	β	–	2003	1994
21. J/95-229	JY712	T	β	–	2003	1994
22. J/95-242	JY712	T	β	–	2003	1994
23. JX/576	JE812	T	β	GM = A2708	1996	1985
24. MP/97-625	**MP/92-35**	**W**	α	**GM: Muziranzara(CIP)**	**2003**	**1996**
				GGM: 65-ZA-5		
25. MP/97-644	**MP/92-35**	**W**	α	**GM: Muziranzara(CIP)**	**2003**	**1996**
				GGM: 65-ZA-5		
26. MP/97-921	**MP/92-154**	**W**	α	**GM: QB/B 92-4**	**2003**	**1996**
27. MP/98-172	**MP/91-83**	**W**	α		2005	**1997**
28. MP/98-31		T	β		2005	1997
29. MP/98-71	**MP/92-30**	**W**	α		2005	**1997**
30. MP/99-322	MP/91-76	T	β		**2005**	**1998**
31. MP/99-406	MP/91-76	T	β		**2005**	**1998**
32. MS/92-1090	Kufri Jyoti	T	β	GM: 3069 d(4)	1998	1991
33. MS/93-1344	MS/81-145	T	β		2000	1992
34. MS/94-1118	TS4	T	β	TS4 (from Peru)	2001	1993
35. MS/94-899	MS/82-638	T	β	GM = JN46	2000	1993
36. MS/95-117	JEX/C-166	T	β		2002	1994
37. MS/95-1309	**MS/83-398**	**W**	α		**2002**	**1994**
38. MS/97-1606	**MS/83-279**	**W**	α		**2003**	**1996**
39. MS/97-621	**27/15**	**A**	ε	**27/15 (from Peru)**	**2003**	**1996**
40. SM/85-50	SLB/J132	T	β	GM = M124-2	1993	1985
41. SM/94-44	HB/83-39	T	β	GM = VB/A-64	2002	1994

Table 1 continued

(b) Advanced hybrid lines -chloroplast andmitochondrial genome type

Name	Maternal parent	Cp-type	Mt-type	Remarks	Year of introduction to AICPIP trials	Year of crossing
42. SM/87-151	Kufri Jyoti	T	β	GM = 3069 d(4)	2000	1987
43. SM/87-55	Kufri Jyoti	T	β	GM = 3069 d(4)	1996	1987
44. SM/88-343	**Kufri Megha**	**W**	α	**GM = SLB/K-37** **GGM = Z-443**	**1997**	**1988**
45. SM/88-991	I 1062 (Sita)	T	β		1997	1988
46. SM/90-45	**HB/82-372**	**W**	α	**GM: VB/A-64**	**2000**	**1990**
47. SM/92-168	**HB/82-372**	**W**	α	**GM: VB/A-64**	**2001**	**1992**
48. SM/93-237	**SS/C-562**	**W**	α	**DM = 22%**	**2002**	**1993**
49. SM/94-137	–	T	β		N.A.	1994
50. SM/95-43	**CP2380**	**W**	γ	**DM = 22%**	**2005**	**1995**
51. SM/94-82	**-**	**W**	α		**N.A.**	**1994**
52. SM/96-127	Kufri Jyoti	T	β	**DM = 22%**; GM = 3069 d(4)	2005	1996

(c) Predicted advanced hybrids -chloroplast and mitochondrial genome type

Name	Maternal parent	Cp-type	Mt-type	Maternal parents pedigree	AICPIP introduction	Year of crossing
1. OP1	**EX/A680-16 selfed**	**A**	ε	**GM = N0.507.14**	**N.A.**	**N.A.**
2. PS/M-75	**Kufri Jawahar**	**W**	α	**GM = Kufri Neelamani**	**1990**	**N.A.**
3. PS/M-98	**Kufri Jawahar**	**W**	α	**GM = Kufri Neelamani**	**1989**	**N.A.**
4. 83-P-12	**Kufri Jawahar**	**W**	α	**GM = Kufri Neelamani**	**1994**	**1983**
5. 85-P-621	Kufri Bahar	T	β	GM = Kufri Red	1993	1985
6. 85-P-670	Kufri Bahar	T	β	GM = Kufri Red	1993	1985
7. 85-P-718	Kufri Bahar	T	β	GM = Kufri Red	1993	1985
8. 83-P-47	Kufri Sutlej	T	β	GM: Kufri Bahar	1994	1983
9. 86-P-111	Kufri Chandramukhi	T	β	GM = Sd.4485	1995	1986
10. 94-P-5	86-P-40	T	β	GM: Kufri Chandramukhi	2001	1994
11. J/93-68	Kufri Pukhraj	T	β	GM = Craig Defiance	2001	1992
12. J/92-159	JN2207	T	β	GM = JF4920	1999	1991
13. JX1	Kufri Jyoti	T	β	GM = 3069 d(4)	1994	N.A.
14. JX23	Kufri Jyoti	T	β	GM = 3069 d(4)	1995	N.A.
15. JX24	Kufri Jyoti	T	β	GM = 3069 d(4)	1994	N.A.

Table 1 continued

(c) Predicted advanced hybrids -chloroplast and mitochondrial genome type

Name	Maternal parent	Cp-type	Mt-type	Maternal parents pedigree	AICPIP introduction	Year of crossing
16. JX14	JE812	T	β	GM = A 2708	1993	N.A.
17. JX123	JE812	T	β	GM = A 2708	1992	N.A.
18. JX161	JE812	T	β	GM = A 2708	1993	N.A.
19. JX216	JE812	T	β	GM = A 2708	1995	N.A.
20. JX254	JE812	T	β	GM = A 2708	1994	N.A.
21. MS/95-117	Kufri Pukhraj	T	β	GM = Craig Defiance	2002	1994
22. MS/85-163	Kufri Lalima	T	β	GM = Kufri Red	1992	1984
23. MS/92-3128	MS/82-638	T	β	GM = JN46	1998	1991
24. MS/72-3146	MS/82-638	T	β	GM = JN46	1999	1971
25. MS/98-1095	**Kufri Jawahar**	**W**	α	**GM = Kufri Neelamani**	**N.A.**	1997
26. MP/97-637	**MP/92-35**	**W**	α	**GM: Muziranzara**	**2003**	**1996**
27. MP/97-699	**MP/91-65**	**W**	α	**GM: POOS16**	**2003**	**1996**
28. MP/90-94	**MEX750826**	**W**	α		**1996**	**1989**
29. MP/91-23(G)	**F6**	**W**	α		**1996**	**1990**
30. HB/82-372	**VB/A-64**	**W**	α	**GM: VB-8**	**1991**	**1982**
31. HB/83-185	**VB/A-132**	**W**	α	**GM: VB-8**	**1993**	**1983**
32. SM/85-50	SLB/J132	T	β	GM: M-124-2	1993	1985
33. SM/85-41	SLB/J132	T	β	GM: M-124-2	1994	1985
34. SM/85-45	SLB/J132	T	β	GM: M-124-2	1995	1985
35. SM/85-60	SLB/J132	T	β	GM: M-124-2	1995	1985
36. SM/87-55	Kufri Jyoti	T	β	GM: 3069D	1996	1987
37. SM/85-162	**VB/A-85**	**W**	α	**GM: VB-8**	**1995**	**1985**
38. SM/92-338	**HB/82-372**	**W**	α	**GM: VB/A-64**	**2001**	**1992**

Bold indicate non tuberosum type cytoplasm

T—*tuberosum* type plastid genome; W— wild type plastid genome; A—*andigena* type plastid genome

GM—Maternal grandmother; GGM—Matrilineal Great Grandmother

DM = dry matter (given only in genotypes not developed specifically for Processing purpose)

N.A. = not available

AICPIP—All India Coordinated Potato Improvement Project

Table 2 Details of markers used for plastid genome/mitochondrial genome analysis

Marker	Sequence	Region amplified	Type
H1 marker	F 5′ GGAGGGGTTTTTCTTGGTTG 3′	*ndhl*C/*trn* intergenic	241 bp cpDNA deletion specific PCR
	R 5′ AAGTTTACTCACGGCAATCG 3′		
H2 marker	F 5′ GCATCGAGCGTGTTGTTGGA 3′	*rbc*L gene	PCR -RFLP (*Hae*III)
	R 5′ AGTCCACCGCGAAGACATTC 3′		
H3 marker	F 5′ CAGGGGTCCATTCCCTTGAC 3′	*ycf*4 and *ycf*10	PCR-RFLP (*Dra*I)
	R 5′ AGAAAGAAATCCACCAGGGC 3′		
NTCP6	F 5′ GGT TCG AAT CCT TCC GTC 3′	*rps*16/*trn*Q intergenic	Chloroplast SSR
	R 5′ GAT TCT TTC GCA TCT CGA TTC 3′		
NTCP8	F 5′ ATA TTG TTT TAG CTC GGT GG 3′	*trn*G intron	Chloroplast SSR
	R 5′ TCA TTC GGC TCC TTT ATG 3′		
NTCP9	F 5′ CTT CCA AGC TAA CGA TGC 3′	*trn*G/*trn*R intergenic region	Chloroplast SSR
	R 5′ CTG TCC TAT CCA TTA GAC AAT G 3′		
NTCP14	F 5′ AATCCGTAGCCAGAAAAATAAA 3′	*psbM*/*trnD* intergenic	Chloroplast SSR
	R 5′ CCGATGCATGTAATGGAATC 3′		
ALM1	5′ CAC AAA TCC ATC TTT GTT TAT GC 3′	*atp*6	$\alpha + \gamma$ types = 1.2 kb Others = nil
ALM3	5′ GCG TTG GCT TAC ACG GAA ACT AG 3′		
ALM4	5′ AAT AAT CTT CCA AGC GGA GAG 3′	*cob*, *rps*10	α type = 2.4 kb, β type = 1.6 kb, Others = nil
ALM5	5′ AAG ACT CGT GAT TCA GGC AAT 3′		
ALM6	5′ AAT TAG GCC CGG CTA GGA ACA 3′	*cob*	γ types = 2.4 kb, Others = nil
ALM7	5′ AAC CCA GTC CCT ATG GTA TCT CCT 3′		

however, ssp. *tuberosum* is known to amplify fragment of 205 bp size, thereby indicating a 241 bp deletion. These were evaluated by 1.6% agarose gel electrophoresis stained with ethidium bromide.

Similarly, in H2 and H3 markers, PCR amplification products were ethanol precipitated before digestion with *Hae*III and *Dra*I respectively. For mitochondrial genome analysis, three mitochondrial DNA specific markers mentioned above were used for PCR amplification and evaluated by 1.2% agarose gel electrophoresis.

Four cpSSR markers were analysed by semi-automated capillary-based electrophoresis. Two cpSSR forward primers, viz., NTCP6 and NTCP8 were labelled with 5′ FAM and the other two forward primers, viz., NTCP9 and NTCP14 with JOE. PCR reactions and GeneScan capillary electrophoresis based genotyping was done on the ABI Prism310 Genetic analyzer as described in our earlier studies (Chimote et al. 2004). The runs were performed at 60°C for 24 min at 15 kV with 5 s injection time. All genotypes were analyzed twice to check reproducibility of results.

Prediction analysis

Information based on organelle genome analysis in the above study and common matrilineal pedigree was used to predict cytoplasm type in 38 other advanced hybrid lines, as shown in Table 1c.

Results

Chloroplast deletion markers

Most of the varieties and advanced hybrids have T cp-type chloroplast DNA, similar to Chilean cultivated potato as revealed by H1 marker. T cp-type 241 bp chloroplast deletion typical of *tuberosum* type was found in 32 varieties and 37 advanced hybrids analyzed. T cp-type deletion was observed in all 17 early bulking hybrids (J series), 3 hybrids for East Indian plains (P series) and two heat tolerant hybrids (HT series). However, this deletion was absent in 6 varieties (Kufri Chipsona-1, Kufri Chipsona-2, Kufri Chipsona-3, Kufri Jawahar, Kufri Megha and Kufri

Himalini), 5 out of 8 processing hybrids (MP series), 7 out of 14 late blight resistant hill hybrids (SM/HB series), 3 medium maturing hybrids for North Indian plains (MS series) and *andigena* clone EX/A 680-16. Exotic processing cultivar Atlantic also showed T-type cytoplasm.

The grouping pattern of varieties and hybrids was the same with the H2 markers (Table 3). All genotypes showing T cp-type yielded two fragments of 193 and 141 bp on *Hae*III digestion with H2 marker, while in non-T cp-type genotypes only an undigested 334 bp fragment was observed. In case of H3 marker, all genotypes yielded 1018 bp fragment except two advanced hybrids (MS/97-621 and EX/A680-16), which gave two fragments of 575 and 443 bp, with rejoined fragment being also observed in them.

Chloroplast microsatellite markers

Four cpSSR markers (NTCP6, NTCP8, NTCP9 and NTCP14) together amplified a total of 6 fragments, with NTCP6 and NTCP9 giving only a single fragment each. These studies classified them into three clear groupings with first major group comprising of T cp-type genotypes, second group comprising genotypes with W cp-type and last group comprising of MS/97-621 and EX/A680-16. The differences in their peak patterns are given in Table 3. Three possible allelic combinations were observed at each loci except NTCP6 (150/151), thereby clustering all genotypes into 3 groups (T, W and A cp-type).

Mitochondrial typing studies

ALM1 + ALM3 primer combination amplified *atp*6 region resulting in 1.2 kb band specific to $\alpha + \gamma$ mt-types in W cp-type (Table 4). ALM4 + ALM5 primer combination yielded 2.4 kb fragment specific to α type in the same genotypes with a sole exception of SM/94-43. SM/94-43 amplified 2.4 kb fragment of *cob* gene with ALM6 + ALM7 primer combination specific to γ mt-type.

Mitochondrial DNA specific ALM4 + ALM5 marker yielded 1.6 kb PCR product in all genotypes with T and A plastid genomes types. There are reports that β mitochondrial type coincides with T-type chloroplast (Rasmussen et al. 2000; Lossl et al. 2000). Based on these reports, all genotypes having T-type plastid genome and yielding 1.6 kb PCR product with ALM4 + ALM5 combinations were assigned β mt-type. These include 32 Indian varieties, 37 advanced hybrids and cv. Atlantic. Similarly, both A-cp-type genotypes, EX/A680-16 and MS/97-621, also yielded 1.6 kb PCR product with ALM4 + ALM5 and were assigned ε mt-type i.e. typical of ssp. *andigena* (Table 4). On this basis, we concluded that out of 90 varieties and hybrids analyzed, 69 are of β type, 17 of α type, 1of γ type and 2 of ε type.

Table 3 Details of band/peak pattern obtained in varieties and hybrids

Cp-type/Marker	H1	H2	H3	NTCP6		NTCP8		NTCP9	NTCP14
				Medium	Large	Small	Large		
Tuberosum (T)	202	193 + 141	1011	150	172	220	253	279	146
Wild (W)	443	334	1011	151	173	222	255	309	147
Andigena (A)	443	334	575 + 443	150	174	218	251	289	148

All figures are in bps

Table 4 Details of PCR amplification pattern with mt-type specific primers

Primers	Amplified region	Mitochondrial genome type
ALM1 + ALM3	*atp*6	$\alpha + \gamma$ types = 1.2 kb; Others = nil
ALM4 + ALM5	*cob*, *rps*10	α type = 2.4 kb; γ type- nil; Others (β/ε) type = 1.6 kb
ALM6 + ALM7	*cob*	γ types = 2.4 kb; Others = nil

Prediction analysis

Matrilineal pedigree similarity based prediction of organelle genome types using above information was used to conclude that 25 out of 38 advanced hybrid lines are T/β type; 12 are of W/α type and one is of A/ε type (Table 1c). All 10 early bulking hybrids (J/Jx series), 6 hybrids for East Indian plains (-P- series) and four medium maturing hybrids (MS series) showed *tuberosum* type cytoplasm. However, T-type deletion was absent in all 4 processing hybrids (MP series), 4 late blight resistant hybrids (SM/HB series) and three other hybrids (OP1, PS/M75, PS/M78).

Discussion

Organelle genome typing in Indian potato varieties/advanced hybrids revealed that divergence was observed only in a few recently released genotypes developed during the last 15 years primarily for specific breeding purposes like processing quality and late blight resistance, which involved wild gene introgression in their maternal pedigree.

Amplification/restriction pattern with H1, H2, H3, NTCP6 and NTCP9 matched with earlier reports (Bryan et al. 1999; Powell et al. 1999; Hosaka 2003; Sukhotu et al. 2004). However, in all the three haplotypes (T/W/A), fragments sizes were 1 bp larger with NTCP8 and 3 bp smaller with NTCP14 than reported (Hosaka, 2003; Sukhotu et al. 2004). This may be due to different electrophoresis techniques used for resolution.

In the present study the majority (73.4%) of genotypes (94 out of 128) had T-type chloroplast and β type mitochondrial genome. Present day potato cultivars have predominantly T-type organelle DNA typical of Chilean germplasm (Powell et al. 1993; Hosaka 1995; Provan et al. 1999). The predominance of T-type cytoplasm in most potato cultivars can be traced back to the 19th century Chilean introduction, Rough Purple Chili and its derived progenies i.e. Garnet Chili and Early Rose. These genotypes exhibit cytoplasmic male sterility so that they were used as female parents in a large proportion of crosses in North American and European potato breeding (Glendinning 1983).

Analysis of Indian potato varieties revealed 32 out of 38 had T-type deletion, with the only exceptions being six recently released varieties (Kufri Chipsona-1, Kufri Chipsona-2 Kufri Chipsona-3, Kufri Jawahar, Kufri Megha and Kufri Himalini). This is contrary to early reports that the Indian varieties are more like *andigena* type than that of *tuberosum* type (Swaminathan 1958; Sinha and Pushkarnath 1964). T-type marker typical of ssp. *tuberosum* was present in almost all improved Indian varieties, except in Kufri Jawahar and Kufri Megha and three landraces i.e., Phulwa, Gulabia, Lalmutti (Spooner et al. 2005). On nuclear SSR analysis, all Indian cultivars and landraces clustered along with Chilean landraces (ssp. *tuberosum*) rather than with Andean *andigena* accessions as expected, suggesting that Indian cultivars are not true *andigena* but are of neo-tuberosum type. Provan et al. (1999) reported that the extreme cytoplasm bottleneck in most European cultivars was not reflected in nuclear divergence analysis, thereby pointing towards their wide paternal base. Most of the modern Japanese cultivars had T-type cytoplasm; with a few W-type and old cultivars of A-type (Hosaka 1993). Mitochondrial genome type analysis revealed that out of 144 German potato varieties characterized, 79, 46 and 19 varieties respectively had β, α and γ mitochondrial genome type (Lossl et al. 2000).

In the present study, 34 non T-type genotypes formed 2 groups, i.e. larger W- type (31) and smaller A-type (3). EX/A680-16, MS97-621 and predicted OP1 have A/ε cp/mt DNA type typical of ssp. *andigena* type. The frequency of A-type cytoplasm was very low (2.34%) as expected because of the negative role played by *andigena* cytoplasmic factors (Maris 1989). These three genotypes showed a pattern typical of A-cp-type with NTCP6 (174 bp), NTCP14 (148 bp) and NTCP8 (218 bp + 251 bp fragment). Further, they also yielded 575 and 454 bp fragments with H3 marker and 289 bp fragments with NTCP9. H3 (575 and 454 bp) and NTCP9 (289 bp) markers were perfectly correlated and are observed in almost all cultivated species (except ssp. *tuberosum* and *S. juzepczukii*) of S-, A- and most C-cp types (Sukhotu et al. 2006).

None of the genotypes studied showed 127 bp band typical of S-cp-type indicating its total absence in matrilineal pedigree of Indian potato breeding. Only a few Indian varieties and hybrids are known to have either *S. phureja* or *S. stenotomum* in their pedigree (e.g. POOS16 (in MP/97-921), I1062 (in

Kufri Himalini) and MEX32, etc). Kufri Jyoti's maternal grandparent GM 2182ef7 traces back to *S. phureja* × *S. demissum* on male side. *S. phureja* shows cytoplasmic barrier with reciprocal differences on crossing with ssp. *tuberosum* (Amoah et al. 1988; Grun et al. 1977).

W/α cytoplasm type was observed in all three chipping varieties (Kufri Chipsona-1, Kufri Chipsona-2, Kufri Chipsona-3) and nine out of twelve hybrids bred specifically for processing purpose. Late blight resistant variety Kufri Himalini and hybrid SM/92-168 with good processing qualities also have W/α cytoplasm type. Interestingly most of them have Central/South American accessions like MEX-750826, F6, Muziranazara, POO16, I1062 and VB/A 64 in their matrilineal pedigree, involving wild species with good processing attributes in pedigree. There is higher variability in wild potato species than in cultivated potatoes for characters like dry matter, starch content, amylose content, mean diameter of starch granules, etc (Jansen et al. 2001). W/α genotypes identified could help in narrowing down selection for introgression of processing quality trait. However, high dry matter and processing attributes are also present in genotypes with T/β type cytoplasm i.e. in Chipping cultivar Atlantic, three processing hybrids (i.e. MP/98-31, MP/99-322 and MP/99-406) and two heat tolerant hybrids (HT-series hybrids and B-420). Atlantic has cv. Lenape cytoplasm with *S. chacoense* in paternal pedigree.

In case of late blight resistance breeding programme, two recent varieties (Kufri Megha and Kufri Himalini) and almost half of SM/HB-hybrids (11 of 23) developed for North Indian hills (where the duration of crop is long and late blight infection is epidemic) had W/α type cytoplasm. Wild species especially *S. demissum*, play an important role in incorporation of late blight resistance in potato breeding. However, most of the earlier Indian varieties (SLB series) with R gene derived late blight resistance had T/β cytoplasm as they are derived from resistant breeding material of Scottish Plant Breeding Station with *S. demissum* in paternal pedigree.

All of the early bulking hybrids studied had T/β type cytoplasm typical of *tuberosum* ssp. This can be explained by the fact that any potato breeding involving wild species/*andigena* results in delayed tuberization and thereby a late maturation crop which defeats the basic purpose of this breeding programme. In potato breeding programme for regular maturation crop in Indo-Gangetic plains, with yield being main objective, 3 out of 7 recent hybrids lack *tuberosum* typical T-type cytoplasm.

Our study highlights the importance of wild gene introgression in potato breeding for specific purposes like late blight resistance and processing. Current diversified uses of potato in processing, demands introgression of wild gene pool for improving quality characters such as cold chipping, high dry matter and starch content of tubers. Desirable genes for many quality traits can be found in Andean potato genotypes, whereas for abiotic and biotic stresses, breeding could well benefit from use of wild species.

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