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Nuclear-cytoplasmic diversity in parental genotypes used for Indian early bulking potato (*Solanum tuberosum* ssp. *tuberosum*) breeding programme

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Abstract

Forty parental lines used for development of early bulking potato varieties were analyzed for genetic divergence using twenty RAPD primers and cytoplasmic base by using six PCR-based markers comprising of two chloroplast DNA deletions based (HI and H3), one plastid SSR (NTCP9) and three mitochondrial SCAR markers. In RAPD divergence studies there were three clear clusters, those with *andigena* pedigree and JF series in pedigree formed two separate clusters from rest of genotypes. AGB69.1 was the most distinct parent followed by the parent-progeny combination (MS/82-638 and MS/92-3128). On plastome/chondriome analysis three *andigena* type cytoplasm possessing parents (Phulwa, AGB69-1 and A98-175) and four wild type cytoplasm possessing parents (i.e. AG14(X37)H, J99-2, A98-165 and PS/M75) were distinct from the rest i.e. *tuberosum type* cytoplasm genotypes. The diverse *andigena* type cytoplasm genotypes can be preferred as parents to broaden the genetic base in early bulking varieties, while those wild type cytoplasm genotypes may contribute to introgression of useful traits like higher dry matter and late blight resistance.The results of present study showed that divergent parents from both paternal and maternal pedigree are being used in Indian breeding programme for early bulking varieties. Divergent parents identified in present study may be useful in planning hybridization programme aimed at effective utilization of heterosis.

Key words: Nuclear, cytoplasmic, diversity, RAPD, potato, organelle genome

Introduction

The commonly cultivated potato (*Solanum tuberosum* L.) is a highly heterozygous tetraploid. The conventional potato breeding programmes depend mainly on identification of promising parental lines for making desired crosses, creation of variability through hybridization and subsequent selection of desirable recombinants for further evaluation and vegetative propagation. The choice of parents in hybridization depends on the objectives of the programme. Breeders prefer to work with parents having good agronomic attributes, adaptation and other desired traits. In general, genetic divergence, combining ability of parents and results of progeny tests are kept in view.

The present-day Indian potato varieties have economically important characters like processing quality and late blight resistance derived from a narrow genetic base. Most of these parents represent tetraploid *S. tuberosum* ssp. *tuberosum* cultivars with negligible representation of *S. tuberosum* ssp. *andigena* and wild/other cultivated species (other than *S. tuberosum*). For reasons of superior agronomic attributes and good adaptability *S. tuberosum* ssp. *tuberosum* has been extensively used in varietal improvement programmes all over the world. However, continuous cycles of breeding and selection for similar economic attributes have resulted in reduced genetic variability in cultivated potatoes. This reduces the chances of recovering high heterosis for economic characters. Since distantly related lines are more complementary and produce heterotic effects, use of genetic distances is recommended for planning crosses in breeding programmes [1-3].

The wild and primitive species are valuable for their resistance to wide range of biotic and abiotic stresses. The underexploitation of wild and primitive cultivated species in varietal improvement programmes is primarily due to long time required in developing desirable parental lines. In cultivated *tuberosum* potato

breeding, male sterility is a serious constraint limiting the choice of pollen parents in hybridization. Only a few cultivated/wild species have been used in varietal improvement programmes to serve as donors (female parents) of only the desired dominant genes controlling traits like processing quality and disease resistance due to difficulties like linkage drag. Narrow maternal base in European cultivars was not reflected in nuclear diversity pointing towards wide paternal base [4]. In this context, organelle genome based markers offer opportunities for studying introgression into cultivated potato gene pool, across the species barriers.

In India, the rice-wheat cropping system is prevalent in 10 million ha of the Indo-Gangetic plains, where potato competes with wheat as a *rabi* crop. In the intervening period (during September to November), a short duration potato crop (60-75 days) is taken in rice-wheat cropping system. Moreover in irrigated western Indo-Gangetic plains, potato is grown in three season *viz*., early autumn, autumn and spring. Therefore, it is an important task to develop high yielding and early bulking varieties. Early potato harvest ensures remunerative prices and also reduces risk from frost which occurs regularly by mid-winter. Present study involved analysis of genetic divergence in forty parental lines frequently used for crossing work for breeding early maturing varieties. Genetic divergence was studied at total genomic level by using Random Amplified Polymorphic DNA (RAPD) markers and at cytoplasmic organelle genome levels by using plastid/ mitochondrial DNA specific markers.

Material and methods

A modified CTAB method [5] was used to isolate genomic DNA from two grams of potato tubers of each of 40 parental lines grown from disease-free tubers in glasshouse under short day winter conditions of Central Potato Research Station, Jalandhar. These parents consisted of 4 Indian varieties, 1 local landrace, 1 imported variety, 5 imported germplasm accessions and rest being lines/breeding lines (Table 1). Spectrophotometer (SmartSpec 3000 of Bio-Rad) was used to quantify as well as to check the quality of each DNA sample. Finally, all the DNA samples were diluted in sterile MilliQ water so that they contained 25ng DNA/µl.

Twenty random decamer primers procured from M/S Operon Technology, Inc. (1000 Atlantic Ave., Alameda, CA 94501, USA) were used in this study for RAPD analysis. The PCR reaction was set up in a Thermal Cycler (Perkin Elmer: Gene Amp PCR System 9700); followed by 1.6% agarose gel electrophoresis, scanned in Fluor S Multiimager (Bio-Rad) and the images were analyzed by the diversity database software package (Bio-Rad) as reported earlier [6].

For diversity analysis, RAPD data was recorded as binary matrix and analyzed under the SIMQUAL module of NTSYSpc ver 2.0 [7] using Dice coefficient [8]. SAHN module based on UPGMA clustering method [9] was used to generate a dendrogram; while Principal co-ordinate analysis (PCA) was performed to get 2-Dimensional scatter-plot graph of genetic divergence using NTSYS software. Genotype index (GI) and diversity index (DI) were calculated for each primer set [10, 11]. Genotype index reveals the proportion of distinct genotype profiles to the total varieties studied per assay. Diversity index (DI) of polymorphic bands/peaks in each assay was calculated as $DI = I\text{-}\Sigma(pg^2)$, where *pg* is the frequency of an individual genotype.

Plastome specific markers used for plastid DNA analysis

A total of 3 polymerase chain reaction (PCR) based markers consisting of two chloroplast deletions based (H1 and H3) [12] and one *Nicotiana tabaccum* plastome sequence based NTCP9 plastid SSR [4] were used in the present study. The PCR was set up as in case of RAPD using marker specific primers. PCR amplification involved 45 cycles (40 in NTCP9), with each cycle consisting of 30 seconds denaturation at 94°C, 30 seconds annealing at 52°C (55°C in NTCP9), and 1 minute (30 seconds in NTCP9) extension at 72°C. The samples were preceded by initial denaturation at 94°C for 5 minutes before the start of first cycle and after the final cycle with 5 minutes extension at 72°C. PCR amplification products of H1 SCAR marker and *Dra*I digested PCR product of H3 CAPS marker were subjected to 1.6% agarose gel electrophoresis, while, cpSSR marker NTCP9 was analyzed by 2% metaphor agarose (LifeTech. Ltd.) electrophoresis. The gel imaging and analysis was done as in case of RAPD analysis.

Mitochondrial DNA analysis

Mitochondrial DNA analysis was done with specific PCR primer pairs i.e. ALM1 + ALM3; ALM4 + ALM5 and ALM6 + ALM7 [13]. The polymerase chain reaction was performed in the same way as in case of cpDNA (plastid DNA) analysis as mentioned above with differences being 1 minute each of denaturation and annealing (at 58°C) and $2^1/_2$ minutes of extension. Amplification products were separated by electrophoresis on 1.2% agarose gel, scanned and images were analyzed.

Table 1. List of parental lines studied

S. No.	Name of potato parental lines	Parentage	Particulars
1.	Kufii Bahar	Kufri Red x Gineke	Old Indian variety
2.	Kufri Ashoka	EM/C-1020 x Tonda de Berlino	New Indian variety
3.	Kufri Pushkar	QB/A9-120 x CP1462 (Spatz)	Recent Indian variety
4.	Kufri Chamatkar	Ekishirazu x Phulwa	Old Indian variety
5.	Phulwa (white)	Old *andigena* landrace	Local landrace
6.	CP1362[AG14(x37)H]	Not available	Accession from USA
7.	CP1909 [B6532-10]	Not available	Accession from USA
8.	CP2013 [Atzimba]	Not available	Accession from Mexico
9.	CP2383 [AGB69-1]	Not available	Accession from Peru
10.	CP3290 [Hope Hely]	Not available	Accession from Hungary
11.	J96-278	Kufri Pushkar x CP 1704	Regular duration line
12.	J93-86	MS/82-638 x Kufri Pukhraj	Advanced early bulking line
13.	J95-242	JY712xKufri Jyoti	Advanced early bulking line
14.	J92-159	JN2207 x Kufri Jyoti	Advanced early bulking line
15.	J99-2	CP 2058 x MS/82-354	Advanced early bulking line
16.	JX118	CP1346 (Krirrne) x MS/78-62	Regular duration line
17.	JX216	JE8 12 x Kufri Jyoti	Regular duration line
18.	JX576	JE812x Kufri Jyoti	Regular duration line
19.	JN2303	JF4928xCP1463	Old line
20.	MS/92-1090	Kufri Jyoti x PH/F 1545	Adv. regular duration line
21.	MS/92-3128	MS/82-638 x MS/80-758	Adv. regular duration line
22.	MS/82-638	J.N. 46 x JLR/A 148	Regular duration line
23.	MS/82-797	JLR/A284 x Kufri Jyoti	Regular duration line
24.	J/93-139	Croft (CP2160) x MS/82-797	Advanced early bulking line
25.	A9S-48	JEX/A318xJEX/A855	A x A line
26.	A98-165	JEX/B 998 x JEX/A 855	A x A line
27.	A98-175	JEX/A 1048 x EX/A 680-16	A x A line
28.	A98-188	JEX/A11 02 x JEX/A 764	A x A line
29.	A98-98	JEX/A44xEX/A680-16	A x A line
30.	A98-47	JEX/A318xJEX/A855	A x A line
31.	JEB/A26	JF4841xCP1362	Regular duration line
32.	PS/M75	JH222 (Kufri Jawahar) x JN2207	Regular duration line
33.	Craig's Defiance	Arose naturally on cv. like Sutton Snowball	-
34.	E4451	Kufri Kuber x Adina	Old line
35.	JV62	JF4708 x JI5857	Regular duration line
36.	JN2231	JF4920xCP1463	Old line
37.	JN2207	JF4920 x CP1463	Old line
38.	JE812	A.2708xCP1362	Old line
39.	JN1197	JF 4928 xCP 1736	Old line
40.	JX108	CP1 346 x MS/78-62	Regular duration line

A x A: Andigena x Andigena

Results and discussion

Eighteen out of the twenty random primers used in RAPD analysis amplified a total of 130 distinct fragments in 40 tetraploid potato parental lines, of which 73 were highly informative (present in 40-60% i.e. 16-24 samples). Seven RAPD fragments were monomorphic and four were unique. These primers could distinguish from 4 to 20 clusters among forty genotypes studied. Maximum of 10 bands were observed with OPD11 and OPE2, however, most informative RAPD primers were OPD14, OPS-1 and OPB16, each capable of distinguishing 38 out of 40 potato genotypes studied whereas, the primer OPC-13, distinguished only four potato lines.

Moderate level of diversity (SI range = 0.51-0.86) was observed in parental genotypes as revealed by dendrogram. Clustering analysis showed two mega-clusters (each with 2 sub-clusters) along with 2 micro-clusters. First mega-cluster consisted of *tuberosum* clones comprising almost all J/JX series lines/Indian commercial varieties/exotic *tuberosum.* Phulwa and its progeny Kufri Chamatkar (having *andigena* type cytoplasm) and AG14(X37)H (with wild type cytoplasmic genes) were also in this mega cluster. The second mega-cluster formed two sub-clusters with distinct genotype grouping i.e. one cluster (SI range 0.65-0.78) had all six andigena lines along with parent progeny-combination II (MS/82-797 and J/93-139); while the second cluster (SI range 0.62-0.86) having many old parental lines with JF series in pedigree. AGB69.1 was the most divergent (SI = 0.51) genotype with a separate micro-cluster on its own. The parent-progeny combination-Ia (MS/82-638 and MS/92-3128) formed another highly divergent micro-cluster (SI = 0.56 with 0.62 among themselves).

The 2D scatter plot placement pattern reflected the divergence/ similarity pattern similar to that derived by clustering analysis. As in case of the first mega-cluster [all J/JX series lines/ Indian commercial varieties/ exotic *tuberosum;* along with Phulwa; Kufri Chamatkar and AG14(X37)H] were placed together in densely packed manner. AGB69.1 was most lonely placed, while the distinct parent-progeny combination Ia (MS/82-638 and MS/92-3128) was placed close to the A-series *andigena* lines along with the parent-progeny combination II (MS/82-797 and J/93-139). The genotypes with all *andigena* pedigree were placed together in the 2D PCO graph. Many genotypes with JF-pedigree (JN2231, JN2207, PS/M75, JV62, JEB/A 26 and to limited extent JN1197) were placed together along with E4451 and exotic cultivar Craig's Defiance. Three genotypes (*viz*., JN1197, JX 108 and JE812) that clustered along with JF-series derived lines were placed in between of JF-derived parental lines and those present in first mega-cluster. On overall analysis of 2D scatter plot, an anti-clockwise development pattern is visible with three decades old JF derived lines placed before around 2 decades old JX-series lines followed by J-series lines (decade old) and finally A-series lines in evaluation phase.

A total of 12 parent-progeny combinations were identified derived from 8 parents as shown in 2D PCO plot (Table 2). Wide range (SI range 0.56-0.73) of variation was observed within these combinations in tree as well as 2D PCO plots. During clustering analysis and 2D plot five parent-progeny combinations were clustered/placed together, however two (IV and VII) pairs were placed far apart and that too on opposite side along both axes. High heterozygosity in potato might have resulted in wide variation in progeny from their parents.

Nine full sib combinations derived from four common parents were observed in the present study (Table 3). Four JN series line were full sibs and formed six combinations among themselves. The tree and 2D plot the divergence pattern varied with many instances where these analyses yielded contrasting results. On clustering analysis (SI range 0.59-0.80) four full-sib combinations were clustered together, while the rest five were clustered separately. IIa combination was placed most closely in all three analyses. All thirteen half-sib combinations (including nine with Kufri Jyoti as a common parent) were clustered together (SI range 0.63-0.76), five belonging to same sub-cluster as well (Table 4). In 2D PCO plots ten (six very close) of them were placed closely. All five Kufri Jyoti sibs are placed together in clustering tree as well as in 2D plot. Parents placed in clearly distinct groups/clusters should be preferred for crossing to get heterotic effect, while those placed closely/clustered together should be avoided to prevent inbreeding depression.

Five basic cpDNA types (T/C/W/S/A) have been identified in cultivated landrace of potato [14]. Similarly there are five chondriome types in potato (α, β, γ, δ and ε-type); of which δ type has not been reported in cultivated potato [13]. Although these organelle genome types are not species specific; however, their frequencies vary in different species. Present day potato cultivars have predominantly T-type organelle DNA typical of Chilean germplasm [4, 15, 16]. In our previous study [17] all 28 early bulking lines studied had T-type cytoplasm, while a few varieties (Kufri Jawahar, Kufri Megha, Kufri Chipsona-1, Kufri Chipsona-2, Kufri Chipsona-3 and Kufri Himalini) and many lines developed for processing (9 out of 12) and late blight resistance (11 out of 23) had W/α type cytoplasm [17].

H1 marker distinguished T-type specific chloroplast DNA deletion in thirty-three genotypes analyzed resulting in a 202 bp band, while other 7 parental lines (*viz*., Phulwa, AGB69-1, A98-175, A98-165, J99-2, AG14(X37)H and PS/M75) yielded a 423 bp band. H3 marker on PCR-RFLP (*Dra*I digestion) distinguished three genotypes (Phulwa, AGB69-1, A98-175) with A cp-type, as these samples yielded 550 + 440 bp fragments, while the remaining genotypes yielded 1010 bp fragment. NTCP9 cpSSR marker

Table 2. Genetic divergence amongst 12 parent-progeny combinations analyzed

Code (Parent-progeny combination)	SI	Tree	2D plot	3D plot
Ia (MS/82-638 + J93-86)	0.62	+	+	+
Ib (MS/82-638 + MS/92-3128)	0.56	-	-	-
II (MS/82-797 + J93-1 39)	0.68	++	+	+
III (Kufri Pushkar + J96-278)	0.69	++	++	++
IV (Kufri Ashoka + MS/82-797)	0.59	-	-	-
Va (AG14(X37)H + JE812)	0.59	-	-	-
Vb (AG14(X37)H + JEB/A26)	0.59	-	-	-
VIa (JE812 + JX216)	0.59	-	-	+
VIb (JE812 + JX576)	0.59	-	-	+
VIIa (JN2207 + J92- 159)	0.59	-	-	-
VIIb (JN2207 + PS/M757)	0.73	++	++	++
VIII (Phulwa + Kufri Chamatkar)	0.70	++	++	+

+ /- indicate relative degree of closeness or distinctness

Table 3. Genetic divergence amongst 9 full sib combinations analyzed

Code (Parent-progeny combination)	SI	Tree	2D plot	3D plot
I (A98-48 + A98-47)	0.68	++	++	++
IIa (JN2231+JN2207)	0.80	++	++	+
IIb (JN2231+JN1197)	0.63	+	-	-
IIc (JN2231+JN2303)	0.59	-	-	-
IId (JN2207 + JN1197)	0.59	-	-	-
IIe (JN2207 + JN2303)	0.59	-	-	-
IIf (JN1197+JN2303)	0.59	-	+	+
III (JX108+JX118)	0.59	-	++	-
IV (JX216+JX576)	0.78	++	++	-

+ /- indicate relative degree of closeness or distinctness

distinguished W type parental lines [A98-165, AG14(X37)H, J99-2 and PS/M75] by yielding a 310 bp fragment. These parents have wild *Solanum* species genes introgressed from maternal side, most likely by repeated backcrossing to reduce the linkage drag. NTCP9 marker did not distinguish the A-and T-type lines, since their 270/280bp bands did not separate enough due to small size differences.

On chondriome type analysis, ALM4+ALM5 primer combination yielded α chondriome specific 2.4 kb fragment in the same four parental lines with W cp-

Table 4. Genetic divergence amongst 13 half sib combinations analyzed

Code (Parent-progeny combination)	SI	Tree	2D plot	3D plot
J92-242 + J92-159	0.65	-	++(-)	-
J92-242 + JX216	0.70	++	++(-)	-
J92-242 + JX576	0.70	++	+(-)	+
J92-242 + MS92-1090	0.76	++	++(-)	++
J92-159 + JX216	0.64	+	+(-)	++
J92-159 + JX576	0.64	+	+(-)	-
J92-159 + MS92-1090	0.64	+	++(-)	-
MS92-1090+JX216	0.70	++	++	-
MS92-1090+JX576	0.70	++	++	+
A98-175+A98-98	0.65	+	++	-
A98-165+A98-48	0.71	++	++	++
A98-165+A98-47	0.67	++	++	++
JE812+JEB-A26	0.63	+	++	-

+ /- indicate relative degree of closeness or distinctness

haplotype [i.e. AG14(X37)H, 399-2, A98-165, PS/M75] and 1.6 Kb fragment in rest of the potato parental lines indicating that they are either of β or ε-haplotype. Similarly γ mtDNA-type specific ALM6 + ALM7 did not amplify any PCR product in any of these genotypes indicating its absence. ALM1 + ALM3 primer combination amplified α + γ types specific 1.2 kb fragment in four genotypes [AG14 (x37), J99-2, A98-165, PS/M75]. Based on reported co-segregation of T cpDNA with β mtDNA and A cpDNA with ε mtDNA, genotypes with 1.6kb band on ALM4+ALM5 PCR analysis and showed T and A cpDNA type, were assigned β and ε mtDNA type, respectively. Therefore there are thirty-three T cp/β and ε mtDNA haplotypes and three A cpDNA/ε mtDNA haplotypes (i.e. Phulwa, AGB69-1, A98-175).

Three andigena type cytoplasm-possessing parents identified (Phulwa, AGB69-1 and A98-175) in the present study can be used as parents for making diverse crosses in early bulking potato breeding programmes. The *andigena* collections have wide range of valuable traits; therefore have been used extensively for neotuberosum programmes and *tuberosum* x *andigena* crosses, resulting in heterosis and vigour. Based on comparative nuclear and cytoplasmic divergence analysis of Indian cultivars and exotic/Andean cultivars it was suggested that the Indian ones are not true *andigena* but rather they are of neotuberosum type [18].

There is higher variability in wild potato species than in cultivated potatoes for processing characters like dry matter, starch content, amylose contents as well as late blight resistance. There are not many parental lines from wild *Solanum* species as it needs long and difficult prebreeding task due to differences in ploidy, undesirable characters associated linkage drags and non-crossability of some cases. In our previous study [17] there was total absence of non T-type cytoplasm in all the early bulking lines, which can be justified by delayed maturity and associated linkage drag. In the same study, W/α cytoplasm type was reported 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 cytoplasm type. Four wild type cytoplasm possessing parents (i.e. AG14(X37)H, 399-2, A98-165 and PS/M75) identified in the present study may contribute useful traits like higher dry matter and late blight resistance to early bulking lines developed from them.

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