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DIVERSITY ANALYSIS OF POTATO ADVANCED HYBRIDS USING MORPHOLOGICAL AND SSR MARKERS

Reena Sharma[1], SK Kaushik[2], Vinay Bhardwaj[1], Dalamu[3], Anupama Singh[1], Poonam[1], Ashwani Kumar[1], CM Bisht[1], SK Chakrabarti[1]

ABSTRACT: The study was conducted to characterize 39 Indian potato advanced hybrids using highly polymorphic 15 SSRs (Simple Sequence Repeats) and to compare the efficiency of DNA based markers with morphological descriptors. The molecular markers detected 3 to 21 alleles with minimum allele count for STU06 and maximum for STM5114 and STM1052, and an average of 13.3 alleles per primer. The average PIC (Polymorphic information content) value for each primer was 0.84, with highest PIC value observed for primer STM5114 (0.92), STM1052 (0.91), STINHW (0.90), POTM1-2 (0.91), and STM0032 (0.90). The highest marker index (MI) was also observed for marker STM5114 and lowest for STM07 with average of 11.6 per marker. The resolving power (RP) was highest for marker STM0032. Based on these parameters, the study identified 5 most informative markers that could distinguish all the 39 Indian potato hybrids, thus adding to the existing information with regard to suitability of markers in identification and fidelity testing in Indian potato cultivars. The hybrids were clustered into 5 and 7 groups using molecular markers and morphological traits based diversity analysis, respectively. The linear correlation between morphological and molecular markers based similarity illustrated no significant correlation indicating that morphological descriptors and pedigree is not merely expression of SSR fingerprints and thus combining information of molecular markers and morphological data will be appropriate for diversity analysis in potato, particularly when the number of molecular markers used in the study are less in number.

KEYWORDS: Molecular characterization, potato, SSR markers

INTRODUCTION

Potato belongs to the family *Solanaceae* and it is an economically important crop with potential to provide 'nutritional food security' worldwide including developing countries especially India, where the humble spud is the staple food of millions. Traditionally, potatoes prefer cool, long days of summer season in the hilly, temperate areas. On the contrary, about 90% of the potatoes are grown in the short days of winter season in Indian sub-tropical plains. The high yielding, indigenous 58 potato varieties and agro-techniques developed by the ICAR-Central Potato Research Institute, Shimla have made tremendous impact on potato production in the country. Consequently, India ranked 2nd globally with an annual production of 48.605 million tons during 2017 (FAO Stat, 2017) with national yield reaching an average of 24 tonnes per hectare. It has been observed that the productivity of major food crops have attained a plateau and conventional efforts by the breeders have, in general, failed to break the yield barriers. Potato is no exception. In fact, potato researchers across the globe are in pursuit of the reasons for stagnant productivity levels for decades. One of the main, unanimously accepted reasons cited in literature is the narrow genetic base of the improved cultivars. One of the oldest landrace Rough Purple Chili believed to be originated from Chili is present in the pedigree of most of the modern American

[1]ICAR-Central Potato Research Institute, Shimla, Himachal Pradesh - 171 001
[2]ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi - 110 012
[3]ICAR-Central Potato Research Station, Kufri - 171 012, Himachal Pradesh, India
E-mail: vinaycpri@gmail.com

varieties (Bethke *et al.*, 2014). Similar is the situation, in the Indian potato varieties where some of the researchers have reported narrow genetic base (Chimote *et al.*, 2004, 2007; Gopal and Oyama, 2005) while Chakrabarti *et al.* (1999) and Pattanayak *et al.* (2002) reported broad genetic base using molecular markers. These studies were undertaken either using commercial cultivars or advanced hybrid selections which were bred keeping in mind late blight resistance which still remains a menace in almost all the potato growing regions of the country. Thus appropriately, we can find Kufri Jyoti as one of the parents in many such selections. However, with time many more germplasm accessions were imported and the repository augmented at ICAR-CPRI, Shimla. Thus providing better and improved parental lines to the breeders possessing resistances to biotic, abiotic stresses quality traits.

It is also well established that heterosis for the traits of interest can be realized when hybridization among diverse parents is attempted. Diversity analysis in potato has been done using both morphological and molecular markers (Chimote *et al.*, 2007; Tiwari *et al.*, 2018, 2019). Morphological markers are often considered to be unreliable being dependent on environment, yet are significant as present day protection of varieties based on Distinctness, Uniformity and Stability (DUS) parameters is done using morphological markers only. Molecular markers like RFLP (Restriction Fragment Length Polymorphism), SSR (Simple Sequence Repeats), ISSR (Inter Simple Sequence Repeats) etc. though considered robust for diversity analysis, yet, are also marred with similar reproducibility concerns like Randomly amplified polymorphism (RAPD) sequence characterized amplified region (SCAR) etc. Moreover, the results may also vary from lab to lab owing to the protocols/instruments/chemicals used. Besides, the molecular markers cover only a small percentage of total genome and thus cannot be a true representative of the complete genetic diversity. Thus, it seems plausible to analyze genetic diversity using both molecular and morphological markers. Simple Sequence Repeats (SSR) are considered an ideal fingerprinting marker system that creates complex banding patterns by simultaneously detecting multiple loci; are economic and possess high reproducibility. SSR markers have been extensively used in potato in different genetic studies including linkage mapping, germplasm surveys and phylogenetic analysis (Veilleux *et al.*, 1995; Milbourne *et al.*, 1998; Ashkenazi *et al.*, 2001; Ghislain *et al.*, 2004; Feingold *et al.*, 2005).

This study aims to evaluate genetic diversity in recent Indian potato advanced hybrids using previously described 15 highly distinctive SSR markers providing information for all potato chromosomes and to compare the efficiency of these DNA based SSR markers with morphological descriptors.

MATERIALS AND METHODS

DNA Extraction

Plant material in this study consisted of 39 potato advanced hybrid lines (**Table 1**) maintained at experimental fields at ICAR-Central Potato Research Station, Kufri, Himachal Pradesh (31.10°N 77.25°E, 2,290 masl). The genomic DNA from leaf tissue of each genotype of the population was extracted with the Gene elute plant Genomic DNA Purification miniprep kit G2N70 (Sigma Aldrich, USA). The extracted DNA was quantified by Spectrophotometery (UV-1700 Pharma Spec. Shimadzu, spectrophotometer) and qualitatively checked on 0.8% Agarose gel.

Table 1. Advanced hybrid lines/ cultivars used for SSR and morphological analysis.

Accession No. at ICAR-CPRI	Variety/ advance hybrid	Parentage	Acc. no	Variety/ advance hybrid	Parentage
CP 4105	MP/98-71	MP/92-30 × MP/90-94	CP 4217	J/99-48	MS/J92 × CP1406
CP 4117	MP/98-172	MP/90-84 × MP/92-35	CP 4218	J/99-242	MS/83-398 × Kufri Sutlej
CP 4126	MP/99-322	MP/91-76 × MP/92-35	CP 4120	MS/00-3740	JE/812 × CP1704
CP4118	MP/99-406	MP/91-76 × MP/92-35	CP 4128	MS/00-3808	JW/160 × MS/89-1095
CP 3853	J/92-159	JN 2207 × Kufri Jyoti	CP 3895	MS/93-1344	MS/81-145 × PH/F-1545
CP 3893	J/93-86	MS/82-638 × Kufri Pukhraj	CP 3898	MS/95-1309	MS/83-398 × JI/1857
CP 3900	J/95-227	JY/712 × Kufri Jyoti	CP 4115	MS/99-1871	PH/F1045 × MS/82-638
CP 3902	J/95-242	JY/712 × Kufri Jyoti	CP 4104	SM/92-338	HB/82-372 × JEX/C-166
CP 4121	J/95-378	CP2359 × CP2383	CP 4106	SM/94-44	HB/83-39 × LT-5
CP3901	J/95-229	JY/712 × Kufri Jyoti	CP 4107	SM/95-43	CP/3280 × CP2132
CP 4110	J/95-144	CP1588 × MS/82-797	CP 4102	SM/96-127	Kufri Jyoti × HB/83-39
CP 4111	J/96-80	CP2383 × Kufri Pukhraj	CP 4108	SM/98-239	CP3255 × HB/83-39
CP 4122	J/96-84	CP2383 × Kufri Pukhraj	CP 3913	DSP-19	MF-1 × TPS-13
CP4112	J/96-149	Kufri Jyoti × CP2383	CP 3912	DSP-7	MF-1 × TPS-13
CP 4123	J/96-171	CP2287 × Kufri Pukhraj	CP 4103	KS/96-725	QB/A × 9-120CP3336
CP4114	J/97-204	Kufri Ashoka × MS/82-797	----------	2000P-55	91P-27 × CP3192
CP 4113	J/96-238	CP2287 × CP2383	CP 4101	PP-2500	Clonal selection from white skin variety from Pahalgaon
CP 4125	J/97-243	Kufri Ashoka × JEX/A-805	CP 4100	PP-48	Clonal selection from Red skin variety from Pahalgaon
CP 4124	J/97-168	CP2359 × CP2383	CP 3871	B-420	CIP 387415.47 × 389746.2

SSR PCR Amplification

The extracted DNA was PCR amplified using selective fifteen highly polymorphic microsatellite markers (**Table 2**) with high polymorphism and distinction ability. Each PCR reaction was performed in a 10 µl reaction volume containing 0.5 µl of 10 µm each forward and reverse primer, 1 µl of 10 × PCR Gold buffer, 0.8 µl of 2.5 mM $MgCl_2$, 1 µl of 10 Mm dNTPs, 0.2 units of AmpliTaq Gold DNA polymerase (Applied Biosystems, USA) and 2 µl of 50 ng genomic DNA. PCR Amplification was carried out using gradient PCR thermocycler system C-1000 (Bio Rad, USA). PCR Product (5 µl) for each marker was first separated and visualized as a single fragment on 2% agarose gel, stained with non-carcinogenic dye (Gel Red, Nucleic Acid stain -Biotium). One µl of PCR product was further separated for allele detection on a chip based nucleic acid separating system Agilent bioanalyser, 2100 (CA, USA) using prepackaged reagents (DNA 1000 Agilent kits). Data was collected and displayed as a gel-like image and/or electropherogram with automated sizing and quantitative information (fluorescence intensity/ versus base pair size/migration time) in a digital format, operated through dedicated software package (The 2100 expert software).

SSR statistical Data Analysis

The PCR profiles produced by SSR markers were scored manually for each allele as the binary data obtained as 1 for presence and 0 for absence for each SSR locus.

Table 2. SSR marker's detail.

Marker/ Locus	Ch	Repeat motif	Primer Sequence(5′-3′)	Appoximate Fragment Size (bp) Agarose Gel (2%)	Anneling temp	Reference
STIKA/ STM1020	III, V	$(T)_{12}(A)_9(ATTCTTGTT(TA)_2CA(TA)_7$	F-TTC GTT GCT TAC CTA CTA R-CCC AAG ATT ACC ACA TTC	216	50	Provan *et al.*, 1996., Milbourne *et al.*, 1998
STINHW/ STM1021	IX	$(CT)_3$ TT$(CT)_8$ $(AT)_9$	F-GGAGTCAAAGTTTGCTCACATC R-CAC CCT CAA CCC CCA TAT C	181	55	Provan *et al.*, 1996
STM07	XII	$(AC_{)9}$	F-GGA CAAGCTGTGAAGTTTAT R-AATTGAGAAAGAGTGTGTGTG	178	54	Milbourne *et al.*, 1998
STU 06/ STM1045	II, XII	$(TGG)_5$	F-GAA GTT TTA TCA GAA TCC R-ATC ACC TCA TCA GCA ATC	181	55	Milbourne *et al.*, 1998
STM0037	X1	$(TC)_5$ (AC)6AA….(AC)7 (AT)4	F-AAT TTA ACT TAG AAG ATT AGT CTC R-ATT TGG TTG GGT ATG ATA	90	55	Ghislain *et al.*, 2004, 2009
STM0031	VII	$(AC)_5$ (AC)3 (GCAC)2	F-CATACG CAC GCA CGT ACA C R-TTC AAC CTA TCA TTT TGTGAGTCG	172	58	Ghislain *et al.*, 2004, 2009
POTM 1-2	ND	$(AT)_{20}$	AATAATACTGTGATGCCACAATGG GTGGCATGTCTTCGAAGGTAC	200-246/221	56	Coombs *et al.*, 2004
STM 1064,	II	(TA)12 (TG)4 GT (TG)5	GTTCTTTTGGTGGTTTTCCT TTATTTCTCTGTTGTTGCTG	204	55	Ghislain *et al.*, 2004, 2009
STM1016,	II	$(TCT)_9$	TTCTGATTTCATGCATGTTTCC ATGCTTGCCATGTGATGTGT	250/243-262	55	Milbourne *et al.*, 1998
STM2013	VII	$(TCTA)_6$	TTCGGAATTACCCTCTGCC R-AAAAAAAGAACGCGCACG	160	55	Milbourne *et al.*, 1998
STM1106	X	$(ATT)_{13}$	TCCAGCTGATTGGTTAGGTTG ATGCGAATCTACTCGTCATGG	156 /145 -211	55	Ghislain *et. al.*, 2004, 2009
STM1052	IX	(AT)14GT (AT)4 (GT)6	CAATTTCGTTTTTTCATGTGACAC ATGGCGTAATTTGATTTAATACGTAA		58	Ghislain *et al.*, 2004, 2009
STM5114	II	(ACC)n	AATGGCTCTCTCTGTATGCT GCTGTCCCAACTATCTTTGA	297 -322	56	Ghislain *et al.*, 2004, 2009
STM0032	XII	(AC)7 (AC)5	GGCTGCAGGAATTATGTGTTC GATGTAAAACACGTGTGCGTG	150-175	54	Milbourne *et al.*, 1998
Stg0016	I	(AGA)n	AGCTGCTCAGCATCAAGAGA ACCACCTCAGGCACTTCATC		55	Ghislain *et. al.*, 2004, 2009

Genetic diversity was calculated at each locus for allelic Polymorphism Information Content (PIC) as described by Nei, 1973. The values for each SSR were estimated by determining the frequency of alleles per locus using the following formula: $PIC=1-\sum x_i^2$ where x_i is the relative frequency of the i^{th} allele of the SSR loci. Markers were classified as informative when PIC was ≥ 0.5. Marker index (MI) was calculated using the formula MI= EMR (Effective multiplex ratio × PIC, where EMR is the product of total number of bands obtained per primer (n) and the fraction of polymorphic bands (β). Resolving power of each marker was calculated using the formula: $Rp=\sum Ib$, where Ib is the band informativeness and Ib = (1-2 X (0.5-p), where p is the proportion of genotype containing the band (Prevost and Wilkinson, 1999).

Morphological data analysis:

Phenotypic data for only 43 DUS (Out of 51) qualitative traits based on guidelines for conduct of DUS for potato germplasm identification were scored from ICAR-CPRI, Shimla germplasm breeder's record for advanced hybrids/variety. The morphological qualitative data were converted to binary data for each trait before subjecting to the statistical analysis.

Data analysis : Cluster analysis was performed with NTSYS pc version 2.1 (Rohlf, 2000) and XLSTAT based on an un-weighted pair group method with Arithmetic means (UPGMA) using SAHN programme (Sequential, algorithm, hierarchical and nested clustering parameters) of this system. SIMQUAL function of this programme was used for obtaining similarity matrix values based on Jaccard's co-efficient for SSR data as well as the morphological data. Excel spreadsheet was run through XLSTAT software to perform genetic relationship of all the 39 advanced hybrids using Principle Co-ordinate Analysis (PCoA).

Correlation test **:** Mental test was performed using MAXCOM function using XLSTAT 2015 to test the correlation between the similarity matrices of the two marker systems i.e. SSR markers and morphological markers.

RESULTS AND DISCUSSION

Marker performance

All the 15 SSR primers used to genotype 39 advanced hybrids produced good quality, reproducible, amplified bands of size 73-482 bps. The study detected 3 to 21 alleles at each locus with minimum allele count for STU 06 and maximum for STM5114 and STM1052, and an average of 13.3 alleles per primer. A total of 205 polymorphic fragments were amplified. Overall marker performance was assessed considering three important parameters: polymorphic information content, marker index and resolving power (**Table 3**). PIC values ranged from 0.52 to 0.92 with highest PIC value for primer STM5114 (0.92) and lowest for STM07 (0.52). Average PIC value per primer was 0.84**.** With a large no of fragments detected by SSR primers, highest effective multiplex ratio depends upon the number for polymorphic bands (β), as observed for marker STM5114. To determine the general usefulness of the markers used, marker index (MI) was calculated. The highest MI was also observed for marker STM5114 and lowest for STM07 with average of 11.6 per marker. There was a positive correlation between the values of MI and PIC (r= 0.8). The resolving power (RP) that determines the discriminatory potential of the primers was highest for marker STM0032. Average resolving power was 6.9 per primer. There was significant correlation between the values of RP and MI (r=0.66). Thus, all the primers selected were considered good for evaluating similarity relationship and genetic diversity in the present study. SSR profiles

of a representative gel of primer STM1052 and STM1064 is shown in **Fig. 1**.

Cluster analysis

The diversity of the 39 potato advanced hybrids was calculated using Jaccard's genetic similarity coefficients with all the fifteen markers using XLSTAT (**Fig. 2**). Pair wise similarity coefficient was lowest (0.08) between accession SM/95-43 & J/96-149 and highest (0.54) between accession SM/94-44 and J/99-242. Taking the threshold cut off point of the similarity coefficient at 0.25; all the genotypes were differentiated into 5 major groups/clusters. The first cluster constituted two accessions, the second cluster comprised of 30 accessions and cluster 3 consisted of 5 accessions. Whereas, cluster 4 constituted one accession and cluster 5 also had only one accession which was an outlier (**Table 4**).

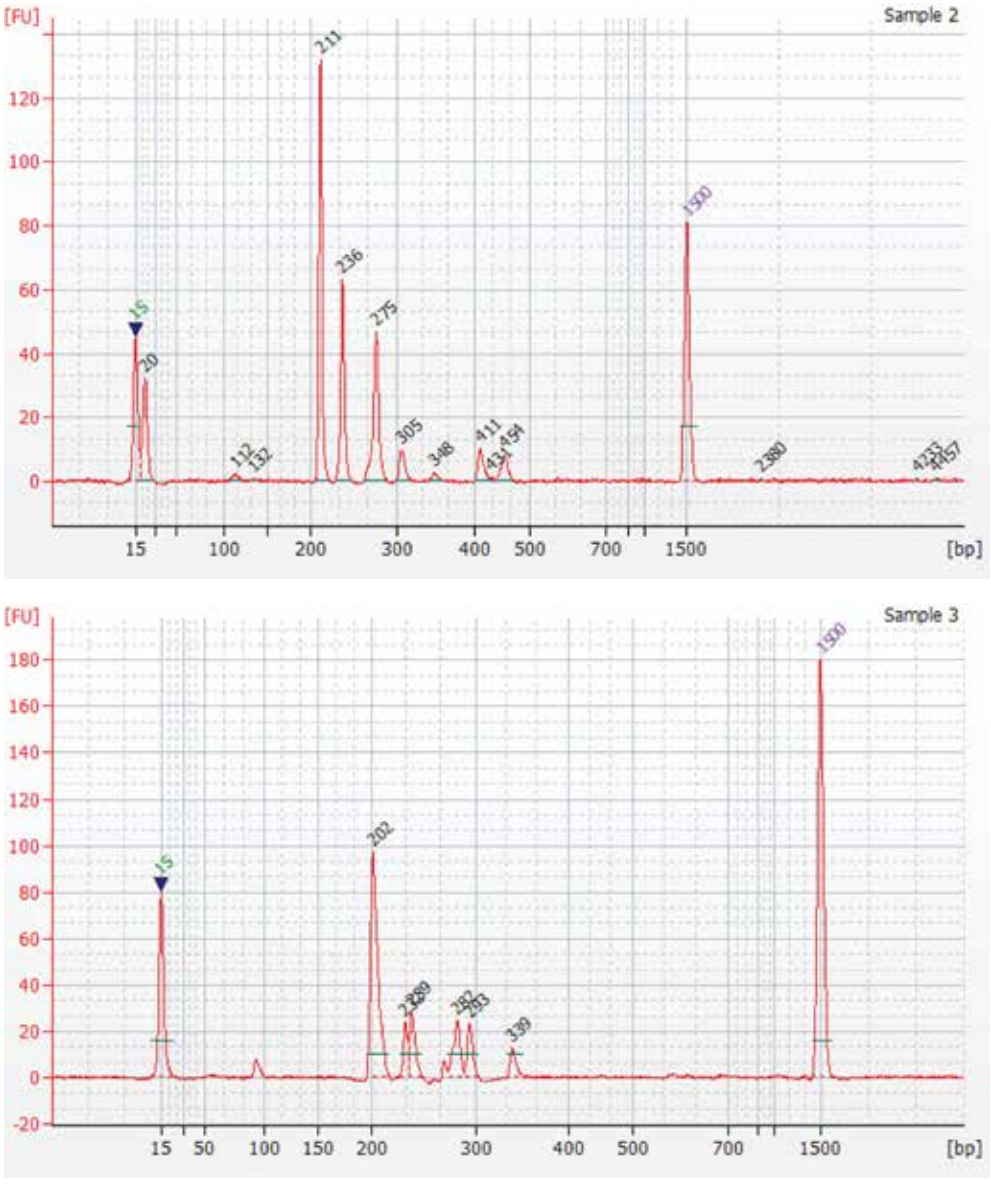

Fig. 1. Gel image electropherogram of SSR marker STM1052, and STM1064 generated through Bioanalyser 2100.

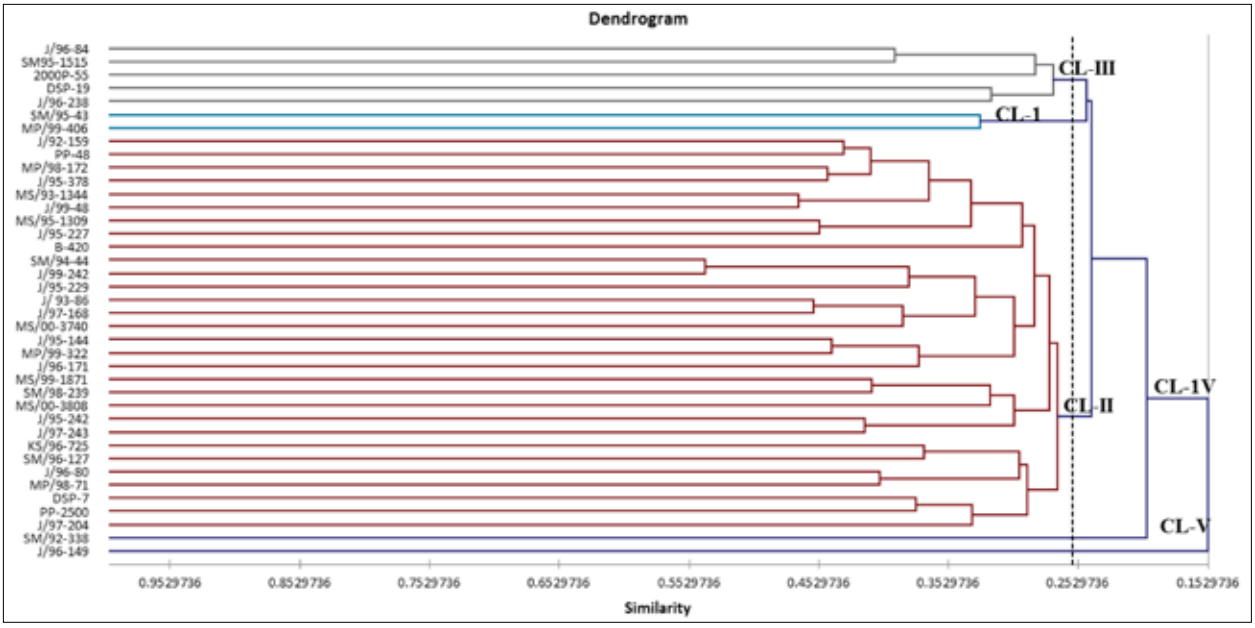

Fig. 2. Genetic diversity analysis dendogram using jaccards similarity coefficient of 39 advanced potato hybrid lines using 15 SSR markers.

Table 3. Marker informativness/performance for 15 SSR Markers.

Primer Name	No of polymorphic alleles (np)	β = np/ (total alleles)	Rate of polymorphism (%)	Effective Multiplex Ratio (np × β)	PIC* : 1-∑fi	Marker index (PIC × EMR)	Resolving Power Rp=∑Ib**
STIKA	8	1	100	8	0.84	6.72	6.20
STM07	3	1	100	3	0.52	1.56	3.12
STU06	6	1	100	6	0.77	4.62	4.56
STINHW	12	1	100	12	0.90	10.8	7.28
STM0031	16	1	100	16	0.88	14.08	7.79
STM0037	5	1	100	5	0.77	3.85	4.61
POTM1-2	18	1	100	18	0.91	16.38	5.48
STM 1064	11	1	100	11	0.82	9.02	6.76
STM 2013	15	1	100	15	0.89	13.35	8.97
STM1016	17	1	100	17	0.87	14.79	5.43
STM1106	17	1	100	17	0.89	15.13	5.48
STM1052	21	1	100	21	0.91	19.11	9.38
STM5114	21	1	100	21	0.92	19.32	9.02
STM0032	16	1	100	16	0.90	14.4	9.69
STg0016	14	1	100	14	0.90	12.6	10.41
Average				13.3	0.84	11.17	6.9

Polymorphic information content (PIC), EMR (Effective Multiplex Ratio), Ib is the band informativeness {1-2 × (0.5-p)}, where p is the proportion of genotype containing the band.

Principal Co-ordinate analysis

To better understand the relationships among the accessions, PCoA was conducted using the genetic similarities data set. The matrix of genetic relationship based upon jaccards distance coefficient visualized by performing PCoA, showed two significant axes. The first three principal axes accounted for 8.69, 7.42 and 6.53% of the total variation, respectively and the cumulative percentage of the eigen values accounted for 22.64% of the variation observed in the genotypes. The two-dimensional plot generated from PCoA was largely congruent with that generated by UPGMA clustering (**Fig. 3**).

Comparative utility of five best SSR markers STIHW (0.90), POTM1-2 (0.91), STM1052 (0.91), STM5114 (0.92) and STM0032 (0.90) having highest PIC value was done by cluster and PCoA. In the SSR dendogram, the genetic similarity coefficient ranged from 0.29 to 0.52 (**Fig. 4**). At cut off similarity coefficient of 0.24, all the 39 cultivars could be differentiated into 9 major clusters. The first cluster contains maximum number of 17 cultivars and rest of the eight groups contains 3, 2, 2, 2, 8, 2, 2, 1 genotypes, respectively (**Fig. 5**). PCoA plot allowed a better and clear separation of genotypes and was overall similar to PCoA performed using all the fifteen SSR markers (**Fig. 7**).

Morphological marker analysis:

Dendrogram tree generated through Similarity analysis data of 43 (DUS) morphological traits was based on jaccards coefficient since the qualitative data was converted into binary data. The similarity coefficient ranged from 0.72 to 0.38. Pairwise highest similarity was found between J/95-144 & J/93-86 (0.72) and lowest between SM/96-127 & J/96-149 (0.14) (**Fig. 5**).At cut off value of 0.38 all the accessions were classified into 7 major clusters. Two major clusters were having 16 and 14 genotypes. One clusters

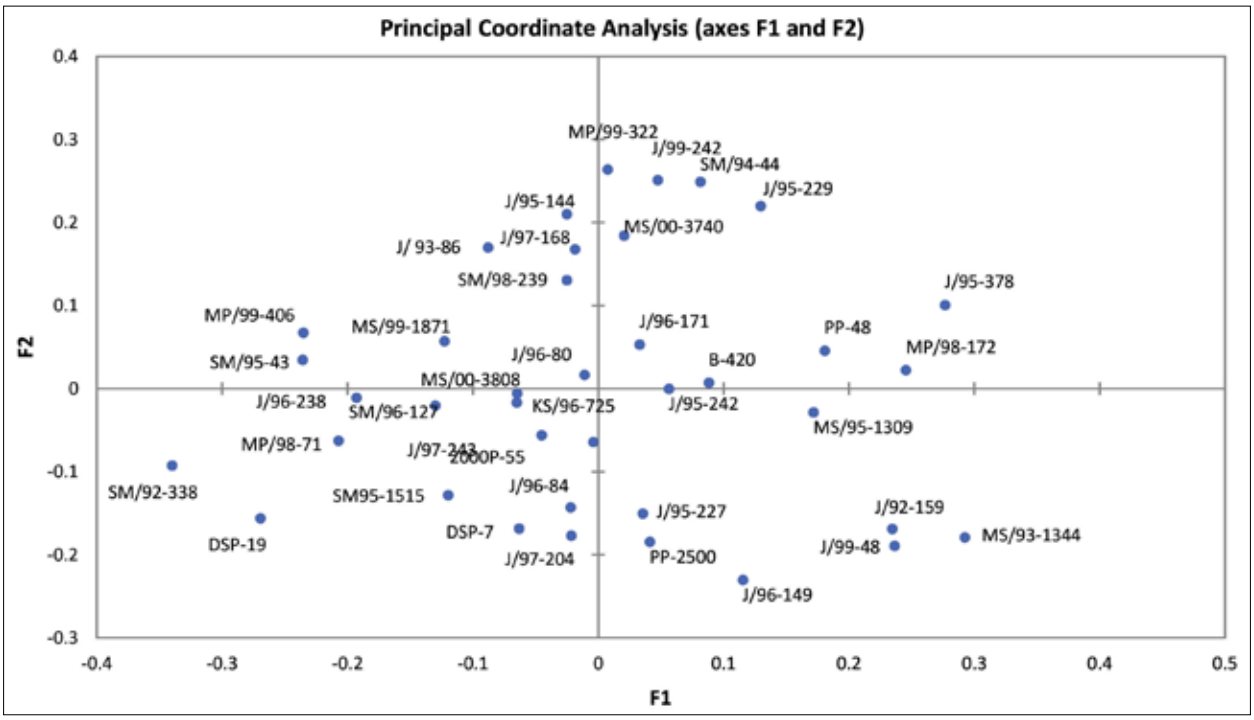

Fig. 3. Principle coordinate analysis (PCoA) for 15 SSR markers as applied on 39 advanced Indian potato hybrids.

with 3 genotypes and two clusters with a pair of genotypes each. Whereas, two clusters comprised single genotype each (**Table 4**). Principal Coordinate analysis (PCoA) showed 10.1, 19.7, and 17.9 of the total variation for the first three co-ordinates that accounted for a Cumulative variation of 27.7 % (**Fig. 6**) and all the hybrids were dispersed on the PCo A plot.

Combined SSR and morphological markers analysis:

The binary data pooled using both the morphological and SSR markers were further assessed for any evident change in divergence or delineation of the studied potato hybrids in different clusters/groups. Dendogram generated depicted a slight change in similarity coefficient range that ranged from 0.51 to 0.31. Pair wise similarity for diversity of the hybrids was lowest **(Fig. 7)** and **(Fig. 8)** between SM/96-127 & J/96-149 (0.14) and highest between J/95-144 - J/ 93-86 (0.51).

Mantel matrix correlation test

The Mantel test computed to test the linear correlation between two (DUS and SSR) similarity proximity illustrated no significant correlation, p= 0.794 i.e. $p > 0.05$. Thus, the degree of morphological difference between the advanced potato hybrids was not related to the genotypic differences using SSR markers.

Currently molecular markers based studies for evaluating genetic diversity in different crops have been published. However, International Union of Plant Variety Protection (UPOV) still considers morphological DUS characters as a major decisive factor for uniqueness, distinction,and stability to settle intellectual property rights issues (Gopal *et al.,* 2007). The aim of the present study was to test highly polymorphic, co-dominant SSR markers for assessment of genetic diversity in recent tetraploid Indian potato advanced hybrid selections *vis-à-vis* morphologically defined DUS characters Hence, in the present investigation we first assessed the efficiency of SSR markers for the detection of genetic diversity (**Fig. 2**) using multivariate analysis. Efficiency of polymorphism was evaluated through various statistical parameters such as polymorphic information content,

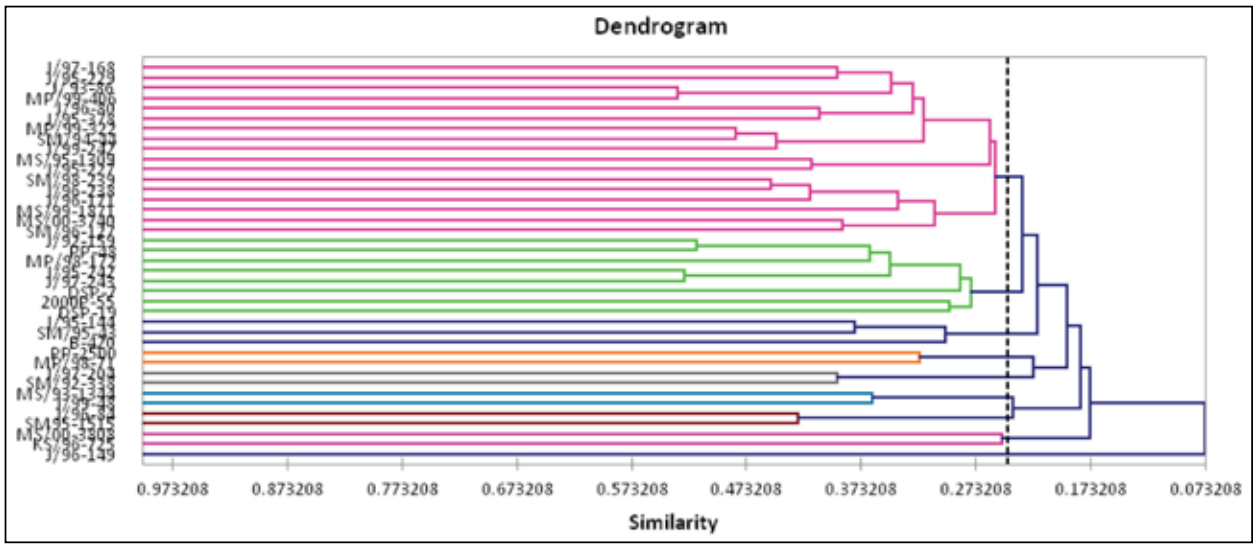

Fig. 4. Genetic diversity analysis dendogram using jaccards similarity coefficient of 39 advanced potato hybrid lines with 5 SSR markers.

markerindex, and resolvingpower. Markers with many alleles, or highly polymorphic markers, tend to be highly informative also. PIC Value of each primer was analysed with mean of all alleles for each SSR marker (Sharma *et al.*, 2014). All the 15 primers used in this study were having PIC value greater than 0.5 indicating high in formativeness. The

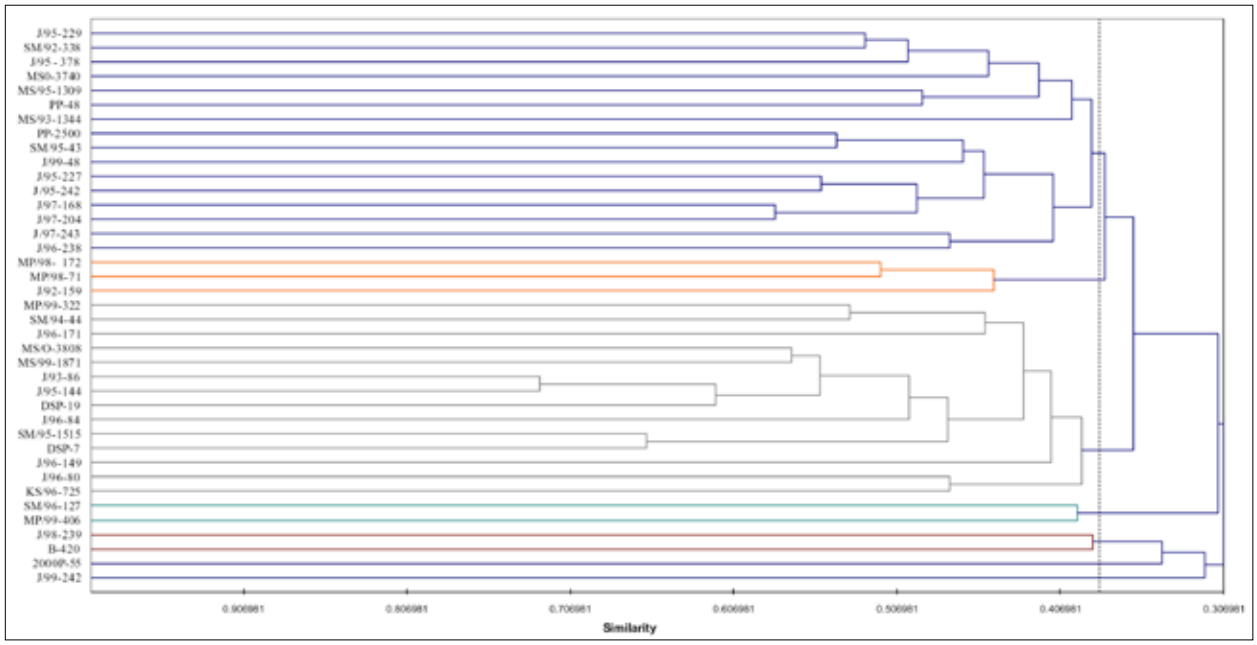

Fig. 5. Genetic diversity analysis dendogram using simple matching coefficient of 39 advanced potato hybrid lines using DUS characters.

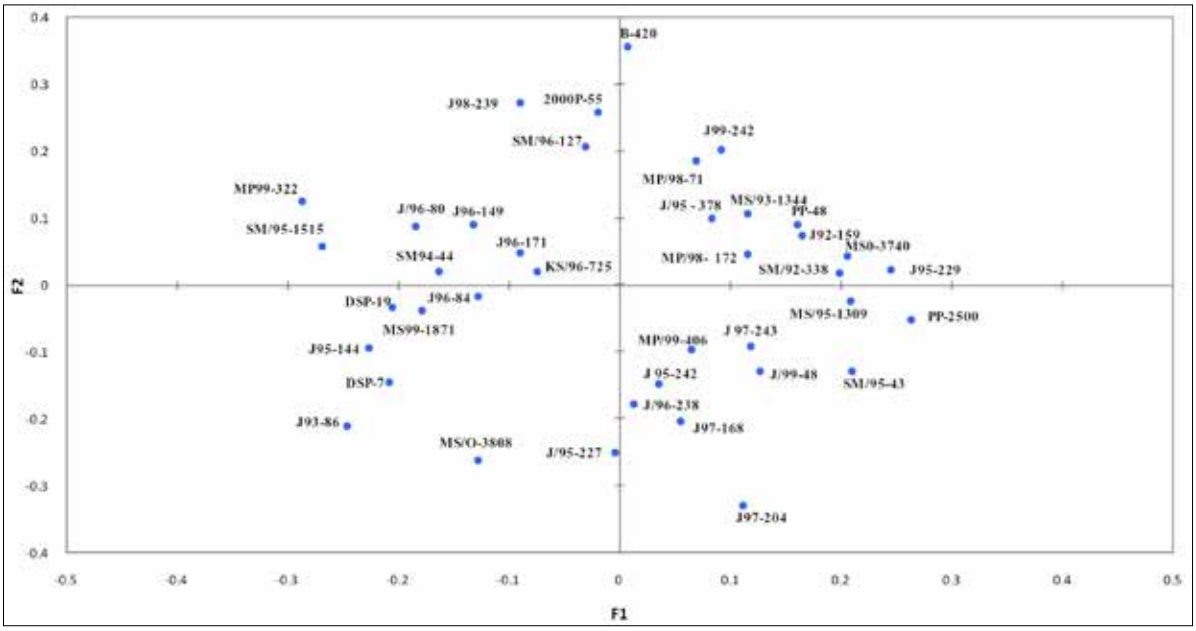

Fig. 6. Principle coordinate analysis (PCoA) for morphological markers as applied on 39 advanced Indian potato hybrids.

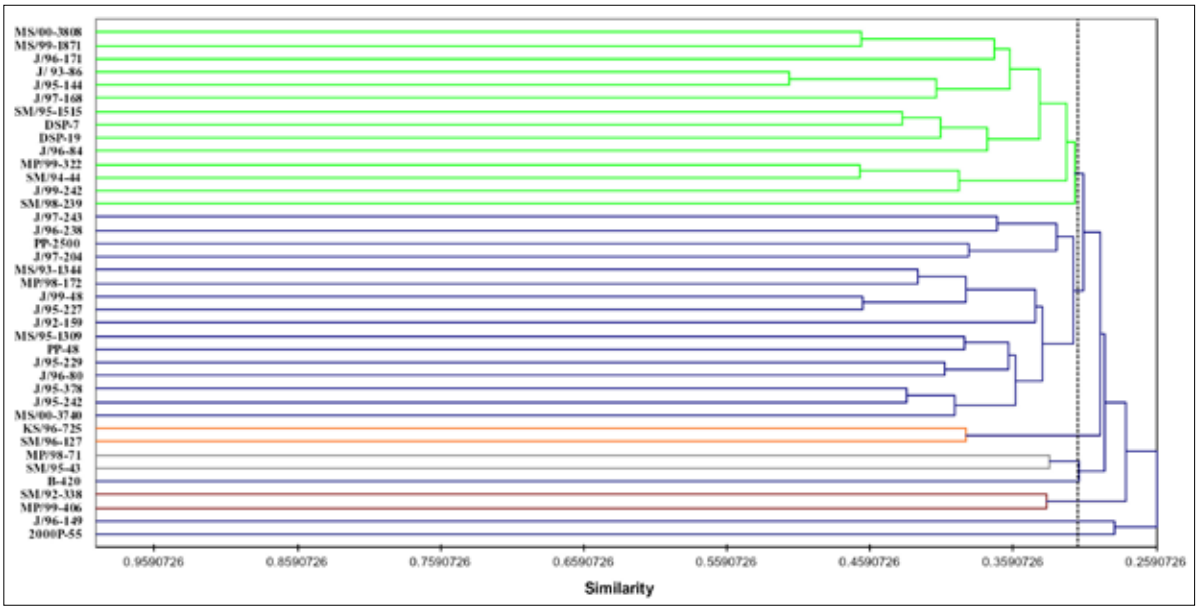

Fig. 7. Genetic diversity analysis dendogram using jaccards coefficient of 39 advanced potato hybrid lines using morphological and dus markers.

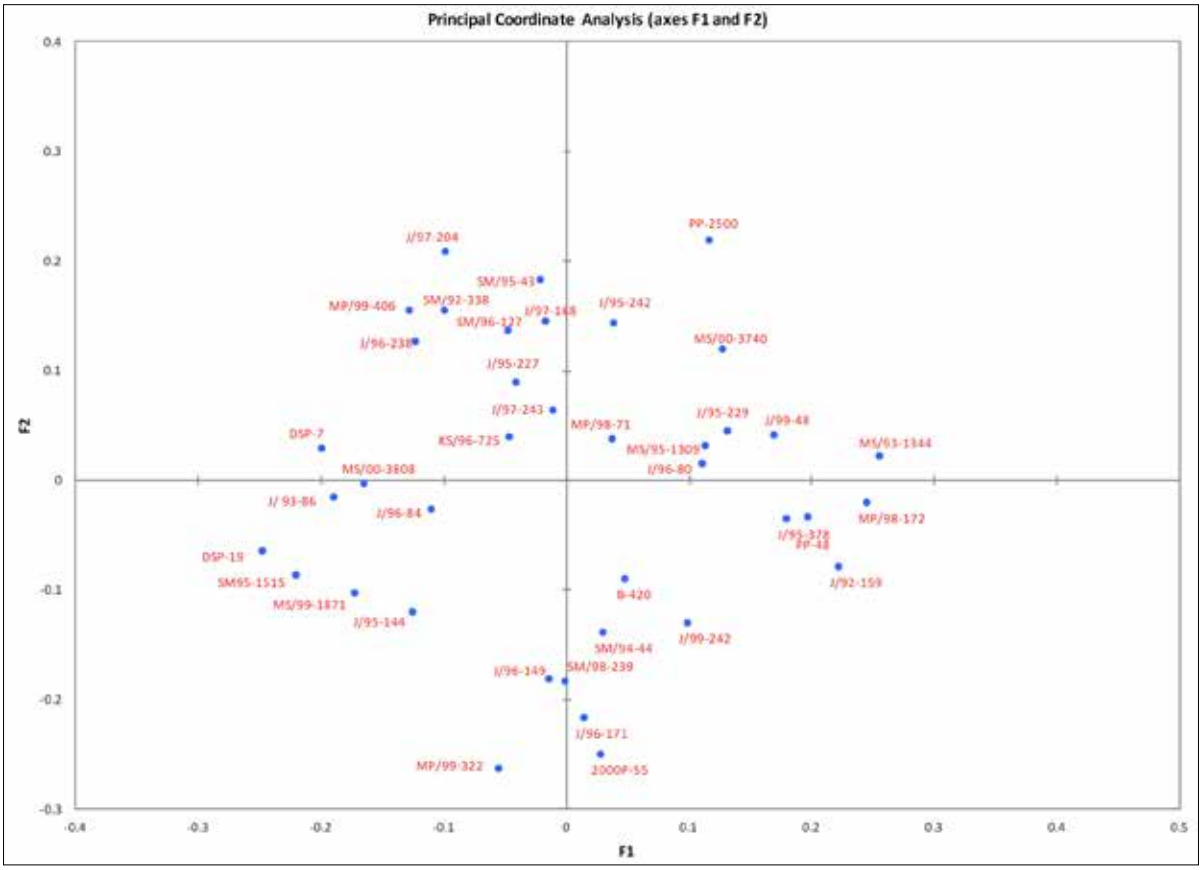

Fig. 8. Principle coordinate analysis (PCoA) for SSR morphological markers as applied on 39 advanced Indian potato hybrids.

Table 4. Cluster grouping in 39 advanced hybrids through SSR and molecular markers.

Cluster Code	SSR Markers	Morphological Markers	SSR_DUS
	Accessions/(No.)	Accessions (No.)	Accessions(No.)
I	MP/99-406,SM/95-43 (2)	MP/99-406,SM/96-127(2)	MP/99-406, SM/92-338(2)
II	MP/98-71, SM/96-127, KS/96-725, J/97-204, PP-2500, J/97-243, J/95-242, MS/00-3740, PP-48, J/95-378, MP/98-172, J/95-227,MS/95-1309, J/99-48, B-420, J/92-159, MS/93-1344, J/96-80, J/95-229, J/97-168, J/99-242, SM/94-44, MP/99-322, J/95-144, J/96-171, SM/98-239, MS/99-1871, MS/00-3808, DSP-7, J/93-86 (30)	SM/95-43, J/96-238, SM/92-338, J/97-204, PP-2500, J/97-243, J/95-242, MS/0-3740, PP-48, J/95-378, J/95-227, MS/95-1309, J/99-48, MS/93-1344, J/95-229, J/97-168(16)	SM/95-43, MP/98-71(2)
III	J/96-238, SM/91-1515, J/96-84, DSP-19, 2000P-55(5)	MP/98-71, MP/98-172, J/92-159(3)	J/96-238, J/97-204, PP-2500, J/97-243, J/95-242, MS/00-3740 PP-48, J/95-378, MP/98-172, J/95-227, MS/95-1309, J/99-48, J/92-159, MS/93-1344, J/96-80, J/95-229 (16)
IV	SM/92-338(2)	KS/96-725, J/96-80, SM/94-44, MP/99-322, J/95-144, J/96-171, MS/99-1871, MS/O-3808, DSP-7 J/93-86, SM/95-1515, J/96-84, DSP-19, J/96-149(14)	SM/96-127, KS/96-725(2)
V	J/96-149 (1)	B/420, J/98-239(2)	B-420(1)
VI		J/99-242(1)	J/97-168, J/99-242, SM/94-44, MP/99-322, J/95-144, J/96-171, SM/98-239, MS/99-1871, MS/00-3808, DSP-7, J/93-86, SM/95-1515, J/96-84, DSP-19(14)
VII		2000P-55(1)	2000P-55(1)
VIII			J/96-149(1)

average PIC value for each primer was 0.84, with highest PIC value observed for primer STM5114 (0.92), STM1052 (0.91), STINHW (0.90), POTM1-2 (0.91), and STM0032 (0.90). Since, the set of primers chosen revealed high polymorphic information content and were located on different potato chromosomes representing large genomic coverage, these were considered for similarity, relationships and diversity analysis for Indian potato advanced hybrids and varieties. The other parameter, marker index is also a good indicator to gauge the efficiency of polymorphism, ranged from 1.58 to 19.36 with average marker index of 11.7. Marker index was derived from effective multiplex ratio. Marker STM5114 was having highest PIC and marker index values. High significant positive correlation was found between PIC and MI values (r=0.81) indicating that both the statistical parameters can be used to evaluate the information content generated by SSR markers. The third parameter that measures the discriminatory potential of SSR marker, the resolving power, ranged from 3.12 to 10.41 with an average of 6.9 per primer suggesting that the set of primers chosen were largely capable of distinguishing among different genotypes. Significant correlation between Marker index and resolving power values was observed (r=0.66). Thus, these parameters can be effectively used for diversity analysis among different genotypes. Based on these parameters, the study could identify 5 most informative markers that could distinguish all the 39 Indian potato hybrids, thus adding to the exiting information with regard to suitability of markers in identification and

fidelity testing in Indian potato cultivars as suggested by earlier workers (Chimote *et al.,* 2005; Chakrabarti *et al.,* 2006). However, Chimote *et. al*. (2007) observed that only two SSR primers were capable of distinguishing all the Indian potato varieties. To test his hypothesis, we added the data generated by primers Stu 06 and Stika in the present study with earlier studies and conducted similarity analysis of 81 (42 potato varieties and 39 hybrids of present study) genotypes which revealed that these two primers could not distinguish all the 81 genotypes (data not shown). This is theoretically expected also as the number of genotypes will increase, increased number of markers will be required to distinguish them. This is probably the reason also that UPOV is not allowing exclusive use of molecular markers for varietal identification, though marker data can be used as supplementary information. Hence, in future, we may still need more number of stable molecular markers to distinguish the increased number of potato genotypes.

In the present study, the pair-wise similarity between advanced hybrid selections ranged from 0.08 to 0.54 based on SSR marker data and 0.16 to 0.72 based on morphological data. When both the data sets were pooled, the pair wise similarity ranged from 0.14 to 0.51. The lower range of similarity is expected from marker data as these represent only very small, conserved regions of the genome. However, the combined results revealed that these advanced hybrid selections are fairly dissimilar to each other as against the notion that the Indian potato breeding programme has resulted in the narrow genetic base of the varieties and advanced hybrids (Chimote *et al.,* 2004; 2007; Gopal and Oyama, 2005). The narrow genetic base of the varieties may be attributed to the fact that till early 1990s, the focus of the breeding programme was to develop mainly late blight resistant high yielding varieties for table purpose only. Consequently, limited numbers of improved parents deriving resistance initially from *S. demissum* and later from *S. tuberosum* ssp. *andigena* were extensively used. However, during the last two decades the breeding objectives were redefined and separate programmes were initiated to develop and deploy region specific improved cultivars including processing cultivars to cater to the specific needs of the processing industry. The set of 39 hybrids used in the present study is thus comprised of hybrids developed for processing purposes, early maturity, possessing late blight moderate resistance for cultivation in plains and high level of resistance for cultivation in the hills. Further, these hybrids are comprised of 7 pairs of full-sibs and 47 half-sibs. The similarity values ranged from 0.24 to 0.42 among full-sibs and 0.14 to 0.38 among half-sibs. This study also is in agreement with the results published earlier by several workers (Kujal *et al.*, 2005; Demeke *et al.*, 1996; Forapani *et al.*, 1999) who could not relate diversity analysis to pedigree of the varieties/ breeding lines. Theoretically, it can be expected also as the highly heterozygous and tetraploid genome of potato provides opportunity for a large number of permutations and combinations to occur during meiosis giving rise to highly segregating progeny. This may probably be the reason that often one popular parent, like Rough Purple Chili in US varieties and Kufri Jyoti in Indian varieties/ advanced breeding lines is found because this type of parent with good general combining ability will continuously produce progenies with desired traits and will result in development of many popular varieties (Shandil *et al*., 2017, Jansky *et al.,* 2018).

CONCLUSIONS

Present study concludes that morphological descriptors and pedigree is

not merely expression of SSR fingerprints and the distribution of potato hybrids was independent of their origin or pedigree. It is also evident from our results that only SSR markers based data revealed less similarity as against only morphological data. Thus, it will be prudent to combine both the data sets for undertaking diversity analysis in potato. The fact that the 39 advanced breeding selections studied are diverse further support our belief that the region specific or requirement specific breeding programmes being practiced at present needs to be further strengthened by integrating marker aided selection for the traits of interest which will result in development and deployment of genetically diverse potato varieties in the country. It is further added that five primers used in this study can clearly differentiate all the potato hybrids thus can serve as a reliable tool for genetic identification at present but the number of primers will need to be increased with the increase in number of genotypes in future.

LITERATURE CITED

Ashkenazi V, Chani E, Lavi U, Levy D, Hillel J and Veilleux RE (2001) Development of microsatellites markers in potato and their use in phylogenetic and fingerprinting analysis. *Genome* **44**: 50-62

Bethke PC, Nassar AMK, Kubow S, Leclerc YN, Li XQ, Muhammad H, Molen T, Bamberg J, Martin M and Donnelly DJ (2014) History and Origin of Russet Burbank (Netted Gem) a Sport of Burbank. *Am J Potato Res* **91**: 594-609.

Bonierbale MW, Plaisted RL and Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. *Genetics* **120**: 1095-103

ChimoteVP, Chakrabarti SK, Pattanayak D and Naik PS (2004) Semi-automated simple sequence repeat analysis reveals narrow genetic base in Indian potato cultivars *Biol Plant* **48** (4): 517-22

Chimote VP, Pattanayak D and Naik PS (2007) Molecular and morphological divergence studies in Indian potato varieties. *Indian J Biotechnol* **6**: 216-223.

Chakrabarti SK, Pattanayak D, Sarkar D, Chimote VP and Naik PS (2006) Stability of RAPD fingerprints in potato: effect of source tissue and primers. *Biol Plant* **50** (4): 531-36

Coombs JJ, Frank LM and Douches DS (2004) An applied fingerprinting system for cultivated potato using simple sequence repeats. *Am J Potato Res* **81**: 243-50

Del Rio A, Bamberg J and Huaman Z (2006) Genetic equivalence of putative duplicate germplasm collections held at CIP and US potato genebanks. *Am J Potato Res* **83**: 279-85

Douches DS, Ludlam K and Freyre R (1991) Isozyme and plastid DNA assessment of pedigrees of nineteenth century potato cultivars. *Theor Appl Genet* **82**: 195-00

Feingold S, Lloyd J, Norero N, Bonierbale M, Lorenzen J (2005) Mapping and characterization of new EST-derived microsatellites for potato (*Solanum tuberosum* L.). *Theor Appl Genet* **111**: 456-66

Gebhardt C, Ritter E, Debener T, Schachtschabel U, Walkemeier B, Uhrig H, Salamini F (1989) RFLP analysis and linkage,mapping in *Solanum tuberosum*. *Theor Appl Genet* **78**: 65-75

Gebhardt C, Blomendahl C, Schachtschabel U, DebenerT, Salamini F and Ritter E (1989) Identification of 2n breeding lines and 4n varieties of potato (*Solanum tuberosum* ssp. *tuberosum*) with RFLP-fingerprints. *Theor Appl Genet* **78**(1): 16-22

Ghislain M, Trognitz B, del Rosario Herrera M, Solis J, Casallo G, Vasquez C *et al*. (2001) Genetic loci associated with field resistance to late blight in offspring of *Solanum Phureja* and *S. tuberosum* grown under short-day conditions. *Theor Appl Genet* **103**: 433-42.

Ghislain M, Spooner DM, Rodrıguez F, Villamon F, Nunez J, Vasquez C Waugh R and Bonierbale M (2004) Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. *Theor Appl Genet* **108**: 881–890

Ghislain M, Andrade D, Rodrıguez F, Hijmans RJ, Spooner DM (2006) Genetic analysis of the cultivated potato *Solanum tuberosum* L. Phureja Group using RAPDs and nuclear SSRs. *Theor Appl Genet* **113**: 1515-27

Ghislain M, Nunez J, Herrera MDR, Pignataro J, Guzman F, Bonierbale M and Spooner DM (2009) Robust and highly informative microsatellite-based genetic identity kit for potato. *Mol Breeding* **23**: 377-388

Gorg R, Schachtschabel U, Ritter E, Salamini F and Gebdardt C (1992) Discrimination among 136 tetraploid potato varieties by fingerprints using highly polymorphic DNA markers. *Crop Sci* **32**: 815-19

Harris PM (1978) The potato crop. Chapman & Hall, London: 730p

Gopal J and Oyama K (2005) Genetic base of Indian potato selections as revealed by pedigree analysis. *Euphytica* **142**: 23-31

Gopal J, Kumar V, Pandey SK, Kumar R, Pande PC and Singh SV(2007) Morphological descriptors for DUS testing of potato varieties. *Potato J* 59-60

Jansky SH and Spooner DM, (2018) The evolution of potato breeding. Plant Breeding Rev. **41**: 169-214

Jones CJ, Edwards KJ, Castaglione S, Winfield MO, Sala F, Van De Wiel C, Breedemeijer G, Vosma B, Matthes M, Daly A, Brettschnedider R, Bettini P, Buiatti M, Maestri E, Malcevschi A, Marmiroli N, Aert R, Volckaert G, Rueda J, Linacero R, Vazquez A and Karp A (1997) Reproducibility testing of RAPD, AFLP, and SSR markers in plants by a network of European laboratories. *Mol Breed* **3**: 381-90

Mc Gregor CE, Lambert CA, Greyling MM, Louw JH and Warnich L (2000) A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. *Euphytica* **113**: 135-44

Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C and Waugh R (1998) Isolation, characterization and mapping of simple sequence repeat loci in potato. *Mol Gen Genet* **259**: 233-45

Moisan-Thiery M, Marhadour S, Kerlan MC, Dessenne N, Perramant M, Gokelaere T *et al.* (2005) Potato cultivar identification using simple sequence repeats markers (SSR). *Potato Res* **48**: 191–200

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proc Natl Acad Sci USA* **70**: 3321-23

Pattanayak D, Chakrabarti SK and Naik PS (2002) Genetic diversity of late blight resistant and susceptible Indian potato cultivars revealed by RAPD markers. *Euphytica* **128**: 183-89

Prevost A and Wilkinson MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor Appl Genet* **98**: 107-12

Provan J, Powell W and Waugh R (1996) Microsatellite analysis of relationships within cultivated potato (*Solanum tuberosum*). *Theor Appl Genet* **92**: 1078-1084

Ross H (1986) Potato breeding - problems and perspectives. Verlag Paul Parey, Berlin: 132p.

Rohlf FJ (2000) Programa NTSYS-pc:numerical taxonomy and multivariate analysis system: version 2.1. Exeter Software, New York: 83p.

Shandil RK, Chakrabarti SK, Singh BP, Sharma S, Sundaresha, Kaushik SK, Bhatt AK and NN Sharma (2017) Genotypic background of the recipient plant is crucial for conferring RB gene mediated late blight resistance in potato *BMC Genetics* **18**: 22 DOI 10.1186/s12863-017-0490-x

Sharma V and Nandineni MR (2014) Assessment of genetic diversity among Indian potato (*Solanum tuberosum* L.) collection using microsatellite and retrotransposon based marker systems. *Mol Phylogenet Evol* **73**: 10-17

Tiwari JK, Ali N, Devi S, Kumar V, Zinta R and Chakrabarti SK (2018) Development of microsatellite markers set for identification of Indian potato varieties. *Sci Hort*. **231**: 22-30

Tiwari JK, Ali N, Devi S, Kumar V. Zinta R and Chakrabarti SK(2019) Analysis of allelic variation in wild potato (*Solanum*) species by simple sequence repeat (SSR) markers. *Biotech* **9**: 262 https://doi.org/10.1007/s13205-019-1785-6

Veilleux RE, Shen LY and Paz MM (1995) Analysis of the genetic composition of anther-derived potato by randomly amplified polymorphic DNA and simple sequence repeats. *Genome* **38** (6): 1153-62.

Spooner DM, Nunez J, Rodriguez F, Naik PS and Ghislain M (2005) Nuclear and chloroplast DNA reassessment of the origin of Indian potato varieties and its implications for the origin of the early European potato. *Theor Appl Genet* **110**: 1020-1026.

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