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Molecular characterization and genetic diversity of Indian potato, (*Solanum tuberosum* L.) germplasms using microsatellite and RAPD markers

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ABSTRACT

Potato (*Solanum tuberosum* L.) is the fourth most important food crop in the world and an important vegetable crop. The genus *Solanum* consists of 220 tuber containing species of which seven tuber-bearing species is used for commercial cultivation. Potato is a self-pollinated crop with cross-pollination up to 2.54 percent. Use of molecular markers to determine genetic variation, genetic diversity and evolutionary relatedness is becoming more popular for the assessment of diversity among cultivars of crop species of from various geographical origins. In the present study, total 42 RAPD markers have been employed, out of which 21 primers were polymorphic with 66.95% average polymorphism and 0.783 average PIC value, indicating higher informativeness of primers. In the present study, total 25 SSR markers have been employed out of these only 4 potent SSR primers were polymorphic. For SSR markers, average polymorphism was 58.33% and mean PIC value was 0.712. The results of current study indicate that RAPD and SSR markers used in the study have very promising polymorphism and PIC values hence, seemed to be good for the molecular characterization and assessing genetic relationship among genotypes of potato.

KEY WORDS: POTATO (*SOLANUM TUBEROSUM* L.), MOLECULAR MARKERS, MICROSATELLITE, RAPD, SSR

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INTRODUCTION

Potato (*Solanum tuberosum* L.) originated from South America is one of the major vegetable crops of the world which is also the fourth most important food crop in the world, after maize, wheat, and rice (Ghebreslassie *et al*., 2016; Majeed and Muhammad, 2018). In India, it was introduced in early seventeenth century possibly by the Portuguese or by the Britishers (Pal and Nath, 1951; Kapuria *et al*., 2016). It is a source of low cost energy to the human diet hence, it is considered as an economy food (Pandey and Sarkar, 2005). Globally, the major potato growing nations are China, India, Russia, Ukraine and United States of America (Bradshaw, 2019). The genus *Solanum* consists of 220 tuber-containing species among which seven tuber bearing species exploited for commercial cultivation (Hawkes and Jackson, 1992). The most important feature in potato taxonomy is the variation in ploidy level. Potato is a self-pollinated crop and the level of cross-pollination is up to 2.54 percent (Kapuria *et al*., 2016; Wang *et al*., 2019).

Knowledge of the genetic diversity and relationships among the varieties/cultivars are very beneficial to recognize the gene pool, to recognize the gaps in germplasm collections and to improve effective conservation and management approaches. Use of DNA markers for the identification of genetic variation, genetic diversity and evolutionary relatedness can be useful in identifying genetic/population structure as well as diversity among cultivars from various geographical origins. Due to their simplicity, reliability and cost effectiveness, PCR based methods are in demand (Parita *et al*., 2018).

Different PCR based techniques have been developed during the last two decades, each with specific benefits and drawbacks. The random amplified polymorphic DNA (RAPD) marker system is rapid, simple and requires no prior sequence information in which one random 10-mer primer is used (Welsh and McClelland, 1990; Williams *et al*., 1990). The dominant nature of inheritance of RAPD is considered as its drawback. Among different classes of molecular markers, simple sequence repeats (SSR)/microsatellites which are tandem repeats of 1-6 nucleotide long DNA motifs gaining importance in plant breeding and genetics due to their co-dominant inheritance, multi-allelic nature, relative abundance, hyper variability, reproducibility, good genome coverage including organellar genomes, chromosome specific location, amenability to automation and high throughput genotyping (Oliveira *et al*., 2006; Walunjkar *et al*., 2013). The PCR based techniques have been used for DNA fingerprinting and genotyping in potato including RAPD (McGregor *et al*., 2000; Ghislain *et al*., 2006; Chimote *et al*., 2007; Rocha *et al*., 2010; Gorji *et al*., 2011; Onamu *et al*., 2016; Ayman *et al*., 2018), SSR (McGregor *et al*., 2000; Braun and Wenzel, 2004; Feingold *et al*., 2005; Ghislain *et al*., 2006; Chimote *et al*., 2007; Ghislain *et al*., 2009; Rocha *et al*., 2010; Favoretto *et al*., 2011; Maras *et al*., 2017; Ahmed *et al*., 2018; Tiwari *et al*., 2019), ISSR (McGregor *et al*., 2000; Bornet *et al*., 2002; Gorji *et al*., 2011; Vanishree *et al*., 2016) and AFLP (McGregor *et al*., 2000; Braun and Wenzel, 2004; Solano Solis *et al*., 2007; Jian *et al*., 2017). The present work was conducted to evaluate the phylogenetic relatedness of Indian potato genotypes using RAPD and SSR markers.

MATERIALS AND METHODS

Plant material used in the study

Plant material consisted of young leaves sampled from young plants of 15 diverse Indian potato germplasms (Table 1). They were grown in Botanical Garden, C.P. College of Agriculture, SDAU, Sardarkrushinagar, Gujarat, India.

Genomic DNA extraction and quantification

Genomic DNA was extracted from tender fresh leaves of each germplasm by using CTAB (Cetyl Trimethyl Ammonium Bromide) method described by Doyle and Doyle, (1990) with minor modifications. The quality and integrity of DNA were checked by electrophoresis using 0.8% agarose gel. The DNA samples were quantified on UV spectrophotometer (BioSpectrometer, Eppendorf, Germany). The stocks were diluted to a final concentration of 30 ng/µl of DNA and used for further work.

PCR amplification

RAPD analysis

Forty two primers were selected and used to ascertain polymorphism among diverse germplasms of potato. The

Table 1. Potato germplasms used in the present study

Sr. No.	Germplasm	Sr. No.	Germplasm	Sr. No.	Germplasm
1	DSP-7	6	DSP-287	11	Kufri Kundan
2	Kufri Chipsona-1	7	Kufri Pushkar	12	Kufri Khyati
3	JX-249	8	Kufri Jyoti	13	Kufri Bahar
4	MF-1	9	Kufri Sutlaj	14	Kufri Anand
5	MS/95-1309	10	Kufri Badshah	15	KCM

RAPD primers used in present study are UBC, OPA, OPB, OPC, OPH, OPG, OPW and OPX series and polymorphic primers used in the analysis are given in Table 2. PCR was carried out as method given by Yasmin *et al*., (2006) with minor modifications in a total reaction volume of 25 µl. The PCR mixture consisted of 1 X PCR buffer, 10 mM dNTPs, 2.0 mM $MgCl_2$, 1 U of Taq DNA polymerase, 20 pmol/µl Primer and 30 ng/µl DNA template. All amplifications were carried out on a eppendorf thermal cycler using PCR conditions initial denaturation at 94°C (4 min) one time, denaturation at 94 °C for 1min, annealing at Tm of primer for 45 sec and extension at 72°C for 2 min for 35 cycles with final extension of 72°C for 7 min.

SSR analysis

PCR was carried out as method given by Ghebreslassie *et al.,* (2016) with minor modifications in a total reaction volume of 25 µl. The PCR mixture consisted of 1 X PCR buffer, 10 mM dNTPs, 2.0 mM $MgCl_2$, 1 U Taq DNA polymerase, 20 pmol/µl of primer pair and 30ng/µl of template DNA. The polymorphic primers used in present study are given in Table 2. PCR conditions consisted of initial denaturation at 94°C (4 min) for first cycle only, denaturation at 94 °C for 30 sec, annealing at Tm of primer for 1 min and extension at 72°C for 1 min for 35 cycles with final extension of 72°C for 6 min.

Resolution of amplified products

The amplified products of RAPD and SSR were resolved on 1.5% and 3.0% agarose gel (Dharajiya *et al*., 2017). The gel was stained with ethidium bromide (10µl/100ml). The standard DNA marker (100 bp) was also run along with the samples. After electrophoresis, the gel was carefully taken out of the casting tray and photographed using AlphaEaseFC 4.0.0 Gel Documentation system (Alpha Innotech Corporation, USA).

Analysis of RAPD and SSR data

Data were scored for computer analysis on the basis of the presence (1) or absence (0) of the amplified DNA fragments. The data were entered into the binary matrix and subsequently analyzed using PAleontological STatistics (PAST) -Version 3.18 (Hammer *et al.,* 2001) was used for genetic diversity evaluation. Coefficients of similarity were calculated by using Jaccard's similarity co-efficient (Jaccard, 1908) and cluster analysis was performed by using the Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) function of PAST version 3.18. The Relationship between the potato genotypes was graphically represented in the form of dendrograms by using the cluster analysis function of the software. In this method the dendrogram and similarity matrix were correlated to find the goodness-of-fit of the dendrogram constructed based on the similarity coefficients. The marker data were then standardized for Principal Component Analysis (PCA). The software program AlphaEaseFC version 6.0.0 developed by Alpha Innotech Corporation, USA was used for determining the Molecular Weight (MW) of bands separated on the gel. The Polymorphism Information Content (PIC) value for each locus was calculated on the basis of allele frequency by the formula given by Anderson *et al.,* (1993). The polymorphism percentage was calculated as per the method suggested by Smith *et al*., (1997).

RESULTS AND DISCUSSION

Molecular marker analysis

Total 42 RAPD primers were used for screening of 15 potato germplasms. Out of 42 primers, 21 were polymorphic which amplified a total of 116 reproducible DNA fragments. Out of total 116 DNA fragments, 79 fragments were polymorphic with the mean number of polymorphic bands per primer was 3.76. The size of amplified fragments varied from 140 to 2059 bp. The highest number of amplified bands (8) was exhibited by primer UBC 31 and UBC 34 whereas lowest number of amplified bands (3) was produced by primer OPW 4. The highest polymorphism (100%) was exhibited by two primer OPA 8 and OPB 18, while the lowest polymorphism (20.00 %) was evinced with OPW 8. The average polymorphism detected by the RAPD loci in the present investigation was 66.95 % (Table 2).

Out of 25 SSR primers used in the study, 4 were polymorphic which amplified a total of 18 reproducible fragments among which 11 fragments were polymorphic. The mean number of polymorphic bands was 2.75. The size of PCR amplified fragments varied from 125 to 1899 bp. The highest number of amplified band (6) was exhibited by primer STWIN12G and STCPKIN 3 and the lowest number of amplified bands (2) was exhibited by primer STPRINPSG. The highest polymorphism (66.66) was exhibited by two primers *viz*., STWIN12G and STCPKIN 3, while the lowest polymorphism (50.00%) was evinced with STPRINPSG and STACCS 3. The average polymorphism detected by the SSR loci in the present investigation was 58.33 % (Table 2).

The distribution of the primers used in the study according to the PIC values and per cent polymorphism is given in the Fig. 1. Most of the RAPD primers (11) have PIC value from 0.7 to 0.8 whereas two SSR primers have PIC value from 0.8 to 0.9. Most of the RAPD primers have per cent polymorphism more than 60 %. It indicates that these primers possess good importance in the diversity analysis in potato.

Table 2. Result of RAPD and SSR analysis in potato genotypes

Sr. No.	Primer name	TB	TPB	TMB	% P	PIC	AL (bp)
RAPD Primers							
1	UBC-34	8	6	2	75.00	0.852	228 to 1800
2	UBC 77	4	2	2	50.00	0.737	152 to 553
3	UBC 88	6	4	2	66.66	0.797	274 to 1888
4	UBC 31	8	4	4	50.00	0.858	309 to 2022
5	UBC 33	5	3	2	60.00	0.782	129 to 493
6	OPH 5	5	4	1	80.00	0.763	276 to 807
7	OPA 7	5	5	0	100.0	0.776	214 to 1227
8	OPA 6	7	4	3	57.14	0.848	140 to 827
9	OPB 18	5	5	0	100.0	0.786	201 to 586
10	OPB 10	4	3	1	75.00	0.732	430 to 1216
11	OPB 6	3	1	2	33.33	0.606	197 to 649
12	OPB 5	6	5	1	83.33	0.801	678 to 2059
13	OPA 19	6	4	2	66.66	0.822	172 to 677
14	OPA 12	5	4	1	80.00	0.790	143 to 638
15	OPA 8	7	7	0	100.0	0.846	154 to 560
16	OPA 3	6	4	2	66.66	0.785	260 to 1030
17	OPA 2	7	6	1	85.71	0.841	195 to 2047
18	OPW 8	5	1	4	20.00	0.776	369 to 1529
19	OPW 5	5	2	3	40.00	0.770	437 to 1526
20	OPW 4	3	2	1	66.66	0.660	824 to 1840
21	OPW 2	6	3	3	50.00	0.823	382 to 1101
Total		116	79	37	-	-	-
Mean		5.52	3.76	1.76	66.95	0.783	-
Range		3 to 8	1 to 7	0 to 4	20 to 100	0.606 to 0.858	143 to 2059
SSR Primers							
1	STACCS 3	4	2	2	50.00	0.709	272 to 529
2	STCPKIN 3	6	4	2	66.66	0.819	212 to 915
3	STPRINPSG	2	1	1	50.00	0.497	165 to 528
4	STWIN12G	6	4	2	66.66	0.821	125 to 1899
Total		18	11	7	-	-	-
Mean		4.5	2.75	1.75	58.33	0.712	-
Range		2 to 6	1 to 4	1 to 2	50.00 to 66.66	0.497 to 0.821	125 to 1899

TB: Total no. of Bands; TPB: Total no. of Polymorphic Bands; TMB: Total no. of Monomorphic Bands; PIC: Polymorphism Information Content; % P: Per cent Polymorphism; MW: Molecular Weight; AL: Amplicon Length; bp: base Pair

Construction of dendrogram

RAPD based dendrogram

Jaccard's co-efficients were used to compare set of variables and to generate similarity matrix. Jaccard's co-efficients for all genotypes as per RAPD analysis are shown in Table 3. Similarity indices were estimated on the basis of twenty one RAPD primers ranged from 0.56 (between DSP-287 and Kufri Chipsona-1) to 0.82 (between Kufri Kundan and Kufri Sutluj). UPGMA (unweighted pair-group method with arithmetic mean) dendrogram was prepared by using Jaccard's similarity co-efficients (Fig 2. (A)). The dendrogram clustered with the data generated by all primers and their amplicons grouped the 15 genotypes into two major clusters *i.e.,* Cluster A and Cluster B. Dendrogram showed two major clusters with co-efficient value 0.7564. The cluster A contained only one genotype, namely, Kufri Pushkar. The cluster B was

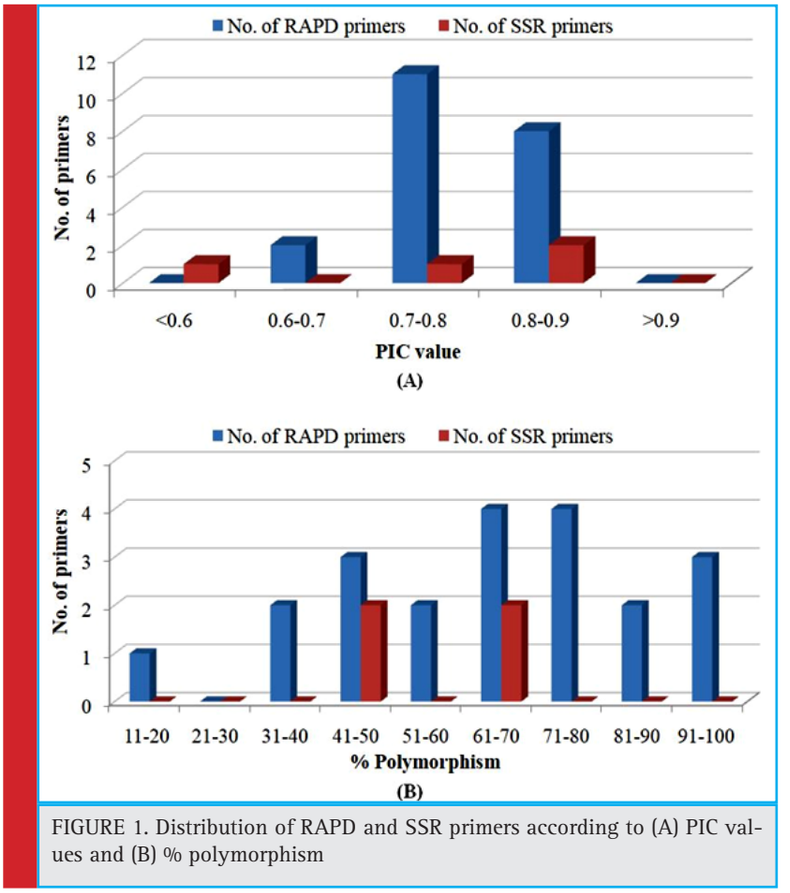

FIGURE 1. Distribution of RAPD and SSR primers according to (A) PIC values and (B) % polymorphism

further grouped in three clusters B1, B2 and B3. The cluster B1 contained 11 genotypes. The cluster B2 and B3 contains two and one genotype, respectively.

SSR based dendrogram

Jaccard's similarity co-efficients (Table 4) were estimated on the basis of four SSR primers, ranged from 0.47 (Kufri Badshah and Kufri Kundan with DSP-287) to 1.0 (between Kufri Kundan and Kufri Badshah). UPGMA dendrogram was prepared by using Jaccard's similarity co-efficients which was grouped into two main clusters *i.e.*, Cluster A and Cluster B (Fig 2. (B)). Dendrogram showed two major clusters with co-efficient value 0.8306. The cluster A was further divided into two clusters A1 and A2. The cluster A1 contained 6 genotypes and cluster A2 contained 8 genotypes. The cluster B was dividing into one group containing only one genotype DSP-287. Genotypes Kufri Badshah and Kufri Kundan had highest similarity.

Principle Component analysis (PCA)

In the PCA plot, derived from the RAPD genotyping data, it can be observed that Kufri Pushkar and Kufri Sutluj is placed farthest from Kufri Anand in the 1st coordinate (X-axis), while Kufri Bahar and MF-1 were placed farthest in the 2nd coordinate (Y-axis) (Fig. 3 (A)). Most of the varieties with moderately resistances were located in 1st coordinate right side in the plot including MF-1, DSP-287 and MS/95-1309 except Kufri Sutlaj.

In the PCA plot derived from the SSR genotyping data, it can be observed that DSP-287 and Kufri Sutluj is placed farthest from Kufri Anand in the 1st coordinate (X-axis), while Kufri Jyoti and DSP-287 were placed farthest in the 2nd coordinate (Y-axis) (Fig. 3 (B)). Varieties with moderately resistance located on the right side of 1st coordinate in the plot including the MF-1 and MS/95-1309 except Kufri Sutluj and DSP-287.

In potato, various studies have been previously reported at molecular level deciphering variation across accession and varieties. Center wise studies also have been performed that indicating within the center diversity. Previously, RAPD analysis has been employed in many literature for checking molecular polymorphism and allelic variation McGregor *et al.,* (2000), Yasmin *et al.,* (2006), Chimote *et al.,* (2007), Onamu *et al.*, (2016) and Ayman *et al.*, (2018). In the present study, total 42

Table 3. Jaccard's co-efficients for potato genotypes by RAPD analysis

	DSP-7	K. Chip-1	JX-249	MF-1	MS/95-1309	DSP-287	K. Pushkar	K. Jyoti	K. Sutlaj	K. Badshah	K. Kundan	K. Khyati	K. Bahar	K. Anand	KCM
DSP-7	1.000														
K. Chip-1	0.644	1.000													
JX-249	0.677	0.667	1.000												
MF-1	0.663	0.634	0.616	1.000											
MS/95-1309	0.663	0.670	0.737	0.670	1.000										
DSP-287	0.670	0.560	0.708	0.660	0.727	1.000									
K. Pushkar	0.663	0.598	0.582	0.586	0.670	0.626	1.000								
K. Jyoti	0.617	0.640	0.711	0.576	0.731	0.633	0.641	1.000							
K. Sutlaj	0.602	0.659	0.691	0.578	0.677	0.667	0.660	0.761	1.000						
K. Badshah	0.635	0.641	0.590	0.677	0.694	0.701	0.696	0.667	0.778	1.000					
K. Kundan	0.674	0.628	0.643	0.598	0.663	0.740	0.663	0.653	0.820	0.780	1.000				
K. Khyati	0.692	0.663	0.733	0.681	0.630	0.653	0.594	0.652	0.725	0.688	0.710	1.000			
K. Bahar	0.656	0.644	0.696	0.580	0.698	0.636	0.645	0.767	0.707	0.670	0.747	0.692	1.000		
K. Anand	0.699	0.600	0.702	0.653	0.687	0.694	0.570	0.714	0.643	0.677	0.698	0.717	0.816	1.000	
KCM	0.619	0.624	0.639	0.643	0.694	0.650	0.592	0.685	0.633	0.684	0.636	0.688	0.725	0.769	1.000

Table 4. Jaccard's co-efficients for potato genotypes by SSR analysis

	DSP-7	K. Chip-1	JX-249	MF-1	MS/95-1309	DSP-287	K. Pushkar	K. Jyoti	K. Sutlaj	K. Badshah	K. Kundan	K. Khyati	K. Bahar	K. Anand	KCM
DSP-7	1.000														
K. Chip-1	0.882	1.000													
JX-249	0.667	0.765	1.000												
MF-1	0.882	0.882	0.765	1.000											
MS/95-1309	0.824	0.824	0.706	0.938	1.000										
DSP-287	0.706	0.611	0.588	0.706	0.647	1.000									
K. Pushkar	0.588	0.688	0.786	0.688	0.625	0.500	1.000								
K. Jyoti	0.824	0.824	0.813	0.824	0.765	0.556	0.733	1.000							
K. Sutlaj	0.647	0.647	0.857	0.647	0.588	0.563	0.769	0.688	1.000						
K. Badshah	0.647	0.750	0.857	0.750	0.688	0.471	0.917	0.800	0.846	1.000					
K. Kundan	0.647	0.750	0.857	0.750	0.688	0.471	0.917	0.800	0.846	1.000	1.000				
K. Khyati	0.722	0.722	0.813	0.824	0.765	0.647	0.733	0.765	0.800	0.800	0.800	1.000			
K. Bahar	0.688	0.688	0.786	0.688	0.625	0.500	0.833	0.733	0.917	0.917	0.917	0.733	1.000		
K. Anand	0.824	0.722	0.706	0.824	0.765	0.750	0.625	0.667	0.800	0.688	0.688	0.875	0.733	1.000	
KCM	0.824	0.824	0.813	0.938	0.875	0.647	0.733	0.875	0.688	0.800	0.800	0.875	0.733	0.765	1.000

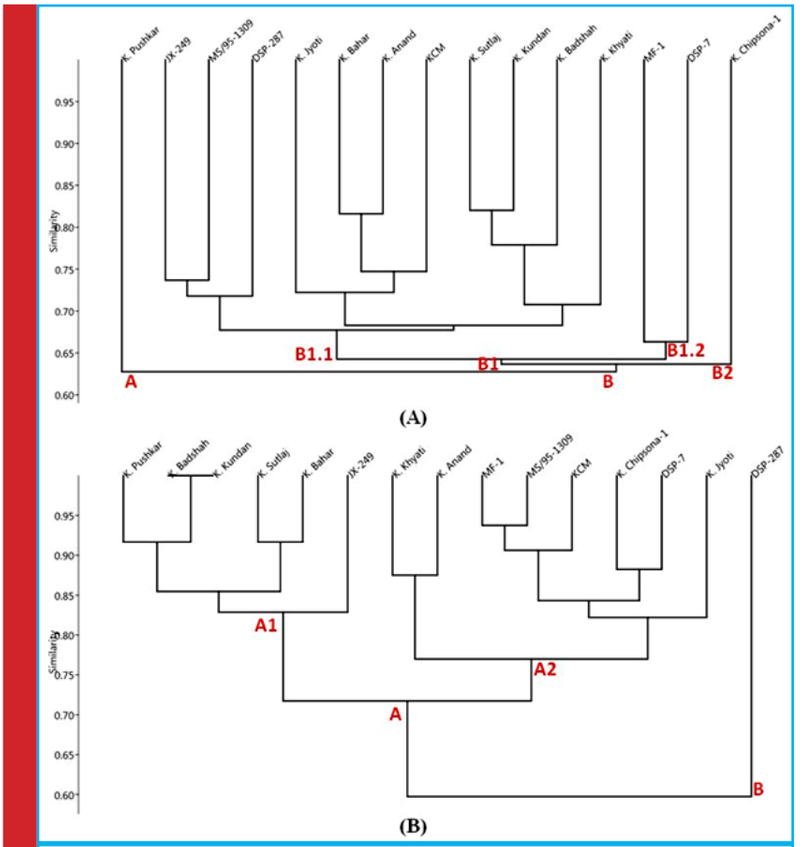

FIGURE 2. Dendrogram potato genotypes constructed from (A) RAPD and (B) SSR based Jaccard's similarity using UPGMA method

RAPD markers have been employed out of which 50% of primers accounts for mean polymorphism greater than 66.95% with PIC 0.783, indicating higher informativeness of primers. Previously, Chimote *et al.,* (2007), Gorji *et al*., (2011) and El Komy *et al.,* (2012) found mean polymorphism about 20%, 31% and 57.4% which was less then present result, indicating more superior polymorphic potential of primers used in the present study and its employment for further research by other in future. Moreover, Gorji *et al*., (2011) found 0.28 mean PIC value which was very less as compared to the mean PIC value of RAPD (0.78) in the present research. Recently, Onamu *et al*., (2018) used 19 RAPD markers for the assessment of genetic diversity among 35 potato accessions and reported 81.45% polymorphism which was higher as compare to the current investigation.

Among DNA markers, microsatellites have been chosen over RAPD because of their co-dominant behavior, multiallelism, reproducibility, and high level of polymorphism detected. In potato, few researchers such as Mc Gregor *et al*., (2000), Feingold *et al*., (2005), Ghislain *et al*., (2006), Chimote *et al*., (2007), Sharma and Nandineni, (2014), Maras *et al*., (2017), Ahmed *et al*., (2018) and Tiwari *et al*., (2019) have successfully used SSR markers. In the present study, total 25 SSR markers have been employed out of these only 4 potent SSR primers were polymorphic. Average polymorphism was 58.33% and mean PIC value was 0.712. Previously, Chimote *et al*., (2007) work with SSR marker for with general morphological character turns to only mean 19.5% polymorphism with almost same genotype indicating more superior polymorphic potential of our SSR marker and its employment for further research by other in future. While, Sharma and Nandineni, (2014) worked with different Kufri varieties by using same set of SSR markers their study revealed more polymorphism (80-90%) then the present study. Biniam *et al*., (2016) found that 97.8% SSR markers were highly polymorphic with an average PIC value of 0.87 which was very promising for the characterization of potato genotypes. In recent times, Duan *et al*., (2019) used 20 SSR markers for the analysis of genetic diversity among 217 potato cultivars and reported 97.99% polymorphism and 0.83 PIC value which were higher as compare to the current investiga-

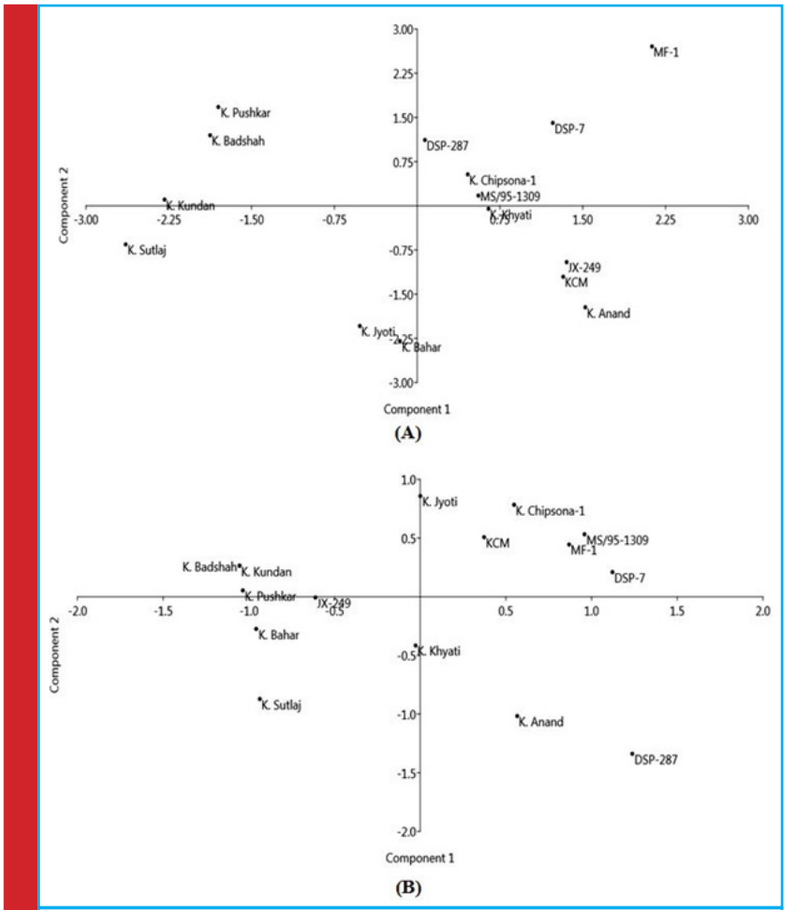

FIGURE 3. PCA analysis of potato genotypes based on (A) RAPD and (B) SSR analysis

tion. The molecular markers with high PIC values can be utilized for the diversity analysis in potato germplasms.

CONCLUSION

The results of current study indicate that RAPD and SSR markers used in the study seemed to be the good for the molecular assay for fingerprinting and assessing genetic relationship among genotypes of potato as they have very promising polymorphism and PIC values. These markers can be utilized for molecular breeding for the improvement of potato.

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