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Assessment of genetic diversity using rapd molecular marker in potato varieties

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Abstract
In this study, Random Amplified Polymorphic DNA (RAPD) marker will be used to study the molecular diversity of 10 popular potato varieties of India. DNA was extracted from tender leaf samples and it was quantified by using UV - Vis spectrophotometer. Total thirteen RAPD primers were used to run test the polymorphism and data was analyzed by DARwin6 software and dendrogram was developed to establish evolutionary relationships among potato varieties. The Polymorphic Information Content (PIC) among varieties ranged between 0.19 and 0.84. All of the RAPD primers had percent polymorphism more than 60 %.

Keywords: potato, molecular diversity, PIC, factorial analysis, RAPD, PCR

Introduction

Potato (*Solanum tuberosum* L.) is a member of Solanaceae a large plant family containing more than 3000 species. Potato originates from a specific origin place that is wild Andes of Peru South America and also the fourth most important foodcrop in the world after maize, wheat and rice. *Solanum* consist of 220 tuber containing species among which seven tuber species are used for commercial cultivation. (Ghebreslassie *et al*., 2016) [9]. Potato is a self-pollinated crop and the level of crosspollination is up to 2.54% (Kapuria *et al*., 2016; Wang *et al*., 2019) [30]. Nutritional composition of potato contains starch (17.5%), total sugar (0.5%), crude fiber (0.7%), total nitrogen (0.32%), crude protein (2.0%), Lipid (0.12%), Glycoalkaloid (3-10%). The Central Potato Research Institute (CPRI) is the premier Institute working on research on potato, and is situated at Shimla in Himachal Pradesh. The varieties released from CPRI have 'Kufri'as their first name.

The global annual production of potato is approximately 300 million tons. It was most cultivated and consumed in Europe, North America until the beginning of 1990 (FAO, 2008). India is the second largest producer of potato (45.34 million metric tons per year) and Uttar Pradesh (UP) is the major potato producing state with 31.26% of total production (Federici *et al.,* 2015) [8]. Potato is a herbaceous plant which is recognized as a single species, *Solanum tuberosum* cultivar varied based on ploidy level with a basic chromosome number n = 12 and maybe diploid (2n = 2x = 24), triploid (2n = 3x = 36), tetraploid (2n = 4x = 48), or pentaploid (2n = 5x = 60) and a genome size of about 844 Mb (Golmirzaie and Ortiz, 2004) [10].

Genetic diversity is used to study the taxonomic relationship among genotype and to choose varieties with good qualities (Barandalla *et al.,* 2006) [2]. Random Amplified Polymorphic DNA (RAPD) are well suited for molecular diversity analysis within and among species Potato is cultivated in diverse climatic zones including temperate region the subtropics and trophic and bothlowland and highland crop (Sharma and Nandineni, 2014) [24]. *Solanum tuberosum* L. includes highly diverse short-duration varieties of potatoes like Kufri Bahar, Kufri Pukhraj, Kufri Himsona, Kufri Mohan, Kufri Neelkanth, Kufri Gaurav, Kufri Lalima, KMC, Bodmlal, Kufri Jyoti etc. Genetic variability has been considered as is a prerequisite for crop improvement programs. The quantification of genetic Diversity made it possible to select diverse parents for successful program hybridization (Badoni and Chauhan, 2010) [15].

Molecular characterization of plant varieties

Molecular characterization is an important biotechnologies tool in plant breeding programs and it is used to determine genetic variation, genetic diversity, and Phylogenetic relationship for assessment of diversity among cultivars of crop species from the various geographical origins (Tosti and Nejri, 2002). The traditional methods based on morphological traits require more time, cost expensive and has a large effect on the environment by the development of a wide range of molecular technique, marker-assisted breeding is now used to enhance the conventional breeding program for crop improvement (Kapuria *et al.,* 2018-2019).

Molecular marker is the most assessment of genetic diversity and identification of varieties (Upadhyay *et al*., 2004) [28]. The development of molecular markers associated with the traits has an advantage that the desirable genotype of plants can be selected. Some molecular techniques have been developed over the years, using RAPD (Randomly Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), RFLP (Restriction Fragment Length Polymorphism), ISSR (Inter- Simple Sequence Repeat), gene-based SSR (Simple Sequence Repeat) marker and SNPs. Among them, RAPD has shown promising results for evaluation and molecular added breeding is quick, and requires no prior sequence information (Williams

et al., 1990; Tabkhkar *et al.*, 2012) [31, 26]. RAPD marker has been used in potato for evaluating genetic diversity. In this study, Random Amplified Polymorphic DNA (RAPD) marker will be used to study the molecular diversity among the different molecular marker RAPD (Randomly amplified polymorphic) are widely and most commonly use marker because they are dominant, technically simple, quick, non-radioactive, largely automatable, relatively inexpensive and require small amounts of DNA, that`s why polymerase chain reaction (PCR) generating RAPD fragments is more understood (Selaocoe *et al.*, 2019). Hence, the present investigation forces molecular diversity analysis of 10 varieties of potato through RAPD markers to identify the divergence genotypes for the potato improvement program.
In this study, Random Amplified Polymorphic DNA (RAPD) marker will be used to study the molecular diversity of 10 popular potato varieties of India.

DNA was extracted from Tender Leaf Samples.

Materials and methods

Collection of plant materials and field experiment

Ten varieties of potatoes were obtained from the Central Potato Research Institute (CPRI), Modipuram, Meerut, UP (table 1). The plant material was grown in the research farm of Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut during Rabi 2019-2020. The tuber potatoes with good quality, disease-free, and healthy conditions were sown in the soil. The row to row distance was taken as 75cm and the distance among the seeds sown was kept at 25cm. All management and agricultural practices were followed for the better quality of seedling.
The Fresh leaves were collected at the 3-4 leaf stage of a plant from all ten potato plants varieties.

Table 1: Details of potato seed varieties collected from CPRI.

S. No.	Name of Varieties	Selection number	Year of release	Parentage
1	Kufri Bahar	E3797	1980	Kufri Red x Gineke
2	Kufri Pukhraj	JEX/C-166	1998	Craigs Defiance
3	Kufri Himsona	MP/97-644	2008	MP/92-35 x Kufri chipsona-2
4	Kufri Mohan	INA	2015	MS/92-1090 × CP1704
5	Kufri Neelkanth	(MS/8-1565)	INA	INA
6	Kufri Gaurav	JX- 576	2012	INA
7	Kufri Lalima	BS/C-1753	1982	Kufrired×AG 14(wis× 37)
8	KMC	INA	INA	INA
9	Bodmlal	INA	INA	INA
10	Kufri Jyoti	SLB/Z389	1968	3069d (4) ×2814a (1)

INA = Information not available

Isolation of genomic DNA from potato leaves of all varieties

The chemicals were purchased from companies like Himedia, Genetix, Merck and Spectrochem (India). Total genomic DNA was extracted. Potato leaves from ten varieties were collected then it was freeze with liquid nitrogen, and store at 80°C until use. Mortar pestle was used for grind 0.2 g of leaves in the presence of liquid nitrogen until finely grind and transferred grind leaf tissue to 2 ml polypropylene centrifuge tubes. The freshly prepared extraction buffer (100 mM Tris-Cl (pH 8), 20 mM of 2 ml were added. EDTA, 1.4 M NaCl, 2% CTAB, and 100 mg PVP/0.1 gm leaf tissue were mixed by inversion and incubated in 60°C oven (with shaking) for 35 min. Tube contains leaf tissue were removed from heat, and let cooled to room temperature for 4 - 6 min. and centrifuged at 14,000 rpm for 10 min. Supernatant was collected and an equal volume i.e. 1ml volume of chloroform: isoamylalcohol (24: 1) were added and mixed by inversion. The mixture was centrifuged at 13,000 rpm at room temperature for 15 min in a tabletop centrifuge. The supernatant was transferred to new 2 ml centrifuge tube and micropipette were used to transfer supernatant and aqueous phase to a new 2 ml Eppendorf tube and 0.7 volume of isopropanol were added and incubated at - 20ºC for overnight. The nucleic acid was precipitated by centrifuged at 13,000 rpm for 10 min. at 4°C. The supernatant was discarded and washed pellet with cold (0-4°C) 80%, ethanol, and spin at 6,000 rpm for 6 min. Pellets was dried in 37°C oven until dried (1 hr). The 300 µl TE buffer was added inside the pellet (10 mM Tris, pH 8, 1 mM EDTA) for overnight at (4-6°C). The 300 µl TE buffer was added inside the pellet (10 mM Tris, pH 8, 1 mM EDTA) for overnight at (4-6°C). The nucleic acid was dissolved with 5µl of RNase (10 mg/ml) and incubated at 37°C for 30 min.

Gel Electrophoresis and quantification estimation of DNA by spectrophotometer

Double-stranded DNA was analyzed on Agarose gel. The electrophoresis was carried out in submarine horizontal agarose slab gel apparatus. Gels of different strength (0.8 to 1.5%) were prepared depending on the size of DNA to be analyzed. An Appropriate amount of Agarose (0.8-1.2%, as per the requirement) was dissolved in 1 X TAE buffer or 1 X TBE buffer for running DNA samples and boiled in the microwave oven to dissolve completely. After cooling to about 55ºC, EtBr (stain) was added at a concentration of 0.5 µg/ml (prepared in sterile water and mixed thoroughly) then agarose solution was poured on a gel casting tray sealed with microspore tape and on which a slot forming comb had been placed at a height of about 2 mm. After gel had solidified, the comb was removed and the gel was kept in the horizontal gel electrophoresis apparatus. Enough electrophoresis buffers 1 X TAE or 1 X TBE was poured just to cover gel approximately 2-3 mm in the gel running tank. About 5µl DNA sample and 3µl loading dye (Bromophenol blue) of each variety was mixed and loaded directly into the wells.
DNA ladder (100bp or 1000bp) was also loaded to compare the size of the DNA fragment. The gel was run at 5V/cm of constant voltage until the bromophenol dye reached almost at the end of the gel. The gel was viewed on a UV transilluminator to visualize the fragment of DNA of different sizes.

Quantification of DNA by spectrophotometer

The DNA was quantified by spectrophotometer as previously used by Yasmin *et al.,* (2006) [32]. A cuvette filled with a TE buffer was taken to calibrate the spectrophotometer. The 10 µl DNA and 1ml water were taken in another cuvette to measured absorbance at 260 nm and 280 nm. The second step has proceeded with all of the 10 potato varieties. If the ratio of absorbance (260 nm and 280 nm) was between the range of 1.8 and 2, then DNA was considered as pure. Quantification of DNA was calculated as – [OD at 260 nm × 50 ng/µl × 100].

PCR using RAPD primer

RAPD primers were selected based on previous works and analyzed to evaluate the molecular polymorphism among the potato varieties (table 2).

Table 2: Primer numbers and sequences.

S. No	RAPD Oligo Name	Molecular Weight	Oligo Sequence (5'3')
1	Primer 22	3004	AATCGGGCTG
2	Primer 23	3019	GGTCCCTGAC
3	Primer 24	2955	GACCGCTTGT
4	Primer 25	3093	TGCGCCCTTC
5	Primer 26	3013	GTCCACACGG
6	Primer 27	3084	TGGGGGACTC
7	Primer 28	3044	CTGCTGGGAC
8	Primer29	3028	GTAGACCCGT
9	Primer 30	3139	GGAGGGTGTT
10	Primer 31	3084	TGGACCGGTG
11	Primer 32	2939	TGTCATCCCC
12	Primer 33	3004	GTTGCCAGCC
13	Primer 34	3077	TGAGCGGACA

PCR was carried out as a method given by Yasmin *et al*., (2006) [32] with minor modifications. The DNA sample treated with RNase at 37º at for 1 hour was taken. The DNA sample was diluted from high concentration i.e. (50µl DNA and 50µl water). The stock solution of 13 primers was prepared

by added Protease, RNase and DNase free water. All the 13stock solutions were vortexed for 10 sec. All the 13stock solutions were centrifuged for 10 sec. The 1.5 ml vials were taken and labelled them as primer 1, 2, 3, 4…… and so on. The 10µl of primer and 90µl of water were added. The vials containing primers were vortexed for 10 sec. All the 13 primers were centrifuged for 10 sec. The master mix for PCR were prepared and the molecular biology water (12µl), 10X assay buffer (1.5µl), 10mM dNTP's (1.5µl), Taq DNA polymerase(0.2µl), Primer (3µl), DNA Template (1µl) and MgCl2 (0.8µl) were taken for 1 Sample. The following reaction was set up for PCR (table 3).

Table 3: Master Mix for PCR.

S. No.	Component for Master Mix	Volume/reaction
1	Template DNA	1µl
2	10 ng/gm Random Primer	3µl
3	10 mM dNTPs	1.5µl
4	Taq DNA Polymerase	0.2µl
5	10X Taq DNA Polymerase buffer	1.5µl
6	50 mM Magnesium Chloride	0.8µl
7	Molecular Biology H2O to make up	12µl

PAGE and band imaging

The amplified fragment was separated on 1% polyacrylamide gel electrophoresis and initial voltage of 60 volts for 30 min, increased up to 120 volts for about 5-6 hour in TBE buffer [Tris (108gm),mM Boric acid (55gm), 500 mM EDTA (9.3gm)] and standard molecular weight marker of 100bpand 1000bp were used as the method adopted by Rocha *et al*. (2010) [19] separated amplified fragments on 10% polyacrylamide gel by electrophoresis at 200 V in TBE buffer (500 mM EDTA pH 8.0, 1M TRIS pH 8.0, 89 mM boric acid) for two hours. The 150 ml of 10 X PAGE solution were prepared on dissolved 0.5 gm APS (Ammonium Per Sulphate) and 75 µl TEMED (Tetramethyl ethylene diamine) in a beaker for prepared one plate. The comb was inserted in electrophoretic plate and allowed the acrylamide to polymerize for 40 min to form a gel. The binder clips, spacer, and comb were removed from the gel assembly and binder clips were used to mount the gel electrophoresis apparatus. The running buffer was poured into the upper and lower chamber of the electrophoresis apparatus and the air bubble and a small piece of gel were removed from the wells under the gel using a syringe. The sample was mixed with loading dye and molecular weight marker in wells. The power supply was turned on, and run the gel until the dye in the sample reached the bottom of the gel. The gel assembly was removed from the electrophoresis apparatus. The spatula was used to remove the gel from the glass plate and prepared for subsequent analysis.

The gel containing DNA was stained with silver nitrate solution to reveal the DNA bands and visualized under the Gel visualization unit to confirm the banding pattern of all plant analyzed. Amplified RAPD profile of all the genotypes with each primer was documented by the Gel Documentation system. The size of RAPD fragments was estimated by comparison with the DNA marker. RAPD fingerprints were recorded in the binary form (0 indicates absence and 1 indicates presence of a band). Primers with a null allele where an amplification product could not be detected were not considered in the analysis.

Analysis and Preparation of Dendrogram

A simple matching coefficient was calculated to construct a similarity matrix and the DARwin6 software was used to perform hierarchical cluster analysis and to construct a dendrogram.

Results and discussion

Gel electrophoresis and quantification estimation of DNA by spectrophotometer

Genomic DNA was extracted by using the CTAB method and the isolation was confirmed by running the DNA samples of agarose gel electrophoresis (figure 1). The genomic DNA was quantified by using the Advanced Microprocessor UV-Vis Single Beam Spectrophotometer LI-295 spectrophotometer (table 4).

Bornet *et al.* (2002) extracted genomic DNA from fresh or frozen leaves by a cetyl tri methyl ammonium bromide (CTAB) protocol. Doyle and Doyle (1987) used the CTAB method to isolate genomic DNA from 1 g of fresh leaves of potato.

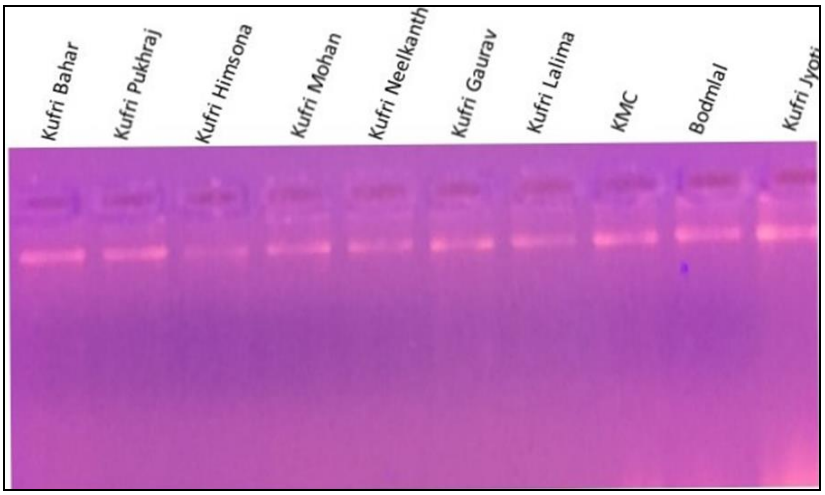

Fig 1: Isolation of genomic DNA was isolated from ten varieties of potato was confirmed by gel electrophoresis.

Table 4: DNA Quantification values of all ten varieties of potatoes.

S. No	Name of varieties	Absorbance at 260 nm	Absorbance at 280 nm	Ratio260/280 nm	Quantification of DNA ng/ml
1	Kufri Bahar	0.421	0.408	1.031	2,105
2	Kufri Pukhraj	0.453	0.436	1.038	2,265
3	Kufri Himsona	0.448	0.421	1.064	2,240
4	Kufri Mohan	0.507	0.471	1.076	2,535
5	Kufri Neelkanth	0.416	0.338	1.0	2,080
6	Kufri Gaurav	0.508	0.466	1.09	2,540
7	Kufri Lalima	0.505	0.465	1.086	2,525
8	KMC	0.52	0.481	1.081	2,600
9	Bodmlal	0.502	0.454	1.105	2,510
10	Kufri Jyoti	0.558	0.494	1.129	2,790

PAGE and band imaging

Amplified RAPD profile of all the genotypes with each primer was documented using a gel documentation system. The size of RAPD fragments was estimated by comparison with the DNA marker as described in figures 2, 3 and 4. RAPD fingerprints were recorded in the binary form (0 indicating absence and 1 indicating the presence of a band) to construct a dendrogram by using DARwin 6. Standard molecular weight markers of 10 bp and 100 bp were used. The material was stained with silver nitrate.

According to Selaocoe *et al.*, (2019) the gel profile for proteins and RAPD was detected with Quantity one firmware version 4.6.9 (Bio-Rad, Hercules, CA) and noted as a present (1) and absent (0). The monomorphic and polymorphic bands were noted, and bands with the same mobility but different intensities on the gel were not distinguished from each other. The data was summarized in a data matrix based on monomorphic and polymorphic fragments (Bassam *et al.*, 1991) [3].

According to Mrutu (2015) [16], the polymorphisms observed between the parents are used as markers for hybrid identification. RAPD Bandage Comparison parent model with respective hybrids, the true hybrids were confirmed. Clear bands in each variety were observed and stored as "0" (absent) or "1" (present) values. Band models ("0", Matrix "1") were tabulated separately for the individual primers and the data were pooled to obtain a combined matrix for the genotypes. NTSYS-pc software version 2.1 (Rohlf and Corti, 2000) [20] was used to calculate binary data ("0", "1") and evaluate genetics associations between genotypes. Pairwise comparisons were made between samples based on the Jaccard similarity coefficient and visualized by cluster analysis, unweighted pair group method with arithmetic averages (UPGMA), and illustrated in a phylogram. The analysis of the main coordinates was carried out based on Jaccard similarity for all individuals and a graph was generated using the first two main coordinates.

According to Kapuria *et al.*, (2019) [15] data were scored for computer analysis based on the presence (1) or absence (0) of the amplified DNA fragments. The data has been entered into the binary matrix and then analyzed using Paleontological Statistics (PAST) -Version 3.18 (Hammer *et al.*, 2001) [13] used for the assessment of genetic diversity. Coefficient of similarity were calculated using the Jaccard similarity coefficient (Jaccard, 1908) [14], and the cluster analysis was performed using the unweighted pair group method with the arithmetic mean function (UPGMA) of past version 3.18. The relationship between the potato genotypes was graphically represented in the form of dendrograms using the cluster analysis function of the Software. In this method, the dendrogram and the similarity matrix were correlated to find the quality of the fit dendrogram built based on the similarity coefficient. The marker data were then standardized for Principal component analysis (PCA). The software Alpha Ease FC version 6.0.0 developed by Alpha Innotech Corporation, USA was used to determine the molecular weight (MW) of the bands separated on the gel. The PIC (Polymorphism Information Content) value for each locus was calculated based on the allele frequency by the formula given by Anderson *et al.* (1993) [1]. The percentage of polymorphism was calculated according to the method suggested by Smith *et al.* (1997) [25].

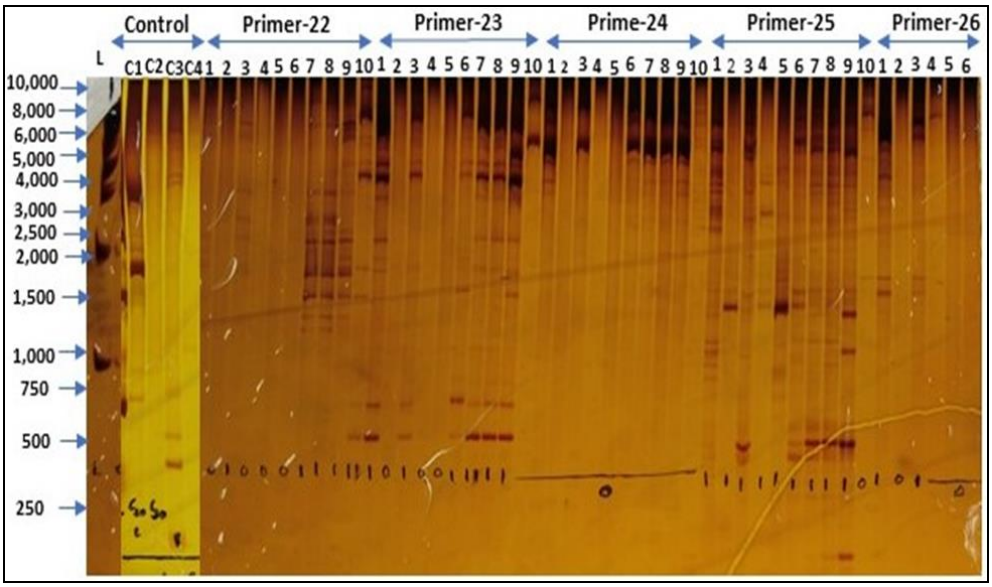

Fig 2: Polyacrylamide gel electrophoresis of amplification products obtained with RAPD primer with control C1, C2, C3, C4 and primers 22, 23, 24, 25 & 26.

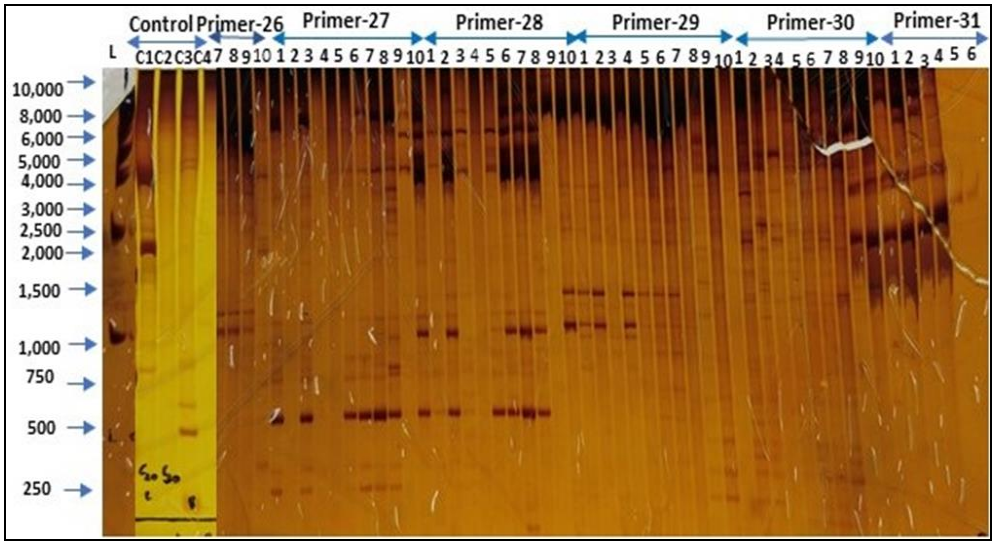

Fig 3: Polyacrylamide gel electrophoresis of amplification products obtained with RAPD primer with control C1, C2, C3, C4 and primers 26, 27, 28, 29, 30 & 31.

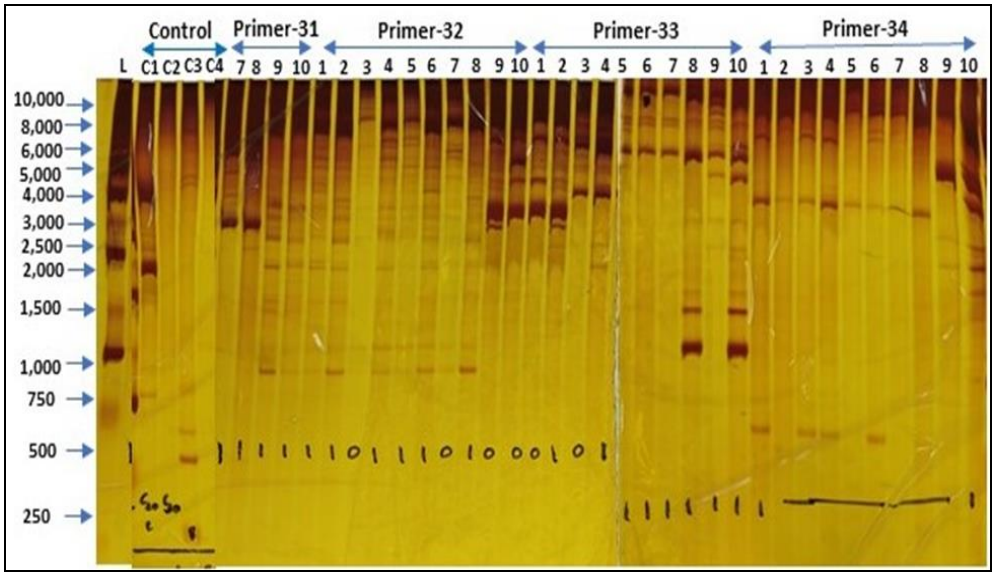

Fig 4: Polyacrylamide gel electrophoresis of amplification products obtained with RAPD primer with control C1, C2, C3, C4, and primers 31, 32, 33 & 34.

Molecular marker analysis

Total 13 primers were selected for screening of 10 different varieties of potato varieties for 130 reactions in which primer 22 showed polymorphism with varieties 3, 7, 8, 9, 10 and primer 23 showed polymorphism with varieties 1, 3, 6, 7, 8, 9 and primer 24 showed polymorphism with 1, 9 and primer 25 showed polymorphism with varieties 1, 3, 4, 7, 8, 9, 10 and primer 26 show polymorphism with varieties 1, 3, 7, 8, 9, 10 and primer 27 show polymorphism with varieties 1, 3, 6, 7, 8, 9, 10 and primer 28 showed polymorphism with varieties 1, 2, 3, 6, 7, 8, 10 and primer 29 showed polymorphism with varieties 1, 3, 4, 6, 7, 8, 10 and primer 30 showed polymorphism with varieties 1, 3, 4, 5, 6, 7, 8, 9 and primer 31 show ed polymorphism with varieties 1, 2, 3, 4, 5, 6, 7, 9, 10 and primer 32 showed polymorphism with varieties 1, 2, 4, 5, 6, 7, 9, 8, 10 and primer 33 showed polymorphism with varieties 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and primer 34 showed polymorphism with varieties 1, 3, 4, 6, 9, 10 although primer 22, primer 23, primer 28, primer 29, primer 30 and primer 33 did not showed monomorphism with any variety wherease primer 24 showed monomorphism with varieties 6, 7, 8 and primer 25 showed monomorphism with varieties 2, 6 and primer 26 showed monomorphism with 4 variety and primer 27 showed monomorphism with only 2 variety and primer 31 showed monomorphism with 8 variety and primer 32 showed polymorphism with 3 variety and primer 34 showed monomorphism with varieties 2, 5, 8. However, primer 25, primer 31, primer 32, primer 33 did not show any morphism with any variety, and primer 22 show no morphism with varieties 1, 2, 4, 5, 6, and primer 23 show no morphism with 2, 4, 5, 10 and primer 24 show no morphism with varieties 2, 3, 4, 5, 10 and primer 26 show no morphism with varieties 2, 5. 6 and primer 27 show no any morphism with varieties 4, 5 and primer 28 show no morphism with varieties 4, 9 and primer 29 show no morphism with 4, 9 and primer 30 show no morphism with varieties 2, 10 and primer 34 show no morphism with variety 7. The distribution of primers used in the study according to the PIC value & percent polymorphism is given in table 5. PIC value was calculated by using the formula as adopted by Saroj *et al.* (2015) [21].

Most of the RAPD primers (13) have PIC value from 30 to 35.

All of the RAPD primers had percent polymorphism more than 60%. It indicates that these primers possess good importance in the diversity analysis in potato (table 6). According to Kapuria *et al*., (2019) [15] the distribution of the primers used in the study according to the PIC values and percent polymorphism and 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.

Table 5: Polymorphism information in ten varieties of potato by pattern analysis of RAPD markers.

S. No.	Primer Name	Monomorphism	Polymorphism	PIC (Polymorphic Information Content)	Polymorphic percentage
1	Primer 22	-	5	0.75	100%
2	Primer 23	-	6	0.64	100%
3	Primer 24	2	2	-	60%
4	Primer 25	2	8	0.19	80%
5	Primer 26	1	7	0.75	87.50%
6	Primer 27	1	7	0.64	87.50%
7	Primer 28	-	9	0.64	100%
8	Primer 29	-	8	0.36	80%
9	Primer 30	-	8	0.64	80%
10	Primer 31	-	9	0.84	90%
11	Primer 32	1	9	0.64	90%
12	Primer 33	-	10	0.36	100%
13	Primer 34	3	6	0.75	66.60%

The most elevated polymorphism (100%) showed by four preliminaries 22, 23, 28, and 33 while the least polymorphism (60%) was manifested with one preliminary 24. What's more, normal polymorphism is 86.27%. Onamu *et al*., (2018) utilized 19 RAPD markers for the evaluation of hereditary assorted variety among 35 potato promotions and revealed 81.45% polymorphism which was lower when contrasted with the current examination. Beforehand, Gorji *et al*., (2011) [11], discovered mean polymorphism about 20%, 31%, and 57.4% which was not exactly the current outcome, showing the more unrivaled polymorphic capability of preliminaries utilized in the current examination and its work for additional exploration by other in future. Besides, Gorji *et al*., (2011) [11] found a 0.28 mean PIC esteem which was less when contrasted with the mean PIC estimation of RAPD (0.78) in the current exploration. Rocha *et al.*, (2010) [19] investigated hereditary decent variety in potato cultivar by RAPD and SSR markers. They notice that genomic DNA of 16 potato cultivars was Intensified with 25 RAPD ground works that produced 92 polymorphic groups. Tiwari *et al.*, (2020) [27] utilized the 24 microsatellite markers from the PGI unit to approve the hereditary decent variety of the 77 Indian Andigena potato center assortments. Altogether, 214 SSR alleles were identified in the center assortment, out of which 208 alleles were polymorphic with supreme frequencies between 2 to 58.

The PIC estimations of SSR loci went from 0.61 to 0.90. Datir (2020) [5] analyzed the allelic variety for 24 SSRs markers with a known situation on the potato hereditary guide incorporating competitor qualities related with sugar digestion in 305 descendants.

Phylogenetic investigation and planning of dendrogram

The 0-1 table was set up in dominate and another duplicate of information was spared in MS-DOS (text)' design. At that point replicated in Darwin Software was used to develop dendrogram (figure 5).

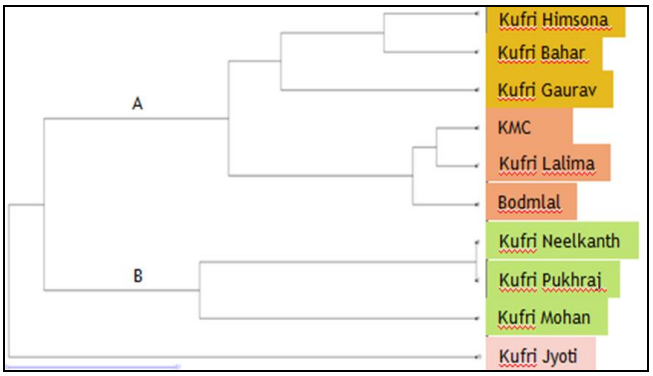

Fig 5: Dendrogram generated from RAPD using DARwin6 software based on RAPD markers in 10 varieties of potato

Determining true genetic dissimilarity between individuals using molecular markers is an important point for clustering which provides a visual idea about variability presented and assure the continued genetic improvement. Dendrogram generated by DARwin 6 software grouped 10 varieties of potato into two distinct clusters A and B Dendrogram were prepared as shown in figure 6. The biggest clusters with more number of genotypes found were Cluster A. In cluster - A it is obvious that Kufri Himsona, Kufri Bahar and Kufri Gaurav showed highest similarity while KMC, Kufri Lalima, and Bodmlal showed the highest similarity among themselves.

On the other in cluster B, it is obvious that least similarity was revealed by Kufri Jyoti. Kufri Neelknath and Kufri Pukhraj showed a closer relationship. Choosing parents/genotypes from these diverse clusters may produce heterosis in segregating generations which can be utilized for the development of good and promising hybrids**.** Wang *et al.* (1999) [29] established phylogenetic tree and dendrogram based on an unpaired group method of arithmetic means (UPGMA) using the software POPGENE (Version 1.31). Serrano *et al*., (2005) [23] generated dendrogram on the basis of the cluster analysis unbiased genetic similarity SSR alleles among cultivars. Hadi and Nurchasanah *et al.*, (2020) [12] developed dendrogram was generated by UPGMA. Ghebreslassie *et al.*, (2016) [9] was generated by UPGMA. The same pattern was observed based on factorial analysis computed using DARWin 6 software that the variety with identities 3, 9, 1, 7, 10, 4, 5 were clustered separately from the other genotypes and they are independent of one another. Mrutu (2015) [16] used factorial analysis to determine closely related varieties among 47 seed samples of maize UH6303.

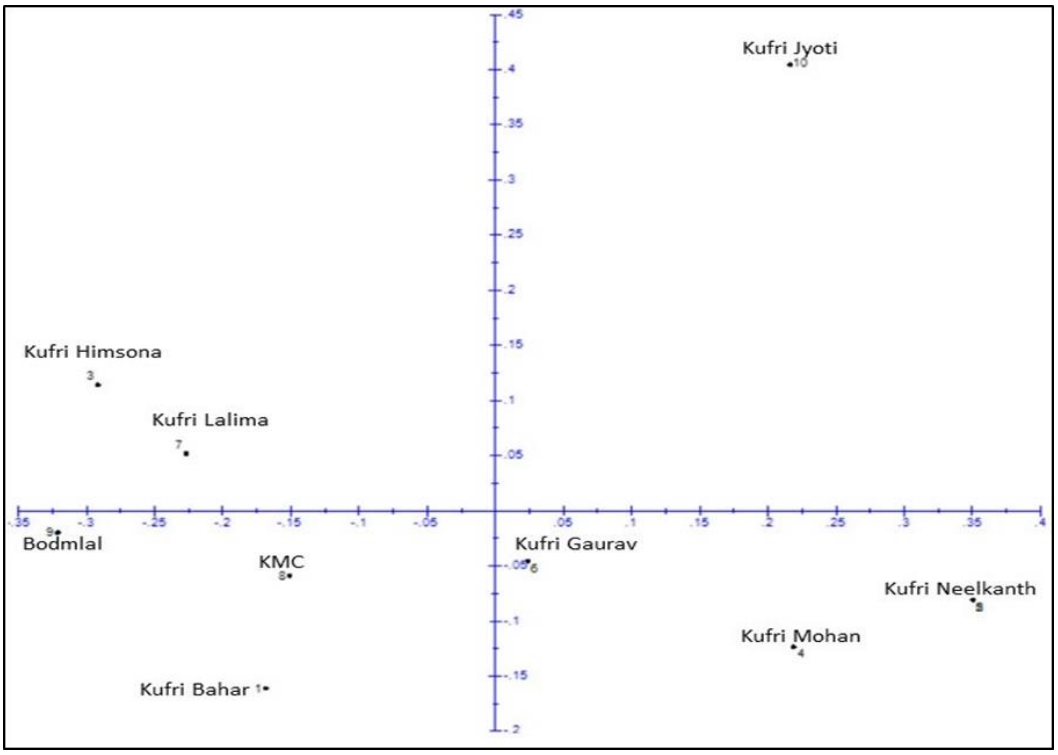

Fig 6: Factorial analysis among 10 varieties of potato-based on similarity matrix computed using DARWin software.

Table 6: Primer wise band size details of different molecular bands obtained in different potato varieties.

						Primer (bp)							
Plant varieties	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**
1	-	4000	3800	4500	3000	3000	5000	1400	-	3000	5800	6000	6000
		3000	3500	4000	4000	1200	1200	1100		3200	5200	3500	3500
		2300		3800	1400	1000	1000	1000			5000	3000	500
		600		2300	1500	740	500				4000		
		5000		1300		500					2500		
				1200		260					2000		
				1000		240							
				850									
				750									

2	-	-	-	1400	-	4000	5500	1400	3000	3000	5500	6200	4000
							1200	1000	2100	3200	5200	3800	
							1000		2000	2200	3800	3000	
							560		800		3000	2400	
									480		2500		
									460		2300		
									250		2200		
											1500		
											800		
3	7000	4000	-	3000	3000	3200	5500	-	2200	6000	8000	5500	7000
	6000	3000		2300	1600	2500	1400		1800	3200		3500	3500
	3500	600			1500	1200	1200		1600	2000			500
	2800	500				1000	1000		1300				
	2500					750	500		250				
						500							
						260							
						240							
4	-	-	3500	600	-	-	7500	1400	4000	8000	7000	5500	6000
			3000				5500	1100	1900	6000	6000	3500	3500
			1400				4000	1000		3000	5500		
			1300							2200	3200		
											2500		
											1800		
											1200		
5	-	-	-	-	-	-	-	1900	-	-	8000	7000	3500
								1400			6000	5200	
											5000		
											1000		
6	-	3000	3000	4000	-	3000	6000	2000	2900	-	5800	8000	6000
		4000		1600		780	4000	1400	1400		5000	6100	5800
		600		1400		740	1000	1000	400		4000		3500
		500		480		500					2700		
				450							2000		
											1800		
											1200		
											1000		
7	7000	4000	3000	5500	1200	8000	5500	4000	4000	4800	5800	7000	-
	3500	3000		3300	1100	4000	1200	2500	3000	3800	5500	5000	
	3200	2300		1500		800	1000	2000	1900	3100	1000		
	2300	600		480		750	500	1400	400				
	1800	500		460		730		1200	260				
	1500					500		900					
	1400					240		740					
8	6000	4000	3000	4500	1200	3900	5500	-	2500	3100	5000	5000	3500
	3500	3000		1500	1100	1200	1200		1600		3500	1400	
	3200	2300		1000		750	1000		1400		2500	1200	
	2300			480		740	500		480		1200		
	1800			460		500					1000		
	1500					240							
	1400												
9	5500	1400	3500	3000	1200	4000	-	5500	-	3500	5800	7000	2500
	2300	600	3000	1400	1100	2700		4000		3400	2500	5000	2200
	1800	500	2500	1300		2500		1400		2800		1500	
	1400			1200		1200		1200		2000			
						1000		260		1800			
						750		240		1400			
						500				1200			
10	3800	-	-	480	4000	5000	1400	480	480	3500	5800	7000	2200
	1400			100	2000	4000	1000	360	360	2500	5500	5000	1800
	600				1900			250	250	2000	4000	4000	1500
	400				1800				240	800	3000	2200	800
					740							1400	
					260							1200	

Conclusion

The present study was performed to demonstrate the study of molecular diversity of potato (*Solanum tuberosum).* All ten varieties of potato indicated presence of bands with one or more RAPD primers. The obtained results indicated that RAPD marker is useful for determining the genetic diversity

of potato varieties. PAGE and band imaging used to determine the primer showing polymorphism and no polymorphism. Dendrogram generated by DARwin6 software, Cluster A. In cluster - A it is obvious that Kufri Himsona, Kufri Bahar and Kufri Gaurav showed highest similarity while KMC, Kufri Lalima, and Bodmlal showed the highest similarity among themselves. On the other in cluster B, it is obvious that least similarity was revealed by Kufri Jyoti. Kufri Neelknath and Kufri Pukhraj showed a closer relationship. Factorial analysis, distribution of RAPD primer, and dendrogram used to determine the most closely related species among potato varieties. The present study is significant in aspect of evaluating the diversity among the different potato varieties.

As morphological and biochemical diversity analysis are not sufficient to differentiate the varieties in terms of their characters, hence molecular diversity analysis presents advantage over these conventional diversity analysis approaches as it gives more accurate information about the difference on genome level which could be useful in developing varieties with enhanced quality in near future.

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