

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.607.437>

Assessing Genetic Diversity of Potato (*Solanum tuberosum*) Genotypes Grown in Tarai Region of Uttarakhand by Using Simple Sequence Repeat (SSR) Technique

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ABSTRACT

This study aimed to evaluate the genetic diversity and identify potato cultivars by SSR markers. The genomic DNA of 50 potato cultivars was amplified with nineteen Simple Sequence Repeats (SSRs) primers. Out of nineteen primers only fifteen primers were amplified and gave polymorphic bands. Therefore, fifteen SSR primers were used in diversity analysis of 50 potato genotypes. Out of fifteen primers, four primers were found to be monomorphic and rests were polymorphic. 11 SSR primers pairs which were amplified polymorphic band that generated 34 polymorphic bands. Most of the primers detected more than one loci and observed to be polymorphic thus able to differentiate different genotypes. The dendrograms generated by cluster analysis distinguished the cultivars genetically. The PIC values demonstrated the high information content of the primers used and 50 potato varieties were identified based on SSR primer pairs. Thus, by means SSR markers the genetic diversity was assessed and the 50 potato genotypes analyzed in this study were identified.

Keywords

Solanum tuberosum,
Micro-satellites,
Genetic diversity.

Article Info

Accepted:
29 June 2017
Available Online:
10 July 2017

Introduction

Genetic diversity is a prerequisite for an effective plant breeding programme. It is a useful and essential tool for parent's choice in hybridization to develop high yield potential cultivars and to meet the diversified goals of plant breeding (Gaur *et al.*, 1978, Hayder *et al.*, 2007 and Shekhawat, 2001).

The cultivated potato have narrow genetic base due to limited introduction of germplasm from their natural range in South America (Rauf *et al.*, 2010). Most of the potato cultivars are autotetraploid ($2n=4x=48$), highly heterozygous and out breeding species,

which suffer from inbreeding depression. Heterosis in potato perpetuates because of its vegetative propagated nature. Therefore, selection of suitable parents for hybridization is crucial and evaluation of germplasm is essential to the development of new potato cultivars. The advantage of detecting polymorphism simply and quickly, but it lacks reproducibility (Chakrabarti *et al.*, 2001), while SSR markers provide high reproducibility and genetic informativeness (Braun and Wenzel, 2005). In the present investigation, the purpose of the study was to evaluate the genetic diversity in 50 potato

(*Solanum tuberosum* L.) genotypes that are being made available to potato growers and to develop a molecular profile using SSR markers.

Materials and Methods

The present investigation was conducted at Vegetable Research Centre (VRC) and Molecular diversity analysis was carried out in the Pantnagar Centre for Plant Genetic Resources (PCPGR) of Govind Ballabh Pant University of Agriculture & Technology, Pantnagar, Udham Singh Nagar, Uttarakhand, India during 2014-15.

Genomic DNA isolation protocol

Modified CTAB (Cetyl Trimethyl Ammonium Bromide) method

Using electronic balance 1.0 g of alcohol sterilized leaf material was weighed for DNA extraction.

A minute quantity of PVP (Poly vinyl pyrrolidone) was added to combat the effect of phenolic compounds

Pre-weighed leaf material was ground in liquid nitrogen to fine powder using pre-chilled pestle and mortar

The powder was transferred to 15 ml polypropylene tubes containing 5 mL, of pre-warmed extraction buffer. Spatula was used to dispense the material completely.

Samples were incubated at 60° C for 30 min with occasional mixing by gentle swirling.

3 ml (double amount of sample) chloroform: iso-amyl alcohol (241) was added and mixed by inversion to emulsify.

Centrifugation was done at 15,000 rpm at room temperature for 10 min.

Aqueous phase was removed with a wide bore pipette and transferred to a clean tube and added with 2/3 volume of isopropanol, mixed by quick gentle inversion and the tubes were kept overnight at -20° C.

The samples were centrifuged for 10 min at 5,000 rpm. 4°C.

The supernatant was poured without disturbing the DNA pellet at the bottom of the tubes.

The DNA pellet was washed with 70% ethanol.

The pellet was air dried and dissolved in 500 µL TE buffer. It was stored at -20° C.

Standardization of PCR conditions

There are number of variables in a PCR which have to be optimized to give target amplification. These parameters are:

Denaturation temperature and time.

Annealing temperature and time.

Amount of template DNA and primer to be taken.

Concentration of MgCl₂ in the assay buffer.

The number of cycles to be performed.

All the SSR markers used in the study were selected based on copy number and absence of stutter band good quality and high diversity index values (Feingold *et al.*, 2005; Milbourne *et al.*, 2008; Kandemir *et al.*, 2010; Kandemir *et al.*, 2006, Rocha *et al.*, 2010 and Sharma *et al.*, 2014.

Detail of SSR used in this experiment was given in table 1.

Procedure of gel electrophoresis

Horizontal gel electrophoresis unit was used for fractionating SSR markers on agarose gel using the following procedure:

The open ends of a clean, dry plastic tray supplied with the electrophoresis were sealed with tape so as to form a mold.

The mold was set on a horizontal section of a bench.

Agarose gel (1.5% and 2.5%) for SSR was prepared by dissolving appropriate amount of agarose in TAE buffer. Agarose was dissolved by heating the solution at 100°C. It is allowed to cool to room temperature.

Added EtBr to a final concentration of (0.5 µL of 10 mg/mL) solution of EtBr to 100 mL gel mixture) and mix well.

After complete setting of the gel, the comb was removed carefully, the tape was removed and the gel was mounted in an electrophoresis tank and PCR amplified products were loaded in each gel along with molecular markers.

A pre-run of 15 minutes at 50 Volt given to the gel.

For each well, DNA loading dye and DNA samples were mixed in 1:5 ratios and loaded in the gel with a micropipette.

Electrophoresis was done at 80-90 volt for 3 hour in TAE electrophoresis buffer.

The gel was visualized in U.V. transilluminator (BioRad, USA) and stored using gel documentation system.

Data analysis

Amplified SSR profile of all the genotypes with each primer was documented using gel

documentation stem. Data were recorded as presence (1) or absence (0) of band throughout the amplified profile with a primer. Similarly data were also recorded for all the primers separately as presence or absence of band. Each amplified band was considered as unique locus. The binary data were used to calculate genetic similarities based on Jaccard's coefficient (Jaccard, 1981) and UPGMA (Unweighted Pair Group Method using Arithmetical Averages) dendrogram was generated to determine the genetic relationship of potato genotypes.

Results and Discussion

Genetic diversity analysis

Pair wise similarity matrix based on Jaccard's coefficient for fifteen SSR primers are presented in table 2 for 50 potato genotypes. Four primers were found monomorphic and rests were found to be polymorphic. The similarity coefficient was ranged from 31 to 100 per cent. Maximum similarity occurred between EM-5 and TPSK-05-06-44 (100%) while TPSK-05-06-95 and Laddy Rosseta also showed 100 per cent similarity with each other (Table 3).

Cluster analysis

The phylogenetic tree was constructed through NTSYSpc cluster analysis software using UPGMA (un-weighted pair group method with arithmetic mean) of SSR markers involving data generated out of fifteen primers on fifty genotypes of potato depicted in figure 1. All 50 genotypes were demarcated at approximately 51 per cent similarity and further divided into two clusters each with approximately 57.5 and 58.5 per cent similarity (Fig.1).

Cluster I comprised of 30 genotypes while cluster II contained Only 20 genotypes.

Table.1 Detailed description of primer sequences of SSR markers for potato

S. No.	SSR Primer Code	Forward Sequence (5'→3')	Reverse Sequence (5'→3')
1.	STM0007	5'-GACAAGCTGTGAAGTTTAT-3'	5'AATTGAGAAAGAGTGTGTGTG-3'
2.	STI24	5'-CGCCATTCTCTCAGATCACTC-3'	5'-GCTGCAGCAGTTGTTGTTGT-3'
3.	STI30	5'-TTGACCCTCCAACATAGATTCTTC-3'	5'-TGACAACCTTAAAGCATATGTCAGC-3'
4.	STI57	5'-CCTTGTAGAACAGCAGTGGTC-3'	5'-TCCGCCAAGACTGATGCA-3'
5.	STP0Ac58	5'TTGATGAAAGGAATGCAGCTTGIG-3'	5'ACGTTAAAGAAGTGAGAGTACGAC-3'
6.	STM1016	5'TTCTGATTTTCAGTCATGTTTCC-3'	5'ATGCTTGCCATGTGATGTGT-3'
7.	STI031	5'AGGCGCACTTTAACTTCCAC-3'	5'CGGAACAAATTGCTCTGATG-3'
8.	STM1104	5'TGATTCTCTTGCTACTGTAATCG-3'	5'CAAAGTGGTGTGAAGCTGTGA-3'
9.	STM1106	5'TCCAGCTGATTGGTTAGGTTG-3'	5'ATGCCGAATCTACTCGTCATGG-3'
10.	STM0037	5'AATTTAACTTAGAAGATTAGTCTC-3'	5'ATTTGGTTGGGTATGATA-3'
11.	STM0030	5'AGAGATCGATGAAAAACACGT-3'	5'GTGGCATTTTGATGGATT-3'
12.	STM0019	5'AATAGGTGTAAGTACTCTCAATG-3'	5'TTGAAGTAAAAGTCCAGTATGTG-3'
13.	STM1049	5'CTACCAGTTTGTGATTGTGGTG-3'	5'AGGGACTTTAATTTGTTGGACG-3'
14.	STM1031	5'TGTGTTTGTCTTCTGTAT-3'	5'TTCAGTCAACTCCTGTTGCG-3'
15.	STU6SNRN	5'GAAGTTTATCAGAATCC-3'	5'ATCACCTCATCAGCAATC-3'
16.	STI005	5'CTACCAGTTTGTGATTGTGGTG-3'	5'AGGGACTTTAATTTGTTGGACG-3'
17.	STGBSS	5'AATCGGTGATAAATGTGAATGC-3'	5'ATGCTTGCCATGTGATGTGT-3'
18.	STWAX-2	5'CCCATAATACTGTCGATGAGCA-3'	5'GAATGTAGGGAAAGATGCATGA-3'
19.	STCAAS3	5'AATTCATGTTTGCAGTAGGTC-3'	5'ATGCAGAAAGATGTCAAATGA-3'

Table.2 Analysis for polymorphism in SSR markers

S. No.	Primer	Sample size	GC content(F)	GC content(R)	Annealing temperature (°C)	Polymorphic bands	Monomorphic bands	Polymorphism (%)
1.	STM0007	48.00	36.84	38.09	58.00	3	0	100
2.	STI24	48.00	52.38	50.00	60.00	4	0	100
3.	STI57	48.00	52.38	55.55	60.00	3	1	75
4.	STP0Ac58	48.00	41.66	41.66	57.00	2	0	100
5.	STM1016	48.00	31.81	45.00	58.00	2	0	100
6.	STM0037	48.00	25.00	33.33	53.00	2	0	100
7.	STM0030	48.00	33.33	38.55	55.00	2	0	100
8.	STM0019	48.00	39.13	33.33	52.00	2	0	100
9.	STI005	48.00	39.13	40.90	58.50	5	0	100
10.	STGBSS	48.00	36.36	45.00	58.80	6	0	100
11.	STM1031	48.00	26.31	50.00	52.60	3	1	75

Table.3 Principal component (PC) score based on the correlation coefficient matrix of 17 quantitative characters of 50 genotypes of potato (2014-15)

Genotype	Vector 1	Vector 2	Vector 3	Vector 4	Vector 5	Vector 6	Vector 7
Kufri Arun	338.441	51.838	132.575	-197.283	-113.521	108.314	107.950
Kufri Jawahar	250.191	9.631	117.667	-147.301	-83.968	70.225	70.526
PNT-1	252.036	6.552	112.538	-145.410	-86.139	59.336	63.608
TPSK-05-06-110	155.756	-48.683	110.550	-98.414	-53.167	21.888	19.748
TPSK-05-06-79	291.725	1.142	134.252	-174.679	-102.380	76.383	72.666
MS/95-1542	277.499	17.028	110.840	-158.730	-106.532	45.449	65.920
EM-2	227.724	-0.596	116.541	-135.529	-81.265	51.261	56.007
TPSK-0506-98	186.515	-42.734	122.380	-113.317	-65.692	36.003	30.390
DPS-19	185.722	-18.471	112.542	-106.607	-60.340	40.466	39.130
M-3	276.990	26.624	128.865	-164.292	-97.837	80.061	82.679
TPSK-05-06-95	84.663	-89.899	106.939	-63.583	-37.529	-10.777	-16.108
Laddy Rossetta	121.689	-66.799	117.273	-79.879	-44.396	12.137	7.733
EM-5	180.412	-24.237	112.302	-114.306	-63.747	35.121	35.298
EM-3	186.060	-21.699	118.425	-118.604	-65.854	41.333	38.082
Kufri Giriraj	304.545	17.640	138.041	-182.105	-115.488	77.774	81.795
Atlanta	109.602	-64.765	110.051	-63.766	-39.632	4.686	-0.358
Kufri Sadabahar	82.454	-81.710	100.798	-57.768	-31.496	-9.595	-13.734
AICRP-07-05	205.315	-41.087	121.602	-125.231	-83.417	25.373	26.630
TPSK-05-06-117	328.164	-5.004	144.379	-205.661	-137.598	65.098	70.130
Kufri Chipsona-2	177.313	-50.884	124.969	-104.974	-72.515	14.518	16.746
MS/93-1344	143.307	-66.670	114.979	-97.428	-57.504	10.826	6.715
TPSK-05-06-105	146.721	-54.289	108.916	-89.967	-61.197	9.411	12.132
Kufri Badsah	160.979	-30.624	113.258	-91.297	-53.574	34.167	29.774
TPSK-05-06-80	151.279	-57.551	111.950	-93.155	-55.525	14.606	12.828
Kufri Pushkar	303.811	11.610	128.645	-183.012	-114.406	74.516	74.100
TPSK -05-06-44	290.809	-30.134	121.613	-174.713	-128.074	28.219	44.189
Kufri Khyati	225.743	-22.409	122.915	-129.453	-84.043	42.688	39.936
Kufri Jyoti	322.296	35.848	131.710	-188.128	-118.069	89.900	95.167
TPSK-05-06-61	136.818	-67.465	108.352	-81.825	-53.788	0.398	2.523
EM-1	162.553	-22.989	109.276	-98.506	-49.827	41.399	35.075
TPSK-05-06-85	191.139	-54.996	123.202	-115.576	-73.715	20.626	17.904
C-11	157.175	-29.005	115.566	-90.143	-49.709	36.870	33.381
DPS-07	250.286	14.034	117.733	-151.936	-83.459	70.777	71.370
TPSK-05-06-86	162.542	-55.807	117.781	-98.920	-61.611	14.155	12.921
MS/99-1871	154.579	-61.727	113.781	-108.766	-61.023	13.438	12.362
MS/0-3740	305.907	2.921	134.991	-198.673	-113.497	78.864	77.162
TPSK-05-06-83	349.377	10.829	145.151	-221.950	-135.450	83.118	86.552
TPSK-05-06-007	264.759	-15.001	135.904	-162.829	-99.677	56.086	56.022
Kufri Himalini	257.414	19.267	117.702	-149.780	-85.868	71.900	73.858

Kufri Surya	263.158	29.143	126.245	-150.200	-81.267	84.695	80.317
MS/0-9808	203.099	-12.524	114.619	-120.664	-68.993	44.887	44.122
PH-3	199.416	-34.361	123.466	-116.757	-76.833	30.265	31.567
P-11	186.919	-27.863	111.185	-111.504	-72.651	30.672	31.465
C-1	133.516	-30.120	85.506	-73.983	-48.510	8.890	19.613
C-10	199.515	-2.025	105.046	-113.916	-70.454	39.144	47.538
Kufri Frysona (C)	272.355	-1.542	129.661	-168.878	-106.508	57.120	62.851
Kufri Chipsona-1 (C)	287.018	26.895	121.061	-165.395	-98.977	72.998	79.969
Kufri Bahar (C)	96.165	-62.732	97.608	-66.726	-33.249	0.423	-1.504
Kufri Guarav (C)	251.535	-14.709	118.747	-148.158	-100.129	40.041	50.464
Kufri Ashoka (C)	307.667	18.192	138.361	-185.396	-109.823	88.379	80.407

Fig.1 Dendrogram depicted the classification of the fifty genotypes of potato constructed using UPGMA method based on SSR markers. The scale in the bottom is Jaccard's coefficient of similarity

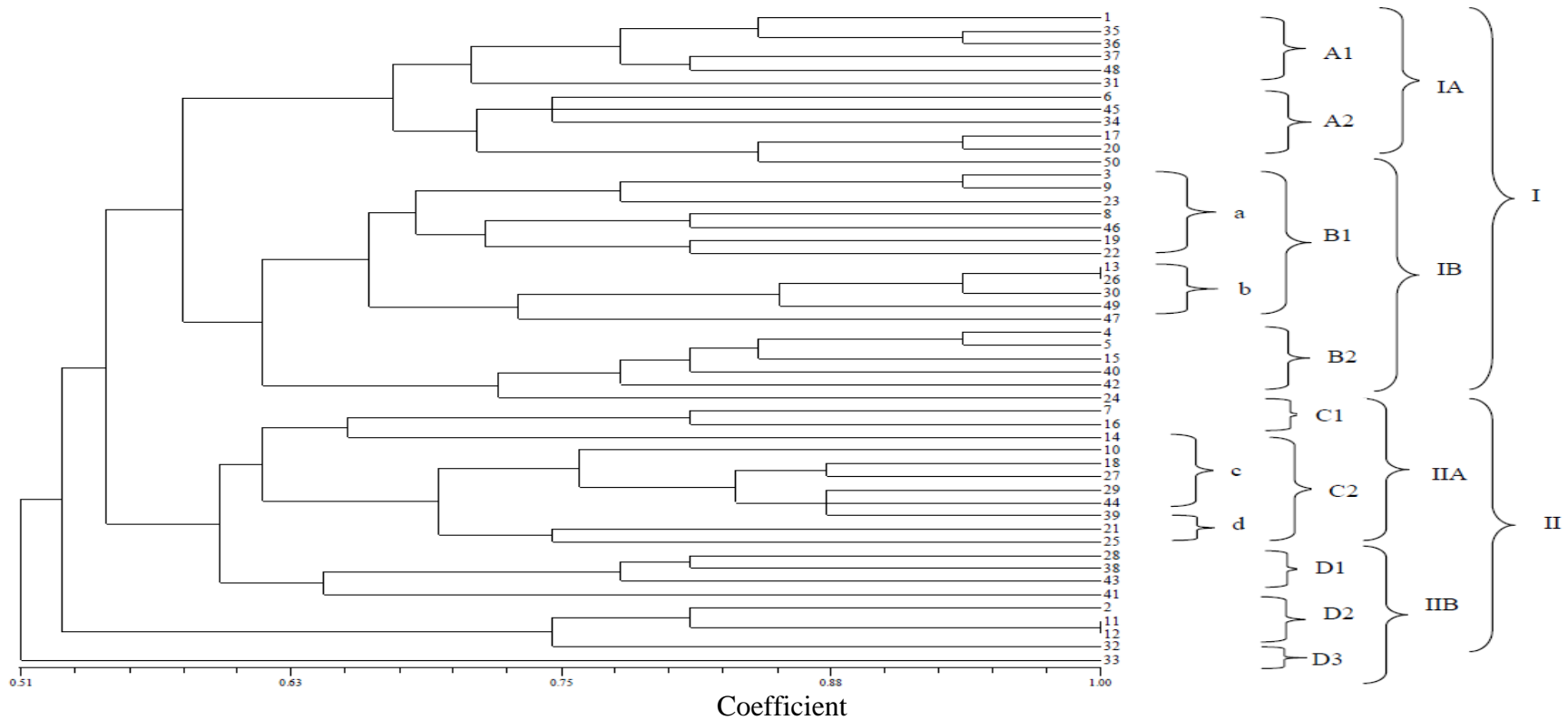


Fig.2 PCR amplification of 50 genotypes of potato by SSR primer STI24

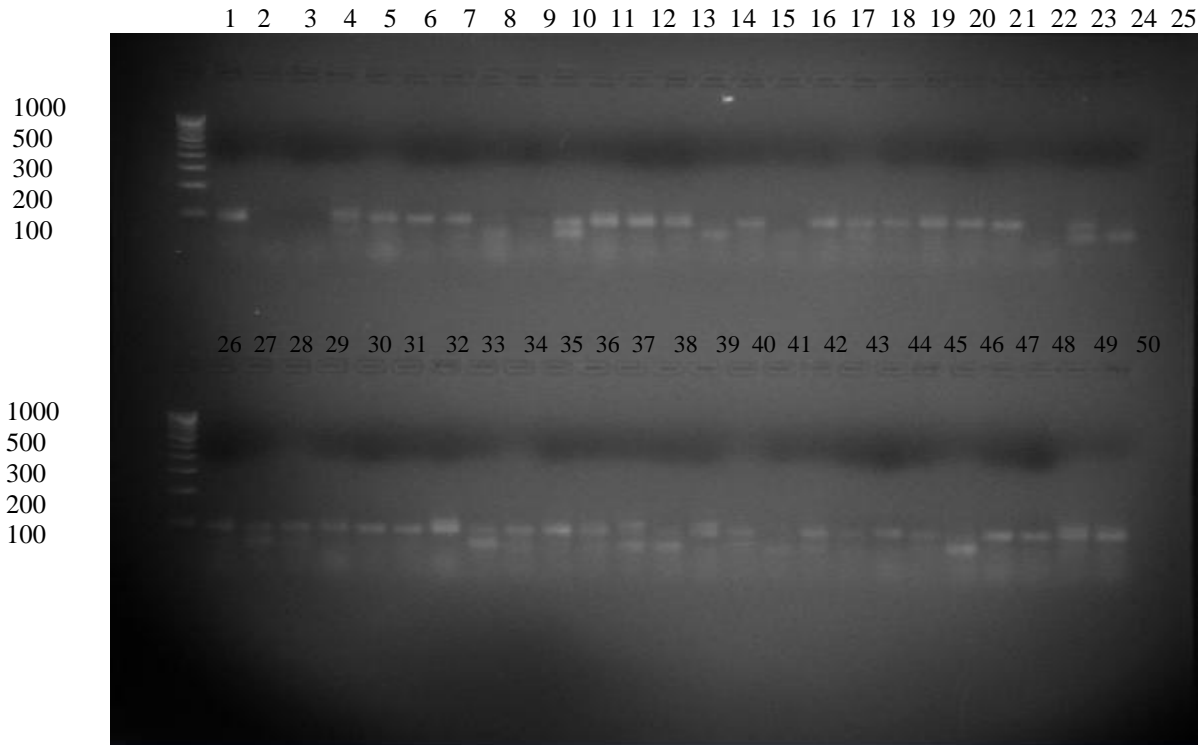


Fig.3 PCR amplification of 50 genotypes of potato by SSR primer STI57

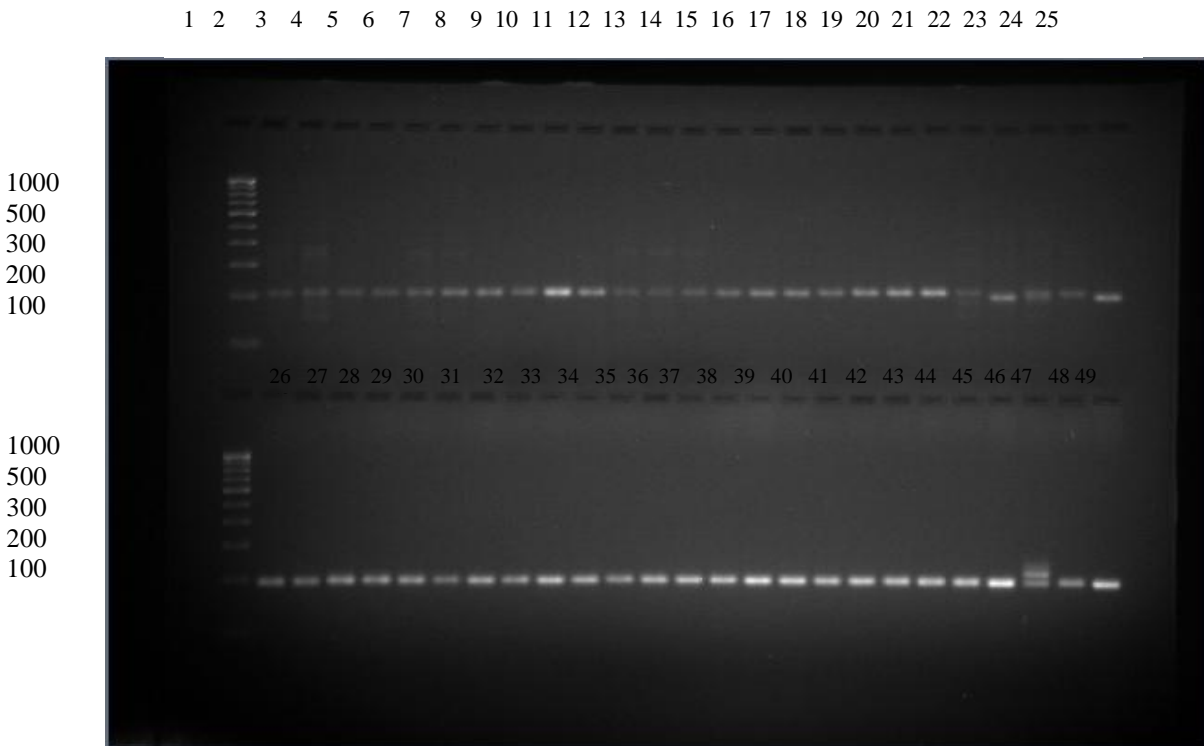


Fig.4 PCR amplification of 50 genotypes of potato by SSR primer STGBSS

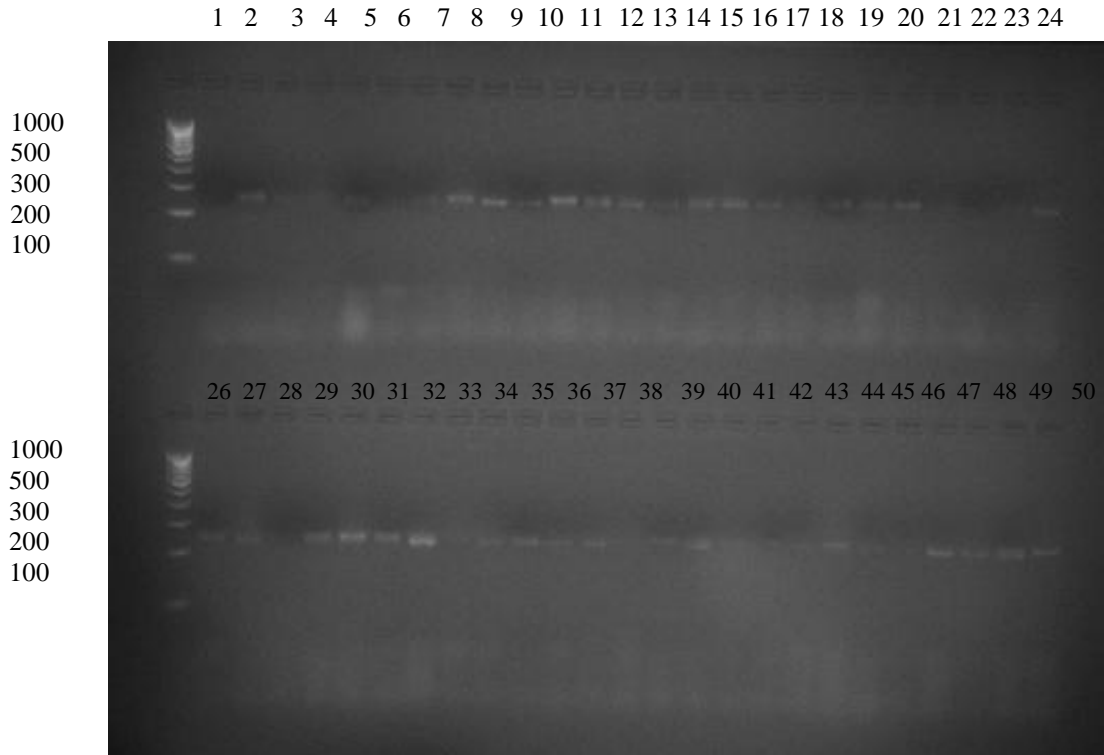


Fig.5 PCR amplification of 50 genotypes of potato by SSR primer STM1016

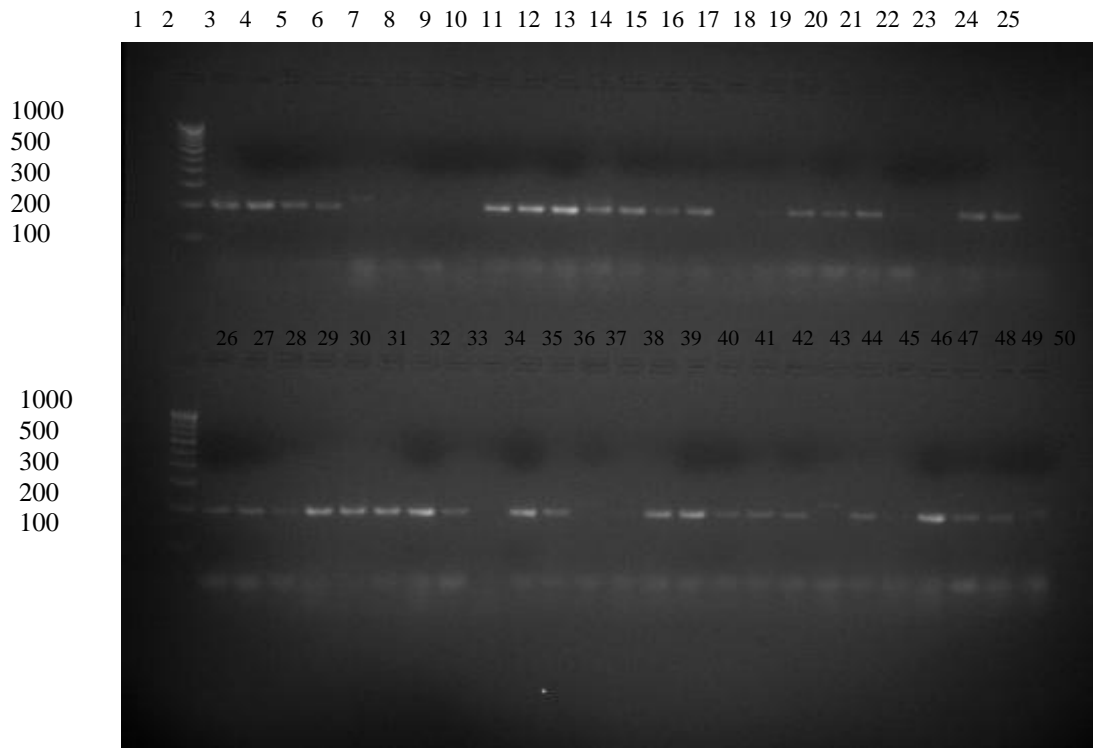


Fig.6 PCR amplification of 50 genotypes of potato by SSR primer STP0Ac58

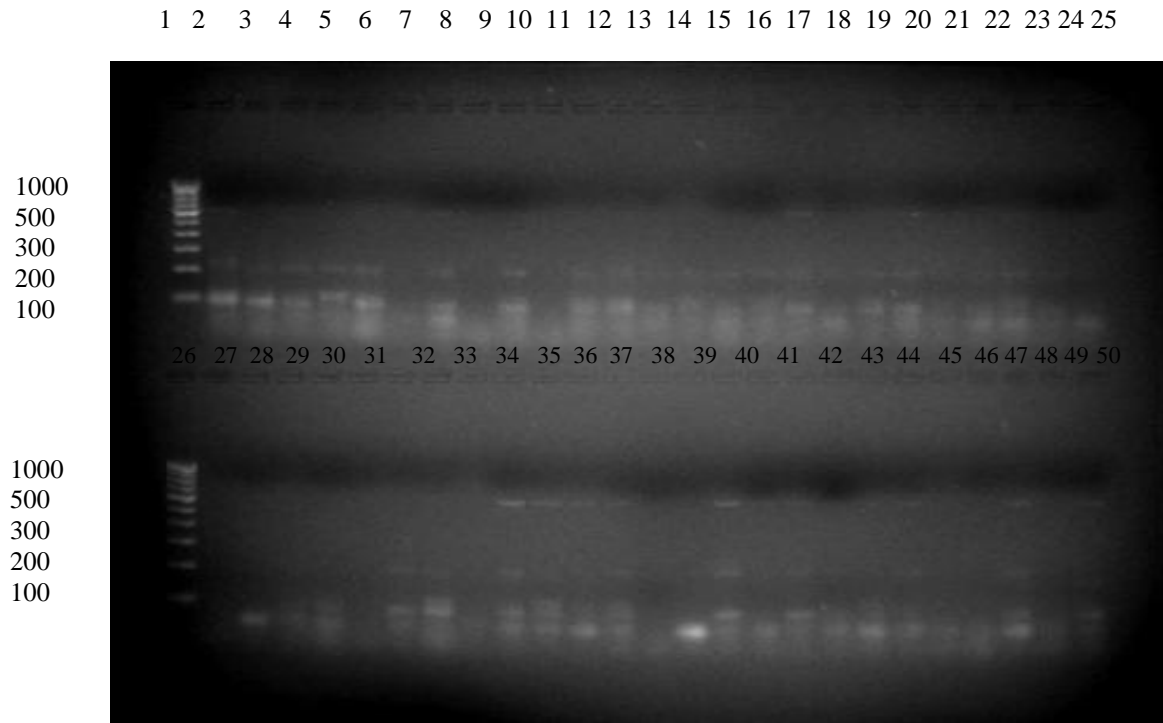


Fig.7 PCR amplification of 50 genotypes of potato by SSR primer STM0019

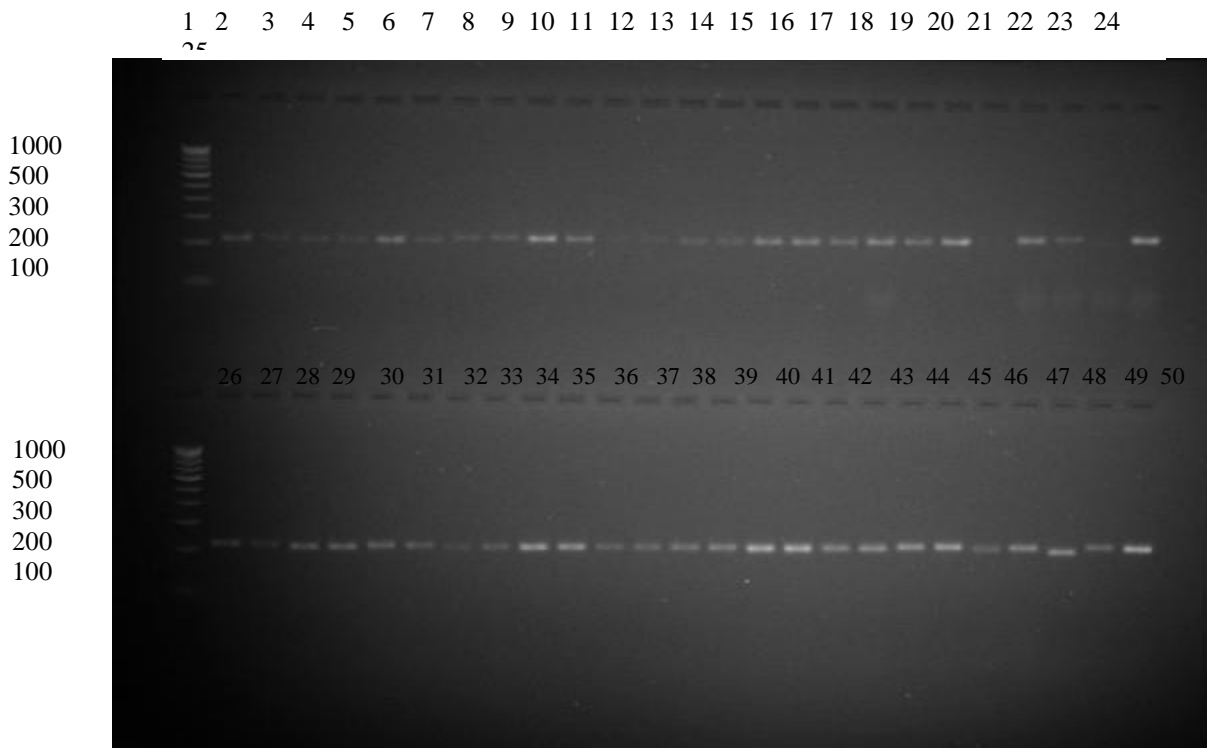


Fig.8 PCR amplification of 50 genotypes of potato by SSR primer STI005

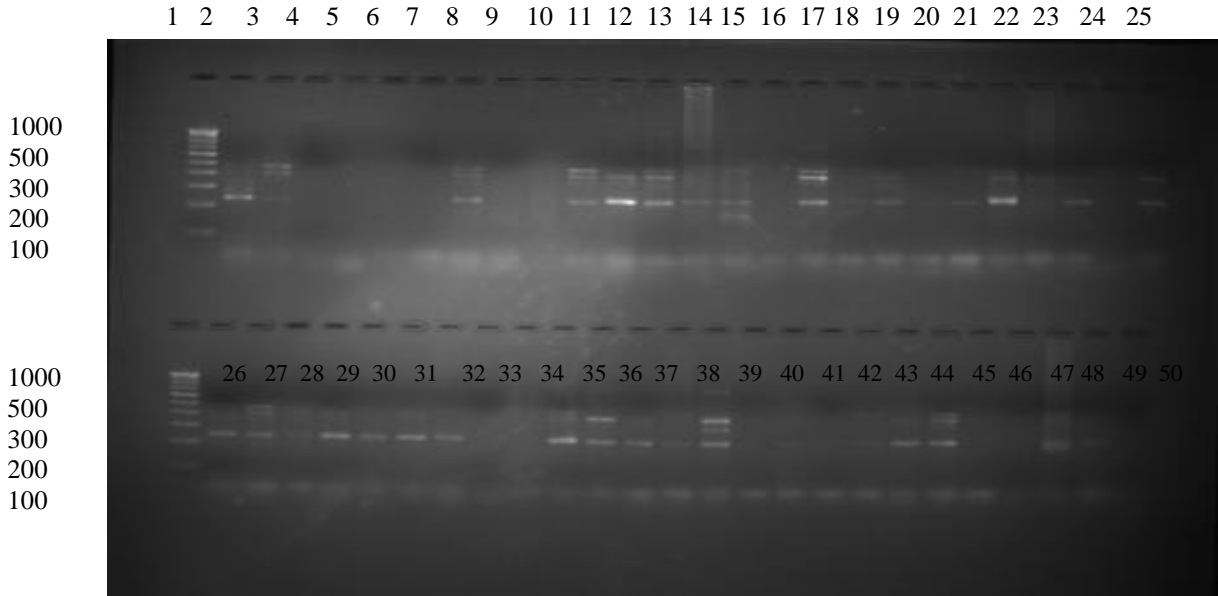
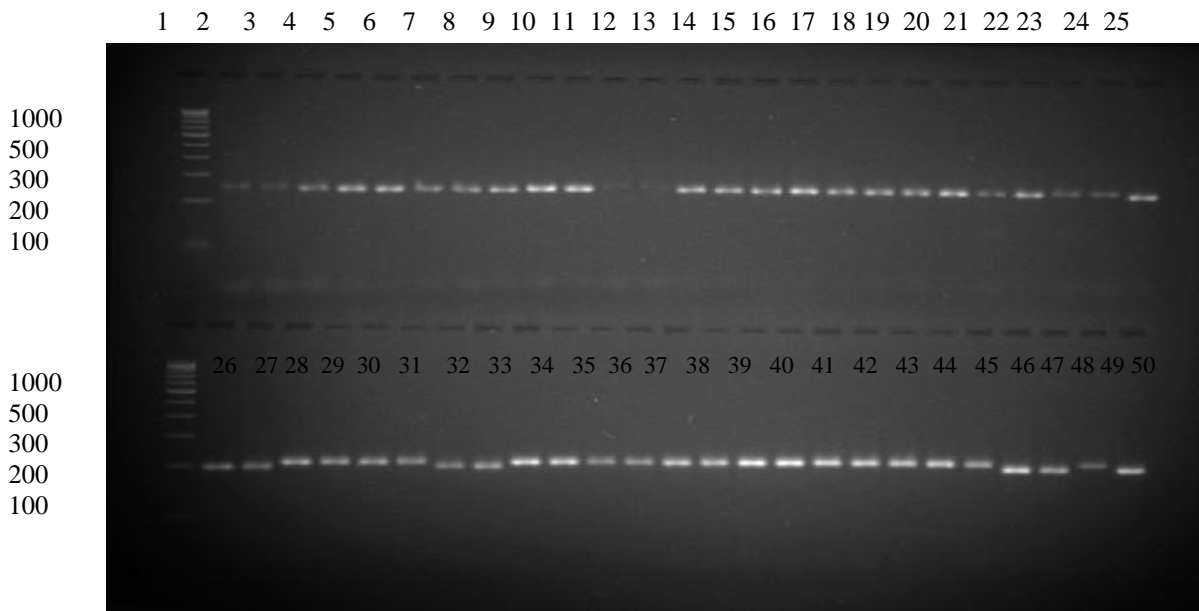


Fig.9 PCR amplification of 50 genotypes of potato by SSR primer STM0030



Cluster I was further subdivided into two sub-clusters IA and IB each with approximately 67 and 61.5 per cent similarity, respectively. Sub-cluster IA was further forked in to two small clusters A1 and A2, cluster A1 genotypes had approximately 70 per cent similarity and contain 6 genotypes i.e. Kufri Arun, MS/99-1871, MS/0-3740, TPSK-05-06-83, Kufri Bahar and TPSK-05-06-85 while cluster A2 also

contain 6 genotypes (MS/95-1542, C-10, TPSK-05-06-86, Kufri Sadabahar, Kufri Chipsona-2, Kufri Ashoka) with approximately 71 per cent similarity. Sub cluster IB divided into two small clusters B1 and B2 with approximately 66 and 72 per cent similarity. Cluster B1 again divided into two super small cluster a and b with approximately 68 and 74 per cent similarity. Cluster a of super small

cluster comprised seven genotypes viz. PMT-1, DPS-19, Kufri Badsah, TPSK-05-06-98, Kufri Frysona, TPSK-05-06-117 and TPSK-05-06-105. Cluster b of super small clusters contains five genotypes EM-5, TPSK-05-06-44, EM-1, Kufri Gaurav and Kufri Chipsona-1. Cluster B2 contain six genotypes i.e. TPSK-05-06-110, TPSK-05-06-79, Kufri Giriraj, Kufri Surya, PH-3 and TPSK-05-06-80.

Main cluster II was further subdivided into two sub-clusters IIA and IIB each with approximately 61.5 and 64 per cent similarity, respectively.

Sub-cluster IIA was further forked in to two small clusters C1 and C2, cluster C1 genotypes had approximately 64.5 per cent similarity and contain 3 genotypes i.e. EM-2, Atlanta and EM-3 while cluster C2 further divided into super small cluster i.e. c and d. Super small cluster c contain six genotypes viz. M-3, AICRP-07-05, Kufri Khyati, TPSK-05-06-61, C-1 and TPSK-05-06-007 with 75.5 per cent similarity and cluster d of super small cluster comprised two genotypes viz. MS/93-1344 and Kufri Pushkar with 74.8 per cent similarity. Cluster IIB divided into two small cluster i.e. cluster D1, cluster D2 and cluster D3 with approximately 64, 74.5 and 51 per cent similarity. Cluster D1 contain Kufri Jyoti, TPSK-05-06-007, P-11 and MS/0-9808. Cluster D2 contain four genotypes i.e. Kufri Jawahar, TPSK-05-06-95, Laddy Rossetta and C-11. Cluster D3 comprised one genotype i.e. DPS-07.

In contrast, SSRs are codominant and give reproducible results because they are mostly developed from introns of genes that is why they are said to be highly specific especially useful for mapping in tetraploid potato. Milbourne *et al.*, 1997 compared different types of PCR derived markers to estimate variability and concluded that microsatellites offer an effective means of analyzing genetic distance between potato varieties. SSRs additional advantages include their ubiquity, distribution across the genome, co-dominant behavior, multiallelism, reproducibility and high level of

polymorphism detected (Milbourne *et al.*, 1997, Mc Gregor *et al.*, 2000 and Spooner *et al.*, 2005). SSRs produced specific patterns, high polymorphism and placed genotypes in many clusters. Similar findings were recorded by Ghislain *et al.*, (2006) and Komy *et al.*, (2012). Thus, by means of SSR markers the genetic diversity was assessed and the 50 potato genotypes analyzed in this study were identified (Figs. 2-9).

Thus, based on the finding of present investigation it can be concluded that significant diversity and variability was present among the genotypes. The SSR markers used in the study were found to produce polymorphic bands and resulted into differentiation among various genotypes under study. SSRs produced specific patterns, high polymorphism and placed genotypes in clusters. Thus, by means of SSR markers the genetic diversity assessed and the 50 potato genotypes/varieties analyzed in this study were identified. The significant variation exists among the genotypes based on molecular characters and with the use of SSR markers, assessment of the genetic diversity can also help us to plan a future breeding programme using the diverse parent.

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How to cite this article:

Rajani, Dharendra Singh and Jeena, A.S. 2017. Assessing Genetic Diversity of Potato (*Solanum tuberosum*) Genotypes Grown in Tarai Region of Uttarakhand by Using Simple Sequence Repeat (SSR) Technique. *Int.J.Curr.Microbiol.App.Sci*. 6(7): 4219-4230.
doi: <https://doi.org/10.20546/ijcmas.2017.607.437>