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Characterization of potato cultivars using EST-SSR and ISSR 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. Use of molecular markers to determine genetic variation, genetic diversity and evolutionary relatedness is becoming more popular for the assessment of diversity among cultivars. EST-SSR amplification of 42 potato cultivars using 61 EST-SSR primers generates 4382 scorable bands with an average of 72 bands per primer. The grouping provided by cluster analysis showed cultivar identification and divergence among potato cultivars. All 42 cultivars were classified into 5 main clusters where Kufri Nilkanth, 20-(P-42-600), 17-(P-35-400), Kufri Khyati, Kufri Kesar, Kufri Ashoka, 19-(P-40-500), 4-(C-20-200), and 1-(C-10-400) most diverge one. Genotypes could be identified based on specific banding pattern. Inter Simple sequence repeat (ISSR) analysis showed ISSR amplification of 42 potato cultivars using 29 ISSR primers generates 6002 scorable bands with an average 207 bands per primer. The cluster analysis showed identification of cultivar and variance among 42 cultivars, grouped the cultivars into 4 clusters where Kufri Himalini, 28-(RH-2-600), Kufri Chandramukhi, 20-(P-43-200) and 26-(P-54-300).

Keywords: Potato, genetic diversity, EST-SSR, ISSR

Introduction
Genetic diversity is key to advancing crop improvement programs. The best way to increase crop diversity is to use germplasm stored in gene banks in plant breeding programs (Liao and Guo, 2013). multiple molecular markers (e.g., RAPD, SCAR, SSR, ISSR) are used to assess genetic diversity, of which SSR is used for pedigree (Sitther *et al.* 2012) [22], genetic mapping (Song *et al.* 2005) [23] and genetic diversity analysis (Baranski *et al.* 2012) [2] because of its high polymorphism, codominance, simplicity, and low cost. In the first study using the potato SSR, we characterized the genetic structure of plants from the anthers of potato clones by searching the potato sequences published by, yielding five polymorphic SSRs. (Veilleux *et al.* 1995) [27]. Then Kawchuk *et al.* (1996) [10] examined 252 *S. tuberosum* sequences, observed 24 alleles, and used the DNA products of to construct a database for cultivar identification. Milbourne *et al.* (1997) [15] used SSR and his three other PCR-based marker systems in to examine genetic relationships in the potato gene pool and identified 16 cultivars. Since then, additional SSR sites have been discovered in the potato genome, including highly informative and user-friendly microsatellites (Ghislain *et al.* 2004) [7]. The SSR marker has been used to identify French potato cultivars (Moisan-Thiery *et al.* 2005) [16], to identify potato germplasm in the INIA Chilean breeding program (Mathias *et al.* 2007) [7], and to SSR developed potato cultivars (Reid and Kerr, 2007) [20] that were used to develop a rapid identification method based on SSR and developed an SSR-based potato genetic identity kit (Ghislain *et al.* 2009) [6]. Identified genetic relationships in Spanish potato cultivars (de Galarreta *et al.* 2011) [3].
ISSR were the first to use to distinguish closely related individuals (Zietkiewicz *et al.* 1994) [28]. Inter Simple Sequence Repeats (ISSRs) are PCR-based dominant markers that do not require prior genome information. This technique is simpler and straightforward than AFLP, involves less developmental cost than SSRs and more reliable than RAPD markers. It can be used to study biodiversity, hybridization, and genetic stability (J. Tiwari *et al.* 2015) [25], gene mapping, and genetic map construction (Gupta *et al.* 2012) [8]. Prevost & Wilkinson, (1999) [19] successfully used the technique to identify, characterize, and estimate genetic divergence among potato cultivars.

Materials and methods

Plant materials

The cultivars used in this study are collected from agriculture and horticultural research station, Khambolaj, Anand Agricultural University.

DNA isolation

The DNA was isolated from leaf samples of 42 potato cultivars collected from tuber grown plants (300 mg) by crushing them into small pieces using Liq N2. We transfer the powdered leaf into 2 ml microcentrifuge tubes and add 500 µl warm buffer (2% PVP, pH 8.0, EDTA 20mM, 5% (W/V) CTAB, Tris-Cl pH 8.0, 100mM, 1.4M NaCl, 2 % mercaptoethanol) to each tube and keep them in 65ºC hot water bath for 45 minutes after which the tubes are shaken every 10 minutes. In a microcentrifuge tube, mix equal volumes of chloroform: isoamyl alcohol (24:1) gently for one minute. After centrifugation at 4°C for 15 minutes at 12,000 rpm, the mixture was collected. The supernatant was transferred into new tubes containing iso-propanol and 3M sodium acetate pH 6.0. The tubes were shaken gently several times and incubated for 10-15 minutes. DNA strands were easily visible after this time. At 4 ºC, DNA strands were centrifuged for 10 minutes at 10,000 rpm for 10 minutes, and the supernatant was gently emptied afterward. Inside the tube, a white DNA pellet was intact. To the tubes containing DNA pellet, 500 µl 70% ethanol was added, and the tubes were centrifuged at 10,000 rpm at 4 C for 5 minutes. After discarding the upper phase, the tubes were placed upside down on absorbent paper so that the deposition dried, and then 50 ml of TE buffer (10 mM Tris-Cl pH 8.0, 1mM 0.5 M EDTA pH 8.0) was added to each. At 37 ºC for 60 minutes, 2 µl of RNAase was added to each tube. RAase enzyme was then inactivated at 65 ºC for 5 minutes (Doyle and Doyle, 1987) [4].

Polymerase chain reaction (PCR)

To assess the genetic diversity of isolates of this species, 29 ISSR primers and 61 EST-SSR primers was selected and were used for conducting the test in polymerase chain reaction. The names are listed in table. The PCR reactions were conducted in the Bio-Rad ® Thermocycler and Applied biosystems thermal cycler.

Expressed sequence tag-derived simple sequence repeat markers (EST-SSRs) reaction conditions

Polymerase chain reaction (PCR) for EST-SSR was performed in 10 µl volume containing 1 µl templates DNA, 8 µl PCR master mix (2x), 1 µl diluted primer, and 3 µl nuclease-free water. The PCR reaction conditions were an initial denaturation of 95 °C for 3 minutes, followed by 35 cycles of 94 °C for 30 seconds, annealing temperature (primer specific) for 30 seconds, 72 °C for 1 min, and a final extension at 72 °C for 5 minutes (Kristamtini *et al.* 2016) [11].

Inter-simple sequence repeat (ISSR) reaction conditions

Polymerase chain reaction (PCR) for ISSR was performed in 10 µl volume containing 1 µl templates DNA, 8 µl PCR master mix (2x), 1 µl diluted primer, and 3 µl nuclease-free water. The PCR reaction conditions were an initial denaturation of 94 °C for 5 minutes, followed by 35 cycles of 94 °C for 1 min, annealing temperature (primer specific) for 75 seconds, 72 °C for 2 min, and a final extension at 72 °C for 10 minutes (Bagherabadi *et al.* 2015) [1].

The amplified fragments were separated by electrophoresis using agarose gel at 2.5% (w/v), 3µl ethidium bromide (10 mg/ mL) with TBE buffer (40 mM Tris-borate, pH 8.0; 1 mM Na_2 EDTA), for 1 h at 120 V, and photographed under UV light.

Statistical analysis

Based on the presence or absence of bands of the amplified DNA fragments, a data matrix of ISSR and SSR alleles was constructed for 42 cultivars. For each SSR and ISSR, we calculated the number of alleles, allele size, frequency, and polymorphic information content (PIC). PIC for each SSR marker was calculated as: PIC = 1 -$\sum Pi^2$,where Pi is the frequency of the ith allele detected in all cultivars (Nei, 1973), and PIC for each ISSR marker was calculated as PIC= $2f(1-f)$, where f is the frequency of the present allele detected in all cultivars. NTSYS-PC 2.21(J., 2006) was used to analyze genetic diversity of dominant ISSR markers. The DARwin 6.0.21 markers software (Perrier *et al.* 2003)[18] was used to analyze the genetic diversity of co-dominant markers. Dendrograms were generated using the unweighted pair-group method (UPGMA) clustering method and the Jaccard coefficient to calculate similarity.

Results and discussion

Inter-simple sequence repeat (ISSR)

The 42 potato cultivars were analysed using 29 ISSR primers. Number of amplified bands, number of polymorphic bands per primer and variety, primer differentiation ability indices are depicted in Table. 2.

The highest number of amplified (282) bands was obtained using primer UBC810 and UBC 842. While number of highest polymorphic bands were obtained using the primer UBC 808 and UBC 846. The primer UBC 840 and UBC 811 gave the highest monomorphic bands.

The number of ISSR alleles among the 42 potato cultivars ranged between 101 (Kufri Bahar) and 187 (Kufri Khyati). In total, 6004 alleles were detected with the number of alleles per locus varying from 92 (UBC 849) to 374 (UBC 811). The 2131 are polymorphic (35.5 %) showed varying degree of polymorphism and 3873 (64.5 %) were monomorphic. They included ISSR HB-10, UBC857980, UBC811660, ISSR LA22:D381, UBC807, UBC808, UBC809, UBC810, UBC811, UBC813, UBC842, UBC846, UBC834, gpd 1, gpd 2, UBC849. The highest PIC value of ISSR locus was observed in gpd2 (0.439), which was followed by ISSR HB-10 (0.436), UBC834 (0.421) and UBC857980 (0.415). The minimum PIC value (0.232) was recorded for ISSR HB-14, UBC836, and UBC849.

The data presented in Table 3 & Fig. 1 shows a dendrogram of 42 varieties based on the total ISSR polymorphism at the Jaccard similarity coefficient value that ranged between 0.275 (Kufri Anand and 16-(P-42-600)) and 0.667 (Kufri Garima and 19-(P-40-500)) (Table.4.8). Setting the cut-off point of similarity coefficient at 0.471, 4 different clusters were distinguished, each of which were further split into different sub clusters. The cluster A grouped Kufri Himalini, 28-(RH-2-600), Kufri Chandramukhi, 20-(P-43-200) and 26-(P-54-300).

Cluster analysis

Cluster B grouped Kufri Lalit, 27-(PH-3-400). Cluster C is

sub divided into four sub clusters. Sub cluster C1 consists of 20-(P-42-600), 17-(P-35-400), 3-(C-18-800), 5-(C-28-200), 13-(P-28-200), Sub cluster C2 consists of 16-(P-34-600), 19-(P-40-500), Kufri Garima, Kufri Khyati, 1-(C-10-400), 7-(P-14-800), C3 of consists of 12-(P-25-600), 24-(P-48-800), 11-(P-24-200), 4-(C-20-200), 14-(P-30-600), Kufri Nilkanth, 9-(P-22-600). Cluster D is sub divided into 2 sub clusters D1 and D2 consists of Kufri Gaurav and 16 Kufri cultivars.

EST-SSR

A total of 62 EST-SSR primers were used to characterize the diversity among 42 potato cultivars. Number of amplified bands, number of polymorphic bands per primer and cultivar, primer differentiation ability indices are depicted in Table. 2.

The highest number of amplified (119) bands was obtained using STI003, STI034. While no of highest polymorphic bands are obtained using the primer STI011. The primer STI017 gave the highest monomorphic bands.

The minimum (90) and maximum (136) number of SSR alleles among the 42 potato cultivars was observed for 24-(P-48-800) and 3-(C-18-800), respectively. In total, 4386 SSR alleles were detected with the number of alleles per locus varying from 42 (STI012, STI018, STI025, STI026, STI050, STI057, STI058) to 119 (STI003, STI034). The 2203 (50.23 %) polymorphic bands showed varying degree of polymorphism, they included STI001, STI002, STI003, STI004, STI005, STI006, STI009, STI015, STI019, STI021, STI024, STI046, and 2183 (49.77 %) are monomorphic. The highest PIC value (1) of EST-SSR locus was observed in STI011, STI016, STI019, STI021, STI024, STI048, STI051, STI053, STI056 and the lowest (0) was observed in STI012, STI018, STI025, STI026, STI044, STI050, STI057, STI058.

Cluster analysis

The data presented in Fig. 2 shows a dendrogram of 42 potato cultivars based on Jaccard similarity coefficient value that ranged from 0.53 (Kufri Gaurav and Kufri Khyati and 1 (26-P-54-300) and Kufri Himalini (Table 4.9). Setting cut of point of similarity coefficient at 0.765, 5 main clusters were distinguished, 3 clusters each of which were further split into different sub clusters. The first cluster A consists of Kufri Nilkanth, Kufri Khyati, 20-(P-42-600), 17-(P-35-400), Kufri Kesar, Kufri Ashoka, 19-(P-40-500), 4-(C-20-200), 1-(C-10-400). Sub cluster B consists of 9-(P-22-600), 3-(C-18-800), 14-(P-30-600), and cluster C consists of Kufri Garima. Cluster D is further sub divided into 1 and 2, sub cluster 1 consists of 20-(P-43-200), Kufri Surya, Kufri Chandramukhi, Kufri Sadabahar, 28-(RH-2-600), Kufri Arun, Kufri Bahar, Kufri Pukhraj, Kufri Gaurav.

Sub cluster 2 is further sub divided into A and B, sub cluster a consists of 12-(P-25-600), 13-(P-28-200), Kufri Chipsona-1, Kufri Lima, 24-(P-48-800). Sub cluster B consists of Kufri Ganga, Kufri Mohan, Kufri Sindhuri, Kufri Lalit, Kufri Lalima, Kufri Anand, 11-(P-24-200), 27-(PH-3-400), 7-(P-14-800), Kufri Jyothi, Kufri Badashah, 26-(P-54-300), Kufri Himalini, 16-(P-34-600), 5-(C-28-200).

Table 1: EST-SSRs primer sequences, allele size ranges, and annealing temperatures

Sr.no.	Primer name		Sequence (5'->3')	Tm	Expected size (bp)	PIC	Total no Bands	Polymormhic Bands
1	STI001	F	CAGCAAATCAGAACCCGAT	54.5	188	0.59	95	57
		R	GGATCATCAAATTCACCGCT	55.25				
2	STI002	F	ACAGGAATCACACCTGCACA	57.3	122	0.61	75	46
		R	TTCAACATCCGCCTGTCATA	55.25				
3	STI003	F	ACCATCCACCATGTCAATGC	57.3	144	0.865	119	103
		R	CTCATGGATGGTGTCATTGG	57.3				
4	STI004	F	GCTGCTAAACACTCAAGCAGAA	58.39	103	0.476	71	34
		R	CAACTACAAGATTCCATCCACAG	58.87				
5	STI005	F	CTAATTTGATGGGGAAGCAGAA	56.53	157	0.159	63	10
		R	CGGAGATAAAACCCAAGTCC	57.3				
6	STI006	F	CTTTAGTCCTTGGCAGAGCTT	57.87	250	0.03	59	2
		R	CGGGCTGATTCTTCTTCATC	57.3				
7	STI007	F	TATGTTCCACGCCATTTCAG	55.25	134	0.0096	63	1
		R	ACGGAAACTCATCGTGCATT	55.25				
8	STI008	F	CATCTCCTTCACCTGCTCCT	59.35	152	0.002	44	0
		R	CGACAAAGGAGGAAATCCAA	55.25				
9	STI009	F	GCGAAAACCTTCACCTGCTCCT	62.12	237	0.598	104	62
		R	CTGCTGTTGCTGTTGATGGTT	57.87				
10	STI011	F	TGGTGTGCACAAACTTAAGAGG	58.39	99	1	104	104
		R	GAGGAGATCACAATTCCTTTGA	56.53				
11	STI012	F	GAAGCGACTTCCAAAATCAGA	55.92	183	0	42	0
		R	AAAGGGAGGAATAGAAACCAAAA	55.3				
12	STI013	F	CCACTTCCTCCACTTCCAAA	57.3	157	0.009	63	1
		R	CCATGGTTGCACCAACTAGA	57.3				
13	STI014	F	AGAAACTGAGTTGTGTTTGGGA	56.5	120	0.198	65	13
		R	TCAACAGTCTCAGAAAACCCTCT	58.87				
14	STI015	F	GCATGTCTTCGAAGGTACGTTTA	58.87	241	0.23	52	12
		R	TTCTTCACAGCAGCAAGGTG	57.3				
15	STI016	F	GAATTGCAGAGAGGACCTGG	59.35	197	1	84	84
		R	ACTCCCTGTTGTCGGAGATG	59.35				
16	STI017	F	TATGGAAATTCCGGTGATGG	55.25	163	0.074	105	8
		R	GACGGTGACAAAGAGGAAGG	59.35				
17	STI018	F	CCACTACTGCTTCCTCCACC	61.4	189	0	42	0

		R	GCAGCAACAACAAGCTCAAC	57.3				
18	STI019	F	TCCCTGTTGCCTTGAACAAT	55.25	126	1	84	84
		R	TGGGAAAAGGTACAAAGACGA	55.9				
19	STI020	F	GACGCAGAACTCATCTTGTTCA	58.3	117	0.476	71	34
		R	GCAAAATTTGAAAAACTATGGATG	54.1				
20	STI021	F	TCATCAAGTCGTCGTCGTCATCAA	61	106	1	84	34
		R	TCGAATGATCCAAAGCTTCC	55.25				
21	STI022	F	TCTCCAATTACTTGATGGACCC	58.39	133	0.095	55	5
		R	CAATGCCATACACGTGGCTA	57.3				
22	STI023	F	GCGAATGACAGGACAAGAGG	59.35	193	0.19	65	12
		R	TGCCACTGCTACCATAACCA	57.3				
23	STI024	F	CGCCATTCTCTCAGATCACTC	59.82	168	1	84	84
		R	GCTGCAGCAGTTGTTGTTGT	57.3				
24	STI025	F	CTGCCGCAAAAAGTGAAAAC	55.25	116	0	42	0
		R	TGAATGTAGGCCAAATTTTGAA	52.8				
25	STI026	F	CAACGCTACTCAATGGCTCA	57.3	182	0	42	0
		R	ACAACTCTAGAACGAGAGGAACA	58.87				
26	STI027	F	CGCAAATCTTCATTCATCCGATTC	59.3	148	0.198	65	13
		R	TCCGGCGGATAATACTTGTT	55.25				
27	STI028	F	ATACCCTCCAATGGGTCCTT	57.3	192	0.58	74	43
		R	CTTGGAGATTTGCAAGAAGAA	53.97				
28	STI029	F	GACTGGCTGACCCTGAACTC	61.4	155	0.58	74	43
		R	GACAAAATTACAGGAACTGCAAA	55.3				
29	STI030	F	TTGACCCTCCAACTATAGATTCTC	59.3	107	0.58	74	43
		R	TGACAACTTTAAAGCATATGTCAGC	58.06				
30	STI031	F	AGGCGCACTTTAACTTCCAC	57.3	138	0.88	101	89
		R	CGGAACAAATTGCTCTGATG	55.25				
31	STI032	F	TGGGAAGAATCCTGAAATGG	55.25	121	0.59	95	56
		R	TGCTCTACCAATTAACGGCA	55.25				
32	STI033	F	TGAGGGTTTTCAGAAAGGGA	55.25	134	0.61	75	46
		R	CATCCTTGCAACAACCTCCT	57.3				
33	STI034	F	CAAGAAACCAAGAGCAAATTTCA	55.3	158	0.85	119	101
		R	TGGCGAATGTGAGAAACAAA	53.2				
34	STI035	F	ACCTTTGAGGAATTGCAGGA	55.25	102	0.47	71	33
		R	CATTGAAGGAGTTCCAGTCC	57.3				
35	STI037	F	GGACAACCAAGTGAGCAACA	57.3	180	0.159	63	10
		R	TGAGGAGAAAGGCACACAAA	55.25				
36	STI039	F	GATTGATCCAATCACGCACA	55.25	236	0.03	59	2
		R	AATTATTCGCGCAATTCGTC	53.2				
37	STI040	F	TCTTTCCCTTTTTATCCTCACTG	57.08	180	0.009	63	1
		R	GGGATTGGGTTTGAAGTAGTTG	58.39				
38	STI041	F	CTCTGTTTCTCTAATCGGCCGTA	60.65	135	0.0022	44	0
		R	AAGCGTTGGCCACCGCCA	60.52				
39	STI042	F	TCACGAGGTGCCCAACTG	58.24	133	0.59	104	61
		R	TCCATTCGTCAACAAGGT	51.41				
40	STI043	F	CAATGCGAATGTTGCTACTGGT	58.39	138	0.97	94	91
		R	ATCCACCAAGACCTCCAGAA	57.3				
41	STI044	F	GAGAACCCACCCACCAA	55.18	162	0	42	0
		R	GGTATTGTGCTTGAACAGCCA	57.87				
42	STI045	F	CTGTACCCATTACTTCTCTGCTGA	61.01	91	0.009	63	1
		R	GCAACTTTGAAGGGTGTTTGC	57.87				
43	STI046	F	CAGAGGATGCTGATGGACCT	59.35	191	0.19	65	12
		R	GGAGCAGTTGAGGGCTTCTT	59.35				
44	STI047	F	ACTGCTGTGGTTGGCGTC	58.24	143	0.19	53	10
		R	ACGGCATAGATTTGGAAGCATC	58.39				
45	STI048	F	CGAGTCCGTGGATCTCACG	60.99	170	1	84	84
		R	GATTCCCGCCGGTAAAGC	61.11				
46	STI049	F	GGAAGTCCTCAACTGGCTG	57.89	157	0.74	105	78
		R	TCAACTCTCTGCCTACTGCCCAA	52.17				
47	STI050	F	TTCCTCTAAGCGGCAAAAGG	50	91	0	42	0
		R	GGAGGAGACTTGGGTTTCTCC	57.14				
48	STI051	F	GGTCTCCATTAGCCCTCTGAG	57.14	170	1	84	84
		R	ACATAAATGGATCACACA	46.85				
49	STI052	F	TCATCACAACGTGACCCCA	56.67	173	0.47	71	33
		R	GGGCTTGAATGATGTGAAGCTC	60.25				
50	STI053	F	TCAGACCGGGTTCGATGG	58.24	160	1	84	84
		R	CGGCTTGAATCATTGCCCA	56.67				

51	STI054	F	GCCACTATGCAAGCCCATTTG	59.82	176	0.095	55	5
		R	GGGTCGATGTTTCGGTTGAG	59.35				
52	STI055	F	CCGTTGATGGGATTGCACA	56.67	220	0.197	65	13
		R	TGATATTAACCATGGCAGCAGC	58.39				
53	STI056	F	GACAGAGAATATGGGACCACCA	60.25	195	1	84	84
		R	GCAGCACCTTAAATGGCTGAC	59.82				
54	STI057	F	CCTGTAGAACAGCAGTGGTC	59.35	196	0	42	0
		R	TCCGCCAAGACTGCATGCA	58.82				
55	STI058	F	CAAGCACGTTACAAACAAGCAA	56.53	121	0	42	0
		R	TTGAAGCACATACACAAACA	51.15				
56	STI059	F	AGACGGGTGCACACGCAC	60.52	141	0.197	65	13
		R	TGCTTGAGTATGACAGCACTTGA	58.87				
57	STI060	F	ACTTCTGCATCTGGTGAAGC	57.3	173	0.58	74	43
		R	GGTCTGGATTCCCAGGTTG	58.82				
58	STI061	F	AGCAACCACCACAGCAGA	55.97	137	0.58	74	43
		R	CCGGCGATTGGATCGACG	60.52				
59	STI062	F	GGGGTCAAGCTCCATACG	58.24	117	0.58	74	43
		R	ACTAAACCACAACCCATGAGC	57.87				
60	STI063	F	GCATTCTATGGCCAACATTGG	57.87	25	0.88	101	89
		R	AGATTCCTCCAATTTCCCAGC	57.87				
61	STI064	F	CAAATTCTCCCCATTTTGGA	53.2	171	0.19	65	12
		R	AACCGATTCAAAAACCCTCA	53.2				

Table 2: ISSR primer sequences, allele size ranges, and annealing temperatures

Sr.no	Primer name	Sequence	Tm (ºC)	Product size (bp)	PIC	Total no alleles	Polymorphic bands
1	ISSR HB-10	GAGAGAGAGAGACC	46.3	400-1500	0.4357	118	51
2	ISSR HB-14	CTCCTCCTCGC	40.03	230-1500	0.2325	92	21
3	ISSR 827	ACACACACACACACACG	52.77	300-1940	0.32943	131	43
4	ISSR 841	GAGAGAGAGAGAGAGATC	53.69	450-1400	0.25057	181	45
5	ISSR ACAa	GTGACAACAACAACAACA	49.13	580-1650	0.28025	275	77
6	ISSRUBC079	TCTCTCTCTCTCTCTCA	50.36	640-920	0.276	116	32
7	UBC864600	ATGATGATGATGATGATG	46.85	716-1565	0.32754	243	80
8	UBC857980	ACACACACACACACACCTG	56.67	430-1500	0.41513	149	62
9	UBC811660	GAGAGAGAGAGAGAGAC	52.77	383-1679	0.3993	220	88
10	ISSR LA22:D381	AGAGAGAGAGAGAGAGC	52.77	370-2025	0.38955	251	98
11	UBC807	AGAGAGAGAGAGAGAGT	50.36	336-2000	0.37859	179	68
12	UBC808	AGAGAGAGAGAGAGAGC	52.77	339-1558	0.41204	267	110
13	UBC809	AGAGAGAGAGAGAGAGG	52.77	380-2190	0.37721	221	83
14	UBC810	GAGAGAGAGAGAGAGAT	50.36	383-1679	0.36142	282	102
15	UBC 811	GAGAGAGAGAGAGAGAC	52.77	350-1050	0.37708	374	141
16	UBC812	GAGAGAGAGAGAGAGAA	5036	250-1700	0.31973	216	69
17	UBC813	CTCCTCCTCCTCCTCTT	55.18	260-2500	0.36943	180	66
18	UBC822	TCTCTCTCTCTCTCTCA	50.36	200-2220	0.34993	167	58
19	UBC835	AGAGAGAGAGAGAGAGCC	55.97	320-1880	0.28345	276	78
20	UBC841	GAGAGAGAGAGAGAGACC	55.97	400-1790	0.31519	164	52
21	UBC842	AGAGAGAGAGAGAGAGTG	53.69	640-2830	0.36142	282	102
22	UBC846	CACACACACACACACAAT	51.41	355-2190	0.41204	267	110
23	UBC818	CACACACACACACACAG	52.77	305-2725	0.31519	164	52
24	UBC834	AGAGAGAGAGAGAGAGCT	53.69	305-2830	0.42074	250	105
25	gpd1	CAACGGCTTCGGTCGCATTG	61.4	400-2180	0.38955	251	98
26	gpd2	GCCAAGCAGTTGGTTGTGC	58.82	390-2000	0.43855	130	57
27	UBC834	AGAGAGAGAGAGAGAGCT	53.69	385-2560	0.2325	92	21
28	UBC840	GAGAGAGAGAGAGAGATT	51.41	305-2770	0.37708	374	141
29	UBC849	GTGTGTGTGTGTGTGTCA	53.69	310-2680	0.2325	92	21

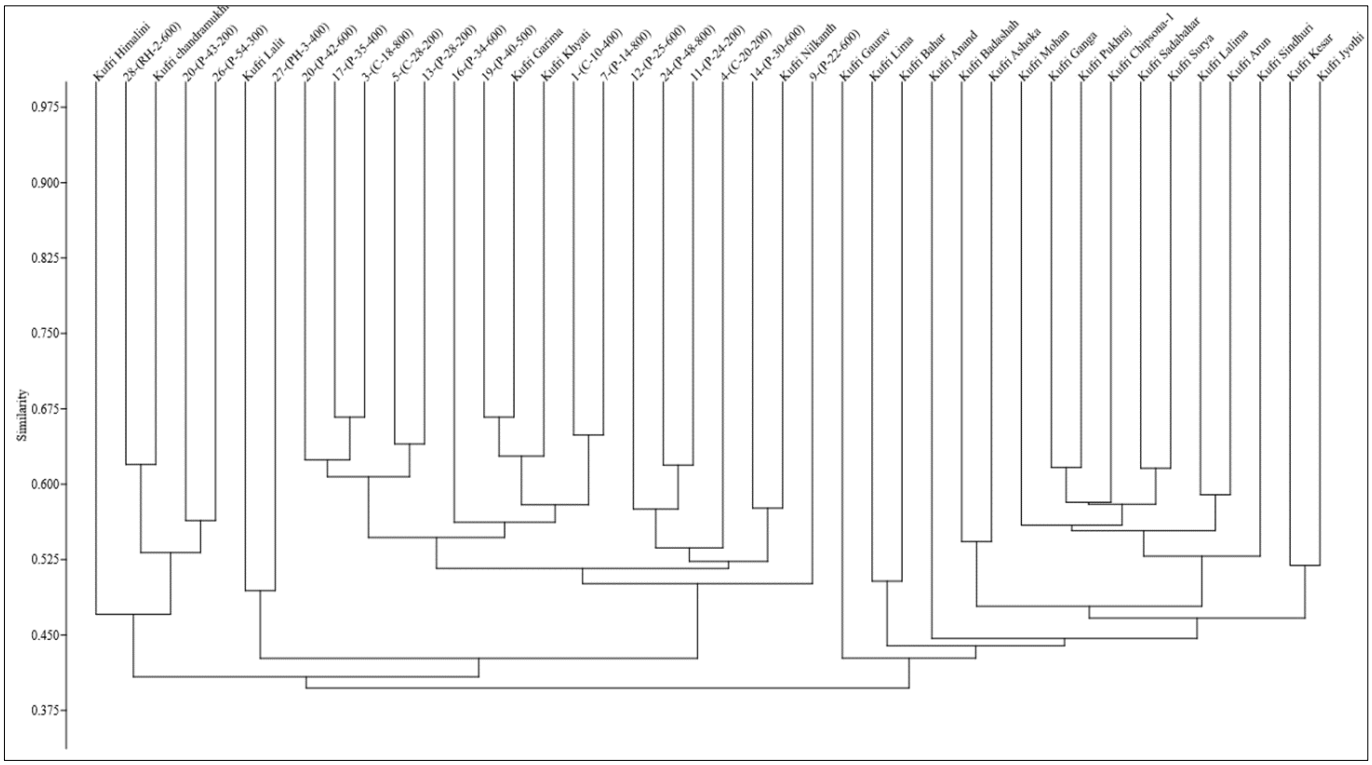

Fig 1: Dendrogram based on ISSR analysis of 42 potato cultivars

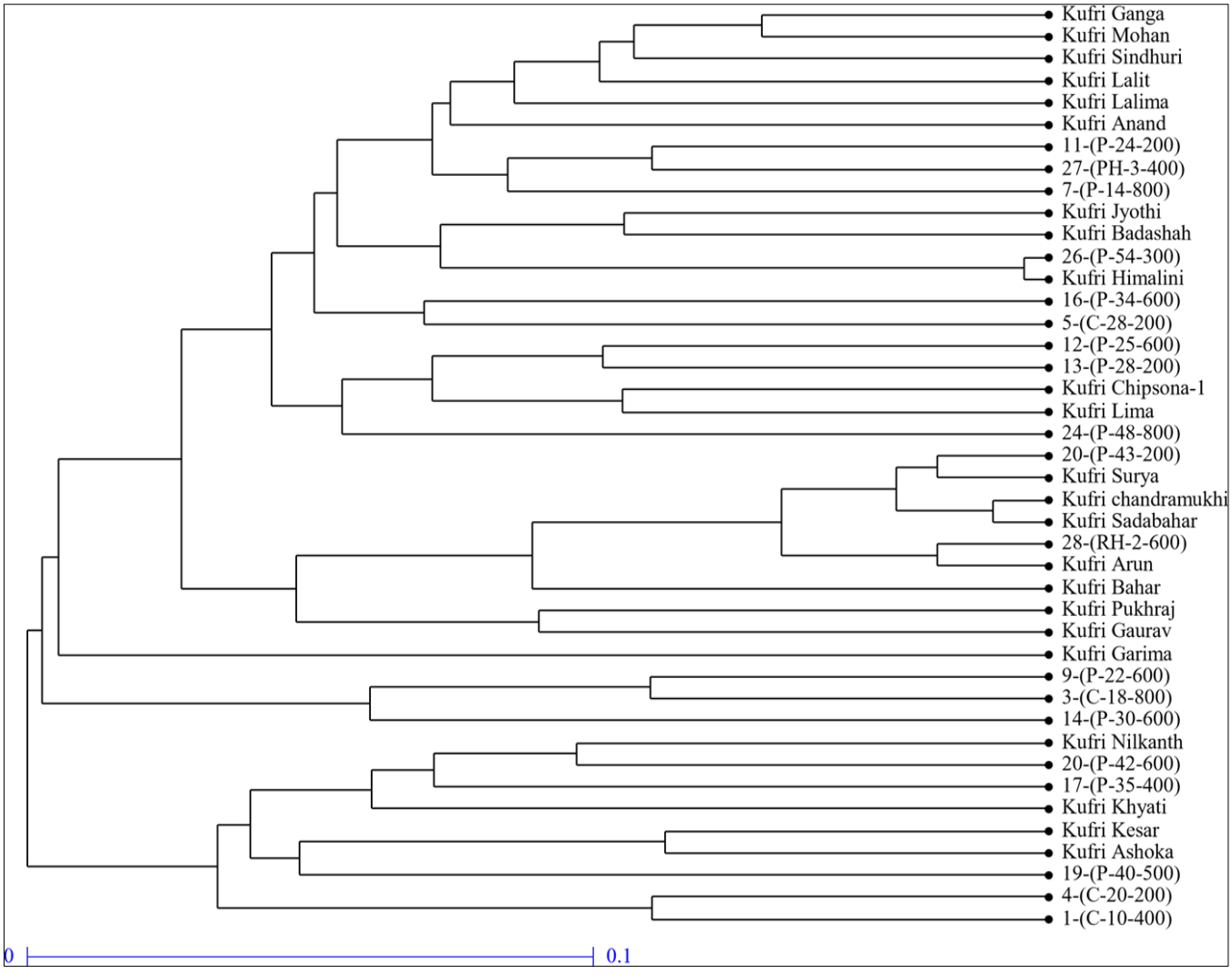

Fig 2: Dendrogram based on EST-SSR analysis of 42 Potato cultivars

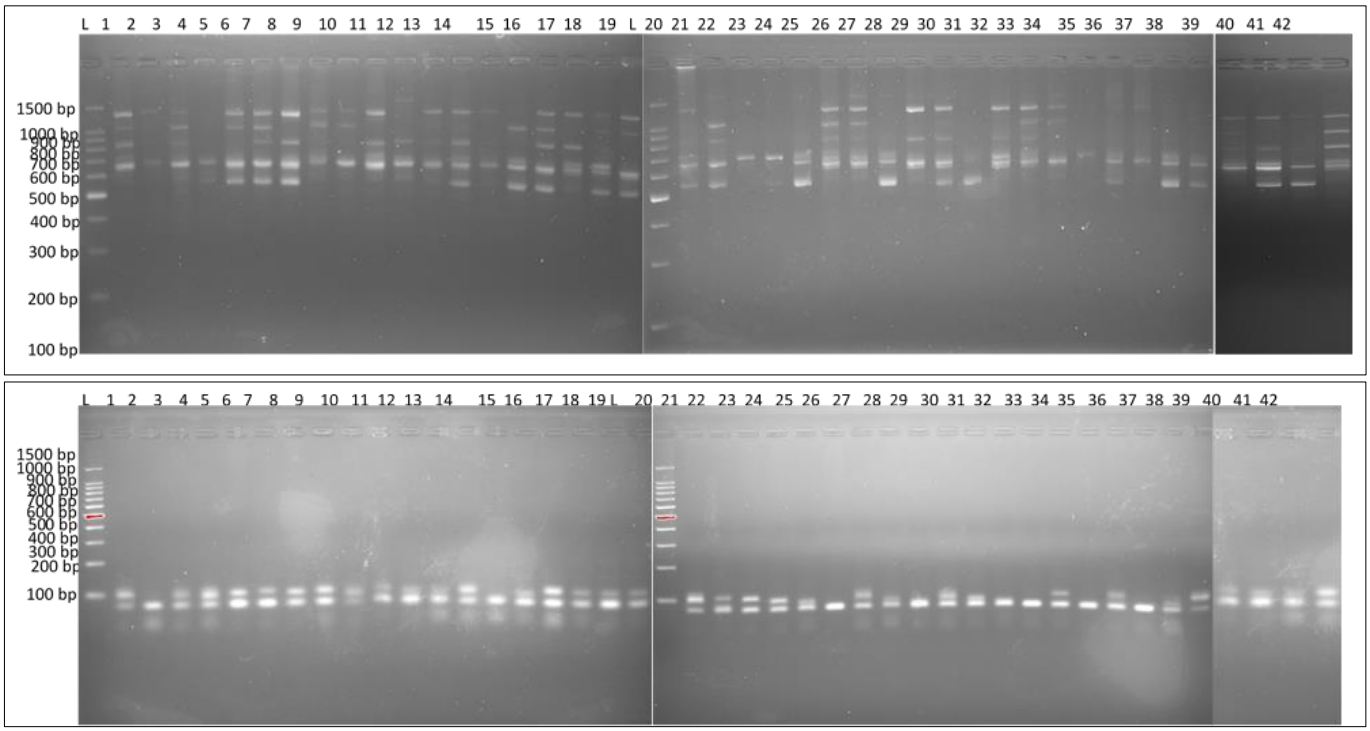

Fig 3: ISSR (UBC 811) & EST-SSR (STI004) banding pattern in different cultivars 1 = 7-(P-14-800); 2 = 1-(C-10-400); 3 = 27-(PH-3-400); 4 = 20-(P-42-600); 5 = 17-(P-35-400); 6 = 5-(C-28-200); 7 = 13-(P-28-200); 8 = 3-(C-18-800); 9 = 9-(P-22-600); 10 = 12-(P-25-600);11 = 14-(P-30-600);12 = 24-(P-48-800);13 = 11-(P-24-200)14 = 4-(C-20-200) 15 = Kufri Nilkanth; 16 = 16-(P-34-600); 17 = 19-(P-40-500); 18 = Kufri Garima; 19 = Kufri Khyati; 20 = Kufri Badashah; 21 = Kufri Ashoka; 22 = Kufri Lalit; 23 = Kufri Kesar; 24 = Kufri Mohan; 25 = Kufri Gaurav; 26 = Kufri Ganga; 27 = Kufri Lalima; 28 = Kufri Sindhuri; 29 = Kufri Pukhraj; 30 = Kufri Lima; 31=Kufri Anand; 32 = Kufri Jyothi; 33 = Kufri Chipsona-1, 34 = Kufri Bahar; 35 = Kufri Arun; 36 = Kufri Sadabahar; 37 = Kufri Surya; 38 = Kufri Himalini; 39 = 28-(RH-2-600); 40 = Kufri Chandramukhi; 41 = 20-(P-43-200); 42 = 26-(P-54-300).

Table 3: Jaccard similarity coefficient data among different cultivars of potato using ISSR markers

Cultivars	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
7-(P-14-800)	1.00																																										
1-(C-10-400)	0.65	1.00																																									
27-(PH-3-400)	0.55	0.56	1.00																																								
20-(P-42-600)	0.59	0.61	0.54	1.00																																						
17-(P-35-400)	0.51	0.58	0.50	0.63	1.00																																				
5-(C-28-200)	0.55	0.58	0.49	0.59	0.64	1.00																																			
13-(P-28-200)	0.60	0.57	0.51	0.62	0.61	0.64	1.00																																		
3-(C-18-800)	0.49	0.59	0.46	0.62	0.67	0.62	0.56	1.00																																	
9-(P-22-600)	0.56	0.52	0.48	0.47	0.52	0.50	0.52	0.53	1.00																																
12-(P-25-600)	0.53	0.52	0.46	0.52	0.55	0.56	0.61	0.56	0.55	1.00																															
14-(P-30-600)	0.51	0.54	0.47	0.56	0.53	0.52	0.57	0.57	0.56	0.56	1.00																														
24-(P-48-800)	0.55	0.59	0.52	0.52	0.53	0.55	0.56	0.54	0.48	0.59	0.55	1.00																													
11-(P-24-200)	0.45	0.48	0.46	0.48	0.46	0.50	0.53	0.49	0.41	0.56	0.53	0.62	1.00																												
4-(C-20-200)	0.50	0.53	0.43	0.49	0.48	0.51	0.53	0.50	0.44	0.54	0.52	0.56	0.51	1.00																											
Kufri Nilkanth	0.53	0.52	0.45	0.49	0.44	0.52	0.57	0.50	0.42	0.51	0.58	0.54	0.48	0.50	1.00																											
16-(P-34-600)	0.58	0.57	0.48	0.49	0.52	0.50	0.51	0.56	0.56	0.52	0.55	0.56	0.53	0.51	0.50	1.00																									
19-(P-40-500)	0.57	0.56	0.46	0.53	0.59	0.50	0.55	0.52	0.47	0.55	0.51	0.49	0.42	0.44	0.40	0.55	1.00																								
Kufri Garima	0.57	0.61	0.48	0.47	0.55	0.55	0.54	0.56	0.54	0.55	0.50	0.50	0.45	0.45	0.52	0.57	0.67	1.00																							
Kufri Khyati	0.59	0.57	0.48	0.53	0.57	0.55	0.62	0.51	0.48	0.55	0.52	0.49	0.48	0.45	0.54	0.55	0.62	0.64	1.00																						

Kufri Badashah	0.43	0.40	0.35	0.40	0.38	0.40	0.41	0.37	0.36	0.37	0.43	0.34	0.31	0.35	0.41	0.32	0.39	0.41	0.37	1.00																						
Kufri Ashoka	0.50	0.44	0.38	0.40	0.37	0.46	0.42	0.38	0.40	0.43	0.39	0.38	0.40	0.35	0.40	0.43	0.38	0.43	0.39	0.54	1.00																					
Kufri Lalit	0.39	0.38	0.49	0.38	0.40	0.42	0.39	0.36	0.42	0.41	0.36	0.35	0.32	0.32	0.29	0.35	0.35	0.39	0.33	0.39	0.41	1.00																				
Kufri Kesar	0.40	0.37	0.38	0.43	0.35	0.43	0.43	0.36	0.36	0.38	0.38	0.32	0.34	0.34	0.42	0.35	0.37	0.35	0.35	0.50	0.49	0.37	1.00																			
Kufri Mohan	0.46	0.42	0.43	0.53	0.53	0.50	0.50	0.49	0.45	0.47	0.44	0.38	0.41	0.42	0.35	0.39	0.43	0.46	0.47	0.51	0.54	0.49	0.44	1.00																		
Kufri Gaurav	0.38	0.37	0.31	0.36	0.38	0.50	0.37	0.37	0.35	0.39	0.38	0.39	0.33	0.39	0.36	0.32	0.31	0.33	0.36	0.39	0.41	0.37	0.39	0.48	1.00																	
Kufri Ganga	0.44	0.40	0.40	0.42	0.43	0.47	0.53	0.41	0.42	0.49	0.43	0.42	0.41	0.45	0.40	0.36	0.37	0.39	0.37	0.47	0.50	0.43	0.51	0.56	0.53	1.00																
Kufri Lalima	0.41	0.37	0.37	0.44	0.43	0.43	0.42	0.47	0.39	0.41	0.44	0.36	0.35	0.44	0.42	0.36	0.39	0.41	0.37	0.51	0.48	0.41	0.47	0.57	0.45	0.61	1.00															
Kufri Sindhuri	0.42	0.42	0.41	0.48	0.48	0.50	0.45	0.47	0.51	0.48	0.48	0.45	0.45	0.44	0.42	0.37	0.38	0.40	0.39	0.48	0.51	0.39	0.41	0.56	0.41	0.56	0.55	1.00														
Kufri Pukhraj	0.42	0.40	0.39	0.41	0.43	0.44	0.48	0.39	0.40	0.48	0.40	0.38	0.42	0.37	0.38	0.34	0.39	0.44	0.38	0.43	0.52	0.43	0.44	0.57	0.46	0.62	0.53	0.58	1.00													
Kufri Lima	0.36	0.34	0.36	0.35	0.36	0.34	0.34	0.37	0.42	0.37	0.45	0.33	0.32	0.35	0.32	0.38	0.38	0.43	0.36	0.44	0.43	0.45	0.41	0.50	0.38	0.46	0.56	0.45	0.46	1.00												
Kufri Anand	0.37	0.33	0.34	0.34	0.40	0.34	0.40	0.33	0.36	0.43	0.37	0.38	0.32	0.34	0.34	0.28	0.36	0.34	0.37	0.49	0.43	0.37	0.39	0.47	0.37	0.49	0.45	0.43	0.43	0.44	1.00											
Kufri Jyothi	0.34	0.37	0.29	0.34	0.43	0.43	0.45	0.41	0.31	0.42	0.36	0.39	0.42	0.38	0.41	0.34	0.34	0.34	0.36	0.44	0.47	0.31	0.52	0.49	0.48	0.55	0.49	0.45	0.43	0.36	0.48	1.00										
Kufri Chipsona-1	0.42	0.39	0.40	0.40	0.45	0.44	0.45	0.41	0.48	0.47	0.38	0.38	0.39	0.45	0.33	0.38	0.36	0.42	0.39	0.45	0.46	0.42	0.41	0.54	0.40	0.60	0.55	0.50	0.56	0.51	0.44	0.46	1.00									
Kufri Bahar	0.38	0.39	0.36	0.35	0.35	0.36	0.34	0.36	0.37	0.38	0.36	0.31	0.32	0.28	0.32	0.32	0.38	0.45	0.36	0.39	0.41	0.40	0.39	0.47	0.31	0.41	0.43	0.35	0.44	0.50	0.35	0.35	0.46	1.00								
Kufri Arun	0.40	0.38	0.35	0.39	0.40	0.41	0.43	0.38	0.36	0.42	0.44	0.39	0.38	0.45	0.44	0.40	0.35	0.40	0.42	0.41	0.48	0.33	0.44	0.48	0.47	0.53	0.59	0.51	0.56	0.46	0.43	0.49	0.52	0.39	1.00							
Kufri Sadabahar	0.40	0.39	0.39	0.40	0.42	0.47	0.44	0.40	0.37	0.44	0.40	0.36	0.39	0.46	0.41	0.34	0.39	0.39	0.39	0.45	0.50	0.41	0.46	0.54	0.47	0.62	0.59	0.47	0.52	0.51	0.44	0.52	0.58	0.48	0.59	1.00						
Kufri Surya	0.41	0.42	0.42	0.40	0.44	0.42	0.45	0.38	0.37	0.45	0.38	0.37	0.37	0.43	0.39	0.34	0.37	0.45	0.41	0.46	0.47	0.45	0.42	0.59	0.43	0.58	0.57	0.50	0.61	0.54	0.44	0.49	0.58	0.43	0.54	0.62	1.00					
Kufri Himalini	0.42	0.37	0.34	0.40	0.45	0.46	0.46	0.43	0.37	0.48	0.43	0.36	0.36	0.40	0.45	0.41	0.38	0.36	0.50	0.38	0.42	0.39	0.43	0.45	0.43	0.51	0.43	0.45	0.44	0.38	0.39	0.47	0.41	0.31	0.53	0.45	0.50	1.00				
28-(RH-2-600)	0.41	0.45	0.37	0.45	0.46	0.48	0.53	0.49	0.36	0.51	0.48	0.46	0.47	0.44	0.47	0.39	0.41	0.43	0.52	0.39	0.44	0.33	0.35	0.48	0.37	0.49	0.44	0.49	0.40	0.39	0.43	0.45	0.44	0.33	0.45	0.44	0.46	0.49	1.00			
Kufri chandramukhi	0.46	0.49	0.39	0.42	0.45	0.47	0.47	0.45	0.36	0.49	0.43	0.45	0.44	0.41	0.49	0.37	0.48	0.49	0.52	0.38	0.45	0.33	0.38	0.48	0.43	0.48	0.44	0.40	0.42	0.41	0.42	0.46	0.42	0.38	0.41	0.48	0.50	0.47	0.62	1.00		
20-(P-43-200)	0.36	0.37	0.28	0.34	0.37	0.38	0.38	0.37	0.27	0.38	0.33	0.33	0.36	0.34	0.34	0.33	0.38	0.42	0.42	0.39	0.37	0.32	0.32	0.39	0.34	0.34	0.33	0.33	0.32	0.36	0.39	0.39	0.34	0.28	0.33	0.34	0.43	0.42	0.52	0.57	1.00	
26-(P-54-300)	0.39	0.40	0.33	0.34	0.39	0.44	0.36	0.42	0.37	0.44	0.39	0.34	0.35	0.36	0.37	0.41	0.43	0.43	0.48	0.39	0.43	0.38	0.33	0.43	0.43	0.41	0.35	0.38	0.36	0.36	0.38	0.40	0.38	0.32	0.38	0.44	0.40	0.50	0.50	0.54	0.56	1.00

Table 4: Jaccard similarity coefficient data among different cultivars of potato using EST-SSR markers

Cultivars	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
7-(P-14-800)	1.00																																									
1-(C-10-400)	0.82	1.00																																								
27-(PH-3-400)	0.83	0.74	1.00																																							
20-(P-42-600)	0.76	0.81	0.74	1.00																																						
17-(P-35-400)	0.71	0.75	0.72	0.84	1.00																																					
5-(C-28-200)	0.79	0.69	0.80	0.76	0.77	1.00																																				
13-(P-28-200)	0.78	0.63	0.81	0.64	0.65	0.75	1.00																																			
3-(C-18-800)	0.63	0.59	0.67	0.62	0.63	0.73	0.68	1.00																																		
9-(P-22-600)	0.68	0.59	0.69	0.63	0.63	0.68	0.74	0.88	1.00																																	
12-(P-25-600)	0.69	0.65	0.81	0.66	0.70	0.80	0.85	0.80	0.78	1.00																																
14-(P-30-600)	0.69	0.60	0.69	0.61	0.62	0.72	0.74	0.76	0.82	0.78	1.00																															
24-(P-48-800)	0.65	0.60	0.72	0.58	0.54	0.62	0.74	0.64	0.70	0.76	0.70	1.00																														
11-(P-24-200)	0.86	0.74	0.86	0.71	0.66	0.74	0.79	0.67	0.75	0.77	0.77	0.77	1.00																													
4-(C-20-200)	0.72	0.85	0.63	0.73	0.66	0.61	0.66	0.60	0.61	0.67	0.65	0.66	0.67	1.00																												
Kufri Nilkanth	0.79	0.85	0.72	0.85	0.79	0.69	0.65	0.59	0.66	0.67	0.66	0.59	0.76	0.77	1.00																											

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STI017 gave the highest number of monomorphic bands. The minimum (90) and maximum (136) number of SSR alleles among the 42 potato cultivars were observed for 24-(P-48-800) and 3-(C-18-800), respectively. A dendrogram of 42 potato cultivars based on Jaccard similarity coefficient value ranged from 0.53 (Kufri Gaurav and Kufri Khyati) to 1 (26-P-54-300 and Kufri Himalini). The superiority of ISSR marker over other techniques due to its fast, high reproducibility and low cost, leading to useful information makes it quite suitable for the analysis of genetic variations. The highest number of amplified (282) bands of ISSR was obtained using primer UBC810 and UBC 842. While highest number of polymorphic bands was obtained using the primer UBC 808 and UBC 846. The number of ISSR alleles among the 42 potato cultivars ranged between 101 (Kufri Bahar) and 187 (Kufri Khyati). The highest PIC value of ISSR locus was observed in gpd2 (0.439).

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Disclosure statement
No potential conflict of interest was reported by the authors.

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