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GENETIC DIVERSITY AND DNA FINGERPRINTING OF POTATO VARIETIES USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS

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ABSTRACT

DNA fingerprinting is a tool for plant breeder rights protection, and variety registration in Plant Breeder Rights Repository. In the present study, we developed a DNA fingerprinting profile of 12 potato cultivars grown in Punjab Pakistan using 214 informative Simple Sequence Repeat (SSR) markers. A total of 1720 alleles were amplified by 214 SSR with an average of 8.04 alleles per marker. Approximately 72% of amplified alleles (1329 alleles) were polymorphic with 6.88 polymorphic alleles per SSR marker. The number of alleles ranged from 1 to 31. Similarly, polymorphic alleles per marker ranged from 0 to 24. A maximum number of alleles and polymorphic alleles were reported by IBR13 marker. The Polymorphic information content (PIC) value ranged from 0 to 0.96. The average PIC value for 214 amplified markers was 0.73. Collectively, 72 SSR markers amplified unique allelic patterns for DNA fingerprinting. Potato varieties Rubby and Sadaf were identified by 15 SSR markers whereas Faisalabad Red and SH-5 were identified by 12 SSR markers. Cluster and structure analysis classify the potato genotypes into two distinct groups. This information will be useful for the variety registration process and will provide a platform for future DNA fingerprinting and genetic diversity studies for the choice of SSR markers.

Keywords: Cluster analysis; Genetic diversity; Polymerase Chain Reaction; Polymorphic Information Content; Variety identification,

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is the fourth most important and non-cereal principal cash crop globally (Devaux *et al*. 2014). It is high yielding and high nutritive vegetable, possessing carbohydrates and several minerals, fibers, fats, and vitamins contributing a total of 390 KJ $100g^{-1}$of baked potatoes (Majeed and Muhammad, 2018). Pakistan is among the large potato producing countries yielding 45.66 tonnes per hectares. According to Economic Survey of Pakistan, in 2020, Pakistan produced a total of 4,609, 600 tones with a total yield of 245,159 kg/ha of potatoes with 95% of production mainly originated from Punjab. Pakistan has been largely exporting potatoes to Afghanistan, Middle East, Russia, and Europe. In 2018, the country exported potatoes worth 120.9 million USD (GOP, 2020).

With ever increasing population and growing food demands (Shahzad *et al.,* 2021a, b) potato varieties are evolving at faster rates with improved taste, enhanced pathogenic resistance, and high yield. Ever-increasing crop varieties need to be distinguished from each other. Traditionally, potato varieties were identified using morphological and physiological traits on the basis of Distinctiveness, Uniformity and Stability (DUS) testing through color, texture, sprouting, growth habit, and disease resistance. These traits are high affected by environment and lead towards false identification hence generating high risk of germplasm mixing (van Eck, 2007). Furthermore, biochemical markers such as isozymes are affected by developmental stages and growth conditions of a plant. Hence, rapid, reliable, and accurate identification of varieties is necessary for development of new cultivars with minimal laboratory instruments (Singh *et al.,* 2019; Jamil *et al.,* 2021a).

Molecular marker or DNA based markers are widely accepted for identification due to their immense benefits over morphological and biochemical markers (Vreugdenhil *et al.,* 2011). Genetic markers are most efficient tool for identification of cultivar and estimation of relatedness. Further, Plant breeders' Rights Rules 2018, issued by Ministry of National Food Security and Research, Pakistan clearly states the necessity of DNA profiling before registration of new variety for varietal protection (Sadaf, 2018; Iqbal *et al*., 2021a). Various PCR based markers including randomly amplified polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP), Inter Simple Sequence Repeat (ISSR), Restriction Fragment Length Polymorphism (RFLP), and Single Nucleotide Polymorphism (SNPs) are the promising tools to assess genetic diversity and fingerprinting (Iqbal *et al.,* 2019; Jamil *et al.,* 2020 a, b).

Microsatellites or SSR (Simple sequence repeats) markers are comprised of short 1-6 bp repetitive DNA motifs that act as a powerful tool for studying genetic diversity, phylogeny, cultivar discrimination,

marker-assisted selection for breeding and genome mapping (Lan *et al.,* 2012; Vieira *et al.,* 2016). They are simple, highly informative, reproducible, abundant, co-dominant, frequently, and randomly spread all over genome and have a specific location on a chromosome that can be easily analysed using PCR (Kalia *et al.,* 2011). SSR was first used for genetic analysis of anther-derived potatoes and later on several studies demonstrated use of SSR markers to differentiate potato cultivars e.g. 38 accessions of potatoes in Brazil were distinguished using SSR markers (Veilleux *et al.,* 1995; Favoretto *et al*. 2011). 589 native Chilean potato accessions were analysed for genetic diversity using SSR markers (Muñoz *et al.,* 2016).

Although various studies relating to potato DNA fingerprinting are reported worldwide but no such report is available in Pakistan to date. In the present study, 214 SSR markers were used to analyse, distinguish, and generate fingerprints of twelve potato varieties in Punjab Pakistan. The SSR markers were selected based on polymorphism and their ability to discriminate. Present study will provide vivid information for breeding programs, genotype verification, cultivar registration, genetic assessment, and variety protection.

MATERIALS AND METHODS

Twelve potato genotypes (Table 1) were surveyed using SSR markers for varietal discrimination and genetic diversity assessment. The cultivars were grown in growth chambers at Agriculture Biotechnology Research Institute (ABRI), Faisalabad, Pakistan under standard growth conditions. The fresh young leaflets were collected and stored at -40℃ till proceeded further.

Table 1. List of Potato genotypes used in the study along with pedigree parentage.

Sr. No.	Name	Pedigree Parentage
1	Faisalabad Red	Desiree × Laal-e-Faisal
2	Faisalabad White	CIP Clone No. 386043 (Introduction)
3	SH-5	Bartina × Cardinal
4	PRI-Red	FD44-24 × FD 12-24
5	Ruby	384636-1 × FD 1-8
6	Sadaf	FD 3-15 × FD 35-36
7	Sialkot Red	SH-5 × Cardinal
8	Sahiwal White	FD 35-36 × SH-5
9	Cosmo	FD 3-15 × FD 35-36
10	Sahiwal Red	FD 3-15 × SH-5
11	Ravi Red	FD 35-36 × SH-5
12	FD 81-1	N-9619 × FD 3-15

Genomic DNA was extracted from 2 grams of young leaflets by grinding samples in liquid nitrogen. Finely powdered samples were preceded according to modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Kanwal *et al*., 2021). The samples were quantified using Nanodrop spectrophotometer (ND200, Thermo Scientific, U.S.A.). The extracted DNA with A260/A280 = 1.80 – 2.0 was considered as pure. Furthermore, the quality and quantity of each extract was assessed by loading DNA 20 ng/ µL on 0.8% (w/v) on agarose gel stained with ethidium bromide. DNA samples were stored at -40℃. 226 polymorphic SSR markers (Table S1) were selected and synthesized according to previous studies (Buteler *et al.,* 1999; Hwang *et al.,* 2002; He *et al.,* 2003; Ghislain *et al.,* 2009; Salimi *et al.,* 2016). Polymorphic SSR markers were used to fingerprint and analyse genetic diversity of 12 potato cultivars.

PCR amplifications were performed in a 25 µL reaction mixture, containing 2.0 µL of 20 ng/µL DNA template, 0.6 µM forward primer, 0.6 µM of reverse primer, 12 µL green master mix and volume was adjusted using double distilled deionized water (d_3H_2O) for each SSR marker. Following PCR program was used: initial denaturation 5 min at 94 °C, 35 cycles of denaturation of 30 s at 94 °C, 1 min of annealing at SSR specific annealing temperature (46-60 °C) and 45 s at 72 °C; with a final extension step of 7 min at 72 °C. The PCR products were stored at 4°C.

All amplified products were fractioned on vertical gel electrophoresis System model POWERPRO-3AMP (cleaver scientific limited) using 6% PAGE performed at 16 watts power using 50 bp DNA ladder as a reference. PAGE gels were further stained by Silver nitrate staining for visualization according to previously described staining protocol (Jamil *et al.,* 2020b). Finally, images were captured using Syngene trans-illuminator.

The data file for SSR markers was constructed in the form of a binary matrix by scoring 0 for absence and 1 for presence of specific amplification of allele. For cluster analysis, a distance matrix was generated using NTSYSpc 2.0 and Un-weighted pair Group Method of Arithmetic Means (UPGMA) was used to construct dendrogram. Moreover, population structure and genetic diversity levels of potato genotypes were estimated using a model-based Bayesian clustering approach implemented in STRUCTURE v. 2.3.4 (Pritchard *et al.,* 2000) by assigning accessions to population based on SSR markers. Genotyping data of 214 microsatellites were used to determine population's structure of various potato varieties. Population structure analysis was performed according to parameters previously mentioned in our recent paper (Jamil *et al.,* 2020b). Further Polymorphic Information contents (PIC) were also calculated for all SSR markers along with number of alleles, polymorphic alleles, and different allelic diversity parameters.

RESULTS

***SSR Polymorphism*:** Among 226 SSR markers used in the study, twelve markers i.e. CB330645, CB330657, IB2·45A, Ib-255F1, IB2-66, Ib3/31, Ib-316, IBC1, IBSSR16, IBSSR23, IBSSR24, and IBSSR25 were not amplified remaining 214 markers were used for fingerprinting and genetic diversity studies. Among 214 amplified markers 21 were monomorphic and 193 were polymorphic. A total of 1720 alleles were amplified with an average of 8.04 alleles per marker. Approximately 72% of amplified alleles (1329 alleles) were polymorphic with 6.88 polymorphic alleles per SSR marker. The number of alleles ranged from 1 (for 23 SSR markers) to 31 (IBR13). Similarly, polymorphic alleles per markers ranged from 0 (15 markers) to 24 (IBR13). The average number of alleles and polymorphic alleles was 8.2 and 6.2 respectively. The PIC value for markers ranged from 0 (12 SSR markers) to 0.96 (IBR13). The average PIC value for 214 amplified markers was 0.73. Whereas size of amplified products ranged from 80 to 1000 bp (Table 2).

***DNA Fingerprinting*:** The 193 polymorphic markers were used for fingerprinting of 12 potato genotypes. Twelve potato genotypes were identified successfully by using different seventy-two markers. SSR markers BU691268 (Fig 1) and IBJ5446 each distinguished three potato genotypes (SH-5, Ruby & Sadaf) and (Faisalabad-Red, Ruby, and Cosmo) respectively. Similarly, 14 markers i.e., STM0019a, STM1052, STM5114, STM5127, Ib-286, IBN-35, IBE-5, CB330283, CB330200, BM878740, BU692896, CB330762, IBE32 and IBJ62 identified two genotypes each as given in Table 3. The genotypes Ruby and Sadaf were uniquely identified by 15 markers whereas Faisalabad Red and SH-5 were identified with 12 markers each. Similarly, Sahiwal White was identified with the help of 11 SSR markers and Faisalabad White by 10 markers. The remaining genotypes i.e., Ravi Red, Sahiwal Red, PRI Red, Sialkot Red, Cosmo, and FD 81-1 were uniquely identified using 6, 4, 3, 2, 2 and 1 SSR markers respectively (Table 3).

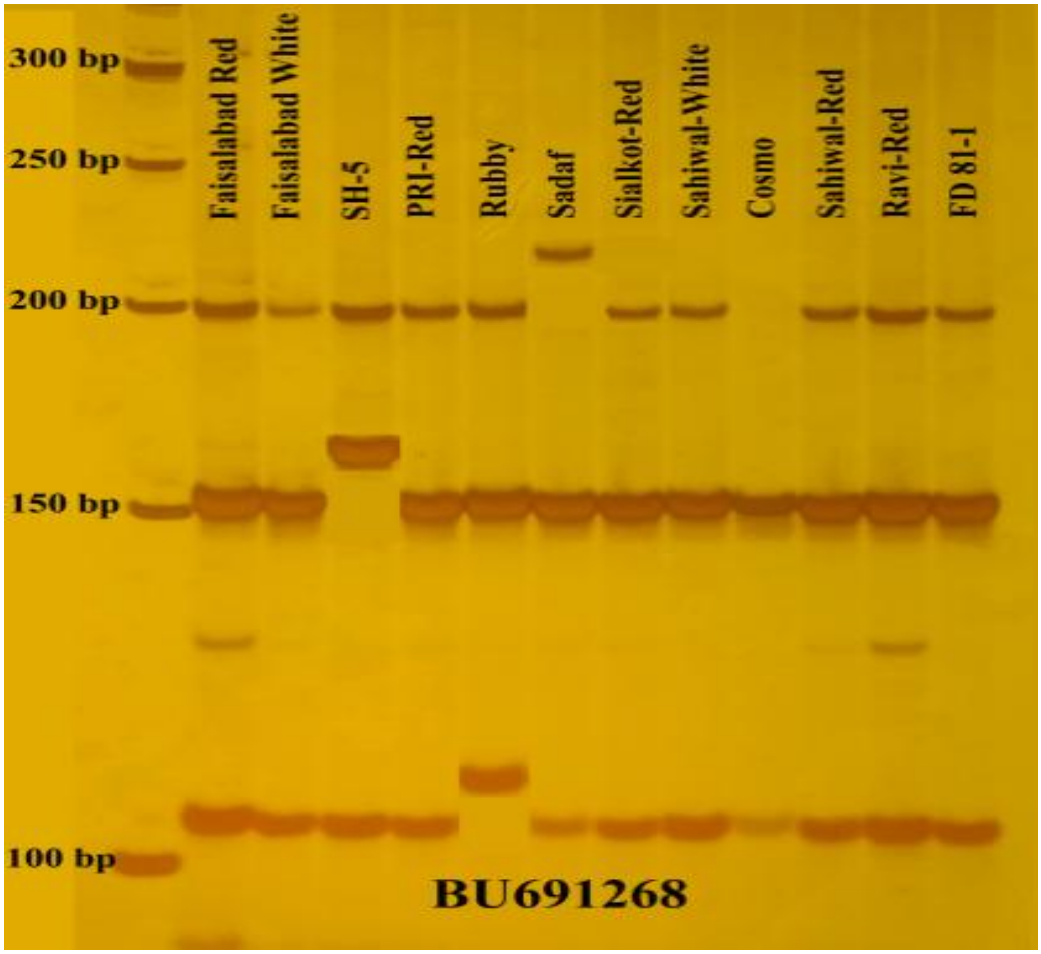

Fig 1. Polyacrylamide gel electrophoresis result of BU691268 SSR marker containing DNA fingerprints of three genotypes i.e., SH-5, Rubby, and Sadaf.

Table 2. List of SSR markers used in the study along with polymorphism status (PS), Polymorphic Information contents (PIC), No. of alleles (NOA), Polymorphic Alleles (PA), and annealing temperature (AT).

Sr. No.	Marker Name	PS	PIC	NOA	PA	TA	Sr. No.	Marker Name	PS	PIC	NOA	PA	TA	Sr. No.	Marker Name	PS	PIC	NOA	PA	TA
1	BM878740	Polymorphic	0.91	14	9	45	73	IB-255	Polymorphic	0.79	5	5	45	144	IBR12	Polymorphic	0.94	19	14	46
2	BM878757	Polymorphic	0.89	11	9	42	74	Ib-275	Polymorphic	0.72	4	1	46	145	IBR13	Polymorphic	0.96	31	24	44
3	BM878879	Polymorphic	0.65	3	1	45	75	Ib-286	Polymorphic	0.89	13	4	42	146	IBR14	Polymorphic	0.74	5	2	46
4	BU690134	Polymorphic	0.96	27	22	42	76	Ib-297	Polymorphic	0.83	7	6	46	147	IBR16	Polymorphic	0.90	12	10	44
5	BU690375	Polymorphic	0.79	6	5	42	77	Ib3/24	Polymorphic	0.79	5	1	44	148	IBR19	Polymorphic	0.75	7	5	44
6	BU690615	Polymorphic	0.72	4	4	42	78	Ib3/28	Monomorphic	0.00	1	0	42	149	IBR20	Polymorphic	0.90	13	10	44
7	BU690708	Polymorphic	0.63	3	3	44	79	Ib-318	Polymorphic	0.64	4	2	44	150	IBR21	Polymorphic	0.82	6	6	46
8	BU690750	Polymorphic	0.93	17	16	44	80	IbC10	Polymorphic	0.77	5	4	48	151	IBS01	Monomorphic	0.00	1	0	44
9	BU690910	Polymorphic	0.68	4	3	44	81	IbC11	Polymorphic	0.80	7	3	42	152	IBS02	Polymorphic	0.68	4	3	44
10	BU690977	Monomorphic	0.00	1	0	46	82	IbC12	Polymorphic	0.79	5	2	44	153	IBS07	Polymorphic	0.62	3	2	46
11	BU691143	Polymorphic	0.91	14	12	42	83	IBC13	Polymorphic	0.73	4	1	44	154	IBS09	Polymorphic	0.78	8	7	46
12	BU691268	Polymorphic	0.89	7	7	42	84	IBC3	Polymorphic	0.47	4	3	44	155	IBS10	Polymorphic	0.77	5	3	46
13	BU691341	Polymorphic	0.92	19	15	42	85	IBC4	Polymorphic	0.52	4	3	44	156	IBS11	Polymorphic	0.63	3	3	50
14	BU691547	Polymorphic	0.93	19	15	42	86	IBC5	Polymorphic	0.47	3	3	46	157	IBS17	Polymorphic	0.88	12	11	44
15	BU691662	Monomorphic	0.00	1	1	42	87	IBC8	Polymorphic	0.74	5	3	44	158	IBS18	Polymorphic	0.70	5	3	44
16	BU691762	Monomorphic	0.00	1	0	42	88	IBC9	Polymorphic	0.77	5	3	46	159	IBSR01	Polymorphic	0.44	2	2	42
17	BU691865	Polymorphic	0.85	8	4	45	89	IBCIP-1	Polymorphic	0.90	12	6	42	160	IBSSR02	Polymorphic	0.32	2	1	46
18	BU691949	Polymorphic	0.85	11	9	42	90	IBCIP-12	Monomorphic	0.80	5	0	46	161	IBSSR03	Monomorphic	0.00	1	0	45
19	BU691984	Monomorphic	0.00	1	1	42	91	IBCIP-13	Polymorphic	0.85	8	6	42	162	IBSSR04	Polymorphic	0.92	12	12	42
20	BU692061	Monomorphic	0.00	1	1	46	92	IBCIP-2	Polymorphic	0.92	16	6	42	163	IBSSR05	Polymorphic	0.82	6	6	45
21	BU692090	Polymorphic	0.85	8	5	45	93	IBCIP-5	Polymorphic	0.87	9	8	42	164	IBSSR06	Polymorphic	0.79	5	5	44
22	BU692095	Polymorphic	0.93	18	12	45	94	IBCIP-7	Polymorphic	0.87	10	7	42	165	IBSSR07	Polymorphic	0.15	2	2	48
23	BU692154	Polymorphic	0.63	5	5	42	95	IBCIP-8	Monomorphic	0.00	1	0	46	166	IBSSR08	Polymorphic	0.84	8	6	42
24	BU692227	Polymorphic	0.85	10	9	46	96	IBCIP-9	Polymorphic	0.87	8	3	46	167	IBSSR09	Polymorphic	0.76	5	4	46
25	BU692248	Polymorphic	0.38	2	2	46	97	IBE1	Polymorphic	0.84	8	8	44	168	IBSSR10	Polymorphic	0.90	11	11	44
26	BU692403	Polymorphic	0.90	11	11	42	98	IBE10	Polymorphic	0.85	9	5	44	169	IBSSR11	Polymorphic	0.86	9	9	44
27	BU692471	Polymorphic	0.67	4	3	46	99	IBE12	Polymorphic	0.90	13	13	42	170	IBSSR12	Polymorphic	0.83	9	9	46
28	BU692496	Monomorphic	0.63	3	3	46	100	IBE14	Polymorphic	0.95	25	20	42	171	IBSSR13	Polymorphic	0.41	3	2	44
29	BU692566	Polymorphic	0.70	4	1	46	101	IBE15	Polymorphic	0.86	10	8	42	172	IBSSR14	Polymorphic	0.44	2	1	50
30	BU692646	Monomorphic	0.00	1	0	46	102	IBE2	Polymorphic	0.63	3	3	48	173	IBSSR15	Polymorphic	0.66	3	2	44
31	BU692658	Polymorphic	0.91	13	10	42	103	IBE24	Polymorphic	0.89	11	9	42	174	IBSSR17	Polymorphic	0.57	3	2	46
32	BU692739	Polymorphic	0.89	13	11	42	104	IBE27	Polymorphic	0.91	15	14	42	175	**IBSSR18**	Polymorphic	0.83	8	7	44
33	BU692763	Polymorphic	0.79	5	4	48	105	IBE28	Polymorphic	0.90	14	14	42	176	IBSSR19	Polymorphic	0.68	5	3	45
34	BU692780	Polymorphic	0.81	6	2	42	106	IBE29	Polymorphic	0.72	4	4	42	177	IBSSR20	Monomorphic	0.00	1	1	46
35	BU692800	Monomorphic	0.00	1	0	46	107	IBE3	Polymorphic	0.64	4	4	44	178	IBSSR21	Polymorphic	0.81	7	7	46
36	BU692858	Polymorphic	0.85	9	7	42	108	IBE30	Polymorphic	0.81	6	4	42	179	IBSSR22	Polymorphic	0.83	6	2	46
37	BU692896	Polymorphic	0.92	17	9	42	109	IBE32	Polymorphic	0.71	6	5	42	180	IBSSR26	Polymorphic	0.90	11	5	48

38	BU692914	Polymorphic	0.91	14	13	42	110	IBE33	Polymorphic	0.89	10	7	42	181	IBSSR27	Polymorphic	0.86	9	9	44
39	BU692940	Polymorphic	0.88	10	10	42	111	IBE34	Polymorphic	0.87	9	7	42	182	IBSSR331	Polymorphic	0.84	7	3	42
40	CB329940	Monomorphic	0.00	1	0	45	112	IBE4	Polymorphic	0.85	9	6	46	183	SSR128	Polymorphic	0.88	11	7	50
41	CB329965	Monomorphic	0.92	12	0	42	113	IBE5	Polymorphic	0.90	14	3	46	184	SSR270	Polymorphic	0.89	12	12	45
42	CB330083	Monomorphic	0.89	9	0	48	114	IBE7	Polymorphic	0.86	12	12	44	185	SSR38	Polymorphic	0.88	10	7	45
43	CB330141	Polymorphic	0.74	6	4	42	115	IBE8	Polymorphic	0.82	8	3	46	186	SSR4	Polymorphic	0.78	6	5	50
44	CB330144	Monomorphic	0.00	1	0	48	116	IBJ1525	Polymorphic	0.92	16	8	44	187	SSR450	Monomorphic	0.80	5	5	45
45	CB330200	Polymorphic	0.87	11	9	46	117	IBJ1798E	Polymorphic	0.90	13	9	45	188	SSR555	Polymorphic	0.90	11	3	45
46	CB330223	Polymorphic	0.63	3	1	48	118	IBJ199	Polymorphic	0.93	18	18	46	189	SSR578	Polymorphic	0.70	5	3	50
47	CB330283	Polymorphic	0.41	4	3	46	119	IbJ206a	Polymorphic	0.60	5	4	42	190	SSR92	Polymorphic	0.64	3	1	45
48	CB330285	Polymorphic	0.84	8	8	48	120	IBJ206b	Polymorphic	0.79	5	1	44	191	STG0001	Polymorphic	0.90	12	12	52
49	CB330296	Polymorphic	0.84	7	3	42	121	IBJ27	Polymorphic	0.72	4	4	42	192	STG0010	Polymorphic	0.59	3	3	55
50	CB330416	Polymorphic	0.86	10	8	42	122	IBJ290	Polymorphic	0.81	6	4	46	193	STG0016	Polymorphic	0.87	9	9	53
51	CB330456	Polymorphic	0.38	2	1	48	123	IBJ302	Polymorphic	0.91	17	15	42	194	STG0025	Polymorphic	0.76	6	5	55
52	CB330471	Polymorphic	0.92	16	16	48	124	IBJ3816E	Polymorphic	0.71	4	2	44	195	STI0001	Polymorphic	0.86	9	9	55
53	CB330554	Polymorphic	0.87	12	10	42	125	IBJ462E	Polymorphic	0.85	7	7	55	196	STI0003	Polymorphic	0.90	11	9	55
54	CB330601	Polymorphic	0.91	16	14	42	126	IBJ530	Polymorphic	0.89	11	7	48	197	STI0004	Polymorphic	0.84	8	7	55
55	CB330627	Polymorphic	0.90	14	14	42	127	IBJ5446b	Polymorphic	0.87	13	10	44	198	STI0012	Polymorphic	0.87	10	9	55
56	CB330636	Polymorphic	0.86	13	9	42	128	IBJ559	Polymorphic	0.90	15	12	44	199	STI0014	Polymorphic	0.76	5	5	55
57	CB330643	Polymorphic	0.74	5	3	42	129	IBJ566	Polymorphic	0.89	11	9	44	200	STI0030	Polymorphic	0.59	3	3	58
58	CB330649	Monomorphic	0.00	1	0	46	130	IBJ62	Polymorphic	0.93	23	21	42	201	STI0032	Polymorphic	0.88	10	9	60
59	CB330675	Monomorphic	0.67	3	0	46	131	IBJ766E	Polymorphic	0.93	17	11	44	202	STI0033	Polymorphic	0.86	10	10	60
60	CB330693	Polymorphic	0.83	10	8	43	132	IBJ90	Polymorphic	0.86	8	6	46	203	STM0019a	Polymorphic	0.83	9	9	54
61	CB330694	Polymorphic	0.89	15	14	44	133	IBN18	Monomorphic	0.67	3	0	42	204	STM0031	Polymorphic	0.89	14	14	48
62	CB330729	Polymorphic	0.82	7	7	42	134	IBN21	Polymorphic	0.91	14	13	42	205	STM0037	Polymorphic	0.75	5	5	56
63	CB330762	Polymorphic	0.88	11	10	42	135	IBN22	Monomorphic	0.00	1	0	44	206	STM1052	Polymorphic	0.85	13	13	48
64	CB330798	Polymorphic	0.57	3	2	42	136	IBN24	Polymorphic	0.82	7	2	44	207	STM1053	Polymorphic	0.73	4	2	60
65	CB330817	Polymorphic	0.93	19	14	42	137	IBN34	Polymorphic	0.85	9	8	42	208	STM1064	Polymorphic	0.82	7	6	45
66	CB330917	Polymorphic	0.91	16	14	42	138	IBN35	Polymorphic	0.81	7	7	44	209	STM1104	Polymorphic	0.88	9	9	60
67	IB 2-38	Polymorphic	0.76	6	5	42	139	IBN36	Polymorphic	0.91	12	12	48	210	STM1106	Polymorphic	0.60	5	3	60
68	Ib2/30	Polymorphic	0.84	7	2	44	140	IBN37	Polymorphic	0.70	4	3	42	211	STM5114	Polymorphic	0.82	9	9	45
69	IB2.45B	Polymorphic	0.64	3	3	42	141	IBR03	Polymorphic	0.66	3	1	46	212	STM5121	Polymorphic	0.87	9	9	48
70	Ib2-27	Polymorphic	0.88	10	7	44	142	IBR04	Polymorphic	0.78	6	5	46	213	STM5127	Polymorphic	0.50	5	5	48
71	Ib-242	Polymorphic	0.92	15	14	42	143	IBR08	Polymorphic	0.83	10	9	44	214	STPoAc58	Polymorphic	0.87	8	2	63
72	Ib2-248	Polymorphic	0.92	12	4	46														

***Genetic Diversity Analysis*:** Based on the results of 193 polymorphic SSR markers, SHAN similarity matrix was used to generate an UPGMA dendrogram to study extent of genetic diversity among 12 potato cultivars (Fig 2). The similarity coefficient between 12 potato varieties varied from 0.69 to 0.84. As shown in Fig 2, the dendrogram divided 12 potato genotypes into two distinct groups. Cluster I is represented by light green color and comprised of five genotypes i.e. Faisalabad Red, Faisalabad White, SH-5, PRI Red, and Rubby. Similarly, Cluster II was represented by light blue color and is comprised of seven genotypes i.e. Sadaf, Sialkot Red, Sahiwal White, Sahiwal Red, Ravi Red, and FD 81-1. Cosmo did not form any cluster with rest of genotypes and remained separated (Fig 2).

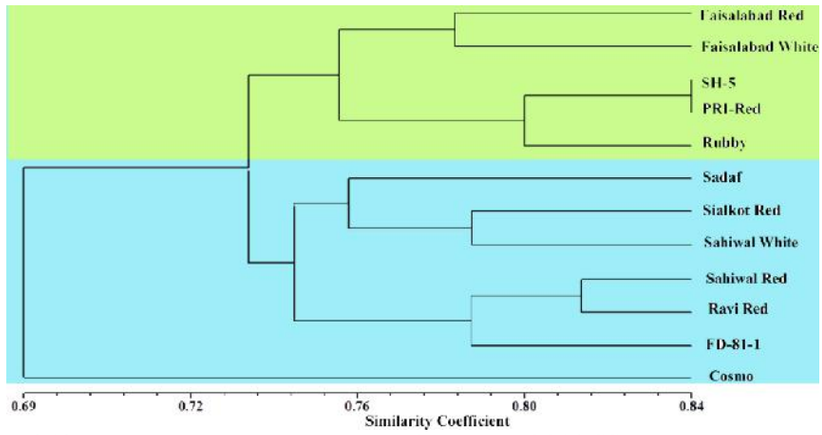

Fig 2. Dendrogram of 12 potato genotypes generated using data of 214 SSR markers through SHAN similarity matrix and unweighted pair group method with arithmetic mean clustering method.

Table 3. List of SSR markers that uniquely identify each genotype.

Genotype Name	No of Markers	Marker
Faisalabad Red	12	STM1106, STM5127, Ib-24, Ib-286, IBE-7, IBS-17, BU691547, IBJ5446, BU690375, CB330200, CB330416, IBJ302
Faisalabad White	10	STM0019a, STM1052, STM5127, SSR38, IBCIP-5, IBR-13, CB330200, BM878740, CB330762, IBE32
SH-5	12	BU692227, IBSSR27, Ib-286, IBC-3, IBE-5, BU691268, IBJ1525, CB330554, BU692896, BU692095, IBE12, IBJ62
PRI-Red	3	STM5114, IBC-8, IBJ530
Ruby	15	BU692566, STM0031, STG0025, STI0012, IBN-35, BU691268, CB330283, CB330625, BU692896, CB330762, BU692914, IBE1, IBE15, IBE32, IBJ62
Sadaf	15	STM0019a, STM1052, STM5114, IBE-1, IBR-19, IBR-20, BU691268, IBJ559, CB330636, CB330771, BM8787757, CB330917, SSR270, IBCIP-1, IBJ5446
Sialkot Red	2	IBE-1, IBE-3
Sahiwal White	11	BU691949, IBR-21, IBE-5, IBE14, BU691143, IBJ559, CB330200, CB330601, BM878740, BU692739, IBN36
Cosmo	2	IBJ5446, BU690750
Sahiwal Red	4	STI0003, IBCIP-2, BU692858, IBN34
Ravi Red	6	IBN-21, CB330693, CB330285, CB330283, IBJ1798E, IBE34
FD 81-1	1	IBN-35

The maximum similarity was observed between SH-5 and PRI Red in cluster I sharing 84% of genetic loci. The lowest genetic similarity was observed between Cosmo and rest of 11 genotypes sharing 69% of genetic loci. There exists a domestic relationship between cultivar distribution and agro-ecological zone as observed in UPGMA dendrogram. Varieties Bred in Sahiwal i.e. Sadaf, Sahiwal White, Sahiwal Red, Ravi Red, Cosmo, FD 81-1 except PRI Red and Rubby shared same cluster. Similarly, varieties bred in Faisalabad i.e. Faisalabad White and Red also shared same clusters whereas varieties bred in Sialkot i.e. Sialkot White and SH-5 did not share same clusters (Fig 2).

In most of cases, dendrogram results fit well with pedigree/parentage information. Sialkot Red and Sahiwal White share a common parent (SH-5) and lie in same cluster. Sahiwal white and Sahiwal Red have both parents in common (FD 3-15 × SH-5). Both these genotypes share a common parent with Ravi Red (SH-5), Cosmo FD 81-1, and Sadaf (FD 3-15) hence all genotypes clustered together. However, in some cases, this was not true i.e. Sialkot Read and SH-5 have a common parent (Cardinal) but both genotypes are in different clusters. Faisalabad White and Faisalabad Red are in same clade but do not have a common parent. Sialkot White, Sialkot Red, Ravi Red, and Sahiwal Red are descendants of SH-5 but SH-5 lies in a separate cluster (Fig 2).

***Population Structure of potato genotypes*:** Model-based cluster analysis using a Bayesian approach was carried out to infer population structure of twelve potato varieties using data of 193 polymorphic SSR markers. The LnP (D) scores for number of populations (K) increased up to 2 and showed inflation point at K2 which divides population into two groups. Similarly, ΔK-value also showed a peak at K = 2, indicating that genotypes

comprised of two populations that are further subdivided into two subgroups. Population 1 (P1) contained five genotypes i.e. Faisalabad Red, Faisalabad White, SH-5, PRI Red, and Rubby. Whereas Population 2 (P2) contained seven genotypes Sadaf, Sialkot Red, Sialkot White, Cosmo, Sahiwal Red, Ravi Red, FD 81-8. Both populations were comprised of further two subgroups. The average distance between individuals in the same cluster (heterozygosity) was high in P2 (0.3008) as compared to P1 (0.2766). However genetic diversity in P1 was high with Fst value 0.16 as compared to P2 with Fst value 0.05. Results for Both K=2 and K=3 are given in Fig 3 for more accurate comparison (Fig 3).

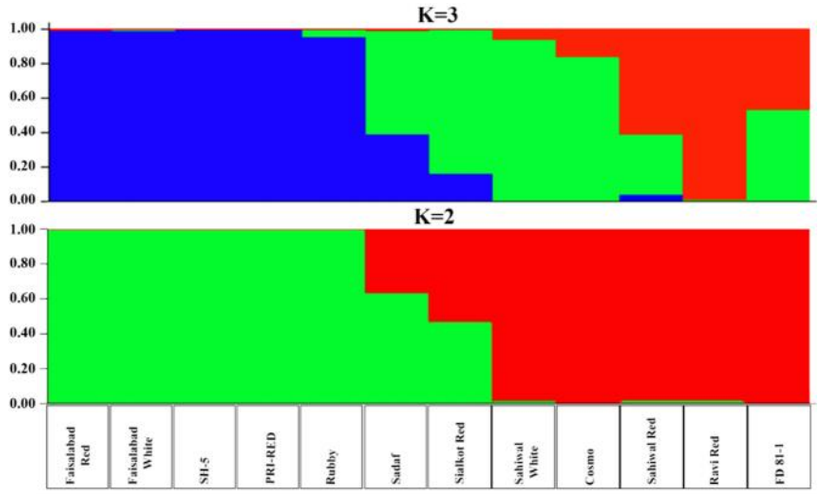

Fig 3. Structure Analysis of Potato varieties grown in Punjab Pakistan. Parameters: no admission model; K = 02; 10,000 Burn-in period; 100000 Rep.

DISCUSSION

Potato cultivar identification is of prime importance for germplasm maintenance and breeder's right protection. Different types of DNA markers i.e. RFLPs, AFLPs, RAPDs, SSRs, and ISSRs were used in past for DNA fingerprinting, marker-assisted selection, phylogenetic and genetic diversity studies of potato (Onamu *et al.,* 2016). Among these, SSR markers are more useful due to high polymorphism, ease of use, and high reproducibility (Jamil *et al.* 2020a, b; Iqbal *et al.,* 2021b). Previously SSR markers have frequently been used for DNA fingerprinting, genetic diversity, and population structure studies of potato (Rodriguez-Bonilla *et al.,* 2014; Song *et al.,* 2016; Jian *et al.,* 2017; Duan *et al.,* 2019; Wang *et al.,* 2019).

Among 226 markers, 193 polymorphic markers were used for further genetic analysis (Table 2). Up to our knowledge, no such studies were conducted in Pakistan for DNA fingerprinting of potato cultivars previously. However one report is available about genetic diversity studies but varieties used in that study are only six and RAPD markers were used which are not reproducible and reliable (Abbas *et al.,* 2008). Unlike previous study of Duan *et al.,* (2019), all varieties used in present study were distinguishable from each other on the basis of 72 SSR markers. Two SSR markers i.e. BU691268 (Fig 1) and IBJ5446 identified three varieties each whereas 14 markers distinguished two varieties each (Table 3). Our results support the findings of Jamil *et al.,* (2020 a, b) that SSR markers are a powerful tool for genotyping and DNA fingerprinting of crops.

In our study alleles per locus (1 to 23 with average 8.04 alleles) and PIC value (0 to 0.96) were different from previous studies (Salimi *et al.,* 2016; Song *et al.,* 2016; Duan *et al.,* 2019; Wang *et al.,* 2019) because of the genetic background of varieties and SSR markers applied. Number of markers used in previous studies (20-30) was not comparable with (193 polymorphic SSR markers) our study as our study offered more genome coverage (Table 2). Duan *et al.* (2019) proposed a set of 11 SSR markers that are sufficient for discrimination of 217 potato cultivars. Similarly, Karaagac *et al.* (2014) proposed a set of six SSR markers for distinguishing of 50 tetraploid potato varieties. Different markers identified in our studies i.e. IBR13 (24 PA), BU690134 (22 PA), IBJ62 (21 PA), IBE14 (20 PA), and IBJ199 (18 PA) (Table 2) are a useful source for DNA fingerprinting and genetic diversity studies in future.

The genetic diversity studies with help of cluster (Fig 2) and population structure analysis (Fig 3) indicated narrow genetic makeup of potato genotypes explored in this study. Except for Cosmo, all other genotypes shared 72% genetic loci. However, Cosmo was dissimilar and shared 69% genetic loci with other 11 genotypes. The reason behind high similarity among genotypes is shared parentage. Except for Faisalabad White and Faisalabad Red, all other genotypes have one parent in common with any other genotype. SH-5 was used as a present in breeding of four genotypes. Similarly, FD 3-15 and FD 35-36 parents were used for breeding of four genotypes each. In extreme cases, Sahiwal Red and Sahiwal White both were evolved from the same cross (FD 35-36 × SH-5) due to colour difference only (Table 1).

At the time of selection breeders usually pay attention to phenotypes (a combination of genetics and environment) without considering genetic makeup (Braun and Wenzel, 2004). This is a leading reason for narrow genetic makeup of cultivated varieties of potato and other crops which expose crops to different disease and insects in the form of pandemics (Jamil *et al.,* 2020c). For any successful breeding program information of genetic diversity in base material should be known and kept in consideration while designing a crossing plan. Crossing among genetically similar but phenotypically different genotypes has narrowed down genetic diversity and stagnant yield potential of cultivated crops including potato (Ray *et al.,* 2012). The practice of using similar

parents in breeding program, again and again, should be discouraged (Jamil *et al.,* 2021b).

Conclusion: DNA fingerprints were developed for 12 potato genotypes grown in Punjab Pakistan. The genetic diversity studies using cluster and structure analysis grouped 12 potato genotypes into 2 distinct groups. Cluster I was comprised of 05 genotypes and cluster II was comprised of 6 genotypes whereas 01 genotype Cosmo did not obey clustering. The results of cluster and structure analysis were complementary to each other. The polymorphism information of 214 amplified SSR markers was also reported in this study. Our study will provide a platform for protection of Potato Breeders Rights and will help in varietal registration in Plant Breeder Rights Registry. We proposed five informative SSR markers i.e., IBR13, BU690134, IBJ6, IBE14, and IBJ199 for future DNA fingerprinting and genetic diversity studies.

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