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Genetic Variability and Population Structure of Pakistani Potato Genotypes Using Retrotransposon-Based Markers

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Abstract: Molecular germplasm characterization is essential for gathering information on favorable attributes and varietal improvement. The current study evaluated the genetic divergence and population structure of 80 potato genotypes collected from Punjab, Pakistan, using polymorphic retrotransposon-DNA-based markers (iPBS). A total of 11 iPBS primers generated 787 alleles with a mean value of 8.9 alleles per primer, of which ~95% were polymorphic across the 80 genotypes. Different variation attributes, such as mean expected heterozygosity (H = 0.21), mean unbiased expected heterozygosity (μHe = 0.22), and mean Shannon's information index (I = 0.32), showed the existence of sufficient genetic diversity in the studied potato genotypes. Analysis of molecular variance (AMOVA) showed that genetic variation within the population was higher (84%) than between populations (16%). A neighbor-joining tree was constructed based on the distance matrices that arranged the 80 genotypes into five distinct groups, and the genotypes FD61-3 and potato 2 had the highest genetic distance. A STRUCTURE analysis corroborated the dendrogram results and distributed the 80 genotypes also into five clusters. Our results determined that retrotransposon-based markers are highly polymorphic and could be used to evaluate genetic diversity between local and exotic potato genotypes. The genotypic data and population structure dissection analysis reported in this study will enhance potato varietal improvement and development.

Keywords: molecular characterization; varietal improvement; heterozygosity; neighbor-joining tree

Citation: Mehmood, A.; Dracatos, P.M.; Maqsood, L.; Yousafi, Q.; Hussain, A.; Jaskani, M.J.; Sajid, M.W.; Haider, M.S.; Hussain, M.M. Genetic Variability and Population Structure of Pakistani Potato Genotypes Using Retrotransposon-Based Markers. *Agriculture* **2023**, *13*, 185. https://doi.org/10.3390/agriculture13010185

Academic Editors: Edyta Paczos-Grzeda, Volker Mohler and Sylwia Sowa

Received: 27 November 2022
Revised: 31 December 2022
Accepted: 4 January 2023
Published: 11 January 2023

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1. Introduction

Among food crops, potato (*Solanum tuberosum* L.) is considered a vital staple food cultivated commercially across temperate and sub-tropical regions [1]. Global potato production was recently estimated at over 370 million metric tons annually and is cultivated over more than 17 million hectares [2]. Potatoes were first grown in modern-day southern Peru and northwestern Bolivia from 5000 to 8000 BC [1]. China is the leading producer of potatoes and produces approximately 22% of global potato production, followed by India, Russia, Ukraine, and the United States [3]. It is estimated that potato is cultivated in Pakistan on approximately 185,360 hectares, with an average production of 4.5 million tons annually, and it is extensively grown in the Punjab region [4].

The potato genome has 12 chromosomes and is an autopolyploid, having different ploidy states, including diploid ($2 \times n = 24$), triploid ($3 \times n = 36$), tetraploid ($24 \times n = 48$), pentaploid ($5 \times n = 60$), and hexaploid ($6 \times n = 72$). The population structure and genetic characterization of potatoes has been well characterized relative to other solanaceous plants [5,6]. Determining the extent and distribution of genetic divergence in distinct gene pools, evaluating germplasm collections, and improving the efficient preservation and

management approaches are all critical factors for successful potato breeding programs. Conventionally, varietal characterization is conducted by assessing morphological traits; however, physical characteristics are often influenced by environmental variability, epistatic interactions, and pleiotropic effects, which restrict only a few traits, leading to low levels of polymorphism [7]. Inherent molecular variation in plant genomes makes it possible to establish and utilize genetic differences between various taxonomic groups, which assists researchers in assessing genetic diversity in gene pools of interest.

Commonly, DNA-based molecular markers are utilized to explore genetic diversity both within and between different crop plant species [8]. The inter-priming binding sites (iPBS) are a retrotransposon-based marker system based in the amplification of a target region incorporated through reverse transcriptase primer binding sites of two adjacent retrotransposons that are in opposite and anti-parallel directions [9]. The iPBS method uses a universal tRNA for primer binding and, therefore, does not rely on pre-defined sequence information, making it an attractive cost-effective proposition for efficient genotyping [10]. The iPBS method has been used for genetic diversity analysis in several crop species, such as date palm, guava, okra, beans, peas, and tobacco [11–16]. The iPBS markers have also been used extensively in genetic diversity studies of Turkish potatoes [17]. Earlier research has confirmed the universality of iPBS markers for molecular and phylogenetic studies and reported that iPBS markers are powerful tools for assessing genetic diversity [11,14,17]. Moreover, iPBS markers have been shown to be consistently more polymorphic than SSR markers [17].

The Pakistani potato is the only germplasm characterized on a morphological basis [18]. Previously, five cultivars were also evaluated for genetic diversity using RAPD markers [19]. These studies suggest that most Pakistani potato accessions need further genetic characterization, to provide vital information for potential use in local breeding programs. The current study has been designed to determine the relationship between the population structure and genetic diversity of 80 potato accessions sourced from the Punjab region in Pakistan using iPBS retrotransposon-based markers. This study will improve the efficacy of incorporating desired traits, such as disease/stress resistance, yield, and environmental adaptability.

2. Materials and Methods

2.1. Plant Material and Genomic DNA Extraction

We collected 80 potato accessions from the Potato Research Institute, Sahiwal; all genotypes and passport data are listed in Table 1. DNA The was extracted from 16-day-old potato leaves using a modified CTAB method [20]. The DNA quality was checked using gel electrophoresis (1% agarose), and quantity was measured with a known λ-DNA concentration.

Table 1. Details of 80 potato (*Solanum tuberosum* L.) genotypes collected from the Potato Research Institute Sahiwal used for genetic diversity analysis in this study.

Accession	Variety Name	Source	Cross	Morphological Characters (Color, Shape, Eyes)
LS 1	FD44-26	Local Strains	385270-163 × Dura	Dark red, oblong, shallow
LS 2	FD48-4	Local Strains	384640-3 × 385270-163	Red, round
LS 3	FD48-54	Local Strains	384640-3 × 385270-163	White, round, shallow
LS 4	FD61-3	Local Strains	Diamant × FD12-24	White, round, shallow
LS 5	FD63-4	Local Strains	384636-1 × FD1-8	White, round, deep
LS 6	FD69-2	Local Strains	FD4-2 × SH-5	Dark red, round, deep
LS 7	FD71-1	Local Strains	FD8-3 × ultimas	Red, round/oval, medium
LS 8	FD73-75	Local Strains	FD35-36 × SH-5	Red, round, shallow
LS 9	FD73-77	Local Strains	FD35-36 × SH-5	Red, round, deep

Table 1. *Cont.*

Accession	Variety Name	Source	Cross	Morphological Characters (Color, Shape, Eyes)
LS 10	FD74-19	Local Strains	9619 × FD49-28	White, round, shallow
LS 11	FD74-33	Local Strains	9619 × FD49-28	White, oblong, shallow
LS 12	FD74-40	Local Strains	9619 × FD49-28	White, round, medium
LS 13	FD74-47	Local Strains	9619 × FD49-28	White, round, deep
LS 14	FD74-50	Local Strains	9619 × FD49-28	White, oval, shallow
LS 15	FD74-51	Local Strains	9619 × FD49-28	White, oblong, shallow
LS 16	FD75-3	Local Strains	FD49-28 × SH-5	White, round, shallow
LS 17	FD75-55	Local Strains	FD49-28 × SH-5	Red, round, deep
LS 18	FD76-13	Local Strains	FD3-15 × SH- 5	Red, oblong, medium
LS 19	FD76-27	Local Strains	FD3-15 × SH- 5	Red, oblong, shallow
LS 20	FD76-35	Local Strains	FD3-15 × SH- 5	Dark red, oblong, shallow
LS 21	FD76-48	Local Strains	FD3-15 × SH- 5	White, round, shallow
LS 22	FD77-62	Local Strains	FD3-9 × SH-5	Dark red, oblong, deep
LS 23	FD80-6	Local Strains	FD3-15 × FD35-36	Light red, round, deep
LS 24	FD51-5	Local Strains	Dura × SH-5	White, round, shallow
LS 25	SL 1-4	Local strains	SH-5 × Red fantasy	Red, oblong, medium
LS 26	SL 1-47	Local strains	SH-5 × Red fantasy	Red, oblong, shallow
LS 27	SL 4-26	Local strains	FD48-4 × SH-5	Dark red, oblong, deep
LS 28	SL13-64	Local strains	SH -5 × FD 48- 54	Red, oblong, shallow
LS 29	SL 13-78	Local strains	FD51-5 × FD69-1	White, oblong, shallow
LS 30	SL14-15	Local strains	Saghitta × SH-5	Red, round, deep
LC 31	FSD white	Local Cultivar	————————	White, round, shallow
LC 32	FSD red	Local Cultivar	————————	Red, round, deep
LC 33	Sadaf	Local Cultivar	FD3-15 × FD35-36	White, round, shallow
LC 34	Ruby	Local Cultivar	384636-1 × FD1-8	Dark red, round, shallow
LC 35	PRI Red	Local Cultivar	FD44-24 × FD12-24	Red, oblong, shallow
NARC 36	Potato 3	NARC Cultivar	—————————	———————-
NARC 37	Potato2	NARC Cultivar	—————————	————————
NARC 38	NARC	NARC Cultivar		
NARC 39	N-13	NARC Cultivar	————————-	Light yellow, oval, medium
NARC 40	N-15	NARC Cultivar	—————————	Light red, oval, medium
NARC 41	N-18	NARC Cultivar	——————	Light red, oval, medium
NARC 42	N-34	NARC Cultivar	———————-	White, oval, medium
NARC 43	N-2005-1	NARC Cultivar	———————	Red, oval, medium
NARC 44	N-2005-4	NARC Cultivar		
NARC 45	N-393619-44	NARC Cultivar	————————	Light red, round, deep
NARC 46	N-4	NARC Cultivar	————————	White, round, medium
EC 47	Aurea	Exotic Cultivar	Lady Rosetta × (Sat-runa × Pentland dell)	White, round, medium
EC 48	Dolly	Exotic Cultivar	Lady Rosetta × Britta	Light red, round, deep
EC 49	Elbieda	Exotic Cultivar	————————	White, oval, shallow
EC 50	Elodie	Exotic Cultivar	80F66.25 × 81F145.14	White, oval, shallow

Table 1. *Cont.*

Accession	Variety Name	Source	Cross	Morphological Characters (Color, Shape, Eyes)
EC 51	Eldorodo	Exotic Cultivar	———————	Red, oval, medium
EC 52	Estima	Exotic Cultivar	Nopol × G3014	Light yellow, oval, medium
EC 53	El-mundo	Exotic Cultivar	——————	White, oval, medium
EC 54	Erora	Exotic Cultivar	———————	White, oval, medium
EC 55	Paramount	Exotic Cultivar	Janat × Dutch seeding	Red, oval, medium
EC 56	Hybrid 202-05-01	Exotic Cultivar	——————-	Light red, oval, medium
EC 57	Simply red	Exotic Cultivar	Asterix × HZ86 AM75	Light red, oblong, medium
EC 58	Santana	Exotic Cultivar	Spunta × VK69-491	White, oblong, shallow
EC 59	Romera	Exotic Cultivar	Belladonna × Laura	Light red, oval, medium
EC 60	Monika	Exotic Cultivar	Krasa × Velox	White, oval, medium
EC 61	Verdi	Exotic Cultivar	Tomnsa × Diana	White, oval, medium
EC 62	Safrane	Exotic Cultivar	——————	White, oval, medium
EC 63	Terka	Exotic Cultivar	Fabula × Pamir	Light yellow, round, shallow
EC 64	Red Valentine	Exotic Cultivar	Mondial × Amadeus	Red, oval, medium
EC 65	Red Sonia	Exotic Cultivar	——————	Red, oval, medium
EC 66	Red Sun	Exotic Cultivar	Inova × Amadeus	Red, oblong, medium
EC 67	Rositta	Exotic Cultivar	——————-	Light red, oval, medium
EC 68	Sagitta	Exotic Cultivar	Gallia × RZ-86-2918	Light yellow, oblong, medium
EC 69	Suzen	Exotic Cultivar	——————	White, oval, medium
EC 70	Sassy	Exotic Cultivar	G82TTT37 × Propmesse	White, round, medium
EC 71	Focus	Exotic Cultivar	Agria × Bru 82-78	Light yellow, round, shallow
EC 72	Florice	Exotic Cultivar	Fanette × Inra72.68.5	Light yellow, oblong, medium
EC 73	HZD-04-684	Exotic Cultivar	——————	Red, oval, shallow
EC 74	Shepody	Exotic Cultivar	Bake king × F58050	White, oval, medium
EC 75	Orchestra	Exotic Cultivar	Maradonna × Cupido	Light yellow, oval, medium
EC 76	Jitka	Exotic Cultivar	M22/12 × Bonita	Light yellow, round, medium
EC 77	Red river	Exotic Cultivar	——————	Red, oval, medium
EC 78	Kuroda	Exotic Cultivar	AR76-199-3 × Konst80-1407	Red, oval, medium
EC 79	KWS-06-125	Exotic Cultivar	—————-	Dark red, oval, medium
EC 80	Pirol	Exotic Cultivar	Agriax × 1.214.226-84	

Abbreviations are as follows: LS, local strains; LC, local cultivars; NARC, National Agriculture Research Centre; EC, exotic cultivars.

2.2. Amplification Profile of Retrotransposon-Based iPBS Primers

Initially, 16 iPBS primers (detailed in Table 2) previously developed and characterized by Kalender et al. [9] were assessed for their polymorphism and utility. A total of 11 primers that gave clear polymorphic bands were selected for molecular profiling of the 80 potato accessions detailed in Table 1. Here, PCR reactions were performed in 20 µL reactions consisting of 11.5 µL double-distilled H_2O, 2 µL 10× Taq buffer with (NH_2) SO_4, (Thermo Scientific, Waltham, MA, USA), 2 µL (20 mM) $MgCl_2$ (Thermo Scientific), 1 µL (2 mM) dNTPs (Deoxyribonucleotide triphosphate), 1 µL iPBS primer (Macrogen, Seoul, Republic of Korea), 0.5 µL Taq polymerase (Thermo Scientific), and 2 µL (10 ng) template DNA. The PCR conditions involved an initial denaturation cycle of 5 min at 94 °C, 35 cycles for 1 min at 94 °C, 1 min at an annealing temperature range between 30–50 °C, 2 min at the temperature of 72 °C, then a final extension of 10 min at a temperature of 72 °C, and storage temperature of 4 °C for 1 hour.

Table 2. List of 16 inter-primer binding site (iPBS) retrotransposon primers with their sequence and annealing temperature used in this study.

Serial No	iPBS Primers	Base Pair Sequence (5′-3′)	Annealing Temp (°C)	GC Content (%)
1	2257	CTCTCAATGAAAGCACCA	46	44
2	2229	CGACCTGTTCTGATACCA	46	50
3	2252	TCATGGCTCATGATACCA	43	44
4	2277	GGCGATGATACCA	46	54
5	2375	TCGCATCAACCA	30	50
6	2376	TAGATGGCACCA	46	50
7	2387	GCGCAATACCCA	46	58
8	2391	ATCTGTCAGCCA	46	50
9	2374	CCCAGCAAACCA	30	58
10	2377	ACGAAGGGACCA	46	67
11	2383	GCATGGCCTCCA	46	66
12	2232	AGAGAGGCTCGGATACCA	48	56
13	2239	ACCTAGGCTCGGATGCCA	50	61
14	2272	GGCTCAGATGCCA	46	62
15	2373	GCTCATCATGCCA	46	54
16	2390	GCAACAACCCCA	46	58

The iPBS primers by Kalender et al. [9] were used in the initial screening.

2.3. Band Counting and Statistical Measurement

To confirm band pattern uniformity, three experimental replicates were performed for each PCR for all iPBS markers on the potato accessions. The PCR bands were examined using a 2% agarose gel using a transilluminator and were scored manually; only clear visible bands were scored with the assumption that bands of the same size represented the same single locus. A binary matrix was constructed for the presence of an allele on a specific locus denoted as '1', and for the absence of an allele marked as '0' for a particular locus. To estimate the polymorphism of each dominant marker, polymorphic information content (PIC) was calculated as PIC = $1 - [f^2 + (1 - f)^2]$, where 'f' indicates the frequency of the marker in the data set. Statistical parameters, such as Shannon's information index (I), heterozygosity (He), unbiased heterozygosity (μHe), number of different alleles, number of effective alleles, and principal coordinate analysis (PCoA), were calculated using GeneAlex 6.5 [21]. The binary matrix was imported to construct a neighbor-joining (NJ) tree using MEGA 7.0.14 [22]. The model-based software STRUCTURE v. 2.3.4 created the population structure and allocated individual genotypes to sub-populations [23]. A Bayesian approach was applied to determine the population structure of the potato genotypes used in this study. Data from 11 distinct iPBS markers were analyzed using STRUCTURE software; using the value of K (10 runs at each K), the highest number of clusters was estimated by running combination data among the population and allelic frequency of 10,000 steps followed by 50,000 simulations of a Monte Carlo Markov chain (MCMC). The most probable K value was determined by measuring the assessed data of log probability of LnP(D), and the value of ΔK was calculated for the rate of change in LnP(D) between consecutive K-values [24]. We used the STRUCTURE HARVESTER for computational analysis of 80 potato accessions based on iPBS markers, and the maximum number of Ln Pr (X|K) was selected for bar plots among all 10 independent runs [25].

3. Results

3.1. Molecular Assessment of iPBS Markers

The 11 primers detailed in Table 3 gave stable, precise, and polymorphic PCR amplicons, and were subsequently selected for further genetic analysis of the 80 potato accessions. The banding pattern of the PCR products of 80 potato genotypes using iPBS primer 2252 is shown in Figure 1. The highest polymorphic band size of 1800 bp was obtained in Ruby and N-34 genotypes for primer 2229. Across 11 primers, 787 alleles were identified, out of which 752 were polymorphic, showing 96% polymorphism. Primer 2229 amplified the

highest number of bands (60), and primers 2390 and 2391 amplified the lowest number of bands (25). The highest PIC value amongst the 11 polymorphic iPBS markers was marker 2277 (0.39), whereas marker 2391 had the lowest PIC value of 0.14 (Table 3). The highest values of Shannon's information index (I = 0.48), heterozygosity (He = 0.33), and unbiased expected heterozygosity (μHe = 0.34) was observed for marker 2229.

Table 3. Detection of polymorphism and summary statistics for mean values of 11 iPBS primers used to assess genetic diversity among 80 potato genotypes.

iPBS Primers	AN	Size Range (bp)	PM	% PM	MM	PIC	Na	Ne	I	He	μHe	f
2229	86	500–2000	85	99	1	0.37	1.83	1.58	0.48	0.33	0.34	0.45
2232	86	550–1350	86	100	0	0.33	1.61	1.40	0.36	0.24	0.25	0.46
2239	86	600–1500	80	93	6	0.24	1.45	1.37	0.33	0.22	0.23	0.35
2252	86	650–2000	85	99	1	0.25	1.63	1.38	0.35	0.23	0.24	0.39
2272	84	500–1750	79	94	5	0.19	0.67	1.11	0.12	0.07	0.08	0.13
2277	80	450–1000	80	100	0	0.39	1.71	1.54	0.45	0.31	0.32	0.47
2374	82	400–1250	60	73	22	0.22	1.25	1.15	0.19	0.11	0.11	0.14
2375	80	350–3500	65	81	15	0.25	1.23	1.2	0.23	0.14	0.14	0.17
2377	83	300–3500	75	90	8	0.36	1.75	1.52	0.44	0.3	0.31	0.45
2390	85	280–1000	80	94	5	0.36	1.66	1.57	0,47	0.32	0.34	0.54
2391	75	250–1000	60	80	15	0.14	1.07	1.08	0.13	0.07	0.07	0.81

Abbreviations are as follows: AN, allelic number; PM, number of polymorphic bands; MM, number of monomorphic bands; PIC, polymorphic information content; Na, number of different alleles; Ne, number of effective alleles; I, Shannon's information index: He, heterozygosity; μHe, unbiased expected heterozygosity; f, frequency.

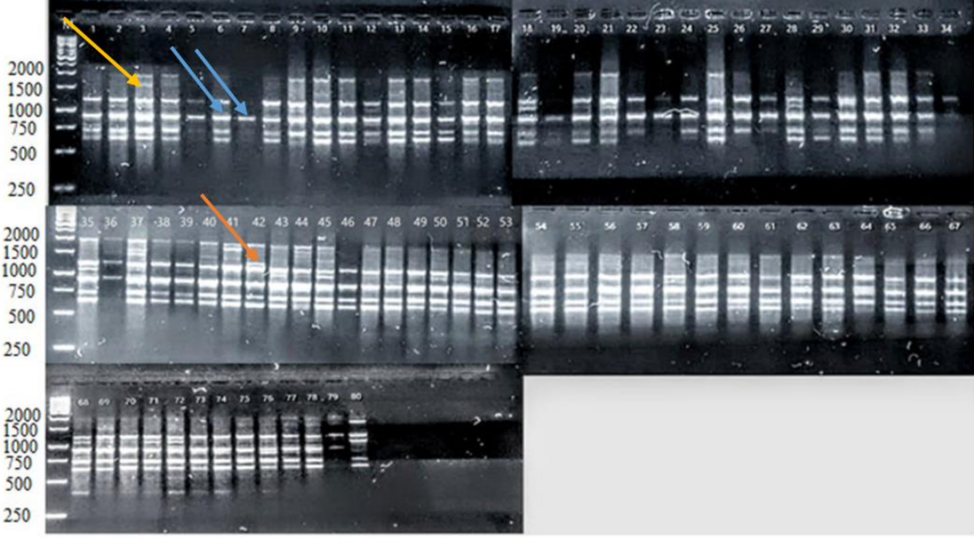

Figure 1. The banding pattern of PCR products of 80 potato genotypes using 2252 iPBS primers. Well 1 includes a 1 kb size ladder, wells 2–30 include local strains, wells 31–35 are local cultivars, wells 36–46 represent NARC accessions, and wells 47–80 contain exotic cultivars. Yellow-colored arrows point to polymorphic alleles of sizes 1700 bp, and blue-colored arrows point to monomorphic alleles of size 900 bp, while the orange color represents a unique allele of size 1300 bp.

3.2. Heterozygosity and Molecular Variance (AMOVA) of 80 Potato Genotypes

Shannon's information index (I) ranged from 0.29 (NARC) to 0.37 (EC), with an average of 0.32. Expected heterozygosity (He) ranged from 0.18 (NARC) to 0.24 (EC) with an average value of 0.21, and the unbiased heterozygosity (μHe) ranged between 0.19 (NARC) and 0.24 (EC) with an average value of 0.22 (Table 4). Among all populations, the population of ECs (exotic cultivars) showed the highest Shannon's information index (0.37), expected heterozygosity (0.24), and unbiased expected heterozygosity (0.24), while genotypes from

the NARC population showed the lowest Shannon's information index (0.29), expected heterozygosity (0.19), and unbiased expected heterozygosity (0.19). The genotypes included in the EC population had the highest genetic distance from the genotypes of the local strain population, local cultivar population, and NARC population.

Table 4. The summary of statistical analysis of genetic diversity across 80 potato genotypes based on 11 iPBS primers.

Groups	N	Na	Ne	I	He	μHe
LS	30	1.56	1.36	0.34	0.22	0.22
LC	5	1.11	1.35	0.29	0.2	0.22
NARC	11	1.36	1.30	0.29	0.19	0.19
EC	34	1.75	1.39	0.37	0.24	0.24
Mean	20	1.45	1.35	0.32	0.21	0.22

Abbreviations are as follows: N, number of sample size; Na, number of different alleles; Ne, number of effective alleles; I, Shannon's information index: He, heterozygosity; μHe, unbiased expected heterozygosity; LS, local strain population; LC, local cultivar population; NARC, NARC population; EC, exotic cultivar population.

Analysis of molecular variance (AMOVA) was performed to determine the diversity both among and between the 80 potato genotypes according to their four geographic regions of origin (Table 5). Results from AMOVA revealed greater molecular variation within populations (85%) relative to between populations (15%).

Table 5. Analysis of molecular variance (AMOVA) of 80 potato genotypes based on 11 iPBS markers presenting the percentage of molecular variance among and within the population.

SV	df	SS	MS	Est. Var.	%	PhiPT
Among Pops	3	166.47	55.49	2.30	15%	0.021 ***
Within Pops	82	1056.16	12.88	12.88	85%	
Total	85	1222.63		15.18	100%	

Abbreviations are as follows: SV, source of variation; df, degrees of freedom; SS, sum of squares; MS, mean square; Est. Var., estimated variance; %, percentage of variation; *** $p < 0.001$.

3.3. Principal Coordinate analysis (PCoA) and Hierarchical Clustering of 80 Potato Genotypes

Principal coordinate analysis (PCoA) depicted the 80 potato genotypes based on their genetic distance (Figure 2). The 2 axis of the principal coordinate accounted for 20.6% of the total molecular variation, which distributed 80 genotypes in thee main groups, while six genotypes, including NARC37, NARC46, LC34, LS7, LS9, EC78, and EC47, were distinct from other genotypes.

Genetic distance was calculated using the dissimilarity index for constructing a NJ tree using 11 iPBS markers. Among the local strain (LS) population, LS9 (22.74) and LS3 (19.72) revealed the highest genetic distance. Among the local cultivar (LC) population, LC36 showed the highest genetic distance (17.35). Cultivars NARC37 (23.97) and NARC46 (19.88) indicated the maximum genetic length among the NARC cultivars population, and EC78 (23.65), EC69 (21.80), EC80 (21.65), and EC77 (21.34) had the maximum genetic distance among the EC (exotic cultivar) population. The NJ dendrogram separated 80 potato accessions into three major clusters with five sub-clusters (Figure 3). Cluster 1 consists of 16 genotypes, including 14 local strains, 1 NARC cultivar, and 1 local cultivar, of which LS14 (FD74-50) and LS10 (FD74-19) showed the closest genetic similarity, while LS7 (FD71-1), LS9 (FD73-77), LC34 (Ruby), and NARC46 (N-4) were more genetically diversified than other genotypes. Cluster 2 consists of 22 potato genotypes, including 17 exotic cultivars (ECs), 3 local strains, and 2 NARC cultivars, of which EC53 (El-mundo) and EC51 (Eldorodo) showed the closest genetic similarity while EC47 (Aurea), EC50 (Elodie), and EC54 (Erora) were genetically distinct. Cluster 3 consists of 17 exotic cultivars, of which EC73 (HZD-04-684) and EC70 (Sassy) had the closest genetic similarity, while EC71 (Focus), EC78 (Kuroda), EC65 (Red Sonia), and EC80 (Pirol) were genetically distinct. Cluster

4 consists of 13 genotypes, including 9 local strains and 4 local cultivars, of which LC32 (FSD red), LS18 (FD76-13), and LS25 (SL1-4) were genetically dissimilar. Similarly, cluster 5 consists of 12 genotypes, including 8 NARC cultivars and 4 local strains, of which LS29 (SL13-78) and LS24 (FD51-5) were closely related, while LS22 (FD77-62) and NARC39 (N-13) were more genetically dissimilar than other genotypes (Figure 3).

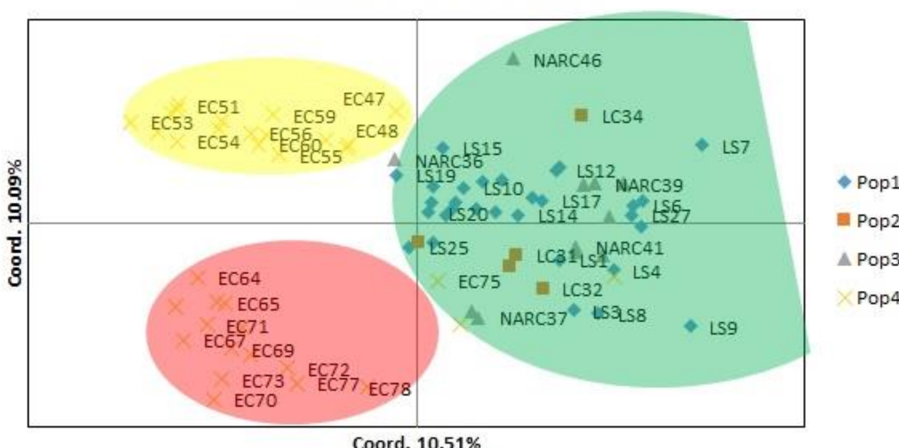

Figure 2. Principle coordinate analysis of 80 potato genotypes indicated the 20.6% variation based on retrotransposon markers; population 1 represents local strains, population 2 represents local cultivars, population 3 represent NARC cultivars, and population 4 represents exotic cultivars.

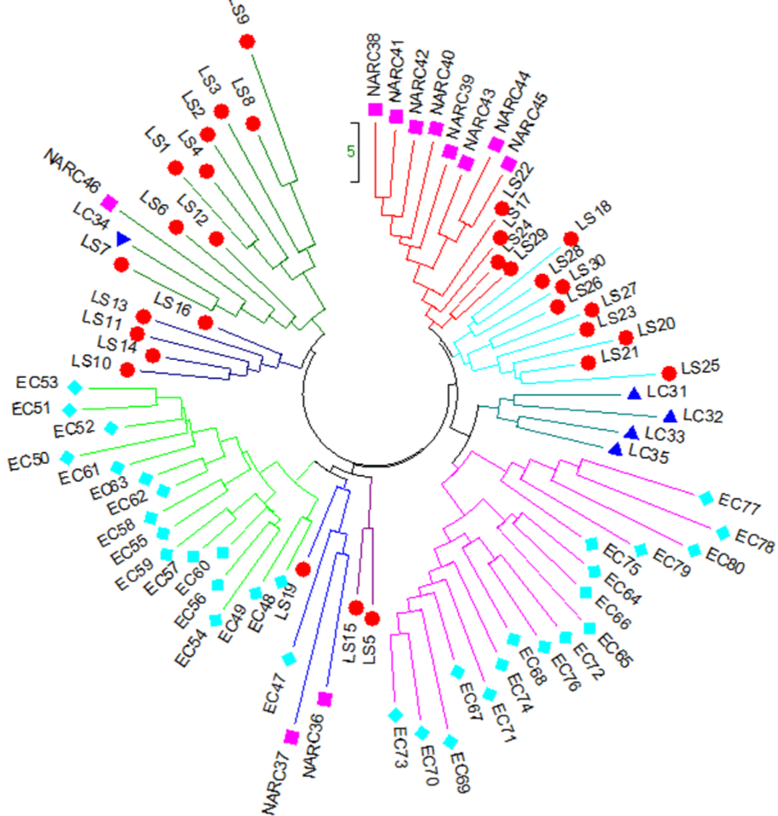

Figure 3. Neighbor-joining tree of 80 genotypes of potato generated with data from 11 iPBS primers showing 5 main clusters with some clusters containing sub-groups. Abbreviations are as follows: LS (●), local strains; LC (▲), local cultivars; NARC (■), and EC (◆), exotic cultivars.

3.4. Genetic Structure of 80 Potato Genotypes

The Bayesian methodology [23] used in STRUCTURE was applied to calculate the genetic structure of binary data extracted from 11 iPBS primers, and the data suggested that an optimum number of K = 5 represents the presence of five main clusters among 80 potato genotypes (Figure 4). A total of 11 iPBS primers distributed 80 potato genotypes into five major groups identified in yellow, red, blue, purple, and green (Figure 5). If a genotype has a member coefficient of 80% in K = 5, it belongs to that population. The result of iPBS data showed that population 1 had 7 genotypes (yellow), population 2 had 14 genotypes (red), population 3 had 7 genotypes (blue), population 4 had 13 genotypes (green), and population 5 had 14 potato genotypes (purple). In terms of the population structure of some the potato genotypes collected, namely LS18, LS28, NARC41, NARC45, EC51, EC52, EC54, EC56, EC59, EC60, EC61, EC62, EC67, EC68, EC70, EC73, and EC74, it was proposed that they do not share a common ancestor and represent pure genetic material. At the same time, genotypes with multiple colors are a mixture from numerous clusters, i.e., LC31, LC32, LC33, LC34, LC35, and LC36.

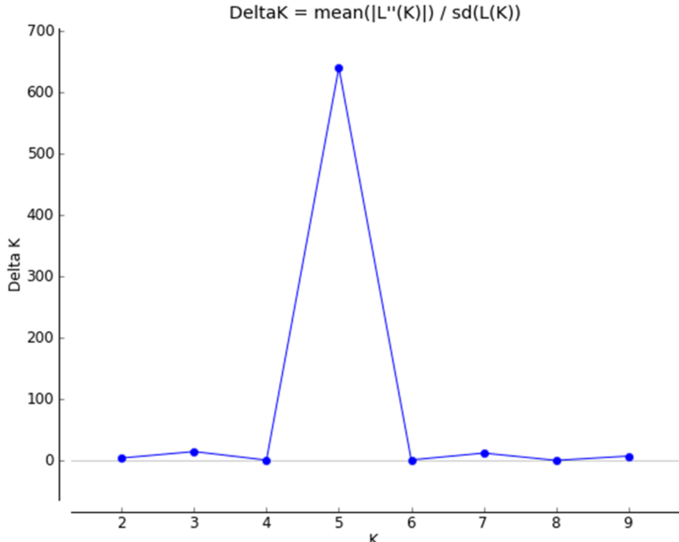

Figure 4. Plot of delta K calculated as the mean of the second-order rate of change in the likelihood of K divided by the standard deviation of the likelihood of K, m(|L″(K)|)/sd[L(K)]. Delta K = 5 is the potential number of genetic clusters that may exist in the overall sample of individuals.

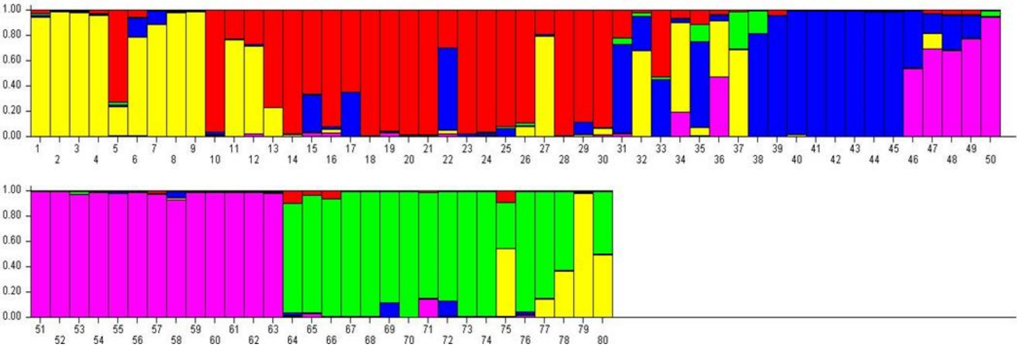

Figure 5. The population structure of 80 genotypes assessed using Bayesian analysis with allelic variation from the 11 iPBS markers. Five populations (yellow, red, blue, purple, and green) were defined using the method described in Evanno et al. (2005). Each vertical line symbolizes an individual multi-locus genotype.

4. Discussion

Despite low labor requirements, potato production in Pakistan is poor relative to neighboring countries, such as India and Bangladesh. This is thought to be due to several biotic and abiotic stresses, as well as the limited allocation of arable land. To overcome biotic and abiotic stresses using a breeding approach understanding the genetic diversity within breeding lines, landraces, and germplasm is critical. According to previous studies, retrotransposons comprise half of the plant genome's repetitive DNA. Potato has been reported to contain 214 Mb of LTR-transposons comprising 30% of total genome size. The iPBS markers are derived from retrotransposons and are not reliant on prior sequence information. Furthermore, iPBS markers have been applied in several different genetic evaluation studies of plant species, such as *Cicer* ssp. [26], *Saussurea esthonica* [27], *Diospyros* ssp. [28], *Myrica rubra* [29], and grape [30]. The iPBS markers typically generate multiple polymorphic bands per locus, are highly reproducible, and are inexpensive compared to other marker systems [30].

A total of 11 polymorphic iPBS markers were effective in characterizing the genetic variation between and within 80 potato genotypes. Unique alleles were found in Red River, Sagitta, FD 61-3, and PRI-RED genotypes with primers 2229, 2232, 2375, and 2239, respectively; these alleles could be sequenced in the future for primer design. The PIC values estimate the marker power of discrimination for a locus and provide the size of alleles. The PIC value recorded for each primer ranged from 0.13 to 0.38, with a mean value of 0.28, which is higher than the PIC value (0.12–0.31) reported in Turkish potato accessions by Demirel et al. [17]. This is likely due to the selection of more specific transposable base iPBS markers in this study into the genetic diversity of the Pakistani potato germplasm. The molecular analysis of 80 genotypes revealed the average recorded heterozygosity (He) for 11 iPBS primers was 0.2. The low He and Shannon's information index (I) values observed in this study are due to using a limited number of iPBS markers, leading to selection bias.

A dendrogram was constructed based on data gathered from 11 iPBS primers by using the NJ method, and this distributed the 80 potato genotypes into five main clusters. Further cluster analysis showed that genetic diversity was higher among and between the potato genotypes due to genetic drift. The dendrogram was built based on dissimilarity coefficient values that showed a wide range of variable values of the similarity index and indicates that iPBS markers can be used effectively in genetic diversity studies. To further enhance the Pakistani potato industry, phylogenetic characterization based on genetic distance could be helpful for crop breeding, facilitating the development new breeding programs. Wild relatives and primitive cultivars of potatoes contribute to diversity in genetic resources for production programs for the potato crop [31–33]. The current study's genetic dissimilarity results showed the highest genetic distance among the genotypes, including LS9 (FD73-77), LS3 (FD48-54), LC36 (Potato 3), NARC37 (Potato2), NARC46 (N-4), EC78 (Kuroda), EC69 (Suzen), EC80 (Pirol), and EC77 (Red River). These results provide a basis for enhanced diversification for parental selection for potato breeding in Pakistan.

Potato has been reported to show higher heterozygosity, as it is a tetraploid outcrossing crop [34]. The present study has shown a high level of genetic diversity in the potato genotypes selected. The heterozygosity results are in agreement with the previously reported studies [34]. Furthermore, heterosis and mutation-positive selection could also be the important factor contributing to the high heterozygosity in potato. Shannon's information index (I) is important in order to understand genetic variation among cultivars, as it is related to genetic differences in uniformity and population combining abundance. The variation in I observed among genetic groups might be due to geographic factors, habitat destruction, restriction in gene flow, and type of breeding system. Further variation could be the result of the inclusion of wild accessions in the present study. These results are in contrast with a Chinese study [35] that showed that I varied from 0.73 to 1.76 among the 149 main potato cultivars of China. Analysis of molecular variance (AMOVA) showed the presence of high variation within potato genotypes, with the percentage of total variance being 85%. It has been previously stated that higher variations in varieties may be due to

reasons, such as selection, adaptation, gene flow, genetic drift, and variation in ecotypes and pollination method [36].

The PCoA approach is a widely used method for assessing genetic diversity based on quantitative and qualitative traits that scale distance data to multidimensional planes for the characterization of genetic diversity. The data acquired from this study of population structure and heterozygosity of potato germplasm indicate that NARC cultivars clustered together in the dendrogram due to their low heterozygosity. Despite their extensive distribution and cultivation, these findings indicate that only a few NARC cultivars have been used in potato breeding programs. Our results showed that the local cultivar population and NARC cultivars tended to be closely related based on their clustering showing minimal genetic diversity that can be exploited for breeding purposes. Individuals with multiple colors, such as LC31, LC32, LC33, LC34, LC35, and LC36, are admixtures indicating the maximum genetic drift and, thus, inform future studies into enhancing potato genetic diversity for germplasm collection and conservation [8]. The genotypes were clustered based on geographical distribution and morphological features to execute a similarity index analysis. The PCoA method has been used previously to study the genetic relatedness among different potato genotypes [37]. The genetic diversity for 26 potato genotypes grown in Turkey was previously analyzed using six AFLP primers, resulting in the production of 191 polymorphic bands which distributed potato genotypes into six distinct subgroups [38]. Another study in Turkey used SSR markers for fingerprinting major potato landraces and varieties grown in Central Anatolia [39]. Among 16 SSR primers, five markers (STM19, STM31, STM3012, STI32, and STI42) distinguished the 15 potato genotypes into five groups [39]. In our study, 11 transposon-based markers distributed the potato accessions into 5 groups according to their genetic structures.

5. Conclusions

The study of the genetic variation of 80 Pakistani potato genotypes using iPBS-based markers provided data about their relatedness and diversity. This data can be submitted to the relevant molecular databases to incorporate new information in the national gene pool. Other techniques, such as genotype-by-sequencing (GBS) and DArT-Seq, can also be used to enhance genetic diversity studies using an increased number of accessions to better assess genetic distances among divergent genotypes. A comprehensive genome-wide association mapping study is required to better understand and further explore genetic diversity studies in a highly diverse collection of potato accessions. Establishing germplasm consisting of a core collection used in breeding programs is necessary. The findings of this study confirm the extent of diversity within the Pakistani potato germplasm. Further molecular diversity, trait dissection, and characterization studies are required for germplasm preservation and crop improvement.

Author Contributions: L.M. and A.M. performed data collection and original draft preparation; P.M.D. performed substantial reviewing, editing and formatting M.S.H. and M.J.J. were also responsible for reviewing and editing; A.M. and A.H. were responsible for software; supervision and lab work data analysis was by M.W.S.; Q.Y. and M.M.H. were responsible for the fieldwork and methodology. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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