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DNA fingerprinting and genetic diversity analysis of world quinoa germplasm using iPBS-retrotransposon marker system

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Abstract: Quinoa is an important staple food crop for millions of impoverished rural inhabitants of the Andean region. Quinoa is considered a good source of protein,vitamins, minerals, and antioxidants. This study aimed to investigate the genetic diversity and population structure of world quinoa germplasm originating from 8 countries through the iPBS-retrotransposon marker system. Molecular characterization was performed using the 11 most polymorphic primers. A total of 235 bands were recorded, of which 66.8% were polymorphic. Mean polymorphism information content (PIC) was 0.410. Various diversity indices including mean effective number of alleles (1.269), mean Shannon's information index (0.160) and gene diversity (0.247) revealed the existence of sufficient amount of genetic diversity in studied germplasm. Bolivia–17 and Mexico–1 were found to be genetically distinct accessions and can be suggested as candidate parents for future breeding activities. Various diversity indices were also calculated among germplasm collection counries and the results clearly showed the existence of higher genetic diversity in Bolivian and Peruvian accessions. The model-based structure, neighbor-joining, and principal coordinate analysis (PCoA) grouped quinoa germplasm according to their collection country. Analysis of molecular variance (AMOVA) revealed that most of the variations (69%) in world quinoa germplasm are due to differences within populations. Findings of this study can be used for deeper understanding of the genetic relationship and in the determination of appropriate breeding and conservation strategies for quinoa.

Keywords: Chenopodium quinoa, grain crop, polymorphism, germplasm characterization, population structure

1. Introduction

Characterization of germplasm provides an opportunity to investigate the genetic diversity and to identify the novel variations that can be employed for various breeding activities (Guliyev et al., 2018; Gramazio et al., 2018). Advancement in molecular marker technology boosts breeding activities through germplasm characterization and identification of novel variations. Various types of molecular markers have been developed according to their efficiency and utilization. Retrotransposons are genetic elements that have the ability to change their location, copy numbers, and are considered and abundant component of the plant genome (Finnegan, 1989). Long terminal repeat (LTR) and non-LTR retrotransposons are 2 types of retrotransposons, however the former is found abundant in plant genome compared to the latter (Kalendar et al., 2010). Application limitation in both LTR and non-LTR retrotransposons leads to the development of a new marker system named inter primer binding site (iPBS) (Kalendar et al., 2010). Kalendar et al. (2010) proposed iPBS a universal marker system that can be utilized for the molecular characterization of any plant and animal species. iPBS-retrotransposon is a PCR-based marker system depending on the presence of tRNA as a reverse transcriptase primer binding site (Kalendar et al., 2010). This marker system has been successfully utilized for the investigation of genetic diversity and population structure of various crops like pea (Baloch et al., 2015), common bean (Aydin and Baloch, 2019; Nemli et al., 2015), ciccer (Andeden et al., 2013), laurel (Karık et al., 2019), and pepper (Yildiz et al., 2019). This universal marker system has also been utilized for the genetic characterization of quinoa germplasm using 17 accessions cultivated in Turkey (Hossein-Pour et al., 2019). However, the size of germplasm evaluated so far represents only a small subset of available quinoa genetic resources.

Quinoa *(Chenopodium quinoa* Willd.) is an important grain crop of *Amaranthaceae* family, having 2n = 4x = 36 chromosomes. Quinoa has an allotetraploid genome size of about 1.5 GB (Jarvis et al., 2017). Quinoa is native to the

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Andean region that is considered an origin, domestication, and diversity hotspot for various crops (Risi 1984; Repo-Carrasco et al., 2003; Fuentes et al., 2009). Quinoa contains higher protein (10–18%) contents and good concentrations of lysine and methionine (Galwey et al., 1989; Rojas, 2015). It also contains good concentrations of fiber, minerals, and antioxidants such as polyphenols (Ando et al., 2002; Hirose et al., 2010). Quinoa is used to make flour, soup, and alcohol and has been extended to pharmaceutical and industrial areas (Bhargava et al., 2006). Quinoa is popular among celiac patients and people with wheat allergies because of lesser gluten content (James, 2009). The United Nations declared 2013 as International Year of Quinoa because of its higher nutritious potential and stress-tolerant characteristics (FAO, 2013). Peru and Bolivia are the main quinoa producing countries. During 2017, quinoa was cultivated in an area of 173.242 hectares in Bolivia, Peru, and Ecuador (FAO, 2017).

Quinoa contains 5 ecotypes including the Andean highlands (Peru and Bolivia), inter-Andean valleys (Colombia, Ecuador, and Peru), Salares (salt lakes; Bolivia, Chile, and Argentina), Yungas (Bolivia), and Coastal/Lowlands (Chile); each of these ecotypes are highly adapted to specific environments and reflects a greater level of genetic variation (Bazile et al., 2016; Salazar et al., 2019). Molecular characterization of quinoa germplasm revealed 2 distinct gene pools: the Andean highlands group (in the highlands of Peru and Bolivia) and the coastal diversity group (in the coastal regions of central and southern Chile) (Fuentes et al., 2009; Jarvis et al., 2017; Salazar et al., 2019).

Various studies have been conducted to explore the genetic diversity and population structure of quinoa germplasm using different molecular marker systems (Fuentes et al., 2009; Maughan et al., 2012; Al-Naggar et al., 2017; Ana-Cruz et al., 2017; Zhang et al., 2017; Salazar et al., 2019; Hossein-Pour et al., 2019). However, limitations in earlier studies such as type, number of germplasm, and marker system leads to conducting a study with a good number of accessions from origin and domestication center of this crop. Therefore, this study aimed to investigate the genetic diversity and population structure of 96 quinoa accessions originating from 8 countries through the iPBS-retrotransposon marker system. Our aim is to provide detailed genetic characterization of large size quinoa germplasm, which will give brief insight into the diversity harbored by the various accessions representing different countries, which are important centers for quinoa diversity and domestication.

2. Material and methods

2.1. Plant material and DNA isolation

A total of 96 quinoa accessions collected from 8 countries were used as plant material in this study. These accessions were obtained from the United States Department of Agriculture (USDA) (Table 1). All quinoa accessions were sown in the greenhouse at Bolu Abant İzzet Baysal University, Turkey, at 25 °C. Fresh, young, and healthy leaf tissues were taken at the proper time for DNA extraction. The DNA extraction was carried out using the fresh leaves from each accession by following the CTAB protocol (Doyle and Doyle, 1990) and a specific protocol suggested by Diversity Arrays Technology (available at https://www.diversityarrays.com/orderinstructions/plant-dna-extraction-protocol-for-dart/). The DNA concentration of each accession was measured using agarose gel (0.8%) and further confirmed with the help of NanoDrop (DS-11 FX, DeNovix Inc., Wilmington, DE, USA). A final concentration of 5 ng/µL for each sample was maitained and stored at –25 °C until the start of polymerase chain reaction (PCR).

2.2. iPBS-retrotransposon PCR amplifications

Primers used in this study were derived from a study by Kalendar et al. (2010). A total of 70 iPBS-retrotransposons primers were screened on randomly selected eight quinoa accessions. Among these 70 screened primers, the 11 most polymorphic primers were selected for PCR amplification of all 96 accessions quinoa accessions (Table 2). Conditions for iPBS-PCR amplification were adjusted by following the methodology described by Kalendar et al. (2010) with slight modifications. The PCR mixture consisted of 20 ng of template DNA, 1x DreamTaq PCR buffer, 2 mM $MgCl_2$, 0.2 mM each deoxyribonucleotide triphosphate (dNTP), 1 mM primer for 12–13-nt primers or 0.6 mM for 18-nt primers and 0.2 U Taq polymerase (Thermo Fisher Scientific Inc., Waltham, MA, USA) in a 20 µL reaction mixture. Amplification reactions in PCR were adjusted as denaturation at 95 °C for 3 min, subsequently followed by 30 denaturation cycles at 95°C for 15 sec, an annealing temperature of 50–65 °C depending on primers used for 1 min; and a final extension at 72 °C for 5 min (Kalendar et al., 2010). The amplified fragments were electrophoresed on agarose gel 2% (w/v) using 0.5x TBE buffer at a stable voltage of 120 V for 220 min. Gel staining was performed with ethidium bromide and graphics were taken by using a UV Imager Gel Doc XR+ system (Bio-Rad Laboratories, Inc., Hercules, CA, USA) light and photographed. A 100 bp+ DNA ladder was used as a molecular weight marker.

2.3. Data analysis

Only strong and clear bands were considered for scoring. iPBS-retrotransposon is a dominant marker system and scoring was performed as binary fashion; 0 or 1 representing the absence and presence of a band respectively, concerning 100 bp+ DNA ladder (Figures 1–3). Popgene ver. 1.32 (Yeh et al., 2000) was used for the estimation of various genetic diversity parameters like

Table 1. Passport data of world quinoa germplasm used in this study.

No	Accession name	Accession no	Donor	Country origin	Plant ID	Continent
1	USA-1	Ames 13719	USDA	United States, New Mexico	27 GR	North America
2	USA-2	Ames 13724	USDA	United States, New Mexico	18 GR	North America
3	USA-3	Ames 13727	USDA	United States, New Mexico	38TES	North America
4	USA-4	Ames 13730	USDA	United States, New Mexico	1ESP	North America
5	USA-5	Ames 13734	USDA	United States, New Mexico	47TES	North America
6	USA-6	Ames 13736	USDA	United States, New Mexico	30TES	North America
7	USA-7	Ames 13737	USDA	United States, New Mexico	2 WANT	North America
8	USA-8	Ames 13739	USDA	United States, New Mexico	29TES	North America
9	USA-9	Ames 13744	USDA	United States, New Mexico	409	North America
10	Bolivia-1	Ames 13747	USDA	Bolivia	APELAWA	South America
11	USA-10	Ames 13751	USDA	United States, New Mexico	21 GR	North America
12	USA-11	Ames 13754	USDA	United States, New Mexico	52ALC	North America
13	USA-12	Ames 13756	USDA	United States, New Mexico	3 UISE	North America
14	USA-13	Ames 13757	USDA	United States, New Mexico	53ALC	North America
15	USA-14	Ames 13758	USDA	United States, New Mexico	29TES	North America
16	USA-15	Ames 13759	USDA	United States, New Mexico	20ALC	North America
17	USA-16	Ames 13762	USDA	United States, New Mexico	47TES	North America
18	USA-17	NSL 86628	USDA	United States, Maryland	537 BK60-B	North America
19	USA-18	NSL 86649	USDA	United States, South Carol	PLANT VIRUS	North America
20	USA-19	NSL 92331	USDA	United States, Washington	JAPANESE STRAIN	North America
21	Bolivia-2	PI 470932	USDA	Bolivia	PasanRalle	South America
22	Mexico-1	PI 476820	USDA	Mexico	Santa Elena 7	North America
23	Bolivia-3	PI 478408	USDA	Bolivia, La Paz	R-64	South America
24	Bolivia-4	PI 478410	USDA	Bolivia, La Paz	R-66	South America
25	Bolivia-5	PI 478414	USDA	Bolivia, La Paz	R-70	South America
26	Bolivia-6	PI 478415	USDA	Bolivia, La Paz	R-71	South America
27	Bolivia-7	PI 478418	USDA	Bolivia, Potosi	R-132	South America
28	Peru-1	PI 510532	USDA	Peru	Quinoa de Quiaca.	South America
29	Peru-2	PI 510538	USDA	Peru	JaroJuira (Aymara), Quinoa Am	South America
30	Peru-3	PI 510539	USDA	Peru	(Span.)	South America
31	Peru-4	PI 510540	USDA	Peru	Grande (Span.).	South America
32	Peru-5	PI 510547	USDA	Peru	Ara Juira (Aymara), Quinoa Sil	South America
33	Peru-6	PI 510548	USDA	Peru	YulajQ'ang'olla (Quechua), Qu	South America
34	Peru-7	PI 510550	USDA	Peru	Q'ello Quinoa (Quechua), Quino	South America
35	Peru-8	PI 510551	USDA	Peru	Quinua (Quechua), Quinoa var.	South America
36	Chile-1	PI 584524	USDA	Chile	QQ056	South America
37	Argentina-1	PI 587173	USDA	Argentina, Jujuy	LP 128	South America
38	Peru-9	PI 596498	USDA	Peru, Cuzco	Rosa Junin	South America
39	Chile-2	PI 614880	USDA	Chile, Los Lagos	QQ065	South America
40	Argentina-2	PI 614881	USDA	Argentina, Jujuy	QQ95	South America
41	Chile-3	PI 614882	USDA	Chile, La Araucania	QQ67	South America

Table 1. (Continued).

42	Argentina-3	PI 614883	USDA	Argentina, Jujuy	QQ101	South America
43	Argentina-4	PI 614884	USDA	Argentina, Jujuy	QQ87	South America
44	Chile-5	PI 614885	USDA	Chile, Bio-Bio	QQ57	South America
45	Chile-6	PI 614886	USDA	Chile, Maule	QQ74	South America
46	Chile-7	PI 614887	USDA	Chile, Bio-Bio	QQ63	South America
47	Chile-8	PI 614889	USDA	Chile, Bio-Bio	QQ59	South America
48	Bolivia-8	PI 614901	USDA	Bolivia, Oruro	CQ 101	South America
49	Bolivia-9	PI 614903	USDA	Bolivia, Oruro	CQ 103	South America
50	Bolivia-10	PI 614905	USDA	Bolivia, Oruro	CQ 105	South America
51	Bolivia-11	PI 614906	USDA	Bolivia, Oruro	CQ 106	South America
52	Bolivia-12	PI 614907	USDA	Bolivia, Oruro	CQ 107	South America
53	Bolivia-13	PI 614908	USDA	Bolivia, Oruro	CQ 108	South America
54	Bolivia-14	PI 614911	USDA	Bolivia, Oruro	CQ 111	South America
55	Bolivia-15	PI 614912	USDA	Bolivia, Oruro	CQ 112	South America
56	Bolivia-16	PI 614913	USDA	Bolivia, Oruro	CQ 113	South America
57	Bolivia-17	PI 614914	USDA	Bolivia, Oruro	CQ 114	South America
58	Bolivia-18	PI 614915	USDA	Bolivia, Oruro	CQ 115	South America
59	Bolivia-19	PI 614917	USDA	Bolivia, Oruro	CQ 117	South America
60	Bolivia-20	PI 614918	USDA	Bolivia, Oruro	CQ 118	South America
61	Bolivia-21	PI 614919	USDA	Bolivia, Oruro	CQ 119	South America
62	Bolivia-22	PI 614920	USDA	Bolivia, Oruro	CQ 120	South America
63	Bolivia-23	PI 614921	USDA	Bolivia, La Paz	CQ 121	South America
64	Bolivia-24	PI 614922	USDA	Bolivia, La Paz	Sayana	South America
65	Bolivia-25	PI 614923	USDA	Bolivia, La Paz	Jamiri	South America
66	Bolivia-26	PI 614924	USDA	Bolivia, La Paz	CQ 124	South America
67	Bolivia-27	PI 614925	USDA	Bolivia, La Paz	CQ 125	South America
68	Bolivia-28	PI 614926	USDA	Bolivia, La Paz	CQ 126	South America
69	Bolivia-29	PI 614927	USDA	Bolivia, La Paz	CQ 127	South America
70	Bolivia-30	PI 614930	USDA	Bolivia, La Paz	CQ 130	South America
71	Bolivia-31	PI 614931	USDA	Bolivia, Oruro	CQ 131	South America
72	Bolivia-32	PI 614932	USDA	Bolivia, Oruro	CQ 132	South America
73	Bolivia-33	PI 614933	USDA	Bolivia, Oruro	CQ 133	South America
74	Bolivia-34	PI 614934	USDA	Bolivia, Oruro	CQ 134	South America
75	Bolivia-35	PI 614935	USDA	Bolivia, Oruro	CQ 135	South America
76	Bolivia-36	PI 614937	USDA	Bolivia, Oruro	CQ 138	South America
77	Bolivia-37	PI 614938	USDA	Bolivia, Oruro	CQ 139	South America
78	Chile-9	PI 634917	USDA	Chile	Pichilemu	South America
79	Chile-10	PI 634918	USDA	Chile	Baer	South America
80	Chile-11	PI 634919	USDA	Chile	Pichaman	South America
81	Chile-12	PI 634921	USDA	Chile	UDEC-2	South America
82	Chile-13	PI 634922	USDA	Chile	UDEC-4	South America
83	Chile-14	PI 634923	USDA	Chile	UDEC-1	South America
84	Chile-15	PI 634924	USDA	Chile	UDEC-5	South America

Table 1. (Continued).

85	Chile-16	PI 634925	USDA	Chile	UDEC-3	South America
86	Peru-10	PI 643079	USDA	Peru, Puno	Pasankalla	South America
87	Australia-1	PI 665272	USDA	Australia	Bianra de Juny	Australia
88	Bolivia-38	PI 665273	USDA	Bolivia, La Paz	Line 2-31	South America
89	Bolivia-39	PI 665274	USDA	Bolivia, La Paz	Line 0291	South America
90	Bolivia-40	PI 665275	USDA	Bolivia, La Paz	Line 0692	South America
91	Bolivia-41	PI 665276	USDA	Bolivia, La Paz	Line 1376	South America
92	Bolivia-42	PI 665277	USDA	Bolivia, La Paz	Line 1599	South America
93	Bolivia-43	PI 665278	USDA	Bolivia, La Paz	Line 1784	South America
94	USA-10	PI 665283	USDA	United States, Colorado	Col. #6197	North America
95	Bolivia-44	PI 674265	USDA	Bolivia, La Paz	Chucapaca	South America
96	Ecuador-1	PI 674266	USDA	Ecuador	DE-1	South America

Table 2. List of 11 iPBS-retrotransposon primers with their sequence and annealing temperature used to elucidate genetic diversity in world quinoa germplasm.

Primer name[1]	Sequence (5′→3′)	Annealing temperature (°C)
2074	GCTCTGATACCA	49.6
2080	CAGACGGCGCCA	63.3
2095	GCTCGGATACCA	53.7
2228	CATTGGCTCTTGATACCA	54.0
2230	TCTAGGCGTCTGATACCA	52.9
2249	AACCGACCTCTGATACCA	51.0
2253	TCGAGGCTCTAGATACCA	51.0
2272	GGCTCAGATGCCA	55.0
2277	GGCGATGATACCA	52.0
2390	GCAACAACCCCA	56.4
2251	GAACAGGCGATGATACCA	53.2

[1]Primers, their sequences, and annealing temperature were derived from Kalendar et al. (2010).

effective alleles number (Ne), Shannon's information index (I), and gene diversity (He). Polymorphism information content (PIC) was calculated for each primer according to criteria suggested by Baloch et al. (2015). Popgene ver. 1.32 was also used for the estimation of various genetic diversity indices among the collection country. Popgene ver. 1.32 was also used to calculate Nei's genetic distance. GenAlExV6.5 (Smouse and Peakall, 2012) was used for the estimation of analysis of molecular variance (AMOVA) and principal coordinate analysis (PCoA). To explore the relationship among 96 quinoa accessions, neighbor-joining analysis was performed using R statistical software (version 3.4.1, Vienna, Austria). The Bayesian clustering model was applied in STRUCTURE software (version 2.3.4, Stanford, CA, USA) to obtain a brief understanding of genetic structure of world quinoa germplasm. The most favorable number of clusters (K subpopulations) were determined according to the protocol of Evanno et al. (2005). A total of 10 independent runs were set for each K value, and each run, the initial burn-in period was set to 5000 with 100,000 MCMC (Markov chain Monte Carlo) iterations. We plotted the number of clusters (K) against logarithm probability relative to the standard deviation (ΔK). The final assignment of individual accessions was based on the magnitude of the membership coefficient being greater than or equal to 50% as suggested by Habyarimana et al. (2016).

3. Results

A total of 11 most polymorphic iPBS-retrotransposon primers were used to characterize the quinoa germplasm. These 11 primers yielded a total of 235 strong and clear bands, with an average of 21.4 bands per primer across 96 quinoa accessions. Out of these 235, 157 (66.8%) were found to be polymorphic, with an average of 14.3 bands per primer (Table 3). The highest (28) and lowest (11) number of scorable bands were observed for primers 2074 and 2272 respectively. The PIC value ranged from 0.663 (2228) to 0.170 (2390) with a mean value of 0.410. Mean polymorphism was 66.8%, which ranged 35–93% for primers 2390 and 2095, respectively. The highest (1.822) and lowest (1.068) number of effective alleles were observed for primers 2251 and 2390, respectively, with an average of 1.269. Maximum (0.451) and minimum (0.041) Shannon's information index was observed for primers 2251 and 2390 respectively, while mean Shannon's information index was 0.160. Maximum gene diversity (0.644) was recorded for primer 2251 while the lowest (0.068) level of gene diversity

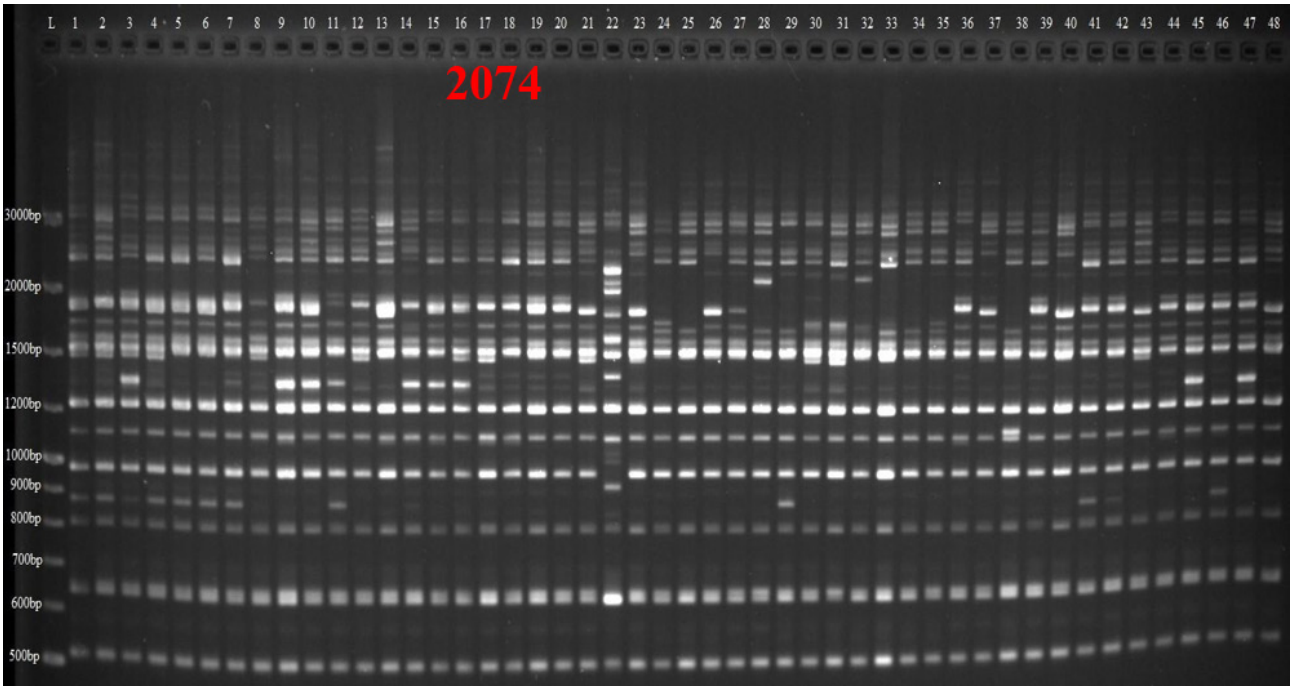

Figure 1. A gel based polymorphism representation in quinoa germplasm with 2074 iPBS-retrotransposon primer.

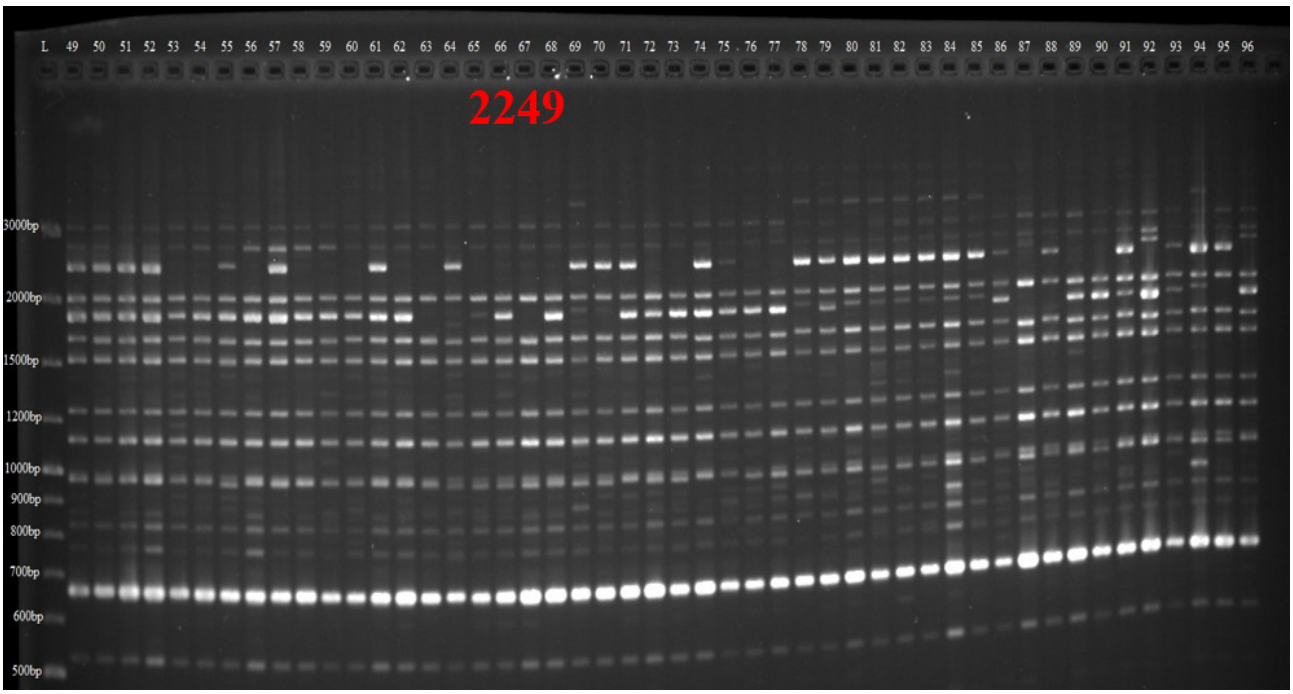

Figure 2. A gel based polymorphism representation in quinoa germplasm with 2249 iPBS-retrotransposon primer.

was observed for primer 2390; mean gene diversity was 0.247. Maximum Nei's genetic distance was 0.5844 present between Bolivia–17 and Mexico–1 accessions, while 0.004 was the minimum genetic distance present between the Chile–13 and Chile–14 accessions.

To explore genetic diversity comprehensively, various diversity indices were calculated at the country level (Table 4). Accessions from Bolivia showed a greater level of diversity by reflecting higher Ne (1.173), gene diversity (0.1053), and Shannon's information index (0.164).

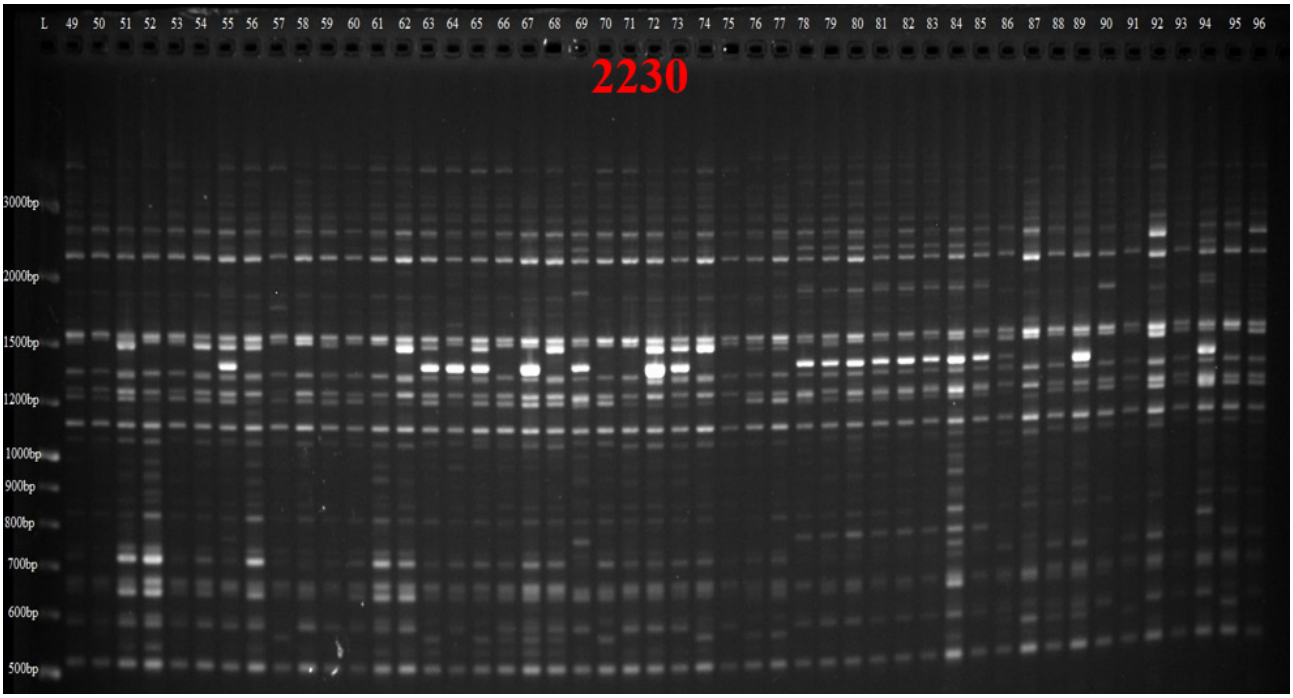

Figure 3. A gel based polymorphism representation in quinoa germplasm with 2230 iPBS-retrotransposon primer.

Table 3. Different diversity indices computed to explore genetic diversity in world quinoa germplasm with the iPBS-retrotransposon marker system.

Primers	Total bands	Polymorphic bands	Polymorphism (%)	PIC	Ne	I	He
2074	28	18	64	0.471	1.206	0.128	0.204
2080	26	22	85	0.520	1.165	0.109	0.188
2095	14	13	93	0.397	1.318	0.187	0.296
2228	22	19	86	0.663	1.272	0.175	0.284
2230	22	12	55	0.394	1.269	0.153	0.229
2249	22	12	55	0.386	1.117	0.075	0.126
2253	15	11	73	0.401	1.388	0.226	0.341
2272	11	5	45	0.257	1.110	0.065	0.107
2277	24	18	75	0.484	1.222	0.143	0.232
2390	26	9	35	0.170	1.068	0.041	0.068
2251	25	18	72	0.362	1.822	0.451	0.644
Mean	21.4	14.3	66.8	0.410	1.269	0.160	0.247
Total	235	157					

PIC: Polymorphism information content, Ne: Effective number of alleles, I: Shannon's information index, He: Gene diversity.

Accessions from Chile reflected minimum values for all investigated diversity indices. Genetic distance was also calculated at the country level, which revealed accessions from Bolivia and Chile contained maximum (0.113) and minimum (0.049) mean genetic distance, respectively.

The model-based structure algorithim divided studied germplasm into 2 populations; 58 accessions (60.4% of the total samples) in population A and, 38 accessions (39.6% of the total samples) in population B, mainly on the basis of collection country (Figure 4). Neighbor-joining

Table 4. Countries based diversity evaluation for world quinoa germplasm using iPBS-retrotransposon marker system.

Country	Ne	He	I	Mean GD
USA	1.113	0.0708	0.1127	0.07
Bolivia	1.173	0.1053	0.1641	0.113
Peru	1.1662	0.1024	0.1574	0.12
Chile	1.0802	0.0505	0.0806	0.049
Argentina	1.1345	0.0755	0.1094	0.11

Ne: Effective number of alleles, He: Gene diversity, I: Shannon's information index, Mean GD: Mean Nei's genetic distance.

clustering also grouped whole germplasm according to collection country (Figure 5). To strengthen our results, PCoA was also performed, which supported the clustering of structure and neighbor-joining analysis by dividing 96 quinoa accessions into 2 main clusters corresponding to the 2 populations (populations A and B), resulting in structure analysis (Figure 6). Analysis of variance (AMOVA) quantified the total variations into 2 strata: variation within populations (69%) and variation among populations (31%) (Table 5). Results of AMOVA were also confirmed by performing the AMOVA among the structure evaluated populations and revealed the existence of higher variation (67%) within populations.

4. Discussion

4.1. iPBS-retrotransposon as a source of polymorphism

During this study, the total number of bands (235) were found higher than reported by Hossein-Pour et al. (2019) using iPBS-retrotransposon markers, Ana-Cruz et al. (2017) using ISSR markers, and Romero et al. (2019) using SSR markers for quinoa germplasm. The average number of bands (21.4) found in this study were also higher than reported by recent studies (Salazar et al. 2019; Romero et al. 2019). Among the total 235 bands, 157 bands were found polymorphic and a number of polymorphic bands found in this study were higher than the reported by Romero et al. (2019). Mean polymorphism (66.8%) reported in this study was found greater than a previous study by Saad-Allah and Youssef (2018). They performed genetic characterization of 5 quinoa accessions and observed a total of 38.89% and 31.47% mean polymorphism with RAPD and ISSR markers, respectively. The PIC value is used to understand the efficiency of polymorphic loci for the identification of genetic diversity (Mir et al., 2012) and to explore the discriminating power of markers among genotypes (Nemli et al., 2015). During this study, mean PIC value was found to be 0.41 and varied 0.17–0.66. The mean PIC value obtained in this study was found higher than reported by earlier studies using various marker systems in quinoa germplasm (Zhang et al., 2017; Saad-Allah and Youssef, 2018; Hossein-Pour et al., 2019). Results presented herein explain the presence of a good level of polymorphism in evaluated quinoa germplasm and also confirm the universal nature of the iPBS-retrotransposon marker system, which can be used for the detection of polymorphism in any species.

4.2. Genetic diversity and population evaluation of world quinoa germplasm

Various diversity indices calculated revealed the existence of higher genetic diversity in world quinoa germplasm. Maximum number of effective alleles represents the

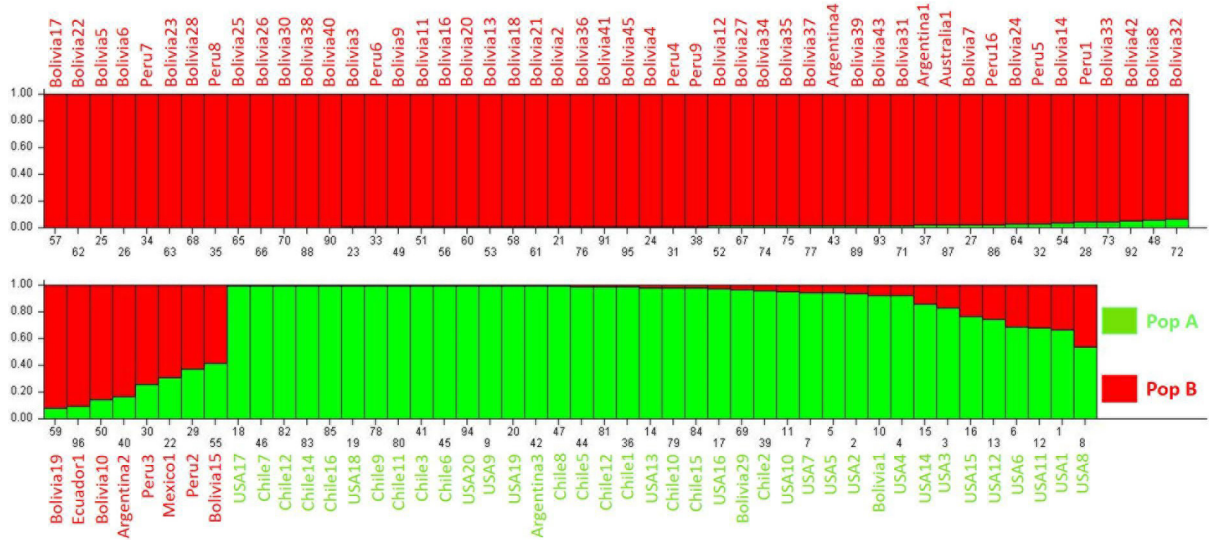

Figure 4. Population structure of world quinoa germplasm revealed by the iPBS-retrotransposon marker system.

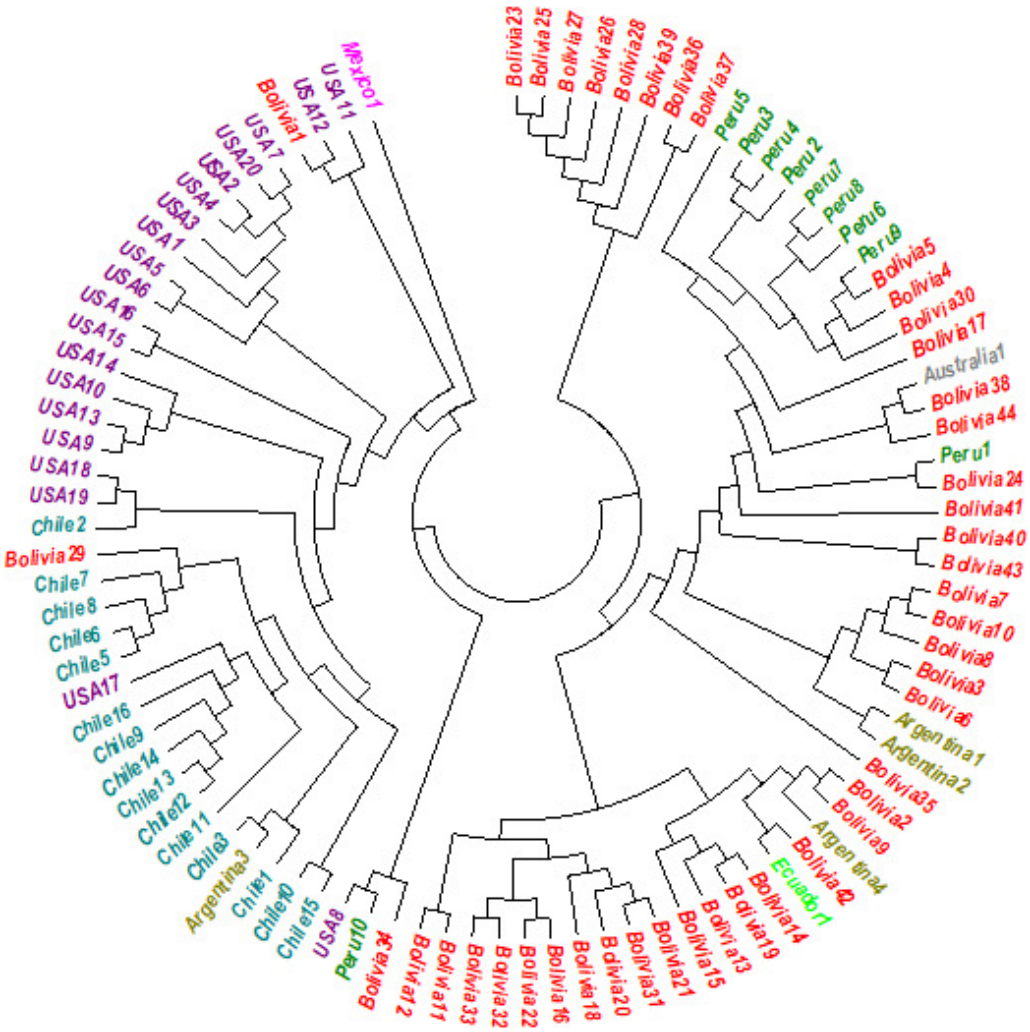

Figure 5. Neighbor-joining analysis of world quinoa germplasm revealed by the iPBS-retrotransposon marker system.

presence of a good level of genetic diversity andalso refers to the alleles having an ability to move into the next generation (Kimura, 1965; Romero et al., 2019). An effective number of alleles is considered an important indicator for the evaluation of markers having a great contribution to variations in a studied germplasm. Mean number of effective alleles (1.26) was found to be lower than Hossein-Pour et al. (2019) using the iPBS-retrotransposon marker system. This might be possible due to differences in studied germplasm. Shannon's information index is an important benchmark to assess variations in studied germplasm as it differentiates the genetic diversity in a population combining abundance and evenness (Yildiz et al., 2019). Mean Shannon's information index was 0.16, which was found lower than reported by earlier studies using different molecular markers for quinoa (Saad-Allah and Youssef, 2018; Hossein-Pour et al., 2019). Mean gene diversity was found lower than Hossein-Pour et al. (2019) using iPBS-retrotransposon marker system. The existence of lower values for these diversity indices may be due to the origin and number of studied germplasm. Mean genetic distance among the 96 quinoa accessions was 0.134. Maximum genetic (0.5844) distance was present between South and North American countries. Bolivia–17 and Mexico–1 were found genetically most distinct accessions reflecting maximum genetic distance. Chile–13 and Chile–14 accessions were found genetically similar because they accounted for minimum genetic distance. As Bolivia–17 and Mexico–1 were found genetically distinct, they can be suggested as candidate parents for the initiation of various breeding activities for quinoa.

Quinoa is a pseudocereal and versatile crop originating from the Andean Plateau, around Lake Titicaca, on the Peruvian–Bolivian border (Heiser and Nelson, 1974; Jacobsen, 2003). Genetic diversity of quinoa is associated with 5 ecotypes: Andean (Peru and Bolivia), inter-Andean

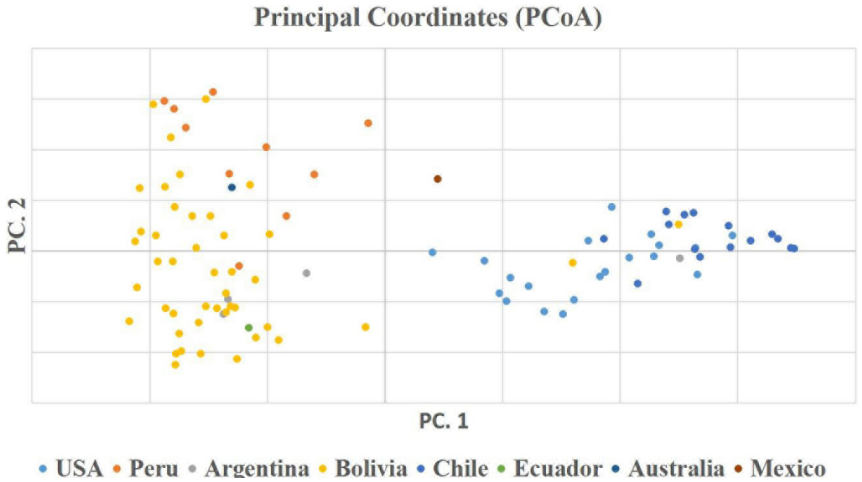

Figure 6. Principal coordinate analysis (PCoA) of world quinoa germplasm revealed by the iPBS-retrotransposon marker system.

Table 5. Analysis of molecular variance (AMOVA) revealing genetic diversity in world quinoa germplasm through the iPBS-retrotransposon marker system.

Summary AMOVA table[1]					
Source	df	SS	MS	Est. var.	%
Among Pops	7	361.837	90.459	4.958	31%
Within Pops	88	955.862	10.862	10.862	69%
Total	96	1317.699		15.820	100%
Summary AMOVA table[2]					
Source	df	SS	MS	Est. var.	%
Among Pops	1	284.600	284.600	5.934	33%
Within Pops	94	1141.077	12.139	12.139	67%
Total	95	1425.677		18.073	100%

[1]: AMOVA at countries basis, [2]: AMOVA at the structure algorithm evaluated populations.

valleys (Colombia, Ecuador, and Peru), Salare (Bolivia, Chile, and Argentina), Yunga (Bolivia), and Coastal (Chile) (Zhang et al., 2017). Results of this study revealed that Bolivian and Peruvian accessions were rich in genetic diversity, while accessions from Chile showed the presence of a narrow level of genetic diversity. These results were further strengthened by calculating the genetic distance at the country level. Maximum mean genetic distance was reflected by Peruvian accessions, while accessions from Chile reflected maximum similarity with each other because of very low genetic distance. Previous studies suggested Bolivia and Peru as a genetic diversity center and confirmed the existence of lower level of diversity in the accessions from other countries (Christensen et al., 2007; Salazar et al., 2019). The higher level of genetic diversity in Bolivian and Peruvian accessions provides an opportunity for leveraging its hardiness and further its wide adaptation. The lower level of diversity in the accessions of Chile might be due to founder effects linked to the distribution of this crop from its origin and diversity center. There is a possibility that human selection for various favorable traits also exerted great pressure on the decrease in genetic diversity of Chilean accessions.

The model-based structure algorithm has been found more informative and precise as compared to other clustering algorithms (Bouchet et al., 2012; Newell et al., 2013). Therefore, structure algorithm was taken as a clustering benchmark in this study. Structure algorithm

divided studied germplasm into 2 main populations: A and B on the basis of collection country (Figure 4). Bolivia, Peru, and Ecuador are origin, domestication, and main genetic diversity centers of quinoa. Accessions from these countries were clustered in population A and reflected their genetic similarity upon membership coefficient. Population B mainly comprised of accessions from the USA and Chile. It was interesting that accessions from Argentina were present in both populations and 2 accessions from Bolivia were also present in population B. Earlier studies confirmed that there are 2 main groups of quinoa in South America; the Andean highlands and Chilean coastal type. The Andean highlands are further divided into northern highlands and southern highlands. Chilean coastal type is comprised of Chile, the USA, and Mexico (Christensen et al., 2007; Maughan et al., 2012; Zhang et al., 2017). Clustering obtained in this study was found to be similar to that reported by Zhang et al. (2017) using InDel markers for quinoa germplasm. In their study, accessions from Peru and Bolivia were present in 1 population and accessions from Chile, Mexico, and the USA in another population, similar to what we have found in this study.

The neighbor-joining analysis mainly divided the studied germplasm according to collection country (Figure 5). Most of the accessions from Bolivia and Peru made their separate subcluster. Accessions from the USA and Chile were present in another cluster. Quinoa is associated with 5 ecotypes including Andean highlands (Peru and Bolivia), inter-Andean valleys (Colombia, Ecuador and Peru), Salares (salt lakes; Bolivia, Chile and Argentina), Yungas (Bolivia), and Coastal/Lowlands (Chile) (Bazile et al., 2016; Salazar et al., 2019). In this study, we also observed that Andean highlands ecotypes (accessions from Peru and Bolivia) were clustered together under the main group and subgroup. Our results also showed the existence of Yungas ecotypes because accessions from Bolivia also made their separate subcluster. In this study, there were a lesser number of accessions from Argentina; however, it can be observed that accessions from Argentina, Chile, and Bolivia were present under the same subcluster, ultimately revealing their genetic association with the Salares ecotype. Clustering of accessions in neighbor-joining analysis was found to be similar to structure algorithm clustering. Accessions from Bolivia and Peru were present in 1 group, while accessions from Chile and the USA were clustered in other clusters similar to structure algorithm.

Principal coordinate analysis (PCoA) also divided the studied germplasm according to collection country (Figure 6). Accessions from Peru, Ecuador, and Bolivia made their separate populations similar to structure analysis, and accessions from the rest of the countries made their separate populations. The small discrepancy between structure clustering and neighbor-joining analysis was also observed. Since these accessions showed full membership coefficients in the model-based structure, the discrepancy observed in neighbor-joining clustering can be explained by its reduced resolution power relative to the model-based structure (Bouchet et al., 2012; Newell et al., 2013).

Analysis of molecular variance (AMOVA) was assessed by considering within- and between-population components. The AMOVA results revealed that most of the variations (69%) are because of individuals within the populations. Among the quinoa populations, there was only 31% variation. Higher variations among the individuals within populations indicates the presence of higher levels of subdivision and hierarchy. These results strongly agreed with the study of Ana-Cruz et al. (2017), as they also find higher genetic variations with the quinoa population using ISSR markers. Moreover, for the confirmation of these results, AMOVA was also run within and among structure evaluated populations. The AMOVA revealed that maximum variations (67%) are due to individuals within populations. Therefore, it can be stated that the great potential of genetic variation is present within the population of studied germplasm, which can be employed for better breeding of this crop in the future.

As is obvious from the previously discussed evidence, the iPBS-retrotransposon marker system revealed the existence of great potential of genetic variations in world quinoa germplasm. Collection point was a key factor for the clustering of accessions under same cluster. Bolivia–17 and Mexico–1 were found to have genetically distinct accessions, and might be suggested as candidate parents for the future breeding activities. Peru and Bolivia reflected higher genetic diversity compared to rest of countries. Results of AMOVA revealed the existence of higher genetic diversity within populations.

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