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Development of DNA Fingerprints and Assessment of Genetic Diversity in 54 Chenopodium quinoa Germplasm Accessions using InDel Markers

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Research Article

Keywords: Quinoa, InDel markers, Genetic diversity, Cluster analysis, DNA fingerprinting

Posted Date: November 6th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11467348/v1

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Additional Declarations: No competing interests reported.

Abstract

To characterize genetic diversity, clarify the relationships between accessions, and build a molecular identification system for quinoa (*Chenopodium quinoa* Willd.), 54 accessions from different sources were phenotyped and genotyped with 45 polymorphic InDel primer pairs that gave clear and repeatable amplification. Ninety stable and scorable band loci were obtained. The mean effective number of alleles, Nei's gene diversity, Shannon's information index, and polymorphism information content (PIC) were 1.7496, 0.4179, 0.6053, and 0.3271, which indicates that the tested collection has moderate genetic diversity. Phenotypic clustering divided the accessions into three groups at a phenotypic distance of about 0.50, whereas molecular clustering separated them into five groups at a genetic similarity coefficient of about 0.57. The accessions from different geographical origins were interspersed in the clusters. The DNA fingerprint was generated by coding the 90 band loci in a fixed order; each accession had a unique code, so that all accessions tested could be distinguished. These results provide a molecular basis for accession identity verification, assessment of genetic relationships, screening for duplicate material, and the selection of parents for crossing.

Introduction

Quinoa (Chenopodium quinoa Willd.) is a member of the Amaranthaceae family. It is an allotetraploid species (2n = 4x = 36) originating in the Andes of South America (Chen and Liu 2022), and it tolerates cold, drought, poor soils, and salinity. Quinoa grain is a pseudocereal. It contains essential amino acids, vitamins and minerals, and has high nutritional value (Navruz-Varli and Sanlier 2016). With the growing global interest in health-oriented diets, quinoa cultivation and consumption have expanded rapidly. DNA fingerprints are stable and readily identifiable, can support cultivar identification and seed purity testing, and can also help protect plant variety rights (Xing et al. 2024).

DNA molecular markers are a powerful way to meet these needs. They are direct markers of genetic variation at the DNA level, and are widely used for molecular breeding, diversity analysis, relationship assessment and heterosis research (Wang 2024). Compared with morphological and cytological markers, they are more abundant and are not affected by environmental conditions (Agarwal et al. 2008). Common markers in plant genetics and breeding include simple sequence repeats (SSRs), also known as microsatellites, which remain useful for fingerprinting and seed-purity testing because of their high polymorphism, codominant inheritance, and established protocols (Cheng et al. 2008); single-nucleotide polymorphisms (SNPs), which are widely used due to their high density, genetic stability and compatibility with automated analysis (Gan et al. 2024); and insertion/deletion (InDel) markers, which have unique genomic features (Zhang et al. 2017).

InDel markers have considerable potential for germplasm identification and diversity studies. An InDel is an insertion or deletion of a nucleotide segment at the same genomic locus among individuals of a species or related species (Das et al. 2015). They have advantages such as broad genomic distribution and high density, with many loci discoverable from whole-genome resequencing data (Tian et al. 2026);

they allow reliable and reproducible genotyping using primers designed in conserved flanking sequences, and the mutation rate of InDel is relatively low (Feng et al. 2025); and they allow low-cost detection by conventional PCR followed by agarose or polyacrylamide gel electrophoresis, without the specialized equipment required for many SNP assays (Cheng et al. 2008; Wang et al. 2026). They can also be adapted to higher-throughput platforms such as KASP and fluorescent capillary electrophoresis (Zhu et al. 2025). They may be particularly useful in polyploid quinoa: some SSR loci produce four or more amplicons, which complicates genotype scoring, whereas InDel loci often have two allelic states, and the banding pattern is clear (Zhang et al. 2017).

InDel markers are used to evaluate crop genetic diversity, build DNA fingerprints, verify the identity of cultivars, and support marker-assisted selection (He et al. 2025; Zhou et al. 2025). This study used 45 InDel primer pairs to analyze 54 quinoa accessions from diverse sources. Specifically, we aimed to (1) evaluate the polymorphism and potential utility of this marker set in quinoa, (2) characterize the genetic relationships among the 54 accessions, and (3) build a DNA fingerprint to assist in the accurate identification, collection management, and use of quinoa germplasm.

Materials and methods

Plant materials

Fifty-four quinoa germplasm accessions were used in this study. Accession codes, names, and sources are listed in Table 1.

Table 1 Accession codes, names, and sources of the quinoa germplasm

Accession code	Accession name	Source	Accession code	Accession name	Source
LMSV-01	Yun 10	Yunnan, China	LMSV-29	X2-7	Tibet, China
LMSV-02	Yun 21	Yunnan, China	LMSV-30	X2-4	Tibet, China
LMSV-03	Yun 29	Yunnan, China	LMSV-31	X1-9	Tibet, China
LMSV-04	Yun 46	Yunnan, China	LMSV-32	X1-176	Tibet, China
LMSV-05	Yun 79	Yunnan, China	LMSV-33	JL-2014	Shanxi, China
LMSV-06	Qingli No. 1	Qinghai, China	LMSV-34	JL-2014-5	Shanxi, China
LMSV-07	Qingbaili No. 1	Qinghai, China	LMSV-35	GSSDQQ-2	Gansu, China
LMSV-08	Y2020-227	Qinghai, China	LMSV-36	HX-9	Qinghai, China
LMSV-09	HS-3	Qinghai, China	LMSV-37	HX-10	Qinghai, China
LMSV-10	HSHL-3	Qinghai, China	LMSV-38	WL-1	Qinghai, China
LMSV-11	XN-13	Qinghai, China	LMSV-39	WL-2	Qinghai, China
LMSV-12	Y2020-287	Qinghai, China	LMSV-40	HXZZ-1	Qinghai, China
LMSV-13	HLQH2014-4	Qinghai, China	LMSV-41	HXZZ-2	Qinghai, China
LMSV-14	HGA-6	Qinghai, China	LMSV-42	HXZZ-3	Qinghai, China
LMSV-15	YS-1	Qinghai, China	LMSV-43	HXZZ-4	Qinghai, China
LMSV-16	2-53	Tibet, China	LMSV-44	HXZZ-10	Qinghai, China
LMSV-17	X2-3	Tibet, China	LMSV-45	DX-2015-1	Qinghai, China
LMSV-18	2-53X1	Tibet, China	LMSV-46	GB-2015	Qinghai, China
LMSV-19	2-53X2	Tibet, China	LMSV-47	Y1-18	Argentina

LMSV-20	2-53-3	Tibet, China	LMSV-48	Y5-7	Argentina
LMSV-21	2-50X	Tibet, China	LMSV-49	Y5-24	Argentina
LMSV-22	X1-8	Tibet, China	LMSV-50	Y6-11	Argentina
LMSV-23	X1-1	Tibet, China	LMSV-51	SL51	Jiangsu, China
LMSV-24	1-25X	Tibet, China	LMSV-52	Zhongli No. 1	Sichuan, China
LMSV-26	1-227X	Tibet, China	LMSV-53	Zhongli No. 3	Sichuan, China
LMSV-27	1-178X	Tibet, China	LMSV-54	Zhongli No. 4	Sichuan, China
LMSV-28	2-53X	Tibet, China	LMSV-55	Limai No. 1	Sichuan, China

Note: LMSV-25 was not included in this study.

Phenotypic data recording

Quinoa was sown in April 2025 at the experimental station of the Qinghai Academy of Agricultural Sciences, at an elevation of about 2,309 m. The mean annual temperature at the site is about 6 °C and the mean annual precipitation about 384 mm. Phenotypic observations were taken at every growth stage. Following the quinoa guidelines for distinctness, uniformity, and stability (DUS) testing issued by the Ministry of Agriculture and Rural Affairs of the People's Republic of China (2025), 28 traits were recorded: growth habit and anthocyanin coloration of the young stem; leaf color, length, width, petiole length, waxiness, and margin incisions; color of the top leaf and flower buds; stem color, stem stripes and stripe color, and number of stem nodes; color, branching pattern, length, and width of the immature and mature panicle; saponin content; grain color; and thousand-grain weight. Representative phenotypic traits are presented in Table 2.

Table 2 Selected phenotypic traits of the 54 quinoa accessions

Accession code	Flowering time(day)	Leaf color	Panicle color (immature)	Plant height (cm)	Grain color	1000-grain weight (g)
LMSV-01	62	Medium green	Green	125	White	1.6
LMSV-02	66	Light green	Green	191	White	2.84
LMSV-03	68	Red	Purple	215	White	2.48
LMSV-04	68	Light green	Purple	203	White	2.88
LMSV-05	91	Medium green	Green	191	White	1.99
LMSV-06	68	Dark green	Purple	170	White	2.81
LMSV-07	59	Light green	Green	157	White	2.71
LMSV-08	68	Dark green	Green	130	White	1.87
LMSV-09	69	Medium green	Red	198	Yellow	2.53
LMSV-10	69	Medium green	Green	156	White	2.84
LMSV-11	80	Light green	Green	180	White	2.93
LMSV-12	80	Light green	Green	197	White	2.6
LMSV-13	73	Medium green	Green	185	Yellow	2.81
LMSV-14	75	Light green	Red	191	Yellow	2.65
LMSV-15	97	Dark green	Red	**215**	White	**0.41**
LMSV-16	65	Dark green	Variegated	181	White	2.5
LMSV-17	70	Light green	Pink	175	White	2.07
LMSV-18	68	Medium green	Green	157	White	2.86

LMSV-19	75	Medium green	Red	204	Yellow	2.68
LMSV-20	77	Medium green	Red	120	Yellow	2.17
LMSV-21	80	Medium green	Yellow	**194**	White	**3.05**
LMSV-22	77	Medium green	Yellow	**188**	Yellow	**2.79**
LMSV-23	70	Medium green	Yellow	201	Yellow	2.5
LMSV-24	74	Medium green	Green	167	White	2.73
LMSV-26	79	Light green	Green	185	Red	3.67
LMSV-27	75	Medium green	Green	181	White	2.33
LMSV-28	69	Light green	Red	178	White	3.02
LMSV-29	66	Medium green	Purple	167	White	2.81
LMSV-30	68	Medium green	Yellow	147	White	2.51
LMSV-31	69	Medium green	Red	154	White	2.91
LMSV-32	67	Medium green	Red	150	White	2.87
LMSV-33	68	Medium green	Green	170	White	2.76
LMSV-34	69	Medium green	Green	170	White	2.7
LMSV-35	72	Medium green	Yellow	163	Yellow	1.7
LMSV-36	64	Medium green	Purple	186	White	1.89
LMSV-37	69	Medium green	Purple	144	White	2.42
LMSV-38	62	Red	Purple	192	White	2.1
LMSV-39	63	Medium green	Red	141	White	2.36

LMSV-40	64	Light green	Green	158	White	2.29
LMSV-41	60	Medium green	Purple	151	Brown	3.29
LMSV-42	55	Light green	Purple	134	White	2.76
LMSV-43	57	Medium green	Red	183	White	3.18
LMSV-44	64	Medium green	Red	154	White	0.95
LMSV-45	79	Light green	Red	201	Yellow	2.59
LMSV-46	67	Medium green	Green	187	Yellow	2.31
LMSV-47	58	Light green	Purple	150	White	3.51
LMSV-48	92	Medium green	Purple	276	Yellow	2.59
LMSV-49	70	Light green	Purple	213	Yellow	2.14
LMSV-50	62	Red	Red	150	Black	3.28
LMSV-51	63	Medium green	White	144	White	1.42
LMSV-52	79	Medium green	Green	160	White	2.51
LMSV-53	72	Medium green	Green	**161**	White	**3.24**
LMSV-54	69	Medium green	Red	**120**	White	**2.47**
LMSV-55	79	Medium green	Red	**93**	White	**2.71**

DNA extraction

Leaves were collected at the seedling stage from five morphologically uniform plants, taken in equal amounts, and pooled. Total genomic DNA was extracted with a modified cetyltrimethylammonium bromide (CTAB) procedure (Jain et al. 2025). DNA concentration and quality were checked with a NanoDrop 2000 spectrophotometer and by 6% denaturing polyacrylamide gel electrophoresis. DNA was diluted to 80 ng μL^{-1} and kept at −20 °C until use.

Primer sources and PCR amplification

Primer sequences for the 45 InDel markers came from Jiang et al. (2024) and from markers developed earlier in our laboratory. Primers were synthesized by Shanghai Jierui Bioengineering Co., Ltd. (Shanghai, China), and their sequences are listed in Table 3. Each 10-μL reaction contained 0.2 μL Taq DNA polymerase, 1 μL PCR buffer, 0.8 μL dNTPs, 1 μL of each forward and reverse primer, 2 μL DNA template, and double-distilled water to a final volume of 10 μL. Amplification followed a thermal profile of 95 °C for 5 min; 35 cycles of 95 °C for 30 s, primer-specific annealing for 30 s, and 72 °C for 2 min; and a final step of 72 °C for 10 min and 4 °C for 10 min. Annealing temperatures were chosen according to the Tm of each primer pair. Amplicons were resolved by 6% denaturing polyacrylamide gel electrophoresis, visualized by silver staining, and documented with a gel imaging system (Bassam et al. 1991).

Data analysis

Bands were scored from each electrophoretic profile as “1” when a clear band was present at the target fragment position and as “0” when it was absent. The scores were assembled into a binary (0/1) matrix from which DNA fingerprints were derived. The number of alleles (Na), effective number of alleles (Ne), Nei’s gene diversity (H), Shannon’s information index (I), and polymorphism information content (PIC) were computed with POPGENE version 1.32 (Yeh et al. 1999). Molecular and phenotypic clustering, together with the dendrograms, were obtained with NTSYS-pc version 2.1 (Rohlf 2000). Fingerprints were then built from combinations of the polymorphic markers.

Results

Primer screening and validation

All InDel primers were first screened against six quinoa accessions chosen to represent geographically distant sources and contrasting morphology. Amplification products were examined by denaturing polyacrylamide gel electrophoresis. Primers that failed to reveal polymorphism were discarded, whereas those giving clear, reproducible bands were kept and the PCR conditions were adjusted accordingly. In the end, 45 InDel primer pairs that produced clear bands, showed polymorphism, and amplified stably were selected and used to genotype the 54 accessions.

Table 3
Primer sequences of the 45 InDel marker pairs

Primer pair	Forward primer (5'–3')	Reverse primer (5'–3')
Indel 1	AGATCGACAAGTATGAATTACTGC	GAAAAGGCTTTGTTGAGTTTCCCT
Indel 2	CTAAGGGGAGTGGAATTCCTATGG	TCCCATGAATTTCCACAAAATGGG
Indel 3	TGGTCTTATACCCACAAGGAAGTC	TGCCATTGCCATGTATTTCGTATC
Indel 4	GACATGGCTAAGTCTGCAAAAGAA	AGTTAGACAAAGGTAGTTTAAGCC
Indel 5	TGTTAGGATGGAACTATGAATGTGA	TAAAAGGTTCTCCACCAGATTTGA
Indel 6	GATTTTTGAATTCAGCATAACT	TCATAAATGAGAGTTGTACTTATCAGGT
Indel 7	CGAAAACTAGCTTAACAAAAGCTAAGG	GCAAATAATCGTGCTTCTTGATCT
Indel 11	GCACAACAGCTTGTTTACTGAGTT	AGTAAAGAAGATATACTTTGGCACC
Indel 15	TTTGTGATGTGATCATCGTTGTCC	TTCCATCGCTGATTAGCTGTTTTC
Indel 17	AAACCAGAAAGACGAAGGCATCAT	ATTTTGGGGATTCCTTTGGTTTGG
Indel 18	AGTCAATACTATGTTTTTACTTGT	ATTGACTCATTGAGCTTGAGCTTG
Indel 19	GGAATGGGAAAGCCAACATCTG	TTAGCAGATCGAGCAGAGTAGAAC
Indel 20	GAGAAGAGCCACCGGATATTTCT	TGAATCTTCTGGTGTAGCTGGAAA
Indel 21	AGTCTCGTCTAACTACAAGATAGGA	ACCCTCTAAAAGCTCTAGACATGC
Indel 23	CTAGCGTATAGTAGCCCCACTTG	CGAGTGCATGCCGAAAGCTA
Indel 24	ACAATGGAGGAGCTATAAAGCAAG	TTTCCATCTTCTAGTCTTCATCCT
Indel 26	TCCTATATTATCCAAATTTGAACCT	TGAATCAAACCACATACGCCTAAT
Indel 29	TTATTTCCCTCCAACCAAACACAC	TCTCAGCTTAAGGTGTTCTCATGC
Indel 32	TAAGAGGGAGGTACGGTTAGTGAT	AGCTTCAATCAGAAATTGAACAGGT
Indel 37	AATCGGGCAAATAAACGAACACAT	GTCGAGCATGAGAGCAAACTTATT
Indel 39	GGGGATACCCCACTTTAACTTTGA	GAAAGGCAAAAGATAGGAAGGTGG
Indel 40	AGAAGCGAAAGTTACTGGGAGATT	TCACCAAATTCCTCCAAAACTTCA
Indel 41	AGATGTATAGTTTTGGCCACCCTT	GCCTAGATGTACTAATCGTGGGAG
Indel 42	ATCTAATTTAAACTTGAATCATGC	CTTAAAAACTGACCTGACCCGAAC
Indel 44	TGGGAAAGTATGCACCTGAAGAAT	TCACCCGGTTCTGTTCAATAAGAT

Note: Primers designated “Indel” were developed previously in our laboratory; primers designated “JAAS” were obtained from Jiang et al. (2024).

Primer pair	Forward primer (5'–3')	Reverse primer (5'–3')
Indel 45	TCGGTAATAAAGGTGTGGTGATGT	ACGAAGTCCTCATTGATATCGTGT
Indel 48	TAGATCGAATTGTCAAGGATCGCA	ACGGATCACAGTTTTACCCTCAAT
Indel 50	TTTGGGAAAACGTCCAAAGTCTTG	TCCAACAGTTGCCCAAAACAAAAT
Indel 53	CTAGCCCACAGGCATCACAG	CACAAGCACCATCGCAACG
Indel 55	TCCTCGGCAAATATAACATTCTCA	AGGAGATCCCTTGTATCCTTATCTCT
Indel 58	AATGTAGATGTGGGATGAACTGCT	GTATGTTTGGGCTGTTAGGTTGAA
JAAS7	ATCCTGTGCTGACGCTGAATCC	AGATTTCGGGCTTCGAGTTGGG
JAAS18	CCTTCAAGGGAACTGGAAACTCC	CAGAACAAACTGTCCCACCTCAG
JAAS20	CGTCCAGCTCAATTACTTCCAAC	GCTCAATGCATCTAACAGAGGTG
JAAS23	CTGACGCTGAATCCTGAGACAAC	TTTGGGTAGATTTCGGGCTTCG
JAAS24	GAAACCTTCTCTCCCACCATAGC	GGAGAATCACCATCACACGAAAC
JAAS26	GACGAGCCATCAACTCAATTCAC	TGCAGTCTGCTGTGCTTTGATC
JAAS30	GCAGCTCCAAGAGAAACTACACC	TGCCAGGATTCAGAAAGGACGAC
JAAS40	GGAGTGGTGAATTCTCGAGTTGG	AGGAATTGAAGGTGTTGGCATTC
JAAS51	TAATGGCGGTGTTCGTGGTAATG	ACTAGCCTTTCCACCATGTAGC
JAAS52	TCAAGGCGTGCAGCAGTAGC	CCACCTAAGCTGTCGCAACAC
JAAS53	TCAGGAGACAGAAGGCAGGATAC	TTGGTGCTTAGTGTTGCTCTGAG
JAAS57	TCCAGGAGTGGACAAGATGATTG	TATCCACCGTAACCTCCTTAGGC
JAAS61	TCGCATCCACTTTGGGTATCCTC	AGTTGGACGAATGTTGTGTCTCC
JAAS62	TCGGAGAATCACTCGTGTCAAGC	TTGCGCGTGGTGAGCTATTATTG

Note: Primers designated “Indel” were developed previously in our laboratory; primers designated “JAAS” were obtained from Jiang et al. (2024).

Genetic diversity

All 45 selected InDel primer pairs were amplified and scored across the 54 quinoa accessions (Table 4). Each accession gave between zero and two amplification bands, and the number of alleles was 2.0000 for every one of the 45 markers. The mean effective number of alleles was 1.7496 (range 1.3717–1.9530), mean Nei’s gene diversity (H) was 0.4179 (range 0.2645–0.4879), mean Shannon’s information

index (I) was 0.6053 (range 0.4225–0.6810), and mean PIC was 0.3271 (range 0.2215–0.3688). These values indicate that the selected primers captured a moderate level of polymorphism.

Table 4
Genetic diversity parameters for the 45 InDel markers

Marker	Alleles (Na)	Effective alleles (Ne)	Nei's gene diversity (H)	Shannon index (I)	PIC
Indel 1	2.0000	1.7132	0.4008	0.5863	0.3158
Indel 2	2.0000	1.8749	0.4657	0.6583	0.3570
Indel 3	2.0000	1.7033	0.3948	0.5791	0.3114
Indel 4	2.0000	1.8459	0.4573	0.6496	0.3525
Indel 5	2.0000	1.6145	0.3735	0.5585	0.3015
Indel 6	2.0000	1.8522	0.4600	0.6526	0.3542
Indel 7	2.0000	1.8336	0.4506	0.6420	0.3480
Indel 11	2.0000	1.8102	0.4472	0.6394	0.3471
Indel 15	2.0000	1.8228	0.4497	0.6416	0.3481
Indel 17	2.0000	1.8556	0.4604	0.6528	0.3542
Indel 18	2.0000	1.5327	0.3357	0.5141	0.2754
Indel 19	2.0000	1.7726	0.4272	0.6165	0.3335
Indel 20	2.0000	1.7821	0.4319	0.6218	0.3367
Indel 21	2.0000	1.6649	0.3884	0.5739	0.3096
Indel 23	2.0000	1.9507	0.4871	0.6801	0.3684
Indel 24	2.0000	1.8280	0.4492	0.6406	0.3473
Indel 26	2.0000	1.8999	0.4728	0.6656	0.3608
Indel 29	2.0000	1.4726	0.3116	0.4869	0.2598
Indel 32	2.0000	1.8593	0.4592	0.6512	0.3530
Indel 37	2.0000	1.6826	0.4054	0.5952	0.3231
Indel 39	2.0000	1.5981	0.3738	0.5608	0.3037
Indel 40	2.0000	1.8453	0.4575	0.6500	0.3527
Indel 41	2.0000	1.8675	0.4619	0.6540	0.3545
Indel 42	2.0000	1.6127	0.3425	0.5127	0.2716
Indel 44	2.0000	1.6765	0.4033	0.5930	0.3219
Indel 45	2.0000	1.3717	0.2690	0.4390	0.2321

Marker	Alleles (Na)	Effective alleles (Ne)	Nei's gene diversity (H)	Shannon index (I)	PIC
Indel 48	2.0000	1.6913	0.4076	0.5973	0.3242
Indel 50	2.0000	1.6759	0.3805	0.5617	0.3011
Indel 53	2.0000	1.7384	0.4181	0.6072	0.3287
Indel 55	2.0000	1.4016	0.2645	0.4225	0.2215
Indel 58	2.0000	1.5440	0.3359	0.5127	0.2741
JAAS7	2.0000	1.8391	0.4562	0.6487	0.3521
JAAS18	2.0000	1.6458	0.3873	0.5743	0.3107
JAAS20	2.0000	1.6511	0.3660	0.5435	0.2901
JAAS23	2.0000	1.9210	0.4788	0.6716	0.3640
JAAS24	2.0000	1.9131	0.4771	0.6701	0.3633
JAAS26	2.0000	1.7761	0.4281	0.6173	0.3339
JAAS30	2.0000	1.8309	0.4524	0.6445	0.3497
JAAS40	2.0000	1.7470	0.4273	0.6185	0.3359
JAAS51	2.0000	1.7420	0.4142	0.6018	0.3250
JAAS52	2.0000	1.9383	0.4837	0.6767	0.3666
JAAS53	2.0000	1.9137	0.4769	0.6698	0.3631
JAAS57	2.0000	1.9530	0.4879	0.6810	0.3688
JAAS61	2.0000	1.8758	0.4657	0.6582	0.3569
JAAS62	2.0000	1.5882	0.3622	0.5457	0.2940
Mean	2.0000	1.7496	0.4179	0.6053	0.3271

Molecular clustering analysis

The 90 bands produced by the 45 primer pairs were used to cluster the 54 accessions (Fig. 1). Genetic similarity coefficients spanned 0.35 to 1.00, which points to substantial diversity among the accessions examined. At a similarity coefficient of about 0.57, the accessions fell into five groups. Group I was the largest and contained 43 accessions from diverse sources, among them 16 from Tibet, 16 from Qinghai, 4 from Sichuan, 3 from Yunnan, 2 from Shanxi, and 1 each from Gansu and Argentina. Group II comprised 7 accessions, 3 from Argentina, 3 from Qinghai, and 1 from Yunnan. Group III contained 2 accessions, 1 from Wulan (Qinghai) and 1 from Jiangsu. Group IV consisted of LMSV-03 alone (Yunnan), and Group V of LMSV-15 alone (Qinghai).

Phenotypic clustering analysis

Cluster analysis of the phenotypic traits is presented in Fig. 2. At a clustering distance of about 0.50, the accessions separated into three groups. Group I contained 52 accessions: 16 from Qinghai, 16 from Tibet, 5 from Yunnan, 4 from Sichuan, 3 from Argentina, 2 from Shanxi, 2 from Golmud, 2 from Ulan, 1 from Gansu, and 1 from Jiangsu. Group II consisted of a single accession, LMSV-50 from Argentina, and Group III of a single accession, LMSV-15 from Qinghai.

Construction of DNA fingerprints

DNA fingerprints based on the InDel markers were constructed for the 54 quinoa accessions from the 45 primer pairs and the 90 band loci they produced (Fig. 3). Every fingerprint consisted of an ordered series of “0” and “1” scores, in which “1” denoted a clear amplification band at a given locus and “0” the absence of a band (Gou et al. 2025). Pairwise comparison of the fingerprint codes identified no identical profiles, so the combined banding patterns of the 45 InDel primer pairs separated all accessions tested. The generated fingerprints can help with accession identity verification, duplicate screening, cultivar identity testing, and standard germplasm management.

Black boxes indicate the presence of an amplification band and white boxes indicate its absence. Rows are accession codes (LMSV-01 to LMSV-55); columns 1–90 represent the 90 DNA band loci generated by the 45 primer pairs.

Discussion

Polymorphism and potential utility of InDel markers

The InDel markers in this study showed moderate polymorphism in the collection, with a mean PIC of 0.3271. About one-third of the markers had PIC values greater than 0.35, which indicates that discriminatory power is relatively high. A similar pattern was reported for cauliflower by (Wang et al.2026). The mean PIC values reported for quinoa SSR studies were 0.366 for 16 primer pairs tested in 41 accessions (Lu et al. 2015) and 0.524 for 65 SSR markers tested in 166 accessions (Sun et al. 2021), both higher than the value observed here. These differences may reflect marker type, sample size, and genetic background. Markers with high polymorphism may be prioritized when assembling a core marker set for quinoa. Some markers had relatively low PIC values, potentially because InDel markers are typically biallelic and because of the genetic backgrounds and composition of the sample, as well as long-term artificial selection (Doebley et al. 2006). Future work should prioritize markers with high PIC values and clear bands to develop a standardized core primer set applicable to a broad range of germplasm.

Relationships between phenotypic traits and molecular variation

Based on phenotypic characteristics and InDel markers, the 54 quinoa accessions differed at both the phenotypic and the molecular level. Phenotypic clustering separated three groups at a distance of about 0.50, whereas molecular clustering separated five groups at a genetic similarity coefficient of about 0.57. Most accessions were concentrated in the main group, and a few were clearly distinct. Accessions from different geographical origins were interspersed among groups, which was consistent with the frequent exchange of quinoa germplasm among regions of China. The two methods singled out different accessions as the most divergent: LMSV-50 and LMSV-15 were the most distinct phenotypically, whereas LMSV-03 and LMSV-15 showed the most isolated molecular relationships, which indicates partial concordance between phenotypic variation and DNA-level variation, although differences were found for some accessions. Phenotypic traits are affected by genetic background and environment, whereas InDel markers mainly reflect genomic variation; the two approaches capture complementary aspects of germplasm diversity. Combining phenotypic assessment with molecular marker analysis can more comprehensively evaluate genetic differences and also inform accession identification, the selection of valuable material, and the choice of breeding parents.

Application of the DNA fingerprints

The 90 band loci of 45 InDel primer pairs were coded in a fixed order, and DNA fingerprints were constructed for 54 quinoa accessions. Pairwise comparison showed no identical codes, which indicates that the combined profiles distinguish all accessions. These fingerprints are a digital resource for germplasm collection management and can help identify accession mix-ups or losses. They can also assist identity verification and the authentication of cultivars, as well as provide molecular information for germplasm databases and plant variety protection. Molecular markers can complement the evaluation of DUS, but they need to be combined with morphological characteristics and field tests (Xu et al. 2022).

Conclusions

45 polymorphic InDel primer pairs were used to evaluate the genetic diversity of quinoa and to construct DNA fingerprints for 54 quinoa germplasm accessions. The 45 primer pairs produced 90 stable, scorable band loci. The mean effective number of alleles, Nei’s gene diversity, Shannon’s information index and PIC were 1.7496, 0.4179, 0.6053, and 0.3271, respectively, which indicated that the tested collection had moderate genetic diversity. Some markers with high polymorphism were suitable for selection in core identification sets. The genetic similarity coefficients of the accessions ranged from 0.35 to 1.00, and the accessions were divided into five molecular groups at a coefficient of approximately 0.57. Phenotypic clustering resolved three groups at a distance of approximately 0.50. Accessions from different geographical origins occurred in different groups, which indicated that genetic relationships and phenotypic differences were not fully consistent with geographic origin. Coding the 90 band loci produced unique DNA fingerprints for all 54 accessions. These findings provide a molecular basis for the evaluation of quinoa germplasm, genetic improvement and utilization.

Declarations

Acknowledgements The authors thank the staff of the experimental station of the Qinghai Academy of Agricultural Sciences for their assistance with field management and phenotypic data collection.

Author Contributions Xiaoling Guo: Conceptualization, methodology, data analysis, and writing—original draft. Yang Liu: Investigation, data curation, and writing—review and editing. Yu Pang: Investigation and writing—review and editing. Cuiping Chen: Supervision, funding acquisition, and writing—review and editing. All authors read and approved the final manuscript.

Funding This work was supported by the project “DUS Testing for Plant Variety Protection Applications.”

Data availability The datasets generated or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations This work does not contain any studies involving human and animal subjects.

Conflict of interest The authors of this work declare that they have no conflicts of interest.

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Figures

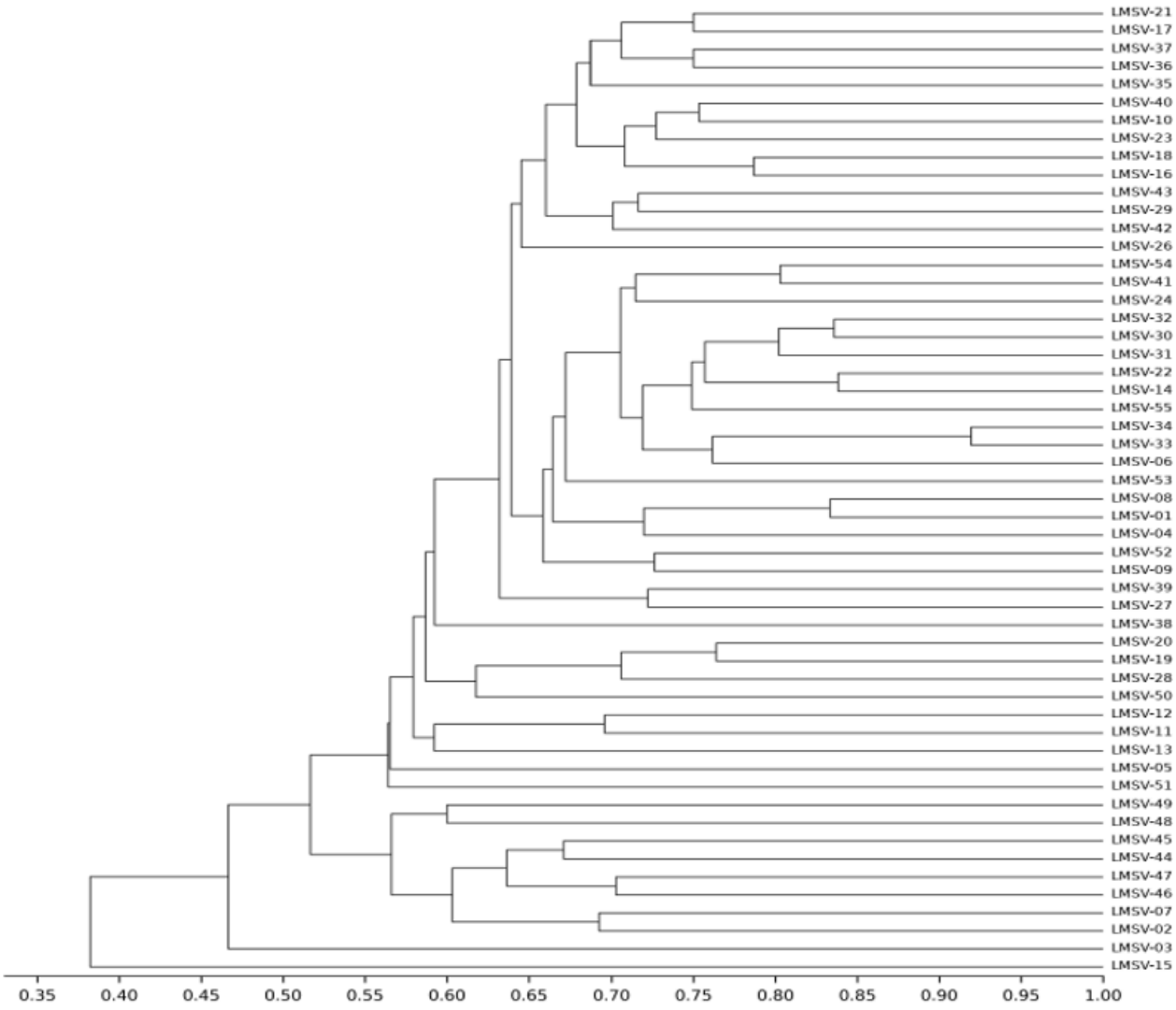


Figure 1

Dendrogram of 54 quinoa accessions based on 45 InDel marker pairs

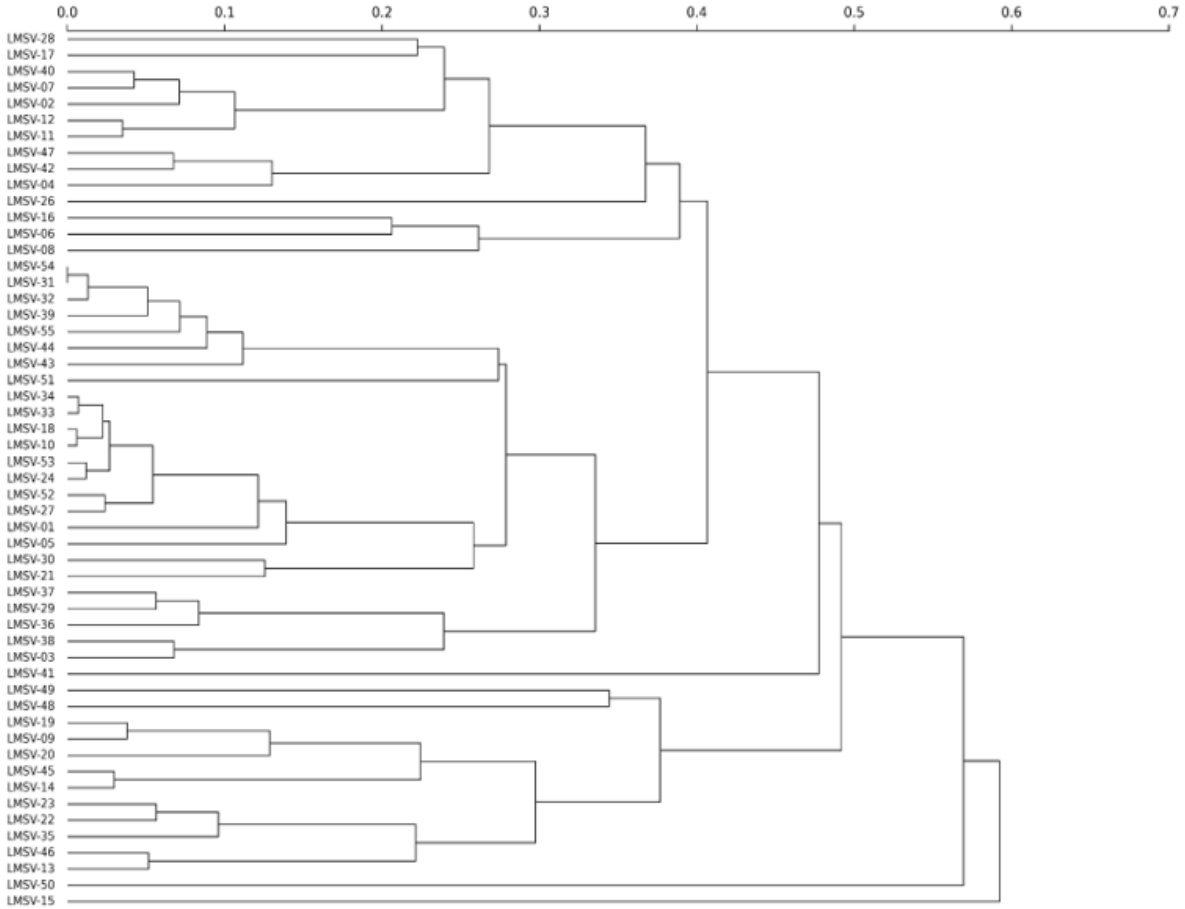


Figure 2

Dendrogram of 54 quinoa accessions based on phenotypic traits



Figure 3

DNA fingerprints of 54 quinoa accessions based on InDel markers

Black boxes indicate the presence of an amplification band and white boxes indicate its absence. Rows are accession codes (LMSV-01 to LMSV-55); columns 1–90 represent the 90 DNA band loci generated by the 45 primer pairs.