Construction of Molecular Identification Card on basis of fluorescence SSR utilizing Proso millet Core Germplasm (Panicum miliaceum L.) origin from Qinghai

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Research Article

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Abstract

Proso millet (*Panicum miliaceum* L.) is one the oldest crops from the Chinese and the Indus Civilization, but presently it has become under-farmed and under-breeding. However, dearth of genomic resources has hindered in effective utilization of proso millet germplasm, therefore the development of Molecular Identification Card for millet is highly needed, when harnessing germplasm resources. The Molecular ID-based on QR-Code is a systematic approach which provides comprehensively details. So, the aim of this study is to construct molecular identification card barcode based on SSRs markers using 149 proso millet germplasm from Qinghai. The genetic diversity parameters; the number of allele (Na) ranged from 3 to 4, the number of effective allele (Ne), 2.0268 to 2.6911, Shannon's information index (I) 0.7287 to 1.0444, expected homozygous (Ho) 0.3694 to 0.4917, expected heterozygous (He) 0.5083 to 0.6306, Nei's genetic diversity index (Nei) 0.5066 to 0.6284, polymorphism information content (PIC) 0.9314 to 0.9616 and major allele frequency (MAF) 0.0872 to 0.1879 values respectively. Duo (RYW18 and RYW37) reported highest number of alleles (Na 4) as compared to others. Phylogeny analysis provided into three populations levels (P1 with millet genotypes 130), (P2 with genotypes 12) and (P3 genotypes 7) is consistent with PC values; PC1, PC2 and PC3; 60.3%, 12.31%, and 7.57% respectively. Moreover, population structure analysis provided millet genotypic distribution into sub-levels, further (ΔK Avanno principled) highest K=3 confirmed this study is effective and reliability. The findings would provide comprehensively solid foundation for molecular characterization and genotypic variability as non-drought and globally food diversification.

Keywords: Proso millet, Molecular ID, SSR, Phylogeny analysis, Germplasm, Ancient crops and Population structure

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1. Introduction

Proso millet (*Panicum miliaceum* L.) is one the oldest minor-seeded cereal crops and edibility processes dating back earlier-Neolithic Age from the Indus Civilization and also in the Chinese Civilization about 8000 BC years in an ancient times (Kheya et al., 2023, Weber & Kashyap, 2016, Khound et al., 2024). Millet belongs to a Gramineae (Poaceae family) with 36 number of chromosomes [4] less water needed crops and non-fertilizers and following with self-pollination which can grow in short period (60-90 days) (Rajput et al., 2016).

Furthermore, the millets can play many divergent functions in crops rotations and agriculture sustainable and it is farmed in semi-arid and arid regions of Asia (East Asia, West and South), Europe and America, (Wang et al., 2016; Habiyaremye et al., 2017) and in China: the regions include (Inner Mangolia, Northwest China, Central China, Tibet Plateau), followed this, duo China and India are fore-front globally producers of the millet (IYM FAO, 2023, R. Wang et al., 2016). Conversely, the significant of millets in Chinese agriculture has waived over time during Ming Dynasty, followed with introducing new crops such as maize and rice accounting for 70 % productions [6].

And millet is consisted of 10 genera and 14 species namely major millet: pearl millet (Pennisetum glaucum), finger millet (Eleusine coracana), proso millet (Panicum miliaceum) and foxtail millet (Setaria italica) and minor millet: fonio millet (Digitaria exilis), kodo millet (Paspalum scrobiculatum) and tef millet (Eragrostis tef) [9]. On the other hand, rapidly desertification and droughts have caused famine conditions, there is scarcity of food in the world particularly developing countries (Dharmoon Bhawani 2022). Therefore the millet offers this viable and dynamic pathways mechanism of cropping due to drought tolerant and stress resistant (IYM FAO, 2023). Millets productions are globally ranking sixth after rice, wheat maize, barely, sorghum (https://oar.icrisat.org/12478/).

Millet being an orphan crop however, it is historically and culturally significant rich in nutrients such as low glycemic hindering diabetes [11], and phenolic compound functions in better digestibility mechanism [12]. In addition, millets are more nutritious than other crops rice and wheat, Fe, Ca, P and Zn that are helpful for health in bones teeth and can be used for therapeutics (Maharjan & Manandhar, 2023: Jacob et al., 2024) and gleutin free viable for celiac illness (Bhatt et al., 2022) and full with amino acids Lys, Met, Cys, and proteins, fats, ash, crude-fiber thymine plus ribloflavin essence for addressing nutritional security [9]. Millet involves in carbon sequestration due to C4 and also acting cropping rotation, making an ideal farming in tropical countries to ensuring biodiversity conservation and better for cropping (Kumari et al.,2024, Upadhyaya, Yadav, et al., 2011).

The ICRISAT Gene Bank has conserved worldly total 120,454 accessions of divergent millets from 144 countries, among them, proso millets-pertaining accessions are almost 849 found in commonly 30 countries (Upahyaya et al., 2009). However, there is inconsistent details whether how many accessions-based on proso millets have been effectively utilized globally. Therefore, exotic genotypic harnessing is highly required for ensuring crop diversification and food security. So, molecular study is dynamic and vibrant approaches [19]. Furthermore, the molecular characterization refers to preservation of genetic diversity is the convenient use of germplasm resources, in-situ conservation and crops breeding [20].

Genotypic harnessing is effectively for breeding, and stable ways in plant germplasm preservation and maintenance (Guo et al., 2022, Li et al., 2016). However, the core germplasms are reported on divergent crops (Yang et al., 2023, Upadhyaya, Ravishankar, et al., 2011, Holbrook & Dong, 2005, Ayour et al., 2025, Ambati et al., 2020, Zhang et al., 2021, Chen, 2025, Mei et al., 2024). Previously several studies have reported on molecular studies using different germplasm approaches (Zhao et al., 2025, Sai Timmarao et al., 2025, X.-H. Chen et al., 2022).

And however, there is non-sufficient study present on proso millet-based on molecular approaches. So, simple sequence repeat (SSR) markers are powerful that could be used in genetic database, molecular breeding, and phylogeny study that act major roles in crops breeding (Kumar, 2024, Hu et al., 2009), and it is microsatellites or short tandem repeats (STRs) effective for crops breeding. The SSR markers-based

on previously studies have demonstrated tangibly effectiveness owing to strong stability and reliability utilized divergent crops finger millet (Eleusine coracana or Geartn) [35], yellow-horn (Xanthoceras sorbifolium) [36], pomegranate (Punica granatum) [37], litchi (Litchi chinensis Sonn.) [38] and Adzuki bean (Vigna angularis) [39]. Based on above-stated details, SSR markers are selected for this study.

However, there is no-sufficient report based on SSR markers for Molecular Identification Card using 149 proso millet core germplasm from Qingahi Province. So, the aim of this study was to (i) construct Molecular ID as barcode method (ii) genetic diversity parameters analysis (iii) Phylogeny analysis, PCA, and population structure analysis to enhance crops breeding effectively genotypic preservation and molecular conservation.

2. Materials and methods

2.1 Materials and plants growth

In this study, we selected proso millet germplasm from Qinghai Province, China, the total number of proso millet accessions 149 were provided by the Institute of Crop Science, Chinese of Academy of Agricultural Sciences for research. The samplings distributions were from different various localities or areas of the Qinghai included: numbers of 51 germplasm from Minhe, 8 germplasm Hualong, 6 germplasm Jianzha, 17 germplasm Ledu, 14 germplasm Huangzhong, 8 germplasm Xiningshi, 3 germplasm Gonghe, 8 germplams Huzhu, 11 germplasm Xunhua, 1 germplasm Guide, 1 germplasm Guinan, 1 germplasm Mindou, 13 germplasm Pingan and 2 germplasm mainly center of Qinghai and also 5 germplasm collectively Qinghai-Xining respectively (Supplementary Table 1).

The plants were farmed three to four seeds per pod under control of green-house with temperature 25.0°C, humidity 37% and soil sandy-loamy at College of Agronomy, Shanxi Agricultural University Taigu, Shanxi province PRC, China (Co-ordinates: “E112° 34, 26.66, N37° 46, 37.16”). Comprehensively proso millet germplasm with details information is provided in (S Table 1). Our experiments were carried out under the local and national regulations followed given guidelines by the Shanxi Agricultural University PRC, China and University of Nebraska Lincoln, the USA. The well grown seedlings around 20 days were harvested and preserved at -80 °C for carrying out more procedures. We grinded leaves applying mortar and pestle with addition of liquid nitrogen converting into fine powder followed with plant genomic DNA isolation was carried out using modified CTAB protocol [40]. Each accession containing leaves took 4 to 6 from seedlings (~2g). The measurement of DNA integrity and purity were conducted in accordance with Nano drop method [41] and assessed its quantity using spectrophotometer (BioDrop, Cambridge). The concentrations of DNA were set 30-60 ng μL and kept at -20°C for moreover use. And 0.8 % agarose gel electrophoresis was conducted when adding 160ml of TBE buffer at 110 voltage and 40 m and finally the gels were visualized through Gel-doc to assess optimized clear bands (Image Lab Software).

2.2 Primer selecting and followed with PCR amplification

149 accessions of proso millet from different geographically collection sites of Qinghai Province (S Table 1) were used for screening of total 10 simple sequence repeat (SSR) markers (Table 2). Randomly samples were selected to screen primers effectively and viably at starting. And finally, the primers were chosen for further procedures. And the SSRs were developed by our Millet Molecular Breeding Lab of

Shanxi Agricultural University, China (*Wang Ruiyun et al.,* 2017). These SSRs markers as developed were assigned fluorescent labeling at the 5 end of each primer was provided by Sangon Biotech with given tutelages (Shanghai Co,. LTD). For DNA amplification, PCR was carried out, by following the procedure: DNA added 20-50µL and 10µL PCR-mixture containing 10µL 2x Taq Master Mix (Dye), 8µL double distilled water (DD water), 0.5µL amplified DNA and 0.8 µL forward and reverse primer each making each sample 20 µL. The PCR initiated with earlier de-naturation at 94 °C for 5 min, 35 cycles for 30 sec for 94 °C for de-naturation, a operation time of 30 s conducted with annealing temperature based on divergent primers and extension within 72 °C for 35 s, the period needed was for extension was assessed as per the length of amplification outcomes. After PCR, further steps taken to perform while diluting 4 µL DNA Ladder (50bp) and 6 µL amplified DNA in the gel Poly-acrimide 33% Gel Electrophoresis (PAGE) was conducted and results obtained were taken in gel form stated modified protocol [33].

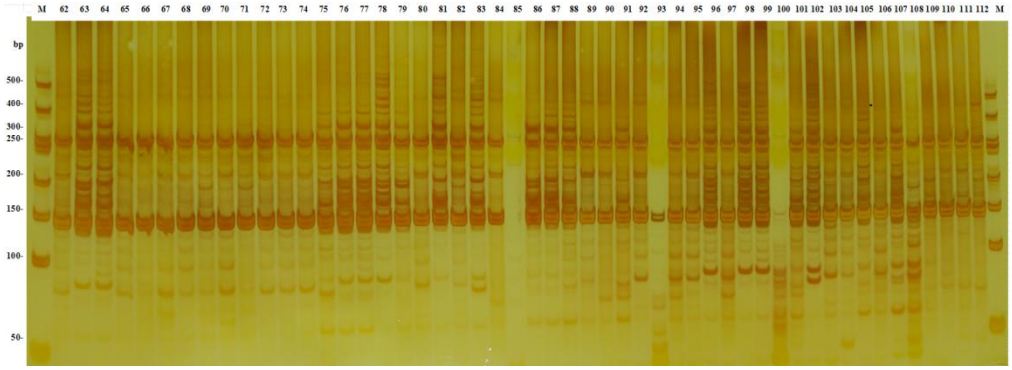

Fig. 1 Polyacrimide electrophoresis diagram of the amplified parts of the RYW12 primer pair material

2.3 Genetic Diversity Analysis

The data in gel form containing (0 and 1) were collected and standardized, here zero indicated absence bands (recessive) and one indicated to present (dominant) from 33% Poly Acrimide Gel Electrophoresis (PAGE). Each base pair (bp) was assigned 50 (bp) to 500 (bp). The genotypic allelic data were converted into binary matrix format before use. After standardizing the genotypic proso millet data, we used for computing genetic diversity parameters analysis using Genetic Population Analysis software (Popgen version 32) (https://sites.ualberta.ca/~fyeh/popgene_download.html). The dynamic M-strategy was used to target and select diverse set of allele for genotypic maximization based on genetic distance method [20] and as results; loci were formed according to the matrix data, the number of allele (Na), number of effective allele (Ne), Shannon information index (I), expected homozygous (Ho) expected heterozygous (He) and Nei's genetic diversity index (Nei). And polymorphism information content (PIC) values and major allele frequency (MAF) were computed using PowerMarker (version 3.25).

2.4 UPGMA analysis, PCA and Population structure analysis

We utilized to select 30% samplings as highly polymorphism and clear bands from entire simple sequence repeat (SSR) to construct un-weighted pair group method on basis of genetic distance via PowerMarker (version 3.25). The UPGMA tree was standardized with using MEGA (version 7) to set in newick format (tree branches length and bootstrap information) for uploading on Interactive Tree of Life (iTOL) (https://itol.embl.de/) for beautifying tree and nodes were distributed in accordance with geographically origins (S Table 1). For principle component analysis (PCA), we computed using similarity coefficient (DICE1945) in NTSYSpc (2.11) and generated 3-D plot and PC values were determined using (SPSS latest version).

Conducting population structure analysis of proso millet 149 germplasm from Qinghai (S table 1), we used grouping based model approach (Bayesian method ΔK) clustering for population analysis. Consequently, it was analyzed into three main populations or groups using STRCUTURE (version 2.3.1). Sub-population structure analysis provided equal millet genotypic distribution. In addition, we determined these population structure analysis had further sub-groupings into 5 and 8 population levels. We employed ad-mixture based model with independent allele frequencies to assess potential groups (K values) ranging from 1 to 10. For optimized assessing sub-populations levels, other indices were at peak levels including 20,000 burns in length and followed with Monte Carlo Markov Chain (MCMC) K running 5 times (Pritchard and Donnelly, 2001). Finally, we used Structure-selector an online tool to determine optimum K values based on ΔK of Avanno approach (https://lmme.ac.cn/StructureSelector/).

2.5 Construction of Molecular Identity Card

For construction molecular identification card of proso millet 149 accessions from divergent areas and localities of Qinghai (S Table 1), we selected Unicode, assigning scientific name with local names and genotypic data were standardized before using to develop Molecular Identification Card based on QR Code or barcode (https://cli.im/). The development of Molecular ID was assessed with other approaches genetic diversity parameters analysis, NJ tree, PCA analysis and Population structure.

3. Results

3.1 Genetic diversity indices

For statistical analysis, the genetic diversity indices of proso millet genotypes 149 from Qinghai (S Table 1), it is already stated that two primers failed to produce viable bands (RW3 and RYW28) both were replaced with other new duo primers (RWY12 and RYW18) (Table 1).

Table 1 Information of 10 fluorescently labeled SSR primers

Primer name	F-Sequence (3-5)	R-Sequence (5-3)	5' End modification	Temprature (°C)
RYW6	AGCCGATTTGCTGTGGAGT	CTGCCTCCGATGAGTTGGT	5`6-FAM	57.4
RYW8	GGGTCAGAGAATACACAGCG	GTAGGGAAGGAGAAGTGGGT	5`6-FAM	55.9
RYW12	ACCATCCCAGCACAAACCA	TGCCTGAAGGAGAAGAGCG	5`6-HEX	57.45
RYW18	CTCCCTCTTTGTCCTCGTT	GCTGCCTCTTCGCTATCTT	5`6-FAM	54.45

RYW37	CATTCCGTTCCTTGTCTTCC	CAGTCTCACTCCTGCGATGT	5`6-FAM	55.3
RYW40	TGCTCTTCGGCTCTTCTCC	ATCAGCTCATCGTGACCCC	5`6-FAM	57.35
RYW42	AGACACCCTGGGCAACATC	CTGGACTGGGCTTCGTTCT	5`HEX	57.8
RYW43	GGAGATGCTTGCTTGGTTG	CAGGAATCGCAAGGAACAG	5`HEX	54
RYW125	TTGACGACGACTGTGTGC	TGTTGGTGGAGTTGAGGAC	5`6-FAM	55.1
RYW146	TGATGCTTCTTGGGTTCG	CGCCGTCCACTTCTGTAT	5`6-FAM	53.6

The genetic diversity parameters analysis (Table 2) results provided comprehensively millet genotypic information, the number of allele (Na) calculated range from mostly 3 to 4 with total 32 allele and mean 3.2 with standard deviation 0.4216, the number of effective allele (Ne) range from 2.0268 to 2.6911 with mean values 2.2499 and standard deviation 0.2589, Shannon information index (I) vary from 0.7287 to 1.0444 containing mean 0.8585 and standard deviation 0.2569, expected homozygous (Ho) range from 0.3694 to 0.4917 with mean 0.4474 and standard deviation 0.0481 values, expected heterozygous (He) vary from 0.5083 to 0.6306 with mean 0.5526 and standard deviation 0.0481, Nei's genetic diversity index (Nei) ranged from 0.5066 to 0.6284 containing mean 0.5506 and standard deviation 0.0479. Lastly, polymorphism information content (PIC) varied at 0.9314 to 0.9616 values with mean 0.0098 and major allele frequency (MAF) from 0.1007 to 0.1879 with mean 0.1248 and standard deviation 0.0318 values. Both (RYW18 and RYW37) indicated highest number of allele (Na 4) among other primers. The results indicated it had viable millet genotypic diversity and molecular genomic variability when showing its gene parameters results.

Table 2. Genetic diversity analysis

Locus	Na	Ne	I	Ho	He	Nei	PIC	MAF
RYW6	3	2.0978	0.7903	0.4746	0.5254	0.5233	0.9376	0.1678
RYW8	3	2.5928	1.0180	0.3833	0.6167	0.6143	0.9430	0.1275
RYW12	3	2.6911	1.0444	0.3694	0.6306	0.6284	0.9555	0.1007
RYW18	4	2.0309	0.7382	0.4904	0.5096	0.5076	0.9509	0.1275
RYW37	4	2.2863	0.9393	0.4355	0.5645	0.5626	0.9314	0.1342
RYW40	3	2.0824	0.7786	0.4784	0.5216	0.5198	0.9583	0.1074
RYW42	3	2.5167	0.9936	0.3953	0.6047	0.6027	0.9427	0.1879
RYW43	3	2.0268	0.7287	0.4917	0.5083	0.5066	0.9570	0.0872
RYW125	3	2.1468	0.8249	0.4640	0.5360	0.5342	0.9450	0.1007
RYW146	3	2.0269	0.7288	0.4917	0.5083	0.5066	0.9616	0.1074
Mean	3.2	2.2499	0.8585	0.4474	0.5526	0.5506	0.9483	0.1248
Total	32		--	--	--	--	--	--
St.Dev	0.4216	0.2569	0.1270	0.0481	0.0481	0.0479	0.0098	0.0318

(Na: number of alleles, Ne: number of effective alleles, I: Shannon's information index, Ho: expected homozygous, He: expected heterozygous, Nei: Nei's genetic diversity index, PIC: Polymorphism information content, and MAF; major allele frequency)

3.2 UPGMA analysis PCA and Population structure

The NJ tree based on genetic distance results (Fig. 1) displayed 149 proso millet genotypes from Qinghai (Table. S1) into three main populations or cluster branches, P1 (containing 130 genotypes), P2 (12 genotypes) and P3 (7 genotypes) respectively. While geographically evolutionary approach, it showed six geographic origins (Fig. 1). The unequal population distribution in grouping indicated higher genotypic variation and molecular evolutionary history from China. Furthermore, the Principle component analysis (PCA) results (Fig. 2) showed dynamic and uniqueness with phylogeny tree, PC1, PC2, and PC3, 60.3%, 12.31% and 7.57% with respect.

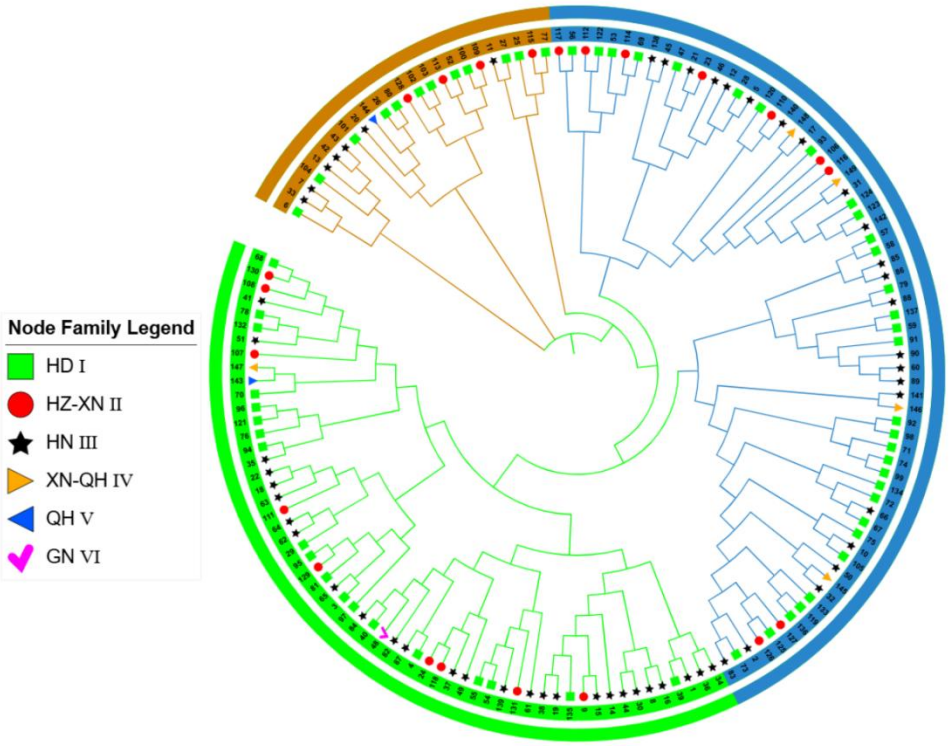

Fig. 1 Phylogenetic linkage of 149 proso millet individual

(Nodes distribution of the tree in accordance with their groups, in the ring of node with different colour display different accessions “Square green HD-I” indicates accessions from Haidong, “Circle red HZ-XN II” refers accessions from Huangzhong-Xining, “Star black HN III” represent accessions from Huangnan, “ right triangle brown XN-QH IV” shows accessions from Xining and partially Qinghai but closer to the Xining, “left triangle blue QH” presents accessions from mainly center Qinghai and subsequently, “Tick-marked reddish GV IV” indicates accessions from Guinan).

For population structure analysis of 149 proso millet germplasm from Qinghai (S Table 1), the results exhibited this into various levels (K=3, K=5 and K=8), K=3 that indicated into three groups (Fig. 3), (first group green) indicated 50 divergent millet genotypes from various sites and places of Qinghai, 33

genotypes from (Minhe) with encompassed 8 genomtypes, 2 genotypes from Hualong, 1 genotype encompassed jianzha, 1 Ledu, 1 encompassed Hungzhong, 2 genotypes Xiningshi, 3 genotypes Gonghe with 1 encompassed, 2 genotypes Huzhu, 2 Xunhua with encompassed 1, 1 genotype Guide place and 1 genotype Guinan. Second red with 50 millet genotypes group referred to 17 genotypes Minhe with encompassed 3 genotypes, 16 genotypes Ledu with 2 genotypes encompassed, 6 genotypes Hualong, 1 genotype Mindou, 6 genotypes Pingan and 4 Xunhua, third blue group with 49 genotypes showed 5 encompassed genotypes from Xunhua, 13 Hunagzhong with 2 genotypes encompassed, 7 genotype Pingan, 6 genotypes Xiningshi, 6 genotypes Huzhu with 1 encompassment, 5 genotypes Jinazha, 2 mainly hub of Qinghai and 5 genotypes Xining-Qinghai respectively.

Population structure analysis results had further population distribution into smaller levels (K=5) and K=8), the K=5 resulted into five, the first blue group and second reddish displayed non-admixture and admixture millet genotypes, include, the number of 18 genotype from Minhe with 15 genotypes overlapping, 1 encompassed genotype Hualong, 1 encompassed genotype Ledu, 1 genotype also Hunagzhog, 1 genotype overlapping Huzhu and the rest are (encompassed predominantly 15 genotypes Minhe, 1 genotype Hualong, 2 genotypes Xiningshi, 3 genotypes Gonghe and 1 Huzhu. Third red and fourth green group showed red predominant 16 genotypes from Minhe containing 4 encompassment, 8 genotypes Hualong with 1 encompassed, 14 genotypes Ledu containing 1 encompassed, 1 genotype Mindou, 6 genotypes Pingan with 2 encompassed, 4 genotypes from Xunhua with 1 genotype overlapping. Fourth green and fifth yellow group displayed millet genotypes 11 from Xunhua with 1 encompassed, 19 genotypes Huangzhong with 3 overlapping, 7 genotypes Mindou with 3 encompassed, 6 genotypes Huzhu with 2 encompassed, 5 genotypes Jinzha, 2 genotypes mainly Qinghai and 5 genotypes Xining-Qinghai having 2 encompassemnt.

(K=8) exhibited into 8 sub-population groups: first green and second brownish group referring 34 millet genotypes from Minhe with non-encompassed 13, 2 genotypes Hualong with 1 non-encompassed, 1 genotype Jinzha, 1 genotype Ledu, 3 genotypes Hunagzhong with 1 free, 4 genotypes Gonghe, 1 genotypes Huzhu, 2 genotypes Xunhua, 1 genotype Guide and 1 genotype Guinan. Third reddish, forth turquoise and fifth brown group mostly showed admixture millet 8 genotypes from Minhe with 2 non-overlapping, 16 genotypes Ledu containing 2 non-overlapping, 6 genotypes Hualong, 7 genotypes Mindou, 4 genotypes Xunhua with 1 non-overlapping genotype. Lastly sixth, seventh and eighth group indicated 5 genotypes from Xunhua, 19 genotypes Huangzhong, 7 genotypes Mindou with 4 non-admixtures, 7 genotypes Huzhu containing 3 non-admixtures, 5 genotypes Jinazha with 2 genotypes, 2 Qinghai with 1 genotypes and 5 Xining-Qinghai with 4 genotypes non-overlapping respectively.

This equal population distribution that indicated rich genotypic variability and pronounced proso millet germplasm, when this referred that K=3 is the apogee level (Fig. 1). This showed better applicability with highest point (Fig. 4a and Fig. 4b) is a more potential than other (K=5 and K=8) for unpacking tangible molecular genomic study. However, this result showed with NJ tree is highly reliable results. Therefore, K=3 results chosen with consistency (Fig. 4a and Fig. 4b) comprehensively.

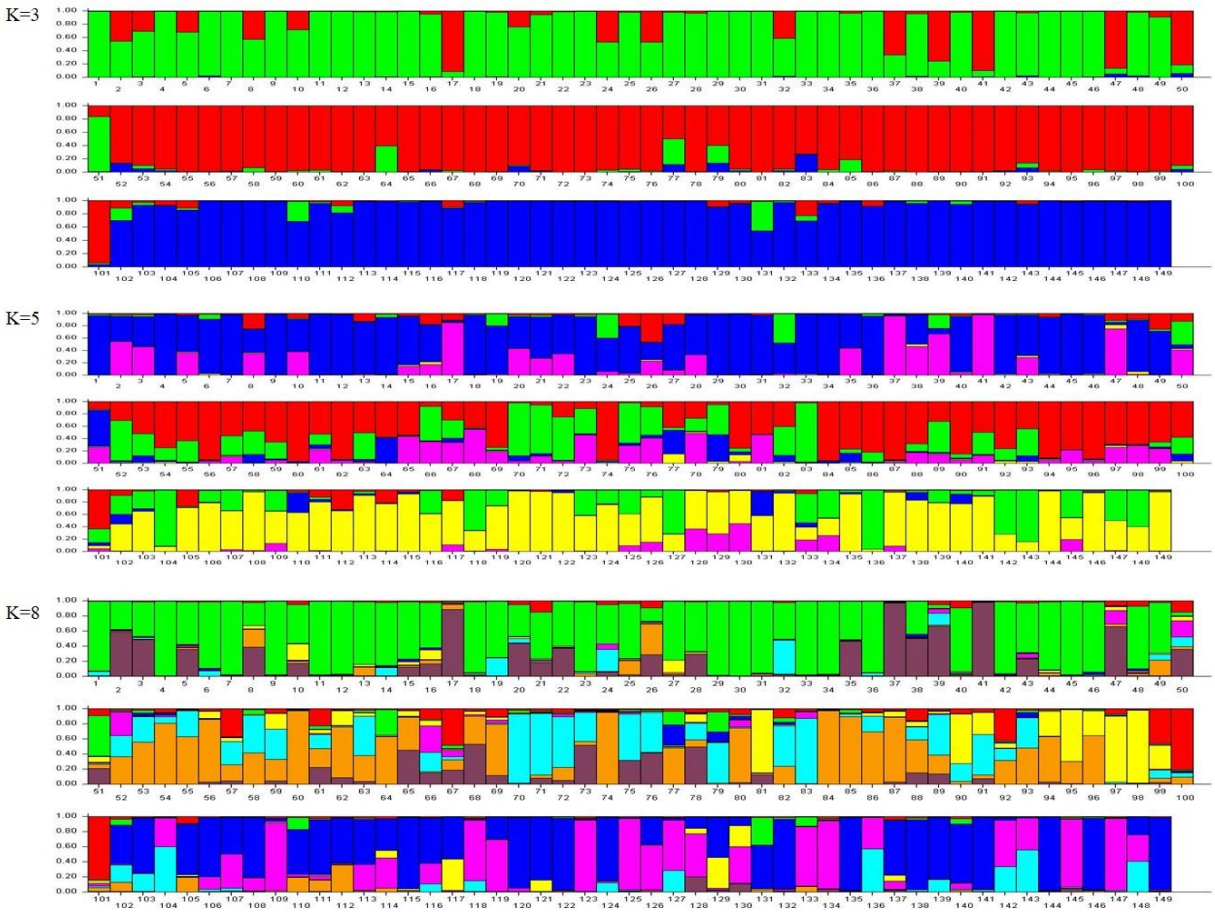

Fig. 3 Population structure analysis of proso millet germplasm

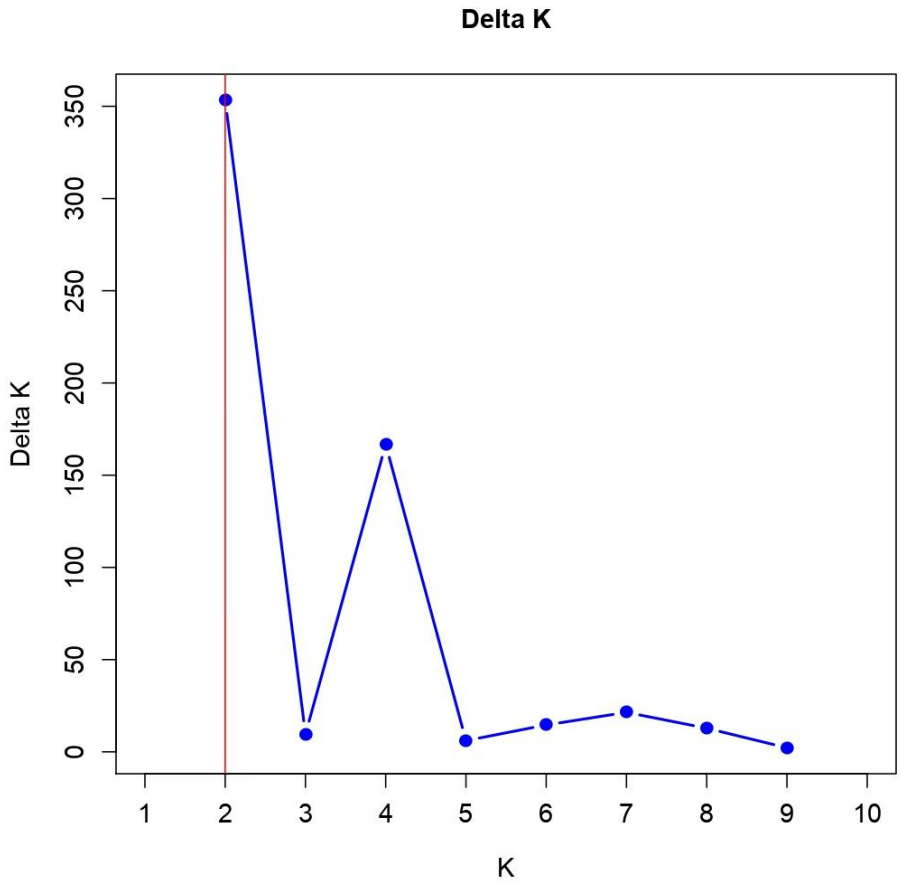

Fig. 4 (a) ΔK values -based Bayesian model for different suggested (K) population analysis on STRUCTURE

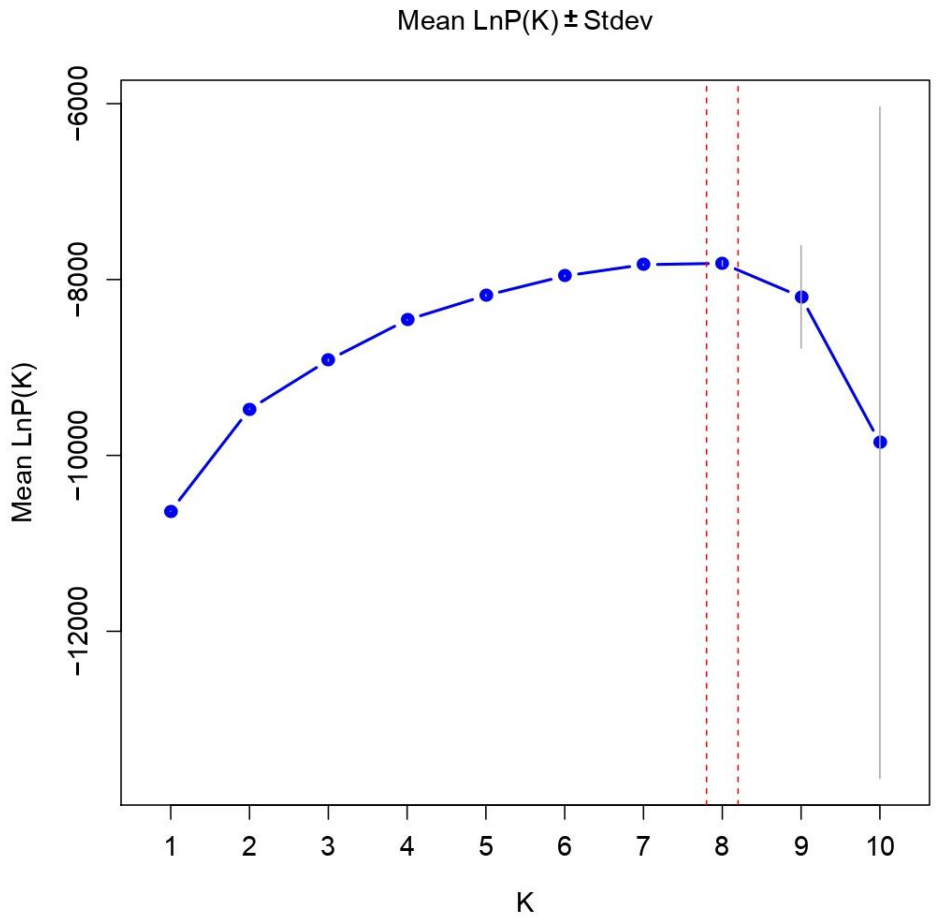

Fig. 4 (b) shows divergent ΔK values comprehensively consistent with (Fig. 4 a)

Name: Jidan mi
Unicode: 4162
Japanica rice: Jidan mi
Province: Qinghai

Name: Shaoshao meizi
Unicode: 4165
Japanica rice: Shaoshao meizi
Province: Qinghai

Name: Bai jidan mi kong
Unicode: 5526
Japanica rice: Bai jidan mi kong
Province: Qinghai

Name: Hemamei
Unicode: 5528
Japanica rice: Hemamei
Province: Qinghai

Name: Millet seed
Unicode: 5530
Japanica rice: Millet seed
Province: Qinghai

Name: Huang meizi
Unicode: 5531
Japanica rice: Huang meizi
Province: Qinghai

Name: Gezaomi
Unicode: 5533
Japanica rice: Gezaomi
Province: Qinghai

Name: Xiaoqingmi
Unicode: 5534
Japanica rice: Xiaoqingmi
Province: Qinghai

Name: Honngmeizi
Unicode: 5535
Japanica rice: Honngmeizi
Province: Qinghai

Name: Heimeizi
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Province: Qinghai

Name: Hongmeizi
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Province: Qinghai

Name: Shaoshaomeizi
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Japanica rice: Shaoshaomeizi
Province: Qinghai

Name: Meizi
Unicode: 5543
Japanica rice: Meizi
Province: Qinghai

Name: Jidanmi
Unicode: 5544
Japanica rice: Jidanmi
Province: Qinghai

Name: Jidanmi
Unicode: 5545
Japanica rice: Jidanmi
Province: Qinghai

Name: Xiaoqingmi
Unicode: 5546
Japanica rice: Xiaoqingmi
Province: Qinghai

Name: Dabami
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Japanica rice: Dabami
Province: Qinghai

Name: Gezaomi
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Province: Qinghai

Name: Gezaomi
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Province: Qinghai

Name: Xiaoqingmi
Unicode: 5558
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Province: Qinghai

Name: Jidanmi
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Japanica rice: Jidanmi
Province: Qinghai

Name: Qingmi
Unicode: 5560
Japanica rice: Qingmi
Province: Qinghai

Name: Qingmi
Unicode: 5561
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Province: Qinghai

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Province: Qinghai

Name: Baigedami
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Province: Qinghai

Fig. 5 Molecular Identification Card based on-bi-dimensional QR-code

3.3 Development Molecular Identification Card and Validation

The constructed Molecular Identification Card of 149 proso millet germplasm from Qinghai (S Table 1) that provided effective QR-code-based method containing entire millet genotypic information (Unicode, scientific name with local names and genotypes collections with divergent races) (Fig. 5). This result with genetic diversity parameters and others NJ tree, PCA and Population structure analysis indicated viable and dynamic for molecular study and validation. The genetic diversity indices showed this results validity.

4. Discussion

For harnessing proso millet germplasm in molecular characterization and breeding programs with new cultivars release as drought stress and agriculture sustainable, so molecular level study is a dynamic and vibrant for ensuring crops rotation and in-situ genomic conservation [31]. This cropping rotations ensure biodiversity conservation and introducing new cultivars release of proso millet with enhancing genetic resources (Kumar et al., 2024). Because M-strategy is a viable approach that is utilized to capture maximum genetic diversity via targeting and selecting divergent set of alleles with enhancing genomic variability for millet genotypic molecular breeding resources [20]. In this regard, this study is about Molecular Identification Card based on microsatellite simple sequence repeat (SSR) using 149 proso millet core germplasm from Qinghai, China is effective and tangible for diversifying global food production and agriculture sustainability with climate adaptability (IYL FAO 2023).

4.1 Genomic diversity of proso millet

Genomic resources are crucial for comprehensively understanding and genotypic utilization, so genetic diversity is important for molecular study [17]. Our findings on genetic diversity parameters, the number of allele (Na) are reported mostly 3 in each and only duo markers (RYW12 and RYW18) suggested 4 allele and total number of allele are 32. This endorsed with earlier study on hog millet [29] which reported convergent 3 number of allele in each SSR is effective for millet genotype harnessing and utilization, however, there is minor difference in number allele reported post de novo study that implied our study more diverse and significant reliability. The genetic diversity indices (Table 2) the number of effective allele (Ne) averaged from 2.5928 to 2.0268 with mean 2.2499 values are consistent with [33]. Shannon information index (I) averaged from 1.0180 to 0.7228 and expected homozygous (Ho) 0.3694 to 0.1917, this supported with germplasm approach report [21] and expected heterozygous (He) 0.5083 to 0.6396 reported in our finding are divergent on earlier report. This may be owing to sampling size and inconsistency millet genotype exchange due to environmental concerns and genotypes. Our polymorphism information content (PIC) reported at 0.9314 to 0.9616 is higher with this SSR-based yellowhorn study [36] which implied our millet-based report is more genomic diverse and molecular levels as compared with yellowhorn crops. Another genomic database on millet provided genetic applicability and molecular resources significant in agriculture (Kumar et al., 2024). Major allele frequency (MAF) varied from 0.0872 to 0.1678 is tangibly effective implying molecular identification is uniqueness for harnessing millet genotypes [34], [44]. However, our study comprehensively is consistent with SSR based reported in Hog millet, Chinese gastrodia and yellowhorn (X.-H. Chen et al., 2022, Mei et al., 2024, Le et al., 2024).

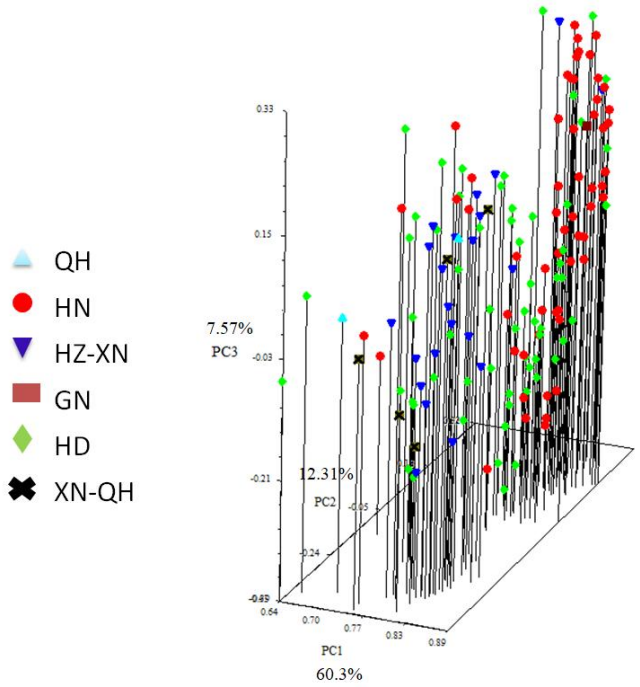

Fig. 2 Principle component analysis (PCA) of proso millet germplasm

4.2 Phylogeny analysis, PCA and population structure

The UPGMA based evolutionary analysis is tangibly an important when harnessing millet genotypic resources for molecular study and genetic variability in order to unpack further genomic resources [28]. NJ tree of proso millet 149 genotypes from Qinghai finding (Fig. 1) and (S Table 1) provided phylogenetic linkage into three populations levels on cluster based or stratified sampling, P1 contains (130 genotypes), P2 (12 genotypes) and P3 (7 genotypes) respectively. Here P refers to population in the phylogeny tree. This un-equal population distribution suggested that had higher phylogenetic linkage, genetic diversity and traits variation, furthermore this implied a solid foundation of genomic millet farming history in ancient times of Bronze age from China [22] who investigated finding millet genomic DNA from the Neolithic Age of China. The NJ tree is consistent with PC values, PC1 converged with this report [29], however PC2 is divergent and PC3 60.3%, 12.31% and 7.57% respectively. Similarly, our PC is implied higher than post de novo report [26] who reported PC1 21.14% and PC2 7.26% that finding insights suggesting our study is more effective and reliable due to the genotypic significant of millet germplasm (Kumar et al., 2024).

Population structure analysis of proso millet 149 genotypes from Qingahi (S Table 1) based grouping model approach (Bayesian method) is effective and better for molecular genomic studies. The population structure analysis provided equally sub-grouping distribution levels, K=3, K=5 and K=8 (Fig. 3), however, (K=3) exhibited into three sub-populations group, first green group provided 50 millet genotypes with minor ad-mixture, millet 33 genotypes from Minhe (8 admixture or encompassment), 2 genotype from Hualong, Jinazha 1 genotype containing admixture, 1 genotype Ledu, Hunagzhong with 1 encompassed, followed by Xiningshi 2 genotype, Gonghe 3 genotype including 1 encompassed, Huzhu 2, Xunhua 2 genotype with 1 encompassed and finally Guide 1 genotype and Guinan 1 genotype respectively. Second red group with 50 millet genotypes that indicated 17 genotypes from Minhe include 3 encompassed, 16 genotypes Ledu with 2 encompassed and finally 6 genotypes from Hualong, 1 genotype Mindou, 6 genotypes Pingan and 4 genotypes from Xunhua. Third blue group with 49 millet genotypes which provided genotypic traits from divergent places of Qinghai, 5 encompassed genotypes from Xunhua, 13 genotypes with 2 encompassed Huangzhong, 7 genotypes Pingan, 6 genotypes Xiningshi, 6 with 1 encompassed genotypes Huzhu, 5 genotypes Jinazha and finally 2 genotypes from hub of Qinghai and 5 genotypes from mainly Xining-Qinghai.

However, the population structure analysis (Fig. 3) has further sub-population grouping hierarchical levels (K=5 and K=8), when deemed smaller genetic population linkages, but K=3 is the most effective and viable that provides reliable and consistent findings when compared with Avanno principled method (ΔK) is the apogee (Fig. 4a and Fig. 4b) implying significantly applicability and reliability. This comprehensively highest K finding suggested to be a viable millet genotype variation and molecular development which endorsed with this study peak K values [29]. Moreover, the (K=3) that is consistent with NJ tree when deemed population distribution (Fig. 1). Moreover, the un-equal millet genotypic distribution in phylogenetic traits which implied broader genomic variation and gene flow as deemed with ad-mixture in population structure analysis [33].

4.3 Development of Molecular Identification Card of Proso millet

Genomic characterization and molecular development using various approaches genomic tools and non-genomic are effective for harnessing millet germplasm in gene bank and introducing new cultivars [31]. Molecular studies are solid foundation for providing easy access of genetic resources origins from simple sequence repeat (SSR) millet core germplasm for molecular development (Kumar et al., 2024). Therefore development Molecular ID Card Barcode-based on SSR markers using 149 proso millet core germplasm from Qinghai is a dynamic for providing easy accessible germplasm, gene editing and genotypic variability in crops production globally. Furthermore, our study is consistent with genetic diversity indices Na, Ne, I Ho He Nei, PIC and MAF (Table 1) and NJ tree (Fig. 1) followed by PC values (Fig. 2) and sub-population structure (Fig. 3) which suggested significant millet genotypic reliability and applicability when harnessing or harvesting genomic resources for pre-breeding and de novo cultivars release.

Therefore our study would provide solid foundation in better accessing proso millet germplasm for breeding and pre-cultivars release in; however, future studies will be required from single nucleotide polymorphism (SNP) or GWAS that will provide further molecular genotypic resources and genomic variability. Because SSRs are multi-allelic in nature that may possibly limit or skip in capturing rare alleles that are uniqueness in order to unpacking millet genotypes for stress resistant and yield.

5. Conclusion

For effective utilization of germplasm is a tangible approach via harnessing proso millet accessions of molecular genotypic study. Millets are rich in nutrient and role in drought stress; however the dearth of genomic resources has hampered in dynamically millet germplasm harvesting and molecular characterization. So, the development of Molecular ID Card Barcode-based on SSR markers applying proso millet (*Panicum miliaceum* L.) would be useful for crop diversification and in-situ genomic resources conservation. In this study, our findings are consistent with genetic diversity parameters, Na, Ne, I, Ho He Nei, PIC and MAF followed with phylogeny tree, PC values, and population structure analysis, respectively. This finding would provide a solid foundation for germplasm harvesting in breeding programs and pre-cultivars release as agriculture sustainable and climate adaptability in ensuring global food security.

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Data availability: Data are present in this article and supplementary materials are available in separate attached file. Furthermore information on this if required then, there should be requested to the author.

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