Geographical differentiation and genetic diversity of Algerian pearl millet assessed with microsatellites

Diferenciación geográfica y diversidad genética del mijo perla argelino evaluadas mediante microsatélites

Diferenciação geográfica e diversidade genética do milheto pérola argelino avaliada por microsatélites

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Abstract

Pearl millet, a drought-tolerant cereal, is a crucial role in food security, in arid and semi-arid regions. Despite its global significance, genetic diversity of Algerian pearl millet populations remains underexplored. This study assessed the genetic diversity of 22 pearl millet genotypes from four distinct Saharan agroclimatic zones in Algeria using 24 SSR markers. A total of 87 alleles were detected, with an average of 3.62 alleles per locus and polymorphic information content (PIC) values ranging from 0.043 to 0.815, indicating substantial genetic variability. Analysis of Molecular Variance (AMOVA) revealed greater genetic variance within individuals than among them. Genotypes from Tamanrasset and In Salah exhibited higher diversity than those from Oued Souf and Adrar, with private and rare alleles underscoring the impact of geographic isolation. Cluster analysis and principal coordinates analysis (PCoA) grouped genotypes by geographic origin, identifying five major genetic groups, confirming patterns of gene flow and local adaptation. Populations from Niger and India were highly genetic distant from Algerian landraces making them promising candidates for heterotic hybrid development. These findings provide insights for broadening the genetic base of breeding strategies targeting yield and stress resilience in pearl millet.

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Resumen

El mijo perla, cereal tolerante a la sequía, es esencial para la seguridad alimentaria en regiones áridas y semiáridas. A pesar de su importancia global, la diversidad genética del mijo perla en Argelia sigue poco estudiada. Este trabajo evaluó 22 genotipos de cuatro zonas agroclimáticas saharianas mediante 24 marcadores SSR. Se detectaron 87 alelos (media= 3,62 por locus), con valores de contenido de información polimórfica (PIC) entre 0,043 y 0,815, lo que evidencia una variabilidad considerable. El Análisis de Varianza Molecular (AMOVA) mostró mayor varianza dentro de los individuos que entre ellos. Los genotipos de Tamanrasset e In Salah presentaron mayor diversidad que los de Oued Souf y Adrar, destacando la presencia de alelos privados y raros por efecto del aislamiento geográfico. Los análisis de conglomerados y de coordenadas principales (PCoA) agruparon los genotipos por origen geográfico, identificando cinco grupos genéticos principales. Las poblaciones de Níger e India mostraron gran distancia genética respecto a las argelinas, lo que las convierte en candidatas prometedoras para el desarrollo de híbridos heteróticos. Estos resultados aportan información útil para ampliar la base genética en programas de mejoramiento orientados a la productividad y la resiliencia al estrés del mijo perla.

Palabras clave: Mijo perla, marcador SSR, diversidad genética, diversidad regional, razas locales argelinas.

Resumo

O milheto pérola, cereal tolerante à seca, é fundamental para a segurança alimentar em regiões áridas e semiáridas. Apesar de sua relevância global, a diversidade genética do milheto na Argélia permanece pouco explorada. Este estudo avaliou 22 genótipos de quatro zonas agroclimáticas saharianas utilizando 24 marcadores SSR. Foram detectados 87 alelos (média= 3,62 por loco), com valores de conteúdo de informação polimórfica (PIC) entre 0,043 e 0,815, indicando elevada variabilidade. A análise de variância molecular (AMOVA) mostrou maior variação dentro dos indivíduos do que entre eles. Genótipos de Tamanrasset e In Salah apresentaram maior diversidade que os de Oued Souf e Adrar, com alelos privados e raros refletindo o impacto do isolamento geográfico. As análises de agrupamento e de coordenadas principais (PCoA) organizaram os genótipos por origem geográfica, identificando cinco grupos principais. As populações do Níger e da Índia mostraram grande distância genética em relação às argelinas, tornando-se candidatas promissoras para o desenvolvimento de híbridos heteróticos. Esses resultados fornecem subsídios para ampliar a base genética em programas de melhoramento voltados à produtividade e à resiliência ao estresse do milheto pérola.

Palavras-chave: Milheto pérola, marcador SSR, diversidade genética, diversidade regional, raças locais argelinas.

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is the sixth most important cereal crop worldwide, after maize (*Zea mays* L.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and sorghum (*Sorghum bicolor* (L.) Moench), and it remains a key staple in arid and semi-arid regions due to its resilience to drought, poor soils, and high temperatures (Shinde *et al*., 2020; Deevi *et al*., 2024). India accounts for about 42 % of global production, followed by Niger, China, and several African countries (Yadav *et al*., 2021).

Pearl millet (*Pennisetum glaucum* [L.] R. Br.) was domesticated in the western Sahel of Africa around 4000-5000 years ago and subsequently spread across arid and semi-arid regions of Africa and Asia (Oumar *et al*., 2008; Manning *et al*., 2011). It has become a vital staple crop in environments where rainfall is scarce, temperatures are high, and soils are poor-conditions under which other cereals rarely succeed (Yadav *et al*., 2021). Its wide ecological distribution has led to the accumulation of considerable phenotypic and genetic variability, reflected in diverse landraces adapted to contrasting environments (Serba & Yadav, 2016). This diversity, shaped by both natural selection and farmer seed exchange, forms a key resource for breeding programs targeting drought tolerance, earliness, and nutritional quality (Satyavathi *et al*., 2021; Govindaraj *et al*., 2020). Morphologically, it exhibits wide variability in panicle size, tillering capacity, and grain color, while physiologically, it displays deep rooting and efficient water use typical of C_4 cereals (Satyavathi *et al*., 2021). In addition, Lemgharbi *et al*. (2023) revealed significant morphological diversity among local, domesticated, and wild forms in Tidikelt region, mainly in plant height, panicle traits, and seed color. These landraces demonstrated strong adaptation to extreme Saharan conditions and high phenotypic variability, suggesting valuable genetic resources for future breeding and conservation programs.

Despite these phenotypic insights, molecular data on North African germplasm remain scarce. Establishing the extent and structure of their genetic variation is therefore essential for understanding evolutionary relationships and guiding breeding strategies adapted to local agroecological conditions.

Genetic diversity assessment of pearl millet is fundamental for breeding programs aiming at yield improvement, stress tolerance, and disease resistance. While morphological traits are influenced by the environment (Kirouani *et al*., 2023), molecular markers provide more reliable evaluations of variability Kirouani *et al*., 2018; Triki *et al*., 2023). SSRs are co-dominant, highly polymorphic, and reproducible, making them valuable tools in plant breeding for assessing genetic relationships and population structure (Choudhary *et al*., 2016). SSRs have been widely used to characterize pearl millet genetic diversity (Stich *et al*., 2010; Nepolean *et al*., 2012; Gupta *et al*., 2018).

Furthermore, assessing genetic diversity in pearl millet landraces is particularly important for hybrid development, as diversity among inbred line enables the identification of parental combinations that contribute beneficial alleles for yield improvement and stress adaptation (Singh & Gupta 2019). However, most previous studies have focused on West African populations (Rhoné *et al*., 2020), whereas little information exists for North African germplasm, especially in Algeria-the northernmost limit of pearl millet distribution in Africa. Algerian landraces have evolved under contrasting Saharan and sub-humid environments, from oasis systems to Mediterranean foothills, potentially harboring unique allelic combinations associated with drought resilience and local adaptation. This work therefore constitutes the first comprehensive molecular characterization of Algerian pearl millet using SSR markers, filling a significant geographical and genetic gap in the species' diversity analysis. The findings provide a national contribution to the understanding of pearl millet diversity and offer a valuable resource for future regional breeding, conservation, and germplasm exchange programs. The objectives of this study were to: (i) assess the genetic diversity among

Algerian pearl millet genotypes and (ii) examine the distribution of allelic variation across distinct geographic zones.

Materials and methods

Plant material

Twenty-two pearl millet (*Pennisetum glaucum* [L.] R. Br.) genotypes (Table 1) were collected from four contrasting Saharan regions of Algeria-Tamanrasset (12 landraces), In Salah (8), Adrar (1), and Oued Souf (1) representing distinct agro-climatic zones (Table 2).

Table 1. Genotypes used in the present study.

Group code	Name	Origin and date of harvest
B1	TM-10Djf(3)	In Salah (Djafou) - 2010
B2	TM-09Djf(3)	In Salah (Djafou) - 2009
B3	TM-23Azzaoui	AinSalah (Djafou) - 2023
B4	TM-21Lakhal(Fo)	In Salah (Ouaini) - 2021
B5	TM-21SoumDjf	In Salah (Djafou) - 2011
B6	TM-1-11FE(Fo)	In Salah (Foggaret Ezzoua) - 2011
B7	TM-21NadjMed	In Salah (Ouaini) - 2021
B8	TM-21Arialla(Liss)	In Salah (Foggaret Ezzoua) - 2021
B9	TM-22Grenet	Oued Souf - 2022
B10	TM-16NadjMed(Fo)	Adrar (ex-Touat) - 2016
B11	TM-21HamdiZerga(Loc)	Tamenrasset (Center) - 2021
B12	TM-21HamdiN/Tam(06)	Tamenrasset (Niger) - 2021
B13	TM-16NadjMed(Mel)	Tamenrasset (ex-Ahagar) - 2016
B14	TM-22Mham(4)	Tamenrasset (India) - 2022
B15	TM-22Nafisse(Mel)	Tamenrasset (In M'guel) - 2022
B16	TM-22LaghnedjMed	Tamenrasset (Abalessa) - 2022
B17	TM-22ZergaTaleb(Loc)	Tamenrasset (In Dalag) - 2022
B18	TM-22LaghnedjAh(Mel)	Tamenrasset (Abalessa) 2022
B19	TM-21Laghnedj(Mel)	Tamenrasset (Abalessa) - 2021
B20	TM-22N/Tam(2)	Tamenrasset (Niger) - 2022
B21	TM-21N/Tam(Soud)	Tamenrasset (Niger) - 2021
B22	TM-21Zerga-Tam(Loc)	Tamenrasset (Center) - 2021

The contrasting environmental conditions are expected to influence morphological and adaptative traits among landraces such as plant height, panicle structure, seed color, and flowering time, reflecting underlying genetic differentiation, as suggested by previous study on African pearl millet populations (Rhoné *et al*., 2020).

DNA extraction and PCR amplification

The experiment was conducted at the Ouamri experimental site Médéa (36°12'18.2"N 2°31'30.0"E), under moderate sub-humid climatic conditions. Seeds of each genotype (three per spike) were sown in field plots during April 2024, at a depth of about 2 cm in clay-loam soil. The soil was irrigated before sowing to ensure uniform germination, and manual watering was provided when needed until seedling establishment. Leaf samples were collected 15 days after sowing, when plants reached the three-leaf stage. Five fresh leaves were placed in labeled glass tubes, kept in a portable icebox (Iceco GO20, China), and transported to the laboratory of molecular biology (University of Médéa) for DNA extraction using CTAB method (Fulton *et al*., 1995). A set of 24 highly polymorphic SSR primers (Figure 1, Table 3) were selected. PCR amplification was conducted in thermal cycler (Bioer Life Eco, China) in a total volume of 25 µL, containing approximately 50 ng of genomic DNA, 2.5 µM of each primer, green reaction buffer including 1.5 mM $MgCl_2$, 1U Taq DNA polymerase (GoTaq, Promega, USA), and 2.5 mM of each dNTP (Thermo Fisher Scientific, USA). Amplified DNA fragments were separated on 2 % agarose gels using an electrophoresis unit (Thermo Fisher Scientific, USA) and fragment sizes were estimated by comparisons with 100 bp DNA ladder (GeneRuler, Thermo Fisher Scientific, USA).

Data analysis

Genetic diversity parameters, including the number of alleles (Na), effective alleles (Ne), Observed heterozygosity (Ho), expected heterozygosity (He), Shannon's index (I), and polymorphism information content (PIC) were calculated using GenAlEx 6.5 (Peakall & Smouse, 2012) haploid and binary genetic loci and DNA sequences. Both frequency-based (F-statistics, heterozygosity, HWE, population assignment, relatedness. Principal coordinate analysis (PCoA) was also performed in GenAlEx 6.5, while UPGMA dendrogram was generated with MEGA 7.0 (Tamura *et al*., 2007) which expands on the existing facilities for editing DNA sequence data from autosequencers, mining Web-databases, performing automatic and manual sequence alignment, analyzing sequence alignments to estimate evolutionary distances, inferring phylogenetic trees, and testing evolutionary hypotheses. Version 4 includes a unique facility to generate captions, written in figure legend format, in order to provide natural language descriptions of the models and methods used in the analyses. This facility aims to promote a better understanding of the underlying assumptions used in analyses, and of the results generated. Another new feature is the Maximum Composite Likelihood (MCL. Additional diversity indices, including major allele frequency, and gene diversity, were obtained with PowerMaker v3.25 (Liu & Muse, 2005). Population structure was assessed with STRUCTURE software v2.3.4 using the ΔK methode (Evanno *et al*., 2005).

Results and discussion

Overall genetic diversity

The 24 SSR markers generated a total of 87 alleles, with an average of 3.62 alleles per locus, demonstrating the effectiveness of the

Table 2. Environmental characteristics of the four collection sites in Algeria.

Study site	Coordinates (DMS)	Altitude (m)	Rainfall (mm·yr^{-1})	Temp. (°C)	Soil type
Tamanrasset	23°48′54″ N, 5°55′56″ E	~1,400	< 100	38–42	Sandy-loam
In Salah	27°11′53″ N, 2°27′47″ E	~270	< 30	> 45	Sandy-stony
Adrar	28°48′14″ N, 0°19′09″ E	~263	< 30	> 45	Sandy
Oued Souf	33°26′08″ N, 6°53′34″ E	~80	< 100	35–40	Sandy

selected primers. Amplicons sizes ranged from 85 bp (IRD 46) to 378 bp (Xpsmp 2001), comparable to values reported in previous studies (Chandra *et al*., 2020; Kumar *et al*., 2020; Gunguniya *et al*., 2023) polymorphism data generated using 42 simple sequence repeat (SSR. PIC values ranged from 0.043 (Xpsmp 2227) to 0.815 (Xpsmp 2070), with a mean of 0.458 (Table 3). Nearly half of the markers (46 %) had PIC > 0.50, confirming their high discriminatory power, consistent with findings in other pearl millet germplasm (Bougma *et al*., 2021).

The number of alleles per locus varied from 2 to 7, and loci with higher allele numbers tended to show higher PIV values, confirming the correlation between allelic richness and informativeness (Kumar *et al*., 2020; Sangwan *et al*., 2019). The observed range was higher than that reported for west African landraces (Singh *et al*., 2013; Bougma *et al*., 2021; Makwana *et al*., 2021) but lower than values found in more diverse international panels (Sangwan *et al*., 2019; Gunguniya *et al*., 2023). Such differences may reflect germplasm origin, marker choice, and resolution of electrophoresis techniques.

The number of effective alleles (Ne) ranged from 1.05 (Xpsmp 2227) to 6.12 (Xpsmp 2070), with an overall average of 2.42 (Table 3). These values indicate substantial genetic variation across loci. The high Ne values correlate with genotypic diversity and are influences by the marker type and the resolution of the fragment separation technique (Kuang *et al*., 2022). Lower values were obtained than those reported by (Bougma *et al*., 2021).

Observed heterozygosity (Ho) averaged 0.411, ranging from 0.045 to 1.000, while expected heterozygosity (He) averaged 0.516, ranging from 0.044 to 0.836. The high He values at several loci highlight the considerable allelic richness of Algerian pearl millet, while the variation in Ho indicated unequal distribution of heterozygotes across loci. These results confirm the presence of substantial genetic diversity. and in line with the cross-pollinating nature of pearl millet. Similar results of heterozygosity have been reported in international pearl millet panels (Chandra *et al*., 2020; Kumar *et al*., 2020; Rani *et al*., 2024). Similar ranges have also been documented in regional studies, particularly in west Africa and Sudan (Bashir *et al*., 2015; Bougma *et al*., 2021). These agreement between regional reports and our results confirms that substantial diversity is maintained across different African pearl millet despite environmental constraints.

The fixation index (F), indicating allelic fixation within populations, averaged 0.225 and ranged from -0.619 to 0.832. These results suggest a moderate inbreeding level in the studied populations. The variation in F values across loci reveals different evolutionary pressures.

Genetic diversity by region

Significant differences were observed among the four studied regions (Table 4). Tamanrasset and In Salah displayed the highest genetic diversity, with 78 and 71 alleles detected, respectively, along with several private and rare alleles, confirming their roles as major

Table 3. Number of Alleles, Major Allele Frequency, Heterozygosity, Genetic diversity, polymorphic information content and genetic differentiation by locus.

Primers	Amplicon size (bp)	Na[1]	Ne[2]	MAF[3]	Ho[4]	He[5]	PIC[6]	F[7]
Xpsmp 2008	194-265	7.000	3.33	0.349	0.524	0.700	0.642	0.259
Psmp 2214	236-267	3.000	1.68	0.750	0.227	0.406	0.370	0.446
Xpsmp 2019	241-270	3.000	2.60	0.500	1.000	0.616	0.542	-0.619
Xpsmp 2237	265-295	3.000	2.38	0.524	0.222	0.580	0.497	0.622
Psmp 2247	207-214	2.000	1.37	0.841	0.045	0.268	0.232	0.832
Xpsmp 2048	210-282	6.000	2.85	0.533	0.400	0.649	0.608	0.393
Psmp 2249	152-188	4.000	1.45	0.818	0.136	0.309	0.280	0.564
Xpsmp 2063	163-270	6.000	4.85	0.295	0.909	0.794	0.764	-0.138
Xpsmp 2043	138-158	3.000	2.36	0.545	0.364	0.577	0.499	0.376
Xpsmp 2070	174-251	7.000	6.12	0.214	0.762	0.836	0.815	0.097
Xpsmp 2201	330-368	4.000	1.63	0.765	0.394	0.386	0.353	-0.012
Psmp 2202	132-149	2.000	1.89	0.621	0.576	0.471	0.360	-0.216
Psmp 2206	190-210	4.000	2.83	0.523	0.439	0.646	0.599	0.327
Psmp 2219	268-275	2.000	1.90	0.614	0.136	0.474	0.362	0.716
Psmp 2220	145-175	3.000	1.98	0.667	0.429	0.495	0.441	0.143
Xpsmp 2227	200-220	2.000	1.05	0.977	0.045	0.044	0.043	-0.016
Psmp 2231	260-279	2.000	1.82	0.659	0.409	0.449	0.348	0.097
IRD 12	190-210	2.000	1.71	0.705	0.591	0.416	0.330	-0.413
IRD 46	85-122	4.000	1.41	0.833	0.258	0.292	0.274	0.126
Xpsmp 2267	131-202	4.000	2.37	0.598	0.412	0.578	0.530	0.297
Xpsmp 2090	148-186	4.000	2.88	0.474	0.684	0.652	0.592	-0.040
Xpsmp 2248	145-184	2.000	1.95	0.579	0.211	0.488	0.369	0.574
Xpsmp 2001	193-378	5.000	3.34	0.465	0.579	0.700	0.660	0.182
Xpsmp 2076	148-169	3.000	2.31	0.556	0.111	0.568	0.489	0.808
Mean		3.625	2.42	0.600	0.411	0.516	0.458	0.225

[1]Number of allele, [2]Number of effective allele, [3]Major allele frequency, [4]Heterozygosity, [5]Genetic diversity, [6]Polymorphic information content, [7]Fixation index

reservoirs of genetic diversity. By contrast, Oued Souf presented only 49 alleles, with reduced heterozygosity and no private alleles. Adrar had the lowest diversity, with 45 alleles, low Shannon's index, and a deficit of heterozygotes, reflecting possible genetic erosion and isolation. These results confirm that southern populations maintain richer and more diverse germplasm than northern desert regions. The presence of private alleles in the south suggests long-term adaptation and restricted gene flow, while the reduced diversity in the north points to genetic narrowing, possibly due to farmer selection practices or limited seed exchange. Such geographic structuring of diversity has also been reported in west African landraces (Bashir *et al*., 2015; Bougma *et al*., 2021).

Table 4. Genetic diversity of landraces of Pearl millet according to geographic origin.

Region	Na	Naa	Ne	He	Ho	I	PIC	Pa	Ra
Adrar	43	1.79	1.64	0.30	0.43	0.45	0.24	0	0
In Salah	71	3.00	2.23	0.47	0.44	0.81	0.41	2	2
OuedSouf	31	1.29	1.27	0.11	0.18	0.17	0.09	0	0
Tamenrast	78	3.25	2.21	0.50	0.40	0.86	0.44	7	5

Na = Number of Alleles, Naa = Number of average alleles, Ne = Number of effective alleles = 1 / (Sum pi^2), He = Gene diversity = 1 - Sum pi^2 Where pi is the frequency of the i^{th} allele for the population, Ho = Observed Heterozygosity = No. of Hets / N, I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), PIC = Polymorphic Information Content, Pa = Private allele, Ra = Rare allele.

Cluster Analysis and PCoA

Based on 24 SSR markers, UPGMA analysis (Figure 1) grouped the 66 pearl millet accessions into five clusters. The first cluster (Blue) included most accessions from Tamanrasset and In Salah which were closely associated with several Nigerian genotypes, highlighting historical connections and gene flow across Sahelian trade and pastoral routes. A second cluster (Red) was formed by the Indian accessions, which remained distinct but showed partial affinity with some Tamenrasset lines, suggesting either introductions of Asian germplasm into Algeria or convergent adaption to arid environments. A third cluster (Green) contains only Oued Souf accessions, which form a distinct group likely due to their geographical isolation near Tunisia and their reduced genetic base. Meanwhile, cluster 4 (Black), included genotypes from Abalessa, Hoggar, In Saleh, and Adrar, showing more genetic convergence, possibly to local adaptation or shared ancestry within the region. Finally, cluster 5 (Light blue) grouped only In Saleh accessions, emphasizing their genetic distinctiveness, probably due to long-term isolation and local adaptation. As highlighted in similar studies Burkina Faso (Bougma *et al*., 2021; Sawadogo *et al*., 2015) and Sudan (Bashir *et al*., 2015), the sharing of seeds through inheritance and informal exchange often result in the same populations appearing in multiple regions. This widespread practice, as explained by Mariac *et al*. (2006); Vom Brocke *et al*. (2003), contributes to low genetic differentiation due to ongoing gene flow. However, Stich *et al*. (2010) noted that regional similarity can also arise from climatic selection pressures, which over time may narrow genetic diversity.

Genetic distance analysis confirmed these patterns, with values ranging from 0.23 between Tamanrasset and In Salah to 0.52 between Tamanrasset and Oued Souf. This demonstrates greater similarity among neighboring southern regions and stronger divergence between the geographically distant northern and southern populations.

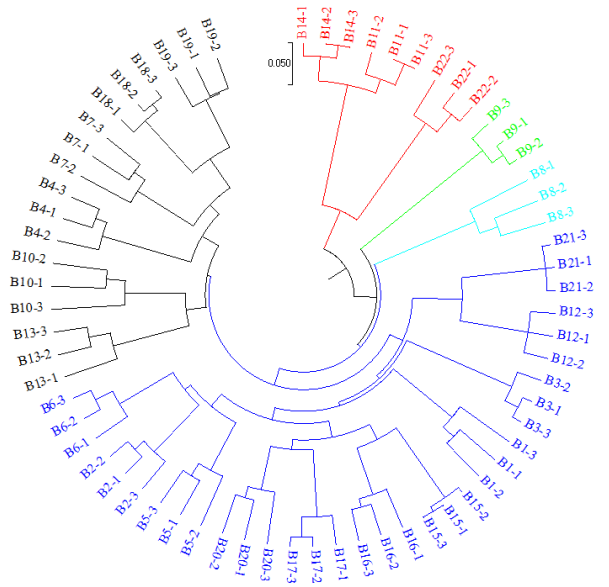

Figure 1. Dendrogram of 66 pearl millet accessions based on banding pattern analysis of 24 SSR markers using Nei 1983 UPGMA method. B1, B2, B3, B5 = In Salah (Djafou). B4, B7 = In Salah (Ouaini). B6, B8 = In Salah (Foggaret Ezzoua). B9 = Oued Souf. B10 = Adrar. B11, B22 = Tamanrasset (Center). B12, B20, B21 = Tamanrasset (from Niger). B13 = Tamanrasset (Hoggar). B14 = Tamanrasset (from India). B15 = Tamanrasset (In M'guel). B16, B18, B19 = Tamanrasset (Abalessa). B17 = Tamanrasset (In Dalag).

Interestingly, high divergence was also observed within Tamanrasset between sites such as the center, Hoggar, and In Dalag, pointing to localized differentiation even within a single region. The information available on genetic distances can be used to predict hybrid performance, as is done previously (Gupta *et al*., 2018).

Principal Coordinates Analysis (PCoA) supported the dendrogram results, explaining 44.10 % of total variation across the first three axes (21.45 %, 9,69 % and 8.96 %, respectively) (Figure 2). PCoA clearly separated Nigerien and Indian accessions from Algerian landraces, while most accessions from Tamanrasset and In Salah clustered close to Nigerien lines. In contrast, Oued Souf and Adrar occupied marginal positions on the scatter plot, confirming their isolation and limited variability.

Analysis of molecular variance (AMOVA) revealed that 66 % of the total variation occurred within individuals, 20 % among individuals, and only 14 % among populations. This result suggests that pearl millet exhibits high intra-individual heterozygosity, likely due its outcrossing nature and extensive gene flow, which allows the maintenance of genetic diversity at the individual level rather than between groups. This findings align with those presented earlier (Ramya *et al*., 2018; Bhardwaj *et al*., 2018; Chandra *et al*., 2020)95 maintainer (B line, and further reinforce the idea that intrapopulation variation plays a dominant role in the genetic structure of pearl millet.

The results of this study provide essential baseline information for the genetic improvement and conservation of pearl millet in Algeria. The detection of significant genetic differentiation among regional populations indicates the presence of locally adapted alleles that could be exploited in breeding programs targeting drought tolerance, earliness, and yield stability under arid and semi-arid conditions.

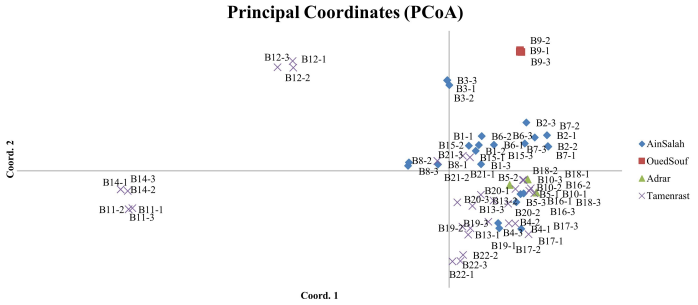

Figure 2. Principal component analysis (PCoA) using 24 SSR markers of 66 accessions from four different regions (In Salah, Oued Souf, Adrar and Tamanrasset) based on Euclidean distance estimates.

The identification of genetically distinct groups also supports the strategic selection of parental lines to broaden the genetic base of national breeding materials. Moreover, the molecular profiles generated here can serve as a reference for germplasm conservation and management, guiding the establishment of a representative national core collection - how is a small subset that minimizes genetic redundancy while preserving the maximum genetic diversity and it is crucial for the efficient management and utilization of germplasm resources - and facilitating the integration of Algerian landraces into regional and international genetic resource networks.

Structure Analysis

Structure analysis identified four genetic subpopulations (K= 4), The bar plot (Figure 3) showed that the first subpopulation (red) grouped accessions from Tamanrasset and India, suggesting a historical exchange of germplasm or convergent adaptation to arid environments. Subpopulation 2 (green) grouped accessions from In Salah and Abalessa, and showed the highest level of admixture, reflecting its central geographic location, and possible role as a zone of gene flow. Subpopulation 3 (blue) encompassed a broader combination of accessions from regions such as Hoggar, In Dalag, Djafou, Adrar and Niger. A low level of admixture was in some Adrar accessions within this group. Subpopulation 4 (yellow) included individuals form Oued Souf, In M'guel, Djafou, and some from Niger, reflecting a high level of shared ancestry among geographically distant populations. The result indicates that, despite its geographical isolation, Oued Souf may have received genetic material through historical seed exchange or migration. These findings are largely consistent with the results obtained from the UPGMA dendrogram and PCoA analysis, which also showed overlapping genetic relationships between Tamanrasset, In Salah, and Niger. However, STRUCTURE analysis adds new insight by showing that Oued Souf is not genetically distinct, but rather part of a mixed gene pool.

Conclusions

The results reveal substantial genetic variability, with greater allele richness and heterozygosity in Tamanrasset and In Salah compared with the narrower diversity in Oued Souf and Adrar. This regional differentiation provides useful information for identifying genetically distant parental lines and strengthening breeding programs aimed at adaptation to arid conditions. The predominance of genetic variance within individuals confirms pearl millet's outcrossing nature and underscores the need to preserve intra-population diversity through targeted conservation strategies. Overall, this study enhances the understanding of Algerian pearl millet diversity and contributes to regional efforts for crop improvement and food security in dryland environments.

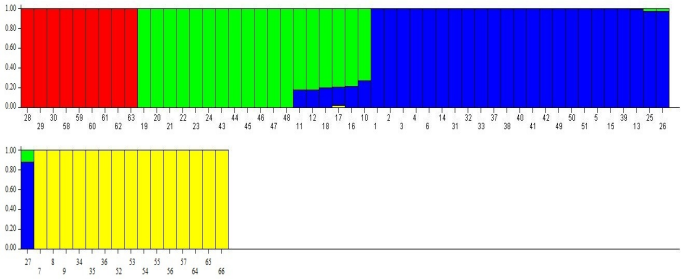

Figure 3. Bar diagram for 66 accessions arranged based on inferred ancestry at K = 4; Color codes represent the four subpopulations. Values in the left indicate the membership coefficient (Q). Proportions of colors in each bar indicate the allelic affiliation of the sub-populations. 1-2-3 (B1), 4-5-6 (B2), 7-8-9 (B3), 10-11-12 (B4), 13-14-15 (B5), 16-17-18 (B6), 19-20-21 (B7), 22-23-24 (B8), 64-65-66 (B9), 25-26-27 (B10), 28-29-30 (B11), 55-56-57 (B12), 31-32-33 (B13), 61-62-63 (B14), 34-35-36 (B15), 37-38-39 (B16), 40-41-42 (B17), 43-44-45 (B18), 46-47-48 (B19), 49-50-51 (B20), 52-53-54 (B21), 58-59-60 (B22).

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Literature cited

Anuradha, N., Satyavathi, C. T., Bharadwaj, C., Nepolean, T., Sankar, S. M., Singh, S. P., Meena, M. C., Singhal, T., & Srivastava, R. K. (2017). Deciphering genomic regions for high grain iron and zinc content using association mapping in pearl millet. *Frontiers in Plant Science, 8*, 412. https://doi.org/10.3389/fpls.2017.00412

Bashir, E. M. A., Ali, A. M., Ali, A. M., Mohamed, E. T. I., Melchinger, A. E., Parzies, H. K., & Haussmann, B.I.G. (2015). Genetic diversity of Sudanese pearl millet (*Pennisetum glaucum* (L.) R. Br.) landraces as revealed by SSR markers, and relationship between genetic and agro-morphological diversity. *Genetic Resources and Crop Evolution, 62*(4), 579-591. https://doi.org/10.1007/s10722-014-0183-5

Bhardwaj, R., Garg, T., Malik, E. A., Vikal, Y., Sohu, R. S., & Gupta, S. K. (2018). Genetic divergence studies in pearl millet [*Pennisetum glaucum* L. (R.) Br.] inbred lines. *Indian Journal of Genetics and Plant Breeding, 78*(3), 382-385. http://oar.icrisat.org/id/eprint/10854

Bougma, L. A., Ouédraogo, M. H., Ouoba, A., Zouré, A. A., Sawadogo, N., & Sawadogo, M. (2021). Genetic differentiation for gene diversity among pearl millet (*Pennisetum glaucum* (L.) R. Br.) landraces as revealed by SSR markers. *International Journal of Agronomy, 2021*(1). https://doi.org/10.1155/2021/6160903

Chandra, S., Singh, S. P., Kaswan, V., Chaudhary, S., Singh, A. M., Sankar, M., Meena, G., Choudhary, M., & Chellapilla, T. S. (2020). Morphological and molecular genetic diversity analysis of pearl millet (*Pennisetum glaucum*) maintainers and restorers. *Indian Journal of Agricultural Sciences, 90*(11), 2083-2089. https://doi.org/10.56093/ijas.v90i11.108564

Choudhary, M., Hossain, F., Muthusamy, V., Thirunavukkarasu, N., Saha, S., Pandey, N., Jha, S. K., & Gupta, H. S. (2016). Microsatellite marker-based genetic diversity analyses of novel maize inbreds possessing rare allele of β-carotene hydroxylase (crtRB1) for their utilization in β-carotene enrichment. *Journal of Plant Biochemistry and Biotechnology, 25*(1), 12-20. https://doi.org/10.1007/s13562-015-0300-3

Deevi, C. K., Swamikannu, N., Pingali, P. R., & Gumma, M. K. (2024). Current Trends and Future Prospects in Global Production, Utilization, and Trade of Pearl Millet. In V. A. Tonapi., N. Thirunavukkarasu., S. Gupta., P. I. Gangashetty., & O. Yadav (Eds.), *Pearl Millet in the 21st Century*. Springer Nature, Singapore. https://doi.org/10.1007/978-981-99-5890-0

Lemgharbi, M., Belhadi, B., Souilah, R., Djabali, D., & Nadjemi, B. (2023). Biodiversity of pearl millet (*Pennisetum glaucum* [L.] R. Br.) in southern Algeria (Tidikelt region). *Biodiversity Journal*, *14*(3), 491-504. https://doi.org/10.31396/Biodiv.Jour.2023.14.3.491.504

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters using the software STRUCTURE: a simulation study. *Molecular Ecology, 14*(8), 2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Fulton, T. M., Chunwongse, J., & Tanksley, S.D. (1995). Microprep protocol for extraction of DNA from tomato and other herbaceous plants. *Plant Molecular Biology Reporter, 13*(3), 207-209. https://doi.org/10.1007/BF02670897

Govindaraj, M., Rai, K. N., Kanatti, A., Upadhyaya, H. D., Shivade, H., & Rao, A. S. (2020). Exploring the genetic variability and diversity of pearl millet core-collection germplasm for grain nutritional traits improvement. *Scientific Reports, 10*, 21177. https://doi.org/10.1038/s41598-020-77818-0

Gunguniya, D. F., Kumar, S., Patel, M. P., Sakure, A. A., Patel, R., Kumar, D., & Khandelwal, V. (2023). Morpho-biochemical characterization and molecular marker based genetic diversity of pearl millet (*Pennisetum glaucum* (L.) R. Br.). *PeerJ, 11*, e15403. https://doi.org/10.7717/peerj.15403

Gupta, S. K., Nepolean, T., Shaikh, C. G., Rai, K., Hash, C. T., Das, R. R., & Rathore, A. (2018). Phenotypic and molecular diversity-based prediction of heterosis in pearl millet (*Pennisetum glaucum* L. (R.) Br.). *The Crop Journal, 6*(3), 271-281. https://doi.org/10.1016/j.cj.2017.09.008

Kirouani, A., Boukhalfoun, L., Ouldkiar, R., & Bouzerzour, H. (2023). Analysis of the effect of GE interaction on the grain yield and its related traits in rainfed Algerian durum wheat (*Triticum turgidum* L. var. durum) grown in contrasting environments. *Revista Facultad Nacional de Agronomia Medellin, 76*(2), 10297-10308. https://doi.org/10.15446/rfnam.v76n2.102517

Kirouani, A., Henkrar, F., Udupa, S. M., Boukhalfoun, L., & Bouzerzour, H. (2018). Genetic diversity in Algerian durum wheat varieties (*Triticum turgidum* L. var. durum) using microsatellite markers. *Bioscience Journal, 34*(6), 1575-1583. https://doi.org/10.14393/BJ-v34n6a2018-42332

Kuang, Z., Xiao, C., Ilyas, M. K., Ibrar, D., Khan, S., Guo, L., Wang, W., Wang, B., Huang, H., Li, Y., Li, Y., Zheng, J., Saleem, S., Tahir, A., Ghafoor, A., & Chen, H. (2022). Use of SSR Markers for the Exploration of Genetic Diversity and DNA Finger-Printing in Early-Maturing Upland Cotton (*Gossypium hirsutum* L.) for Future Breeding Program. *Agronomy, 12*(7), 1513. https://doi.org/10.3390/agronomy12071513

Kumar, S., Hash, C. T., Singh, G., Basava, R. K., & Srivastava, R. K. (2020). Identification of polymorphic SSR markers in elite genotypes of pearl millet and diversity analysis. *Ecological Genetics and Genomics, 14*, 100051. https://doi.org/10.1016/j.egg.2019.100051

Liu, K., & Muse, S. V. (2005). PowerMaker: An integrated analysis environment for genetic maker analysis. *Bioinformatics, 21*(9), 2128-2129. https://doi.org/10.1093/bioinformatics/bti282

Makwana, K., Tiwari, S., Tripathi, M. K., Sharma, A. K., Pandya, R. K., & Singh, A. K. (2021). Morphological characterization and DNA finger printing of pearl millet (*Pennisetum Glaucum* (L.) germplasms. *Range Management and Agroforestry, 42*(2), 205-221.

Manning, K., Pelling, R., Higham, T., Schwenniger, J.-L., & Fuller, D. Q. (2011). 4500-year-old domesticated pearl millet (*Pennisetum glaucum*) from the Tilemsi Valley, Mali: New insights into an alternative cereal domestication pathway. *Journal of Archaeological Science, 38*(2), 312-322. https://doi.org/10.1016/j.jas.2010.09.007

Mariac, C., Luong, V., Kapran, I., Mamadou, A., Sagnard, F., Deu, M., Chantereau, J., Gerard, B., Ndjeunga, J., Bezançon, G., Pham, J. L., & Vigouroux, Y. (2006). Diversity of wild and cultivated pearl millet accessions (*Pennisetum glaucum* [L.] R. Br.) in Niger assessed by microsatellite markers. *Theoretical and Applied Genetics,114*(1), 49-58. https://doi.org/10.1007/s00122-006-0409-9

Nepolean, T., Gupta, S.K., Dwivedi, S. L., Bhattacharjee, R., Rai, K. N., & Hash, C. T. (2012). Genetic diversity in maintainer and restorer lines of pearl millet. *Crop Science, 52*(6), 2555-2563. https://doi.org/10.2135/cropsci2011.11.0597

Oumar, I., Mariac, C., Pham, J.-L., & Vigouroux, Y. (2008). Phylogeny and origin of pearl millet (*Pennisetum glaucum* [L.] R. Br.) as revealed by microsatellite loci. *Theoretical and Applied Genetics, 117*, 489-497. https://doi.org/10.1007/s00122-008-0793-4

Peakall, R., & Smouse, P. E. (2012). GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics, 28*(19), 2537-2539. https://doi.org/10.1093/bioinformatics/bts460

Ramya, A. R., Ahamed, M. L., Satyavathi, C. T., Rathore, A., Katiyar, P., Bhasker Raj, A. G., Kumar, S., Gupta, R., Mahendrakar, M. D., Yadav, R. S., & Srivastava, R. K. (2018). Towards defining heterotic gene pools in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Frontiers in Plant Science, 8*, 1-11. https://doi.org/10.3389/fpls.2017.01934

Rani, R., Khandelwal, V., Jhunjhadia, S., Ambawat, S., Bhanwariya, S., Singh, A., & Kumar, V. (2024). Quantifying genetic diversity based on morphological and molecular analysis in pearl millet [*Pennisetum glaucum* (L.) R. Br.] inbred lines. *Annals of Arid Zone, 63*(3), 105-110. https://epubs.icar.org.in/index.php/AAZ/article/view/151562

Rhoné, B., Defrance, D., Berthouly-Salazar, C., Mariac, C., Cubry, P., Couderc, M., Dequincey, A., Assoumane, A., Kane, N. A., Sultan, B., Barnaud, A., & Vigouroux, Y. (2020). Pearl millet genomic vulnerability to climate change in West Africa highlights the need for regional collaboration. *Nature Communications, 11*, 5274. https://doi.org/10.1038/s41467-020-19066-4

Sangwan, S., Yashveer, S., Kumar, R., Hemender, Sharma, S., & Redhu, N. (2019). Multi trait analysis reveals substantial diversity in pearl millet [*Pennisetum glaucum* (L.) R. Br.] inbred lines. *Journal of Experimental Biology and Agricultural Sciences, 7*(4), 358-375. https://doi.org/10.18006/2019.7(4).358.375

Satyavathi, C. T., Ambawat, S., Khandelwal, V., & Srivastava, R. K. (2021). Pearl millet: A climate-resilient nutricereal for mitigating hidden hunger and providing nutritional security. *Frontiers in Plant Science, 12*, 659938. https://doi.org/10.3389/fpls.2021.659938

Sawadogo, N., Nanema, R. K., Bationo/Kando, P., Traore, R. E., Nebie, B., Tiama, D., Sawadogo, M., & Zongo, J. D. (2015). Évaluation de la diversité génétique des sorghos à grains sucrés (*Sorghum bicolor* (L.) Moench) du Nord du Burkina Faso. *Journal of Applied Biosciences, 84*(1), 7654. https://doi.org/10.4314/jab.v84i1.3

Serba, D. D., & Yadav, R. S. (2016). Genomic tools in pearl millet breeding for drought tolerance: Status and prospects. *Frontiers in Plant Science, 7*, 1724. https://doi.org/10.3389/fpls.2016.01724

Shinde, H., Dudhate, A., Anand, L., Tsugama, D., Gupta, S. K., Liu, S., & Takano, T. (2020). Small RNA sequencing reveals the role of pearl millet miRNAs and their targets in salinity stress responses. *South African Journal of Botany, 132*, 395-402. https://doi.org/10.1016/j.sajb.2020.06.011

Singh, A. K., Rana, M. K., Singh, S., Kumar, S., Durgesh, K., & Arya, L. (2013). Assessment of genetic diversity among pearl millet [*Pennisetum glaucum* (L.) R Br.] cultivars using SSR markers. *Range Management and Agroforestry, 34*(1), 77-81. https://publications.rmsi.in/index.php/rma/article/view/459/426

Singh, S., & Gupta, S. K. (2019). Formation of heterotic pools and understanding relationship between molecular divergence and heterosis in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *PLoS ONE, 14*(5), e0207463. https://doi.org/10.1371/JOURNAL.PONE.0207463

Stich, B., Haussmann, B. I. G., Pasam, R., Bhosale, S., Hash, C. T., Melchinger, A. E., & Parzies, H. K. (2010). Patterns of molecular and phenotypic diversity in pearl millet [*Pennisetum glaucum* (L.) R. Br.] from west and central Africa and their relation to geographical and environmental parameters. *BMC Plant Biology, 10*, 216. https://doi.org/10.1186/1471-2229-10-216

Tamura, K., Dudley, J., Nei, M., & Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution, 24*(8), 1596-1599. https://doi.org/10.1093/molbev/msm092

Triki, T., Bennani, L., Boussora, F., Tlahig, S., Ben Ali, S., Gasmi, A., Yahia, H., Belhouchette, K., Loumerem, M., & Guasmi, F. (2023). Characterization and trait association analysis of 27 pearl millet landraces in Southern Tunisia. *Agronomy, 13*(8), 2128. https://doi.org/10.3390/agronomy13082128

Vom Brocke, K., Christinck, A., Weltzien, R. E., Presterl, T., & Geiger, H. H. (2003). Farmers' seed systems and management practices determine pearl millet genetic diversity patterns in semiarid regions of India. *Crop Science, 43*(5), 1680-1689. https://doi.org/10.2135/cropsci2003.1680

Yadav, O. P., Gupta, S. K., Govindaraj, M., Sharma, R., Varshney, R. K., Srivastava, R. K., Rathore, A., & Mahala, R. S. (2021). Genetic gains in pearl millet in India: Insights into historic breeding strategies and future perspective. *Frontiers in Plant Science, 12*, 645038. https://doi.org/10.3389/fpls.2021.645038

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