



Genetic diversity and population structure of Saharan bread wheat (*Triticum aestivum* L.) landraces from Algeria revealed by SSR markers

Mohamed Adel Berrezoug^{1*}, Amina Bellatreche², Sameh Rahmani Mnasri³,
Abdel Ilah Amrouche⁴, Yassine Moustafa Mahdad^{5,6}

¹Laboratory of Functional Agrosystems and Agronomic Chain Technology, Department of Agronomy, Faculty of Natural and Life Sciences, Earth and Universe, Abou-Bekr-Belkaïd University. 22, Rue Abi Ayad Abdelkrim, Fg Pasteur B.P 119, Tlemcen 13000, Algeria

* Corresponding Author Email: masteragronomie@gmail.com - ORCID: 0009-0007-3276-2877

²Laboratory of Applied genetic in agriculture, ecology and public health, Department of Agronomy, Abou-Bekr-Belkaïd University. Department of Biology, Faculty of Natural and Life Sciences, Earth and Universe. 22, Rue Abi Ayad Abdelkrim, Fg Pasteur B.P 119, Tlemcen 13000, Algeria

Email: amina_agronomie@yahoo.fr - ORCID: 0000-0003-0040-7444

³National Gene Bank of Tunisia, Street Yasser Arafet, 1080 Chargaia 1. Tunis, Tunisia

Email: mnasrisameh@yahoo.fr - ORCID: 0000-0002-9838-8618

⁴Laboratory of Sustainable Management of Natural Resources in Arid and Semi-arid Areas, SNV Department, Institute of Sciences, University Center of Salhi Ahmad, P.O. Box 66, Naâma 45000, Algeria

Email: abdelillahamrouche@gmail.com - ORCID: 0000-0001-8701-3821

⁵Laboratory of Applied genetic in agriculture, ecology and public health, Department of Agronomy, Abou-Bekr-Belkaïd University. Department of Biology, Faculty of Natural and Life Sciences, Earth and Universe. 22, Rue Abi Ayad Abdelkrim, Fg Pasteur B.P 119, Tlemcen 13000, Algeria

⁶Laboratory of Sustainable Management of Natural Resources in Arid and Semi-arid Areas, SNV Department, Institute of Sciences, University Center of Salhi Ahmad, P.O. Box 66, Naâma 45000, Algeria

Email: ya.crag@gmail.com - ORCID: 0000-0002-5993-1825

Article Info:

DOI: 10.22399/ijcesn.4896

Received: 05 December 2025

Revised: 27 January 2026

Accepted: 07 February 2026

Keywords

Genetic diversity
Population structure
Saharan landraces
SSR markers
Arid-zone adaptation

Abstract:

Bread wheat (*Triticum aestivum* L.) cultivation in the Algerian Sahara relies on traditional landraces that have evolved to withstand extreme hyper-arid environmental conditions. These local populations represent a vital yet under-utilized genetic resource for modern breeding programs aiming to improve drought and heat tolerance. This study investigated the genetic diversity and population structure of 16 local landraces collected from the Adrar and Tamanrasset regions in southern Algeria. Genotyping was performed using 13 polymorphic simple sequence repeat (SSR) markers selected for their reliable genome coverage. Genetic diversity parameters were estimated using standard metrics, while population structure was analyzed using Bayesian clustering approaches and Principal Coordinate Analysis (PCoA). Results revealed a total of 72 alleles across the studied loci, with a mean of 5.54 alleles per locus. The genetic diversity indices indicated a moderate level of diversity, with a mean polymorphic information content (PIC) of 0.57, a mean expected heterozygosity (H_e) of 0.573, and a mean observed heterozygosity (H_o) of 0.271. Analysis of Molecular Variance (AMOVA) revealed that the majority of genetic variation resided among individuals (56%) and within individuals (32%), with a significant 12% attributed to genetic differentiation between the two geographic populations. Bayesian STRUCTURE analysis and PCoA consistently identified two distinct genetic pools that largely corresponded to the geographic origin of the accessions, although some admixture was observed. Overall, the results demonstrate substantial genetic variability and a clear structured population pattern within Saharan bread wheat. These landraces represent a valuable genetic resource for conservation strategies and for future research and breeding efforts targeting wheat improvement under water-limited environments.

1. Introduction

Climate change poses an escalating threat to global agricultural productivity, particularly in arid and semi-arid regions where abiotic stresses such as water scarcity, extreme heat, and soil salinity severely limit crop potential. To maintain food security in these vulnerable environments, enhancing crop resilience has become a priority. This requires the identification, conservation, and effective utilization of genetic resources that possess adaptive traits for marginal conditions. Traditional landraces, which have evolved over centuries under the pressure of local environments and farmer selection, represent unique reservoirs of genetic variability. Unlike modern high-yielding varieties, which often have a narrow genetic base due to intensive selection for favorable conditions, landraces retain allelic diversity that confers tolerance to biotic and abiotic stresses [1] ; [2]. Consequently, characterizing these genetic resources has gained increasing importance in breeding programs targeting drought-prone agroecosystems [3].

Bread wheat (*Triticum aestivum* L.) is the most widely cultivated cereal worldwide, occupying over 17% of global arable land and providing nearly 20% of the daily caloric and protein intake for the human population [4] ; [5]. Despite its critical role in food security, wheat yield stagnation has become a major concern, especially as global food demand is projected to rise by 40% by 2030 and could potentially double by 2050 [6] ; [7] ; [8]. The situation is particularly critical in North Africa, a region identified as a climate change "hotspot," where wheat productivity remains heavily constrained by recurrent drought, erratic rainfall, poor soil fertility, and limited success in genetic improvement programs adapted to local constraints [9] ; [10].

In Algeria, wheat is a strategic commodity and a dietary staple; however, national production is far from meeting domestic needs. Average yields remain low, reaching only 1.4 t/ha in 2021, while wheat imports are projected to exceed 7 million tons in 2024 [11] ; [12]. Reducing this import dependency requires a shift towards more resilient agricultural systems and the valorization of local germplasm. In this context, the southern Saharan regions of Adrar and Tamanrasset offer a unique opportunity. These hyper-arid regions maintain traditional *T. aestivum* landraces cultivated under ancient oasis-based systems. These populations have been naturally selected for survival under extreme heat and water stress, exhibiting phenotypic traits such as early maturity and stability under harsh conditions [13] ;

[14]. Understanding and conserving these Saharan landraces is therefore not only a matter of heritage preservation but a crucial component of a broader strategy to increase national crop resilience. Despite their proven agronomic potential, these local varieties remain poorly characterized at the molecular level. While phenotypic evaluations have been conducted, genetic studies on Algerian bread wheat remain limited [15].

Molecular markers, particularly Simple Sequence Repeats (SSRs), are powerful tools for assessing genetic diversity and population structure in self-pollinating crops due to their codominant inheritance, high polymorphism, and genome-wide distribution [16] ; [17]. Although a pioneering study by [18] provided preliminary insights, a comprehensive analysis of the genetic architecture of landraces from the hyper-arid South is still lacking. To bridge this gap, it is essential to analyze the genetic variation of this germplasm, particularly using markers located near genomic regions associated with drought tolerance, to better understand their adaptive potential.

Recent Genome-Wide Association Studies (GWAS) have successfully identified SNP loci and candidate genes associated with drought-related traits in bread wheat [19] ; [20]. Such genomic analyses are therefore crucial not only to understand the evolutionary dynamics and adaptive potential of these genotypes, but also to provide concrete genomic targets to inform breeding strategies for improving wheat resilience in arid and marginal environments.

Therefore, the present study aims to characterize the molecular diversity of Saharan bread wheat landraces to support their conservation and utilization. Specifically, the objectives were to: (i) assess SSR-based genetic diversity and population structure of landraces collected from Adrar and Tamanrasset; and (ii) identify the most informative SSR loci for discriminating this germplasm. By providing a detailed genetic baseline, this study intends to facilitate the integration of these resilient landraces into future breeding efforts targeting wheat improvement for arid and water-limited environments.

2. Material and Methods

2.1 Plant material

Sixteen bread wheat (*Triticum aestivum* L.) landraces were collected from the Adrar and Tamanrasset regions (Table 1). For molecular analysis, genomic DNA was isolated from fresh

young leaves of 7-day-old seedlings obtained via *in vitro* germination.

2.2 DNA isolation and SSR analysis

Total genomic DNA was extracted from fresh young leaves at the 3-leaf stage using the CTAB method described by [21]. For each accession, leaf tissue was pooled from multiple individual plants to obtain a representative bulk sample. DNA isolation was conducted at the National Gene Bank of Tunisia (BNG). The quality and concentration of the DNA were determined through 0.8% agarose gel

electrophoresis and with a Nanodrop spectrophotometer (BioSpec-nano; Shimadzu Biotech).

Genetic profiling was performed using thirteen SSR primers (Table 2) selected from the International Wheat Microsatellite Consortium (WMC). These primers are highly polymorphic and provide wide genome coverage, spanning chromosomes 1A, 1B, 1D, 2A, 2D, 3B, 4B, 5A, 5D, 6D, 7A, and 7D. Additionally, specific primers were selected from genomic regions previously associated with drought- and heat-related quantitative trait loci (QTLs) [22] and [23]

Table 1. Origin, collection sites, and local names of 16 Saharan bread wheat (*Triticum aestivum* L.) accessions from Algeria

Region	Accession	Collection site	Longitudes (E)	Latitudes (N)	Altitude (m)
Adrar	Omroukba	Zaouiat Kounta	0°12'00.52	27°13'00.28	189
	Sabagha	Touat	0°14'12.69	27°51'25.92	269
	El Farh	Tilouline	0°08'24.00	27°05'48.00	170
	Bemmebrouk	Aguil	0°17'38.00	27°52'27.40	258
	Amouch	Deldoul	0°15'34.77	29°01'10.40	282
	Belmebrouk	Zaouiat	0°14'18.29	29°15'41.81	288
	El Menea	El Habla Tsabit	0°13'06.97	28°21'01.45	260
	Bahamoud	Zaouiat	0°14'18.29	29°15'41.81	288
Tamanrasset	Bourabraa	Abalessa	4°04'10.36	22°11'16.00	601
	Bent M'barak1	Tazrouk	6°15'40.92	23°25'17.12	1814
	Manga3	Abalessa	4°04'10.36	22°11'16.00	601
	Tarouzi	Abalessa	4°04'10.36	22°11'16.00	601
	Hribcha	Aïn Amguel	3°25'19.93	24°32'28.27	633
	Manga El Beyda	Idless	5°56'03.64	23°49'03.80	1398
	Labyadh	Tazrouk	6°15'40.92	23°25'17.12	1814
	Manga	Idless	5°56'03.64	23°49'03.80	1398

2.3 PCR amplification and electrophoresis

PCR amplification was performed in a 20 µL reaction volume containing 50 ng genomic DNA, 4.0 mM MgCl₂, 200 µM of each dNTP, 0.25 µM of each primer, 0.4 U Hot-start Taq polymerase, and 1× PCR buffer. Amplifications were carried out in a thermal cycler under the following conditions: initial denaturation at 95°C for 3 min; followed by 35 cycles of 95°C for 30 s, primer-specific annealing temperature (Table 2) for 1 min, and 72°C for 30 s; with a final extension at 72°C for 10 min. PCR products were separated on 3% agarose gels in 1× TAE buffer, stained with ethidium bromide, and visualized under UV light. Fragment sizes were estimated by comparison with 50 bp and 100 bp DNA ladders.

2.4 Analysis of molecular data

Allelic profiles of the 16 bread wheat genotypes, generated using 13 SSR markers, were compiled into a matrix based on allele sizes for each microsatellite locus. This dataset was analyzed using GenAlEx v6.5 [24] to calculate the number of alleles per locus (Na), effective number of alleles (Ne), Shannon's information index (I) [25], observed heterozygosity (Ho), expected heterozygosity (He), and fixation index (F) [26]. GenAlEx was also employed to perform Principal Coordinate Analysis (PCoA) based on inter-individual relationships derived from Nei's unbiased pairwise genetic distance matrix. Molecular variance within and among populations was assessed through Analysis of Molecular Variance (AMOVA). Additionally, the informativeness of the SSR primers was evaluated using Cervus 3.0 software [27], which calculated the polymorphic information content (PIC) for each marker [28]. The sizes of the SSR amplified bands

were utilized to compute Jaccard's dissimilarity coefficients. The correlation between Jaccard and Dice distance matrices was assessed by a Mantel test with 1,000 permutations, revealing a strong correlation ($r = 0.99$, $p < 0.001$). The Jaccard matrix subsequently informed the construction of phylogenetic tree topologies using the neighbor-joining (NJ) method. This analysis was conducted with 1,000 replications, employing DARwin software version 6.0.010 [29].

Genetic structure was inferred using a Bayesian clustering algorithm implemented in STRUCTURE

v2.3.4 [30]. The analysis was conducted using an admixture model with uncorrelated allele frequencies, suitable for local populations where historical gene flow may have occurred. Ten independent runs were performed for each value of K ($K = 1-10$), with a burn-in period of 100,000 iterations followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations. The most probable number of genetic clusters (K) was inferred using the ΔK method of [31].

Table 2. Characteristics of 13 SSR primers used for genetic diversity analysis of 16 Saharan bread wheat (*Triticum aestivum* L.) landraces

Primer	Sequences	Chromosome location	Annealing temperatures	Alleles size (pb)
Gwm131 F Gwm131 R	AATCCCCACCGATTCTTCTC AGTTCGTGGGTCTCTGATGG	1B	55	130 – 300
Gwm325 F Gwm325 R	TTTCTTCTGTCGTTCTCTTCCC TTTTTACGCGTCAACGACG	6D	52	138 – 152
Gwm165 F Gwm165 R	TGCAGTGGTCAGATGTTTCC CTTTTCTTTCAGATTGCGCC	4B	52	180 – 290
Gwm129 F Gwm129 R	TCAGTGGGCAAGCTACACAG AAAACCTTAGTAGCCGCGT	5A	52	130 – 400
Gwm174 F Gwm174 R	GGGTCCTATCTGGTAAATCCC GACACACATGTTCCCTGCCAC	5D	54	210
Gwm99 F Gwm99 R	AAGATGGACGTATGCATCACA GCCATATTTGATGACGCATA	1A	54	120 – 165
Gwm111 F Gwm111 R	TCTGTAGGCTCTCTCCGACTG ACCTGATCAGATCCCCTCG	7D	54	130 – 250
Barc11 F Barc11 R	GCGATGCGTGTAAGTCTGAAGATGA GCGTCCATGGAGCTCTGTTTTATCTGA	2D	55	80 – 300
Barc101 F Barc101 R	GCTCCTCTCACGATCACGCAAAG GCGAGTCGATCACACTATGAGCCAATG	3B	55	60 – 200
Barc108 F Barc108 R	GCGGGTCGTTTCCTGGAAATTCATCTAA GCGAAATGATTGGCGTTACACCTGTTG	7A	55	100 – 260
Barc126 F Barc126 R	CCATTGAAACCGGATTTGAGTCG CGTTCATCCGAAATCAGCAC	7D	56	200 – 250
Wmc177 F Wmc177R	AGGGCTCTCTTTAATTCTTGCT GGTCTATCGTAATCCACCTGTA	2A	57	115 – 295
Wmc179 F Wmc179R	CATGGTGGCCATGAGTGGAGGT CATGATCTTGCCTGTGCGTAGG	1D	56	110 – 300

3. Results and Discussions

3.1 Results

3.1.1 Genetic diversity parameters

The analysis of genetic diversity using 13 SSR markers revealed significant allelic variation among

the 16 bread wheat accessions. A total of 72 alleles were detected across the analyzed SSR loci, reflecting a high level of polymorphism among Saharan wheat accessions. The number of alleles (Na) per locus ranged from 1 (*Barc126*, *Wmc179*) to 11 (*Gwm174*, *Gwm111*), with a mean of 5.54. The effective number of alleles (Ne) varied from 1.000 to 8.182 (mean = 3.341). Shannon's information index (I) ranged from 0.000 to 2.231, with an average of 1.221.

Observed heterozygosity (Ho) ranged from 0.000 to 1.000 (mean = 0.271), while expected heterozygosity (He) ranged from 0.000 to 0.878 (mean = 0.573). The mean Ho was lower than He, which is consistent with the self-pollinating nature of wheat, although some loci (e.g., *Gwm111*) displayed high heterozygosity. The Polymorphism Information Content (PIC) ranged from 0.000 to 0.870, with a mean value of 0.570, indicating that the markers used were highly informative (Table 3).

Table 3. Genetic diversity parameters revealed by 13 SSR markers in 16 Saharan bread wheat (*Triticum aestivum* L.) accessions.

Locus	Na	Ne	I	Ho	He	F	PIC
Gwm131	8	4.225	1.698	0.692	0.763	0.093	0.760
Gwm325	5	3.846	1.471	0.200	0.740	0.730	0.740
Gwm165	6	2.273	1.167	0.733	0.560	-0.310	0.560
Gwm129	3	1.744	0.765	0.000	0.427	1.000	0.420
Gwm174	11	5.844	2.044	0.667	0.829	0.196	0.820
Gwm99	6	3.449	1.484	0.154	0.710	0.783	0.710
Gwm111	11	8.182	2.231	1.000	0.878	-0.139	0.870
Barc11	4	2.048	0.939	0.077	0.512	0.850	0.510
Barc101	6	3.261	1.430	0.000	0.693	1.000	0.690
Barc108	4	2.390	1.055	0.000	0.582	1.000	0.580
Barc126	1	1.000	0.000	0.000	0.000	NaN	0.000
Wmc179	1	1.000	0.000	0.000	0.000	NaN	0.000
Wmc177	6	4.172	1.594	0.000	0.760	1.000	0.760
Total	72	43.435					
Mean	5.538	3.341	1.221	0.271	0.573	0.077	0.570

Na = No. of Different Alleles; Ne = No. of Effective Alleles ($1 / \sum p_i^2$); I = Shannon's Information Index ($-\sum p_i \times \ln(p_i)$); Ho = Observed Heterozygosity (number of heterozygotes / N); He = Expected Heterozygosity ($1 - \sum p_i^2$); F = Fixation Index; PIC = Polymorphism Information Content; NaN: Not Calculated (monomorphic locus)

3.1.2 Analysis of molecular variance (AMOVA)

The Analysis of Molecular Variance (AMOVA) was performed to assess the partitioning of genetic diversity between the two geographic regions (Adrar and Tamanrasset). The results indicated that only 12% of the total genetic variance was explained by differences between these regions (Table 4). The majority of the genetic variation (56%) was found among individuals within populations, while 32% was attributed to variation within individuals. This high level of intra-population diversity suggests significant genetic heterogeneity within the local wheat landraces cultivated in these oases.

3.1.3 Principal Coordinate Analysis (PCoA)

The Principal Coordinate Analysis (PCoA), based on Nei's genetic distance, showed that the first three axes accounted for 19.19%, 16.21%, and 11.46% of the total genetic variation, respectively. Collectively, the first two axes explained 35.40% of the variation,

while the total diversity explained by the three axes reached 46.86%. The PCoA plot differentiated the bread wheat genotypes into two main groups (Figure 1). The first group, positioned on the left side of the plot, included most accessions from the Adrar region. Within this group, two subclusters were observed: one comprised *Amouch*, *El Menea*, and *Bemmebrouk*, and the second included *Sabagha* and *El Farh*. The second group, on the right side, consisted mainly of accessions from Tamanrasset, with two subclusters: one included *Tarouzi*, *Bourabraa*, *Bahamoud*, and *Belmebrouk*; the other grouped *Manga3*, *Bent M'barak1*, *Manga*, *Labyadh*, and *Manga El Beyda*. Two accessions showed distinct positions: *Omroukba* (from Adrar) clustered among Tamanrasset accessions, while *Hribcha* (from Tamanrasset) appeared isolated. Comparison with STRUCTURE results suggests that these atypical positions may reflect potential admixture or distinct genetic backgrounds for these specific accessions.

Table 4. Analysis of molecular variance (AMOVA) among 16 Algerian Saharan bread wheat (*Triticum aestivum* L.) accessions using 13 SSR markers.

Source	Df	SS	MS	Est. Var.	%
Among Pops	1	16.313	16.313	0.588	12
Among Indiv	14	96.563	6.897	2.683	56
Within Indiv	16	24.500	1.531	1.531	32
Total	31	137.375		4.803	100

**Figure 1.** Principal Coordinates Analysis (PCoA) of 16 Saharan bread wheat accessions based on SSR data and Nei's genetic distance. The first three axes explained 46.86% of the total variation, revealing a partial separation among accessions from different regions

3.1.4 Genetic relationships and population structure

Bootstrapping analysis of the neighbor-joining (NJ) tree revealed clear differentiation among all accessions, with genetic identity values ranging from 0.023 to 0.93. The highest similarity was observed between *Manga* and *Labyadh*, while the lowest was between *Hribcha* and *El Farh*. The neighbour-joining dendrogram (Figure 2) revealed three main clusters, supported by bootstrap values ranging from 20% to 100%. Cluster I included three subclusters: *Manga3* with *Bent M'barak1*, *Tarouzi* with *Bourabraa*, and *Bahamoud* with *Belmebrouk*. Cluster II consisted of four subclusters: *Manga* with *Labyadh*, *Manga El Beyda*, *Hribcha*, and *Omroukba*. Cluster III comprised *El Menea* with *Amouch*, *Bemmebrouk*, *El Farh*, and *Sabagha*.

Regarding population structure, the Bayesian clustering analysis performed in STRUCTURE provided complementary insights. Consistent with the clustering approach described by [32] and using the method of [31], a clear peak in ΔK was observed at $K = 2$ (Figure 3). Support for higher K values was substantially lower, as ΔK dropped sharply beyond $K = 2$, suggesting no further significant substructure.

This indicates that the optimal number of genetic clusters is two. This result was supported by the bar plot of population structure (Figure 4), where individuals were assigned to two distinct gene pools. Group 1 (red), predominantly representing the Adrar region, included *Omroukba*, *Sabagha*, *El Farh*, *Bemmebrouk*, *Amouch*, and *El Menea*, along with three accessions from Tamanrasset (*Belmebrouk*, *Bahamoud*, and *Hribcha*), suggesting potential gene flow. Group 2 (green) consisted exclusively of accessions from Tamanrasset, comprising *Bourabraa*, *Bent M'barak1*, *Manga3*, *Tarouzi*, *Manga El Beyda*, *Labyadh*, and *Manga*. Overall, this clustering pattern was largely consistent with the groupings observed in PCoA and NJ analyses, although *Omroukba* and *Hribcha* showed the most atypical positions across the different methods.

3.2 Discussions

3.2.1 Genetic diversity patterns and marker utility

Understanding the genetic structure and diversity of Saharan bread wheat landraces is essential for conserving adaptive variation and supporting

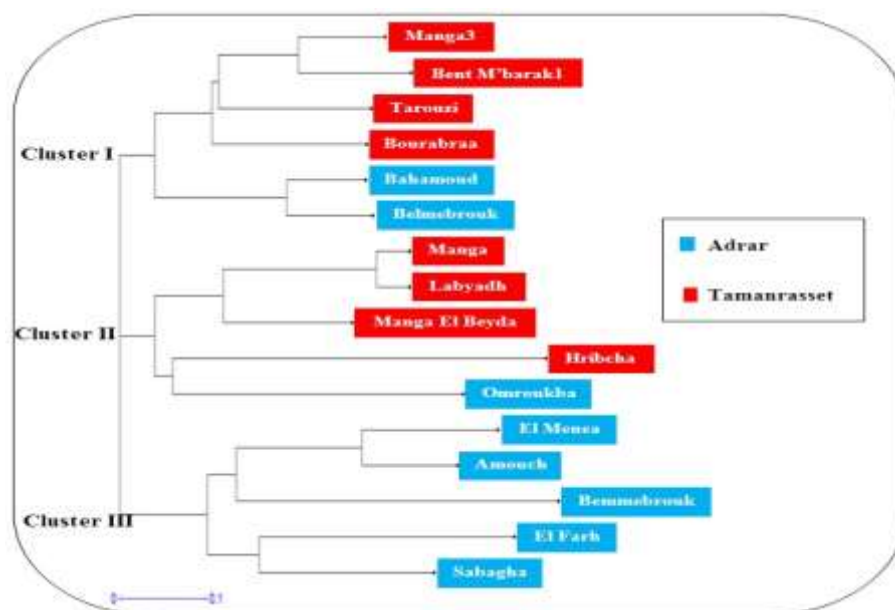


Figure 2. Neighbor-joining (NJ) tree of 16 Saharan bread wheat accessions based on Jaccard's genetic distances

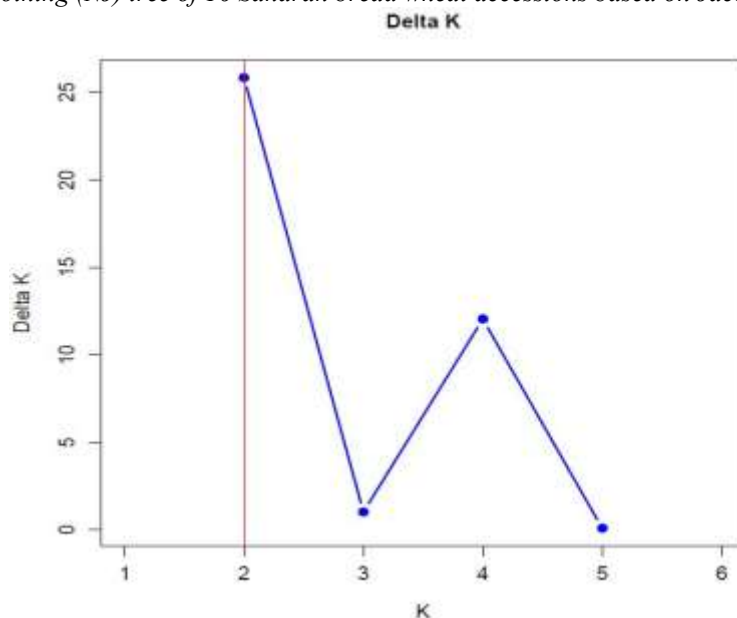


Figure 3. Determination of the optimal number of genetic clusters (K) based on the method of [31]. The clear peak in ΔK observed at $K = 2$ indicates the most probable number of genetic groups within the studied accessions

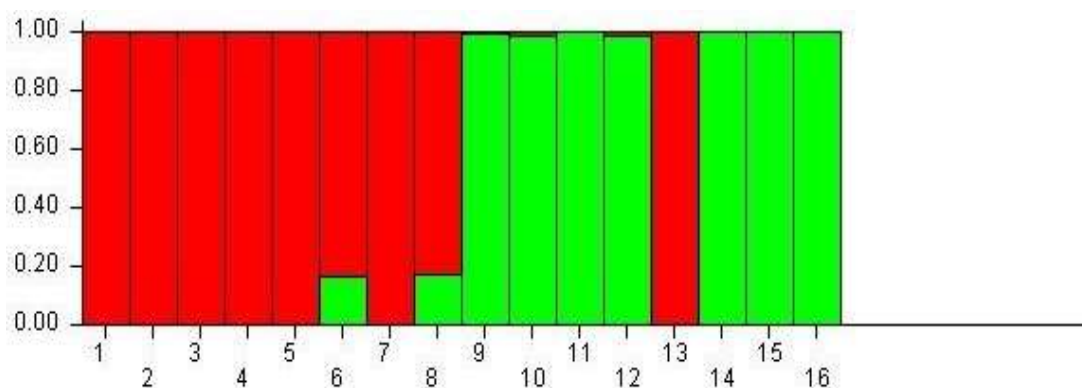


Figure 4. Bar plot of population structure for 16 Saharan bread wheat accessions inferred by STRUCTURE analysis at $K=2$. Each vertical bar represents a single individual, and the colors indicate the proportional membership to the two identified gene pools (Group 1: Red; Group 2: Green).

breeding efforts aimed at improving drought resilience [33] ; [34]. The findings of this study reveal that despite the harsh environmental conditions of the Algerian Sahara, marked by prolonged drought and extreme temperatures, local wheat populations maintain a remarkably high level of genetic diversity.

The analysis revealed a moderate level of genetic diversity across the population, with a mean number of alleles (N_a) of 5.54, a mean effective number of alleles (N_e) of 3.34, and a mean expected heterozygosity (H_e) of 0.573. However, considerable variation was observed among markers, with some loci such as *Gwm111* reaching high values ($H_e = 0.878$). *Gwm111* has previously been identified as highly informative in wheat genotypes evaluated under drought-prone environments [35], confirming its high discriminatory power. These diversity levels are higher than those previously reported for Saharan germplasm [36] ; [37] and align with recent findings in similar marginal environments [38], suggesting that these landraces have retained substantial allelic richness. This may be attributed to traditional farming practices, such as seed exchange among farmers, which counteract the effects of regional isolation and genetic drift [39]. It is worth noting that the genetic diversity observed in this study ($H_e = 0.573$) is comparable to, or even higher than, diversity levels reported in some global collections of bread wheat. This implies that while modern elite cultivars often suffer from genetic erosion due to intensive selection [40], Saharan oases act as reservoirs of allelic variation. Regarding marker utility, the Polymorphism Information Content (PIC) indicated that the selected SSRs were highly informative, with a mean value of 0.57. This mean PIC value is slightly lower than values reported for larger, multi-country panels [41] ; [42], but reflects the high genetic differentiation of local populations. While *Gwm174* is generally regarded as weakly polymorphic in broader wheat collections, it exhibited notable variability in the present study. This suggests that specific loci may be hyper-variable in Saharan populations due to local adaptation or distinct evolutionary histories, making them particularly useful for discriminating germplasm in arid regions [43].

The fixation index (F) varied significantly across loci. As expected for a predominantly self-pollinating species like wheat, many markers (e.g., *Gwm129*, *Barc101*) showed high levels of homozygosity ($F \approx 1$). However, the negative F values observed for other loci indicate an excess of heterozygotes. This heterozygosity might be

maintained by occasional outcrossing events, which are known to occur in landraces, or could be a signature of artificial selection favoring specific heterozygous genotypes in traditional farming systems.

3.2.2 Population structure and local adaptation

The analysis of molecular variance (AMOVA) and Bayesian clustering revealed a clear genetic structure organized into two main gene pools, largely corresponding to the geographic regions of Adrar and Tamanrasset. Comparable spatially structured patterns have been reported in other stress-prone environments [44], supporting the idea that environmental gradients play a major role in shaping genetic diversity. This geographic differentiation was further supported by the presence of private alleles specific to each region, highlighting the unique contribution of each population to the overall genetic diversity. However, the AMOVA also revealed a substantial proportion of variation within individuals (32%). Although bread wheat is primarily autogamous, this result suggests the presence of residual heterozygosity, likely resulting from the heterogeneous nature of landrace seed stocks rather than recent mutation.

Interestingly, the genetic clustering observed here mirrors morphological differentiation reported in previous studies, where Adrar and Tamanrasset populations differed in spike architecture and grain size [45]. This congruence between molecular and phenotypic divergence suggests that the observed genetic structure is not merely a result of isolation by distance, but likely reflects genetic isolation and potentially selection pressures exerted by specific local micro-environments and farmers in these two oases. The identification of admixed individuals (e.g., *Hribcha*) further points to historical or recent seed exchange routes between these seemingly isolated regions.

3.2.3 Implications for conservation and breeding

Given the distinct genetic structure identified between Adrar and Tamanrasset, a conservation strategy focusing on a single location would arguably fail to capture the total diversity of Algerian Saharan wheat. Therefore, our results support an *in situ* conservation approach to maintain these landraces in their respective oases, allowing continued evolution under changing climatic conditions and preventing the genetic erosion threatening traditional germplasm [40]. From an applied perspective, these results provide valuable insights for wheat improvement programmes. The identification of private alleles and structured gene

pools offers opportunities for the development of pre-breeding populations that incorporate drought resilience traits. Integrating molecular markers such as *Gwm111* and *Gwm174* into breeding pipelines could accelerate the selection of superior genotypes [46]. However, further work is required to confirm marker-trait associations through phenotyping under controlled water-deficit conditions [47] and to determine whether these allelic patterns are region-specific or reflect broader adaptation mechanisms [48]. Finally, since SSR markers are neutral, future research should prioritize genotyping with high-density markers, such as SNPs, to enable Genome-Wide Association Studies (GWAS). Integrating these genomic tools with precise phenotypic data, such as the agronomic evaluations by [45] under drought stress, will be crucial to pinpoint the genomic regions responsible for adaptation and to facilitate marker-assisted selection for climate-resilient cultivars [46].

3.2.4 Limitations and future perspectives

Finally, we acknowledge certain limitations of this study. The sample size (16 accessions) and the number of markers (13 SSRs), while sufficient for a baseline assessment of diversity, limit the power of fine-scale population structure inference. Additionally, the use of agarose gel electrophoresis, although cost-effective, has lower resolution than capillary electrophoresis, potentially underestimating the total number of alleles. Future studies should prioritize high-throughput genotyping (e.g., SNPs) and exhaustive sampling to validate these findings. Furthermore, while the observed structure correlates with geographic origin, the link to drought adaptation remains hypothetical and requires validation through physiological assays.

4. Conclusions

This study provides a comprehensive assessment of the genetic diversity and population structure of Saharan bread wheat (*Triticum aestivum* L.) landraces. The results reveal substantial allelic richness and moderate genetic diversity. In particular, the marker *Gwm174*, often reported as low-polymorphic in global collections, exhibited high informativeness in this study, highlighting the specific genetic variability maintained in these Saharan populations. The Bayesian clustering analysis (K=2) identified two distinct gene pools largely corresponding to the Adrar and Tamanrasset regions, suggesting that environmental gradients and traditional seed management play a major role in shaping genetic differentiation. While the observed variation reflects population history rather than proven functional adaptation, these landraces

represent a crucial genetic reservoir. Conserving this germplasm is essential to prevent genetic erosion, and future research integrating high-density genotyping with phenotypic screening will be key to unlocking their potential for breeding drought-resilient varieties.

Author Statements:

- **Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.
- **Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
- **Acknowledgement:** The authors express their gratitude to the Team Leader of the laboratory at the Laboratory for the Sustainable Management of Natural Resources in Arid and Semi-Arid Zones at the University Center Salhi Ahmed Naama, Algeria, for his support, guidance, and contribution. The authors also thank the National Gene Bank (BNG) of Tunisia, represented by its General Manager, for his support. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
- **Author contributions:** All authors contributed to the study conception, design, and data analysis, and read and approved the final manuscript.
- **Funding information:** The authors received no specific funding for this work.
- **Data availability statement:** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

- [1] S. Ceccarelli, «Efficiency of Plant Breeding,» *Crop Science*, vol. 55(1), pp. 87-97, 2015, DOI: 10.2135/cropsci2014.02.0158.
- [2] Halewood M., Chiurugwi T., Hamilton R. S., Kurtz B., Marden E., Welch E., Michiels F., Mozafari J., Sabran M., Patron N., Kersey P., Bastow R., Dorius S., Dias S., McCouch S. & Powell W., «Plant genetic resources for food and agriculture: opportunities and challenges emerging from the science and information technology revolution,» *New Phytologist Foundation*, vol. 217(4), pp. 1407-1419, 2018, DOI:10.1111/nph.14993.
- [3] Lopes M.S and Reynolds M.P., «Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology,»

- Journal of Experimental Botany*, vol. 63(10), p. 3789–3798, 2012, DOI: 10.1093/jxb/ers071.
- [4] Shi X. & Ling H-Q., «Current advances in genome sequencing of common wheat and its ancestral species,» *The Crop Journal*, vol. 6(1), pp. 15-21, 2017, DOI:10.1016/j.cj.2017.11.001.
 - [5] Bapela T., Shimelis H., Tsilo T.J. & Mathew I., «Genetic Improvement of Wheat for Drought Tolerance: Progress, Challenges and Opportunities,» *Plants*, vol. 11(10), p. 1331, 2022, DOI: 10.3390/plants11101331.
 - [6] Reynolds M., Foulkes J., Furbank R., Griffiths S., King J., Murchie E., Parry M. & Slafer G., «Achieving yield gains in wheat,» *Plant, cell & Environment*, vol. 35(10), pp. 1799-1823, 2012, DOI: 10.1111/j.1365-3040.2012.02588.x.
 - [7] Dixon J., Braun H-J., Kosina P. & Crouch J., «Wheat facts & futures 2009,» CIMMYT, Mexico City, Mexic, 2009.
 - [8] Li Y-C., Korol A.B., Tzion F. & Eviatar N., «Microsatellites within genes: structure, function, and evolution,» *Mol Biol Evol*, vol. 21(6), pp. 991-1007, 2004, DOI: 10.1093/molbev/msh073.
 - [9] Nielsen N.H., Gunter B., Jens S., Stig U.A. & Jahoor A., «Genetic diversity and population structure analysis of European hexaploid bread wheat (*Triticum aestivum* L.) varieties,» *PLOS ONE*, vol. 9, 2014, DOI:10.1371/journal.pone.0094000.
 - [10] Kara K., Rached-Kanouni M., Mnasri S. & Ben Nacer M., «Genetic variability assessment in bread wheat (*Triticum aestivum*) grown in Algeria using microsatellites SSR markers,» *Biodiversitas*, vol. 21(6), pp. 2638-2644, 2020, DOI:10.13057/biodiv/d210635.
 - [11] FAO, «Food and Agriculture Organization,» 2023. [En ligne]. Available: <http://faostat.fao.org>.
 - [12] B. Benarba, «Enhancing food security through scientific research in Algeria. A novel vision and unified national strategy,» *Nor. Afr. J. Food Nutr. Res*, vol. 8(18), p. 68 – 69, 2024, DOI:10.51745/najfnr.8.18.68-69.
 - [13] Oumata S., Amouroux P., De Rouw A. & Kane N. A., «Oasis wheats of the South of Algeria: landraces, cultural practices and utilization,» *Genetic Resources and Crop Evolution*, vol. 67, p. 211–230, 2020, DOI:10.1007/s10722-019-00874-7.
 - [14] Oumata S, Monneveux P., Zaharieva M., Mekliche-Hanifi L. & Jacques D, «Variation of morphological traits among wheat (*Triticum aestivum* L.) landraces from two regions of the Algerian Sahara: Potential interest for wheat breeding,» *Genetic Resources and Crop Evolution*, vol. 70, p. 235–250, 2023, DOI:10.1007/s10722-022-01429-z.
 - [15] Bellatreche A., Mahdad M.Y., Kaouadji Z. & Gaouar S.B.S., «Agro-morphological diversity of some accessions of bread wheat (*Triticum aestivum* L.) in western Algeria,» *Biodiversitas*, vol. 18, p. 409–415, 2017.
 - [16] Sehgal S.A., Tahir R.A. & Nawaz M., «Molecular Characterization of Wheat Genotypes Using SSR Markers,» *Bio International Journal Automation*, vol. 16(2), pp. 119-128, 2012, DOI:10.1007/s12298-017-0492-1.
 - [17] Vieira M.L.C., Santini L., Diniz A.L. & Munhoz C.F., «Microsatellite markers: What they mean and why they are so useful,» *Genetics and Molecular Biology*, vol. 39(3), p. 312–328, 2016, DOI:10.1590/1678-4685-GMB-2016-0027.
 - [18] Bellatreche A., Mnasri S.R., Ben Naceur M. & Gaouar S.S.B., «Study of the Molecular Biodiversity of the Saharan Bread Wheat in Algeria,» *Cereal Research Communications*, pp. 1-16, 2019, DOI:10.1556/0806.47.2019.39.
 - [19] Rabieyan E., Bihamta M.R., Esmaeilzadeh M., Alipour H., Mohammadi V., Azizyan K., Javid S., «Analysis of genetic diversity and genome-wide association study for drought tolerance related traits in Iranian bread wheat,» vol. 23(431), 2023, DOI:10.1186/s12870-023-04416-3.
 - [20] Nouraei S., Mia M.S., Liu H., Turner N.C. and Guijun Y, «Genome-wide association study of drought tolerance in wheat (*Triticum aestivum* L.) identifies SNP markers and candidate genes,» *Molecular Genetics and Genomics*, vol. 299(1), p. 22, 2024, DOI: 10.1007/s00438-024-02104-x.
 - [21] Saghai-Marroof M.A., Soliman K.M., Jorgensen R.A & Allard R.W., «Ribosomal DNA sepaer-length polymorphism in barley: mendelian inheritance, chromosomal location, and population dynamics,» *P. Natl. Acad. Sci*, vol. 81, p. 8014–8019, 1984.
 - [22] Alhosein H., Miyuki Ni., Shuhei., Kenji., Masaya F., Hitoshi M & Yutaka O., «Novel QTLs for growth angle of seminal roots in wheat (*Triticum aestivum* L.),» *Plant Soil*, p. 395–405, 2012, DOI: 10.1007/s11104-011-1075-5.
 - [23] Ashraf S., Shahzad A., Karamat F., Iqbal M. & Ali G.M., «Quantitative trait loci (QTLs) analysis of drought tolerance at germination stage in a wheat population derived synthetic hexaploid and opata,» *J. Anim. Plant. Sci*, vol. 25(2), p. 539–545, 2015, DOI: 10.5555/20153154245.
 - [24] Peakall R. & Smouse P.E., «Smouse, P.E. GenALEX 6.5: Genetic analysis in Excel. Population genetic software for teaching and research an update,» *Bioinformatics*, vol. 28, p. 2537–2539, 2012, DOI: 10.1093/bioinformatics/bts460.
 - [25] C. Shannon, «Weaver, W. A mathematical theory of communication,» *Bell Syst. Tech. J*, vol. 27, p. 379–423, 1948.
 - [26] Wright, «The genetical structure of populations,» *Ann. Eugen*, vol. 15, p. 323–354, 1951.
 - [27] Kalinowski S., Taper M.L. & Marshall T.C., «Revising how the computer program Cervus

- accommodates geno-typing error increases success in paternity assignment,» *Mol. Ecol.*, vol. 16, p. 1099–1106, 2007, DOI: 10.1111/j.1365-294X.2007.03089.x.
- [28] Botstein D., White R.L., Skolnick M. & Davis R.W., «Construction of a genetic linkage map in man using re-striction fragment length polymorphisms,» *Am. J. Hum. Genet.*, vol. 32, p. 314–331, 1980.
- [29] J. Felsenstein, «Confidence limits on phylogenies: An approach using the bootstrap,» *Evolution*, vol. 39(4), p. 783–791, 1985, <https://doi.org/10.2307/2408678>.
- [30] Pritchard J.K., Stephens M., Rosenberg N.A. & Donnelly P., «Association mapping in structured populations,» *AJHG*, vol. 67, p. 170–181, 2000.
- [31] Evanno G., Regnaut S. & Goudet J., «Detecting the number of clusters of individuals using the software STRUCTURE: assimilation study,» *Mol Ecol*, vol. 14, p. 2611–2620, 2005.
- [32] Rosenberg N.A., Pritchard J.K., Weber J.L., Cann H.M., Kidd K., Zhivotovsky L.A. & Feldman M.W., «Genetic structure of human populations,» *Science*, vol. 298, p. 2381–2385, 2002.
- [33] S. Ceccarelli, «Efficiency of Plant Breeding,» *Crop Science*, vol. 55(1), pp. 87-97, 2015, doi:10.2135/cropsci2014.02.0158.
- [34] Lopes M.S and Reynolds M.P., «Stay-green in spring wheat can be determined by spectral reflectance measurements (normalized difference vegetation index) independently from phenology,» *Journal of Experimental Botany*, vol. 63(10), n° %110, p. 3789–3798, 2012, DOI: 10.1093/jxb/ers071.
- [35] Faheem M., Talat M., Ghulam S., Naeem A., Alvina G.K. & Abdul Mujeeb K., «Assessment of D-genome Based Genetic Diversity in Drought Tolerant Wheat Germplasm,» *Int J Agric Biol*, vol. 17(4), p. 791–796, 2015.
- [36] Bellatreche A., Mnasri S.R., Ben Naceur M. & Gaouar S.S.B., «Study of the Molecular Biodiversity of the Saharan Bread Wheat in Algeria,» *Cereal Research Communications*, pp. 1-16, 2019, doi:10.1556/0806.47.2019.39.
- [37] Kara K., Mnasri-Rahmani S., Zerrouki A., Rached-Kanouni M., Hamidechi M.A., Saddoud-Dabbabi O., Beldjazia A. & Missaoui K., «Molecular Characterization, Assessment of Bread Wheat Genotypes Triticum aestivum L. Grown in Algeria using Microsatellites SSR Markers,» *AMA*, vol. 55(10), pp. 1-15, 2024.
- [38] Türkoğlu A., Haliloğlu K., Mohammadi S.A., Öztürk A., Bolouri P., Özkan G., Bocianowski J., Pour-Aboughadareh A. & Jamshidi B., «Genetic Diversity and Population Structure in Türkiye Bread Wheat Genotypes Revealed by Simple Sequence Repeats (SSR) Marker,» *Genes*, vol. 14, p. 1182, 2023, DOI: 10.3390/genes14061182.
- [39] V. Sork, «Gene flow and natural selection shape spatial patterns of genes in tree populations: implications for evolutionary processes and applications,» *Evol Appl.*, vol. 9(1), pp. 291-310, 2014, DOI: 10.1111/eva.12316.
- [40] Sall A.T., Chiari T., Legesse W., Seid-Ahmed K., Ortiz R., van Ginkel M. & Bassi F.M., «Durum wheat (*Triticum durum* Desf.): Origin, cultivation and potential expansion in Sub-Saharan Africa,» *Agronomy*, vol. 9(5), p. 263, 2019, DOI: 10.3390/agronomy9050263.
- [41] Özlem A.S. & Begüm T., «Characterization of some bread wheat genotypes using molecular markers for drought tolerance,» *Physiol Mol Biol Plants*, vol. 24(1), p. 159–166, 2018, DOI: 10.1007/s12298-017-0492-1.
- [42] Mohi-Ud-Din M., Alamgir H.M., Motiar R.M., Nesar Uddin. M., Sabibul Haque. M., Eldessoky S.D., Alqurashi M. & Salman A., «Assessment of Genetic Diversity of Bread Wheat Genotypes for Drought Tolerance Using Canopy Reflectance-Based Phenotyping and SSR Marker-Based Genotyping,» *Sustainability*, vol. 9818(14), pp. 1-19, 2022, DOI: 10.3390/su14169818.
- [43] Sehgal S.A., Tahir R.A. & Nawaz M., «Molecular Characterization of Wheat Genotypes Using SSR Markers,» *Bio Inter-national Journal Automation*, vol. 16(2), pp. 119-128, 2012, DOI:10.1007/s12298-017-0492-1.
- [44] Urquijo-Zamora L., Pereira-Lorenzo S., Romero-Rodríguez Á., Lombardero-Fernández M., Ramos-Cabrer A.M. & Fernández-Otero C.I., «Genetic Diversity of Local Wheat (*Triticum aestivum* L.) and Traceability in the Production of Galician Bread (Protected Geographical Indication) by Microsatellites,» *Agriculture*, vol. 15(1), p. 51, 2025, DOI: 10.3390/agriculture15010051.
- [45] Oumata S, Monneveux P., Zaharieva M., Mekliche-Hanifi L. & Jacques D, «Variation of morphological traits among wheat (*Triticum aestivum* L.) landraces from two regions of the Algerian Sahara: Potential interest for wheat breeding,» *Genetic Resources and Crop Evolution*, vol. 70, p. 235–250, 2023, DOI: 10.1007/s10722-022-01429-z.
- [46] Zhang H., Zhang F., Li G., Zhang S., Zhang Z. & Ma L., «Genetic diversity and association mapping of agronomic yield traits in eighty six synthetic hexaploid wheat,» *Euphytica*, vol. 213, p. 1–13, 2017, DOI: 10.1007/s10681-017-1887-3.
- [47] Ahmed H.G.M., Khan A., Kashif M. & Khan S., «Genetic mechanism of leaf venation and stomatal traits for breeding drought tolerant lines in wheat,» *Bangl J Bot*, vol. 46(1), pp. 35-41, 2017.
- [48] Devi J., Dubey R.K., Sagar V., Verma R.K., Singh P.M. & Behera T.K., «Vegetable peas (*Pisum sativum* L.) diversity: An analysis of available elite germplasm resources with relevance to crop improvement,» *Span J Agric Res*, vol. 21(2), pp. 1-13, 2023, DOI: 10.56093/ijas.v93i9.139173.