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RESEARCH ARTICLE

Harnessing genetic diversity in Sudanese sorghum wild relatives for stay-green drought tolerance via microsatellite (SSR) marker assessment

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Abstract Sudan is the birthplace of sorghum, and vast genetic diversity exists among its wild relatives. To assess the genetic potential of Sudan wild sorghum accessions, we used 41 stay-green-specific microsatellite (SSR) markers to analyze the genetic variability and population structure of 256 accessions. Overall, 17 SSR markers were polymorphic, with 55 alleles on average 3.3 per locus. The polymorphic information content (PIC) ranged from 0.49 to 0.57, with an overall mean of 0.53, indicating the potential of these markers for capturing the genetic construction of wild sorghum. Linkage disequilibrium analysis identified the two most informative markers, *Xcup05* and *Xtxp212*. Accordingly, the Nei gene diversity of the populations varied from 0.032 to 0.127, with an overall mean of 0.083. Molecular variance analysis (AMOVA) demonstrated that 99% and 1% of the genetic variations were within and among populations (Fst = 0.066; P 0.001), respectively. However, gene flow (Nm) values varied from 0.058 in populations 1 and 2 to 1.018 in populations 2 and 3. Neighbor-joining trees identified from 21 Sudanese wild sorghum accessions clustered closely to the universally drought-tolerant landrace B35. Structural analysis generated the highest Delta K value (58.2) at K = 2, revealing two distinct subpopulations. While this work provides valuable information about the potential of sorghum wild relatives from Sudan as sources for stay-green drought tolerance, further research should be directed toward identifying the exact mechanisms and genes underlying this stay-green trait using advanced molecular omics techniques. In conclusion, this study highlights the potential role of Sudanese sorghum accessions as reservoirs of ready-to-use stay-green genes for the design of climate-resilient sorghum cultivars in drought-prone areas of Sudan and beyond. However, these wild relatives would require extensive pre-breeding and validation efforts before their genes can be effectively incorporated into elite cultivars.

Keywords Biodiversity · Climate-resilient crop · Molecular marker · *Sorghum bicolor* · Stay-green trait

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Introduction

Climate change and environmental stress are major drivers of biodiversity loss and food insecurity at the global, regional, and local scales. Global warming is one of the most pressing drought-driven factors threatening sustainable food production worldwide.

Recent reports from the Intergovernmental Panel on Climate Change (IPCC) emphasize the need for urgent action to mitigate climate change and adapt sustainable crop production for food security (IPCC 2023). Sorghum (*Sorghum bicolor* (L.) Moenches) is a climate-resilient and stable food crop for more than 500 million people in developing countries, particularly in dry and semi-dry areas where drought is a significant obstacle (Abreha et al. 2022). Hadebe et al. (2017) reported that Sub-Saharan Africa faces a water deficit and limited food access and approximately 43% of the land classified as arid or semi-arid, small-scale rainfed agriculture is the primary source of livelihood. The increasing demand for food puts pressure on sorghum farming, which must often cope with limited water supplies. Although sorghum is known for its adaptability and ability to thrive under low-input conditions, it remains vulnerable to drought during the critical anthesis and grain-filling stages of growth (Blümmel et al. 2015; Borrell et al. 2014a, b; Thomas & Ougham 2014). Water stress during the vegetative and reproductive stages significantly decreased yield by more than 36% and 55%, respectively (Assefa et al. 2010).

Efforts have focused on assessing the effects of water stress on sorghum cropping and performance in water-deficient environments. This information is crucial for developing drought- and stress-tolerant sorghum cultivars. In response to drought stress, sorghum plants employ various survival mechanisms, such as shortening their life cycle, enhancing water uptake, reducing transpiration, increasing tissue tolerance to dehydration, and undergoing biochemical changes involving proline and other metabolites. (Abreha et al. 2022; Hadebe et al. 2017; Liaqat et al. 2024).

Stay-green is an adaptation mechanism that helps sorghum maintain its green leaf area and functional photosynthesis during water-limited conditions, particularly during the post-flowering stage. Postponed leaf senescence during grain filling is an emergent outcome of dynamics that occur earlier in sorghum growth and is essentially due to an enhanced balance between water supply and demand, as well as the efficiency with which the sorghum plant converts water to biomass and grain yield (Borrell et al. 2014a, b; Thomas & Ougham 2014). Such functional "stay-green" (*Stg*) individuals retain the green leaf area (GL) for a more extended period following the onset of a "drought spell," which can be expected to result in more stable grain yield performance across sites and years in their zones of adaptation (Kamal et al. 2019). Thomas and Ougham (2014) described stay-green phenotypes as delaying senescence (type A), reducing the senescence rate (type B), retaining chlorophyll (type C), maintaining greenness through rapid death (type D), and resulting in a naturally greener phenotype. The functionality of stay-green traits relies on sorghum production in areas with limited water availability. Functional stay green refers to the ability of leaves to perform photosynthesis, whereas cosmetic stay green refers to the enhancement of photosynthesis and greenness. However, not all functional enhancements positively affect an organization's output rate. Therefore, selecting stay-green traits and grain yield in breeding programs is essential because observations of delayed senescence are related to sink demands.

Previous research on sorghum identified four quantitative trait loci (QTL) related to stay-green characteristics: *Stg1*, *Stg2*, *Stg3*, and *Stg4* (Borrell, van Oosterom, et al., 2014; Kamal et al. 2019; Ochieng et al. 2021). The mapped QTLs explained 54% of the phenotypic variance of sorghum genotypes with the "stay green" character. The QTLs *Stg1* and *Stg2* were discovered on chromosome 3 via chromosome mapping. *Stg3* and *Stg4* were also found on the 2 and 5 chromosomes, respectively (Borrell, van Oosterom, et al., 2014; George-Jaeggli et al. 2017). These loci are associated with increased grain yield (Jordan et al. 2012) and improved fodder quality (Blümmel et al. 2015). Most investigations used B35/BTx642 as a prominent source of stay green, whereas few used SC56 and E36-1. In contrast, Ochieng et al. (2021) identified other sources of stay-green traits in wild accessions. These included wild accessions GBK045827, GBK016114, GBK048922, GBK016109, and GBK047293, which fell into a different cluster from B35 and E36-1. These results indicate that wild sorghum accessions represent potential new sources of stay-green drought tolerance that can be used for breeding programs.

Sudan is the motherland of sorghum, and wild sorghum has substantial genetic variation. Therefore, it is crucial to identify and characterize new sources of stay-green germplasm from wild and weedy sorghum to incorporate these genetic variations into breeding strategies for improved drought tolerance. To achieve

this, we aimed to identify genetic variations among 256 Sudanese wild sorghum accessions using 17 simple sequence repeat (SSR) markers associated with stay-green QTLs based on the B35 landrace.

Materials and methods

Plant material

We collected 256 wild and weedy Sudanese sorghum species, locally known as Adar, from the border regions between Sudan, Eritrea, and Ethiopia. We assume that sorghum was first domesticated in these areas 8000 years ago because of the substantial genetic diversity of its wild relatives (Abdelhalim et al. 2019). Wild sorghum accession seeds were collected in 2013, and five subsequent cultivation cycles were conducted using a single-seed descent breeding method to ensure homogeneity and purity. The landrace sorghum B35, a universal donor for the stay-green trait, is a BC1 derivative of the Ethiopian Durra line IS12555 (Subudhi et al. 2000) and was included as a positive control. The Sudanese drought-sensitive sorghum cultivar Tabat was used as a negative control.

DNA extraction

Five seeds from each genotype were randomly selected, planted in plastic trays, and placed in a designated section of a lab house at the Biotechnology and Biosafety Research Center, Agricultural Research Corporation (ARC), Sudan. The soil mixture comprised a 1:1 ratio of Shambat cotton clay to sand. Tissue samples were harvested from the leaves of three plants of each genotype in the second week after anthesis.

The collected leaves were placed in Ziplock plastic bags and stored on silica gel at room temperature until DNA extraction. Total DNA was extracted from silica gel-dried leaves using modified cetyl-trimethyl ammonium bromide (CTAB) (Handayani & Cahyani 2021) in an option solution containing M Tris–HCl (pH 8.0), 5 M NaCl, 0.5 M EDTA, 2% 2β-ME, and 2% CTAB. Genomic DNA quality was determined by mixing 3 µL of this sample with 7 µL of 1% agarose gel and applying it at 100 V for 40 min. The concentration of each genomic DNA sample was increased to 100 ng/µL using double-distilled sterilized water. This step was performed to prepare for PCR amplification of DNA. The samples were then frozen at – 20 °C.

Genotyping

Forty-one stay-green-specific SSR primers were used for PCR amplification of genomic DNA from Sudanese wild sorghum accessions (Vadez et al. 2013). PCR optimization and testing of SSR primers were performed using two contrasting controls: B35, a stay-green donor, and Tabat, a Sudanese drought-released cultivar. Of the 41 SSR markers linked to stay-green trait QTLs, 17 were polymorphic and produced a clear amplicon (Bhattramakki et al. 2000). Table 1 lists the primer names, chromosome locations, and allele sizes of these samples. A 20 µL PCR master mix, 4 µL of Solis BioDyne 5×Blend Master Mix Buffer, 0.5 µl of the genomic DNA sample, 0.4 –0 µl of forward primer, 0.4 µl of reverse primer, 14.7 µl of ddH2O. Amplification was performed in a Biometra thermal cycler under the following conditions: The first step involved denaturing the samples at 94 °C for 4 min. In the first step, the samples were denatured at 94 °C for 30 s, and the annealing temperature was then varied from 50 to 60 °C according to the primer leaflet for 30 s. The third step included 35 extension cycles at 72 °C for 1 min. The fourth step involved a final extension step at 72 °C for 7 min, following which the samples were stored at 4 °C until gel electrophoresis. On a 1% agarose gel, 10 µL of the PCR products were loaded with 20 mL of TBE/100 mL, 10 µL of Red Safe (nucleic acid staining solution 20.0000), and 80 mL of deionized water. To assess the size of the PCR bands, a 100-bp DNA ladder was used. To isolate the PCR products, gel electrophoresis was performed on Bio-Rad at 100 V and 400 mA for 90 min in tris-based ethoxybenzyl (TBE) buffer (54 mmol/l tris, 27. 5 g of boric acid, and 20 mmol/l EDTA). acid(20 ml EDTA/1000).

Data analysis

The sizes of all PCR-amplified microsatellite regions were estimated using a Syngene ultraviolet documentation system with a 100-bp standard ladder. We measured genetic diversity parameters, such as the number of alleles (Ne), major allele frequencies

Table 1 Primer names, chromosomes with identified stay-green QTLs, primer sequences, annealing temperatures, and allele sizes of controls detected at 17 microsatellite loci in 256 Sudanese wild sorghum accessions

Markers names	Chromosomes with stay-green QTLs	Sequences		Annealing temperature	Allele sizes in checks bp	
		Forward	Reverse		Tabat	B35
Xtxp 088	SBI-01	ATATGGAAGGAAGAAGCC GG	AACACAACATGCACGCATG	57.4	135	121
Xtxp 014	SBI-05	GTAATAGTCATGACCGAGG	TAA TAG ACG AGT GAA AGC CC	53	165	156
Xcup 24	SBI-01	AAACTGGATGCCACACCA AG	AGCTATACCAACACGGGC AG	58.8	210	191
Xsb AGB 03	SBI-02	GTGTGTGTAGCTTCTTGGG	ACGTAGGAGTAGTTTCTA GGATT	55.8	205	200
Xtxp 286	SBI-02	AGCAGCAGCAGCAACAG	GCGTGGTCTTTGTGGTTC	57	210	215
Xtxp 43	SBI-01	AGTCACAGCACACTGCTT GTC	AATTTACCTGGCGCTCTGC	57.3	190	175
Xcup 05	SBI-04	GGAAGGTTTGCAAGAACA GG	CCAGCCCAACAAGTGCTATC	57	210	200
Xtxp 41	SBI-04	TCT GGC CAT GAC TTA TCA C	AAA TGG CGT AGA CTC CCT TG	56.2	280	300
Sb AGA 01	SBI_03	CGAACCATGATAAATGACTG	ATCCGTTTCACAAAAAAA GT	50	100	110
Xtxp274	SBI-06	GAA ATT ACA ATG CTA CCC CTA AAA GT	ACT CTA CTC CTT CCG TCC ACA T	57.2	350	380
Xtxp 114	SBI_03	CGTCTTCTACCGCGTCCT	CATAATCCCACTCAACAA TCC	57.8	260	251
Xtxp 212	SBI-04	TTTCCCCTCTTTCTTGTGTC	CTCGGCGTCGTCGTA	52	150	180
Xtxp 445	SBI-02	GCCAGTTGAATCCGCTACAT	GAATTGCAATACATAAGC ACACC	57.2	250	238
Xtxp6	SBI-06	ATCGGATCCGTCAGATC	TCTAGGGAGGTTGCCAC	53	110	140
xtxp034	SBI_03	TGGTTCGTATCCTTCTCT ACAG	CATATACCTCCTCGTCGCTC	60.2	380	360
xtxp031	SBI_03	TGCGAGGCTGCCCTACTAG	TGGACGTACCTATTGGTGC	59.5	205	200
xtxp019	SBI-02	CTTTCAATCGGTTCCAGAC	CTTCCACCTCCGTACTC	56.2	295	300

(MAF), gene diversity (h), and polymorphism information content (PIC), using Power Marker version 3.25 software (Liu & Muse 2005). However, the observed heterozygosity (Ho), expected heterozygosity (He), and Shannon's Information Index (I) (Sherwin et al. 2006), and the Hardy–Weinberg equilibrium (HWE) was measured using GenAlex version 6.5 (Peakall & Smouse 2006). Additionally, we conducted a molecular variance analysis (AMOVA) (Meirmans 2006) after grouping accessions based on cluster evaluation. The GenAlex software was used to measure pairwise population genetic distances, and gene flows. We used the neighbor-joining method and Jaccard's genetic similarity coefficients based on the genetic distance matrix to construct a dendrogram. Using R statistical software, we measured the kinship matrix of Jaccardn distances for the 256 Sudanese wild sorghum accession genotypes based on SSR markers. Packages *vegan* and *ggplot2* in R statistical software were used to calculate the kinship matrix of Jaccard distances for the 256 Sudanese wild sorghum accession genotypes based on SSR markers.

The population structure was calculated via Bayesian analysis using the STRUCTURE (version 2.3.4) program to estimate the number of hypothetical subpopulations (K) and the membership probability of each genotype to the identified subpopulations (Pritchard et al. 2000). The model-based Bayesian clustering approach

used Markov chain Monte Carlo (MCMC) algorithms to test hypotheses from one to ten subpopulations. The burn-in and number of iterations of the MCMC algorithm were set to 9,999. This process was independently repeated 10 times using the admixture model. The log-likelihood of the observed data Pr (X|K) for each value of K was retrieved from the structure output (Pritchard JK et al. 2007). The ad hoc quantity analysis was based on the second-order rate of change of the likelihood probability function presented by Evanno et al. (2005). Delta K produced the highest outcome within the Bayesian clustering approach, with a maximum K-value of 2, indicating that the population was grouped into two subpopulations.

Results

Characteristics of SSR markers across *sorghum* accessions

The allelic sizes of the SSR markers in sorghum specific to the stay-green trait ranged from 110 bp for *Sb AGA 01* to 380 bp for *Xtxp274* for landrace B35, which is a universal stay-green donor that can handle drought. On the other hand, for the susceptible check Tabat, the allelic size values ranged from 100 bp for the marker (*Sb AGA 01*) to 380 bp for the marker (*Xtxp034*). Out of 41 SSR-specific stay green markers, 17 were polymorphic and generated 55 alleles, with an overall average of 3.3 per locus. Alleles per locus ranged from 3 to 5, with the highest number (5.0) observed for marker *Xtxp_114*. The study found that the major significant alleles (MAF) had a frequency range of (0.43) for the *Xtxp031* marker to (0.50) for the *Xcup24*, *Xtxp286*, *XsbAGB01*, *Xtxp274*, *Xtxp212*, and *Xtxp445* markers, with a mean frequency of (0.48) per locus (Table 2). Regarding Shannon's information index, the range was from 3.48 for the markers (*Xcup05*, *Xtxp445*, and *Xtxp6*) to 3.5 for the markers (*Xtxp41*, *XsAGB01*, *Xtxp212*, *Xtxp3.5*, and *Xtxp031*), with an overall mean of 3.49. The genetic diversity among the different genetic locations varied from 0.58 (*Xtxp_41*, *Xtxp274*, and *Xtxp212*) to 0.64 (*Xtxp_114* and *Xtxp031*), with an average of 0.60 per location. The expected heterozygosity (He) of each locus differed slightly, ranging from 0.297 (*Xtxp274*)

Table 2 Genetic diversity index summary statistics of 17 simple sequence repeat (SSR) loci across 256 Sudanese wild Sorghum accessions

Marker	MAF	Na	I	GD	He	Ho	PIC	PHWE
Xtxp_088	0.49	3	3.5	0.58	0.3	0.3	0.49	0.000***
Xtxp_014	0.48	3	3.49	0.62	0.44	0.44	0.54	0.000***
Xcup_24	0.5	4	3.49	0.62	0.49	0.49	0.55	0.000***
Xsb_AGB_03	0.49	3	3.49	0.62	0.45	0.45	0.54	0.000***
Xtxp_286	0.5	3	3.49	0.6	0.39	0.39	0.52	0.000***
Xtxp_043	0.46	3	3.49	0.61	0.41	0.38	0.53	0.000***
Xcup_05	0.45	3	3.48	0.6	0.35	0.29	0.51	0.000***
Xtxp_41	0.49	3	3.5	0.58	0.39	0.32	0.49	0.000***
Xsb_AGB_01	0.5	3	3.5	0.61	0.48	0.42	0.53	0.000***
Xtxp274	0.5	3	3.49	0.58	0.3	0.33	0.5	0.000***
Xtxp_114	0.46	5	3.49	0.64	0.37	0.46	0.57	0.000***
Xtxp_212	0.5	3	3.5	0.58	0.38	0.33	0.5	0.000***
Xtxp_445	0.5	3	3.48	0.6	0.33	0.41	0.52	0.000***
Xtxp6	0.48	3	3.48	0.62	0.46	0.45	0.54	0.000***
xtxp034	0.49	4	3.5	0.61	0.33	0.39	0.53	0.000***
xtxp031	0.43	3	3.5	0.64	0.37	0.46	0.57	0.000***
xtxp019	0.45	3	3.48	0.63	0.39	0.43	0.55	0.000***
Mean	**0.48**	**3.2**	**3.49**	**0.6**	**0.39**	**0.4**	**0.53**	
Min	**0.43**	**3**	**3.48**	**0.58**	**0.3**	**0.29**	**0.49**	
Max	**0.50**	**5**	**3.5**	**0.64**	**0.49**	**0.49**	**0.57**	

Bold values indicate marker names and summary statistics (Mean, Min, Max)
*** indicates significance at $p < 0.001$

to 0. 491 (*Xcup_24*), with a mean of 0. 389. Similarly, the heterozygosity levels ranged from 0. 293 (*Xcup_05*) to 0. 491 (*Xcup_24*), resulting in an average of 0. 397 per locus. Markers *Xsb_AGB_01* and *Xtxp_41* exhibited the highest levels of informativeness, as indicated by an information index of 3.503 (Table 2). The PIC values ranged from 0.49 (*Xtxp_088* and *Xtxp_41*) to 0.57 (*Xtxp_114* and *Xtxp031*), with an average of 0.53. All 17 SSR markers showed highly significant ($p < 0.0001$) deviations from the Hardy–Weinberg equilibrium (HWE). According to the results presented in Fig. 1, markers *Xtxp212* and *Xcup05* were most useful.

Genetic relationships between and within populations

Population 1 had the highest number of individuals (117), followed by Population 2 (88), and Population 3 had the lowest number of individuals (52) (Table 3). The number of alleles in all populations was 2.00 (data not shown). Population 3 had the highest effective allele frequency (1.69) and Shannon information index (0.59). In contrast, Population 1

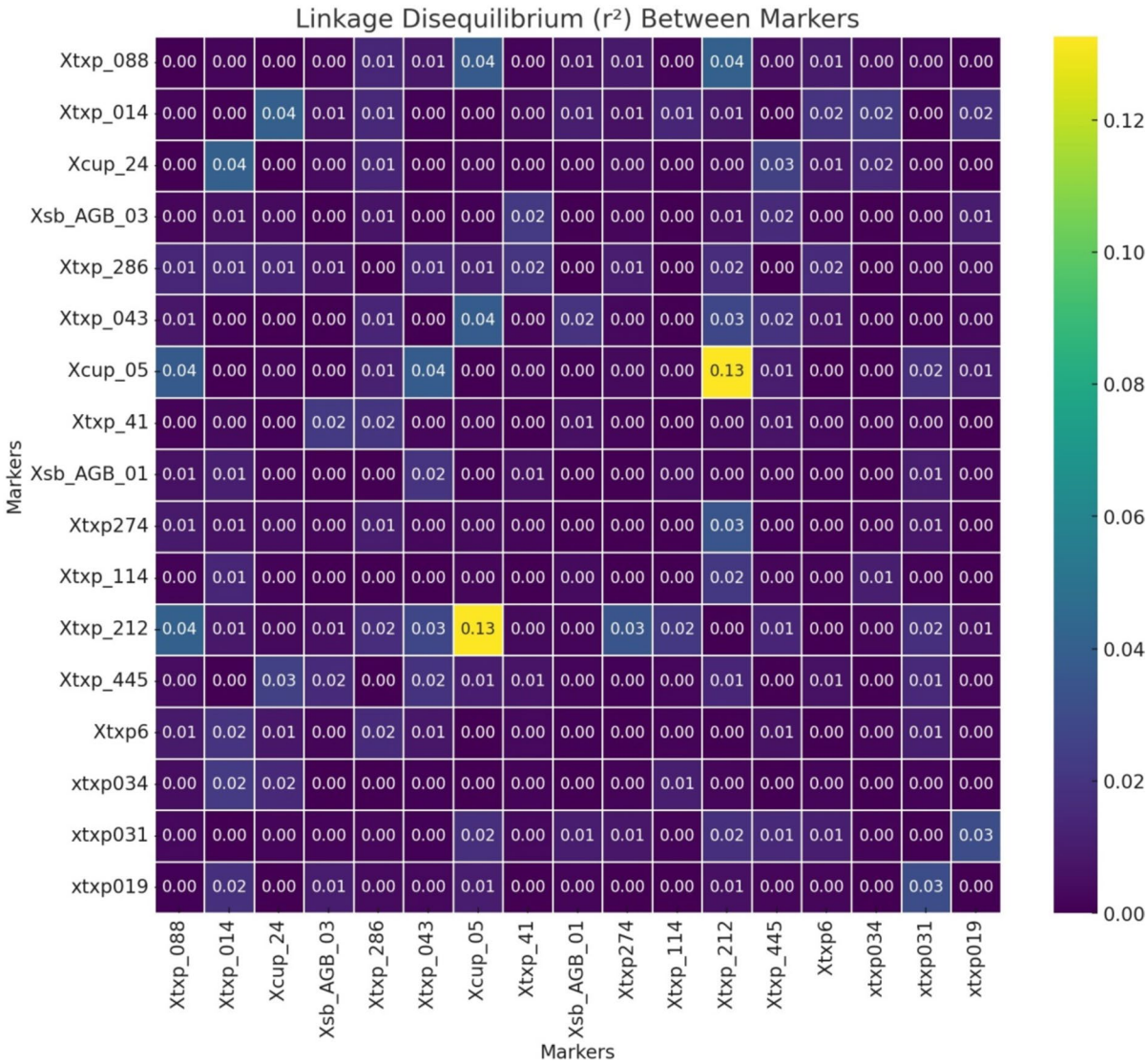


Fig. 1 Linkage disequilibrium analysis shows the most informative markers across 17 simple sequence repeat (SSR) markers

Table 3 Allelic patterns and diversity indices of Sudanese wild sorghum germplasm populations compared with 17 stay-green-specific simple sequence repeat (SSR) loci

Population	Size	Ne	NPA	NLCA	I	h	PPL
Population1	117	1.61	0.00	0.00	0.54	0.090	100%
Population2	88	1.65	0.00	0.00	0.57	0.127	100%
Population3	52	1.69	0.00	0.00	0.59	0.032	100%
Mean	85.7	1.651	0.00	0.00	0.57	0.083	

Na = Observed number of alleles; Ne = Number of effective alleles; NPA = Number of Private Alleles (i.e., the number of alleles unique to a single population); NLCA = Number of Locally Common Alleles (< = 25% and < = 50%), (frequency 5%) found in 25% or fewer populations; I = Shannon's information statistic; h = Nei's genetic diversity; and PPL = the Percentage of Polymorphic Loci

Table 4 Pairwise Nei genetic distances measured using Fst (A) and gene flow (Nm) values (B) among three Sudanese wild sorghum populations

	Population 1	Population 2	Population 3
A			
Population 1	0.000		
Population 2	0.007	0.000	
Population 3	0.010	0.002	0.000
Probability P(rand > = data) based on 999 permutations			
B			
Population 1	0.000	0.944	0.920
Population 2	0.058	0.000	1.018
Population 3	0.083	0.000	0.000

had the lowest number of effective alleles (1.61) and the lowest Shannon information index (0.54). These results indicate private or localized common alleles in a single population. Population 2 had the highest Nei genetic diversity (0.127), and population 3 had the lowest (0.032). The percentage of polymorphic loci per population was 100% (Table 3).

Genetic differentiation, distance, and gene flow

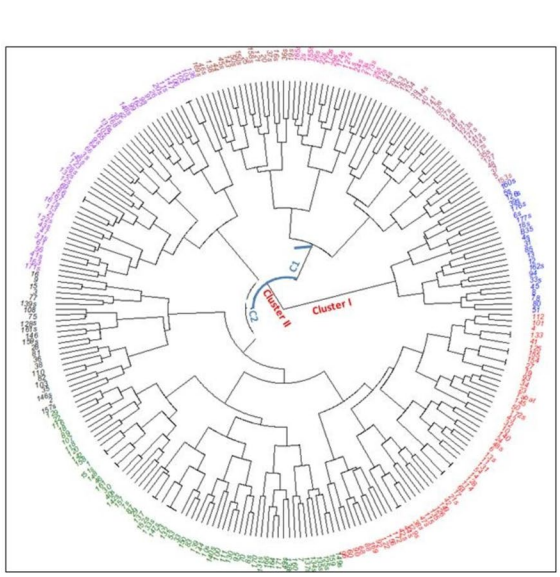
Pairwise genetic differentiation between Sudanese wild sorghum populations for the stay-green trait ranged from 0.002 to 0.010 (Table 4). Populations 3 and 1 exhibited the highest degrees of population differentiation (Fst = 0.010), followed by populations 2 and 1 (Fst = 0.007). Populations 3 and 2 show the lowest population differentiation levels (Fst = 0.002). The gene flow (Nm) between each population and the other populations ranged from 0.058 to 1.018 (Table 4). Table 4 presents the highest gene flow (1.018) between populations 2 and 3, followed by populations 1 and 2 (0.994), and the lowest gene flow (0.58) between populations 1 and 2. AMOVA for the stay-green trait revealed that variability among and within populations accounted for 1% and 99% of the total genetic variation, respectively (Table 5). The overall fixation index value used to measure population differentiation was moderate (Fst = 0.066).

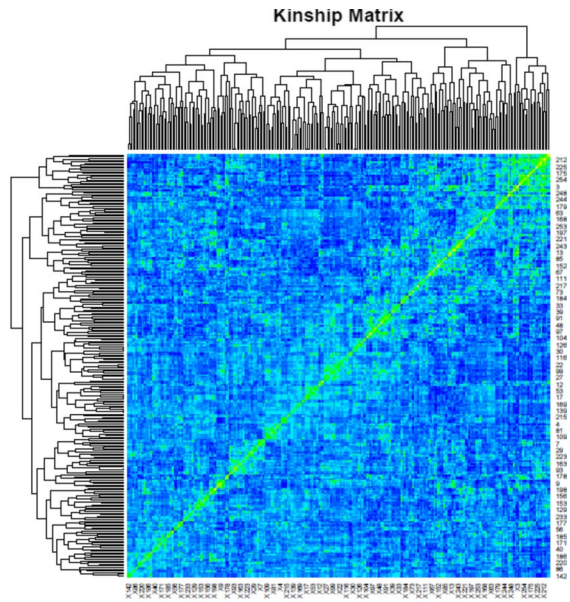
Cluster analysis

The 256 samples of Sudanese wild sorghum used for neighbor-joining cluster analysis were divided into two distinct genetic groups (Fig. 2a). Cluster I comprised 21 accessions, including the widely recognized drought-tolerant landrace B35. On the other hand, we further divided Cluster II into smaller clusters C1 and C2. The sub-cluster C1 consisted of 57 accessions, whereas the sub-cluster C2 consisted of 177 accessions, one of which was the drought-sensitive cultivar

Table 5 Analysis of molecular variance (AMOVA) showing the partitioning of genetic variation within and among populations using 17 simple sequence repeat (SSR) markers

Source	Degrees of freedom	Sum of squares	Mean squares	Est. Var	%	P value
Among Pops	2	32.3	16.15	0.06	1%	0.001
Among Indiv	253	1825.1	7.21	0.00	0%	0.985
Within Indiv	256	1922.0	7.51	7.51	99%	0.910
Total	511	3779.4		7.56	100%	





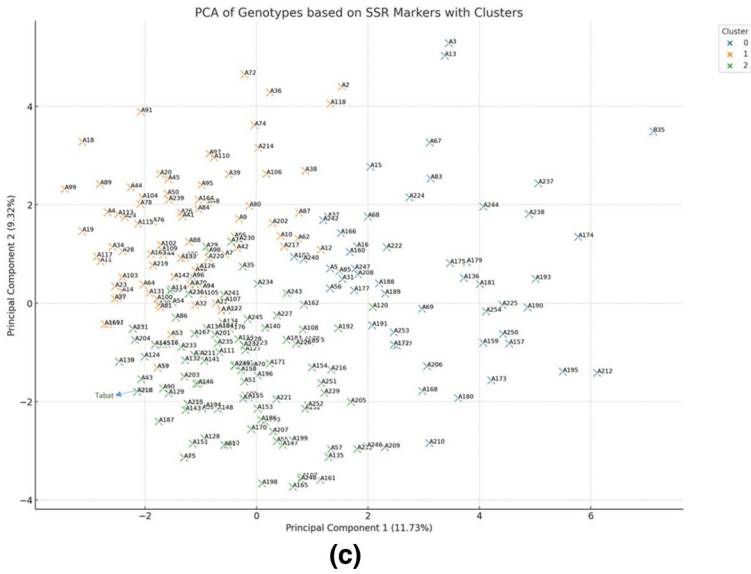


Fig. 2 **a** Neighbor-joining cluster analysis showing the clusters of 256 accessions of Sudanese wild sorghum based on stay green trait SSR markers. **b** Kinship map of wild sorghum accessions based on stay green trait-specific simple sequence repeat (SSR) markers. **c** Principal Component Analysis (PCA) of 256 Sudanese wild sorghum accessions based on the 17-SSR-specific stay green trait. Samples coded with the same color belong to the same population

Tabat. This distinction underscores the genetic differences between drought-tolerant and drought-vulnerable accessions. According to the results of the neighbor-joining cluster analysis (Fig. 2b), the kinship map confirmed that the accessions were split into two separate groups. Consistency between methodologies reinforces the dependability of established genetic classifications. Principal component analysis (PCA) revealed genetic similarities between sorghum accessions (Fig. 2c). The first two main component axes explain 21.05% of total genetic variation. The first and second axes contributed 73% and 9.32%,

respectively (Fig. 2c). PCA confirmed the identified genetic clusters, emphasizing gene diversity in Sudanese wild sorghum populations.

The population structure evaluation revealed that the mean log-likelihood values varied from the lowest of − 5077.2 for K = 1 to the highest for K = 8 (− 4152.0). However, increasing the value of K increased the mean likelihood until K = 8. Subsequently, a slight reduction in the mean log-likelihood from K = 9 was observed (Table 6). Higher K values exhibited a higher standard deviation of log-likelihood, suggesting higher instability in the clustering solution. We observed the highest log-likelihood difference (266.2) between K = 1 and K = 2 and the lowest difference (− 68.7) between K = 8 and 9. Eventually, the highest Delta K value (58.2) was recorded at K = 2, revealing that two distinct subpopulations probably existed among the 256 Sudanese wild sorghum accessions tested based on the 17 stay-green-specific SSR markers. Clumpak's results (bar plot) detected a genetic admixture based on this value; hence, there was clear genetic-based structuring of accessions (Fig. 3a–c).

Discussion

This study aimed to evaluate the genetic diversity of Sudanese wild sorghum using microsatellite SSR markers, focusing on the stay-green trait that contributes to drought tolerance. By using 41 SSR markers, of which 17 were polymorphic, this study identified 55 alleles with an average of 3.3 alleles per marker, highlighting the utility of these markers in detecting genetic diversity in wild sorghum accessions. The two most informative markers, *Xtxp212* and *Xcup05*, were particularly promising for marker-assisted selection (MAS) programs aimed at breeding drought-tolerant sorghum cultivars. Identifying these markers aligns with the findings of Rajarajan and Ganesamurthy (2011), who demonstrated that SSR markers specific to the stay-green trait are highly effective for breeding drought-tolerant sorghum lines.

The stay-green trait, a form of delayed senescence, allows sorghum plants to maintain green leaves under post-flowering drought conditions, contributing to improved grain filling and overall yield stability under water-stress environments. Previous investigations, such as those by Vinodhana and Ganesamurthy (2013) and Ngugi et al. (2013), have emphasized the importance of this trait in breeding programs aimed at enhancing drought tolerance in arid and semi-arid regions. These investigations highlighted the genetic diversity within sorghum accessions and its potential to drive future breeding efforts. In particular, the presence of polymorphic markers associated with stay-green QTLs in Sudanese wild sorghum suggests that this germplasm can be utilized to develop resilient sorghum cultivars tailored to drought-prone environments.

The genetic diversity observed in the three populations of wild sorghum analyzed in this study is crucial for breeding programs. Despite having the smallest number of individuals (58), Population 3 exhibited the highest genetic variation, with more alleles and a higher Shannon's information index than the other populations. This finding aligns with other research, such as Ouedraogo et al. (2017), which demonstrated that even small populations can harbor valuable genetic diversity, mainly when sourced from

Table 6 Mean log-likelihood, standard deviations for log-likelihood, log-likelihood differences, and delta K for structural analysis

K	Reps	Mean LnP (K)	Stdev LnP (K)	Ln'(K)	|Ln''(K)|	Delta K
1	10	− 5077.2	0.1	NA	NA	NA
2	10	− 4811.0	1.6	266.2	93.7	58.2
3	10	− 4638.5	2.6	172.5	10.3	3.9
4	10	− 4476.3	18.2	162.2	51.3	2.8
5	10	− 4365.4	12.8	110.9	22.4	1.8
6	10	− 4232.1	5.8	133.3	54.4	9.3
7	10	− 4153.2	35.1	78.9	77.7	2.2
8	10	− 4152.0	42.2	1.2	69.9	17
9	10	− 4220.7	222.8	− 68.7	133.7	0.6
10	10	− 4155.7	102.9	65.0	NA	NA

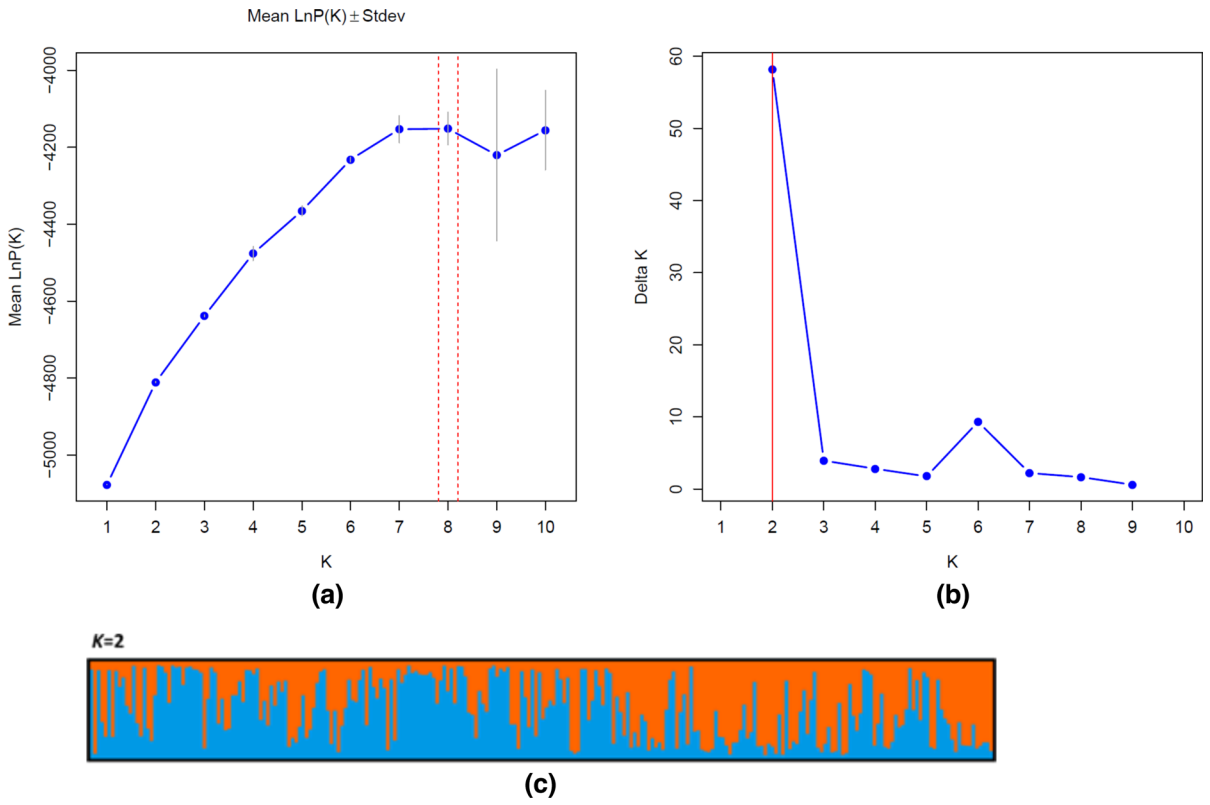


Fig. 3 **a** Mean log-likelihood values of the population structure of Sudanese wild sorghum accessions. **b** Estimated top delta K values obtained by the method proposed by Evanno et al. (2005) estimated the Bayesian population structure for $K=2$. **c** Different colors (blue and orange) represent genetic groups or subpopulations designated by the Structure Harvester. **b** Population structure of sorghum accessions representing SWS populations

underutilized wild relatives. In addition to its genetic diversity, the study highlighted the importance of population structure analysis, which distinct clustering of accessions based on SSR markers, mainly the grouping of the drought-tolerant B35 and drought-susceptible Tabat cultivars with wild-relative accessions, reinforces the potential of wild sorghum for breeding programs. However, this suggests that wild sorghum populations share genetic traits with cultivated drought-tolerant lines, making them valuable for introgression breeding. These results are consistent with research by Haussmann et al. (2002), who identified stable stay-green QTLs across multiple environments in wild and cultivated sorghum populations.

One of the critical components of successful breeding programs is understanding gene flow between wild and cultivated populations. In this study, gene flow (Nm) ranged from 0.58 to 1.018, with the highest gene flow between Populations 2 and 3, which is significant because gene flow can enhance the introgression of valuable traits, such as drought tolerance, from wild relatives into cultivated cultivars. The findings are consistent with Sagnard et al. (2011), who documented gene flow between wild sorghum and cultivated *guinea margaritiferum* sorghum in West Africa. Understanding these gene flow patterns is essential for optimizing breeding strategies, particularly for maintaining genetic diversity while ensuring the stability of introduced traits.

Further investigation into the direction and extent of gene flow, as suggested by this study, will enhance our understanding of how wild and domesticated sorghum populations interact. Mutegi et al. (2010) also highlighted the importance of understanding habitat overlaps and ecological interactions in shaping gene flow between domesticated and wild sorghum. This interdisciplinary approach, involving ecologists, plant breeders, and geneticists, is necessary to create sustainable

breeding programs that capitalize on natural gene flow while preserving genetic diversity.

Our study identified *Xtxp212* and *Xcup05* as highly informative SSR markers that provide a practical tool for marker-assisted selection (MAS) in sorghum breeding. Previous research has shown that MAS can expedite breeding by allowing breeders to select specific traits, such as drought tolerance, at the molecular level rather than relying solely on phenotypic selection. For example, Priyanka et al. (2023) demonstrated that MAS using SSR markers for stay-green QTLs successfully improved drought tolerance in locally adapted sorghum cultivars. However, this study notes that further research is needed to validate these findings under various stress conditions through large-scale field trials. Comparative transcriptomics, metabolomics, and proteomics research will also be crucial for identifying the underlying genetic regulation networks associated with drought tolerance and stay-green traits, as highlighted by Altaf et al. (2023) and Liaqat et al. (2024) that omics technologies can provide deeper insights into the molecular pathways involved in stress responses, offering a more comprehensive understanding of drought resistance mechanisms.

The results of this study have far-reaching implications, particularly for regions like Sudan, where climate change and water scarcity are significant challenges to agricultural productivity. By integrating the genetic diversity found in wild sorghum populations into breeding programs, it may be possible to develop more resilient sorghum cultivars that can withstand the increasing frequency of droughts. However, successfully incorporating these wild relatives into elite cultivars necessitates extensive pre-breeding and validation efforts. The transfer of traits from wild germplasm often introduces undesirable characteristics, which must be meticulously addressed through well-designed breeding programs. In addition, Developing climate-resilient crops is essential for food security in Sudan and globally, as sorghum is a key staple in many drought-prone regions, including sub-Saharan Africa and South Asia.

Conclusion

This study underscores the critical role of Sudanese wild sorghum in enhancing drought tolerance in sorghum breeding programs. The genetic diversity identified, particularly in population 3, and the effectiveness of SSR markers such as *Xtxp212* and *Xcup05* offer valuable resources for developing drought-resistant sorghum cultivars. Future efforts should focus on integrating this wild germplasm into breeding programs, conducting large-scale trials, and exploring the molecular mechanisms underlying drought tolerance to ensure the successful development of climate-resilient sorghum cultivars.

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Declarations

Competing Interests The authors declare no competing interests.

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