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RESEARCH ARTICLE

Exploring the genetic variability of sudanese wild sorghum (*sorghum bicolor* (L.) moench) germplasm for post-attachment *striga hermonthica* resistance mechanisms using single sequence repeat (SSR) primers

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Abstract *Striga hermonthica*, a parasitic weed, poses a significant threat to sorghum production in sub-Saharan Africa, potentially causing yield losses of up to 100%. Identifying sources of *Striga* resistance trait within the diverse wild sorghum accessions is imperative to developing resistant sorghum cultivars. This study analyzed the genetic variation of 255 Sudanese wild sorghum accessions using simple sequence repeat (SSR) markers associated with post-attachment *Striga* resistance mechanisms: nine SSR markers linked to mechanical barrier resistance and two to hypersensitive resistance. We found moderate polymorphism in the *Striga* resistance loci among the Sudanese wild sorghum accessions, with polymorphic information contents (PIC) ranging from 0.03 to 1.92 for hypersensitivity and 0.37 for mechanical barrier resistance mechanism markers. Overall, 18 alleles were detected within the loci for mechanical barrier markers and four for hypersensitive markers. A population structure and cluster analysis revealed that several accessions were closely linked to the resistant checks N13 (mechanical barrier) and Framida (hypersensitivity response). Analysis of molecular variance (AMOVA) showed substantial polymorphism within the population (99% for mechanical barriers and 94% for hypersensitivity), thus indicating that these wild sorghum accessions harbor ready-to-use genes for improving *Striga* resistance in sorghum. Our findings highlight the merits of Sudanese wild sorghum germplasm for post-attachment *Striga* resistance mechanisms, indicating their possible use in sorghum breeding efforts to develop *Striga* resistant cultivars.

Keywords Genetic markers · Post-attachment resistance mechanisms · *Striga hermonthica* · *Sorghum bicolor* · Wild sorghum accessions

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is a staple food crop for millions in sub-Saharan Africa (SSA). Various biotic and abiotic stresses significantly constrain its production. Among the biotic factors, the parasitic weed *Striga hermonthica* poses a considerable threat, potentially causing yield losses of up to 100% (Ejeta 2007). Alarmingly, over 50% of the cultivated area in SSA that supports cereal production is under *Striga* infestation (Rodenburg et al. 2016), threatening the livelihood and food security of millions of families in the face of growing population pressure (Giller 2020).

Domestication and breeding of wild plant species result in genetic diversity within a crop's gene pool. Therefore, the genetic diversity of elite cultivars is generally lower than that of landraces and wild populations. Consequently, lower genetic diversity in the cultivated gene pool increases the risk of biotic and abiotic stress. Similarly, research has shown that cultivated sorghum is highly susceptible to *Striga* (Begna 2021). Well-documented evidence demonstrates that crop wild relatives can serve as valuable reservoirs for alleles of genes that regulate significant traits, such as resistance to biotic factors (Rich et al. 2004). Wild sorghum accessions resistant to *Striga* have been identified, although the genetic basis of this resistance is unclear (Muchira et al. 2021). Hence, there is a need to expand the genetic base to identify novel sources of *Striga* resistance to cope with the increasing impact of *Striga* on sorghum production. Mbuvi et al. (2017) reported that Sudanese wild sorghum accessions exhibited remarkable post-attachment resistance to *Striga*, as revealed through laboratory and field assays. Given that Sudan is a center of origin for both sorghum and *Striga* and harbors extensive genetic diversity (Teferi 2019; Teressa 2019), screening the gene pool of Sudanese wild sorghum is crucial for elucidating resistance mechanisms and identifying new *Striga*-resistant genotypes that can be used in breeding programs to enhance sorghum's resistance to *Striga*.

Crop wild relatives offer a diverse genetic pool for breeding resistance traits. According to Kuiper et al. (1998), several native African savanna grass species show high resistance to *Striga hermonthica*. Interspecific hybrids derived from these grasses showed variable responses at different stages of *Striga* infection. During the initial seed germination stage, these hybrids did not demonstrate resistance, and *Striga* was able to attach to the sorghum seedlings. However, in the later stages of development, the *Striga* seedlings either failed to develop further or showed significant impairment when growing on these grass hybrids (Kuiper et al. 1998). Rich et al. (2004) screened approximately 55 wild sorghum accessions within the primary sorghum gene pool for resistance to *S. asiatica*. They discovered that PQ-434, a Sudanese wild sorghum accession of *Sorghum bicolor drummondii*, had low germination stimulant and haustorium factor (LHF) qualities. Also, Gobena et al. (2017) found that three wild Sudanese sorghum accessions (WSA-1, WSE-1, and WSA-2) strongerresistance to *Striga* compared to the sorghum cultivar N13. These wild sorghum accessions had the lowest *Striga* biomass and number of attached *Striga* individuals. Furthermore, WSA-1, WSE-1, and WSA-2 accessions hindered *Striga* haustorium's entry into the host endodermis, indicating the accumulation of large amounts of secondary metabolites that formed a dark coloration at the interface (Mbuvi et al. 2017). The results suggest these sorghum wild relatives could be a source of the *Striga* resistance trait for developing sorghum cultivars resistant to *Striga* infestation, which could be very helpful for developing sorghum cultivars resistant to *Striga* by directly introducing specific quantitative trait loci (QTLs). Ngugi et al. (2015) reported that they used molecular marker-assisted selection (MAS) to directly introgress and select three *Striga* resistance QTLs (QTL A, B, and J2) located on different chromosomes from the mechanical barrier resistance cultivar N13 to the susceptible cultivar Ochuti.

The hemiparasitic weed *Striga* attaches to the host roots through a specialized haustorium organ, enabling it to drain the host's water and nutrients (Stanley et al. 2021). While *Striga* management employs agronomic and cultural practices (Kanampiu et al. 2018), their effectiveness is limited due to copious seed production (approximately 0.5 million seeds per plant) (Yoneyama et al. 2010), long seed viability (David et al. 2022), and high labor requirements. Furthermore, the ease with which *Striga* seeds disperse as fine dust facilitates their widespread distribution, and rapid evolution enables *Striga* to overcome host resistance mechanisms (Jhu et al. 2023). Thus, understanding the resistance mechanisms through which hosts ward off *Striga* is critical for elucidating host-parasite interactions and integrating natural resistance into multi-pronged management strategies (Mwangangi et al. 2021).

Resistance to parasitic weeds can occur before (pre-attachment) or after (post-attachment) *Striga* attachment to the host roots. However, pre-attachment resistance mechanisms are the first line of defense, resulting in low germination rates and low host root attachment (Fishman & Shirasu 2021; Jhu & Sinha 2022). In this case, Gobena et al. (2017) reported that low germination stimulant-1 (LGS-1) mutations changed strigolactone biosynthesis, switching the production of *5-deoxystrigol*, which is very effective

in stimulating *Striga*, to *orobanchol*, which is not as effective at stimulating *Striga*. As a result, the extent of *Striga* seed germination in response to host root exudates is a key indicator of resistance (Mallu et al. 2021). Furthermore, resistant hosts can exude secondary metabolites that interfere with haustoria-inducing factor biosynthesis, thereby preventing haustorium initiation and development (Rich et al. 2004). Post-attachment resistance is a strategy employed by the sorghum in response to Striga infestation, where the host plant impedes the parasite's development after it has attached to the host roots, often resulting in the death or stunted growth of the parasite (Kavuluko et al. 2021). Post-attachment resistance in sorghum can be further delineated into mechanical and biochemical mechanisms. Mechanical barriers, such as lignification or suberization of cell walls, physically impede the intrusion of *Striga* haustoria into the host vascular system (Mbuvi et al. 2017).

On the other hand, hypersensitivity, a biochemical mechanism, involves a rapid, localized cell death response at the site of parasite attachment, effectively isolating and starving the parasite. This hypersensitive response is often accompanied by the production of phenolic compounds and reactive oxygen species, contributing to the host's defense (Mutuku et al. 2019). Understanding these distinct mechanisms is crucial for developing effective *Striga* management strategies in sorghum breeding programs.

Molecular markers can be effectively used to identify genomic regions linked to *Striga* resistance and investigate how hosts and parasites interact and co-evolve, which allows the development of resistant sorghum cultivars by identifying genes linked to *Striga* resistance (Badu-Apraku et al. 2020). Yoshida and Shirasu (2012) suggested that molecular markers can generate genetic information that provides a platform for understanding the evolutionary and parasitic aspects of host-parasite interactions. In addition, marker-assisted selection is an effective method to identify, validate, and introgress *Striga* resistance genes from resistant donors into elite sorghum cultivars (Gasura et al. 2022). Grenier et al. (2007) identified two genes linked to a hypersensitivity-like resistance mechanism in sorghum (HR1 and HR2). Later, Yohannes et al. (2015) used marker-assisted selection (MAS) to transfer five quantitative trait loci (QTLs) from the resistant Indian donor N13 to the susceptible cultivar Hugurtay. Different molecular markers on the sorghum genetic map associate these genes and QTLs with *Striga* resistance.

Furthermore, Pfunye et al. (2021) demonstrated the effectiveness of MAS by validating molecular markers for *Striga* resistance, which can be incorporated into their selection process. This study aims to understand the *Striga* resistance regulating genetic variants among wild relatives of Sudanese sorghum. In addition, it is focused on elucidating the simple sequence repeat (SSR) loci variation regulating the post-attachment resistance mechanisms of sorghum, including the mechanical barrier and hypersensitive resistance against *Striga hermonthica*.

Materials and methods

Plant materials

In total, 255 wild Sudanese sorghum accessions, locally known as Adar, were collected from the border areas between Sudan, Eritrea, and Ethiopia, where sorghum is domesticated. In addition, the *Striga*-susceptible cultivar Tabat, the universal Indian donor for mechanical barrier N13, and the hyper-sensitive resistant cultivar Framida were included to represent the susceptible and resistant checks (Table 1S).

DNA extraction, simple sequence repeat markers (SSR), and PCR work

Genomic DNA was extracted from the fresh leaves of 10-day-old plants of the 255 accessions using the modified Cetyl-trimethyl Ammonium Bromide (CTAB) method (Mace et al. 2003). The quality of extracted DNA was checked by mixing three µl of the DNA with two µl of blue loading dye, loaded onto a 1% agarose gel, and electrophoresis at 100 V for 40 min. The remaining DNA extracted from each sample was stored at − 20 °C until further use.

In total, 17 SSR markers associated with the mechanical barrier resistance mechanism were tested on the resistant Indian cultivar N13 (positive control) and the susceptible cultivar Tabat (negative control), 9 markers representing five QTLs were polymorphic. The only two available SSR markers linked to HR1 and HR2 resistance genes were used to detect polymorphisms among sorghum accessions for mechanical barrier and hypersensitive *Striga* resistance

Table 1 Characteristics of the 11 simple sequence repeat (SSR) primers used in the study

No.	Primer name	Primer sequence	Chromosome position	Fragment Size (bp)
1	Xisep 0327	F:CTGTTTGTGCTTGCAACTCC R: TCATCGATGCAGAACTCACC	SBI-1	205–210
2	Xtxp 298	F:GCATGTGTCAGATGATCTGGTGA R:GCTGTTAGCTTCTTCTAATCGTCGGT	SBI-2	200–250
3	Xtxp 201	F:GCGTTTATGGAAGCAAAAT R:CTCATAAGGCAGGACCAAC	SBI-2	195–202
4	Xtxp 065	F:CACGTCGTCACCAACCAA R:GTTAAACGAAAGGGAAATGGC	SBI-5.1	145–150
5	Xtxp 014	F:GTAATAGTCATGACCGAGG R:TAATAGACGAGTGAAAGCCC	SBI-5.2	150–170
6	Xtxp 015	F: CACAAACACTAGTGCCTTATC R: CATAGACACCTAGGCCATC	SBI-5.2	200–210
7	Xtxp 045	F: CTCGGCGGCTCCCTCTC R: GGTCAAAGCGCTCTCCTCCTC	SBI-6	190–220
8	Xtxp 057	F:GGAACTTTTGACGGGTAGTGC R: CGATCGTGATGTCCCAATC	SBI-6	248–255
9	Xtxp 145	F: GTTCCTCCTGCCATTACT R: CTTCCGCACATCCAC	SBI-6	219–249
10	Xtxp 96	F:GCTGATGTCATGTTCCCTCAC R: CATTCGTGGACTCTGTCGG	LG02	170–190
11	SbKAFGK1	F:AGCATCTTACAACAACCAAT R:CTAGTGCACTGAGTGATGAC	LG05	140–145

mechanisms, respectively (Anteyi & Rasche 2020; Bhattramakki et al. 2000; Ejeta 2007). The primers' name, sequence, chromosome position, and observed allele fragment size in the base pairs (bp) of the SSRs are listed in Table 1.

PCR was performed using Eppendorf Master Cycler (Eppendorf, Hamburg, Germany). The total volume of the reaction mixture was 20 µl containing 0.5 µl DNA, 4 µl of Solis BioDyne 5×Blend Master Mix Buffer, 12.5 mM $MgCl_2$,1×PCR solution -2.5 Mm $MgCl_2$ µM.1mMdNTPs of each 1XPCR solution -200 µM dATP,200 µM dCTP, 200 µM Dgtp, and 200 µM DttpBSA, Migration equivalent to 3.5–4.5kbDNA fragment. Yellow dye migration is comparable to a 35–45 bp DNA fragment., 0.4 µl forward primer, 0.4 µl reverse primer, and 14.7 µl dd H2O.

The PCR conditions were as follows: initial denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing (45 °C-60°C) for 30 s, and extension at 72 °C for 1 min, and final extension at 72 °C for 7 min. The annealing temperatures of the primer pairs were varied based on their melting temperatures. The amplified products were stored at 4 °C until electrophoresis. Electrophoresis of the amplified PCR products was performed on 2% agarose gels.

Data analysis

Genetic polymorphism analyses were performed using GenAlex v6.5 (Peakall and Smouse 2006). The following genetic parameters were estimated: total number of alleles per locus (Na), number of effective alleles per locus (Ne), expected heterozygosity (He), and Shannon's Information Index (I). Polymorphic information content (PIC) was estimated according to the method described by Smith et al. (1997). The Hardy–Weinberg equilibrium (HWE) test was also computed using GenAlEx version 6.5 (Peakall & Smouse 2006).

The K-means clustering analysis was conducted using the *stats* package for the *k-means* function in the R environment to identify and group the wild sorghum accessions based on specific resistance loci.

The accessions were then grouped according to their K-means clustering analysis and used to analyze the molecular variance (AMOVA). The hierarchical clustering heatmaps were created using the *pheatmap* package in R and the Principal Component Analysis (PCA) of M.B. data was performed using Python's *scikit-learn* library, with the first two principal components visualized with *matplotlib* (Pedregosa et al. 2011). In addition, PCA for HR was performed on the SSR marker data using the *prcomp*() function in R, and the results were visualized using *ggplot2* (Wickham & Wickham 2016).

The population structure and admixture patterns were determined by using an admixture model-based Bayesian algorithm implemented in STRUCTURE software version 2.3.3 (Pritchard et al. 2000). Based on the theory that an individual's genome is a mixture of K ancestral populations, an admixture model with correlated allele frequencies was used. To estimate the best (K), 100,000 burn-in period and 250,000 Markov chain Monte Carlo (MCMC) replications were performed for each K, ranging from 1 to 10. The best K value was predicted using the web-based STRUCTURE HARVESTER software version 0.6.92 (Earl & VonHoldt 2012) following the simulation method of Evanno et al. (2005).

Results

SSR markers and resistance-related allelic variation

Allelic size values for SSR markers linked to mechanical barrier resistance in the resistant cultivar N13 ranged from 145 bp (Xtxp 065) to 250 bp (Xtxp 298), while in the susceptible cultivar Tabat, they ranged from 150 bp (Xtxp 065) to 250 bp (Xtxp 057). For markers linked to hypersensitivity resistance, allelic sizes in the resistant cultivar Framida varied from 145 bp (SbKAFGK1) to 170 bp (Txp 96), whereas in the susceptible check (Tabat), they ranged from 140 to 150 bp (Table 2).

Across 255 sorghum accessions, nine SSR markers linked to mechanical barriers showed two alleles each, totaling 18 alleles. The major allele frequency (MAF) for these markers ranged from 0.502 (Xisep

Table 2 The resistance-related allelic variation indices summary of the 11 simple sequence repeat (SSR) loci across populations of Sudanese wild Sorghum accessions

Marker	MAF	Na	Ne	I	GD	He	F	Ho	PIC	PHWE
Mechanical barrier										
Xisep 0327	0.502	2.00	1.87	0.66	0.50	0.46	1.00	0.002	0.37	0.000***
Xtxp 298	0.549	2.00	1.79	0.63	0.50	0.44	0.73	0.118	0.37	0.000***
Xtxp 201	0.633	2.00	1.93	0.70	0.46	0.48	0.46	0.265	0.37	0.000***
Xtxp 065	0.533	2.00	1.79	0.63	0.50	0.44	0.82	0.074	0.37	0.000***
Xtxp 014	0.559	2.00	1.87	0.66	0.49	0.45	0.67	0.150	0.37	0.000***
Xtxp 015	0.614	2.00	1.92	0.70	0.47	0.48	0.37	0.305	0.37	0.000***
Xtxp 045	0.502	2.00	1.55	0.50	0.50	0.34	1.00	0.000	0.37	0.000***
Xtxp 057	0.510	2.00	1.70	0.60	0.50	0.41	0.91	0.031	0.37	0.000***
Xtxp 145	0.520	2.00	1.88	0.70	0.50	0.46	0.91	0.046	0.37	0.000***
Mean	0.547	2.00	1.81	0.64	0.49	0.44	0.76	0.110	0.37	
Hyper-sensitive										
Xtxp 96	0.90	2.00	1.20	0.23	0.164	0.130	1.000	0.000	1.92	0.008**
SbKAFGK1	0.74	2.00	1.70	0.60	0.380	0.384	1.000	0.000	0.03	0.000***
Mean	0.82	2.00	1.45	0.42	0.272	0.257	1.000	0.000	0.98	

Where MAF = Major allele frequency, Na = Number of alleles, Ne = Effective number of alleles, I = Shannon's Information Index, GD = Gene diversity, He = Expected heterozygosity, F = Fixation Index = (He—Ho) / He = 1—(Ho / He), Ho = observed heterozygosity, PIC = Polymorphic information content, PHWE = P-value for deviation from HWE, ns = not significant, * $P < 0.05$ = significant, ** $P < 0.01$ = high significant., *** $P < 0.001$and hence = highly significant

0327) to 0.633 (Xtxp 201), with an average of 0.547. The highest effective number of alleles (1.93) and expected heterozygosity (0.48) were observed in Xtxp 201, while Xtxp 045 had the lowest values (1.55 and 0.34, respectively). Observed heterozygosity ranged from 0.0 (Xtxp 045) to 0.305 (Xtxp 015), with a mean of 0.11. All nine markers deviated significantly from Hardy–Weinberg equilibrium (HWE) ($p < 0.0001$).

Four alleles were observed for the two markers linked to hypersensitivity with an average MAF of 0.82 per locus. The highest effective number of alleles (1.7) and gene diversity (0.38) were found in SbKAFGK1. Both markers showed significant deviation from HWE, with PIC values ranging from 0.03 (SbKAFGK1) to 1.92 (Txp 96) (Table 2). These results highlight the significant genetic variation associated with mechanical and hypersensitivity resistance in sorghum, providing valuable markers for breeding programs targeting *Striga* resistance.

Resistance variation within and between populations

Based on the K-means clustering analysis, the group 2 population showed the highest number of effective alleles (1.874), Shannon's information index (0.655), and expected heterozygosity (0.463) (Table 3). In contrast, the Group 4 population had the lowest number of effective alleles (1.767), Shannon's information index (0.608), and expected heterozygosity (0.422). No private alleles were unique to a single population. The percentage of polymorphic loci per population was 100% for all the populations. On the other hand, hypersensitive group 2 had a lower number of alleles (1.500), while the different groups had 2.000 alleles, indicating variation in allele numbers across populations (Table 3). The Group 3 population showed the highest number of effective alleles (1.7), Shannon's information index (0.57), and expected heterozygosity (0.39). In contrast, the group 2 population had the lowest number of effective alleles (1.19), Shannon's information index (0.22), and expected heterozygosity (0.139). Most populations had 100% polymorphic loci, except for Group 2 (50%).

Genetic differentiation, distance, and gene flow based on resistance-related SSR loci

Table 4 presents the genetic differentiation from zero ($P \leq 0.001$) among the population groups for mechanical barrier and hypersensitive resistance, measured by Fst values. For the mechanical barrier, the Fst values range from 0.009 to 0.043, indicating varying degrees of genetic differentiation, with Group 5 showing the highest divergence from the other groups. In hypersensitive resistance, Fst values range from 0.001 to 0.338, highlighting a significant genetic distinction between Group 2 and the other populations. These values reflect the genetic structure and differentiation within and between the groups, which are crucial for understanding population dynamics and evolutionary relationships.

The pairwise Nei's genetic distance and gene flow (Nm) of each population from the other populations ranged from 0.012–0.075 and 0.928–0.988,

Table 3 Allelic patterns and diversity indices across populations averaged over the 11 simple sequence repeat (SSR) loci

Where N = number of samples, Na = Number of alleles, Ne = Effective Number of alleles, NPA = Number of Private Alleles, I = ' 'Shannon Information, Index, He = Expected heterozygosity, NPL = Number of polymorphic loci, PPL = percentage of polymorphic loci

Population	N	Na	Ne	NPA	I	He	NPL	PPL
Mechanical barrier								
Group 1	124	2	1.81	0	0.63	0.44	2	100
Group 2	49	2	1.87	0	0.66	0.46	2	100
Group 3	41	2	1.80	0	0.63	0.44	2	100
Group 4	30	2	1.77	0	0.61	0.42	2	100
Group 5	10	2	1.78	0	0.62	0.43	2	100
Mean	50.8	2	1.81	0	0.63	0.44	2	100
Hypersensitive								
Group 1	173	2	1.37	0	0.43	0.27	2	100
Group 2	6	2	1.19	0	0.22	0.14	1	50
Group 3	17	2	1.70	0	0.58	0.39	2	100
Group 4	59	2	1.38	0	0.35	0.23	2	100
Mean	63.7	2	1.41	0	0.40	0.26	1.87	87.5

Table 4 Population genetic-resistance differentiation measured by Fst ($P >= 0.001$) among different genetic groups for mechanical barrier and hypersensitive resistance

	Group 1	Group 2	Group 3	Group 4	Group 5
Mechanical barrier					
Group 1	–				
Group 2	0.012	–			
Group 3	0.009	0.008	–		
Group 4	0.017	0.020	0.009	–	
Group 5	0.043	0.024	0.025	0.021	–
Hypersensitive resistance					
Group 1	–				
Group 2	0.338	–			
Group 3	0.001	0.060	–		
Group 4	0.013	0.325	0.007	–	

Table 5 Pairwise Nei's genetic distance (below diagonal) and gene flow (Nm) values (above diagonal) among sorghum populations from Sudan for mechanical barrier and hypersensitive

	Group 1	Group 2	Group 3	Group 4	Group 5
Mechanical barrier					
Group 1	–	0.980	0.985	0.973	0.928
Group 2	0.020	–	0.986	0.970	0.960
Group 3	0.015	0.014	–	0.988	0.959
Group 4	0.027	0.031	0.012	–	0.970
Group 5	0.075	0.040	0.042	0.030	–
Hypersensitive resistance					
Group 1	–	0.000	0.500	0.500	
Group 2	0.000	–	0.500	0.500	
Group 3	0.693	0.693	–	0.000	
Group 4	0.693	0.693	0.000	–	

respectively (Table 5). The populations of Groups 5 and 1 exhibited the highest genetic distance (0.075) and lowest gene flow (0.928) (Table 5). The populations of Groups 3 and 4 showed the lowest genetic distance (0.012) and highest gene flow (0.988) (Table 5). The populations of Groups 3 and 5 exhibited the second-highest genetic distance (0.042) and second-lowest gene flow (0.959).

For the hypersensitive population, pairwise genetic differentiation between the populations ranged from 0.001 to 0.338 (Table 4). Groups 2 and 1 showed the highest population differentiation (Fst = 0.338), followed by groups 4 and 2 (Fst = 0.325). Groups 3 and 1 showed the lowest population differentiation (Fst = 0.001) (Table 4). For each population, pairwise Nei's genetic distance and gene flow (Nm) showed low (0.00) to intermediate (0.5) values (Table 5).

Analysis of molecular variance

The mechanical barrier AMOVA revealed that variability among and within populations accounted for 1% and 99% of the total genetic variation, respectively. The overall fixation index value, used as a measure of population differentiation, was moderate (Fst = 0.073) (Table 6). In contrast, for hypersensitive resistance, (Table 6) shows variability among and within populations accounted for 6% and 94% of total genetic resistance variation, respectively. The overall fixation index (Fst) was 0.076, indicating moderate genetic resistance differentiation among the populations.

Table 6 Analysis of molecular variance of 255 wild sorghum accessions using 11 simple sequence repeat (SSR) markers for mechanical barrier and hypersensitive resistance mechanisms

Source	Degrees of freedom	Sum of squares	Mean squares	Est. Var. %	Fixation index
Mechanical barrier					
Among Pops	4	24.055	6.014	1%	0.073
Within Pops	503	2106.153	4.187	99%	
Total	507	2130.209		100%	
Hypersensitive resistance					
Among Pops	3	4.652	1.551	6%	0.076
Within Pops	506	134.061	0.265	94%	
Total	509	138.714		100%	

Cluster analysis and population structure

We performed hierarchical clustering analysis on 255 sesame accessions to investigate potential resistance mechanisms against Striga infestation (Fig. 1). Analysis of the mechanical barrier (Fig. 1a) revealed three main clusters represented in a heat map, with colors indicating resistance levels from high (dark blue) to susceptible (red). The clusters comprised an upper cluster (approximately 47.9%, 122 accessions) dominated by resistance, a middle cluster with moderate resistance, and a bottom cluster (approximately 13.7%, 35 accessions) showing higher susceptibility. For hypersensitive response (Fig. 1b), two major clusters emerged: a large upper red cluster containing the majority of accessions (approximately 67.1%, 171 accessions), representing those with higher hypersensitive response, and a blue lower cluster (approximately 32.9%, 84 accessions), indicating a lower hypersensitive response. This distribution suggests two distinct patterns of hypersensitive response among the accessions.

We further investigated the genetic relatedness among sorghum accessions using principal component analysis (PCA) based on the mechanical barrier (PCA) (Fig. 2a). The first and second principal component axes showed 51.81% and 21.66% of total variation among the accessions. Furthermore, we used principal component analysis (PCA) based on hypersensitive response to investigate the genetic relatedness among sorghum accessions (Fig. 2b). PCA revealed 100% of the variations explained by the first two axes, accounting for 69.83% and 30.17%, respectively.

Population structure analysis revealed that the maximum delta K exhibits a sharp peak at K = 3, suggesting three clusters according to the mechanical barrier markers. Based on this value, Clumpak's result (bar plot) detected a genetic admixture; hence, there was clear genetic-based structuring of accessions (Fig. 3a). In contrast, the population structure analysis for HR revealed that the maximum delta K becomes a sharp peak at K = 4, suggesting four clusters. Based on this value, Clumpak's result (bar plot) detected a genetic admixture; hence, the accessions had a clear genetic structure (Fig. 3b).

Discussion

Sudan is recognized as the center of origin and diversity of sorghum, anticipating substantial genetic diversity among wild sorghum germplasms. Consequently, exploring the gene pool in Sudanese wild sorghum for post-attachment mechanisms of *Striga* resistance has significant merit. The genetic variation

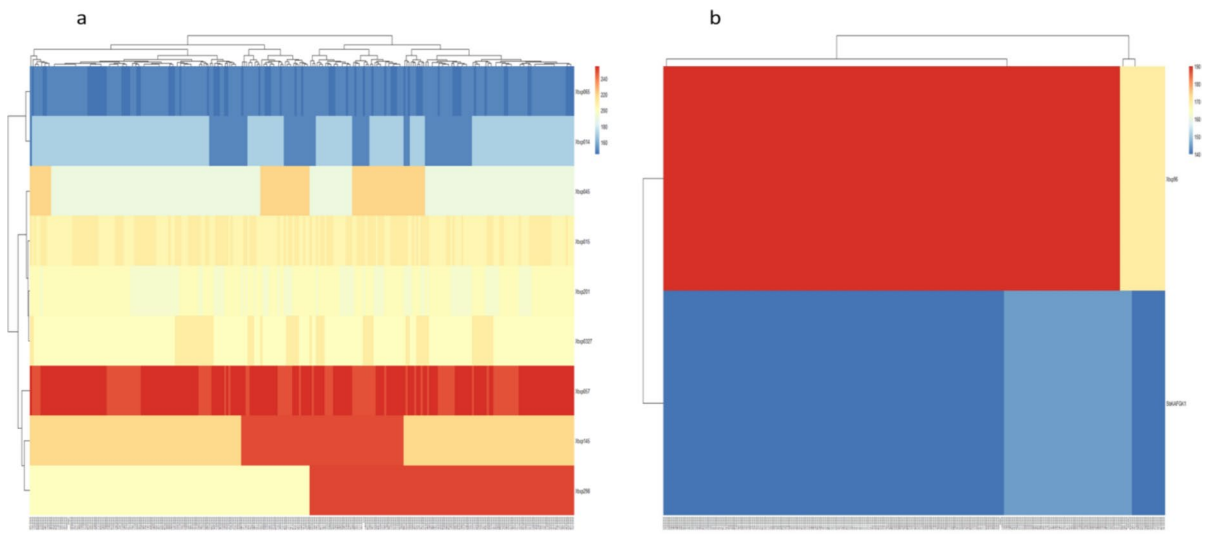

Fig. 1 Unweighted pair group method with arithmetic mean heatmap dendrogram showing genetic relationships among wild sorghum accessions for mechanical barrier resistance (**a**) and hypersensitive resistance (**b**)

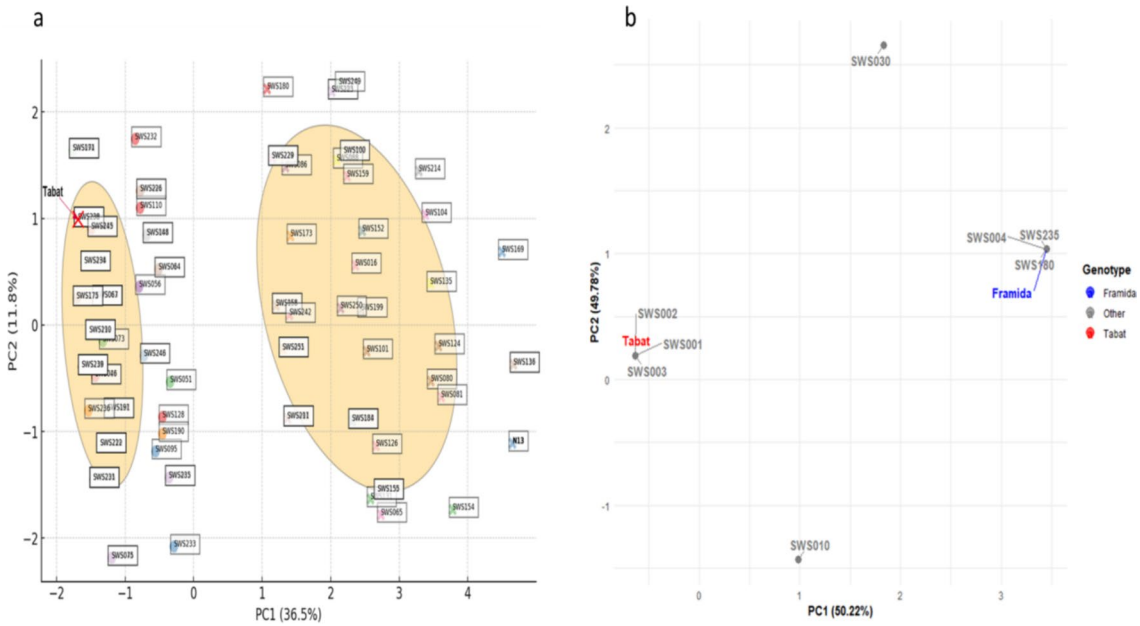

Fig. 2 Principal component analysis of sorghum accessions using simple sequence repeat (SSR) markers, (**a**) based on Mechanical barrier markers (**b**) based on Hypersensitive markers

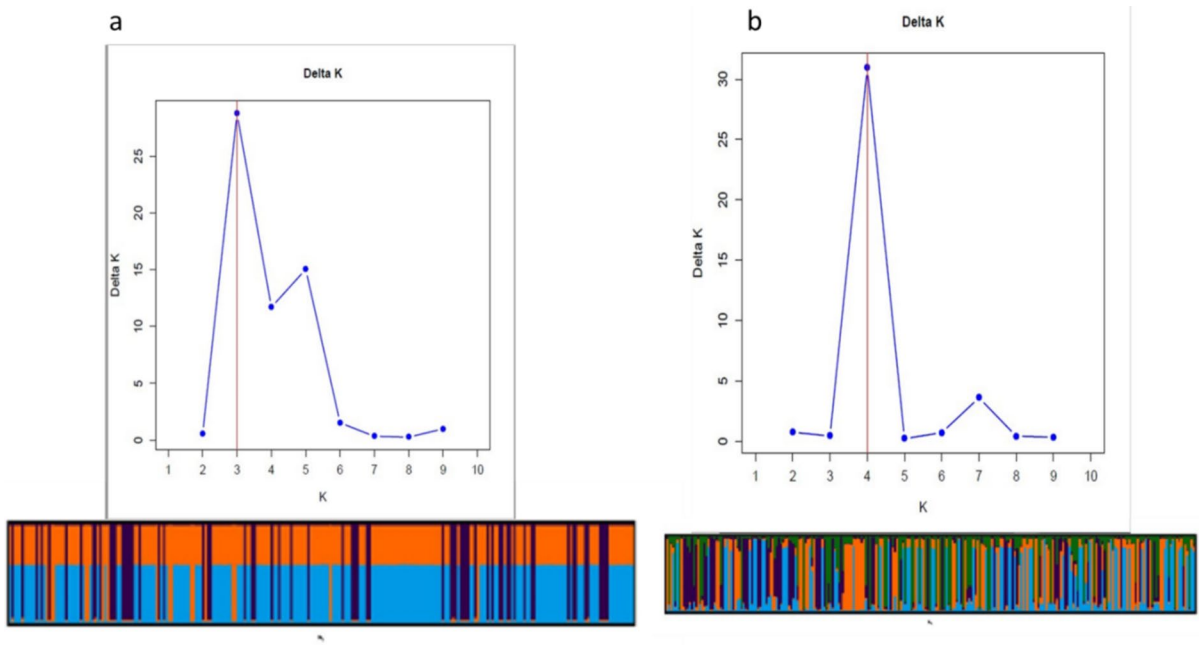

Fig. 3 (**a**) Population structure of sorghum accessions representing Sudanese wild sorghum populations. The best delta *K* value was estimated using the Evanno et al. (2005) method and Bayesian model-based estimated population structure for $K=3$, according to resistance. The different colors (blue and orange) represent genetic groups or sub-populations designated by the Structure Harvester. (**b**) Population structure of sorghum accessions representing SWS populations. Bayesian model-based estimated population structures for $K=4$ according to resistance. The different colors (blue and orange) represent genetic groups or subpopulations designated by the Structure Harvester

of 255 Sudanese wild sorghum accessions was examined using 11 SSR markers. This study focused on loci for post-attachment *Striga* resistance mechanisms, including mechanical barriers and hypersensitive. Our results provide essential information regarding these accessions' genetic structure and differences in the loci for post-attachment resistance, which could benefit sorghum breeding programs in developing *Striga*-resistant cultivars.

According to Maiti et al. (1984), mechanical barriers are physiological mechanisms that prevent *Striga* from penetrating the host cell wall via cell wall thickening caused by silica and lignin deposition. We used the *Striga resistant* Indian cultivar N13, known for its high lignin and suberin deposition, as a resistant check. In contrast, the high-yield, farmer-preferred cultivar Tabat served as a susceptible control. We screened 255 Sudanese wild sorghum accessions for *Striga* resistance genes using 11 SSR markers. Based on the mechanical barrier mechanism, 9 SSR markers were polymorphic and associated with mechanical barriers *Striga* resistance. We obtained the same results as those of Haussmann et al. (2004) reported after using RAPD and SSR markers to identify several quantitative trait loci (QTLs) linked to *Striga* resistance in groups of resistant N13 and susceptible E103 cultivars. Their results concluded that SSRs are highly polymorphic and co-dominantly inherited markers that are widely used in plant breeding, and their abundance in the genome and ease of use make them ideal for QTL mapping and marker-assisted selection. SSR markers flanking the N13 QTL can facilitate the selection of resistant genotypes in breeding programs. In this study, 18 alleles have been detected with an average of two loci. However, the limited number of SSR markers is possibly due to low genetic variation in the loci related to *Striga* resistance.

The mean resistance-related allelic variation observed in this study (0.50) is consistent with Yohannes et al. (2015), who reported a value of 0.57 in Eritrean sorghum genotypes. However, it was lower than the 0.78 reported by Nemera et al. (2022), possibly due to differences in the number and origin of the sorghum accessions investigated. Populations from Group 2 showed higher variation than the grand mean, suggesting the existence of prime candidates for identifying novel sources of resistance for future breeding efforts. The AMOVA results showed that 99% of the genetic-resistance variation was within populations, which is similar to the finding of Tirfessa et al. (2020), who reported a high variation within populations (99.6%) and high gene flow ($Nm = 11.881$) among populations indicating substantial genetic exchange to maintain resistance diversity. The universal Indian-resistant donor cultivar N13 and at least 17 wild sorghum accessions from Sudan were in the same cluster, with an additional 42 accessions in adjacent clusters. However, this is the first report to explore the genetic-resistance variation among Sudanese wild sorghum accessions for *Striga* resistance based on the mechanical barrier mechanism.

The observed genetic-resistance groups in this study represent distinct clusters within Sudanese wild sorghum accessions, highlighting their potential as sources of *Striga* resistance traits in breeding programs. Accessions grouped with resistant checks, such as N13, may share key adaptive traits developed under environmental conditions with high *Striga* infestation or due to human intervention through selection pressures. This clustering suggests the presence of genetic-resistance mechanisms specific to the groups that contribute to enhanced *Striga* resistance.

The findings of this study align with those reported by Mbuvi et al. (2017), who conducted both laboratory and field experiments to evaluate the responses of seven wild sorghum accessions. These accessions were selected from three distinct taxonomic groups: (*Sorghum aethiopicum*), (*Sorghum drummondii*), and (*Sorghum arundinaceum*). The research aimed to determine how these wild accessions reacted to the N13 quantitative trait locus (QTL) associated with resistance to Striga. Their results determined that three wild sorghum accessions exhibited significantly higher resistance than N13, underscoring the significance of wild sorghum as a reservoir for the *Striga* resistance trait, which could broaden the genetic foundation of cultivated sorghum.

Necrosis at the infection site plays a crucial role in hypersensitive resistance mechanisms. According to Ejeta (2007), the cultivar Framida exhibits significant necrosis at the attachment site, which effectively inhibits the development and penetration of Striga roots into the host tissue. Grenier et al. (2007) reported that two genes, *HR1* and *HR2*, control hypersensitive resistance. However, Ejeta (2005) and Anteyi and Rasche (2020) observed a link between these genes and SSR markers *Txp96* and *SbKAFGK1*.

Therefore, we could only investigate hypersensitive resistance mechanisms in sorghum using *Txp96* and *SbKAFGK1*. These two SSR markers generated an average of 2.00 alleles per locus, lower than the 4.8 reported by Yohannes et al. (2015). This variation could be due to the high resistance diversity of sorghum accessions and the differences in SSR marker polymorphisms used in the previous study. According to Botstein et al. (1980), markers with PIC values above 0.5 are considered highly informative, and the mean PIC value of the SSR markers in this study was 0.97, indicating high informativeness. The SSR marker results placed five wild sorghum accessions within the same cluster as the resistant cultivar Framida, whereas 17 accessions were located in an adjacent cluster. The AMOVA results showed that 94% of the genetic-resistance variation exists within populations, which indicates a high resistance variation within populations. The findings are supported by Mamo et al. (2023), who reported substantial genetic variation within populations, quantified at 97.6%, and values greater than 2.0 indicate high gene flow, where the observed gene flow of 2.8 among most population pairs likely reflects the significant genetic resistance variability among the wild sorghum accessions investigated.

Rich et al. (2004) identified Sudanese wild sorghum PQ-434, belonging to *drummondii*, which possessed low germination stimulants and haustorium factor. The ultimate goal was combining multiple Striga resistance mechanisms into a single cultivar using marker-assisted backcrossing breeding. This study aimed to investigate the genetic variation within loci related to the ability of different wild sorghum accessions to resist low germination stimulants and low haustorium factor. Therefore, further investigations, for instance, using in vitro techniques, are necessary to identify promising sorghum wild genotypes for *Striga* resistance. In addition, applying advanced molecular methods, such as transcriptomic profiling and omics, to understand the physiological processes that cause post-attachment *Striga* resistance would be critical for efficiently utilizing the germplasm. Although few transcriptomic or proteomic research is underway to identify susceptibility or resistance genes in *Striga*, the increasing availability of sorghum microarrays should address this gap.

The findings of this study have significant implications for developing resilient sorghum cultivars through breeding programs in Sudan and other regions facing *Striga-related* agricultural challenges. Identifying resistant accessions and their genetic markers can facilitate marker-assisted selection (MAS) in breeding. Incorporating diverse resistance mechanisms from wild sorghum into cultivars could enhance *Striga* resistance, reduce yield losses, and contribute to food security. The genetic insights from this study can inform targeted breeding strategies, harnessing mechanical barriers and hypersensitive responses to develop robust sorghum cultivars adapted to high *Striga* pressure environments. Moreover, this study highlights the potential of Sudanese wild sorghum as a valuable genetic resource for *Striga* resistance. Future research should focus on the detailed characterization of resistance mechanisms and integrating these traits into breeding pipelines to develop improved sorghum cultivars that are resilient against *Striga* and other biotic stresses.

Conclusions

This study screened the resistance variation among Sudanese wild sorghum accessions concerning post-attachment *Striga* resistance mechanisms using SSR markers linked to mechanical barriers and hypersensitivity responses. Some accessions were closely related to the resistant checks N13 and Framida, indicating their potential for introducing *Striga* resistance genes into elite sorghum cultivars. Significant genetic variation was found within the population, suggesting that wild accessions harbor valuable genetic material for *Striga* resistance. Further research is needed on the promising genotypes identified and exploring their responses to *Striga* using advanced omics techniques. Incorporating these resistance traits into breeding programs is essential for developing *Striga*-resistant sorghum cultivars, which could enhance food security and livelihoods in sub-Saharan Africa.

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Data availability Data will made available upon request.

Declarations

Conflict of interest The authors declare no competing interests.

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