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Identification of Striga resistance QTLs regions in some wild sorghum accessions

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Abstract: *Sorghum (Sorghum bicolor (L.) Moench.) is one of the most important cereal crops in the semi-arid areas. S. hermonthica is widely distributed and causes serious damage to sorghum and millets the main staple food crops in Sudan. Different control strategies for the parasite were developed, but they were either expensive or complex for farmers to afford or adopted. Striga resistant sorghum genotypes can be developed through hybridization or gene introgression between wild and cultivated sorghum. Sorghum wild relatives are recognized as the main genetic resource for novel genes to tackle intractable problems. The present study aimed to identify the QTLs regions in wild sorghum accessions resistant to Striga using SSR markers. The present study was undertaken to identify Striga resistance QTL in nine wild sorghum accessions. Sixteen identified SSR markers associated with Striga resistance in five QTL regions were used to screen wild accessions. Four QTLs were found in wild accession WSB-2, three QTLs in WSA-1 and WSA-4, two QTLs in WSB-1 and WSB-3 and a single QTL in WSA-3 and WSD-1. Results indicated that wild sorghum relatives showed not only strong heritable Striga resistance but also different resistance mechanisms from those in cultivated sorghum. This will definitely lead to a durable resistance and with advances in genomics, can lead to cloning of resistance genes for future inter/ intra specific gene transfer.*

Keywords: *Striga*, resistance, QTLs, wild sorghum.

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1. INTRODUCTION

Sorghum is the major staple food crop in sub-Saharan Africa. The parasitic weed, *Striga hermonthica* (Del.) Benth., is the main biotic factor that reduces crop production. Different control strategies for the parasite were developed, but they were either expensive or complex for farmers to afford or adopted. Resistance crop cultivars are found to be the most economical and effective control option. Sorghum wild relatives are recognized as the main genetic resource for novel genes to tackle untractable problems.

Gene flow, in its broadest sense, is the dispersal of genes (by way of pollen grains, seeds, or vegetative reproduction units) from one population of a taxon to another and results in the introduction of new alleles or change in the frequency of alleles within a population [1]. Gene flow between cultivated and wild/weedy relatives occurs when they occupy the same or overlapping locations, are sexually compatible, share a common pollination mechanism and have overlapping flowering times [2]. Crosses between resistance sources and adaptable elite varieties could result in genotypes with combined characteristics of high productivity, acceptable grain characteristics and other agronomic traits, as well as resistance/tolerance to both biotic and abiotic stress.

Molecular markers are used to characterize the genetic differences between organisms or species and to identify novel genes, accelerate backcrossing and pyramiding of genes and estimate genetic relatedness in breeding programs [3].

Simple Sequence Repeat (SSR) markers are widely used in plants because of their technical simplicity, relative abundance, hyper-variability, reproducibility, multi-allelic nature, co-dominant inheritance, good genome coverage and suitability for high-throughput analysis [4].

Molecular markers were significantly used in mapping/tagging of genes/QTLs controlling Striga resistance in sorghum, which resulted in an increase in the efficiency and effectiveness of conventional breeding by indirect selection of the desirable plants in segregating populations [5]. Significant progress has been made to identify molecular markers associated with *Striga* resistance and to construct sorghum genetic linkage maps using different methods [6]. Five genomic regions (QTLs) associated with stable *Striga* resistance in sorghum variety, N13, have been identified [7]. These QTLs were located on linkage groups 1, 2, 5 (2 QTLs) and 6, using the revised linkage group designation as proposed by reference [8], in two independent samples of a mapping population involving this resistance source [7]. The present study was undertaken to identify Striga resistance QTL in wild sorghum accessions.

2. MATERIALS AND METHODS

Nine sorghum wild relatives' accessions resistant to Striga were collected from Sorghum Research Program at Agricultural Research Corporation, Wadmedani, Sudan. Three accessions of *Sorghum bicolor* (L.) Moench (WSB-1, WSB-2 and WSB-3); five accessions of *Sorghum arundinaceum* (Desv.) Stapf. (WSA-1, WSA-2, WSA-3, WSA-4 and WSA-5) and accession of *Sorghum bicolor* subsp. *drummondii* (Nees ex Steud.) de Wet & Harlan. (WSD-1)

2.1 DNA EXTRACTION

High-throughput mini- DNA extraction protocol was followed for DNA extraction from the wild sorghum accessions using a modified CTAB method described by reference [9]. Young leaves (2-3), 3 to 4cm long, were harvested from 10-day old seedlings; leaf tissue, 70-100mg, was placed in a 96 deep-well plate together with two 4 mm stainless steel grinding balls (SpexCerti Prep, USA). For each sample 450 µl of preheated (at 65ºC for half an hour) extraction buffer [100 mMTris-HCl (pH-8, 1.4 M NaCl, 20mM EDTA, CTAB (2-3% w/v), ß-mercaptoethanol] was added and secured with eight strip caps. Samples were homogenized in a Geno Grinder 2000 (SpexCertiPrep, USA), following the manufacturer's instructions, at 500 strokes/min 5 times at 2-minute interval. The plates were fitted into locking device and incubated at 65˚C for 10 min and shaken periodically. For each sample 450µl of chloroform-isoamyl alcohol (24:1) was added and mixed thoroughly by inverting the plate twice; the plate was then centrifuged at 5500 rpm for 10 min. The aqueous layer

(300µl) was transferred to fresh strip tubes (Marsh Biomarket, USA); 0.7 vol. (210µl) of isopropanol (stored at –20˚C) was added to each sample and the tube was inverted gently to mix and then kept at −20˚C for 10 minutes. The samples were centrifuged at 6200 rpm for 15 minutes, the supernatant decanted and remaining pellets air dried for 20 minutes. The samples were treated with 30µg RNase in 200 µl low salt TE at 37˚C for 30 minutes. Solvent extraction was done by adding 200µl of phenol: chloroform: IAA (25:24:1) mixture and centrifuged at 5000 rpm for 10 minutes. The aqueous layer was transferred to fresh tubes and the step was repeated with chloroform: IAA (24:1) mixture. The aqueous layer was again transferred to new tubes and a total of 315µl ethanol-acetate solution (30 ml ethanol, 1.5 ml 3M NaOAc (pH-5.2)) was added to each sample and placed in -20˚C for 5 min. The samples were centrifuged at 6200 rpm for 15 minutes. The supernatant was decanted and pellets washed with 200µl of 70% ethanol and centrifuged at 5000 rpm for 5 minutes. The supernatant was decanted and the pellets air dried for one hour. Finally, the samples were dissolved in 100µl TE (T10E1) and kept at 4˚C. DNA was quantified by running the samples on 0.8% agarose gel containing 0.5µl/10ml Ethidium bromide (10mg/ml) with 0.5X TBE (Tris borate EDTA) buffer at a constant voltage (80 V) for 20 minutes and the gel visualized under UV illumination. The DNA was normalized to 5ng/µl by visually comparing diluted DNA samples with the standard defined λ DNA, molecular weight markers of 5 ng/µl and 10 ng/µl, by running on 0.8% agarose gel with 0.5X TBE (Tris borate EDTA) buffer at a constant voltage (80 V) for 20 minutes. The images of gels were visualized and documented under UV illumination using Uvi Tech gel documentation system (DOL-008.XD, England). Samples with low concentrations were re-extracted.

2.2 Screening with SSR markers

A total of sixteen sorghum SSR primer pairs, expected to detect QTL loci distributed across *Striga* resistant regions of the sorghum genome screened by reference [10] were selected (Table 1). Primers designed with an optimal Tm of 60˚C (54-65˚C), GC content of 30- 70% with a low chance of dimer or hair loop formation and with 100-300bp amplicon size, were used for parental polymorphism and then for screening RILs. Linked SSR markers were used for amplification of the sorghum DNA using polymerase chain reaction (PCR) conditions as mentioned in references [11] and [10]. PCR was performed in 15µl reaction volume with final concentrations of 7.5ng DNA using GoTaq® green master mix (Promega, Madison, USA). The following PCR conditions were used: initial denaturation at 94°C for 15 min (to activate Taq DNA polymerase) then 10 cycles of denaturation at 94°C for 15 sec, annealing at 61°C for 20 sec (temperature reduced by 1°C for each cycle), and extension at 72°C for 30 sec. This was followed by 34 cycles of denaturation at 94°C for 10 sec, annealing at 54°C for 20 sec, and extension at 72°C for 30 sec with the final extension of 10 min at 72°C. Amplification products were separated using the DNA Screening Kit in the capillary electrophoresis device QIAxcel Advanced Instrument (QIAGEN, Netherlands) following the manufacturer's protocol.

Table 1. Markers used in vicinity for *Striga* resistant QTLs in N13, Tabat, AG8 and wild sorghum accessions.

Marker	Position	Amplification product	Forward	Reverse	Origin
Xisep0949	SBI-1	100-109	CAGTGCCAATAAGCTCGTCTC	CATCGATCTCTGCTTCTGCTT	ICRISAT_Ramu
XmSbCIR347	SBI-1	181-183	GAACATCAGAGGGTTTACCA	GAACCAACTACGCTTGTGTC	From CIRAD
Xtxp340	SBI-1	209-211	CACGACGTTGTAAAACGACAGAACTGTGCATGTATTCGTCA	AGAAACTCCAATTATCATCCATCA	(Bhattramakki et al., 2000)
XmSbCIR223	SBI-2	123-133	CACGACGTTGTAAAACGACCGTTCCAATGACTTTTCTTC	GCCAATGTGGTGTGATAAAT	From CIRAD
Xiabtp515	SBI-2	214-218	TGCCACATCGATCTTGTCAC	AGGCAGTCACCCACACTACC	ICRISAT
Xtxp065	SBI-5.1	214-218	CACGTCGTCACCAACCAA	GTTAAACGAAAGGGAAATGGC	(Bhattramakki et al., 2000)
Xisep1129	SBI-5.2	200-204	CCTCCAGCCTACAACTCTGC	TGCCTTATTGGCTTTCTGCT	ICRISAT_Ramu
Xtxp015	SBI-5.2	233-236	CACAAACACTAGTGCCTTATC	CATAGACACCTAGGCCATC	(Law et al., 2000)
Xtxp262	SBI-5.2	167-170	TGCCTGCCCGACCTG	TTGCTGTCTCCGCTTTCC	(Bhattramakki et al., 2000)
Xtxp014	SBI-5.2	139-149	GTAATAGTCATGACCGAGG	TAATAGACGAGTGAAAGCCC	(Law et al., 2000)
Xtxp317	SBI-6	175-177	CCTCCTTTTCCTCCTCCTCCC	TCAGAATCCTAGCCACCGTTG	(Bhattramakki et al., 2000)
Xtxp057	SBI-6	264-267	GGAACTTTTGACGGGTAGTGC	CGATCGTGATGTCCCAATC	(Bhattramakki et al., 2000)
Xtxp176	SBI-6	177-180	CACGACGTTGTAAAACGACTGGCGGACATCCTATT	GGAGAGCCCGTCACTT	(Bhattramakki et al., 2000)
Xtxp045	SBI-6	179-192	CACGACGTTGTAAAACGACCTCGGCGGCTCCCTCTC	GGTCAAAGCGCTCTCCTCCTC	(Bhattramakki et al., 2000)
Xtxp145	SBI-6	204-207	CACGACGTTGTAAAACGACGTTCCTCCTGCCATTACT	CTTCCGCACATCCAC	(Bhattramakki et al., 2000)
Xisep0443	SBI-6	197-206	CACGACGTTGTAAAACGACTCATGTACAGAGGCGACACG	AGGTCGCAACAGACACCTTC	ICRISAT_Ramu

3. Result and discussion

Saturation of *Striga* resistance QTLs regions in wild sorghum accessions

Five *Striga* resistance QTLs regions on linkage groups SBI-01, SBI-02, SBI-05a, b and SBI-06 were identified and mapped by reference [7] with wide field resistance. Associated SSR markers, 16, mapped by reference [12] were used to compare sorghum variety N13 resistance with wild accessions. Markers were used to screen for polymorphism between the wild sorghum accessions and *Striga* resistance variety N13. The SSR markers, 16, linked to *Striga* resistance QTLs were screened against the template DNA of the nine wild sorghum accessions selected for the *Striga* resistance identified QTL regions with N13.

At linkage group SBI-01, sorghum variety N13 was polymorphic with wild accessions WSB-1, WSB-2, WSB-3, WSA-4 and WSD-1 by SSR marker Xisep 0949 (Fig. 1, Table 2). SSR marker Xtxp340 showed polymorphism of N13 with accessions WSB-1, WSB-2, WSB-3, WSA-2, WSA-3 and WSD-1 (Fig. 1, Table 2). Accession WSA-1 was polymorphic with the N13 by SSR marker XmSbCIR347 (Fig. 1, Table 2).

Polymorphic with N13 on linkage group SBI-02 observed by accessions WSA-1 and using SSR markers XmSbCIR223 (Fig. 2, Table 3). The SSR marker Xiabtp515 showed polymorphic only between wild accession WSA-1 and the N13 (Fig. 2, Table 3).

SSR marker Xtxp015 on linkage group SBI -05a provided polymorphism of N13 with WSB-2 (Fig. 3, Table 4). The SSR marker Xisep1129 showed polymorphic of WSB-1, WSB-2, WSA-4, WSA-5 and WSD-1 with the N13 on linkage group SBI-05b (Fig. 4, Table 5).

Linkage group SBI-06, SSR maker Xtxp317 presented polymorphic among the N13 and WSA-1, WSB-2, WSA-3, WSB-3, WSA-4, WSA-5 and WSD-1 (Fig.5, Table 6). SSR marker Xtxp057 showed polymorphic of WSB-2 and WSA-4 with the N13 (Fig. 5, Table 4). SSR marker Xtxp045 indicated polymorphic of WSA-2 with the N13 (Fig. 5, Table 6). SSR marker Xisep0443 presented polymorphic between the wild accessions WSB-2 and WSB-3 and the N13 (Fig. 5, Table 6).

No polymorphism between wild accessions and N13 on linkage group SBI-05a, SBI-05b and SBI-06 using SSR markers Xtxp065, Xtxp014, Xtxp262, Xtxp176 and Xtxp145.

The wild sorghum accessions showed resistance to *Striga* germination according to field evaluation in *Striga* infested field. Reference [13] suggested that mechanical barriers are the *Striga* resistance mechanism in the N13. The wild accessions that are polymorphic with the N13 are suggested to have the same and/ or *Striga* resistance mechanisms. The wild accessions that are not polymorphic with the N13 might have other resistance mechanisms such as low stimulant production, hypersensitive and incompatible response. Wild sorghum accession P47-121(*S. bicolor* subsp. *verticilliflorum* race *arundinaceum*) identified by reference [14] as high germination stimulator and haustorial initiator, but studies by reference [15] proved that it has hypersensitive and incompatible response to *Striga*.

The wild sorghum accession with four QTLs was WSB-2 at SBI-01, SBI-05a, SBI-05b and SBI-06. WSA-1 and WSA-4 showed three QTLs at SBI-01, SBI-02, SBI-05a and SBI-06 and at SBI-01, BI-05b and SBI-06, respectively. Two QTLS were observed at SBI-01 and SBI-05b for WSB-01 and at SBI-01 and SBI-06 for WSB-3. WSA-3 and WSD-1 showed single QTL at SBI-06 and SBI-01, respectively.

In this study, wild sorghum accessions are the source of resistance to *S. hermonthica*. Wild sorghum WSA-1 and WSA-2 displayed low *Striga* germination index and low Striga germination distance, WSB-1 and WSB-2 exhibited low *Striga* germination distance. These accessions could provide valuable source of resistance to *S. hermonthica*. The resistance mechanism in wild sorghum could be the same as resistant varieties or different from it. The resistance genes could transfer to cultivated sorghum cultivars. Resistance mechanisms in wild sorghum are different between species.

Reference [10] saturated the *Striga* resistance QTL regions identified by reference [7] addition of seventeen SSR markers.

Reference [16] also reported that one accession of *S. aethiopicum* and two accessions of *S. arundunicum* comparable to the N13 and could be integrated into adapted farmers- preferred varieties as well as could be used in the identification of *Striga* resistance loci. Reference [17] reported that genetic distances can vary between maps but marker locus order should remain the same between the maps of a single species.

In wild sorghum, *Striga* resistance is controlled by major recessive and some minor genes [18]. *S. arundinaceum* that exhibited hypersensitive response (HR) when crossed with cultivated varieties, led to the discovery that the resistance trait is controlled by two nuclear genes, HR1 and HR2, all in genetic linkage map being associated with different markers [15].

It concludes that sorghum wild relatives showed not only strong heritable *Striga* resistance but also showed different resistance mechanisms than those in cultivated sorghum. This will definitely lead to a durable resistance and with advances in genomics can lead to cloning of resistance genes for future inter/intra-specific gene transfer.

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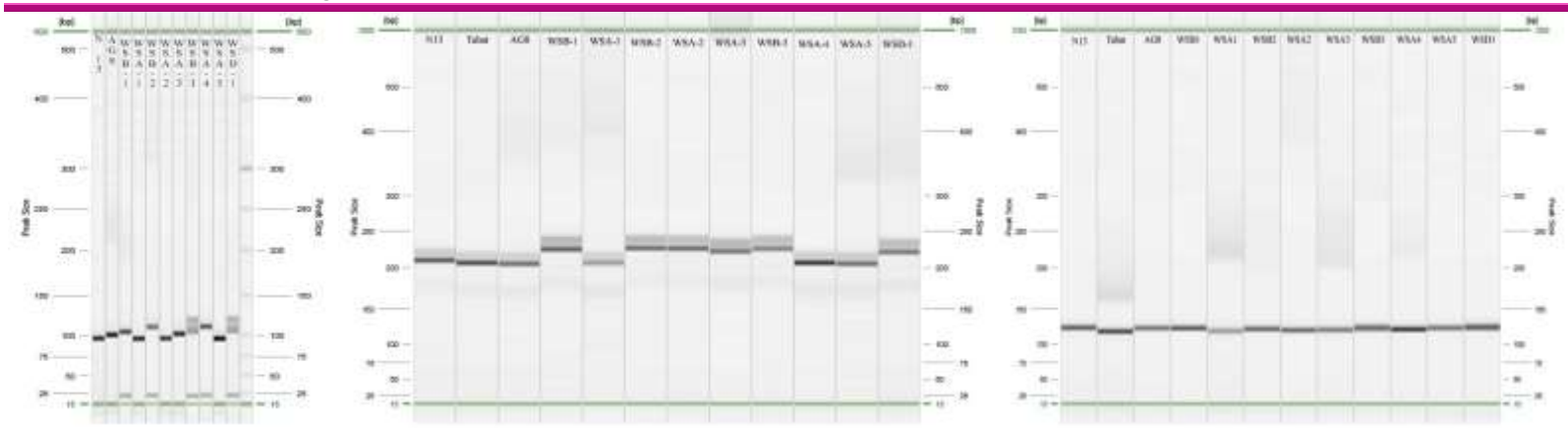

Xisep0949 Xtxp340 XmSbCIR347

Fig.1. The allele's size of genome DNA of N13, Tabat, AG8 and wild sorghum accessions using SSR markers on QTL SBI-01.

Table 2. Polymorphic SSR markers and their allele sizes at linkage group SBI- 01 for nine wild sorghum accessions, N13, Tabat and AG8

Linkage group	Marker name	Allele size (bp)											
		N 13	Tabat	AG8	WSB-1	WSA-1	WSB-2	WSA-2	WSA-3	WSB-3	WSA-4	WSA-5	WSD-1
SBI-01	Xisep 0949	96	---	100	22	96	23	96	101	23	23	96	23
	Xtxp340	209	206	205	225	165	226	226	222	225	206	205	221
	XmSbCIR347	122	117	121	122	23	120	118	119	122	120	122	122

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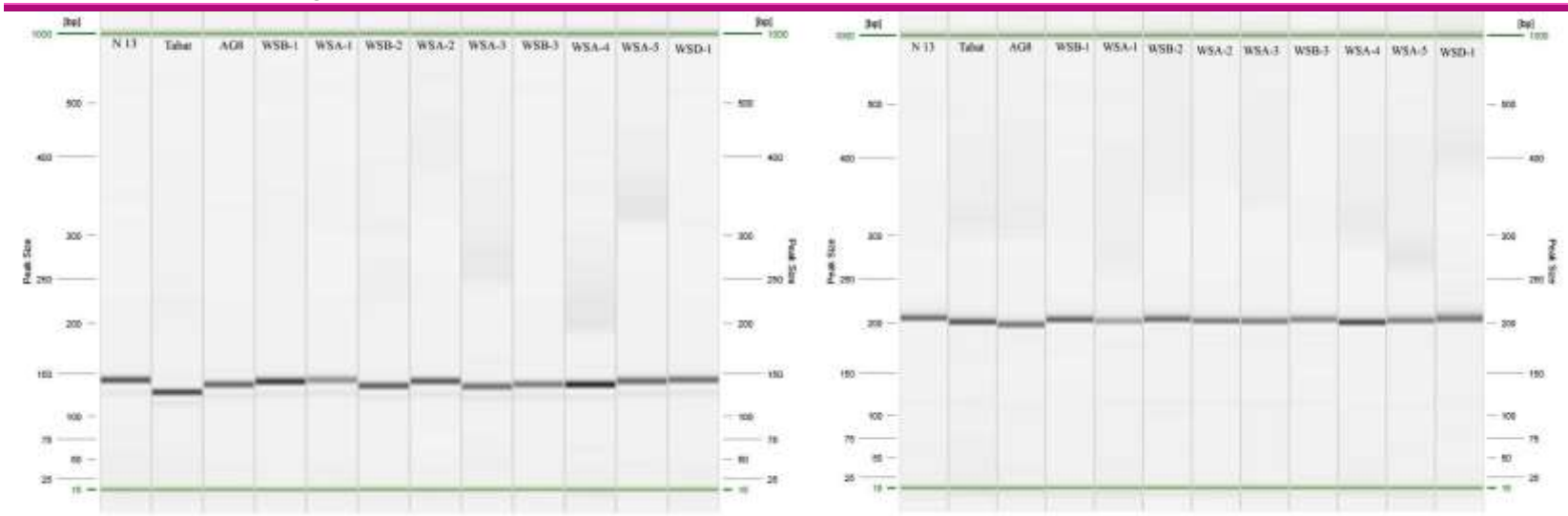

XmSbCIR223 Xiabtp515

Fig. 2. The allele's size of genome DNA of N13, Tabat, AG8 and wild sorghum accessions using SSR markers on QTL SBI-02.

Table3. Polymorphic SSR markers and their allele sizes at linkage group SBI- 02 for nine wild sorghum accessions, N13, Tabat and AG8

Linkage group	Marker name	Allele size (bp)											
		N13	Tabat	AG8	WSB-1	WSA-1	WSB-2	WSA-2	WSA-3	WSB-3	WSA-4	WSA-5	WSD-1
SBI-02	XmSbCIR223	141	127	136	140	126	135	140	134	123	137	140	142
	Xiabtp515	205	201	198	203	22	204	202	201	203	201	202	204

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Fig. 3. The allele's size of genome DNA of N13, Tabat, AG8 and wild sorghum accessions using SSR marker Xtxp015on QTL SBI-05a.

Table 4. Polymorphic SSR markers and their allele sizes at linkage group SBI- 05a for nine wild sorghum accessions, N13, Tabat and AG8

Linkage group	Marker name	Allele size (bp)											
		N13	Tabat	AG8	WSB-1	WSA-1	WSB-2	WSA-2	WSA-3	WSB-3	WSA-4	WSA-5	WSD-1
SBI-05a	Xtxp015	219	217	213	209	207	98	206	215	213	212	217	216

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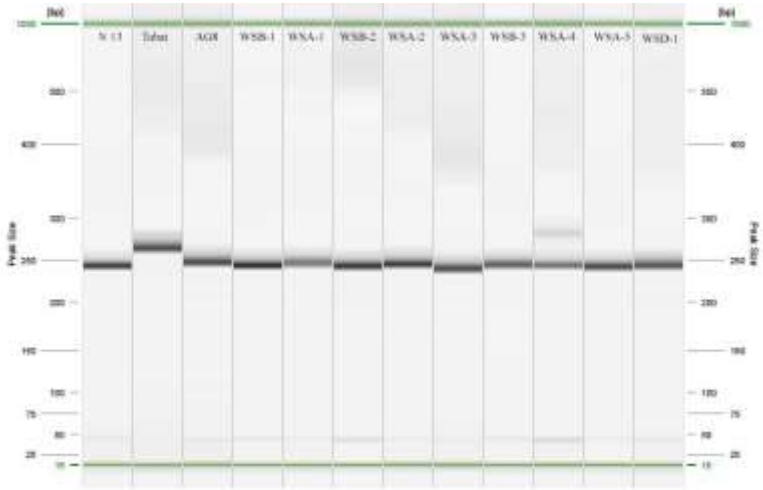
Fig. 4. The allele's size of genome DNA of N13, Tabat, AG8 and wild sorghum accessions using SSR marker Xisep1129 on QTL SBI-05b.

Table 5. Polymorphic SSR marker and its allele sizes at linkage group SBI- 05b for nine wild sorghum accessions, N13, Tabat and AG8

Linkage group	Marker name	Allele size (bp)											
		N13	Tabat	AG8	WSB-1	WSA-1	WSB-2	WSA-2	WSA-3	WSB-3	WSA-4	WSA-5	WSD-1
SBI-05b	Xisep1129	22	21	21	162	22	164	21	21	21	162	166	163

Xtxp317



Xtxp057

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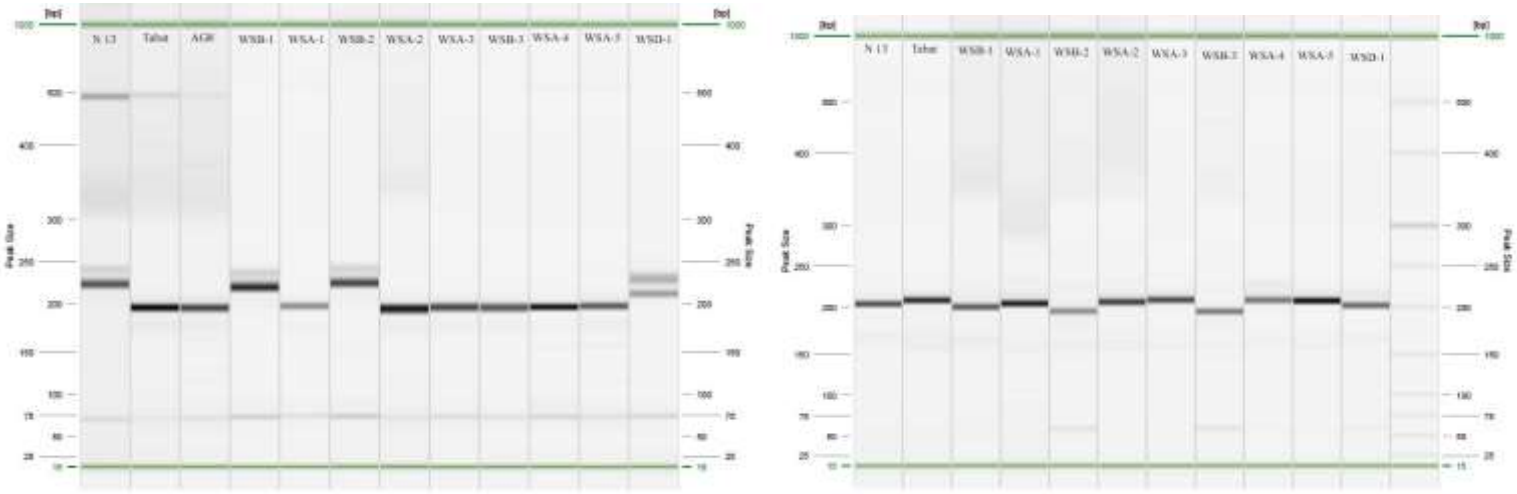

Xtxp045 Xisep0443

Fig. 5. The allele's size of genome DNA of N13, Tabat and wild sorghum accessions using SSR markers on QTL SBI-06.

Table 6. Polymorphic SSR markers and their allele sizes at linkage group SBI- 06 for nine wild sorghum accessions, N13, Tabat and AG8

Linkage group	Marker name	Allele size (bp)											
		N13	Tabat	AG8	WSB-1	WSA-1	WSB-2	WSA-2	WSA-3	WSB-3	WSA-4	WSA-5	WSD-1
SBI-06	Xtxp317	160	59	58	164	59	59	161	58	60	61	61	163
	Xtxp057	243	264	247	244	246	42	245	239	244	42	242	243
	Xtxp045	69	195	71	72	74	74	194	73	73	73	73	73
	Xisep0443	203	208	-----	200	204	58	205	208	59	208	207	202

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