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INTROGRESSION OF STRIGA RESISTANCE INTO POPULAR SUDANESE SORGHUM VARIETIES USING MARKER ASSISTED SELECTION

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ABSTRACT

Witchweed (*Striga*spp.) is one of the most important cereals production constraints globally and is projected to worsen with anticipated climate change. It is especially a devastating parasitic weed in Sub-Saharan Africa and parts of Asia. Integrated management strategies that depend mainly on host plant resistance provide the most effective control mechanism for *Striga*. We used molecular marker-assisted backcrossing to introgress Striga resistance from a resistant genotype, N13, into agronomically important genetic backgrounds (Tabat, Wad and Ahmed). Backcross populations BC_3S_3 were generated and genotyped using Simple Sequence Repeat (SSR) and Diversity Arrays Technology (DArT) markers. A total of 17 promising backcross progenies were selected and screened in *Striga* infested field alongside their parents. The Area Under *Striga* Progress Curve (AUSPC) showed significant decrease in *Striga* count (920-7.5) resulting in a 97-189% increase in yield under *Striga* pressure. Our results demonstrate the practical application of marker assisted selection (MAS) to generate farmer-preferrd *Striga* resistant lines in Sudan.

Key word: Witchweed, QTL, MAS, SSR and DArT, AUSPC.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is a diploid grass (2n=20) and the fifth most important cereal crop world-wide (FAOSTAT, 2010). It serves as a good source of food and nutrition to millions of people in the semi-arid regions of the world Reddy *et al.* (2010). Sorghum is also increasingly gaining importance as a source of livestock feed and biofuel (Zhang *et al.*, 2010). It is grown in at least 86 countries, on an area of 47 million hectares, with annual grain production of 69 million tonnes and average productivity of 1.45 t/ha. Sorghum is ranked second, after maize as the most important cereal crop in drought prone areas, particularly in sub Saharan Africa where it originated. In Sudan, sorghum accounts for 73% of the cropped area.

Sorghum grain yields in farmer's fields in Africa are generally low (500 to 800 kg/ha) compared to yield levels of up to 7t/ ha in developed countries. There are many factors contributing to the low production in Africa but drought and witchweed (*Striga* spp.) remain the major abiotic and biotic constraints respectively. *Striga* is a parasitic weed of most cereal crops and a major threat to smallholder farmers in rain fed agricultural areas of the semi-arid tropics, where yield losses can range between 22-100% (Ejeta and Butler, 1993; Riches and Orr, 2010). The main agriculturally important *Striga* species are *S. hermonthica* and *S. asiatica. S. hermonthica* is the most damaging of all the *Striga* species (Parker, 2009) and a major threat to staple grain crops production in Sudan with up to 100% losses in heavily infested soils (Ejeta and Butler, 1993).

Striga parasitizes its host by competing for nutrients while attached to the host. The host plant phytochemicals are responsible for the activation of this complex relationship by stimulating both germination of the *Striga* and initiation of its haustorium. Although research on *Striga* in Africa has a long history, efforts to promote *Striga* management have had limited success among smallholder farmers. A number of *Striga* control measures (cultural, chemical and bio-control measures) have been suggested but are of limited value to subsistence farmers. Although conventional breeding has been successful in introgression resistance to sorghum, it is a difficult process because of the quantitative nature of the trait and strong influence of the environment on its expression (Ejeta, 2007). Overall, no sole method is effective to control the parasite and *Striga* management resides on integrated approach for which resistant crop cultivars are the backbone (Joel *et al.*, 2007).

Recent advances in crop genomics have facilitated the identification of molecular markers associated with target trait(s) that can be deployed to select a superior line in a breeding programme. The invention of molecular markers has significantly enhanced the effectiveness of breeding for *Striga* resistance (Ejeta, 2007). Significant progress has been made to identify molecular markers associated with *Striga* resistance in sorghum under field conditions. A total of five genomic regions (QTLs) associated with stable *Striga* resistance in sorghum variety N13 have been identified

across a range of 10 field evaluation trials in Mali and Kenya, in 2 years, and in 2 independent mapping population samples (Haussmann *et al.*, 2000). The individual QTLs explain between 14 and 44% of the total phenotypic variation and contribute to partial, quantitative *Striga* resistance, which is expected to be durable.

Recently, the molecular marker density around these Striga QTLs has been increased in sorghum in order to improve the accuracy of Marker Assisted Selection (MAS) (Haussmann *et al.*, 2000). Marker-Assisted Selection (MAS) defines the selection of plants carrying genomic regions that are involved in the expression of traits of interest through molecular markers (Babu *et al.*, 2004; Foolad and Sharma, 2004). Marker-assisted selection is effective in enhancing prospects of breeding especially when ergonomically important traits are difficult to assess due to environment interactions (Robert *et al.*, 2001), as is the case for Striga. In breeding programs, MAS can be used to monitor introgression of the target QTL regions as well as to accelerate the recovery of the recurrent parent genome (Visscher *et al.*, 1996; Charcosset, 1997; Robert *et al.*, 2001). MAS has the potential to greatly increase screening efficiency for complex traits (Kassahun, 2006).

Integration of MAS in *Striga* resistance breeding can greatly accelerate the breeding progress since field screening is difficult, complex, and often unreliable. Some *Striga* resistance genes are also recessive, increasing the time required for, and difficulty of convectional backcross schemes. The objective of this study was to improve sorghum productivity in regions of Sudan that are highly infected with *Striga*. A backcross scheme was initiated aiming at introgression of the QTL regions controlling Striga into two farmer preferred sorghum varieties in Sudan (Tabat& Wad Ahmed) through MAS.

MATERIAL AND METHODS

Plant material: Two populations of backcross-derived lines (BC_3S_3, BC_4F_1) were generated from crosses between N13 (used as a donor) with two farmer preferred sorghum cultivars, Tabat and Wad Ahmed. Genotype N13 has been released for *Striga* resistance in India while both Tabat and Wad Ahmed are high- yielding, mature early but *Striga* susceptible The two populations were developed using backcrossing scheme shown in Fig.1

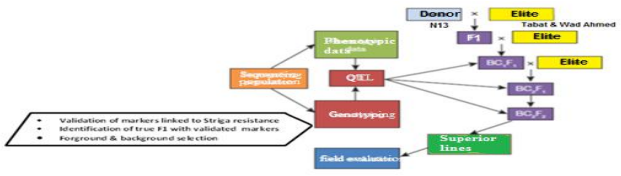

Fig.1: Backcrossing scheme used to generate the BC populations (MABC) Molecular Markers.

A total of 26 Simple Sequence Repeat (SSR) (Table 1) markers linked to *Striga* resistance QTL were were used for selection. carrying the 'N13' allele for Striga resistance to make backcross progenies possessing the QTL genomic region from the resistant donor genotype 'N13' (foreground selection) (Table 1). To estimate the recovery of the recurrent parent genome, a total of 19 SSR markers from the carrier linkage group were used for analyzing and the select genotypes with resistance. (Table 2) and DArT markers were also used.

Backcrossing: The two target cultivars (Tabat & Wad Ahmed) were used as female parents to make independent crosses (Tabat ×N13 ; Wad Ahmed ×N13). The F_1 plants were used as pollen parents to make the first backcross. In order to derive three more backcrosses (2nd and 3rd fourth), selected BC1F1 and BC2F1 plants from each cross were used as pollen parents after foreground selection. Further, BC_2F_1 and BC3F1and BC_4F_1plants were selfed to obtain segregating backcrossed F_2 (BC_2F_2 and BC3F2). Such F_2 generations were subjected to selection of homozygotes and *Striga* resistance screening (Fig. 1).

DNA extraction, PCR and Marker genotyping: DNA was extracted from parental lines, BC_3S_3 and BC_4F_1 individuals of 10-day old seedlings using a modified cetyl trimethyl ammonium bromide (CTAB) extraction method as described by Mace *et al.* (2003). DNA quality and quantity were checked on 0.8% agarose gels and DNA concentration normalized to ~20 ng/µl for further genotyping with linked markers (Table 2).

Linked SSR markers were used for amplification of the sorghum DNA using polymerase chain reaction (PCR) conditions as mentioned in Ramu *et al.* (2009). PCR was performed in 5µl reaction volume with final concentrations of 2.5 ng DNA, 2 mM $MgCl_2$, 0.1 mM of dNTPs, 1x PCR buffer, 0.4 pM of each primer, and 0.1U of Taq DNA polymerase (AmpliTaq Gold®, Applied Biosystems, USA) in a GeneAmp® PCR System 9700 thermal cycler (Applied Biosystems, 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 20 min at 72°C. In addition, fluorescent dye phosphoramidite, either 6-FAM (blue), VIC (green), NED (yellow), PET (red) were used in the PCR reaction mixture for detection of the amplified product on ABI 3700/3130.

Genotyping with SSR markers: Amplification was confirmed by running 2µl of the PCR products on a 2% (w/v) agarose gel stained with GelRed® (Biotium, USA) and visualized under UV light. Amplification products (1.5µl – 3.5µl of each) were loaded together with the size standard GeneScan™ –500 LIZ® (Applied Biosystems) and Hi-Di™ Formamide (Applied Biosystems), and separated by capillary electrophoresis using an ABI Prism® 3730 Genetic analyzer (Applied Biosystems). Allele calling was performed with GeneMapper 4.0 (Applied Biosystems). The scores of all polymorphic EST-SSR markers were converted into genotype codes ('A', 'B', 'H', 'O' and '-') according to the scores of the parents

Marker	Position	Amplification product	Forward	Reverse	Origin
Xisep0949	SBI-1	100-109	CAGTGCCAATAAGCTCGTCTC	CATCGATCTCTGCTTCTGCTT	ICRISAT_Ramu
XmsbCIR268	SBI-1	219-221	CACGACGTTGTAAAACGACGCACCAAAATCAGCGTCT	CCATTTACCCGTGGATTAGT	From CIRAD
Xisep0327	SBI-1	200-210	CTGTTTGTGCTTGCAACTCC	TCATCGATGCAGAACTCACC	ICRISAT_Ramu
XmSbCIR347	SBI-1	181-183	GAACATCAGAGGGTTTACCA	GAACCAACTACGCTTGTGTC	From CIRAD
Xtxp340	SBI-1	209-211	CACGACGTTGTAAAACGACAGAACTGTGCATGTATTCGTCA	AGAAACTCCAATTATCATCCATCA	(Bhattramakki *et al.*, 2000)
Xisep1028	SBI-1	100-103	CACGACGTTGTAAAACGACCAGCGACCATGAGGATGAC	TGGCATGCATCAAACAAGAT	ICRISAT_Ramu
Xtxp197	SBI-2	179-180	CACGACGTTGTAAAACGACGCGTCAATTAATCCAAACAGCCTC	GAGTTCCTATTCCCGTTCATGGTGAT	(Bhattramakki *et al.*, 2000)
XmSbCIR223	SBI-2	123-133	CACGACGTTGTAAAACGACCGTTCCAATGACTTTTCTTC	GCCAATGTGGTGTGATAAAT	From CIRAD
Xtxp080	SBI-2	196-204	CACGACGTTGTAAAACGACGCTGCACTGTCCTCCCACAA	CAGCAGGCGATATGGATGAGC	(Bhattramakki *et al.*, 2000)
Xiabtp515	SBI-2	214-218	TGCCACATCGATCTTGTCAC	AGGCAGTCACCCACACTACC	ICRISAT
Xtxp298	SBI-2	200-205	GCATGTGTCAGATGATCTGGTGA	GCTGTTAGCTTCTTCTAATCGTCGGT	(Bhattramakki *et al.*, 2000)
XmSbCIR329	SBI-5.1	202-206	CACGACGTTGTAAAACGACGCAGAACATCACTCAAAGAA	TACCTAAGGCAGGGATTG	From CIRAD
Xgpsb017	SBI-5.1	188-179	CATGGTTGGTCAGGAAG	GAATAAGGTCACTAAAGCAG	From CIRAD
Xiabtp420	SBI-5.1	302-305	CACGACGTTGTAAAACGACACATGCATGCTTGGAAGTTG	CTCTAGCATGGACCTGCACA	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)
Xtxp225	SBI-5.2	181- 183	TTGTTGCATGTTGGTTATAG	CAAACAAGTTCAGAAGCTC	(Bhattramakki *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

Table1. Markers used for foreground selection in BC3S4& BC4F1 Populations.

Marker	Forward	Reverse
Xtxp050	TGATGTTGTTACCCTTCTGG	AGCCTATGTATGTGTTCGTCC
Xtxp065	CACGTCGTCACCAACCAA	GTTAAACGAAAGGGAAATGGC
Xcup033	GCGCTGCTGTGTGTTGTTC	ACGGGGATTAGCCTTTTAGG
Xtxp274	GAAATTACAATGCTACCCCTAAAAGT	ACTCTACTCCTTCCGTCCACAT
Xtxp013	TCTTTCCCAAGGAGCCTAG	GAAGTTATGCCAGACATGCTG
Xtxp197	CACGACGTTGTAAAACGACGCGTCAATTAATCCAAACAGCCTC	GAGTTCCTATTCCCGTTCATGGTGAT
Xtxp225	TTGTTGCATGTTGGTTATAG	CAAACAAGTTCAGAAGCTC
Xiabtp515	TGCCACATCGATCTTGTCAC	AGGCAGTCACCCACACTACC
XmsbCIR268	CACGACGTTGTAAAACGACGCTTCTATACTCCCCTCCAC	TTTATGGTAGGATGCTCTGC
Xcup037	CCCAGCCTTCCTCCTGATAC	GTACCGACTCCAATCCAACG
Xiabtp500	CACGACGTTGTAAAACGACTTGTGCTGGTAGACGTGGTC	GCATTGGTATCCAACTGCAA
Xtxp014	GTAATAGTCATGACCGAGG	TAATAGACGAGTGAAAGCCC
Xtxp56	TGTCTTCGTAGTTGCGTGTTG	CCGAAGGAGTGCTTTGGAC
Xtxp296	CACGACGTTGTAAAACGACCAGAAATAACATATAATGATGGGGTGAA	ATGCTGTTATGATTTAGAGCCTGTAGA GTT
Xtxp080	CACGACGTTGTAAAACGACGCTGCACTGTCCTCCCACAA	CAGCAGGCGATATGGATGAGC
Xtxp317	CCTCCTTTTCCTCCTCCTCCC	TCAGAATCCTAGCCACCGTTG
Xisep346	CACGACGTTGTAAAACGACCGCTCCTCAGGCTCCTCT	TCCTCGAGCACCTGGTTG
Xiabtp444	CACGACGTTGTAAAACGACCCTTCTTCCACCTCCGTTCTC	GGGAGAGAGAGAGGGTCCATA
XmsbCIR223	CACGACGTTGTAAAACGACCGTTCCAATGACTTTTCTTC	GCCAATGTGGTGTGATAAAT

Table 2. SSR markers used for background selection in BC3S4& BC4F1 Populations.

Genotyping with DArT markers: High quality genomic DNA of recurrent parents and 17 BC_3S_4 populations was used for DArT analysis. Genomic representation from the 17 BC_3S_3 and parental lines was used to develop a DArT array with approximately 7,000 clones. Genomic representation was prepared based on complexity reduction method described by Wenzl *et al.* (2006). Representation fragments were cloned, amplified and printed onto poly-L lysine coated slide before array was processed. The representation was fluorescently labeled and hybridized with the DNA. The slide was then washed, scanned using a focal laser scanner (Tecan LS300) and scored. A software package developed by DArT P/L and DarTsoft (Australia) was used to automatically analyze each batch of image pairs generated.

Field evaluation of BC_3S_3 lines: The standard cultural practices for sorghum were followed during the evaluation process in two consecutive seasons at Gezira Research Farm. Land was prepared by disc ploughing, disc harrowing, leveling and ridging in irrigated sites and by disc- harrowing in rain- fed sites. Treatments were blocked in a complete randomized design with three replicates. Planting was made during the first two weeks of July on ridges in irrigated sites and on flat beds in rain-fed sites. Sub-plot size was 5m x 8 m, at spacing of 80 cm between rows and 30 cm between plants and 3 plants/hole (population density of 125000 plants/ha). In irrigated trials, 40 kg/ha of urea was applied. For artificial infestation, *Striga* seeds were mixed with soil at 1mg/kg and the mixture planted at 5g/hole. The crop was kept weed-free and irrigated every two weeks or whenever necessary. Assessments were made in the central rows of each plot. Data was collected for *Striga* counts. At harvest, heads were cut, sun-dried, threshed, and seed weight determined. General linear Method (GLM) was used for statistical analysis.

RESULTS

Marker Assisted backcrossing (MABC): Marker-assisted backcrossing with elite varieties including crossing, backcrossing and selfing was undertaken as mentioned in Fig. 1. As a first step, the *Striga* resistant donor genotype 'N13' was used as the male parent and crossed individually with two susceptible varieties ('Tabat' and Wad Ahmed). The F1 was selfed and other backcrosses and selfed generations were developed. Plants selected in each generation for marker screening (heterozygous and homozygous for the donor and recurrent parents alleles), selected (17) BC3S3 progenies were used for SSR and DArTs genotyping.

Genotyping of BC_3S_3 with SSR markers*:* For foreground selection template DNA was screened with 26 markers for selecting homozygous progenies for the donor parent alleles at QTL regions, results indicated that, the highest number of the progenies possesses QTLs on SBI-01 and SBI-02 (9, 7), respectively, followed by progenies with QTL on SBI-05a and SBI-06 QTL (6, 5) whereas, the lowest number for SBI-05b QTL (4), (Table 3).

For background selection, nineteen markers were used to select homozygous progenies for the recurrent parent alleles in much of the non-QTL regions (Table 4), results indicated that 76% of the progenies possess high frequency of Tabat allele, while only 24% possess high frequency of Wad Ahmed alleles.

Genotyping of BC_3S_3 with DArT markers*:* On the genotype panel of the DArT clones scored, 115 and 85 DArT clones scored presence and absence for N13 allele, respectively. The genotyping data aligned to linkage map data, it revealed that there were six backcross progenies with SBI-01 QTL introgression; nine similar backcross progenies with SBI-02 and SBI-05b QTL introgression; four backcross progenies with SBI-05a QTL introgression; and five with SBI-06 QTL. In summary, results of SSR markers combined with DArTs of

QTL1 (SBI-1)						
	Xisep0949	XmsbCIR268	Xisep0327	XmSbCIR347	Xtxp340	Xisep1028
N13	100	216	210	160	199	200
Tabat	104	212	207	154	196	195
Wad Ahmed	102	214	205	151	192	197
QTL3(SBI-2)						
	Xtxp197	XmSbCIR223	Xtxp080	Xiabtp515	Xtxp298	
N13	154	134	287	204	202	
Tabat	150	133	284	202	200	
Wad Ahmed	151	130	281	202	200	
QTL3(SBI-5a)						
	XmSbCIR329	Xgpsb017	Xiabtp420	Xtxp065		
N13	131	208	302	132		
Tabat	128	205	301	131		
Wad Ahmed	126	203	300	128		
QTL3(SBI-5b)						
	Xisep1129	Xtxp015	Xtxp225	Xtxp262	Xtxp014	Xtxp317
N13	210	215	165	167	149	158
Tabat	209	212	166	165	142	156
Wad Ahmed	209	213	164	162	145	157
QTL3(SBI-6)						
	Xtxp057	Xtxp176	Xtxp045	Xtxp145	Xisep0443	
N13	261	161	202	231	190	
Tabat	256	157	203	228	185	
Wad Ahmed	254	159	200	229	188	

Table 3. Markers used for foreground selection in BC3S4.

Marker	**N13**	**Tabat**	**WadAhmed**
Xtxp050	126	117	NA
Xtxp065	148	144	NA
Xcup033	179	182	NA
Xtxp274	179	183	NA
Xtxp013	138	132	138
Xtxp197	178	179	178
Xtxp225	183	181	183
Xiabtp515	204	199	204
XmsbCIR268	211	213	NA
Xcup037	205	203	205
Xiabtp500	178	178	169
Xtxp014	126	126	129
Xtxp56	312	322	325
Xtxp296	162	165	162

Table 4. Markers used for background selection.

BC_3S_4 genotyping data showed that all the five QTLs were present in different progenies. When data was aligned to specific LG data, it revealed that ten progenies with QTL1and QTL2, six progenies with QTL5, six progenies with QTL3, four progenies with QTL4 and 3 progenies with QTL5 were introgressed.

The backcross progenies were also assigned to their recurrent parent based on SSR and DArT clones polymorphism, Moreover, DArT clones were uniquely used to differentiate between N13 and the recurrent parent pairs, resulting five progenies possess high frequency of Wad Ahmed alleles, while 12 progenies possess high frequency of Tabat alleles (Table . 5).

Field evaluation of BC_3S_3 lines: BC_3S_4 lines (17), *Striga* resistance QTL introgression lines, along with their parents and appropriate checks, were evaluated in *Striga* artificially infested field. Results revealed that the selected BC3S4 lines can be divided into three groups, 1) seven progenies along with N13 and SRN39 with low Area Under *Striga* Progress Curve (AUSPC) which were considered resistant, 2) six progenies with the recurrent parent WadAhmed, with moderate range of AUSPC were considered moderate resistance and 3) four progenies with Tabat and IS9830 with high AUSPC were considered susceptible (Table6). BC_3S_4 lines with two or more

Progeny	T_1	W_2	W_1	T_2	W_3	T_6	T_7	T_8	T_9	T_{10}	T_{11}	W_{12}	T_{13}	T_{14}	T_{15}	T_{16}	T_{17}
QTL1	√	√		√	√		√		√		√			√		√	
QTL2	√						√			√	√		√	√			√
QTL3	√	√	√	√		√			√			√					
QTL4			√					√					√		√		
QTL5	√	√		√		√									√		

Table 5. Fore ground & background selectionin17BC_3S_3 progenies using SSR & DarT markers.

Resistance		Moderate		susceptible	
Progeny	AUSPC	Progeny	AUSPC	Progeny	AUSPC
1	7.5	W A	465	Tabat	920
2	67.5	5	502.5	16	967.5
14	172.5	6	562.5	15	975
13	210	12	607.5	9	1140
8	217.5	4	637.5		
N13	222.5	10	690		
SRN39	232.5	IS9830	877.5		
7	352.5	3	885		
11	375				

Table 6. Area under Striga Progress Curve (AUSPC) for BC3F4 Progenies.

Striga resistance QTLs were selected. *Striga* resistant and agronomically superior genotypes were advanced together with Wad-Ahmed, Tabat, N13, SRN39 and IS9830 as checks for multi-location trials. Based on field evaluation, results indicate that there are (2) BC_3S_4 lines are as *Striga* resistant as the resistant checks, N13, SRN39 and IS9830 and high yielding (1185.0 and 1035.9 kg/ha for (W2 BC_3S_4 and T1 BC_3S_4) than the checks (Table 7 & Fig 2).

Progeny	*Striga* emerged plants	Kg/ha
T1 BC_3S_4	9.30b	1035.9
W1 BC_3S_4	9.98b	789.1
W2 BC_3S_4	7.01b	1185
T2 BC_3S_4	9.51b	784.4
N13	6.29a	633.6
SRN39	22.06a	597
Wad Ahmed	18.53a	620.6
IS9830	6.25a	693.2
Tabat	22.06a	358.7
CV	4.5	15.4
SE+	76.13	70.7

Table 7. Mean Striga emerged plants and grain yield (kg/ha) f selected entries.

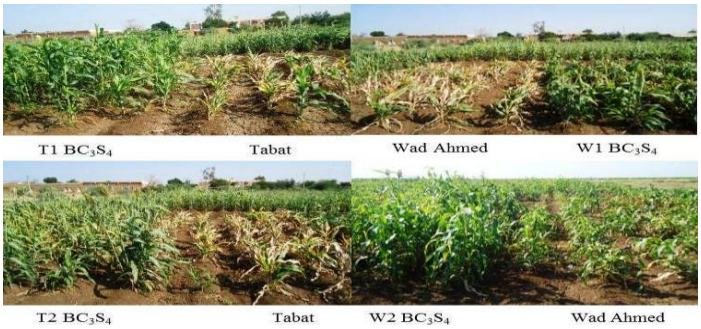

Fig 1. Phenotypic evaluation of BC3S4lineswiththe parent in artificially Striga infested plot.

Characterization of BC_4F_1 populations: The selected BC3F4 lines were backcrossed to the recurrent parents to generate BC4F1 progenies. High quality DNA of 145 progenies was extracted and normalized to 5ng/µl. Twenty six polymorphic SSR markers were optimized. BC4F1 lines (145) along with the parents (3) were screened with 26 SSRs; data revealed that all QTLs (5) were introgressed. QTLs (25) were introgressed in only 15 progenies (Fig. 2).

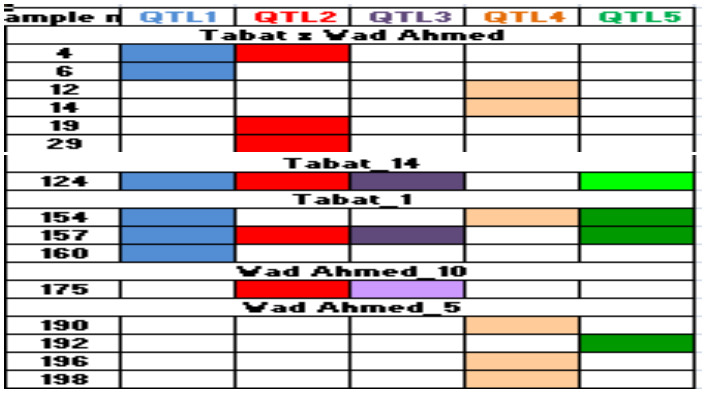

Fig 2. QTLsintrogressedinBC4F1population.

Discussion

Striga is the most limiting biotic constraint for sorghum production by small farmers in rain fed agricultural areas of the semi-arid tropics (Satish *et al.*, 2012). Crop yield losses due to Striga might reach up to 100% in heavy infested soils (Ejeta and Butler, 1993). Climate change scenarios which may adversely affect sub-Saharan Africa make utilization of *Striga* resistant varieties of increasing importance. Breeding for Striga resistance in the field is difficult because of the quantitative nature of the trait and strong influence of the environment on its expression (Ejeta, 2007). Marker-assisted backcrossing has been proven to be a quick way to improve

one or two traits in existing preferred cultivars in several crops (Varshney *et al.*, 2010). The invention of molecular markers has implications on *Striga* resistance trait, in both understanding host- parasite interactions and improving productivity. The use of DNA-based markers for the genetic analysis and manipulation of important agronomic traits has become an increasingly useful tool in plant breeding. One of the greatest potentials of molecular markers is to accelerate the rate of gain from selection of desirable genotypes and in the manipulation of quantitative trait loci (QTLs) that condition complex economic traits. Marker-assisted selection involves the selection of genotypes carrying a desirable gene(s) via linked markers, through marker-assisted selection (MAS); more rapid transfer of traits from donor parents to more elite locally-adapted crop cultivars is possible. Recently utilization of molecular markers in breeding programs has received considerable attention using different crossing schemes.

The identification of the molecular markers for specific *Striga* resistance mechanisms facilitates faster introgression and pyramiding of genes controlling this important trait. In the few studies that relate to the other *Striga* resistance mechanisms, Haussmann *et al.* (2004) identified and mapped QTLs associated with Striga resistance in the sorghum variety, N13, where mechanical barrier is the suggested mechanism of Striga resistance. Based on a series of field evaluations of two independent RILs, (Haussmann *et al.*, 2004) also confirmed the position and the stability of the identified the QTLs.

A high proportion of DArT markers distributed in all linkage groups indicated that DArT markers were more frequent than SSRs. In consistent with what reported by (Vuylsteke *et al.*, 1999), that DArT markers may have a stronger tendency than genomic SSR and AFLP markers in particular to map to such gene-rich regions. For foreground and background selection, markers have been investigated by Groen and Smith (1995) and Visscher *et al.* (1996) who reported a case that a QTL is an estimated gene with unknown position, introgressing a favorable allele of the QTL by recurrent backcrossing could be powerful for improvement, provided that the expression of the gene(s) is not reduced in the recurrent genomic backgrounds. Generally, molecular markers can very effectively increase the efficiency of backcrossing by background selection for the genotype of the recurrent parent, with or without foreground selection for the donor parent alleles at markers in the region of the genome controlling the target trait. This usually ensures a rapid progress in breeding and results in a high frequency of progenies with the trait of interest recombined by the desirable features of the recurrent parent. This is also confirmed with the results of Stam and Zeven (1981), Young and Tanksley (1989) and (Frisch and Melchinger, 2001) who indicated that without background selection, the introgressed segment(s) could remain fairly long over a large number of backcross generations, hence contributing to the presence of non- target parts of the donor genome in the final breeding product, and increasing problems associated with linkage drag.

Selection based on markers could facilitate manipulation of quantitative traits without affecting other important agronomic traits by allowing evaluation of breeding progeny for the presence of target gene(s). Comparing the outcome of a MAS program requires several levels of success: 1) Recovery of the target trait from the donor parent, 2) Recovery of recurrent parent genotype or phenotype for characters not directly associated with the introgressed target trait and 3) Comparison of the effectiveness of MAS relative to conventional selection.

Inconsistent with Haussmann *et al.* (2000) who showed that two major QTLs contributing from 14% - 94% of the trait, the lines (BC_3S_4) with only two major QTLs, have the same level of resistance as the donor parent, and as the lines with four QTLs (including the 2 major QTLs). Targeting these 2 major QTLs will make map based cloning possible and ease inter and intra specific gene transfer. This is also confirmed by field evaluation, progenies with 2 or more major QTLs have the same level of *Striga* resistance as the donor parent (N13). This might be explained by whether one of the flanking markers identified by (Haussmann *et al.*, 2004) positioned within the genes or in close vicinity. That why neither the gene nor the markers were lost after all these recombination cycles.

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