Ali, R., Hash, C., Damaris, O., Elhussein, A., & Mohamed, A. (2016). Introgression of striga resistance into popular sudanese sorghum varieties using marker assisted selection. *World Journal of Biology and Biotechnology, 1*(1), 49-56
DOI: https://doi.org/10.33865/wjb.001.01.0006

(Online) ISSN 2522-6754 | 2016 | (Print) ISSN 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Volume: 01 | www.sciplatform.com | Issue: 01

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

[a] Rasha Ali, [b] Charles. T. Hash, [c] Odeny Damaris, [d] Adil Elhussein [e] Abdalla H. Mohamed
[a] Agricultural Research Corporation (ARC), P.O. Box 126, Wad Madani, Sudan,
[b] International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) Sahelian, Center Niamey, Niger,
[c] International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Nairobi, Kenya,
[d] University of Khartoum, Faculty of Science, Khartoum, Sudan
[e] International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Addis Ababa – Ethiopia
*Corresponding email address: yarashaya@gmail.com

## ABSTRACT

Witchweed (*Striga*s pp.) 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 and Wad 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

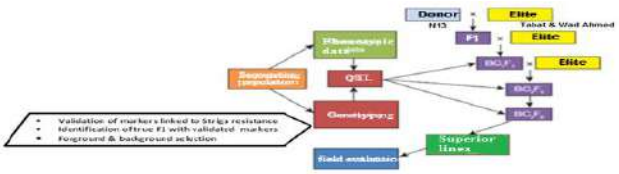


Figure 1: Backcrossing scheme used to generate the BC populations (MABC) Molecular Markers.

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 $BC_4F_1$plants 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 |

| 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 selectionin17$BC_3S_3$ 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.

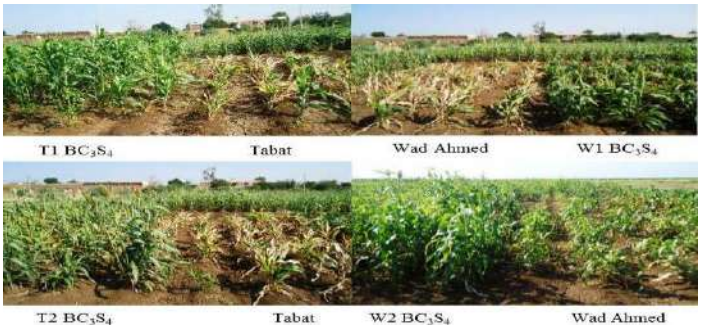


Figure 1. Phenotypic evaluation of BC3S4lineswiththe parent in artificially Striga infested plot.

**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).

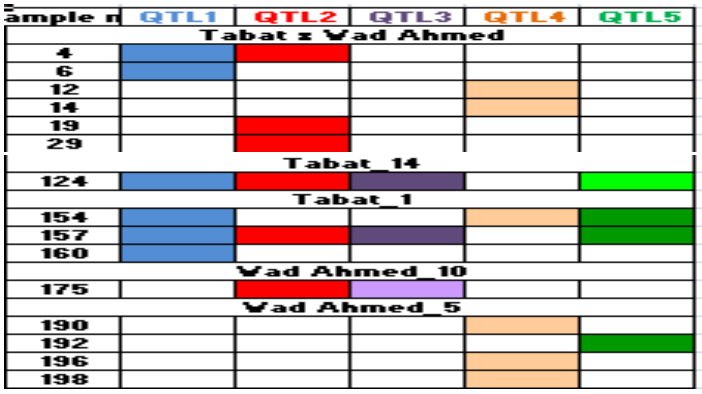


Figure 2. QTLsintrogressedinBC4F1population.

**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.

**ACKNOWLEDGMENT**

The authors would like to thank the Association for Strengthening Agricultural Research in Eastern and Central Africa, (ASARECA), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and Biosciences eastern and central Africa (BecA-ILRI Hub), ABCF fellowship, for providing financial support for this work.

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Date Published (D-M-Y): 15-4-2016