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COMBATING STRIGA WEED IN SORGHUM BY TRANSFERRING RESISTANCE QUANTITATIVE TRAIT LOCI THROUGH MOLECULAR MARKER ASSISTED INTROGRESSION

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A B S T R A C T

Sorghum, the second most important cereal crop in Kenya is often attacked by *Striga hermonthica* weed with grain yields being reduced up to 100%. In the marginal and semi-arid areas, there is urgent need to enhance the genetic resistance to *Striga hermonthica* in local varieties. The aim of this study was to introgress Striga resistance from a documented resistant donor line N13, into Ochuti, a susceptible farmer preferred variety through molecular marker assisted selection (MAS). Two backcross populations namely, BC_2F_1 and BC_3F_1 were generated by crossing N13, the donor parent to Ochuti, the recurrent parent line and the resultant backcrossed Striga resistant progenies were subjected to phenotypic selection initially. At the BC_3F_1 stage, fore-ground selection for the Striga resistance Quantitative Trait Loci (QTLs) was conducted through Polymerase Chain Reaction (PCR) and N13 and Ochuti alleles sized through capillary electrophoresis. Eleven polymorphic markers identified at least three Striga resistance QTLs, in five plants of $BC_3F_{1}//F_2$ generations. Eight progenies from BC_2F_1 and BC_3F_1 backcross populations were evaluated in field trials under artificial Striga inoculation in two locations and for two seasons. The backcrossed genotypes with Striga resistance allowed fewer Striga plants to germinate though in certain cases Ochuti genotypes performed equally the same. Marker assisted Selection (MAS) can successfully be utilized to transfer Striga resistance QTLs from a resistant donor source to a susceptible sorghum variety but the transfer should be complimented by field evaluation of the resistant progenies under artificial Striga infestation over several seasons, locations and replications.

Keywords: Fore-ground Selection, Marker Assisted Selection , QTL, *Striga hermonthica*, Sorghum bicolor.

INTRODUCTION

Sorghum (*Sorghum bicolor (L)* Moench) is the fourth most important cereal in the world (FAOSTAT, 2008) but in Kenya, it is the second most important staple cultivated in both high rainfall and semi-arid areas of Kenya. At the farm level, sorghum production hardly raises beyond 0.8 tons/ha-^{1} due to a number of constraints that include stalk borers, shoot fly, drought stress, smut and anthracnose. However soil water deficits and damage by *Striga hermonthica* (Del.) Benth weed are the two most important factors limiting sorghum productivity (Ejeta *et al*., 2007)). In sub-Saharan Africa, Striga is a serious problem in over 40% of potential sorghum areas that have poor soil fertility and that are continuously cultivated (Ejeta and Butler, 1993). In the Nyanza and Western Provinces of Kenya, 76% of land under sorghum is infested with *S. hermonthica* causing annual losses of about $40.8 million (Kanampiu *et al*., 2002). Annually, about 100 million people lose half their sorghum crop to Striga especially during prolonged drought periods. Sauerborn, (1991) estimated that out of a total area of 79 million hectares in sub-Saharan Africa under cereal production, Striga infested 44 million hectares. *S. hermonthica*, an obligate parasite that damages its

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host underground before emerging is difficult to control through conventional means. Each Striga plant produces a large number of minute seeds which remain viable in the soil for many years (Bebawi *et al.,* 1984). Mechanical and chemical control measures have proven to be expensive or ineffective against the Striga weed and in many parts of Africa farmers simply abandon crop fields or change into another crop as a way of overcoming the hazard (Ejeta *et al.,* 2007). There is need to control Striga in the infested areas, prevent its spread and raise sorghum productivity. One control strategy is breeding for Striga resistant varieties that would result into reduced labour and herbicide. However variations in the field and in the parasite, lack of a precise and screening reliable method coupled together with fact that the resistance against the parasite comes in different forms of mechanisms, have hindered the progress in breeding for resistance (Ejeta *et al.,* 2007). Recent advances utilizing Simple Sequence Repeat (SSR) molecular markers and MAS, have mapped five Striga resistance QTL and their locations as; Chromosome A /linkage group 1, Chromosome J1/ linkage group 5, Chromosome B /linkage group 2, Chromosome I /linkage group 6 and Chromosome J2/ linkage group 5 (Haussmann *et al.*, 2004; Kim *et al*., 2004). Each of these QTL is reported to account for 12 -30% of the total variation observed for Striga resistance (Haussmann *et al*., 2004, Kim *et al.*, 2004) and they have been shown to be stable across locations (Haussmann *et al*., 2004; Grenier *et al*., 2001). In this study, Striga resistance QTL in a characterized donor line, N13 from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) was crossed to a recipient Kenyan farmer preferred cultivar, Ochuti in series of backcrosses and the resistance QTL selected with eleven polymorphic SSR markers.

MATERIALS AND METHODS

Generating back-crosses in the greenhouse: N13, the male parental line that has mechanical resistance to Striga was crossed to a pure line of Ochuti variety as the female recipient line in March 2009 at Kenya Agricultural Research Institute (KARI) Katumani Research Centre and the subsequent F_1 generations backcrossed to Ochuti to finally generate BC_2F_1 and BC_3F_1 progenies. In each cycle of hybridization, N13 was sown one week after Ochuti in order to synchronize flowering and two months after sowing the head of the selected plants were bagged. Emasculation in Ochuti was done once the flowers opening had reached about half the panicle and the anthers were carefully removed in order not to destroy the stigma. The plants were then bagged overnight and pollination was done early the next morning with pollen from N13 plants. The date of pollination was indicated and the bags were pinned firmly on the plant. In the F_1 generation, ten true breeding F_1 plants were selected phenotypically as those that inherited the characteristics of the two parental lines. The ten selected F_1 plants were then sown together with Ochuti in May 2010, and the subsequent BC_1F_1 seed harvested in July 2010. Twenty five plants in the BC_1F_1 generation were selected again phenotypically as those heterozygous for the two parental lines and were backcrossed to Ochuti to generate BC_2F_1 in October 2010. In the next cycle, twenty BC_2F_1 were sown and backcrossed to Ochuti in March 2011 to generate BC_3F_1.

DNA extraction and PCR Analysis: Fourteen day old harvested leaves from twenty five BC_3F_1/F_2 generations were placed into labeled eppendorf tubes containing 90% alcohol, placed in cooler box containing ice at -4^{0}C and transferred to -80^{0}C freezer in the laboratory. DNA was extracted using the Cetyl-trimethyl Ammonium Bromide (CTAB) mini-prep method as developed by Mace *et al*. (2003). The leaf samples were placed into a Geno-Grinder 2000 (Spex CertiPrep, USA) into which two steel beads were added in each of the wells and the plates were then placed in a bucket with liquid nitrogen in order to make the leaf material brittle to grind. 450µl Preheated (65^{o}C) of extraction buffer (3% (w/v) CTAB, 1.4M NaCl, 0.2 % (v/v) β-Mercapto-ethanol and 20 mM EDTA) was added to the leaf samples and ground using the Genogrinder. The macerated leaves were incubated with 450µl Chloroform: isoamyalcohol mixture at a ratio of 24:1, for 15 minutes at 65^{o}C with and mixed occasionally by inversion. Then the tubes were centrifuged at 12000 rpm for 10 minutes at 24^{o}C and the upper portion transferred into fresh tubes (about 400µl). About 0.7 volumes of iso-propanol (stored at -20^{o}C) was added and inverted once and the tubes were then centrifuged at 12000rpm for 15 minutes in order to precipitate the crude DNA pellet. Decanting of the

supernatant was done and the pellet air dried for 30 minutes. 200µl Low salt TE buffer (1mM Tris and 0.1mM EDTA [PH 8]) with 3µl RNase A (10mg/ml) was added to each sample and incubated at 37°C in a water bath to remove the RNA. A second solvent extraction was done by adding 200 µl chloroform: isoamyalcohol (24:1) to each tube and inverting twice to mix and centrifuged. The aqueous layer was then transferred into fresh tubes. DNA was purified by adding 315µl ethanol and 1/10 volume of 3M sodium acetate solution (PH 5.2) to each sample and then the samples were placed in -20°C for 5 minutes for the DNA to precipitate. The tubes were then centrifuged at 12000rpm for 5 minutes and the supernatant decanted. 200µl of 70% ethanol was added and centrifuged at 3500 rpm for 5 minutes. DNA pellet was air-dried for one hour. The pellet was then re-suspended in 100µl low salt TE [10mM Tris, 1mM EDTA (PH 8)] buffer and stored at 4°C.

The SSR markers used in the PCR reactions were M13 forward primers labeled with FAM, NED, VIC and PET (PE-Applied Biosystems) fluorescent dyes (Table 1). A total of 25 BC_3F_1 /F_2 plants were genotyped. The PCR components for a 10 µl reaction were: 2 mM $MgCl_2$, 0.20 µM reverse primer, 0.04 µM forward primer, 0.04 mM of each of the four dNTPs and 0.2 U AmpliTaq Gold DNA polymerase (AmpliTaq® with GeneAmp® Taq DNA polymerase: Applied Biosystems), 30 ng template DNA. The reaction was topped up to 10 µl reaction volume, with double distilled water. Temperature cycling was carried out using the GeneAmp PCR systems 9600 (PE-Applied Biosystems) with the following protocol: 15 min at 94°C, 40 cycles of 1 min at 94°C, 1 min at 50°C and 2 min at 72°C, with a final extension of 20 min at 72°C. The PCR products were run on 2% (w/v) agarose gel electrophoresis to order to verify the amplification and the quality.

Table 1. Eleven11 SSR markers used to screen Striga resistance QTLs, their dye labels, target alleles sizes, repeat composition, chromosomal location and linkage group characteristics.

Primer Name	Primer type	Dye	Repeat Motif	Size of N13 allele	Size of Ochuti allele	Chromosome/ Linkage group
Xtxp 302	Directly labeled	VIC	(TGT)8	237	196	Chromosome A/ Linkage Group 1
Xtxp 303	Directly labeled	NED	(GT)13	150	152	Chromosome J1/ Linkage Group 5
Xtxp 201	Directly labeled	VIC	(GA)36	183	188	Chromosome B/ Linkage Group 2
Xtxp 015	Directly labeled	NED	(TC)16	217	219	Chromosome J2/ Linkage Group 5
Xtxp 208	Directly labeled	FAM	(GGA)8	260	257	Chromosome A/ Linkage Group 1
Xtxp 304	M13 – Tailed	FAM	(TCT)42	323*	231*	Chromosome B/ Linkage Group 2
Xtxp 225	M13 – Tailed	NED	(CT)9(CA)8CCC(CA)6	183*	187*	Chromosome J2/ Linkage Group 5
Xtxp 145	M13 – Tailed	PET	(AG)22	262*	232*	Chromosome I/ Linkage Group 6
Xtxp 057	M13 – Tailed	PET	(GT)21	261*	268*	Chromosome I/ Linkage Group 6
Xtxp 065	M13 – Tailed	VIC	(ACC)4+(CCA)3CG(CT)8	149*	151*	Chromosome J1/ Linkage Group 5
Xtxp 050	M13 – Tailed	NED	(CT)13(CA)9	316*	314*	Chromosome B/ Linkage Group 2

*These markers have 19 extra base pairs from their actual allele size as they are M13-tailed. (Haussmann et al., 2004 and Bhattramakki et al., 2000).

Genotyping: Genotyping by fragment analysis using fluorescent fragment detection system was done on the ABI-3730 DNA Sequencer at the Biosciences Eastern and Central Africa (BecA) laboratory in Nairobi. PCR products were loaded together with a set of three markers. Samples for genotyping were prepared as follow; 0.125 µl of GeneScan™ LIZ 500 internal lane size standard (Applied Biosystems) and 8µl of HI-DI™

Formamide (Applied Biosystems) were added in each co-loaded sample. Liz standard and HI-DI™ mixture was prepared in the ratio of 49:1. Liz standard was used to size DNA fragments whereas HIDI™ was used to ensure that the DNA fragments stayed single stranded after they were denatured. The PCR products were determined by the type of dye used and the strength of the band as seen on the 2% agarose gel. For weaker dyes such as PET and NED more PCR product with a minimum of 3.0 µl and a maximum of 3.5µl was picked as they fluoresce less whereas for stronger dyes such as VIC and FAM that fluoresces more, the PCR product consisted of a minimum of 1.8 µl and a maximum of 2.5 µl. The mixture was denatured at 94°C for 5 minutes then cooled on ice immediately. The denatured DNA fragments were size-fractioned on the ABI 3730 Capillary DNA Sequencer (PE-Applied Biosystems) using the default parameters but with an injection time of 40 seconds. The peaks were sized and the alleles analyzed with Gene-Mapper Version 4.0 software (Applied Biosystems, 2005).

Striga inoculated field trials at Alupe and Kibos sub-stations: Eight genotypes arising from the selfing of BC_2F_1 generations, of lines 11 and 34 and another eight genotypes arising from the selfing of BC_3F_1 of lines 33 and 87 were sown in randomized complete block design of three replications together with N13 and Ochuti as checks, during October 2010 - March 2011 and May - October 2011 rainy seasons at Alupe and Kibos substations of KARI. Each plot consisted of four rows, 3 metres long, with a spacing of 75 cm between rows and 20 cm between plants. The Striga inoculum was prepared by mixing 10g Striga seeds with 5kg of fine sand. The planting holes were infested with one table spoon scoop of Striga seed and sand mixture consisting approximately 3000 Striga seeds (IITA, 1997). Striga infestation count was scored at two week intervals from day 42 to day 99 after sowing. A scale of 1-5 was used to score field resistance as; 1-very resistant, 2-fairly resistant, 3-average, 4-below average and 5-very susceptible according Hausmann *et al*. (2000). The Area under Striga Progress Curve (AUSPC) also known as the Area under the above ground Striga Number Progress Curve (AUSNPC) was calculated in order to provide a quantitative measure of Striga infestation over the entire season as according to Rodenburg *et al*. (2005). This was done by summing the product of Striga plant counts and the number of days between observations, five times at different stages giving rise to AUSNPC1, AUSNPC2, AUSNPC3, AUSNPC4 and total AUSNPC. AUSNPC was calculated as outlined by (Haussmann *et al*., 2000; Rodenburg *et al*., 2005) using the formula:

$$\text{ASNPC} = \sum_{I=0}^{n-1} \left[\frac{Y_i + Y_{(i+1)}}{2}\right] \left(t_{(i+1)} - t_i\right)$$

Where,

n = the number of Striga assessment dates.

Y_i = the Striga count at the i^{th} assessment date.

t_i = the days after planting. at the i^{th} assessment date.

t_0= the days after planting to Striga emergence minus 1.

Y_0 = is 0.

Agronomic data was recorded from the middle two rows as follows:

i. seedling vigor; measured 14 days after sowing was given a score of between 1-5, where 1 indicated the strongest vigour and 5, the weakest
ii. dates to flowering; was recorded as the number of days from planting to when 50% of the plants in each plot flowered
iii. days to Striga emergence; was scored as the date of first Striga emergence in each plot.
iv. number of Striga plants; was count of the number of Striga plants in each plot counted two weeks after emergence of the first Striga plant in the trial and repeated every two weeks
v. number of Striga plants flowered; was the count of the number of Striga plants that flowered in each plot at the flowering stage of the Striga , 90 days after sowing
vi. number of Striga plants forming capsule; was measured as the number of Striga plants with capsules counted in each plot, 105 days after sowing
vii. dry panicle weight; was the dry weight of all harvested panicles
viii. grain weight; was measured as grain from the panicle heads in each plot, harvested, sun dried, threshed, weighed in grams initially but converted to t ha^{-1}.
ix. 100 seed weight; was measured as the number of 100 seeds in each sample and weight expressed in grams
x. number of plants lodging; was measured as total number of plants in the plot that lodged due to Striga infestation.

Broad sense heritability was calculated according to Falconer and Mackay (1996) as; $H^2 = \delta^2_g / \delta^2_p$. The field data was subjected to analysis of variance (ANOVA) using Genstat ® 12th edition and Pearson's correlations were calculated with Proc corr SAS ® program and means were compared with the Bonferroni test at $\alpha = 0.05$ significance level.

RESULTS

The gel image in Figure 1 shows that amplification was successful for most of the markers but though markers Xtp 225, Xtp 303 and Xtp 304 did not amplify well in agarose, their sizes were resolved in the capillary electrophoresis.

Table 2 shows the results of fore-ground selection in the backcrosses. From a total of 20 BC_3F_1 and 25 BC_3F_2 backcrosses genotyped only five plants had introgressed *Striga* resistance QTL in one way or another. Genotype BC_3F_1/L133/p25 introgressed three resistance QTL namely, QTLA, QTLB and QTL J2. QTLA was selected by flanking markers Xtp 208 and Xtp 302 whereas QTLJ2 was selected by flanking markers Xtp 225 and Xtp 015. QTLB could only be identified by one marker, Xtp 304. Nevertheless, the three QTLs were heterozygous for the two parental alleles.

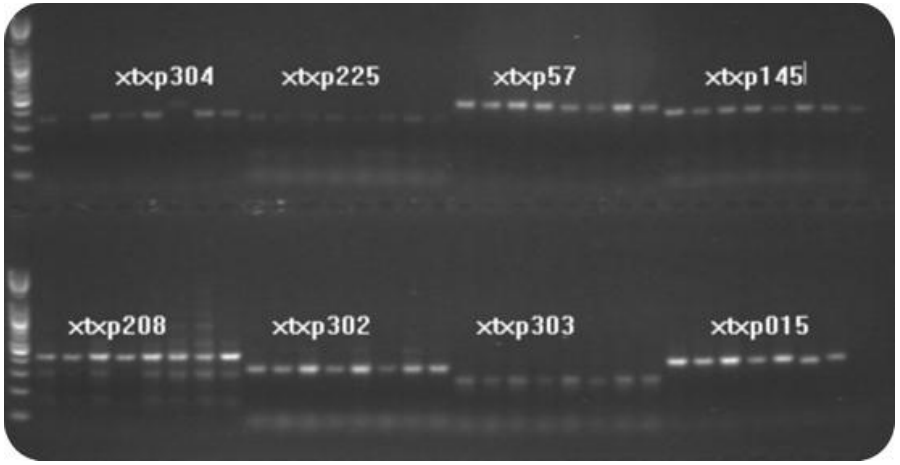

Figure 1. Gel image of 8 markers run in 2%agarose for 30 min at 110 V.

Table 2. Fore-ground Selection of Striga resistance QTL in backcross generations between resistant donor N13 and farmer preferred recipient line, Ochuti.

QTL/linkage group	QTLA-SBI-01						QTLB-SBI-02				QTLI-SBH-06				QTLJI-SBH05				QTLJ2-SBH-05			
Marker	Xtp208		Xtp302		Xtp050		Xtp201		XTP 304		Xtp 145		Xtp 057		Xtp065		Xtp 303		Xtp225		Xtp015	
Alleles/size	N	O	N	O	N	O	N	O	N	O	N	O	N	O	N	O	N	O	N	O	N	O
Backcross Generation																						
BC_3F_1/L133/p25	260	257	237	196					323	231		232		151		151	150		183	187	217	219
BC_3F_2/L133p/p25p7	260		237		317		202		324		260		260		150		164					
C_3F_2/L133p/p25p13	160			197		315	207		324	232			266		150	152	169	171	208	188	236	238
BC_3F_1/L133/p27							183		323	231	262	232					150				217	219
BC_3F_2/L133p/p27/16	60			197		315	207	202	324	232			266		152		171	169	208	188	236	238

Key: N- N13 allele; O- Ochuti.

Genotype BC_3F_2/L133p/p25p13 also introgressed three heterozygous QTLs namely, QTL B, QTLJI and QTLJ2. QTL B could only be selected by one marker Xtp 304, whereas QTLJ1 and QTLJ2 were selected by flanking markers Xtp065,/Xtp 303, and Xtp 225/ Xtp 015 respectively. Genotype BC_3F_2/L133p/p27/16 introgressed three heterozygous QTLs namely, QTL B selected by flanking markers Xtp 201 /Xtp 304, QTLJ1 selected by only one marker Xtp 303 and QTLJ2 selected by flanking markers, Xtp 225 and Xtp015. Genotype BC_3F_2/L133p/p25p7 had introgressed four QTLs namely, QTL A, QTLB, QTLI and QTLJ1 that contained N13 alleles only in all QTL positions and was not heterozygous for both alleles. Genotype BC_3F_1/L133/p27 had three QTLs introgressed in heterozygous state.

In Table 3, the host damage score for BC_2F_1 genotypes varied significantly from that of Ochuti. Resistant donor line, N13 gave the lowest score of 1.75 an indication of its resistance whereas Ochuti gave the highest score of 3

confirming that it is susceptible to *Striga*. In the backcross genotypes, most of the scores ranged between 2 to 2.625 but genotype S4/L11/H2 gave a score almost similar to that of N13, again an indication of Striga resistance QTL having been transferred into Ochuti.

Similarly, the backcross genetypes scored higher grain yields than N13 though not signficantly so and were closer to those of Ochuti except genotype S4/L34/H1 which gave higher yields than Ochuti (Table 3). Dry panicle weight and 100-seed weight in the backcross generations did not differ significantly from those of N13 and Ochuti.

Figure 2, shows all the measurements of AUSNPC for two seasons at Alupe substation only. The Striga counts were lowest for N13 but highest for Ochuti, whereas the BC_2F_1 backcross generation lines (S4/11/H1, S4/L11/H2, S4/L11/H3, S4/L34/H1, S4/L34/H2, S4/L34/H3, S4/L34/H4, S4/L34/H5) had scores that were between the two parental lines. Among the BC_2F_1 generations, genotype, S4/L34/H3 gave the lowest scores, followed by S4/L11/H2, S4/L11/H3 and S4/L34/H1.

Table 3. The performance of BC_2F_1 backcrosses under Striga infestation at Alupe sub-station during Oct 2010-March 2011 season.

Genotype	Dry panicle wt. (Kg)	100-seed wt(gm)	Grain Yield (Kg/M^2)	Host damage score
N13	0.3	2.9	1.31	1.75
Ochuti	0.7	2.5	3.94	3
S4/L11/H1	0.7	2.175	3.94	2.25
S4/L11/H2	0.525	2.425	2.84	1.875
S4/L11/H3	0.3	2.25	1.64	2.375
S4/L34/H1	0.9	2.425	5.03	2.5
S4/L34/H2	0.7	2.325	3.72	2.625
S4/L34/H3	0.625	2.525	2.95	2.5
S4/L34/H4	0.5	2.4	2.73	2.25
S4/L34/H5	0.625	2.525	3.72	2
Mean	0.588	2.445	3.18	2.312
S.E	0.3119	0.258	1.806	0.304
LSD	0.64	0.53	3.706	0.624

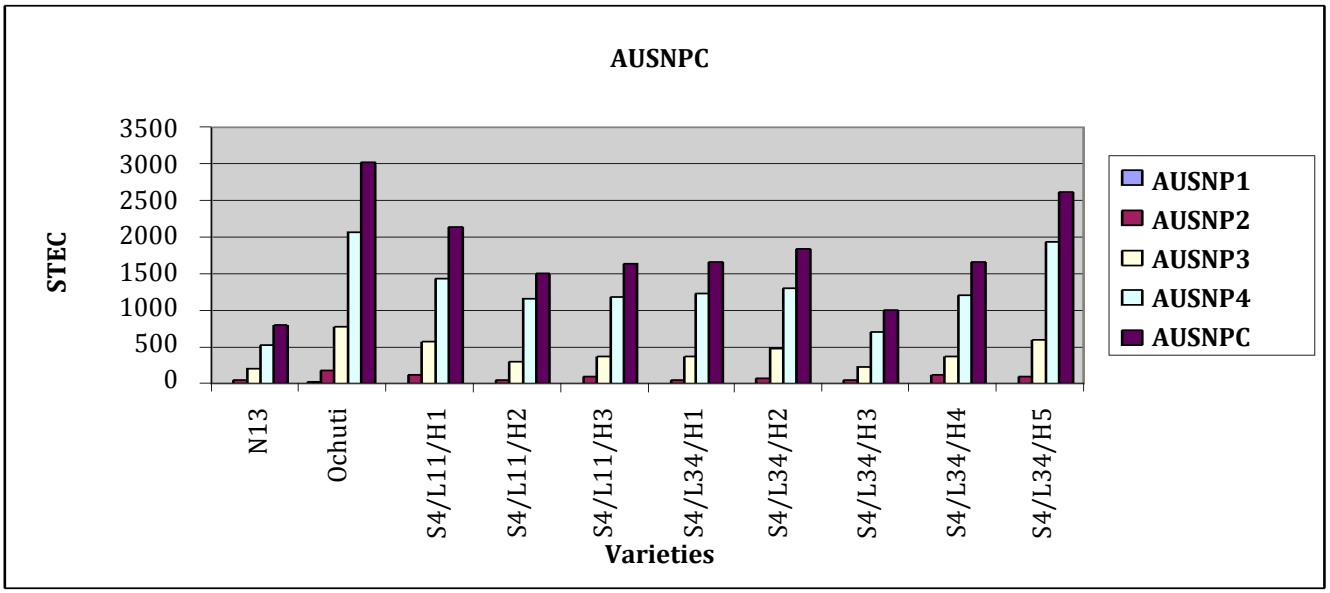

Figure 2. AUSNPC scores in parental lines and in BC_2F_1 backcross generations at Alupe sub-station during Oct 2010-March 2011 seasons.

Table 4, indicates that Striga capsule formation and flowering were highly positively significantly correlated with all the measurements of AUSNPC. Host damage was also highly positively significantly correlated with AUSNPC values and in both cases, the correlations were stronger with AUSNPC 3, AUSNPC 4 and total AUSNPC values confirming that Striga causes more damage to sorghum plant as the crop matures. The results also indicate that scoring AUSNPC at any growth stage is a useful measure of Striga damage, though the best score should be taken later rather than earlier in the crop growth cycle. Reduction in grain yield due to Striga damage appeared to be higher during the early stages of Striga infestation but was progressively lesser at later growth stages as shown by the negatively significant correlation with AUSNPC values.

Table 4. Correlation coefficients between grain yield, host damage, Striga capsule formation and flowering in BC_2F_1 backcross generations.

	AUSNPC1	AUSNPC2	AUSNPC3	AUSNPC4	Total AUSNPC
Striga capsule formation	0.539**	0.687**	0.778**	0.769**	0.779**
Striga flowering	0.517**	0.666**	0.759**	0.753**	0.761**
Yield	-0.348*	-0.325*	-0.289*	-0.240	-0.269
Host damage	0.384**	0.418**	0.444**	0.431**	0.443**

*indicates P value at 99%, ** P value significant at 99.9%

Figure 3, shows total AUSNPC scores in BC_3F_1 generations from two locations in two seasons. The Striga counts for N13 were distinctly lower in both locations during the two seasons. However, the Striga scores for Ochuti and those of the BC_3F_1 backcrosses were indistinguishable. Striga counts for Kibos appeared to be lower than those for Alupe in all genotypes except those for N13 at Alupe during May-October season. BC_3F_1 backcrosses (L33 and L87 lines) shown in Table 5, N13 had the lowest total area under Striga at 5.38 in Kibos and at 4.3 in Alupe. Nevertheless, Ochuti and the backcrosses scored higher AUSNPC values at Alupe than at Kibos as collaborated by the results shown in Figure 3. At Kibos however, Striga capsule formation and flowering did not differ significantly between N13 and the other genotypes, including Ochuti, but these two traits differed significantly at Alupe between N13 and the other genotypes.

Table 5. The reaction of 8 BC3F1 backcross generations to Striga at Kibos and Alupe sub-stations during May 2010 to October 2010 season.

Generation	Striga capsule formation (Kibos)	Striga flowering (Kibos)	Total AUSNPC (Kibos)	Striga capsule formation (Alupe)	Striga flowering (Alupe)	Total AUSNPC (Alupe)
BC3S1L33/4H1	2.79	3.31	6.72	1.56	1.33	8.73
BC3S1L33/4H2	2.66	3.33	6.29	0.96	0.98	8.52
BC3S1L33/4H3	2.31	3.15	6.77	1.5	1.85	8.78
BC3S1L87/4H1	1.25	2.52	5.6	1.25	1.55	8.32
BC3S1L87/4H2	1.44	2.49	5.83	1.2	1.52	8.25
BC3S1L87/4H3	1.58	2.3	5.72	2.31	2.52	8.38
BC3S1L87/4H4	1.77	3.16	6.35	1.19	1.32	8.28
BC3S1L87/4H5	2.77	3.45	6.23	1.48	1.6	8.96
OCHUTI	1.67	2.68	5.72	1.77	2.12	8.76
N13	1.67	2.45	5.48	0	0	4.3
Mean	1.99	2.88	6.07	1.32	1.48	8.13
LSD	1.79	1.39	1.26	1.01	1.28	1.94
CV	43.1	22.3	8.6in	31.1	39.8	11.4

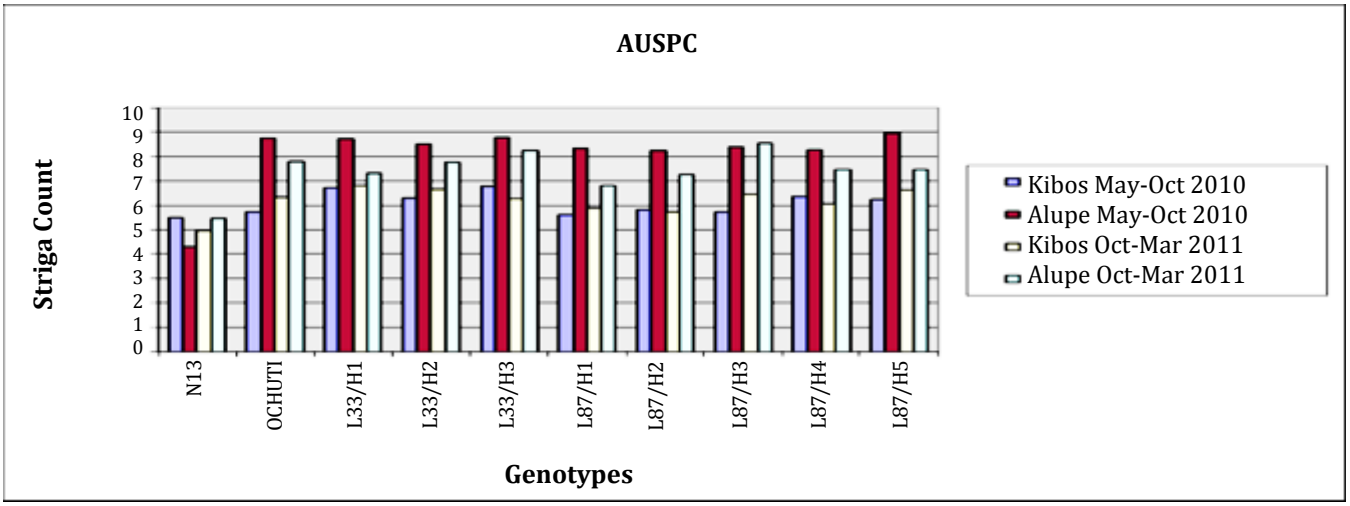

Figure 3. AUSNPC scores in parental lines and in BC_3F_1 backcross generations at Alupe and Kibos sub-station during Oct 2010-March 2011 seasons.

Table 6 shows that genotype x environment interactions were highly significant for all AUSNPC measurements except for AUSNPC1 but these interactions did not differ significantly for Striga capsule formation and flowering in the two locations.

Table 6. Genotype x Environment reactions of 8 BC_3F_1 back-cross generations in two locations (kibos and Alupe) during May 2010 – October 2010 season.

		Trait						
Sources of variation	Df	AUSPC 1	AUSPC 2	AUSPC 3	AUSPC 4	AUSPC Total	Striga Capsule formation	Striga flowering
Environment	1	61.05*	38.90*	65.91*	71.37*	63.39*	6.70*	29.62*
Env*rep	4	10.23*	6.05*	3.72*	5.52*	5.63*	4.51*	3.80*
Genotype	9	6.16*	14.28*	5.64*	2.86*	3.74*	1.10NS	0.71NS
GxE	9	2.37NS	2.72*	4.26*	2.29*	2.51*	0.99NS	1.24NS
Heritability(H^2)	-	0.8	0.9	0.7	0.7	0.7	0.6	0.5

From the table, it appears that AUSNPC3 and AUSNPC4 values are as good indicators of Striga damage as total AUSNPC and that Striga damage could be assessed before flowering stage with certainty. AUSNPC trait, showed higher H^2 values than time to capsule formation and flowering, indicating that AUSNPC is a more heritable trait and easier to select for in the case of Striga resistance than the other two traits.

DISCUSSION

In the BC_3F_1/F_2 progenies genotyped for Striga resistance, only five plants had introgressed the resistance QTL. The number of selected plants appear to be fewer than desired because during the phenotypic selection starting with, the F_1 and BC_1F_1 generations, most plants that did not breed true were rigorously eliminated and secondly, the number of phenotypically identified plants per generations were not more than ten, resulting in fewer plants with resistance QTL being sampled in each cycle of selection. According to Ribaut and Hoisington , (1998) the minimum number of individual plants needed to be screened in a backcross generation during PCR in order to introgress one QTL with 95% ($p>0.05$) confidence should not be less than thirty. Other workers (Semagn *et al*., 2006) have reported that the backcross population being sampled should consist of 1050 plants in order for one to be able to capture between 3-5 of the Striga resistant QTL (Hospital, 2005; Collard *et al*., 2005). This meant that the smaller the population and the lesser the number with resistance genes was being selected the lesser was the chance of selecting Striga resistance per se or the QTL. Indeed, Frisch *et al*. (1999) concluded that selection of QTL through MAS is efficient in large rather than in small populations. But having said that, in this study the fact that at least three genotypes namely, BC_3F_1/L133/p25, BC_3F_2/L133p/p25p13 and BC_3F_2/L133p/p27/16 introgressed three QTLs from different chromosomes and each of these QTL is reported to account for between 10-30% of the Striga resistance variation, these progenies if selfed, would provide resistance for advanced backcrosses. The eleven SSR markers used in foreground selection proved to be polymorphic for parental lines and backcrosses (Fig 1). QTL A, QTL J, QTLJ1, QTLJ2 and QTL B were selected by flanking markers and were therefore likely fixed. However, the distance between these flanking markers is reported to range from 20-50 cM (Hausmann *et al*., 2004). This distance is too large to allow for effective selection of the Striga resistance, since recombination between the marker and the QTL would be inevitable. To be effective in selection of the resistance QTL, markers need to be tightly linked to the QTL and the ideal distance should be at 5-20cM (Semagn *et al*., 2006). The addition of a third marker would have greatly improved the selection further and reduced the loss of the favourable allele due to multiple recombination events (Hash and Senthilvel, 2008). Figures 2 and 3 show the measurement of the area under Striga count using AUSNPC in both BC_2F_1 and BC_3F_1 progenies. A lower value of AUSNPC was an indication of lesser Striga on a genotype and therefore a measure of resistance. Higher values of AUSNPC are expected to incur more damage on the host as shown by the highly positively significant correlation coefficients between AUSNPC values and Striga capsule formation and flowering in Table 4. However, higher grain yield loss in sorghum due to Striga attack appear to have occurred much earlier perhaps before flowering and capsule formation as shown by the progressively lower

negatively significant correlation coefficients of AUSNPC in Table 4. In Fig 2, out of the four counts, the latest count to be scored, AUSNPC 4, as expected had the highest values while AUSNPC 1, 2 and 3 values were lower since they were scored earlier during plant growth. As expected N13 had the lowest AUSNPC values while Ochuti had the highest and the backcrosses varied in between. Indeed the resistance of backcross genotype BC_3F_1-S4/L34/H4 was not significantly different from that of N13 as shown in Table 3 and was more or less at the same level. This is an indication of the mendelian nature of the Striga resistance inheritance. In the BC_3F_1 generations (Fig 3) while the AUSNPC values for N13 were lower and significantly different from those Ochuti and the backcrosses, the AUSNPC values for Ochuti and the backcrosses were not distinct from each other. Indeed, the results shown in Table 4 confirm that when more locations and seasons were considered the values for Striga resistance were more complex. Table 5 and Fig 3 show that there was more Striga damage at Alupe than at Kibos, perhaps because the number of Striga plants germinating at Alupe was higher than that at Kibos as also reported by Hausmann *et al*. (2001). These variations could also have been due to differences in Striga pathenogenicity in the two locations. In the case of BC_3F_1, screening for Striga resistance in the field was most likely influenced by factors such as the heterogeneity of the natural infestation, environmental factors effects on Striga emergence, flowering and capsule formation and interactions between host, parasite and the environment. The fields trials at Kibos and Alupe where these experiments were artificially inoculated with Striga had in the past years been trial sites for screening Striga by other research organizations and so one would expect that over and above the natural infestation, these earlier activities definitely might have contributed to the variations in the Striga populations. The situation was further compounded by the fact that the two trial sites have different soil types and climatic conditions with Kibos being wetter than Alupe at the time of evaluation. Again the AUSNPC values for Kibos were lower than those of Alupe in the two seasons (Table-5) indicating that germination and flowering conditions for Striga were more favoured at Alupe. These factors contributed to the highly significant genotype x environment interactions shown in Table 6. Micro variability of soil fertility and the variation in the natural level of Striga population and interactions between Striga anatagonists such as *Fusarium oxysporum* have been reported to cause variation in the number of emerged Striga plants *(*Haussmann *et al*., 1999).

The results reported here show that screening for Striga resistance among segregating populations by inoculating the field with Striga seeds may be successful if factors such as inclusion of resistant and susceptible checks in multi-locational testing is considered, as inferred by Haussmann *et al*. (2001). In order to assess the complex genotype x environment interactions expected for quantitative traits such AUSNPC and the variability in traits of the Striga weed such as the number of seeds emerging, powerful experimental designs that incorporate many replications are necessary to be able to unravel the Striga resistance per se and dissect the resistance.

CONCLUSION

Three to four QTLs were transferred from a donor line N13 into a farmer preferred sorghum variety successfully with eleven polymorphic SSR markers in BC_3F_1/F_2 backcross generations. Three of the QTLs were fixed and were heterozygous for the two parental alleles. Under artificially inoculated field conditions in two locations, AUSNP values for the Striga resistant backcross genotypes and the check, N13 were lower than those in the susceptible genotypes and in the local check, Ochuti. Striga causes more grain yield loss in sorghum in the early stages of crop growth before flowering. AUSNPC is a genetic trait that is under strong genotype x environment interactions and would require to be evaluated in many locations, seasons and under replications.

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