Field Crops Research 173 (2015) 22–29

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Marker-assisted introgression improves *Striga* resistance in an Eritrean Farmer-Preferred Sorghum Variety

Tadesse Yohannes[a,f], Abraha Tesfamichael[b], Dan Kiambi[c], Rolf Folkertsma[d], C. Tom Hash[e], Kahiu Ngugi[f], Eunice Mutitu[f], Negusse Abraha[a], Mussie Weldetsion[a], Charles Mugoya[g], Clet W. Masiga[g], Santie de Villiers[h,i,*]

[a] *National Agricultural Research Institute, PO Box 4627, Asmara, Eritrea*
[b] *Hamelmalo Agricultural College, PO Box 397, Keren, Eritrea*
[c] *African Biodiversity Conservation and Innovations Centre, PO Box 100882-00101, Nairobi, Kenya*
[d] *Monsanto Holland B.V., Bergschenhoek, The Netherlands*
[e] *ICRISAT-Sahelian Center, BP 12404 Niamey, Niger*
[f] *University of Nairobi, PO Box 39063-00623, Nairobi, Kenya*
[g] *Association for Strengthening Agricultural Research in Eastern and Central Africa, PO Box 765, Entebbe, Uganda*
[h] *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), PO Box 39063-00623, Nairobi, Kenya*
[i] *Pwani University, PO Box 195-80108, Kilifi, Kenya*

ARTICLE INFO

Article history:
Received 7 October 2014
Received in revised form
13 December 2014
Accepted 13 December 2014

Keywords:
Marker-assisted selection
SSR
Sorghum
Striga
Eritrea

ABSTRACT

The parasitic weed *Striga hermonthica* hampers the production of sorghum, the most important cereal crop in Eritrea. This weed has a complex mode of infestation that adapts to many hosts and environments, complicating conventional breeding for resistance, which is the only form of crop improvement available to Eritrean breeders, but has failed. This study aimed at improving resistance against this parasite by transferring 5 *Striga* resistant Quantitative Trait Loci (QTLs) from resistance donor N13 to *Striga* susceptible Farmer-Preferred Sorghum Variety (FPSV) Hugurtay from Eritrea. The method involved backcrossing using marker-assisted selection (MAS) and evaluation of the best introgressed lines for *Striga* resistance in artificially infested fields. Foreground selection was performed with up to 11 polymorphic simple sequence repeat (SSR) markers linked to *Striga* resistance QTLs, while background selection was conducted in the BC_3F_2 generation with 27 polymorphic unlinked SSR markers to identify the best recovery of the recurrent parent (RP) genetic background. Out of 84 BC_3F_3 lines, L2P3-B, L1P5-A and L2P5P35 performed best with respect to both grain yield and reduced *Striga* infestation. These lines were more resistant to *Striga* than Hugurtay, but less resistant than N13. The three lines yielded twice as much as N13, with Area Under *Striga* Number Progression Curve (AUSNPC) values on average 18% higher than that of N13 and 38% lower than that of Hugurtay. This suggests that the introgressed QTLs conferred significant *Striga* resistance and yield advantage to these BC_3F_3 backcross progenies under *Striga* pressure. These lines have good potential for future release and demonstrate that when MAS is available to conventional breeders, even in countries with no genotyping facilities, it is a useful tool for enhancement, expediency and precision in crop improvement.

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the world's fifth most important cereal crop after wheat, maize, rice, and barley (FAOSTAT, 2011). Sorghum forms an important dietary component of many people globally, especially in the arid and semi-arid parts of Africa and Asia. In Eritrea sorghum grain is a major staple food prepared as 'enjera' (leavened bread) or as thick porridge, and the stover is used for animal feed. Sorghum is widely grown in the lowland and mid-highland regions of Eritrea where rainfall is too low for cultivation of most other cereals (Tesfamichael et al., 2013). Nationally, sorghum is grown on more than 200,000 ha annually and accounts for 50% of total food grain production, but with average productivity of less than 1 t ha^{-1} (Ministry of Agriculture, 2010).

The major biotic constraint to sorghum production in Eritrea is *Striga hermonthica*, which affects the majority of farmers in the western part of the country in areas such as Goluj and Shambiko

* Corresponding author at: Pwani University, PO Box 195-80108, Kilifi, Kenya. Tel.: +254 729369758.
E-mail address: s.devilliers@pu.ac.ke (S. de Villiers).

http://dx.doi.org/10.1016/j.fcr.2014.12.008
0378-4290/

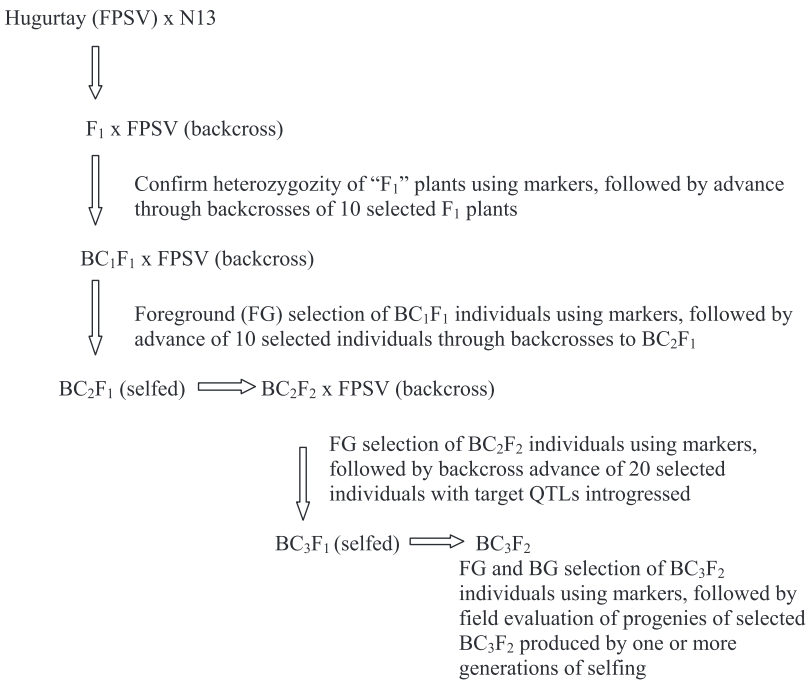

Fig. 1. Scheme of crossing, backcrossing, selfing and marker-assisted selection to generate *Striga* resistant introgression lines in Farmer Preferred Sorghum Variety, Hugurtay, background.

sub-regions. It is prevalent where continuous mono-cropping is practiced and where wild sorghum is prevalent, and its infestation is aggravated by frequent droughts. Yield losses due to *Striga* in Eritrea are estimated at 20–50% of annual sorghum production depending on the rainfall situation.

Efforts to control *Striga* by conventional breeding for resistant varieties are time consuming and have met with few successes unless aided by laboratory screening methods in advanced laboratories (Ejeta and Gressel, 2007). MAS is a useful tool in plant breeding programs for more efficient selection (Lammerts van Bueren et al., 2010) and involves the identification of genotypes carrying desirable alleles, using linked genetic markers. Breeders practice MAS when an important trait that is difficult to assess phenotypically, is tightly linked to a Mendelian trait or molecular markers that can be easily scored (Lammerts van Bueren et al., 2010). The use of molecular markers to breed for *Striga* resistance in sorghum has recently been shown to be possible (Ejeta and Gressel, 2007; Gammar and Mohamed, 2013; Mohamed et al., 2014). Haussmann et al. (2004) identified molecular markers linked to *Striga* resistance QTLs having mechanisms over and above the "low germination stimulant" trait. These authors reported 5 genomic regions (QTLs) associated with stable *Striga* resistance from resistant variety N13 that were identified based on screening across a series of field trials in Mali and Kenya. Three QTLs were identified, one each on linkage groups SBI-01, SBI-02 and SBI-06 and 2 QTLs on linkage group SBI-05 using the revised linkage group designations proposed by Kim et al. (2004).

Although MAS is an accepted and often routine technology in modern breeding programs with access to state-of-the-art genotyping facilities, breeders in many developing countries including Eritrea improve crops using only phenotypic selection for a trait like *Striga* resistance, this entails challenging every generation in infested fields to identify superior individuals. Seeds of these individuals then must be increased for replicated field trials to confirm that the phenotype is true. Conventional backcross breeding takes at least 6 or more generations including the initial crossing generation (Hospital and Charcosset, 1997), as well as two generations of selfing following production of the BC_6F_1 generation (to produce BC_6F_3 seed on BC_6F_2 individuals), and at least one generation of progeny-based testing to identify the desired introgression(s) homozygotes. An important advantage of MAS is that it can reduce the number of backcross generations needed to arrive at the target variety with the introgressed trait(s) (Hospital and Charcosset, 1997; Frisch et al., 1999). For sorghum, with adequate numbers of linked markers, the desired introgression can be achieved within 2–4 backcross generations.

This study aimed to provide Eritrean breeders access to MAS technology in a collaborative experimental design that made use of genotyping facilities and expertise at ICRISAT-Nairobi. All of the field-based work of crossing and advancing generations was done in Eritrea and genotyping was done at ICRISAT-Nairobi with the ultimate objective to transfer resistance alleles at the 5 identified *Striga* resistance QTLs from N13 to Hugurtay, a popular but *Striga*-susceptible Farmer-Preferred Sorghum Variety (FPSV) in Eritrea, using marker-assisted backcrossing (MABC) followed by evaluation of the introgressed lines for improved *Striga* resistance under artificially infested conditions in the field.

2. Materials and methods

Hugurtay (FPSV) was crossed with N13 (resistance donor) and three backcross generations were generated with Hugurtay as RP (Fig. 1). All the breeding activities, selection and collection of tissue samples for DNA extraction were done in Eritrea, and DNA sample preparation and genotyping, and final field evaluation under enhanced *Striga* pressure, were done in Kenya.

2.1. SSR genotyping

Tissue samples were collected in Eritrea from individual plants and shipped to ICRISAT-Nairobi for genotyping. DNA extraction was performed following Mace et al. (2003) with exclusion of the phenol extraction step. The DNA quantity and quality was checked using a Nanodrop® spectrophotometer (Thermo Scientific) and electrophoresis on agarose gels (0.8%, w/v) stained with GelRed (Biotium, USA).

Table 1
Polymorphic SSR markers and their allele sizes at *Striga* resistance QTL positions that were used in foreground screening for QTL introgression in N13 × Hugurtay progenies.

Linkage group (QTL name)	Marker name	Physical map position (Mbp)	Allele size	
			N13	Hugurtay
SBI-01 (QTL01)	Xtxp208	–	260	249
	Xtxp302	9.048	237	229
SBI-02 (QTL02)	Xtxp050	5.080	317	323
	Xtxp201	–	202	Absent
	Xtxp304	5.703	324	318
SBI-06 (QTL06)	Xtxp057	57.419	260	269
	Xtxp145	49.285	260	220
SBI-05 ($QTL05_1$)	Xtxp303	5.729	169	180
	Xtxp065	1.907	150	145
SBI-05 ($QTL05_2$)	Xtxp225	–	184	194
	Xtxp15	42.050	236	238

Key: SBI = *Sorghum bicolor* linkage group.

SSR genotyping was done in the F_1, BC_1F_1, BC_2F_2 and BC_3F_2 generations using 5 to 11 markers linked to the *Striga* resistance QTLs (Haussmann et al., 2004) that were polymorphic between Hugurtay and N13 (Table 1) for foreground genotyping, and using 27 polymorphic markers that were distributed across the remainder of the sorghum genome (Table 2) for background genotyping in the BC_3F_2 generation. Forward primers had an additional 19 bp phage M13 sequence at the 5′ end (CACGACGTTGTAAAACGAC) to allow the incorporation of fluorescent dyes (FAM, NED, PET or VIC) following the method described by Schuelke (2000). PCR reaction mixtures contained 2 mM $MgCl_2$, 0.20 μM reverse primer, 0.04 μM forward primer, 0.16 μM labeled M13 forward primer, 0.04 mM of each of the four dNTPs and 0.2 U DNA polymerase (Sibenzyme) and 30 ng template DNA in a 10 μl reaction volume. Amplification was done on a GeneAmp PCR systems 9600 thermocycler (Applied Biosystems) using initial denaturation at 94 °C for 15 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 1 min and extension at 72 °C for 2 min. Final extension was at 72 °C for 20 min.

Table 2
Polymorphic SSR markers and their allele sizes that were distributed across all ten sorghum linkage groups and were used in background screening to assess recovery of the Hugurtay genotype background in BC_3F_2 individuals homozygous for *Striga* resistance QTL alleles from donor parent N13.

Linkage group	Marker name	Physical map position (Mbp)	Allele size	
			N13	Hugurtay
SBI-01	Xtxp032	–	157	153
	Xcup053	72.905	205	214
	Xtxp357	23.806	260	268
SBI-02	Xtxp214	60.443	242	294
	Xtxp298	57.081	343	294
SBI-03	Msbcir276	55.555	246	250
	Xisp0307	–	361	378
	Xtxp183	–	Absent	296
	Xtxp285	67.824	270	218
SBI-04	Xtxp024	58.547	173	189
	Xisep0228	9.994	113	215
SBI-05	Msbcir248	4.746	138	148
SBI-06	Xtxp057	57.419	260	268
SBI-07	Msbcir300	58.286	135	130
	Xtxp295	61.172	177	191
	Xtxp040	0.861	161	156
SBI-08	Msbcir240	4.468	125	129
	Xtxp250	51	267	270
SBI-09	Xcup002	8.144	221	213
	Xgap015	6.113	129	134
	Xtxp010	47.917	153	158
	Xtxp287	4.242	343	373
	Xtxp289	0.024	342	291
SBI-10	Xcup016	57.842	254	252
	Msbcir283	18.1	134	160
	Xgap001	54.507	273	271
	Xtxp141	58.245	155	183

Key: Mbp = Mega basepair.

Fragment analysis was carried out by capillary electrophoresis using an ABI PRISM 3730 (Applied Biosystems), automated sequencer as described by Folkertsma et al. (2005). Up to 4 markers were analyzed simultaneously by combining 1.5–2.5 μl of each labeled PCR product with formamide and 0.16 μl Genescan Liz 500 molecular weight standard (Applied Biosystems). Allele scoring was done using GeneMapper® Software V4.0 as described by Hayden et al. (2008).

2.2. Evaluation of BC_3F_2-derived progenies for Striga resistance in artificially infested fields

Following marker-assisted selection, progenies from selfed BC_3F_2 individuals with 1–4 *Striga* resistance QTLs, were evaluated for improved *Striga* resistance in the field. During the first season, 84 BC_3F_2-derived lines (BC_3F_3 generation) were screened using an augmented design at Alupe, Kenya. During the second season, selfed progenies of 30 BC_3F_3 lines (BC_3F_4 generation) that performed better than Hugurtay in the field during the first season were evaluated at two locations in Kenya (Alupe 00°29′ N, 34°08′ E, 1189 m altitude and Kibos 00°04′ S, 34°48′ E, 1214 m altitude) in an alpha-lattice design, replicated three times with parents N13 and Hugurtay included as checks. At sowing, each hill was infested with *Striga* seeds, prepared by mixing 5 g of seeds with 5 kg of washed sand and applying 1 tablespoon of inoculum to each hill (Jamil et al., 2012). At 21 days after sowing, seedlings were thinned to 0.2 m between plants and one plant per hill. First weeding, excluding *Striga*, was done by hoe with subsequent weeding done by hand to avoid disturbing emerging and established *Striga* plants.

In all three experiments, progenies were evaluated for days to 50% flowering, plant height, grain yield, days to *Striga* emergence, emerged *Striga* count, number of *Striga* plants flowered, number of *Striga* plants forming seed capsules, and *Striga* vigor on a scale of 1–9 adopted from Haussmann et al. (2000). Successive *Striga* counts were used to calculate the Area Under *Striga* Number Progress Curve (AUSNPC) as described by Haussmann et al. (2000).

2.3. Field data analysis

Data on agronomic performance and *Striga* parameters were subjected to analysis of variance (ANOVA) using Genstat® 15th Edition (http://www.vsni.co.uk). Treatment means were separated using the least significance difference test. Relationships among parameters were tested using Pearson's correlation coefficient. On Striga counts, logarithmic transformations ($\log(X+c)$, where X is the original individual observation and $c = 1.0$) were applied as described by Rodenburg et al. (2005).

3. Results

3.1. Foreground and background genotyping

DNA extraction yielded good quality genomic DNA of 5.2–13.7 μg per sample. Polymorphism screening between N13 and Hugurtay for both foreground and background markers allowed

Table 3
Number of individuals genotyped in the F1 and subsequent backcross (BC) generations.

Generation	Individuals genotyped	Individuals with QTLs introgressed	Individuals advanced to next generation
F_1	10	10	4
BC_1F_1	23	23	10
BC_2F_1	0	–	17
BC_2F_2	58	42	20
BC_3F_1	0	–	11
BC_3F_2	341	216	84

Key: QTL = quantitative trait locus.

selection of a total of 11 foreground SSRs (Table 1) and 27 background SSR markers (Table 2), which were used to screen one or more generations of the backcross progenies. Foreground SSRs are markers that are linked to the target QTL, which enable identification and selection of plants having the alleles of the donor parent at the target loci. Background SSRs are markers that are spread across the genome and not linked to the target QTL, which help to assess the recovery of the recurrent parent genotype.

For genotyping the F_1, BC_1F_1 and BC_2F_2 generations, only 7 polymorphic foreground SSR markers were identified that allowed indirect selection for 4 *Striga* resistance QTLs: Xtxp208 and Xtxp302 (linked to QTL01), Xtxp050, Xtxp201 and Xtxp304 (linked to QTL02), Xtxp303 (linked to $QTL05_1$) and Xtxp225 (linked to $QTL05_2$). No markers were available to screen for QTL06. The number of individuals genotyped and selected during each generation, excluding the parents, is shown in Table 3.

In F_1, 10 plants were genotyped with 6 foreground markers to confirm that they were true hybrids of N13 and Hugurtay. In the BC_1F_1 generation 23 plants were screened with the 7 polymorphic foreground markers, of which 12 BC_1F_1s showed introgression of 1 QTL, 9 of 2 QTLs and 2 of 3 QTLs (Table 4). Ten representative BC_1F_1 plants with different QTL introgression combinations were selected and advanced to BC_2F_1. Seventeen plants of BC_2F_1 were selfed and subsequently, foreground genotyping in BC_2F_2 was done on 58 individuals with the same 7 markers as used for the BC_1F_1 plants. From these, 42 plants were identified with introgressed QTLs of which 23 plants showed homozygous donor QTL alleles (Table 4) and 20 of these plants were selected for advance to BC_3F_1.

Selfed seeds from 11 BC_3F_1 plants were sent to ICRISAT-Kenya where the BC_3F_2 plants were genotyped for QTL introgressions and recurrent parent genome background recovery. By this point additional polymorphic markers had been identified, of which at least 2 were linked to each of the 5 QTLs, as indicated in Table 1. A total of 341 BC_3F_2 individuals were screened using the 11 polymorphic foreground SSR markers. Of these, 216 were found to have from 1 to 4 homozygous introgressed QTLs as shown in Table 5.

Table 4
Summary of the various combinations of QTLs introgressed in heterozygous state in BC_1F_1 and in homozygous state in BC_2F_2.

QTL combinations[a]	Backcross generation	
	BC_1F_1	BC_2F_2
Single introgressions		
QTL01	0	3
QTL02	0	0
$QTL05_1$	0	1
$QTL05_2$	15	3
Double introgressions		
QTL01, QTL02	0	3
QTL01, $QTL05_2$	0	1
QTL02, $QTL05_2$	5	0
$QTL05_1$, $QTL05_2$	1	2
Triple introgressions		
QTL01, QTL02, $QTL05_1$	0	1
QTL01, QTL02, $QTL05_2$	0	1
QTL01, $QTL05_1$, $QTL05_2$	0	8
QTL02, $QTL05_1$, $QTL05_2$	1	0
4 QTLs introgressed		
QTL01, QTL02, $QTL05_1$, $QTL05_2$	1	0
Total individuals with QTLs	23	23

[a] QTL combinations that were not identified in any individuals were not included here. QTL06 was not screened for as polymorphic markers linked to this locus were not available at the time.

3.2. Field evaluation of BC_3F_3 lines under Striga infestation

Combined ANOVA for grain yield across the 2 locations in the second season of field screening in Kenya under *Striga*-infested conditions revealed that genotypes, environments and genotype × environment (G × E) interaction components of variance were significant (Table 6). However, magnitude of the G × E interaction variation was small compared to that for genotype.

Table 5
Summary of QTL introgressions fixed in BC_3F_2 lines.

QTL combination	No. of BC_3F_2 lines	BC_3F_2 lines with QTLs introgressed			
		1 QTL	2 QTLs	3 QTLs	4 QTLs
QTL02	31				
QTL06	13				
$QTL05_1$	4				
$QTL05_2$	93	141			
QTL01, QTL02	1				
QTL01, QTL06	1				
QTL01, $QTL05_2$	3				
QTL02, QTL06	25				
QTL02, $QTL05_2$	13				
QTL06, $QTL05_2$	8				
$QTL05_1$, $QTL05_2$	5		56		
QTL01, QTL02, QTL06	3				
QTL01, QTL06, $QTL05_2$	4				
QTL01, $QTL05_1$, $QTL05_2$	3				
QTL02, QTL06, $QTL05_2$	5				
QTL02, $QTL05_1$, $QTL05_2$	2			17	
QTL01, QTL02, QTL06, $QTL05_2$	2				2
Sub-total	216				
Individuals with no introgressed QTLs	125				
Total genotyped BC_3F_2 individuals	341				

Table 6
Combined analysis of variance for grain yield across locations.

Fixed term	Wald statistic	n.d.f.	*F* statistic	d.d.f.	*F* pr
Env	11.19	1	11.19	26.1	0.003
Gen	10,515.9	31	334.57	90.9	<0.001
Env × Gen	129.36	31	4.12	91	<0.001

Key: Env = Environment, Gen = Genotype, n.d.f. = numerator degrees of freedom, d.d.f. = denominator degrees of freedom, F pr = F probability.

Genotypes differed significantly ($P < 0.05$) for all the observed traits (Table 7). The G × E interaction was also significant for parameters like plant height, grain yield, days to *Striga* emergence, AUSNPC, number of *Striga* plants that flowered and *Striga* vigor. G × E interaction was not significant for days to 50% flowering and number of *Striga* plants forming capsules/seeds.

Parameters used to evaluate improved backcross genotypes for *Striga* resistance were grain yield under *Striga* pressure, followed by comparisons with resistant parent N13 in both locations for low AUSNPC, days to *Striga* emergence and number of *Striga* seed capsules formed, as presented in Table 7, following the recommendations of Haussmann et al. (2000). Taller plants and better grain yield as well as low AUSNPC, days to *Striga* emergence, days to *Striga* flowering and number of capsules formed was observed for genotypes 23 (L2P3-B), 12 (L2P5P35), 22 (L1P5-A), 24 (L2P6-A) and 25 (L2P7-A). All of these genotypes had either 3 or 4 QTLs fixed in different combinations of QTLs 01, 02, 05_2 and 06 as indicated in Table 7.

Table 8
Pearson's correlation coefficients for various Striga resistance parameters measured at Alupe and Kibos during September 2012–February 2013.

Correlated traits	Alupe	Kibos	Combined
Days to *Striga* emergence vs AUSNPC	−0.6028**	−0.7865**	−0.8172**
Days to *Striga* emergence vs grain yield	0.2482	0.5319*	0.4759*
Days to *Striga* emergence vs plant height	0.3708*	0.2172	0.3209
AUSNPC vs grain yield	−0.5402**	−0.6006*	−0.6118**
AUSNPC vs plant height	−0.5737**	−0.1489	−0.4104*
AUSNPC vs days to flowering	−0.0200	−0.1565	−0.1366
Plant height vs grain yield	0.3448	0.2408	0.3469
AUSNPC vs *Striga* flowering	0.6613**	0.8234**	0.8448**
AUSNPC vs *Striga* capsules	0.8039**	0.8021**	0.8550**

* Significant.
** Highly significant at $P < 0.05$.

Days to *Striga* emergence was positively correlated with plant height and grain yield in both locations (Table 8). Therefore, as the days to *Striga* emergence was delayed, plant height and grain yield increased. On the other hand, days to *Striga* emergence showed strong negative correlation with the number of *Striga* plants that emerged so that, the earlier the *Striga* shoots emerged, the higher was the eventual density of the *Striga* plants observed with a concomitant increase in AUSNPC (Table 8). AUSNPC was negatively correlated with grain yield in both locations. *Striga* plants flowering

Table 7
Across-location means for agronomic and Striga-related parameters from field trials at two locations, September 2012–February 2013.

No.	Genotype	QTL type	NHRPLR	DF	PH	GY	DSE	AUS NPC	NSF	NSC	SV
1	L1P2P13	02, 06	18	70.7	143.3	0.95	43.8	55.9	10	7.7	5.7
2	L2P1P7	02, 06	12	69.7	130.7	1.05	48.2	50.4	9.2	7.2	5.7
3	L2P1P22	02, 05_2, 06	16	71.2	141.3	1.78	45	46.4	8.2	7	5.2
4	L2P2P4	02, 05_2	7	71.7	158.6	0.91	41.3	54.3	9.3	7.8	5.2
5	L2P2P8	02, 05_2	15	69.8	135.9	1.18	43	49.1	8.7	6.7	5
6	L2P2P16	2	17	71.2	140.5	1.44	43	57.4	9.3	7.5	6.3
7	L2P5P10	05_2, 06	18	70.2	129.7	1.37	46.2	53.9	9	6.8	5
8	L2P5P11	02, 05_2, 06	16	70.5	139.1	1.71	48.2	46.1	8.3	6.3	4.2
9	L2P5P15	02, 05_2,	16	70.2	133.6	1.47	47.8	51.0	8.5	6	5
10	L2P5P22	06, 05_2	19	70	148.2	1.48	48.8	50.2	7.5	6	5
11	L2P5P25	02, 05_2, 06	15	70.2	134.7	1.37	47.3	49.0	7.7	6.2	4.3
12	**L2P5P35**	**02, 05_2, 06**	**19**	**70.2**	**138.3**	**2.06**	**49.3**	**44.7**	**6.5**	**5**	**4.2**
13	L2P6P9	05_2, 06	18	72	140.3	1.8	48.7	39.9	8.5	6.5	4.8
14	L2P6P14	02, 05_2	15	68.7	153.8	1.45	45	49.2	9.8	7.2	5
15	L2P6P29	02, 05_2, 06	16	69.7	151.8	1.89	50	44.1	6.3	5	4
16	L2P6P38	02, 06	15	70.3	134.8	1.4	40.8	51.6	9.2	6.8	5.3
17	L3P1P4	02, 05_1, 05_2	14	68.5	150.5	1.04	51.3	43.8	6	5	4.2
18	L2P1P15	01, 02	16	70.7	151.5	1.25	45.2	51.5	6.8	5.5	5.2
19	L3P1P14	01, 05_1, 05_2	14	71.2	152.9	1.79	53	45.1	6.3	4.7	4
20	L3P1P23	02, 05_2	18	70.2	151	1.8	44.7	47.6	7.7	5.7	5.3
21	L1P2-A	01, 02, 06	12	70	132	1.04	49	41.4	5.7	4.3	4.5
22	**L1P5-A**	**01, 02, 06**	**18**	**69.2**	**152.7**	**2.02**	**50.5**	**41.8**	**5.3**	**5**	**4**
23	**L2P3-B**	**01, 02, 05_2, 06**	**17**	**72**	**156.3**	**2.09**	**53.3**	**36.2**	**5.8**	**4.3**	**3.8**
24	**L2P6-A**	**01, 05_2, 06**	**18**	**73.3**	**146.7**	**1.95**	**54.2**	**40.1**	**5.5**	**4.5**	**4.2**
25	**L2P7-A**	**01, 02, 05_2, 06**	**15**	**69.7**	**154.7**	**1.91**	**54.3**	**36.5**	**6.2**	**4.5**	**3.8**
26	L3P3-A	01, 05_1, 05_2,	18	71.1	146	1.93	50.7	44.7	7.8	5.7	4.3
27	L2P6P10	01, 05_2	18	71.7	152.9	1.42	44	48.3	8.3	7.2	5.2
28	L2P6P36	6	16	69.2	143.3	0.84	47.5	56.0	10.3	7.7	6
29	L2P4P31	06, 05_2	16	69.7	145.8	0.85	45	50.2	7.2	5.8	4.8
30	L2P5P30	06, 05_2	15	70.2	143.7	1.26	46.5	52.6	10.3	8.2	5.7
31	Hugurtay			70.5	123.2	0.69	40.8	62.6	14	11.1	7.5
32	N13			70.2	146	1.04	56.3	36.3	4	3.2	3.2
		F.Pro.G		0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
		F.Pro.G × E		0.452 ns	0.036	<0.001	0.018	0.002	0.016	0.58 ns	0.046
		G.Mean		70.41	143.9	1.445	47.59	47.73	7.917	6.187	4.859
		CV (%)		2.11	8.4	3.355	10.339	10.15	23.01	27.51	12.719

Key: NHRPLR = number of homozygous recurrent parent loci recovered from 27 SSR background markers analyzed, QTL = quantitative trait locus, DF = days to 50% flowering, AUNSPC = area under striga number progress curve, PH = plant height in cm, GY = grain yield in tons/ha, DSE = days to *Striga* emergence, NSF = Number of *Striga* flowered, NSC = number of *Striga* capsule, SV = *Striga* vigor, F.pro.G = F probability of genotype, F.pro.G × E = F probability of genotype by environment interaction, G.mean = Grand mean, CV = coefficient of variation, ns = non-significant.
Figures in bold indicate the introgression lines that performed best in terms of yield compared to the check varieties under *Striga* pressure.

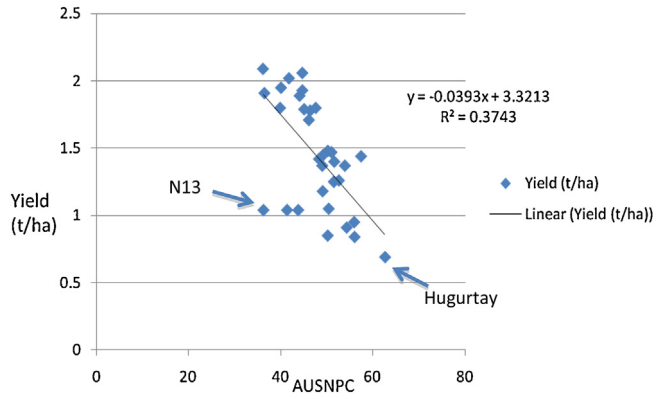

Fig. 2. Relationship between Area Under Striga Number Progress Curve (AUSNPC) and grain yield for combined data from Alupe and Kibos.

and number of *Striga* seed capsules formed both exhibited strong positive correlation with the number of emerged *Striga* plants (AUSNPC), confirming that with higher *Striga* counts, weed productivity was high and more *Striga* plants flowered and produced seed capsules. Fig. 2 shows the relationship between across-site entry mean AUSNPC and grain yield values for the 32 trial entries. As the number of *Striga* plants increased, grain yield reduced significantly to below 1 t ha^{-1}. The equation $y = -0.0393x + 3.3213$ indicates that for every unit increase of AUSNPC, the grain yield was expected to decrease by about 0.0393 t ha^{-1}. The regression R^2 indicated that a moderate but significant proportion of variation in mean grain yield was explained by the variation in mean cumulative *Striga* counts (AUSNPC) across the two sites.

4. Discussion

Use of MAS in modern breeding is not new and has been applied for single and multiple traits introgression over the past decade, often in cereals such as barley, rice and wheat (Collard and Mackill, 2008). However, this tool is most successfully exploited by multinational breeding companies and state-of-the art public research institutions. In contrast, the current study was conducted in two geographically distant developing countries, Kenya and Eritrea. All breeding activities were conducted in Eritrea whilst genotyping and final field evaluation was done at ICRISAT-Nairobi since Eritrea has no DNA extraction or genotyping facilities. The appropriate leaf samples were collected and preserved in Eritrea and sent to Kenya for genotyping.

4.1. SSR genotyping

DNA extracted from leaf samples that reached ICRISAT-Nairobi in good condition (F_1, BC_1F_1 and BC_2F_2) or that were grown from seeds sent to ICRISAT-Nairobi (BC_3F_2) was of sufficiently good quality and quantity for the required genotyping for foreground and background screening, since SSR analysis require relatively small amounts of DNA (Powell et al., 1996; Semagn et al., 2006).

During foreground screening of the early generations (F_1, BC_1F_1 and BC_2F_2) for *Striga* resistance QTLs introgression, limited polymorphic markers were available for some target QTLs (none for QTL06 and only one each for $QTL05_1$ and $QTL05_2$). However, during the BC_3F_2 generation, more markers were available from the completed whole genome sequence of sorghum (Paterson et al., 2009) and subsequent identification of numerous genome-wide SSR markers that were reported by Ramu et al. (2010) and Billot et al. (2013). Two polymorphic flanking markers each were used for QTLs 01, 05_1, 05_2 and 06, and 3 markers for QTL02; enabling more accurate selection for QTL introgression as the focus was to select plants homozygous for the marker alleles of the donor parent flanking each target locus as described by Hospital (2003).

Results for the 341 BC_3F_2 samples genotyped (Table 5) confirmed homozygous (fixed) introgression of resistance alleles at 1 QTL in 141 samples, at 2 QTLs in 56 samples, at 3 QTLs in 17 samples, 4 QTLs in 2 samples and 125 samples with homozygous resistance alleles introgressed at none of the 5 target QTLs. All 5 individual target QTLs were introgressed into at least 15 plants although all introgressions of QTL01 were in combination with at least 1 other QTL. These results allowed the selection of 84 selfed BC_3F_2 plants with different individual QTLs and/or QTL combinations fixed for background screening and field evaluation.

In background (BG) screening, the focus was to identify the BC_3F_2 individuals that had homozygous resistance alleles introgressed at one or more QTL and also achieved the best recovery of the RP genome in the genomic regions not linked to the QTLs (Semagn et al., 2006). BG screening is most effective in the BC_1F_1 generation, when relatively large numbers of plants are genotyped and when large numbers of markers are available that can screen the whole genome at short intervals, which aids identification of linkage drag as well as individuals where such linkage has been overcome that helps to eliminate potentially deleterious genes being introduced from the donor parent (Hospital, 2003). In this study, BG genotyping of the selected 84 BC_3F_2 progeny indicated that 56 individuals exhibited homozygous RP alleles at 15–19 of the 27 SSR loci analyzed, indicating a higher rate of RP recovery compared to the other individuals. This was lower than the expected theoretical RP recovery at BC_3 of 93.75% (Ram and Mishra, 2010), probably due to the simultaneous selection for multiple target QTL introgressions, which will have increased both the amount of linkage drag and the proportion of donor parent allele heterozygosity retained in BC_1F_1, BC_2F_2 and BC_3F_2 individuals selected for generation advance. Physical examples of high levels of RP recovery in sorghum has not been reported, but Kim et al. (2008) succeeded in 88% recurrent background recovery at BC_3 for soybean. It is this ability to select for the RP recovery outside of the target locus that greatly reduces the number of generations required to develop lines that possess the desired introgression(s) in a given elite background (Hospital, 2003). In this study, 3 backcross generations were sufficient to recover lines that closely resembled Hugurtay RP for morphological traits, instead of the average of 6 generations that are routinely considered necessary, and the selected 84 lines were considered good candidates for field evaluation.

4.2. Field evaluation of BC_3F_3 lines

In this study, the results of MAS were verified, after genotyping of the BC_3F_2 generation and selection of the 84 best candidate lines, by field evaluation of the selfed progenies (BC_3F_3) to identify the genotypes with improved resistance to *Striga* infestation combined with agronomic traits of the recurrent parent. Haussmann et al. (2000) suggested that the number of emerged *Striga* plants, *Striga* vigor, number of flowering *Striga* plants, number of *Striga* plants with seed capsules, days to *Striga* emergence and yield of the crop under infestation conditions were the most important parameters to consider amongst other agronomic characters of the host.

For all the parameters measured in the field trial, five lines—L2P3-B (QTL01, QTL02, $QTL05_2$ and QTL06), L2P6-A (QTL02, $QTL05_2$ and QTL06), L2P7-A (QTL01, QTL02, $QTL05_2$ and QTL06), LP1P5-1 (QTL01, QTL02 and QTL06), and L2P5P35 (QTL02, $QTL05_2$ and QTL06)—consistently performed best with regard to the *Striga* resistance parameters discussed below. All of these five lines had incorporated QTL06 and QTL02, most often in combination with QTL01, $QTL05_2$ or both of these (in 2 out of these 5 lines) (Table 7). Both lines with 4 QTLs had the same introgression pattern. $QTL05_1$ was not present in any of these 5 lines, indicating that $QTL05_1$ may

contribute least to the resistance. Gammar and Mohamed (2013) reported that introgression of $QTL05_1$ and $QTL05_2$ improved field resistance more than the other QTLs in sorghum in Sudan.

Days to first *Striga* plant emergence was significantly different among the lines evaluated. Emergence of the parasite was slow and delayed by about two weeks in lines L2P3-B (QTL01, QTL02, QTL06 and $QTL05_2$), L2P6-A (QTL02, QTL06 and $QTL05_2$), and L2P7-A (QTL01, QTL02, QTL06 and $QTL05_2$) compared to the emergence in the susceptible control, Hugurtay (Table 7). Late *Striga* emergence may be an indication of late attachment of *Striga* to the sorghum host. Such late attachments could be attributed to the mechanical resistance mechanism of the N13 donor conferred by its *Striga* resistance QTL alleles. Gebremedhin et al. (2000) also reported delayed emergence of *Striga* on resistant sorghum compared to a susceptible variety. The genetic differences between the sorghum genotypes have been reported to affect the time of parasite attachment, with resistant varieties showing later attachment and parasite emergence than susceptible cultivars (Ezeaku and Gupta, 2004; Rodenburg et al., 2006). The observed delay in *Striga* emergence may also be due to reduced or delayed haustorium initiation and attachment. Alternatively, a less competent match between the parasite and the host roots may have resulted in the progeny with newly acquired resistance QTL, reducing the likelihood of a successful parasitic relationship that manifested in the later emergence of successful parasites.

The AUSNPC as described by Haussmann et al. (2000) is considered an appropriate measure for *Striga* resistance as it incorporates emergence time and numbers of emerged *Striga*. Omanya et al. (2004) previously noted that the AUSNPC is under strong genetic control and offers a suitable measure of progressive *Striga* emergence in the field and the authors encouraged its use in screening.

In the current study, the combined ANOVA (Table 7) indicated that *Striga* infestation over the cropping season, expressed as the AUSNPC, was significantly reduced in the introgressed BC_3F_4 lines compared to the control variety, Hugurtay. *Striga* counts increased steadily for most lines during crop growth. However, lines L2P3-B, L2P7-A, which each had 4 QTLs fixed and lines L1P5-A, L2P6-A, L2P5P35, which each had 3 *Striga* resistance QTLs fixed, maintained the lowest *Striga* infestation levels compared to other backcross lines. The RP (and susceptible check) variety, Hugurtay supported the highest number of *Striga* plants. Haussmann et al. (2000) considered genotypes as resistant when they supported significantly fewer emerged *Striga* plants than the susceptible check and Rodenburg et al. (2006) reported that the number of emerged *Striga* plants recorded above ground was significantly correlated with the number of *Striga* plants attached to the roots in sorghum. The results of this study supported both these trends (Table 8).

Sorghum growth, measured by plant height and grain yield as indicated in the analysis (Table 7) was significantly different among the lines. In a previous report, Ayangowa et al. (2010) noted a reduction of sorghum growth and stunting as dominant symptoms of *Striga* infestation. Stunted growth is likely to result in reduced grain yield. In this study, the combined ANOVA for the two locations showed the highest mean grain yield of 2.09 t ha^{-1} was recorded for line L2P3-B, followed by L2P5P35, L1P5-A and L2P6-A with 2.06, 2.02 and 1.95 t ha^{-1} respectively. All of these lines, besides having three to four *Striga* resistance QTL alleles introgressed and fixed, also had a higher number of the recurrent parent homozygous alleles at the BG marker loci with lines L2P3-B, L1P5-A, L2P6-A that exhibited 17–18 homozygous loci and line L2P5P35 that exhibited 19 out of 27 loci that were screened. These genotypes also closely resembled Hugurtay morphologically, indicating successful genome recovery of the recurrent parent through the successive backcrossing generations. However, Hugurtay yielded poorly at 0.69 t ha^{-1}, due to the *Striga* pressure and its susceptible nature. On the other hand, even though N13 supported fewer *Striga* shoots (Figure 2) its lower yield could be due to its inherent low yield potential (Ejeta, 2007). Lines with entry numbers 23 (L2P3-B), 12 (L2P5P35), 22 (L1P5-A) and 24 (L2P6-A) were the best performers that also showed good stability across the two locations. In these four lines, the emergence of *Striga* was also delayed compared to Hugurtay, possibly explaining the higher grain yields obtained.

The lower *Striga* counts and higher grain yields in the introgressed BC_3F_2-derived introgression lines compared to the RP, Hugurtay, demonstrated that MABC was successful for the introgression of the *Striga* resistance QTLs from the donor parent, N13. It allowed precise identification of the individuals in each generation that had resistance alleles of the QTLs introgressed and therefore allowed targeted advance of only small numbers of selected individuals in each generation – without the need for phenotypic screening of each generation. These introgressed QTLs in the selected backcross progenies were correlated with delayed emergence, reduced numbers of *Striga* shoots and improved grain yield. Gammar and Mohamed (2013) also reported reduced *Striga* emergence and increased sorghum yields after introgressing *Striga* resistance QTL from N13 into Tabat, a FPSV in Sudan.

The number of *Striga* plants flowering and those forming capsules are vital to estimate the productivity of the parasite. In this study, the number of *Striga* plants that flowered and those that formed capsules on the introgressed lines were few compared to the number of emerged *Striga* shoots in each plot (Table 7). This could reduce the multiplication rate of the *Striga* and slow down the accumulation of seeds in the soil. Rodenburg et al. (2006) also identified host resistance as an important determinant of *Striga* reproduction. However, the very large seed production capacity of *Striga* means that there may still be capacity for the *Striga* population to rapidly adapt to the resistance QTLs being deployed as even the reduced number of flowering plants may be enough to maintain soil seed banks at levels sufficient for significant levels of infestation of a resistant host unless resistance deployment is accompanied by removal of emerged *Striga* plants prior to seed dispersal, or other weed management measures (Rodenburg et al., 2006).

Visual evaluation of *Striga* vigor at maturity varied from approximately 4 (*Striga* height 21–30 cm and number of branches on *Striga* plant $\leq$5) to 8 (*Striga* height >40 cm and number of branches on *Striga* plant $\leq$10) (Table 7). In the majority of backcross lines, *Striga* plant vigor was on average, less than 40% of that of the recurrent parent Hugurtay. Rodenburg et al. (2006) suggested that *Striga* plant vigor influences mortality and seed production capacity. In this study *Striga* vigor and capsule number were positively correlated (Table 8).

5. Conclusions

The results of this study clearly demonstrated the potential of using exotic donor germplasm to improve adapted local material in combination with marker-assisted selection and backcrossing. Up to four *Striga* resistance QTLs were fixed in genotypes L2P5P35, L2P3-B, L2P6-A, L1P5-A and L2P7-A, all of which had also recovered relatively high proportion of the recurrent parent, Hugurtay, genome at the background SSR loci analyzed. The identification of these five genotypes is expected to have a positive impact on improving sorghum productivity in the *Striga* prone areas of Eritrea, provided that the introgressed resistance QTLs are indeed effective against *S. hermonthica* populations in those regions.

QTL pyramiding can be done in the future by crossing the introgressed genotypes L2P3-B and L3P3A to incorporate the fifth *Striga* resistance $QTL05_1$ to further improve the results obtained from this study. Genotype L2P3-B had introgressed four QTLs namely QTL01, QTL02, QTL06 and $QTL05_2$ and genotype L3P3-A three QTLs namely QTL01, $QTL05_1$ and $QTL05_2$. These genotypes also

performed among the best agronomically. Furthermore, the best genotypes in this study will in future be invaluable to plant breeders in the Eastern and Central Africa region in their sorghum improvement programs.

Acknowledgments

This work has been undertaken as part of the CGIAR Research Program on Dryland Cereals and the ABCIC *Striga* Resistance Evaluation Project with funding from BMZ and ASARECA.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2014.12.008.

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