Sciknow Publications Ltd.
Journal of Renewable Agriculture
©Attribution 3.0 Unported (CC BY 3.0)

JRA 2013, 1(1):6-10
DOI: 10.12966/jra.04.02.2013

Introgressing *Striga* Resistance from a Mapped Donor Source into a Rwandan Adapted Sorghum Variety

Theogene Niyibigira[1, 2], Kahiu Ngugi[2, *], Santie de Villiers[3], Dan Kiambi[4], Eunice Mutitu[2], Sarah Osama[7], Abigail J. Ngugi[2], Mohamed Abdalla[3], Rasha Ali[5], Charles Mugoya[6], Clet Masiga[6], Daphrose Gahakwa[1]

[1]Rwanda Agriculture Board, P.O.BOX: 5016 Kigali, Rwanda
[2]University of Nairobi, P.O.BOX: 29053-00625 Nairobi, Kenya
[3]International Crops Research Institute for the Semi-Arid Tropics, P.O.BOX: 39063-00623 Nairobi, Kenya
[4]African Biodiversity Conservation and Innovations Centre, P.O.BOX: 100882-00101 Nairobi, Kenya
[5]Agricultural Research Corporation, P.O.BOX: 126 Wad Medani, Sudan
[6]Association for Strengthening Agricultural Research in Eastern and Central Africa, P.O. BOX 765 Entebbe, Uganda
[7]Biosciences eastern and central Africa, P.O.BOX: 3709-00100 Nairobi, Kenya

*Corresponding author (E-mail: kahiukahiu@gmail.com)

Abstract – Sorghum is the world's fifth most important cereal, in terms of both production and area planted. *Striga hermonthica* is one of the major constraints of sorghum production globally and particularly so in Eastern Africa. This study aimed at transferring five *Striga* resistances Quantitative Trait Loci (QTL) located on linkage groups SBI-01, SBI-02, SBI-05 and SBI-06 from a genetically mapped donor source line N13 into a locally adapted farmer preferred variety, IS8193 using Simple Sequence Repeats (SSRs). Nine polymorphic SSR markers were used to identify F_1 generations and the subsequent BC_1F_1 progenies carrying *Striga* resistance QTL. Sixteen F1 progenies and twelve BC_1F_1 were found to have incorporated one to three *Striga* resistances QTL. The twelve BC_1F_1 lines with *Striga* resistance QTL were subsequently backcrossed to IS8193 to produce BC_2F_1 generation for further fore-ground and back-ground selection in the future. This work was conducted during March 2010 to August 2011 at University of Nairobi and at Biosciences eastern and central Africa (BecA)-Nairobi Kenya.

Keywords - Molecular markers, Quantitative Trait Loci, Simple Sequence Repeat (SSR) Markers, *Striga hermonthica*

1. Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is one of the most important cereal crops globally after wheat, maize, rice, barley and has an important role in the food and fodder security for millions of rural families in arid and semi-arid regions of the world [1]. Sorghum is consumed both as whole grain and in form of flour and is also used to make beer in the local brewing industry in Rwanda. In addition to being a food security crop, sorghum is a source of income for the small-holder farm households [2]. Despite its great role, sorghum is affected by many biotic and abiotic factors [3]. Among these constraints, the parasitic weed, *Striga hermonthica* is a major biotic limitation to sorghum production [4]. In Rwanda, sorghum production is affected by *Striga hermonthica* mostly in the Eastern low altitude zones. *Striga* presents a particular threat to production since the parasitic plant kills the plant before it emerges. The parasite evokes different plant responses depending on its life cycle biology that results in various defence mechanisms. Furthermore, a flowering *Striga* plant produces numerous seeds that last for long periods in the soil even under harsh environmental conditions [5]. *Striga hermonthica* infection can cause yield losses of 10% up to complete crop failure. All these factors make it difficult to control this parasitic plant. Nearly 30 million people are affected by *Striga* in Sub-Saharan Africa and up to 50 million hectares of area under crops show varying degree of infestation of *Striga* in the continent [5, 6].

In the infected region of Rwanda controlling *Striga* through some of the conventional methods such as hand-weeding, has not been possible. As shown by studies in other crops, one most strategic ways of controlling *Striga* is to develop *Striga*-resistant varieties through a combination of conventional breeding and Marker Assisted Breeding (MAB) [7, 8]. In this study, *Striga* resistance Quantitative Trait Loci (QTL) was introgressed from a characterized resistant donor sorghum line, N13 into an adapted Rwandan sorghum variety, IS8193 through MAB. Markers associated with *Striga* resistance have been identified by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Equally the five genomic regions controlling *Striga* resistance in the resistant line, N13 were identified from a range of ten field trials conducted in Mali and Kenya in two

consecutive years by Haussmann et al. 2004. The data also indicated that the *Striga* resistance QTL is stable. These QTL identified on SBI-01, SBI-02, SBI-05 and SBI-06 genomic regions [9] are the ones that were targeted for selection with eleven SSR markers in the present study.

2. Materials and Methods

2.1. F_1, BC_1F_1 and BC_2F_1 Generations

The seeds of donor line N13 and of the recipient farmer preferred IS8193 line were sown in greenhouse at University of Nairobi in March 2010 and 14 days after germination leaf samples were collected and conserved in 70% ethanol for DNA extraction. At flowering stage, IS8193 was hand emasculated and fertilized with pollen from N13. The resulting F_1 seeds were sown in greenhouse, and again 14 days old leaf samples were harvested for DNA extraction and genotyping. The selected F_1 individuals carrying *Striga* resistance QTL were again hand emasculated at flowering stage and backcrossed to IS8193 to produce BC_1F_1 progenies. The BC_1F_1 seeds were sown and again 14 days old leaf samples were collected for DNA extraction and genotyping for *Striga* resistance QTL. The selected BC_1F_1 progenies were finally back-crossed to IS8193 to obtain BC_2F_1 progenies.

2.2. DNA Extraction

Individual leaf samples were collected and the genomic DNA samples were extracted from 14 days old sorghum leaves following the protocol described by Mace et al. (2004) [10] with minor modification. The leaf samples were cut into small pieces and placed in 12×8-wells strip tubes containing two stainless steel grinding balls. A volume of 450 µl of preheated extraction buffer containing CTAB (3%, w/v), 1.4M NaCl, 0.2 % (v/v) β-Mercapto-ethanol and 20 mM EDTA was added to each of the samples and then ground using the Geno-grinder. The samples' homogenates were incubated at 65 °C for 30 min with occasional mixing and then advanced to a solvent extraction step using 450 µl of chloroform: isoamylalcohol (24:1) to each sample and mixed twice by inversion. The tubes were then centrifuged at 4500 rpm for 20 minutes at 24°C and the upper aqueous layer was transferred into fresh tubes. To precipitate the crude DNA pellet, 0.7 volumes of iso-propanol (stored at -20°C) were added and inverted once to mix and then centrifuged at 4500 rpm for 35 minutes after 30 minutes of incubation at -20 °C. The supernatant was decanted and the pellet air dried for 30 minutes. Then 200 µl low salt TE buffer (1mM Tris and 0.1mM EDTA [PH 8]) with 3 µl RNase A (10mg/ml) were added to each sample and incubated at 37°C for 30 min. After that, 200 µl of chloroform: isoamylalcohol (24:1) was added to each tube and inverted twice to mix and centrifuged at 4000 rpm for 15min and then an upper solution was transferred to fresh tubes. To purify the DNA, 315 µl ethanol and 1/10 volume of 3M sodium acetate solution (PH 5.2) were added to each sample then placed in -20°C for 30 minutes to allow for precipitation which was followed by centrifugation at 3800 rpm for 20 min. After decantation and pellet air dried, 200 µl of 70% ethanol were added and centrifuged at 4500 rpm for 5min in order to wash the DNA pellet. Finally the decanted supernatant was air dried and then resuspended in 100 µl low salt TE (10mM Tris, 1mM EDTA [PH 8]) buffer and stored at 4°C. After DNA extraction the quality and the quantity of extracted DNA was determined by 0.8% (w/v) agarose gel electrophoresis stained with GelRed(5 µl 100 µl-1) and a Nanodrop (Thermo Fisher Scientific) spectrophotometer.

2.3. PCR Optimization

Forward and reverse SSRs primers labeled with fluorescent dyes (6-FAM, NED, PET, VIC) were used for PCR amplification and separated with a capillary electrophoresis method. The PCR components of 10µl reaction contained 2mM $MgCl_2$, 1x $MgCl_2$-free PCR buffer, 0.04 mM of each of the four dNTPs, 0.2 U of Amplitaq Gold DNA polymerase, 0.04 µM of M13-tagged forward primer, 0.16 µM fluorescent-labelled M13 forward primer (6-FAM, VIC, PET or NED), 0.20 µM of labelled reverse primer and 30 ng of template DNA. A 10µl final volume was obtained by adding distilled water. The amplification was carried out using the GeneAmp PCR systems 9600 (PE-Applied Biosystems) under the following conditions: 15 min at 94 ℃ followed by 40cycles of 30 seconds at 94 ℃, 1 min at 50 ℃ and 2 min at 72 ℃, with a final extension of 20 min at 72 ℃. After PCR reaction, 4 µl of samples selected randomly from each primer were mixed with 2µl lodging dye and then run in a 2% agarose gel (made from 2g of agarose dissolved in 100 ml of TAE or TBE) and stained with 2.5µl of GelRed to examine the success of the amplification.

2.4. Determination of Allele Sizes Using Capillary Electrophoresis

Genotyping was carried out using an ABI 3730 and 3130 DNA Sequencers (Applied Biosystems). PCR products were co-loaded after PCR and they were based on dye label, fragment size and fluorescence to reduce the unit cost of genotyping. To each PCR product, a volume of 8µl from a mixture composing GeneScan Liz 500 internal molecular weight size standard (orange) (Applied Biosystems) and Hi-Di formamide in the ratio of 1/50 and 49/50 respectively was added. Denaturation was done using the GeneAmp PCR systems 9600 (PE-Applied Biosystems) for 5min at 95 ℃ and cooling immediately on ice.

2.5. Data Analysis

The electropherogram peaks were sized and the alleles scored using the Gene-Mapper version 4.0 software. The scoring of the true allele sizes of the parental lines, the F_1s and the BC_1F_1s were scored on the basis of the heterozygosity between the two parents. For every SSR marker, the DNA of two parental lines was included with either that of F_1s or of BC_1F_1s samples as a positive control.

3. Results

3.1. DNA Quality and Quantity Determination

The quantity of the DNA extracted was ranging between 22.84ng/µl and 745.15ng/µl. Concerning the quality, two hundred eighty seven out of three hundred and seven samples extracted DNA including the parents had the ratio of absorbance (260nm/280nm) ranging between 1.8 and 2;

while fifteen had the ratio of absorbance below 1.8 and five had the ratio of absorbance above 2.

3.2. SSR Foreground Markers Screening for Polymorphism

Eleven markers were screened for polymorphism between the two parents N13 and IS8193. The following are the markers used and their linkage groups respectively; xtxp208 and xtxp302 linked to SBI-01 QTL (QTL1), xtxp50, xtxp304, xtxp201 linked to SBI-02 QTL (QTL2), xtxp65 and xtxp303 linked to the first QTL of SBI-05 (QTL3), xtxp225 and xtxp15 tightly linked to the second QTL of SBI-05 (QTL4) and xtxp145 and xtxp57 linked to SBI-06 QTL (QTL5). After the PCR and separation of amp icons by capillary electrophoresis, ten out of eleven markers were polymorphic as indicated by Table 1.

Table 1. Simple Sequence Repeat markers used in screening for *Striga* QTL polymorphism among parents

Marker	IS8193	N13	Linkage Groups
xtxp208	260	257	SBI-01
xtxp302	237	246	SBI-01
xtxp50	314	312	SBI-02
xtxp304	301	209	SBI-02
xtxp201	178	181	SBI-02
xtxp65	146	148	SBI-05
xtxp303	151	163	SBI-05
xtxp225	184	188	SBI-05
xtxp15	-	-	SBI-05
xtxp145	262	234	SBI-06
xtxp57	260	266	SBI-06

3.3. Foreground Selection for F_1s Generation

Out of twenty F_1 progenies screened, sixteen had from one up to three *Striga* resistance QTL introgressed. As shown by Table 2, three genotypes, namely C13P1, C19P6, and C5P14 were heterozygous for three QTL while eight genotypes namely, C16P1, C16P4, C19P1, C19P10, C19P5, C21P3, C5P17 and C5P22 were heterozygous for two QTL. Furthermore, five genotypes namely, C13P3, C13P8, C16P9, C25P6 and C9P2 were heterozygous for only one QTL.

Table 2.F_1 progenies showing heterozygozity at SBI-1, SBI-2, SBI-5 and SBI-6 linkage groups

Sample name	SBI-01 (QTL1)				SBI-02 (QTL2)						SBI-05 (QTL3)				SBI-06 (QTL5)			
	xtxp208		xtxp302		xtxp50		xtxp304		xtxp201		xtxp65		xtxp303		xtxp145		xtxp57	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
C13P1	252	255	235	241	312	314	209	301	178	181	146	148	-	-	234	262	259	264
C13P3	252	255	235	241	312	314	209	301	178	-	146	148	-	-	234	-	259	264
C13P8	252	-	235	241	312	314	209	301	178	-	146	148	151	163	-	-	-	-
C16P1	252	255	235	241	312	314	209	301	178	-	-	-	-	-	234	262	259	264
C16P4	-	-	-	-	-	-	-	-	-	-	146	148	151	163	234	262	259	264
C16P9	252	-	235	241	312	314	209	301	178	-	146	148	-	-	234	262	259	264
C19P1	252	255	235	241	312	314	209	301	-	-	146	148	-	-	234	262	259	264
C19P10	252	-	235	241	312	314	209	301	178	181	146	148	151	163	234	-	259	264
C19P5	252	255	235	241	312	-	209	301	178	181	-	-	-	-	234	262	259	264
C19P6	252	255	235	241	312	314	209	301	178	-	146	148	151	163	234	262	259	264
C21P3	252	-	235	241	312	314	209	301	178	181	146	148	-	-	234	262	259	264
C25P6	252	-	235	241	312	314	209	301	178	181	-	-	-	-	234	262	-	-
C5P14	252	255	235	241	312	314	209	301	178	181	-	-	-	-	234	262	259	264
C5P17	252	255	235	241	312	314	209	301	178	-	146	148	-	-	234	262	259	264
C5P22	252	-	235	241	312	314	209	301	-	-	146	148	151	163	234	262	259	264
C9P2	252	255	235	241	312	314	209	301	178	-	146	148	-	-	-	-	259	264

3.4. Foreground Genotyping of BC_1F_1 Progenies

A total of one hundred fifty nine BC_1F_1 genotypes were screened and out of them twelve lines were found to contain one to three *Striga* resistance QTL. The twelve genotypes were from three lines C13P1, C19P6 and C5P17 as presented by Table 3.4.1. One genotype, namely BC13C1P8 was heterozygous for three QTL while height genotypes namely, BC13C1P2, BC13C1P3, BC13C1P6, BC19C6P3, BC5C17P1, BC5C17P2, BC5C17P3 and BC5C17P5 were heterozygous for two QTL. In addition, three genotypes, namely BC13C1P1, BC13C1P4 and BC13C1P5 were heterozygous for one QTL.

Table 3.BC1F1 progenies heterozygous at SBI-1, SBI-2, SBI-5 and SBI-6 linkage groups

Sample name	SBI-01 (QTL1)				SBI-02 (QTL2)				SBI-05 (QTL3)				SBI-06 (QTL5)			
	xtxp208		xtxp302		xtxp50		xtxp201		xtxp65		xtxp303		xtxp145		xtxp57	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
BC13C1P1	246	-	-	235	306	-	-	181	143	146	151	163	234	-	-	254
BC13C1P2	246	249	229	235	-	308	-	181	143	146	151	163	234	262	-	254
BC13C1P3	246	249	229	235	306	308	175	181	143	146	-	163	234	-	-	254
BC13C1P4	246	249	229	235	-	-	175	181	-	-	-	-	-	-	-	-
BC13C1P5	246	249	229	235	-	-	175	181	-	146	-	163	234	-	-	254
BC13C1P6	246	-	229	235	-	308	-	181	143	146	151	163	234	262	248	254
BC13C1P8	246	249	229	235	306	-	175	181	143	146	151	163	234	262	248	254
BC19C6P3	246	249	229	235	-	-	175	181	-	146	151	-	234	262	248	254
BC5C17P1	246	249	229	235	-	308	-	181	143	146	151	163	234	262	-	254
BC5C17P2	246	249	229	235	-	-	175	181	143	146	151	163	-	262	-	-
BC5C17P3	246	249	229	235	-	-	175	181	-	146	-	163	234	262	248	254
BC5C17P5	246	249	229	235	306	308	175	181	-	146	151	163	234	-	248	-

4. Discussion

The small quantity of extracted DNA was enough to run fifty PCR reactions using eleven foreground markers as one PCR reaction required 0.03µg. As has been reported by other workers [7, 8, 11] SSR markers require very little DNA unlike other types of molecular markers. In addition, the quality of extracted DNA was as high as 93% of the samples had the ratio of absorbance (260nm/280nm) ranging between 1.8 and 2, about 5% of the samples had the ratio of absorbance (260nm/280nm) below 1.8 and 2% had the ratio of absorbance (260nm/280nm) above 2.

In conducting parental foreground selection, one should select those polymorphic markers that are tightly linked to the *Striga* resistance QTL. In this case, polymorphism should be determined by the differences in allele sizes among the parents after allele scoring [12]. In the results obtained here, about 90.9 % of the screened markers were polymorphic indicating that they could be used to select the *Striga* resistance QTL. SSR markers in this study were effective in detecting polymorphism as has also been reported by Korzun (2003).

In the F_1 generation, nine polymorphic markers were used to select the four *Striga* resistances QTL located on SBI-01, SBI-02, SBI-05 and SBI-06 linkage groups. Out of twenty F_1 samples, sixteen were heterozygous for the tightly linked markers in SBI-01, SBI-02, SBI-05 and SBI-06 linkage groups. Three F_1 samples exhibited heterozygous alleles for three QTL in SBI-01, SBI-02, SBI-05 and SBI-06 linkage groups. Eight F_1 lines were heterozygous for two QTL on SBI-01, SBI-02, SBI-05 and SBI-06 linkage groups. Also five F_1 progenies were heterozygous for one QTL located on SBI-01, SBI-02, SBI-05 and SBI-06 linkage groups. In his study, Gitau et al. (2008) [13] reported forty six BC_1F_1 genotypes carrying one or two QTL for *Striga* resistance in sorghum using three foreground primers and based on poly-acrylamide gel electrophoresis analysis.

Also in BC_1F_1 generation, polymorphic markers were used to identify progenies carrying *Striga* resistance QTL. Twelve lines with one to three *Striga* resistances QTL were found on SBI-01, SBI-02, SBI-05 and SBI-06. Three samples were heterozygous for one QTL located on SBI-01 and SBI-05 linkage groups. Introducing even a single QTL in plant breeding using MAB can be beneficial in terms of its contribution to the phenotypic variance of the trait [12]. Eight samples showed heterozygous alleles for two QTL in SBI-01, SBI-02, SBI-05 and SBI-06 linkage groups. One sample exhibited heterozygous alleles for three QTL in SBI-01, SBI-05 and SBI-06 linkage groups.

5. Conclusion

The study demonstrated that one to up to three *Striga* resistances QTL were introgressed successfully in the IS8193 sorghum variety grown in Rwanda. Needless to say that more advanced backcrosses, preferably up to BC_4F_1, are needed in order to stabilize the *Striga* resistance QTL. With the realization of BC_4F_1 *Striga* resistant lines, it will be possible to produce BC_4S_1 seeds for distribution to farmers in Rwanda and this will subsequently lead to increased smallholder farmers' income.

6. 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), Biosciences eastern and central Africa (BecA) and the University of Nairobi (UoN)

for providing financial assistance for this work. The first author would wish also to thank Rwanda Agriculture Board for choosing him as a candidate to implement this project.

References

[1] G. Srinivas, K. Satish, R. Madhusudhana, R. Nagaraja Reddy, S. Murali Mohan, and N. Seetharama: Identification of quantitative trait loci for agronomically important traits and their association with genetic-microsatellites markers in sorghum. *Theoretical and Application Genetics*, 118, 1439-1454. 2009.

[2] ISAR: Sorghum program. Retrieved from http://ww.isar.rw/spip.php?article15 accessed June 11, 2011.

[3] J. M. Poehlman: Breeding Field Crops. 3rd ed. AVI Pub Co., Westport, Connecticut. 1987.

[4] J. Sauerborn: The economic importance of the phytoparasites Orobanche and Striga. In: J. K. Ransom et al. (ed.) Fifth International Symposium of Parasitic Weeds. CIMMYT, Nairobi, Kenya, pp. 137-143. 1991.

[5] G. Ejeta: The Striga scourge in Africa: A growing pandemic, pp. 1-16. In Ejeta and Gressel (eds). Integrating New Technologies for Striga Control: Towards Ending the Witch-hunt, World Scientific Press Co. Ltd., Singapore. 2007b.

[6] G. Ejeta: Breeding for Striga resistance in Sorghum: Exploitation of intricate host-parasite biology. *Crop Science* 47: S216-227. 2007a.

[7] K. Semagn, A. Bjornstad, and M. N. Ndijiondjop: Progress and prospects of marker assisted backcrossing as a tool in crop breeding programs. *African Journal of Biotechnogy*, 5, 2588-2603. 2006a.

[8] K. Semagn, A. Bjornstad, and M. N. Ndijiondjop: An overview of molecular marker methods for plants. *African Journal of Biotechnogy*, 5, 2540-2568. 2006b.

[9] B. I. G. Haussmann, D. E. Hess, G. O. Omanya, R. T. Folkertsma, B. V. S. Reddy, M. Kayentao, H. G Welz, and H. H. Geiger: Genomic regions influencing resistance to the parasitic weed Striga hermonthica in two recombinant inbred populations of sorghum. *Theoretical and Applied Genetics* 109: 1005-1019. 2004.

[10] E. S. Mace, K. B. Kutokshi, K. B. Hutoshki, and J. H. Crouch: A high-throughput DNA markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 142: 1, 169-196. 2004.

[11] V. Korzun: Molecular markers and their application in cereals breeding [online]. In: Marker Assisted Selection: A fast track to increase genetic gain in plant and animal breeding session I: MAS in plants. Available from: http://www.fao.org/biotech/docs/Korzun.pdf. Date accessed: 25 June 2011. 2003.

[12] B. C. Y. Collard, M. Z. Z. Jahufer, J. B Brouwer, and E. C. K. Pang: An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, 142, 169–196. 2005.

[13] Z. K. Gitau, E. C. Ngugi, R. W. Musyoki, and J. G. Gethi: Arresting the scourge of Striga in Sorghum by combining the strengths of Marker-Assisted Backcrossing and farmer participatory selection. In: proceedings of the 10th KARI Biennial Scientific Conference. Nairobi-Kenya. 2008.