



Research Article

Introgression of *Striga* Resistance Genes into a Sudanese Sorghum Cultivar, Tabat, Using Marker Assisted Selection (MAS)

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ABSTRACT

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Resistant crop cultivars provide the most economical, practical and sustainable method of *Striga* control. However, the development of resistant sorghum cultivars slowed by the complexity of the environment and the host/parasite interactions, which made field resistance erratic and unreliable. Recent adventure of molecular markers to tag gene(s) that confer important traits offers new hope for *Striga* control. Significant progress has been made to identify molecular markers linked to *Striga* resistance in sorghum variety, N13. Five genomic regions (QTLs) associated with *Striga* resistance were identified. Flanking simple sequence repeats (SSRs) markers to each QTL were also identified and made available.

The aim of this study was to transfer *Striga* resistance from, N13 to an elite farmer preferred sorghum cultivar, Tabat. Generations of F1, BC1F1, BC1S1 and BC2F1 populations were developed. F1 and BC1F1 generations were first genotyped and progenies with two or more QTLs were backcrossed to generate BC1F1 and BC2F1, respectively. BC2F1 were further genotyped and progenies with two different QTLs were intercrossed for foreground selection. BC2F1 progenies were selfed to generate BC2S1, BC2S2 population. 19 progenies, BC1S1, with *Striga* resistance QTLs were tested in *Striga* artificial infested plots. Progenies with the two or more QTLs showed high levels of *Striga* field resistance, confirming the effectiveness of marker assisted selection (MAS). Significant differences were observed among the progeny tested in the level of *Striga* resistance and other agronomical traits.

INTRODUCTION

Sorghum [*Sorghum bicolor* (L) Moench, $2n = 20$] is one of the most important cereal crops and well adapted to harsh environments characterized by drought and high temperatures. It was first domesticated in the region of North East Africa (Doggett, 1988).

The witch-weed (*Striga spp.*), an obligate root parasite of various crops, is the major constrain to sorghum production in rain-fed areas of semi-arid tropics (Ejeta, 1992). The parasite result a considerable crop damages, especially in the semi arid-tropic of the world (Rich, et al., 2004). *Striga* causes severe reduction to the host crops. Losses in crop yield due to *Striga* infestation are estimated in millions of tones annually (Olivier, 1995). In Sorghum yield, losses may reach 100% on heavily infested soils (Parker and Riches, 1993).

The parasite control is difficult, but it is not impossible. In the last few decades, efforts have been devoted to develop methods for *Striga* control. The most promising way for controlling the parasite resides on the development of resistant cultivars (Ejeta, 1991). However, breeding efforts were hampered by the lack of knowledge for the genetics of resistance and lack of reliable and rapid screening methods (Ejeta, 1992). Screening for *Striga* resistance in the field has failed and slowed due to the quantitative nature of the trait and the influence of the environment. Screening genotypes in *Striga* infested fields is often inefficient due to the host complexity, parasite and environment interactions (Ejeta 1992). Integrated *Striga* management approach was also suggested by Ramiah (1987). He suggested the use of resistant lines, in combination with cultural practices and chemical inputs. The integrated management will not only provide effective *Striga* control but also suppresses development of virulent strain of the parasite and reduces the build up of seeds in the soil seed bank (Ejeta, et al., 1991).

Molecular Markers or genetic markers are DNA sequences associated with certain parts of the genome and they have well defined phenotypes. They can also be defined as, DNA pieces that flag the presence or absence of particular traits (Paterson, 1996). Considering their abundance, molecular markers are powerful tools that speed and increase the precision and the effectiveness of plant breeding (Paterson, 1996). Molecular markers allow selection for traits on basis of a simple laboratory tests on a small plant tissue, rather than direct measurements of the character itself. Molecular makers also showed great potential in selection and manipulation of genotypes for desirable quantitative traits loci (QTLs) that condition complex economic traits (Ejeta, et al., 2000).

Marker assisted selection (MAS) is defined as the process of using the results of DNA testing to assist in the selection of desirable individuals, for specific trait, to become the parents for the next generation (Eenennaam, 2004). Moreover, the

development of molecular markers for genetic analysis expanded the circle of knowledge of genetics and understanding of the structure and behavior of organism genomes (Korzun, 2005). To date, most of modern crop varieties are developed through marker assisted breeding and genetic engineering (Ejeta, 1992). In the last few decades, rapid progress has been made in molecular techniques and great amount of polymorphic markers are generated, sufficient enough to build dense linkage maps (Korzun, 2005). Significant progress has been made in identifying markers and quantitative trait loci (QTLs) associated with *Striga* resistance. Three major QTLs that associate with *Striga* resistance were identified in sorghum cultivar, N13.

The objective of this study was to transfer *Striga* resistance from N13 into farmer preferred sorghum cultivar 'Tabat' using the strength of marker assisted selection and to verify the effectiveness of the marker assisted selection as a breeding tool and generation of elite lines for future use.

MATERIAL AND METHODS

Germplasm

The *Striga* susceptible sorghum cultivar 'Tabat' was selected as recipient parent and *Striga* resistance sorghum variety, N-13, as donor parent. Backcrossing method of breeding was used. Achieving effective backcrossing and optimum population size; 8 F₁s (Four of them were reciprocal cross), 168 BC₁F₁, 168 BC₂F₁, BC₂S₁ progenies were generated (Fig.2). Confirming the effectiveness of marker assisted breeding 19 Tabat BC₁S₁ lines carrying different QTLs were field tested for foreground and background selection.

Mapping Of *Striga* Resistance Genes

This study was based on a published sorghum genetic linkage map constructed by Haussmann, et al. (2001b) (Fig.1).

Genotyping

The genotyping work was carried out at Biosciences for Eastern Central Africa (BecA) molecular biology laboratory located at the International Livestock's Research Institute (ILRI), Nairobi. DNA extraction method was used, as described by Mace et al., (2004).

Genotyping process which can be defined as laboratory method used to detect and amplify a known polymorphic DNA nucleotides sequence, marker, in the generated population individuals. The parents; N-13, and Tabat and his F₁s, BC₁ and BC₂, derived progenies were screened with 11 SSRs markers tpx201, tpx15, tpx225, tpx65, tpx302, tpx303, tpx145, Xtpx57, tpx208, tpx50 and tpx304, that have close association with *Striga* resistance regions QTLs. SSRs amplification was performed according to the method

described by Haussmann, et al., 2001. Then the amplified samples (PCR products) were prepared with formamide Hidi and 500liz standard size, 1000 μ l to 12 μ l respectively, of which 0.7 μ l of the amplified DNA transferred to the sequencer. Scoring of peaks achieved using gene-mapper v.4.0 software program.

Bc1s1 Field Testing

Selected BC1S1 (19 lines) derived progenies were screened under *Striga* artificially infested plot at ARC, Gezira Research Station. The plot artificially infested as follows: viable *Striga* seeds mixed with soil (1g of *Striga* seeds/ 1kg soil). The mixture evenly distributed in holes on top of 80 cm ridges (1g soil seed mixture/ m²). Few days following the artificial infestation, sorghum was planted inter-row spacing 1/10cm. Number of emerged *Striga* plant was evaluated 45 days after crop emergence. The progenies evaluated for days to 50% flowering, plant height and weight of 100 seed. Randomized complete block design (RCBD) with three replicates was used.

RESULTS

Introgression of Resistance Genes

The presence of the regions, QTLs, in association with *Striga* resistance is detected through the presence or absence of the associated polymorphic flanking markers. Eight F1s (Tabat x N-13) progenies were generated then 168 BC1F1 progenies were generated and screened with 10 SSRs polymorphic markers. The screened progenies, 145, showed the absence of regions, QTLs, associated *Striga* resistance. Which distributed as, 52 showed no reaction with primers, 93 progenies showed the reaction but the absence of polymorphisms in the flanking markers. 23 progenies showed the presence of one or more of the three major regions, QTLs, linked to *Striga* resistance. Indicating the presence of at least one region associated with *Striga* resistance in these progenies. 18 of the progenies showed the presence of the QTLs, flanking markers in heterozygous status. Only 5 progenies showed the presence of the QTLs, polymorphic flanking markers, as homozygous status.

Progenies of BC2F1 with the target regions, QTLs, were advanced to generate BC1F1. 168 derived progenies, BC2F1, were generated. Progenies were screened with 7 SSRs polymorphic markers to detect the presence of *Striga* resistance regions, QTLs. 115, showed the presence of the major three QTLs, in close association with *Striga* resistance, which distributed as; 36 progenies showed the presence of QTLs, polymorphic flanking markers, as heterozygote and 79 progenies showed the presence of, QTLs, polymorphic flanking markers, as homozygous status, indicating the presence of at least one region associated with *Striga* resistance in these progenies. 53 progenies showed the absence of these regions, QTLs.

Foreground Selection

The recurrent susceptible parent, Tabat and 19 BC1S1 derived lines (Table 1) selected from the genotyping results carrying different type of resistance (different letters), four parameters were majored; 1) *Striga* count 2) plant height 3) 50% Flowering and; 4) 100 seed weight.

Field testing results showed significant differences in maturity among BC1S1 progenies and the susceptible parent Tabat. However, no significant differences were observed between progenies BC1S1-144-D and BC1S1-127-R (Table 1). The least significant differences (LSD) at P=0.05 was used to separate means.

Striga Count: No significant differences were observed between the susceptible progeny BC1S1-127-R and Tabat (two controls), and BC1S1-121-H, BC1S1-62-D, BC1S1-96-H, BC1S1-75-D and BC1S1-94-H. Significant differences were observed for *Striga* count among these progenies, BC1S1-107-H, BC1S1-67-H, BC1S1-110-H, BC1S1-16-H, BC1S1-24-H, BC1S1-144-D, BC1S1-138-D, BC1S1-55-H, BC1S1-52-H, BC1S1-19-D, BC1S1-63-H, BC1S1-122-H and BC1S1-2-D, (Table 1). The least significant differences (LSD) at P=0.05 was used to separate means.

Plant Height (cm): The results showed significant differences in all selected progenies (treatments), comparing with the susceptible checks, Tabat and BC1S1-127-R (Table 2). The least significant differences (LSD) at P=0.05 was used to separate means.

Weight of 100-Seed (g): results of the hundred-seed weight showed no differences among the BC1S1-62-D, BC1S1-196-H, BC1S1-144-D, BC1S1-19-D, BC1S1-63-H and BC1S1-122-H progenies. However, progenies, BC1S1-121-H, BC1S1-107-H, BC1S1-67-H, BC1S1-110-H, BC1S1-16-H, BC1S1-75-D, BC1S1-24-H, BC1S1-138-D, BC1S1-55-H, BC1S1-52-H, BC1S1-94-H and BC1S1-2-D, showed significant differences comparing with the susceptible checks (Table 1). The least significant differences (LSD) at P=0.05 was used to separate means.

DISCUSSIONS

Breeding for resistance in sorghum is recognized as the most feasible method for *Striga* control (Ejeta, 1992). However, developing crop cultivars with durable *Striga* resistance is slowed for the lack of a reliable screening method. Field screening is affected by many confounding environmental factors. In this era of biological sciences efforts were made to track important gene(s) that govern important traits. Genetic linkage maps were constructed; molecular markers and QTLs were placed on these maps for most crops. In sorghum, great efforts were made to construct consensus linkage maps, as well as to identify molecular markers and QTLs with close association to *Striga* resistance genes. These markers and QTLs will not only ease the screening for *Striga* resistance, but also help tracking important *Striga* resistance genes.

Indeed, in this study 11 markers were used for foreground selection. SSRs alleles were scored for

absence and presence to detect *Striga* resistance QTLs. Each QTL region should be flanked at least by two markers to confirm its presence. Consistent with the literature that each chromosome should be checked with two to three markers and that would be efficient to control the confidence intervals and minimize the risk of losing the target alleles QTLs (Hospital and Charcosset, 1997).

The backcrossing method is generally used in plant breeding programs to transfer target allele(s) from a donor genotype, which mostly possesses undesirable and poor agronomic traits, into preferred genotypes (Allard, 1960). The method drawbacks, some of the donor parent inferior trait alleles transfer with the target alleles. The invention of marker assisted selection increased the precision of the backcrossing method to transfer certain alleles and reduce the probability of transferring the untargeted trait alleles.

To reduce the cost, F1 generation was not genotyped; the seed color of the hybrid was used as a physiological marker to detect F1 plant generation (spreader gene). 168 BC1F1 and BC2F1 progenies were developed for detecting the presence of the major *Striga* resistance QTLs in these derived progenies. This population size corresponding with Hospital and Charcosset (1997) suggested population size. They stated that, introgression of three QTLs carrying chromosomal segments has proved to be possible by marker assisted selection with reasonable population size. Backcrossing scheme advanced to BC2, which recover only 87.5% of recurrent parent genome. This is not recovering most of recurrent parent genome but also reduce the chance of losing the maker in the recombination process in each generation, considering the large confidence interval in our mapping population. This is consistent with marker assisted background selection that achieved on BC2 and BC3 progenies by Bouchez et al. (2002).

Introgressing *Striga* resistance genes (QTLs) into Tabat BC1F1 derived progenies showed 20 progenies with the resistance one or more QTLs, which vary from 18 hybrids (heterozygous) five all donor parent QTLs and no QTLs were in 94 progenies. The few number of plant carrying all the target alleles might be due to crossing over, or might be associated with the based genetic map in which the flanking markers confidence interval vary from 25 to 40cm, this distance is long enough to allow passing the markers without the QTLs. Other studies stated that this reduction in expected frequencies over generations increased with the number of QTLs and the confidence interval between flanking markers. Tabat BC2F1 derived progenies results showed, 115 plants carrying *Striga* resistance QTLs, 79 progenies heterozygous QTLs, 36 donor resistant status QTLs. The QTLs are distributed over the A, B, I and J chromosome and 52 plants with no *Striga* resistance. This number is higher than the expected frequencies, it might also due to the above mentioned factors.

Reports suggested foreground selection is efficient for markers associated with three target regions without the help of phenotypic assays

(Bouchez, et al., 2002). At each backcross generation, the selection of the individuals that carry the allele of interest at the specific QTL is based on their genotype at neighboring markers(s) and then among these individuals one or several individuals carrying the most alleles of recipient type at markers are selected in the next step (Hospital and Charcosset, 1997). The information from genetic map can be used to identify, manipulate and complement traits to the maximum advantage (Paterson, 1996).

To confirm the effectiveness of marker assisted selection in introgressing of *Striga* resistance genes, 19 BC1S1 derived progenies were tested, under *Striga* artificial infestation, with the donor parent, Tabat, as a susceptible control. The susceptible control, Tabat, showed no flowering even after one hundred and twenty days from germination; it was wiped out by end of the season. The results confirmed the high susceptibility of the cultivar, Tabat, and high level of infestation in the artificially infested plot. BC1S1-127-R derived progeny, with no *Striga* resistance QTLs, showed levels of infestation comparable to that of Tabat. Consistent with early reports, sorghum yield losses may reach 100% on heavily infested soils (Parker and Riches, 1993). The results also indicated significant differences in 50% flowering in the other 17 progenies carrying different *Striga* resistance QTLs in homozygous and heterozygous form (D and H form of resistance). BC1S1-144-D derived progeny showed earliest 50% flowering value. It might be generally stated that high levels of *Striga* infestation, delay and/or inhibit genotypes flowering. Susceptible genotypes early flower, intermediate genotypes with delayed flowering and resistant genotypes normal flowering. Emerged *Striga* count is considered as one of the field measurements that determine host plant resistance. Our data indicated that the susceptible checks, Tabat and BC1S1-127-R, have the lowest number of the emerged *Striga* plants in the genotypes tested. This is mainly due to poor development of the two checks as result of *Striga* damage, where they were completely wiped out at the end of season. Genotypes that carry different genetic resistance (QTLs), showed different numbers of emerged *Striga* plants. Significant differences were observed in plant height among these genotypes; the susceptible ones are poorly developed and stunted, although genotypes with resistance have normal development and plant height. These results correspond with many previous studies concerning *Striga*-host interactions. The study also measuring the hundred seed weight (which considered as one of the yield component), the results (Table1) showed no seeds for the susceptible parent Tabat (highly affected with *Striga* damage), 6 of the tested lines showed no significant differences, nevertheless, 12 lines carrying different genetic types of resistance showed a significant difference.

Appearance of the lines carrying group 5 (chromosome J-2QTLs-) as considerable amount and showing field resistance, this can give more attention for this region. In contrast with the study in Kenyan

which minor genes showed more important with the Kenyan *Striga* seeds (Haussmann et al, 2001a).

The study confirmed the effectiveness of marker assisted selection (MAS) as screening for *Striga* resistance tool in sorghum. Tabat-derived progeny BC1S1-127-R (classified as susceptible by the genotyping data) showed the same level of susceptibility as the recurrent parent, Tabat. Tabat-derived progenies BC1S1-67-H, BC1S1-110-H and BC1S1-16-H (classified as resistance by the genotyping data) showed not only high *Striga* resistance levels but also superior agronomic performance.

The data indicated that the Heterozygous (H) status, showed effective transferring and expressing of *Striga* resistance genes as the homozygous (D), which confirm the dominant nature of *Striga* resistance in the donor parent, N13.

Field results showed stronger *Striga* resistance reactions in group 5 (2QTLs) than the other

QTLs groups. This indicates that marker assisted selection might also be reliably used not only to transfer QTLs regions but also might be used to weigh the contribution of each QTL to the trait of interest, even in a fairly saturated linkage map.

Striga field resistance is affected by many environmental factors as indicated by many reports. Our data also showed emerging *Striga* plant count to determine the level of *Striga* infestation in the field might be misleading unless it combined with other agronomic traits and yield components. This also showed the urgency to use MAS which is environment insensitive and for more precise screening to boost breeding for *Striga* resistance.

Pyramiding of *Striga* resistance genes into elite backgrounds is becoming possible through foreground and background selection in MAS breeding.

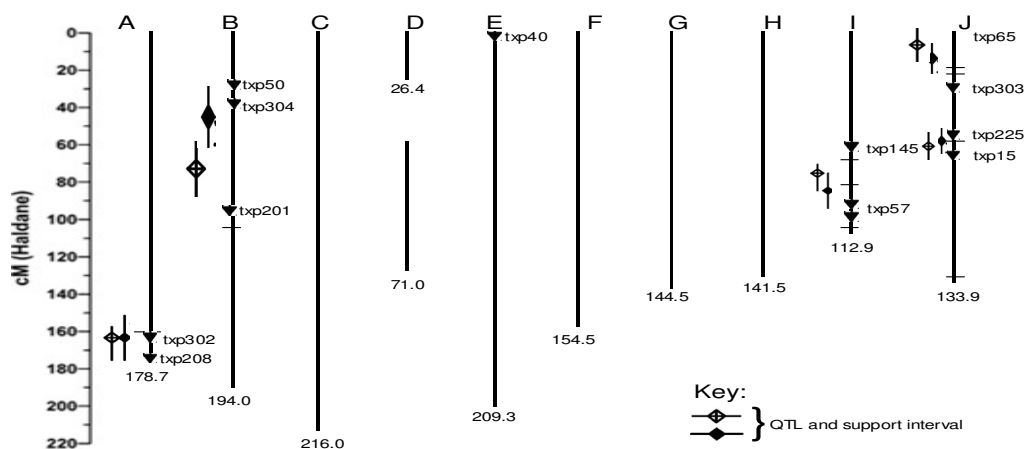


Figure (1) Major QTLs and SSR markers associated with *Striga* resistance in sorghum

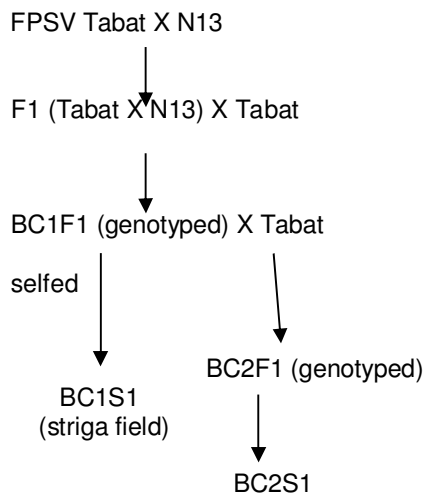


Figure (2) Crossing scheme:

Table (1) Tabat BC1S1 field result

Entries/treat	Days to 50% Flowering	<i>Striga</i> Count (plant/20m ²)	Plant Height (cm)	Weight of 100-Seed (g)
Tabat	0.0	58	52.1	0.00
BC1S1-127-R	0.00	4	49.3*	0.00
BC1S1-121-H	79*	84	109.0*	2.30*
BC1S1-62-D	77*	77	111.6*	1.07
BC1S1-107-H	74*	164*	70.4*	2.27*
BC1S-96-H	56*	85	74.8*	0.00
BC1S1-67-H	62*	214*	147.0*	3.60*
BC1S1-110-H	63*	250*	142.1*	3.00*
BC1S1-16-H	69*	132*	139.9*	2.87*
BC1S1-75-D	71*	72	112.8*	2.60*
BC1S1-24-H	46*	164*	103.9*	2.37*
BC1S1-144-D	0.0	94*	66.8*	0.00
BC1S1-138-D	78*	180*	81.5*	2.37*
BC1S1-55-H	74*	484*	127.7*	3.37*
BC1S1-52-H	77*	148*	111.4*	3.07*
BC1S1-19-D	79*	107*	92.2*	1.33
BC1S1-63-H	86*	202*	100.0*	0.77
BC1S1-94-H	75*	56	100.8*	2.60*
BC1S1-122-H	54*	100*	99.6*	1.30
BC1S1-2-D	83*	206*	98.7*	2.30*
LSD	14.36	81.40	20.41	0.56
C.V%	25.0	88.5	32.0	45.6
Overall Mean	62.6	143.7	99.6	1.9

* Significant at 0.05 probability level

Key:

D= Presence of the donor parent *Striga* resistance region in homozygous status, H= Presence of the donor parent *Striga* resistance regions in heterozygous status, R= Absence of the donor parent *Striga* resistance region

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