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SATURATION OF STRIGA RESISTANCE QTLS IN SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH)

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ABSTRACT

Sorghum bicolor (L.) Moench is the 5th most important cereal crop worldwide. The main biotic constraint to sorghum production is the parasitic weed *Striga hermonthica* (Del.) Benth. *Striga* is controlled using a combination of cultural, chemical and bio-control measures which cannot be afforded by farmers. A cost-effective alternative is to breed varieties resistant to *Striga*. Previous studies involving development of a sorghum genetic linkage map and mapping genomic regions contributing to *Striga* resistance have shown that resistance is a complex trait controlled by at least five QTLs. Genetic linkage maps provide an important genomic resource for understanding genome organization and evolution, and genetic basis of quantitative traits, and provide useful information for identifying and isolating genes responsible for a given phenotype. Therefore there is need for identification of molecular markers closely linked to these resistance QTLs to improve efficiency of marker-assisted selection (MAS) to accelerate development of *Striga*-resistant varieties. The aim of this study was to saturate genomic regions of *Striga* resistance QTLs using SSRs and DArT markers. QTL regions associated with *Striga* resistance were well saturated and confidence intervals for these QTLs were reduced: 27 SSR markers, two morphological markers, along with 175 DArTs markers were added to the previously mapped skeleton linkage map on linkage groups SBI-01, SBI-02, SBI-05a, SBI-05b and SBI-06 at intervals of 3-5 cM. Identified markers would be useful in marker-assisted selection for Introgression of this trait into susceptible sorghum cultivars. Addition of markers tightly linked to *Striga* resistance QTLs are not only advantageous for MAS application, but also assisted in saturating the sorghum linkage map.

Key word: SSR, DArT, Linkage map, Striga and Sorghum.

INTRODUCTION

In east and central Africa (ECA) Sorghum is ranked second after maize (*Zea mays* L.) as the most important cereal crop. It is generally used as food and feed grain, and has potential to be used as fuel source. Sorghum has a great potential to tolerate many biotic and abiotic stresses, making it as an ideal crop for marginal areas. With climate change scenario projections, sorghum remains a crop of hope to meet the food demands of the increasing human population, especially in sub-Saharan Africa. The true potential of sorghum can only be realized through concerted genetic improvement programs (Kim *et al.*, 2004; Varshney and Tuberosa, 2007). In sorghum molecular genetics maps have been developed and positions of various DNA markers have been reported (Patil *et al.*, 2010). Genetic linkage maps of sorghum harbouring RFLP markers (Xu *et al.*, 2000) et al., 2000), AFLP (Boivin *et al.*, 1999), SSR (Bhattramakki *et al.*, 2000), RAPD (Tuinstra *et al.*, 1996; Tuinstra *et al.*, 1997) and EST-SSR (Reddy *et al.*, 2008) markers have reported. The use of SSR markers for the genetic analysis and manipulation of important agronomic traits is becoming increasingly useful in sorghum improvement. Molecular markers have been used in sorghum to identify quantitative trait loci (QTL) for many complex traits, including resistance to the parasitic weed Striga. Five QTLs representing the genomic regions associated with stable Striga resistance in sorghum variety N13 (IS 40609) have been identified and mapped on linkage groups 1, 2, 5 (2 QTLs) and 6, using the revised linkage group designation proposed by Kim *et al.* (2004) with at least 30 cM confidence interval (Haussmann *et al.*, 2004). Identification of QTLs associated with Striga resistance and their subsequent transfer to elite backgrounds, has potential to reduce losses due to Striga and ultimately provides a solid foundation for improving Striga resistance. However, utilizing these QTLs had been limited until recently by the lack of a standard sorghum genetic map (Varshney and Tuberosa, 2007). This study aimed at marker saturation of sorghum genomic regions associated with Striga resistance QTLs, to improve precision of Marker Assisted Selection (MAS), to increase the speed of selection and to enhance the efficiency of resistance genes transfer.

MATERIALS AND METHODS:

Saturation of Striga resistance QTLs: Five previously identified QTLs for Striga resistance from the donor N13 (Haussmann *et al.*, 2004) were targeted. The Recombinant Inbred Line (RIL) population based on cross (N13 × E36-1) was genotyped with 169 SSR markers.

Mapping population: A RIL population consisting of 183 (F2:7) lines was used for this study. The population was developed from the cross of N13 (resistant) × E36-1 (susceptible), a single F1 plant was selfed and F2 plants

advanced by modified single-seed descent to F7 generation. Earlier generations of this population were used in the QTL mapping studies reported by Haussmann *et al.* (2004).

A high-throughput mini-DNA extraction protocol was followed for extraction of the DNA from parental lines and RIL population using a modified CTAB method (Mace *et al.*, 2003).

Mapping of EST-SSR markers: A set of 169 sorghum primer pairs, expected to detect EST-SSR loci distributed across regions of the sorghum nuclear genome were selected. The primer pairs were checked for amplification on six sorghum lines including E36-1 and N13. DNA samples from the parents, previously used to map host plant resistance to the parasitic weed Striga hermonthica (Haussmann *et al.*, 2004), were used as template for PCR amplification.

PCR components and Conditions: As ICRISAT lab protocols, PCR reactions were conducted in 96 well plates in PE 9700 Perkin DNA thermocycler. Reactions were set up in 5µl reaction volumes consisting of 1µl of 5ng DNA template, 0.25µl of 2 mM dNTPs, 0.5µl of (1 pmole/µl M13 tailed forward primer: 2 pmole/µl reverse) primer and 0.5µl of 2 pmole/µl of M13 labeled primer, 0.1 U (0.2µl of 5Uµl) of Taq DNA polymerase (SibEnymes, Russia), 0.5µl of 10X PCR buffer (Sib Enzymes, Russia), 0.25µl of 50 mM $MgCl_2$ (Sib Enzymes, Russia). In addition, fluorescent dye phosphoramidites, [6-FAM (blue), VIC (green), NED (yellow), or PET (red)] were used in the PCR reaction mixture for detection of the amplified product on an ABI 3700/3130 analyzer. The cycling conditions for PCR on a GeneAmp® PCR System 9700 (PE-Applied Biosystems) thermal cycler were optimized to initial denaturation of 15 min. at 94°C, followed by 10 cycles (touchdown) of 94°C for 15 sec, annealing touchdown temperature reducing from 61 to 51°C for 20 sec over 10 cycles, with extension at 72°C for 30 sec. This was followed by denaturation at 94°C for 10 sec, annealing at 54°C for 20 sec, and extension at 72°C for 30 sec for 34 cycles, followed by final extension of 20 min at 72°C to insure amplification to equal length for both DNA strands.

Confirmation of primers amplification: The PCR products together with a 100 base pair ladder were separated on 1.2% agarose gel containing 0.5µl /10ml ethidium bromide (10 mg/ml) by running at a constant voltage of 90V for 30 min. The amplified products were visualized under UV illumination using Uvi Tech gel documentation system (DOL-008.XD, England).

Electrophoresis/SSR fragment analysis: After confirming the PCR amplification on agarose gel electrophoresis, post-PCR multiplex sets were constructed based on the allele size range estimates and the type of forward primer label of the markers. Markers that had different labels and allele size ranges were considered for a set. For post-PCR multiplexing, 1.5µl PCR product of each of FAM, VIC, NED and PET-labeled products were pooled (according to above mentioned criteria) and mixed with 7µl of Hi-Di formamide (Applied Biosystems, USA), 0.25µl of the LIZ-500 size standard (Applied Biosystems, USA) and 1.5µl of distilled water. The pooled PCR amplicons were denatured and size fractioned using capillary electrophoresis on an ABI 3700 automatic DNA sequencer (Applied Biosystems, USA). This sequencer also requires the input text file of plate record containing details of the sample and markers used in each of the wells during PCR. The microsatellite loci that are amplified by PCR using fluorescently labeled forward and unlabeled reverse primers are separated by size using electrophoresis. Detection of specific alleles with respect to their known sizes present in electrophoretic data thus obtained was done using Genemapper® software version 4.0 (Applied Biosystems, USA). Data collection and analysis: The data was collected automatically by the detection of the different fluorescence and analyzed by Genemapper v4.0 software (Applied Biosystems). The scores of all polymorphic EST-SSR markers were converted into genotype codes ('A', 'B', 'H', 'O' and '_') according to the scores of the parents after genotyping of mapping populations with EST_SSR markers. Linkage analysis: Segregation data was used to place the new markers on the (N13 × E36-1) population linkage map. Linkage analysis was conducted with the Kosambi mapping function using the software application Mapmaker/EXP, ver.4.0 (Lincoln *et al.*, 1993), CentiMorgan (cM) distances were calculated and the markers were placed to the established linkage groups with the 'try' and 'compare' commands with a minimum LOD of 4.0 and a recombination frequency of 0.5 (Kosambi, 1944). Linkage map was drawn using MapChart 2.2. (Voorrips, 2002).

RESULTS

Genotyping of RIL population: Parental polymorphism between Striga resistant sorghum variety N13, and Striga susceptible variety E36-1 was established with 169 SSRs, using capillary electrophoresis followed by fluorescence detection. 69 (40%) SSR primers were monomorphic while 100 (59.2%) SSRs were polymorphic. The remaining primer pairs (15.7%) failed to produce an amplification product. For non-labeled SSRs Poly Acrylamide Gel Electrophoresis (PAGE) was used; most of the primer pairs gave the amplicon sizes in the range of 100-300bp.

Marker segregation: In this study, marker segregation patterns of the 100 selected polymorphic SSR markers in the RILs population were checked for fit to the expected Mendelian ratio (1:1). Calculated (χ^2) values at 1 degree of freedom were compared with tabulated value for each marker locus, 97 marker loci segregated in the expected 1:1 ratio. Markers with highly distorted segregation were removed from the data. The phenomenon of segregation distortion can be one of the limitations in map construction as it may affect both the establishment of linkage groups and estimation of recombination frequencies. Selected polymorphic SSR primer pairs (97) were screened against the template DNA of sorghum RILs (183). PCR products were checked for successful amplification on 1.2% agarose gel. Fluorescent-labeled products were separated using capillary electrophoresis and further analyzed with Genemapper 4.0

software, 63 (64.9%), where primer pairs showed reliably-scorable polymorphism. Among the polymorphic markers, 41 were mapped in the vicinity of previously detected Striga resistance QTLs. Genetic linkage map construction: The genotypic data generated on the population of 183 RILs with the 63 polymorphic SSR markers linked to Striga resistance QTLs, was integrated with previous genotypic data, thus providing a total of 277 markers: 100 SSRs, 175 DArTs and 2 morphological markers that were used to construct the genetic linkage map, using Mapmaker/EXP, ver.4.0 software. Seventeen SSR markers closely linked to Striga resistance were added to the QTL regions identified by (Haussmann *et al.*, 2004) (Fig. 1). The number of additional SSR marker loci mapped to each Striga resistance QTL region, ranged from 2 to 8 for QTL4 and QTL1, respectively (Table 1).

SBI	Length (cM)	LOD value	No. of SSRs	No. of DArTs	No. of morph.	Total no. of markers
1	256.6	-1265.06	9	26	1	35
2	299.8	-1623.18	17	13	0	30
3	272.6	-1603.09	12	22	1	34
4	206.1	-1268.8	7	24	0	31
5a	249.6	-1208.13	11	12	0	23
5b	68.8	-537.02	2	9	0	11
6	267.9	-1245.45	10	21	0	31
7	239.1	-1124.05	8	13	0	21
8	183.8	-1092.34	6	16	0	22
9	153.8	-829.91	7	8	0	17
10	164.8	-885.78	5	11	0	16
Total	2362.9		94	175	2	271

Table 1. Mapped markers in different linkage groups, LOD values and length of linkage groups.

Linkage group SBI-01 consisted of 35 markers [SSRs (9) and DArTs (26)], spanning a distance of 256.6 cM, with an average inter- marker distance of 8.9 cM. Markers mapped on this chromosome were Xisep0949, Xisep0327, Xisep0839, Xtxp340, Xtxp037 and Xisep1028, mapped exactly between Xtxp061 and XmsbCIR268 that flank *Striga* resistance QTL1, substantially increasing the length of this linkage group. Linkage group SBI-02 consisted of 30 markers [SSRs (17) and DArTs (13)], spanning a distance of 299.8 cM, with an average inter-marker distance of 19.7 cM. Markers mapped on SBI-02 were Xtxp080, XmsbCIR223, Xiabtp346, Xiabtp500, Xiabtp444, Xtxp013 and Xtxp298. They filled the large gap between Xtxp197 and Xtxp201 flanking *Striga* resistance QTL2. Linkage group SBI-05 was comprised of 34 markers [SSRs (13) and DArTs (21)] spanning 318.4 cM, with an average inter-marker distance of 9.6 cM. Markers mapped on SBI-05 were Xiabtp420, XmsbCIR329, Xisp10258, Xgpsb017, SbPB16049, SbPB16911, SbPB15837, SbPB19771, SbPB18557, SbPB13628 and SbPB14357 in the gap between *Striga* resistance QTL3-flanking markers *Xtxp065* and *Xtxp303*. In the gap between QTL4- flanking markers *Xtxp015* and *Xtxp225*, on the same linkage group, only *Xtxp014* and *Xtxp262* were mapped. Linkage group SBI-06 was composed of 31 markers [SSRs (10) and DArTs (21)], spanning a distance of 267.9 cM, with an average inter-marker distance of 8.9 cM. Markers mapped on this linkage group were Xtxp045, Xtxp317, Xtxp219, Xisep0443, Xtxp176, SbPB15430, SbPB17601, SbPB19263 and Xisep0422, between the QTL5-flanking markers Xtxp145 and Xtxp057 (Table 2).

DISCUSSION

The SSR and DArT markers were evenly distributed across the ten sorghum linkage groups where the map resolution was greatly improved as many of the added markers were mapped to the gaps in the previously existing map. SSRs have been widely used to construct high-density linkage maps and marker assisted breeding programmes in recent years (Patil *et al.*, 2010). Distribution of SSRs on the genetic map will show the distribution of genes in the genome. Another important feature of the genic SSR markers is that, unlike genomic SSRs, they are transferable among related species and genera (Yu *et al.*, 2004; Varshney *et al.*, 2005).

This constructed linkage map is highly advantageous over previously constructed maps, and can be used for further exploitation mainly due to its relatively large population size (183) compared with other sorghum published maps (Bhattramakki *et al.*, 2000; Haussmann *et al.*, 2004). This large population size will not only improve the estimation of marker orders, but also will improve the resolution of QTL mapping of the trait. This is in agreement with Chalmers *et al.* (2001), who reported that the distribution of markers across the full length of the genome is required for detection of contributing loci. The present map distance is longer than the previously reported sorghum maps by Bhattramakki *et al.* (2000) (1141 cM); Haussmann *et al.* (2004) (1410 cM); Menz *et al.* (2002) (1713 cM), Haussmann *et al.* (2004) (1,599 cM); Moens *et al.* (2006)(997.5 cM) Mace *et al.* (2009) and (1603.5 cM). The greater map distance can be attributed to the increase in the recombination frequencies due to the population size and the use of RILs rather than the earlier generations of this same mapping population used in the linkage maps of Haussmann *et al.* (2004) and also because of increase in marker density (Ramu *et al.*, 2009).

***Striga* resistance QTLs SSR associated markers**	**(Mbp)**	**distance(cM)**	**LG length (cM)**
SSRs flanking to *Striga* resistance QTLs on SBI-01			
Xisep0949	6	3.9	256.6
Xisep0327	7	8.7	
Xtxp340	70	1.1	
Xtxp061	66	0	
Xtxp037	56	7.2	
Xisep839	5	0	
XmsbCIR268	9	37.4	
Xisep1028	53	13.4	
SSRs flanking to *Striga* resistance QTLs on SBI-02			
Xtxp080	4	39.5	299.8
XmsbCIR223	5	3.8	
Xiabtp346	13	47.8	
Xiabtp500	19	6.2	
Xiabtp444	56	6.4	
Xtxp013	56	3.7	
Xtxp298	58	30.7	
SSRs flanking to *Striga* resistance QTL on SBI-05a			
Xtxp065	2	0	249.6
Xiabtp420	4	9.3	
Xgpsb17	5	7.2	
Xtxp015	43	4.8	
Xtxp014	43	4.8	
Xtxp225		23.3	
SSRs flanking to *Striga* resistance QTL on SBI-05b			
Xtxp262	58	2.6	68.8
Xtxp123	58	18.3	
SSRs flanking to *Striga* resistance QTL on SBI-06			
Xtxp145	50	0.0	267.9
Xtxp045	45	0.0	
Xtxp317	51	3.5	
Xtxp219	52	3.9	
Xisep443	56	6.9	
Xtxp176	56	2.1	
Xisep0422		7.4	
Xtxp057	58	1.9	

Table 2. SSR markers mapped in the vicinity of Striga resistance QTLs.

Fig.1: Constructed sorghum genetic linkage map for RIL population based on cross (N13 × E36-1).

***Striga* resistance QTLs SSR associated markers**	**(Mbp)**	**distance(cM)**	**LG length (cM)**
SSRs flanking to *Striga* resistance QTLs on SBI-01			
Xisep0949	6	3.9	256.6
Xisep0327	7	8.7	
Xtxp340	70	1.1	
Xtxp061	66	0	
Xtxp037	56	7.2	
Xisep839	5	0	
XmsbCIR268	9	37.4	
Xisep1028	53	13.4	
SSRs flanking to *Striga* resistance QTLs on SBI-02			
Xtxp080	4	39.5	299.8
XmsbCIR223	5	3.8	
Xiabtp346	13	47.8	
Xiabtp500	19	6.2	
Xiabtp444	56	6.4	
Xtxp013	56	3.7	
Xtxp298	58	30.7	
SSRs flanking to *Striga* resistance QTL on SBI-05a			
Xtxp065	2	0	249.6
Xiabtp420	4	9.3	
Xgpsb17	5	7.2	
Xtxp015	43	4.8	
Xtxp014	43	4.8	
Xtxp225		23.3	
SSRs flanking to *Striga* resistance QTL on SBI-05b			68.8
Xtxp262	58	2.6	
Xtxp123	58	18.3	
SSRs flanking to *Striga* resistance QTL on SBI-06			
Xtxp145	50	0.0	267.9
Xtxp045	45	0.0	
Xtxp317	51	3.5	
Xtxp219	52	3.9	
Xisep443	56	6.9	
Xtxp176	56	2.1	
Xisep0422	__	7.4	
Xtxp057	58	1.9	

Table 3. SSR markers mapped in the vicinity of Striga resistance QTLs

This later reason is consistent with the findings of Hayden *et al.* (2005) and Boivin *et al.* (1999), that the improved SSR density on the current sorghum map was facilitated by fluorescence-based marker detection and capillary electrophoresis. The assay platform used provides higher resolution for small allelic size differences and multi- allelic markers compared to the PAGE, which has been used in previous mapping studies (Karakousis *et al.*, 2003). However, the map marker order is essentially the same as in previously existing maps Bhattramakki *et al.* (2000); Haussmann *et al.* (2004) Kim *et al.* (2004); Moens *et al.* (2006) and Moens *et al.* (2006), whereby, a few tightly linked markers were observed. This is in agreement with Kim *et al.* (2005), who reported that the genetic distances can vary between maps but marker locus order should remain the same between the maps of a single species. So far, most existing maps in sorghum were developed using both RFLP and SSR markers (Bhattramakki *et al.*, 2000; Haussmann *et al.*, 2004); In these SSR linkage maps each linkage group represents a chromosome; this is consistent with the findings of Menz *et al.* (2002) and Kim *et al.* (2005).

CONCLUSIONS

The saturated molecular marker-based genetic maps in sorghum promised rapid progress towards the improvement of sorghum. This linkage map with these saturated *Striga* resistance regions will definitely enhance transferring of *Striga* resistance genes from donor to recipient parents. More importantly, the markers mapped in the vicinity of *Striga* resistance QTL regions could be candidates for marker-assisted introgression of *Striga* resistance genes into adapted, farmer-preferred local cultivars and will also ease gene cloning for inter and intra specific transfer.

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