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Exploiting rice–sorghum synteny for targeted development of EST-SSRs to enrich the sorghum genetic linkage map

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Abstract The sequencing and detailed comparative functional analysis of genomes of a number of select botanical models open new doors into comparative genomics among the angiosperms, with potential benefits for improvement of many orphan crops that feed large populations. In this study, a set of simple sequence repeat (SSR) markers was developed by mining the expressed sequence tag (EST) database of sorghum. Among the SSR-containing sequences, only those sharing considerable homology with rice genomic sequences across the lengths of the 12 rice chromosomes were selected. Thus, 600 SSR-containing sorghum EST sequences (50 homologous sequences on

each of the 12 rice chromosomes) were selected, with the intention of providing coverage for corresponding homologous regions of the sorghum genome. Primer pairs were designed and polymorphism detection ability was assessed using parental pairs of two existing sorghum mapping populations. About 28% of these new markers detected polymorphism in this 4-entry panel. A subset of 55 polymorphic EST-derived SSR markers were mapped onto the existing skeleton map of a recombinant inbred population derived from cross N13 × E 36-1, which is segregating for *Striga* resistance and the stay-green component of terminal drought tolerance. These new EST-derived SSR markers mapped across all 10 sorghum linkage groups, mostly to regions expected based on prior knowledge of rice–sorghum synteny. The ESTs from which these markers were derived were then mapped *in silico* onto the aligned sorghum genome sequence, and 88% of the best hits corresponded to linkage-based positions. This study

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demonstrates the utility of comparative genomic information in targeted development of markers to fill gaps in linkage maps of related crop species for which sufficient genomic tools are not available.

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. In addition to being an important source of calories for millions of poor people living in the semi-arid tropics of Africa and Asia, and an essential livestock feed there and elsewhere, sorghum has been identified as a potential source of bio-fuel (Antonopoulou et al. 2008). Sorghum is a model organism for tropical grasses having the 'C₄' photosynthetic pathway and is a logical complement to the 'C₃' grass *Oryza sativa* (Kresovich et al. 2005), which was the first monocot with a completely sequenced genome (Goff et al. 2002; Yu et al. 2002; International Rice Genome Sequencing Project 2005). Sorghum has a relatively small genome (~760 Mb; Arumuganathan and Earle 1991) that is less complex than those of other major C₄ crops like maize (*Zea mays* L.) and sugarcane (*Saccharum officinarum*). Genome sequencing of sorghum was initiated at the end of 2005 through the 'Community sequencing program of Department of Energy-Joint Genome Institute, USA' and during the course of this study the complete aligned genome sequence (8×) information for this species was made available to public use (Paterson et al. 2009; <http://www.jgi.doe.gov/sorghum>). This has provided an opportunity to better understand genome organization in sorghum, its wild relatives including Johnson grass (*Sorghum halepense*), and even maize.

Sorghum is the first species in the crop improvement mandate of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) for which a reasonable amount of genomic tools are available that can be exploited for applied crop improvement. Construction of molecular marker-based linkage maps in sorghum started during the 1990s with restriction fragment length polymorphism (RFLP) markers (Hulbert et al. 1990; Pereira et al. 1994; Chittenden et al. 1994; Ragab et al. 1994; Xu et al. 1994; Dufour et al. 1997; Tao et al. 1998a; Peng et al. 1999) followed by maps adding other marker systems like amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), and recently diversity array technology (DArT) markers (Boivin et al. 1999; Kong et al. 2000; Bhatramakki et al. 2000; Klein et al. 2000; Subudhi and Nguyen 2000; Haussmann et al. 2002a; Menz et al. 2002; Bowers et al. 2003; Mace et al. 2008). Several genomic regions associated with important agronomic traits including

a range of abiotic stress tolerances [primarily mid-season and terminal drought tolerance (Tuinstra et al. 1996, 1997; Crasta et al. 1999; Subudhi et al. 2000; Tao et al. 2000; Xu et al. 2000; Kebede et al. 2001; Haussmann et al. 2002b; Sanchez et al. 2002; Harris et al. 2007) and aluminum tolerance (Magalhaes et al. 2004)], host plant resistances to hemi-parasitic weeds [*Striga* spp. (Haussmann et al. 2004)], insect pests [aphids (Agrama et al. 2002; Katsar et al. 2002; Nagaraj et al. 2005; Wu et al. 2007), midge (Tao et al. 2003), and shoot fly (Folkertsma et al. 2003)], and diseases [of both foliage (Oh et al. 1996; Tao et al. 1998b; Nagy et al. 2007) and panicles (Klein et al. 2001)], and a range of traits related to grain quality (Lijavetsky et al. 2000), crop phenology [height and maturity (Lin et al. 1995; Pereira and Lee 1995; Klein et al. 2001; Feltus et al. 2006a)], and yield components (Hart et al. 2002; Hicks et al. 2002) have been mapped. Now high-density genetic maps are available for sorghum (Klein et al. 2000; Bowers et al. 2003). However, these maps have some significant gaps and many genomic regions are covered by only AFLP and RFLP markers. At the onset of this study there were circa 300 publicly available primer pair sequences for mapped sorghum SSR loci (Brown et al. 1996; Taramino et al. 1997; Kong et al. 2000; Bhatramakki et al. 2000; Menz et al. 2002; Schloss et al. 2002).

SSR markers have clear advantages over RFLP and AFLP markers in terms of technical simplicity, throughput level and automation (Varshney et al. 2005). SSRs are the preferred marker system for many breeding applications. Hence, enriching the existing sorghum linkage map with more SSR markers is a valuable objective for the sorghum breeding community globally. Conventional SSR marker development is a costly and time-consuming process. Thanks to the availability of genomic or expressed sequence tag (EST) sequences in public databases and the recent advent of bioinformatics tools, SSR marker development has become easier and more cost-effective (e.g., Jayashree et al. 2006). In the past, SSR markers have been successfully developed by mining EST databases in several crops (reviewed in Varshney et al. 2005). EST-SSRs are a highly valued marker system as they are developed from transcribed portions of the genome and often target functional diversity. They are also superior in terms of cross-species transferability, and thus are well suited for application in phylogenetic analysis and comparative genome mapping. The transfer rate of EST-SSR markers from sorghum to paspalum (*Paspalum* spp.) and to maize was 68 and 61%, respectively (Wang et al. 2005).

The rice genome exhibits substantial collinearity with the genomes of other grasses, such as sorghum, maize, wheat (*Triticum aestivum* L.), and barley (*Hordeum vulgare* L.) (Ahn et al. 1993; Chen et al. 2002; Devos and Gale 1997; Tarchini et al. 2000). A direct comparison of the

genetic linkage maps of sorghum and rice has been performed by Ventelon et al. (2001) using the mapping information of a common set of RFLP probes. Klein et al. (2003) indicated that the overall architecture of sorghum chromosome 3 and rice chromosome 1 has remained largely intact with the exception of one major rearrangement. The sorghum genome has now been reasonably aligned with that of rice through comparative genetic, cytogenetic, and physical mapping approaches (Draye et al. 2001; Bowers et al. 2005, Kim et al. 2005a, Paterson et al. 2009). In this study, we attempted to develop a set of EST-SSR markers that are expected to provide coverage for gene-rich regions of the entire nuclear genome using comparative genomic information from rice as the sorghum genome sequence information was not available when the study was initiated.

Materials and methods

Data mining of sorghum ESTs

Sorghum EST sequences were downloaded in FASTA format from the J. Craig Venter Institute [formerly, The Institute for Genome Research (TIGR)] database (<http://www.tigr.org>) as of May 2004. The identification of microsatellites from these EST sequences was carried out using SSRIT (www.gramene.org/db/searches/ssritool), and simple scripts (written in Visual Basic) to parse SSRIT output into the relational database. CAP3 (Huang and Madan 1999) was used to identify the non-redundant EST sequences. Non-redundant sorghum EST sequences containing SSRs were then obtained from this local database and used in this study.

Selection of candidate ESTs and primer design

Non-redundant sorghum EST sequences containing microsatellites were BLAST searched against the rice genome sequence available in the GRAMENE database (<http://www.gramene.org/db/searches/blast>) as of May 2004. Each rice chromosome was divided into five arbitrary regions viz., 'top', 'middle', 'bottom', 'between top and middle', and 'between middle and bottom'. The ten top-scoring sorghum EST sequences for each region of the rice chromosome were picked. Thus, a total of 600 SSR-containing EST sequences of sorghum (10 each for each region of the 12 rice chromosomes) having sequence homology with rice sequences were identified. These 600 candidate EST-SSR sequences were expected to sparsely cover the entire nuclear genome of rice, and thereby the corresponding sorghum genomic regions. Primer pairs were designed from the selected 600 non-redundant sorghum EST-SSR

sequences using Primer3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi) after masking the repeat units. The primer pairs were designed with the following conditions: primer length (min-18nt, opt-22nt, max-24nt), T_m (min-54°C, opt-57°C, max-60°C), and GC content (min-45, opt-50, max-60%).

Primer optimization and polymorphism assessment

PCR conditions for the EST-SSR primer pairs were optimized using template DNA of genetically diverse parental pairs of two sorghum mapping populations available at ICRISAT, Patancheru viz., N13 × E 36-1 and BTx623 × IS 18551. N13 is *Striga* resistant while E 36-1 is stay-green, drought tolerant, and *Striga* susceptible. BTx623 is an elite hybrid parental line chosen for genome sequencing (Paterson et al. 2009). It is susceptible to shoot fly and being used as the recurrent parent for ICRISAT's exploratory shoot fly resistance marker-assisted backcrossing program. IS 18551 is a shoot fly resistant donor parent being used by ICRISAT for shoot fly resistance QTL mapping and marker-assisted backcrossing. DNA of parental lines was isolated using a high-throughput DNA extraction protocol as reported by Mace et al. (2003) and normalized to a working concentration of 5 ng/μl.

PCR was performed in 5 μl reaction volume with final concentrations of 2.5 ng DNA, 2 mM MgCl₂, 0.1 mM of dNTPs, 1× PCR buffer, 0.4 pM of each primer, and 0.1 U of *Taq* DNA polymerase (AmpliTaq Gold[®], Applied Biosystems, USA) in a GeneAmp[®] PCR System 9700 thermal cycler (Applied Biosystems, USA) with the following cyclic conditions: initial denaturation at 94°C for 15 min (to activate *Taq* DNA polymerase) then 10 cycles of denaturation at 94°C for 15 s, annealing at 61°C for 20 s (temperature reduced by 1°C for each cycle), and extension at 72°C for 30 s. This was followed by 34 cycles of denaturation at 94°C for 10 s, annealing at 54°C for 20 s, and extension at 72°C for 30 s with the final extension of 20 min at 72°C. Amplified PCR products were resolved on 6% native polyacrylamide gels coupled with silver staining as described by Tegelstrom (1992).

Mapping of EST-SSRs

The polymorphic markers were subsequently mapped using recombinant inbred lines (RILs) of N13 × E 36-1 (Haussmann et al. 2004). The markers polymorphic between N13 and E 36-1 were surveyed on a subset of 94 F₃-derived F₅ RILs. The segregation data were used to place the new markers on to the existing skeleton map with 164 previously mapped RFLP, AFLP, RAPD, and SSR markers (Haussmann et al. 2002a, 2004) at LOD score of 3.0 using Mapmaker 3.0v (Lander et al. 1987). The Haldane mapping

function (Haldane 1919) was used to convert recombination frequency into linkage map distance. The linkage groups were oriented and designated following Kim et al. (2005b).

In silico mapping of ESTs

Aligned sorghum genome sequence information (Sbi 1.4) for elite sorghum inbred BTx623 was made available for public use (<http://www.jgi.doe.gov/sorghum>) during the course of this study (Paterson et al. 2009). This aligned sorghum genome sequence information was downloaded and formatted into a database. All mapped SSR-containing EST sequences (55 markers) were searched against this database using Paracel BLAST (Altschul et al. 1990) on a Paracel High Performance Computing system. These sequences were then aligned on to the sequence-based physical map according to their hit location on each linkage group.

Results

Data mining of sorghum ESTs

In the TIGR database, about 187,282 sorghum EST sequences were available when this study was initiated. We identified 39,106 EST sequences with SSRs using the SSRIT tool. These sequences were clustered using the CAP3 program to identify 10,044 non-redundant EST sequences containing microsatellites.

A set of 1,486 non-redundant sorghum EST sequences containing microsatellites were BLAST searched against the rice genome in the GRAMENE database. From the sequence similarity search, 150 sorghum EST sequences had no hits on rice genome sequence; 180 sequences had homology with sequences on rice chromosome 1; 130 on chromosome 2, 243 on chromosome 3, 98 on chromosome 4, 115 on chromosome 5, 77 on chromosome 6, 67 on chromosome 7, 66 each on chromosomes 8 and 9, 71 on chromosome 10, 144 on chromosome 11, and 79 on chromosome 12. On each chromosome, 50 sorghum SSR-containing EST sequences with highest BLAST search score that provided coverage across the entire length of each rice chromosome were identified. Thus, 1,486 non-redundant EST sequences were BLAST searched against the rice genome to identify 600 sequences (50 per rice chromosome). Primer pairs flanking the SSR motifs were designed using Primer3. These primer pairs were designated as 'ICRISAT Sorghum EST Primers (ISEP)' (listed as electronic supplementary information S1). Among the selected 600 SSR-containing sorghum EST sequences, 470 (78%) sequences have putative annotations. The majority of these are classified as transcription factors or DNA binding proteins.

Out of the 600 sorghum EST-SSR markers developed, 63 were di-nucleotide repeats (10.5%); 425 were tri-nucleotide repeats (70.8%); 78 were tetra-nucleotide repeats (13.0%); and 34 were penta-nucleotide repeats (5.6%). The most abundant repeats among the selected EST sequences were AG and CCG. A total of 79 SSR motifs (13%) were of Class I type of SSRs (≥ 20 nucleotides in length) and 496 SSR motifs were Class II type of SSRs (>12 but <20 nucleotides in length), and remaining 25 SSR motifs were 10 nucleotides in length. Further details on SSR frequency and distribution in the set of EST sequences used here have been published by Jayashree et al. (2006).

Primer optimization and polymorphism assessment

PCR conditions for the 600 primer pairs were optimized using template DNA from the four inbred parental lines of the two mapping populations, and parental polymorphism was simultaneously scored. Out of 600 primer pairs, 457 (76.1%) amplified the template and 386 (84.5%) produced simple and easy to score amplification products, whereas the remaining 15.5% of template-amplifying primer pairs produced multiple fragments that were difficult to score. Most of the primer pairs that produced no amplification or gave non-specific amplification were targeting tri-nucleotide repeats. Of the 386 primer pairs that produced good amplification profiles, 133 primer pairs (34.5%) detected polymorphism between N13 and E 36-1 and 140 primer pairs detected polymorphism between BTx623 and IS 18551 (listed as electronic supplementary information S1). In both crosses, di-nucleotide repeats were found most polymorphic (30–38%), followed by tetra-nucleotide repeats in the N13 \times E 36-1 cross, and penta-nucleotide repeats in the BTx623 \times IS 18551 cross.

Mapping of EST-SSRs

The polymorphic markers were surveyed on 94 RILs derived from the cross N13 \times E 36-1 and the segregation data were scored. A total of 55 EST-SSR markers were added (Table 1) to the existing skeleton map of this mapping population. These markers mapped to all 10 sorghum linkage groups and were well-distributed within each linkage group except in the case of SBI-06 for which the newly mapped markers clustered along the bottom of the linkage group in a region corresponding to the long arm of the sorghum chromosome. Among these 55 newly mapped EST-SSR markers, 9 mapped to SBI-09, 8 markers each mapped to SBI-02 and SBI-03, 7 mapped to SBI-01, 6 markers each mapped on SBI-04 and SBI-07, 3 markers each mapped on SBI-05, SBI-06, and SBI-10, and 2 markers mapped on SBI-08, with an average of 5.5 EST-SSR markers per linkage group. The linkage map augmented with these 55

Table 1 Details of 55 genetically mapped sorghum EST-SSR markers and their corresponding physical map locations on the aligned sorghum genome sequence

Marker	Sorghum EST sequence ID	Repeat motif	Forward primer sequence (5'–3')	Reverse primer sequence (5'–3')	Approx. amplicon size in BTX623 (bp)	Alignment position on rice genome sequence	BLAST score	Linkage group	Physical map position
<i>Xisep0101</i>	AW679887	TG(9)	CAGATCTCCGGTTGAAGAGC	TGAGCCGAGCTCAACATACA	205	chr1: 1931190-12847810	1350	SBI-03	SBI-03
<i>Xisep0102</i>	AW565338	AAG(4)	CGCTGGAGTACACAGAGGAAG	AACAAAATCCGAGCCTGTGTG	680	chr1: 419375-22780478	968	SBI-03	SBI-03
<i>Xisep0107</i>	AW744864	TGG(4)	GCCGTAACAGAGAAAGGATGG	TTTCCGCTACCTCAA AAAACC	205	chr1: 4946397-4946862	711	SBI-03	SBI-03
<i>Xisep0110</i>	BE360507	CG(6)	GAGGGAAAGCTGGAGACC	TCAAGTGTACAACGCATCCAG	480	chr1: 810589-31327180	1821	SBI-09	SBI-03
<i>Xisep0138</i>	AW923239	TA(6)	GAGATCGAGAGGCACCTTGG	CAGGCACAA GCCAATACCA	210	chr1: 40999721-41283498	1349	SBI-03	SBI-03
<i>Xisep0202</i>	AW679616	TGA(7)	CAACCTGTGATTGACCCATT	AAACATGTCCAGATTCATCAAGG	195	chr2: 3957694-3958376	1235	SBI-04	SBI-04
<i>Xisep0224</i>	AW563693	CTG(4)	ACTGGGTTCCCTTTTCCTGT	TCCTGATTTCCCTCTCTTT	200	chr2: 13206311-26037486	824	SBI-04	SBI-04
<i>Xisep0228</i>	BE356910	GAGG(3)	GACATGGCCAGCTAAGAGGA	CCATGCAGTGATCGTTGTGT	220	chr2: 7635368-30560994	746	SBI-04	SBI-04
<i>Xisep0310</i>	AW286133	CCAAT(4)	TGCCTTGTGCTTGTTTTATCT	GGATCGATGCCTATCTCGTC	200	chr3: 10479597-10480017	711	SBI-02	SBI-02
<i>Xisep0314</i>	BE358851	GCC(4)	GTTCCGACGACGACGACCT	GTCCTCGAACCCGACCTT	405	chr3: 1696130-28812187	730	SBI-10	SBI-01
<i>Xisep0327</i>	AW680959	GTT(4)	CTGTTTGTGCTTGC AACTCC	TCATCGATGCAGA ACTCAC	200/210	chr3: 16826707-30714737	730	SBI-01	SBI-01
<i>Xisep0328</i>	BE359052	AAG(4)	CATCTTCTCCGTC AACCAT	ATCCTGCGACCTTCTCAC	300	chr3: 20756868-33479601	1419	SBI-07	SBI-07
<i>Xisep0422</i>	AW285124	GCAT(3)	TGCCCGTAA TTAAGCCCAATA	CCCACGTCTCCAGGTAAGAA	300	chr4: 2810717-30050335	410	SBI-06	SBI-06
<i>Xisep0439</i>	AW745664	GAC(4)	TAGTCAAGCAGCAGTCGTG	GTGTTCAAAGTTTCAGCAGCA	620	chr4: 31792724-31832792	863	SBI-07	SBI-07
<i>Xisep0443</i>	BE355584	GCA(7)	TCATGTACAGAGCCGACACG	AGGTCCCAACAGACACCTTC	190	chr4: 29104052-29122456	734	SBI-06	SBI-06
<i>Xisep0449</i>	BE357875	TCA(7)	CCGCTCATCAGTCATCACAT	ACAAAATCCATCCCAACAG	200	chr4: 365957-34743646	1808	SBI-06	SBI-06
<i>Xisep0506</i>	AW922528	AACG(3)	CGTGCAAGTTTGGAA TTTGTC	CGGGCAGGTATAAGGTGTG	225/430	chr5: 2076388-4402866	697	SBI-09	SBI-09
<i>Xisep0513</i>	AW745388	GGC(5)	GAGGGAAGAAGAAA ACCCAGA	AGCCTCTCTCTCTCTCTCT	300	chr5: 803034-17097487	1053	SBI-09	SBI-09
<i>Xisep0522</i>	BE361646	CAG(8)	TCA TGGACCGTGTCA TCG	GCGTACTGTCTCCACCTCTC	330	chr5: 15943072-22166226	1110	SBI-04	SBI-02
<i>Xisep0523</i>	BE358885	TGC(4)	ACGACATGGACGACATCAGA	AACAAAACACACGGGAAGG	220/305	chr5: 17631682-17632292	911	SBI-09	SBI-09
<i>Xisep0550</i>	AW746901	GA(11)	GCGCGAGAGAGAGAGTTTC	CGAGTTGATCTTCTCGTTGA	185	chr5: 3039650-25338018	670	SBI-09	SBI-09
<i>Xisep0607</i>	AW923923	AGA(4)	CACGAGATTTCACCAAACC	TGCAGGTGTTCCGAAA TAGGA	195	chr6: 2238520-2240631	543	SBI-10	SBI-10
<i>Xisep0608</i>	BE355471	AGA(4)	TTTCA CCAAACCAAGCTAAGG	GTA GAGGCA GCCCTTCTCT	205	chr6: 2238520-2240631	543	SBI-04	SBI-10
<i>Xisep0632</i>	BE355933	CATG(4)	AGAGAGGAGTCCCAAATGC	TTAAGGCCCAA ACAA AACTGG	195	chr6: 8908340-28128503	729	SBI-08	SBI-08
<i>Xisep0639</i>	BE357831	TCT(6)	TCGGACGGAGTCA TCAGATA	GCCTTCTGTCTTCTGTCTCT	200	chr6: 26216943-26460093	628	SBI-10	SBI-10
<i>Xisep0701</i>	BE360023	TTCTT(3)	CGGTGGGAGAGACAGAGAGA	CCAA TCAATACCACTCTCTGTGA	200	chr7: 4061086-4061447	1228	SBI-02	SBI-02
<i>Xisep0704</i>	AW680310	GT(5)	CAAGTCCGTCTGTGCTAGAG	CCCTTTAATTAAGCCCAAACA	200	chr7: 5247630-5247943	908	SBI-07	SBI-07
<i>Xisep0733</i>	BE357808	TGTA(5)	GTGATGCATCTCA CCGACAG	GCAAGTCTGATGCTTGTCT	400	chr7: 22294109-26985575	1443	SBI-02	SBI-02
<i>Xisep0747</i>	BE357135	TCC(5)	AGGACGCTGCTTATCACAA	ACAAGCTCAGGTGGGTGGT	210	chr7: 372151-26726597	897	SBI-02	SBI-02
<i>Xisep0809</i>	AW747322	TATG(4)	GGAAACTCTTGTGGTTGGA	TTGACCTCTTACAAA TGA TCCAC	200	chr8: 1022442-1022570	221	SBI-02	SBI-02
<i>Xisep0824</i>	AW564458	CCG(4)	TCCTGAAAGAAA ACCCACACA	GAGGAGGTGTGGAGGTGTA	200	chr8: 13585814-13586034	428	SBI-08	SBI-08
<i>Xisep0829</i>	BE125697	AG(6)	CGTGCCAAA A TCTAAGCTC	CACGTTGGTCA C ATCAGAAG	205/243	chr8: 27738244-27738554	1225	SBI-07	SBI-07
<i>Xisep0831</i>	BE360943	AAAAG(3)	TCCA TGACCTTGTAGGAGGAG	TTGAAGCAGGAC AACACACC	200	chr8: 24781619-24781723	943	SBI-07	SBI-07
<i>Xisep0839</i>	BE357549	ATTAC(4)	TACGCA TAGCCCTTTCAAT	ATTTCAITATGCCGGTCTCG	200	chr8: 27711931-27712047	378	SBI-01	SBI-01
<i>Xisep0841</i>	BE358373	GCA(10)	TAGGAATGACGACCA CCA	CAAA GCGAAGGGTTTGGCTA	210	chr8: 24665284-24665519	299	SBI-02	SBI-02

Table 1 continued

Marker	Sorghum EST sequence ID	Repeat motif	Forward primer sequence (5'–3')	Reverse primer sequence (5'–3')	Approx. amplicon size in BTx623 (bp)	Alignment position on rice genome sequence	BLAST score	Linkage group	Physical map position
<i>Xisep0843</i>	BE357146	AGTG(3)	CCCAAACATTCACCACGTAAC	GTGAACAGAGGAGGCAGAGG	200	chr8: 2991852-24982703	993	SBI-03	SBI-03
<i>Xisep0844</i>	BE356742	CGT(4)	GTGTTCAAGTTCGACGAGCA	TAGTCGAAGCAGCATCGTG	600	chr8: 1641413-25156453	941	SBI-07	SBI-07
<i>Xisep0938</i>	AW747241	TGGGT(6)	TGCTGTTCTTGAACGTTGTTG	TTTTGCACAAGTTGGGTGT	225	chr9: 17210486-17210944	779	SBI-02	SBI-02
<i>Xisep0948</i>	BE358472	TA(5)	AGGCCAATCACATAATGG	AGTCATGAACAGGGCATC	205	chr9: 20568536-20568726	297	SBI-04	SBI-04
<i>Xisep0949</i>	AW564537	GCA(5)	CAGTGCCAAATAAGTCGTCTC	CATCGATCTCTGCTTCTGCTT	100	chr9: 19365772-19365933	271	SBI-01	SBI-01
<i>Xisep1008</i>	AW285562	CAG(7)	GATCGGCAAGCAGAACAAG	CAGCAATGGAATAGCTCAGG	340	chr10: 2491921-13066504	632	SBI-09	SBI-01
<i>Xisep1013</i>	BE360610	CGG(7)	CGGTTACGGCGGATTATTAC	ATGGTGGCGATGCAGACTA	220	chr10: 4414394-16362660	402	SBI-02	SBI-02
<i>Xisep1014</i>	AW283055	GT(5)	ACCGCCGACGTCATAGTAAG	GGCAGTAACATAGCATCCATCA	240	chr10: 13864191-13864421	391	SBI-09	SBI-09
<i>Xisep1025</i>	AW672031	GCG(5)	ACCTCTCGTCTCTGTCCTC	AGAACATGACCCGGATCGAAG	490	chr10: 8526382-21813965	931	SBI-02	SBI-01
<i>Xisep1028</i>	AW747772	GCA(4)	CAGCGACCATGAGGATGAC	TGGCATGCATCAACAAGAT	200	chr10: 11501109-20941969	638	SBI-01	SBI-01
<i>Xisep1031</i>	BE355884	CGG(6)	TGCTCTGCCCTCGTTCTC	TAGTCTCGGTGCGACTCCAT	690	chr10: 5554237-21765954	326	SBI-03	SBI-03
<i>Xisep1032</i>	BE356267	TTCAG(3)	GCAAGCTTACGGGATCTTC	GCAGCTGGAAAATAATCGAAA	200	chr10: 14058773-21609683	1532	SBI-01	SBI-01
<i>Xisep1039</i>	AW287556	CCTG(5)	GTGGATTCAAATCCGGTGCAC	GGCAAATTTGGCAAGCAAT	200	chr10: 13165407-17730031	492	SBI-01	SBI-01
<i>Xisep1047</i>	AW285966	TGCGT(4)	TCTTTGCCCTCCCGCTAT	TCCATGAAATGAGCAAACGA	160	chr10: 18901923-18902174	235	SBI-03	SBI-03
<i>Xisep1107</i>	BE125211	GCA(6)	GGATAATCTGCAGGCGACTT	CCATCTGCTGCTCTGACTTG	210	chr11: 5341585-5362168	830	SBI-05	SBI-05
<i>Xisep1111</i>	AW286876	CGAG(3)	CCTCTCTCTCAATCCCTCT	ATGGGTAGCGGGTTTCTTG	220	chr11: 1392730-1393672	711	SBI-05	SBI-05
<i>Xisep1128</i>	AW565976	AT(6)	GGGGGAAAAAGTTCCTTA	CGCACACCCATTTCATTC	220	chr11: 14249874-23849881	793	SBI-09	SBI-05
<i>Xisep1133</i>	AW564774	CCA(5)	CGATGACGCTCCAACTCATA	GTGTATGTCGCCGAAGTGG	220	chr11: 2943934-21608323	350	SBI-05	SBI-05
<i>Xisep1213</i>	BE358492	GAA(5)	AGGTCAGGCTTTGCAATCT	ACAAAATTGAAAGGCGGAGAG	202	chr12: 1658936-21046139	315	SBI-01	SBI-01
<i>Xisep1241</i>	BE360849	AGCTG(5)	GAGGGGAGACAGAGGAGAT	CTACCTTTGAGCCCAACCGTA	190	chr12: 24812252-24812523	288	SBI-09	SBI-09

Markers conflicting in physical and linkage map positions are highlighted in bold

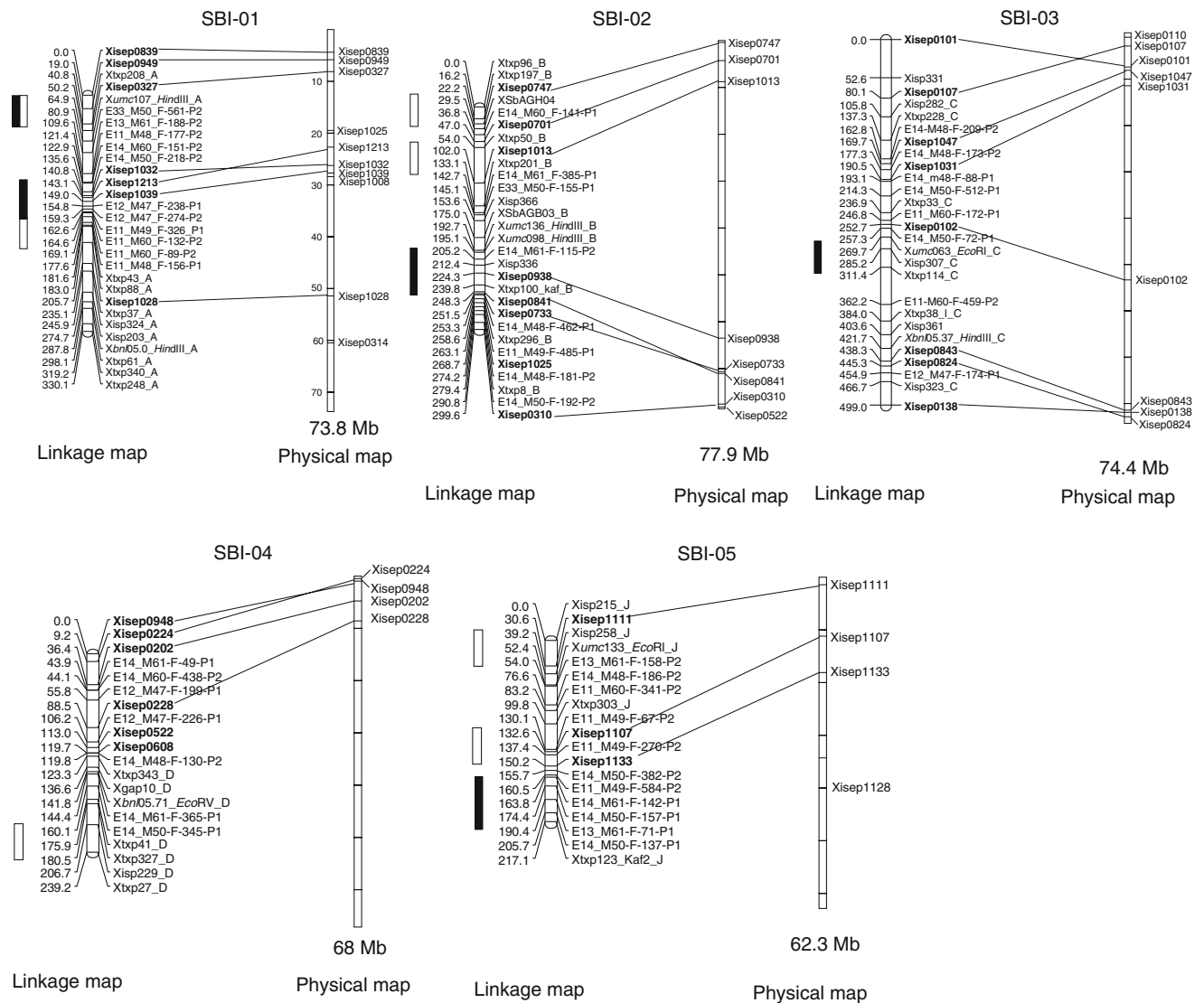


Fig. 1 Distribution of EST-SSR markers across sorghum linkage groups. Linkage group nomenclature follows Kim et al. (2005b). Newly added markers are highlighted in bold font. *Striga* resistance QTL and stay-green QTL

newly mapped loci spanned 2,838 cM (Fig. 1). Markers such as *Xisep0841*, *Xisep0733*, and *Xisep0938* on SBI-02, and *Xisep0138*, *Xisep0824*, and *Xisep0843* on SBI-03, mapped to the vicinity of previously reported stay-green QTLs (Haussmann et al. 2002b) whereas *Xisep0747*, *Xisep0701*, and *Xisep1013* on SBI-02 mapped in the vicinity of previously reported *Striga* resistance QTLs (Haussmann et al. 2004).

In silico mapping of SSR containing ESTs

All genetically mapped SSR-containing ESTs were then mapped physically by BLAST search against the aligned sorghum genome sequence. These markers were then aligned on to the sorghum physical map (Fig. 1). Physical map positions were essentially as expected from the genetic

linkage analysis of these markers, except in case of *Xisep0110*, *Xisep0314*, *Xisep0522*, *Xisep0608*, *Xisep1008*, *Xisep1025*, and *Xisep1128*. Physical and linkage map positions for all 55 mapped markers are listed in Table 1.

Discussion

The nature and frequency of SSRs in sorghum EST collection has been comprehensively discussed in Jayashree et al. (2006). Generally, EST-derived SSR markers are found to be less polymorphic than genomic SSRs. In this study, only 28% of the EST-SSR markers developed were polymorphic among the four sorghum genotypes initially screened. A similar percentage of polymorphism (25%) was reported for EST-SSRs in durum wheat (Eujayl et al. 2002), whereas

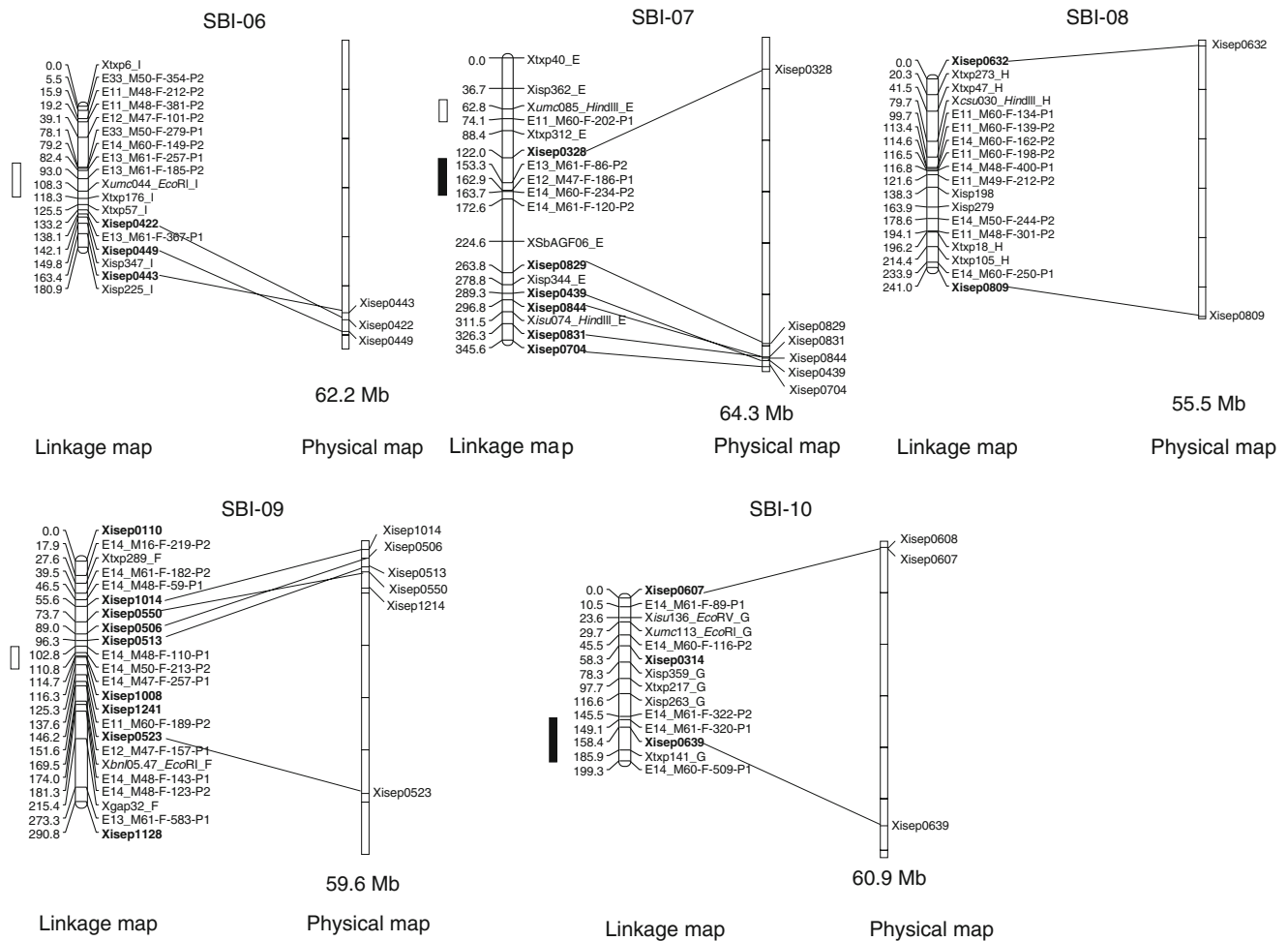


Fig. 1 continued

Thiel et al. (2003) reported 8–54% polymorphism on three different mapping population parental line pairs in barley. Di-nucleotide repeats were more polymorphic (listed as electronic supplementary information S1) than other repeat classes, as reported by Thiel et al. (2003) in barley. In our study, a total of 169 EST-SSR markers (28%) detected polymorphism between parental lines of two mapping populations. Among these, 104 EST-SSR markers were polymorphic in both populations whereas 36 and 29 detected polymorphism specific to the shoot fly and the *Striga* resistance mapping populations, respectively.

Sequence-based alignment studies accelerate the filling of gaps on linkage maps of related species and can be effectively used for comparative genome mapping across species (Klein et al. 2003). When searching the homology of 1,486 non-redundant sorghum SSR-containing EST sequences against the rice genome, the highest alignment scores were obtained for those aligning to the long arms of rice linkage groups (listed as electronic supplementary information S1). Rice chromosome 3 had the highest number of hits followed by chromosomes 1 and 11. In the

sequence similarity search, 150 of these sorghum EST sequences did not have any hit on the rice genome. Klein et al. (2003) also found that 10% of the sorghum ESTs studied have no homologs in the rice genome. Interestingly, this agrees well with the observation that 7% of the predicted genes from the aligned 8× shotgun sequence of sorghum inbred BTx623 have no apparent homologs in *Arabidopsis*, rice or poplar (Paterson et al. 2009).

After incorporating the new markers, the total map distance for the 94-entry (N13 × E 36-1)-based RIL population was 2,838 cM, which is similar to that reported by Tao et al. (2000) using 152 RILs and 306 markers. However, the present map is longer than sorghum linkage maps previously reported by several authors (Hausmann et al. 2002a; Pereira et al. 1994; Chittenden et al. 1994). The variation in map length of different mapping populations is generally attributed to differences in population size, the type of population, and the genetic distance between the parents, which strongly affect observed recombination rates, and thus, genetic distances (Menz et al. 2002). A comparison of the present map with that of Hausmann et al. (2002a), which

was based on a larger sample of progenies from the same mapping population, revealed that 94 out of 113 (83%) common markers had the same linear order in both maps. Some possible rearrangements were detected, which mostly involved alternative orders for closely linked loci. Such rearrangements are likely due to the use of a sub-sample of 94 lines from this mapping population in the current study. The only major change observed in the order of markers was in the upper part of linkage group SBI-04, inverting the order of two markers separated by 9 cM. These EST-SSR markers, however, filled gaps or marker-rare regions in the existing linkage map of sorghum targeted based on rice-sorghum sequence similarity.

The newly developed EST-SSR markers have shown practical significance by virtue of their positions. For example, *Xisep0138*, *Xisep0843*, and *Xisep0824*, which were mapped to SBI-03, are located in the vicinity of a stay-green QTL reported by Haussmann et al. (2002b) (Fig. 1). Three of the markers mapped to SBI-05 (*Xisep1111*, *Xisep1107*, and *Xisep1133*) are located in close proximity to *Striga* resistance QTLs (Haussmann et al. 2004). These markers are currently being used by ICRISAT and our national program partners in the introgression of stay-green and *Striga* resistance QTLs into a wide range of farmer-preferred sorghum genotypes through marker-assisted backcrossing. Two markers [*Xisep0107* (SBI-03) and *Xisep0310* (SBI-02)] were selected based on their linkage map positions and used by the Generation Challenge Programme (GCP) in large-scale SSR-based diversity analysis of over 3,000 sorghum accessions in a global composite germplasm collection. Across this very broad range of wild and cultivated sorghums, a total of nine alleles were noted for *Xisep0310* and six alleles for *Xisep0107* (data not shown). Such small allele numbers with EST-SSRs in such a large and diverse set of germplasm, as compared to genomic SSRs agrees with expectations that EST-SSRs are less polymorphic than genomic SSRs, as the former are derived from conserved and expressed regions of the genome. Although this makes EST-SSRs less useful than genomic SSRs for fingerprinting purposes, this lower level of variability is helpful in diversity analysis, in which relationships between accessions are much more difficult to assess when there are large numbers of rare alleles. Hence, these EST-SSRs have potential for use in assessing functional diversity among different genotypes, as well as use as flanking markers for foreground selection in marker-assisted breeding programs.

In silico mapping of the ESTs (from which these new SSRs were developed) on the aligned sorghum genome sequence (Paterson et al. 2009) gave very similar positions as the conventional linkage analysis, both in terms of chromosome arm location and order. The order of these EST-SSRs from the *in silico* mapping agrees with the linkage

map for all ten sorghum linkage groups provided that the latter are oriented with the short chromosome arm at the top, as per the suggestion of Kim et al. (2005a). Only seven (12.7%) out of 55 mapped EST-SSR markers added to the N13 × E 36-1-based skeleton map were mapped *in silico* to sorghum chromosomes other than those expected. Some of these differences are likely because the ESTs have been drawn of multi-gene families with members distributed on more than one chromosome as a result of ancestral genome duplication events or transposition. For example, *Xisep1025* mapped *in silico* to SBI-01 where as it is mapped to SBI-02 based on linkage analysis. However, the sorghum EST sequence (which had a best hit on rice chromosome 1) from which the primer pair for *Xisep1025* was derived also has a low e-value hit on SBI-02. This may be due to presence of duplicate loci on SBI-01 and SBI-02. Recently, 5,012 genomic SSRs were mapped *in silico* on to the aligned sorghum genome sequence assembly (Yone-maru et al. 2009).

The sorghum genome sequencing project has revealed that predicted gene density in sorghum is much higher at the ends of each chromosome and that heterochromatic regions near the centromere are essentially devoid of predicted genes (Paterson et al. 2009). *In silico* positions for all mapped EST-SSRs are located at the ends of sorghum chromosome arms, away from the centromeric regions, in agreement with the predicted genomic distribution of sorghum genes. The *in silico*-predicted physical map positions of candidate markers (including EST-SSRs that have not yet been mapped) can help to choose markers to fill gaps and better saturate linkage maps. Thus the availability of genome sequences and other marker information can help in selecting or developing markers for targeted mapping not only in a given crop but also in related crops for which sufficient genomic tools are not yet available in the public domain.

On-going and future sequencing projects will contribute many more genomic and genic sequences to publicly available databases, facilitating development of different types of markers at lower cost. As economically important grasses have more conserved regions across taxa than found in other crop lineages, EST-SSRs should become an extremely powerful tool for better understanding of relationships between the grass species and for genetic mapping. Our initial attempt to develop EST-SSRs based on sorghum-rice synteny was successful in filling important gaps in the existing sorghum linkage map. We propose to investigate further the large number of SSR-containing sorghum EST sequences to develop a dense linkage map based on these functional markers. The newly developed markers in hand, which are tightly linked to genomic regions controlling *Striga* resistance and the stay-green component of terminal drought tolerance, can be used in functional

analysis of these traits. These markers are being used by ICRISAT, along with the newly reported CISP markers (Feltus et al. 2006b), for mapping of sorghum resistance to shoot fly, stem borers, and grain mold. Through this study, we have demonstrated the value of a comparative sequence similarity approach for targeted development of PCR-compatible molecular markers for practical applications in crop genetics and breeding. It has previously been reported that more than half (57%) of EST-SSR markers developed from sorghum ESTs show cross-species transferability (Wang et al. 2005), hence these markers can also be successfully used in other related grass species for which insufficient numbers of PCR-compatible markers are available. Primer pairs for non-redundant sorghum EST-SSRs are available from our database (<http://intranet.icrisat.org/gt1/ssr/ssrdat-abase.html>).

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