RESEARCH ARTICLE

Development of SSR markers and construction of a linkage map in jute

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Abstract

Jute is an important natural fibre crop, which is only second to cotton in its importance at the global level. It is mostly grown in Indian subcontinent and has been recently used for the development of genomics resources. We recently initiated a programme to develop simple sequence repeat markers and reported a set of 2469 SSR that were developed using four SSR-enriched libraries (Mir *et al.* 2009). In this communication, we report an additional set of 607 novel SSR in 393 SSR containing sequences. However, primers could be designed for only 417 potentially useful SSR. Polymorphism survey was carried out for 374 primer pairs using two parental genotypes (JRO 524 and PPO4) of a mapping population developed for fibre fineness; only 66 SSR were polymorphic. Owing to a low level of polymorphism between the parental genotypes and a high degree of segregation distortion in recombinant inbred lines, genotypic data of only 53 polymorphic SSR on the mapping population consisting of 120 RIL could be used for the construction of a linkage map; 36 SSR loci were mapped on six linkage groups that covered a total genetic distance of 784.3 cM. Hopefully, this map will be enriched with more SSR loci in future and will prove useful for identification of quantitative trait loci/genes for molecular breeding involving improvement of fibre fineness and other related traits in jute.

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Introduction

Jute is an eco-friendly and biodegradable natural fibre, which is only second to cotton in its importance and competes with the synthetic fibres in the international market; its properties and utility have been widely discussed elsewhere (Hazra *et al.* 2004). Jute belongs to the genus *Corchorus* comprising ~50–60 species distributed throughout the tropics, including the tropical regions of Africa, America (including Brazil, Mexico, Bolivia, Venezuela and West Indies), Australia, China, Taiwan, India, Sri Lanka, Japan, Java, the Malayan peninsula, Marian Islands, the Philippines, Thailand (Kundu 1951). Genus *Corchorus* belongs to the family Sparrmanniaceae, which was earlier a part of Malvaceae *sensu lato* (Heywood *et al.* 2007; Benor *et al.* 2009) and largely comprises diploid species ($2n = 14$). The jute cultivars without exception belong to only two species, namely *Corchorus olitorius* L. (tossa jute) and *Corchorus capsularis* L. (white jute), and were mainly developed through conventional breeding including pure line selection (Ghosh 1983). Despite its relatively small genome size (Benor *et al.* 2011; Sarkar *et al.* 2011), genomics research in this crop was initiated rather late (Islam *et al.* 2005).

Importance of molecular markers and the choice of SSR as preferred markers in all major crops have been widely

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discussed (Rafalski *et al.* 1996; Staub *et al.* 1996; Mohan *et al.* 1997; Kumar 1999). SSR markers have also been extensively used for genome mapping both in plants and animals (Weising *et al.* 1998) and attempts have been made to assign SSR loci to linkage groups in a variety of plant genomes including those of rice (Wu and Tanksley 1993; Yang *et al.* 1994; Temnykh *et al.* 2000), bread wheat (Roder *et al.* 1998), barley (Liu Y. G. *et al.* 1996), maize (Senior *et al.* 1996), soybean (Morgante *et al.* 1994) and chickpea (Winter *et al.* 1999; Nayak *et al.* 2010). However, in jute, due to nonavailability of sufficient number of molecular markers, no serious effort could be made so far for construction of a linkage map.

Earlier, we developed a set of 2469 SSR in jute (Mir *et al.* 2009). In the present communication, we describe 607 additional new SSR, of which 374 were used to study polymorphism between the two parental genotypes of a mapping population for fibre fineness. A preliminary framework linkage map of jute was also prepared using the available polymorphic SSR with the hope that more SSR markers will be added later to prepare a high-density map, which can then be used in conducting QTL analysis for agronomically important traits in jute. Apart from this, some functional markers were also identified through similarity search using SSR containing genomic sequences from jute against cotton EST with known function. Therefore, the objective of the present study was to (i) develop additional new SSR markers (including identification of some functional markers) to enrich the repertoire of SSR markers already available in jute; (ii) to assess polymorphism of these markers between parental genotypes of a mapping population for fibre fineness; and (iii) to construct a framework linkage map in jute using a population of 120 F_6 recombinant-inbred lines (RIL).

Materials and methods

Plant material

A RIL mapping population for fibre fineness was derived from a cross between two jute genotypes of *C. olitorius* (JRO 524 and PPO4), for construction of a genetic linkage map to be used for QTL interval mapping. The mapping population was developed at Central Research Institute for Jute and Allied Fibres (CRIJAF), Barrackpore, India, following single seed descent (SSD) method and consisted of 120 F_6 RIL. Of the two parents used for mapping population, JRO 524 (coarse fibre) was derived from the cross Sudan green × JRO 623, whereas PPO4 (fine fibre and high tensile strength) was a selection from the jute accession OIJ-154 maintained at CRIJAF.

Out of a large collection (~2600) of jute genotypes maintained at CRIJAF, a set of 140 diverse jute genotypes belonging to *C. olitorius* was used to conduct polymorphism survey using a set of newly developed 30 SSR.

DNA extraction

DNA was extracted from 10-day-old seedlings following modified CTAB method (Saghai-Maroof *et al.* 1984) and was purified by phenol : chloroform extraction followed by quantification using UV-spectrophotometer (model UV5704SS, Hyderabad, India).

Simple sequence repeat-enriched DNA libraries

During the present study, four SSR enriched libraries ($(AC)_n$, $(AG)_n$, $(AAC)_n$ and $(AAG)_n$), earlier developed on contract by Genetic Identification Service (GIS; Chatsworth, USA) using genomic DNA of *C. olitorius* cv. JRO 524 were utilized (a part of these libraries was also used by us earlier; Mir *et al.* 2009). Transformation, selection of recombinants, isolation of plasmid DNA and sequencing of the cloned inserts were performed at Interdisciplinary Centre for Plant Genomics and Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi, India. The details of the methods used for developing SSR from these SSR-enriched libraries were described earlier (Mir *et al.* 2009).

Sequence data assembly, SSR mining, classification and designing of primers

The cloned sequences were processed using Phred (Ewing *et al.* 1988) and trimmed to exclude low quality and vector sequences by Cross-Match (P. Green, unpublished; University of Washington Genome Center, http://www.genome.washington.edu/UWGC). Sequences with Phred score >20 were assembled using PHRAP (http://www.phrap.org) into contigs, whereas other sequences remained as singletons. Contigs and singletons were further used for SSR mining using web-based SSRIT software (http://www.gramene.org/db/markers/ssrtool); SSR that were only ≥12 nucleotide long were selected. SSR structure was defined in terms of four categories: (i) simple repeats of the form $(N_1N_2)_X$ or $(N_1N_2N_3)_X$; (ii) compound repeats of the form of $(N_1N_2)_X(N_3N_4)_y$ or $(N_1N_2N_3)_X(N_4N_5N_6)_y$ having two or more adjoining repeat motifs; (ii) perfect repeats that do not have spacers between motifs; and (iv) imperfect repeats of the form $(N_1N_2)_X(N_3)_X(N_1N_2)_X$ having spacers between motifs that were repeated several times. Using the web-based program Primer 3 v 0.4.0 (http://frodo.wi.mit.edu/primer3/), primers were designed, that were later synthesized by IDT® (Integrated DNA Technologies, Coralville, USA).

Similarity search analysis for functional SSR

For identification of clones containing functional SSR (SSR within genes), BLASTn and BLASTx search was performed against nonredundant EST and protein databases of cotton available at NCBI. Blast results showing *E*-value less than −10 were considered good match.

PCR amplification for identification of polymorphic SSR and genotyping

A set of newly developed 374 SSR were screened for polymorphism between two parental genotypes (JRO 524 and PPO4) of the fibre fineness mapping population. Only 66 polymorphic SSR were available. Due to a high segregation distortion, 33 of the above 66 SSR and another set of 20 polymorphic SSR (19 SSR developed by us earlier (Mir *et al.* 2009) and a solitary SSR (HK-60) reported by Akter *et al.* (2008)) were used for genotyping the mapping population.

A set of 30 SSR, that showed amplification when tested on the two parental genotypes (JRO 524 and PPO4) of the fibre fineness mapping population were also used for genotyping 140 diverse lines of *C. olitorius.* PCR amplification was conducted using a total volume of 20 μL containing 50 ng of extracted genomic DNA (2 μL), 2 μM each primer (4 μL), 200 μM dNTPs (0.5 μL), 1× PCR buffer (2 μL), 0.5 unit of *Taq* polymerase (0.1 μL), and 11.4 μL double distilled water. DNA was amplified in Applied Biosystems Veriti 96 well thermal cycler (California, USA) under the following protocol: 94°C for 5 min; 36 cycles of 94°C for 1 min, 50°C for 1 min (depending on the T_m of the primer), 72°C for 1 min and final extension at 72°C for 10 min. For separation of the PCR products, 6% nondenaturing polyacrylamide gel in 0.5× TBE was used in a CBS Scientific electrophoresis unit with MEGA-GEL High Throughput Vertical Unit of CBS Scientific Co. (CBS Scientific, Del Mar, USA). To the running buffer (0.5× TBE), 100 μL ethidium bromide was added and gels were subjected to prerun for 2 h at 250–300 V for a uniform distribution of ethidium bromide throughout the gel. After the prerun, the PCR products were loaded onto the gel and separated at 250–300 V for 2 h. The gels were viewed under High Performance UV Transilluminator (Ultra Violet Products, Cambridge, UK). Polymorphic SSR bands were identified and scored manually from the gel pictures.

Polymorphic information content

Polymorphic information content (PIC) was calculated using the following formula: $\text{PIC} = 1 - \Sigma\,(P_i)^2$, where P_i is the proportion of genotypes carrying the *i*th allele (Botstein *et al.* 1980).

Map construction

Linkage maps were constructed using MAPMAKER v3.0 (Lander *et al.* 1987) with the 'GROUP' command. Mapping was done at a LOD of 3.0 and a maximum of 50% recombination. Marker position within a linkage group was determined with the 'RIPPLE' command and the best marker order of the linkage group was identified using the 'GROUP' command. Centimorgan units were calculated using the Kosambi's mapping function (Kosambi 1944).

Results

SSR enriched libraries

Four independent SSR enriched libraries [$(AC)_n$, $(AG)_n$, $(AAC)_n$ and $(AAG)_n$] reported earlier (Mir *et al.* 2009), were used as a source of 4088 new recombinant clones that were sequenced, to select SSR-containing sequences (table 1).

Identification of SSR

Out of the above 4088 cloned sequences, 2377 sequences were assembled into 642 contigs, the remaining 1711 being

Table 1. SSR discovery in different SSR-enriched libraries of jute (*C. olitorius*) cv. JRO 524.

Item	Library				Total
	$(AC)_n$	$(AG)_n$	$(AAC)_n$	$(AAG)_n$	
Total number of clones sequenced	929	953	994	1212	4088
Total number of singletons	209 (23)[a]	429 (163)[a]	503 (182)[a]	570 (256)[a]	1711 (624)[a]
Total number of clones assembled in contigs	720 (159)[b]	524 (136)[b]	491 (162)[b]	642 (185)[b]	2377 (642)[b]
Number of new contigs	23 (72)[c]	11(44)[c]	26 (71)[c]	18 (41)[c]	75 (228)[c]
Total number of SSR containing sequences (singletons + contigs)	30 (49)[d]	98 (142)[d]	107 (197)[d]	158 (219)[d]	393 (607)[d]
Total number of clones with 1 SSR	16	65	51	109	241
Clones with 2 SSR	11	25	34	40	110
Clones with 3 SSR	1	6	16	7	30
Clones with 4 SSR	2	1	2	1	6
Clones with 5 SSR	–	1	2	1	4
Clones with 6 SSR	–	–	2	–	2
Total number of class-I SSR	35	76	61	69	241
Total number of class-II SSR	14	66	136	150	366

[a]Number of new singletons in parenthesis; [b]total number of contigs in parenthesis; [c]number of clones assembled into new contigs in parenthesis; [d]total number of SSR in parenthesis.

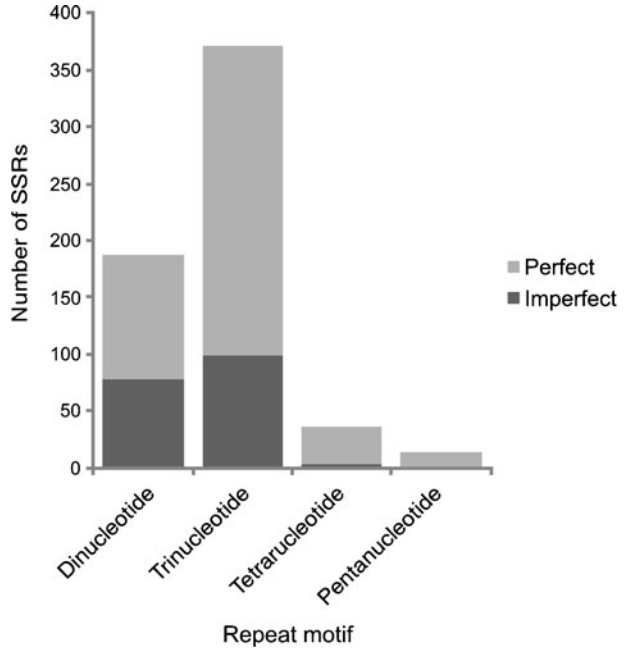

Figure 1. Frequency of SSR based on type of repeat motifs in SSR enriched library of jute.

singletons. Out of 642 contigs, 567 were repetitive with those identified earlier, leaving only 75 (11.68%) new contigs (table 1), of which 49 (63.63%) contained a total of 88 SSR (data not shown). Similarly, out of 1711 singletons, 624 (36.46%) were new (table 1), which included 344 (55.12%) singletons containing 519 SSR (data not shown). Thus, a total of 607 novel SSR were identified from 393 SSR containing sequences (contigs and singletons); these SSR included 241 (39.7%) class-I SSR and 366 (60.29%) class-II SSR (table 1).

Among the above 607 SSR, trinucleotide repeats (371, 61.12%) were predominant (figure 1) followed by dinucleotide repeats (187, 30.80%), tetranucleotide repeats (36, 5.93%) and pentanucleotide repeats (13, 2.14%). The dinucleotide, trinucleotide and tetranucleotide repeats also had imperfect repeats, which accounted for 78 (41.71%), 99 (26.68%) and 3 (2.85%) SSR, respectively; the pentanucleotide repeats comprised only perfect repeats (figure 1). As expected from enriched libraries, the most common repeats observed in the study were AG/TC (118, 63.10%) and AC/TG (46, 24.6%) among dinucleotide repeats and AAG/TTC (109, 29.38%) and AAC/TTG (48, 12.93%) among trinucleotide repeats; tetranucleotide, pentanucleotide and some compound repeat motifs were also observed (figure 2).

Designing and evaluation of primers

Out of 393 SSR containing sequences (with 607 SSR), 323 (82.18%) had more than 25 bp of flanking sequence at each of the two borders of the SSR, making them suitable for designing primers. These 323 sequences carried 417 SSR, for which primer pairs could be designed. The remaining 70 (17.81%) SSR containing sequences could not be used for designing primers, although these could be used for designing primers for anchored SSR (Singh *et al.* 2006). Primers were synthesized for only 374 SSR (for details, see

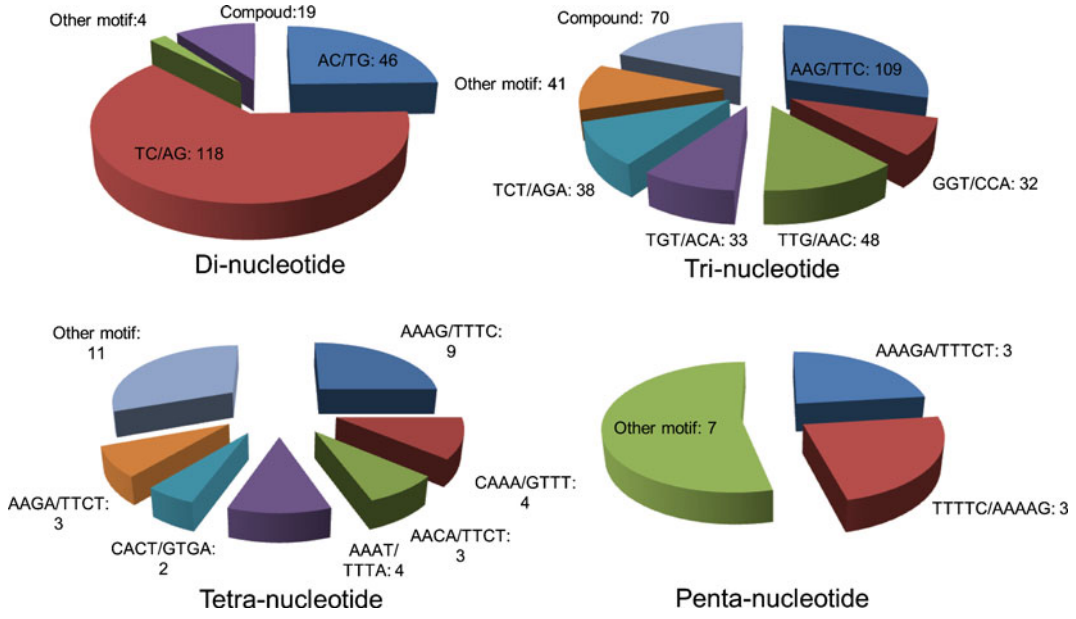

Figure 2. Distribution of different motifs of dinucleotide, trinucleotide, tetranucleotide and pentanucleotide repeats in newly isolated jute SSR.

Table 2. Detailed study of different SSR primers and the amplification and polymorphism across two jute genotypes for different SSR types.

Type	No of primer pairs screened	Average				Amplification efficiency (%)	Polymorphic between JRO 524 and PPO4 (%)
		GC content (%)	Primer length (bp)	T_m (°C)	Product size (bp)		
All SSR	374	46.52 (24–68)[a]	20 (19–25)[a]	59.75 (57.18–62.27)[a]	199.65 (106–250)[a]	320 (85.56)	66 (20.62)
Classification of SSR by SSR complexity							
Perfect SSR	232	51.85 (32.66–61.46)[a]	20.59 (20–31)[a]	59.77 (57.4–61.46)[a]	199.42 (106–250)[a]	198 (85.34)	41 (20.7)
Imperfect SSR	142	48.64 (34–55)[a]	20.63 (19–24)[a]	59.70 (58.32–60.76)[a]	200.09 (131–249)[a]	122 (85.91)	25 (20.49)
By SSR type							
Simple SSR	286	46.57 (24.5–64.32)[a]	20.61 (19–31)[a]	59.75 (57.39–61.46)[a]	199.34 (106–250)[a]	250 (87.41)	50 (20.6)
Compound SSR	88	46.31 (33.39–55.55)[a]	20.56 (19–24)[a]	59.69 (58.32–60.86)[a]	200.83 (139–249)[a]	70 (79.54)	16 (22.85)
By SSR motif							
Dinucleotide SSR	103	47.22 (34–64.32)[a]	20.66 (19–23.5)[a]	59.69 (58.49–60.58)[a]	203.12 (112–250)[a]	92 (89.32)	15 (16.30)
Trinucleotide SSR	240	46.33 (24.5–60)[a]	20.52 (19.5–26)[a]	59.77 (61.46–57.81)[a]	198.62 (106–250)[a]	200 (83.33)	46 (23)
Tetranucleotide SSR	23	45.17 (34.8–52.5)[a]	20.56 (20–23.5)[a]	59.63 (57.39–59.63)[a]	193.62 (150–238)[a]	21 (91.30)	4 (19.04)
Pentanucleotide SSR	8	48.44 (39.89–57.5)[a]	20.3 (20–21.5)[a]	59.77 (58.96–60.34)[a]	200.5 (194–236)[a]	7 (87.5)	1 (14.28)
By repeat length							
Class-I ($\geq$20 bp)	236	46.31 (24.5–64.32)[a]	20.59 (19–24.5)[a]	59.70 (57.815–60.76)[a]	200.59 (131–250)[a]	202 (85.59)	49 (24.25)
Class-II ($>$ 12 $<$20 bp)	138	46.87 (24.5–60)[a]	20.52 (19.5–26)[a]	59.80 (57.39–61.46)[a]	197.77 (106–250)[a]	118 (85.50)	17 (14.40)

[a]Figures in parenthesis represent the range of values.

table 2), 232 (62.03%) of these being perfect SSR with uninterrupted motif length; of these, 223 SSR (96.12%) were simple SSR and 9 SSR (3.88%) were compound SSR with different motifs. Out of the remaining 142 imperfect repeats, 79 (23.53%) were compound SSR and 63 (44.37%) were simple SSR.

Identification of functional markers

As many as 305 sequences containing 374 SSR were also used for BLAST search against nonredundant EST of cotton, available at NCBI. Only eight sequences showed significant similarity with cotton EST. These included one sequence (for primer *MJM1351*) belonging to the library enriched with $(AAC)_n$, three sequences (for primers *MJM1061*, *MJM1195* and *MJM1202*) belonging to the library enriched with $(AG)_n$ and four sequences (for primers *MJM1145*, *MJM1158*, *MJM1217* and *MJM1220*) belonging to the library enriched with $(AAG)_n$. Functional analysis of these eight EST showed that these EST encode the following proteins: 24-sterol C-methyltransferase, RNA-binding protein AKIP1-like, STYLOSA protein and five other predicted proteins (table 3). Out of the eight primers synthesized from the above eight SSR containing sequences only one (*MJM1195*) was polymorphic between the two parental genotypes (JRO 524 and PPO4) of the fibre fineness mapping population.

Polymorphic study

Primer pairs for 374 SSR were tested for their ability to amplify DNA and detect polymorphism between the parental genotypes (JRO 524 and PPO4) of the mapping population for fibre fineness belonging to *C. olitorius*. Of these, only 320 (85.56%) primer pairs produced distinct amplification products within the expected range of size; 66 (20.62%) of these also detected polymorphism. There was no correlation between successful amplification and primer length, GC content and expected product size. However, the percentage of successful amplification was highest for simple SSR and tetranucleotide repeats (table 2). The 66 polymorphic SSR included of 4 SSR belonging to $(AC)_n$ enriched library, 10 SSR belonging to $(AG)_n$ enriched library, 15 SSR belonging to $(AAC)_n$ enriched library and 37 SSR belonging to $(AAG)_n$ enriched library. Again, among the 66 polymorphic SSR, 41 SSR are perfect repeats (6 compound SSR and 35 simple SSR), the remaining 25 being imperfect repeats (10 compound SSR and 15 simple SSR). SSR with AG/TC repeat motif (53.3%) were more polymorphic followed by SSR with AAG/TTC (39.13%) and AGA/TCT (15.21%) repeat motifs (data not shown). Polymorphism due to length variation was observed in 35 (53%) SSR and due to null alleles in 31 (47%) SSR (data not shown). A representative banding pattern of 10 SSR showing polymorphisms on two jute genotypes and that of a solitary SSR (*MJM1182*) in 25 RIL are shown in figure 3, a&b. Sequences of primer pairs of all the 66

Table 3. Similarity search results of sequences of SSR containing jute genome DNA clones against cotton EST sequences.

Jute genomic DNA clone id	Primer name	Cotton EST acc. no.	*E*-value	Similarity	Protein product of cotton EST sequence
Jute_13_III_UF_A05_A_033.ab1	*MJM1195*	EV492961.1	3.00E-25	88%	24-Sterol C-methyltransferase (*Gossypium hirsutum*)
Jute_13_IV_UF_G08_A_056.ab1	*MJM1202*	DT557703.1	2.00E-57	85%	*Populus trichocarpa* predicted protein, mRNA
Jute_13_VII_UF_D05_A_042.ab1	*MJM1061*	DW236257.1	2.00E-18	91%	Predicted protein (*Populus trichocarpa*)
Jute_14_IV_UF_E06_A_039.ab1	*MJM1217*	ES816810.1	5.00E-77	80%	RNA-binding protein AKIP1-like (*Solanum tuberosum*)
Jute_14_IV_UF_G11_A_084.ab1	*MJM1220*	ES793265.1	5.00E-52	83%	*Populus trichocarpa* predicted protein, mRNA
Jute_14_VIIIrep_UF_G11_A_084.ab1	*MJM1145*	DW516241.1	9.00E-17	83%	Hypothetical protein (*Vitis vinifera*)
Jute_14_V_UF_C06_A_038.ab1	*MJM1158*	DW500959.1	3.00E-26	89%	Predicted protein (*Populus trichocarpa*)
Jute_11_VIIIR_D04_A_030.ab1	*MJM1351*	DT549122.1	1.00E-89	87%	STYLOSA protein (*Antirrhinum majus*)

polymorphic SSR are listed in table 1 in electronic supplementary material at http://www.ias.ac.in/jgenet/.

Polymorphic survey of SSR on diverse jute germplasm

A set of 30 SSR primers, all showing amplification when tested on the two genotypes (JRO 524 and PPO4) of the fibre fineness mapping population were used to assess polymorphism in 140 diverse jute genotypes belonging to the cultivated species *C. olitorius*. As many as 23 SSR markers detected polymorphisms amongst 140 diverse jute genotypes (table 4). For 30 SSR loci, a total of 66 alleles were amplified with an average number of 2.2 alleles per locus (range 1–4). The PIC values for individual SSR ranged from 0 to 0.386 with an average of 0.135. Most of the polymorphic SSR loci contained dinucleotide (39.13%) and trinucleoide (52.17%) repeats and only two (8.7%) had tetranucleotide and pentanucleotide repeats.

SSR linkage maps

Due to a very high degree of segregation distortion in jute, the genotypic data of only 53 SSR using the mapping population for fibre fineness could be used for the construction of linkage map. Two SSR (*MJM1277* and *MJM1288*) had duplicate loci, so that 55 SSR loci were identified. Significant deviation ($P < 0.05$) from the expected Mendelian segregation ratio (1:1) was observed at 35 (66.63%) of the 55 SSR loci. A total of 36 SSR loci including 22 of the 35 SSR loci showing segregation distortion and 14 of the remaining 20 SSR loci (showing no segregation distortion) were mapped on six linkage groups (figure 4). The remaining 19 loci could not be mapped as they showed no linkage among themselves or with the 36 mapped loci. The six linkage groups were designated as LG1 to LG6 and covered a total genetic distance of 784.5 cM, with the smallest linkage group of 53.1 cM and the longest being 216.3 cM. The average marker distance between adjacent markers in the map is 21.8 cM.

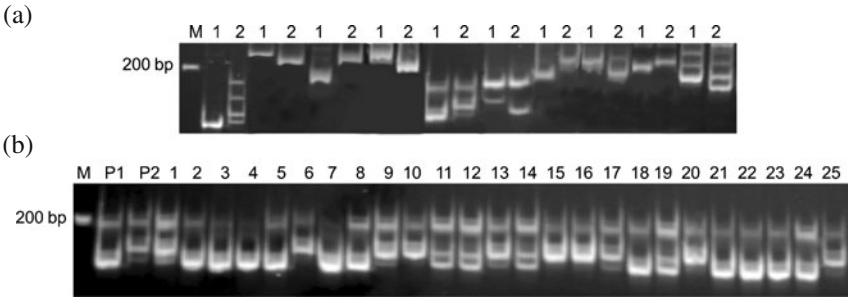

Figure 3. (a) Amplification pattern of 10 SSR (*MJM1305*, *MJM1150*, *MJM1134*, *MJM1120*, *MJM1182*, *MJM1262*, *MJM1401*, *MJM1130*, *MJM1148*, *MJM1140*), showing polymorphism between the two parental genotypes (JRO 524 (1), PP04 (2)) of jute (*C. olitorius*); M, 100-bp ladder. (b) Amplification pattern of SSR MJM1182 in a segregating RIL mapping population of jute (*C. olitorius*) derived from the cross JRO 524 × PPO4; P1, P2, inbred parents; 1–25, RILs; M, 100-bp ladder.

Table 4. SSR markers, their GenBank acc. no., primary motifs, expected product sizes, allele numbers (N_a) and polymorphic information content (PIC).

SSR name	GenBank acc. no.	Primary motif	Product size	N_a	PIC
MJM1032	JN699746	$(tc)_{12}(tg)_{15}$	231	1	0
MJM1042	JN699748	$(ct)_{10},(ct)_7$	229	5	0.3188269
MJM1047	JN699749	$(ag)_{13},(ga)_4$	246	1	0
MJM1051	JN699750	$(tg)_{13}(ga)_{16}$	246	2	0.3523028
MJM1053	JN699751	$(ag)_{22}$	249	2	0.0325024
MJM1054	JN699752	$(ag)_{13},(ga)_{11}$	171	3	0.1217275
MJM1055	JN699753	$(ag)_5,(ga)_3,(ga)_{25}$	231	1	0
MJM1057	JN699754	$(ag)_{28}$	216	3	0.0601186
MJM1060	JN699756	$(ct)_{18},(tc)_3$	193	2	0.3634451
MJM1061	JN699757	$(tc)_{15}$	174	2	0.0425125
MJM1063	JN699758	$(tg)_3,(ga)_{13}$	171	2	0.0425125
MJM1071	JN699762	$(ag)_{16},(ga)_{13}$	247	2	0.0461017
MJM1081	JN699766	$(aca)_{13}$	187	1	0
MJM1084	JN699767	$(ttg)_9$	205	1	0
MJM1091	JN699769	$(tag)_9(gtt)_5,(ttg)_5,(ttg)_4$	151	1	0
MJM1095	JN699770	$(aaca)_8$	150	3	0.095986
MJM1099	JN699772	$(cag)_8$	197	4	0.3578446
MJM1101	JN699773	$(tgtgt)_{11}$	232	2	0.2011142
MJM1109	JN699774	$(ccg)_3,(caa)_7(cag)_3$	216	2	0.3492336
MJM1114	JN699775	$(caa)_8$	207	2	0.1948242
MJM1118	JN699777	$(gaa)_8$	192	1	0
MJM1129	JN699780	$(aga)_{41}$	210	2	0.1110805
MJM1130	JN699781	$(gaa)_{15}$	226	3	0.0567993
MJM1139	JN699788	$(gaa)_7$	201	2	0.1238546
MJM1140	JN699789	$(ttc)_{28}$	185	3	0.2362275
MJM1141	JN699790	$(aag)_7,(aga)_3$	188	2	0.2570024
MJM1142	JN699791	$(ttc)_{10}$	193	3	0.3865023
MJM1147	JN699792	$(ttc)_{12}$	182	2	0.058287
MJM1148	JN699793	$(gaa)_9,(gat)_3$	155	3	0.2208322
MJM1149	JN699794	$(aga)_{11},(aag)_9$	202	3	0.0308838

[a]Commas indicate gap between the two motifs.

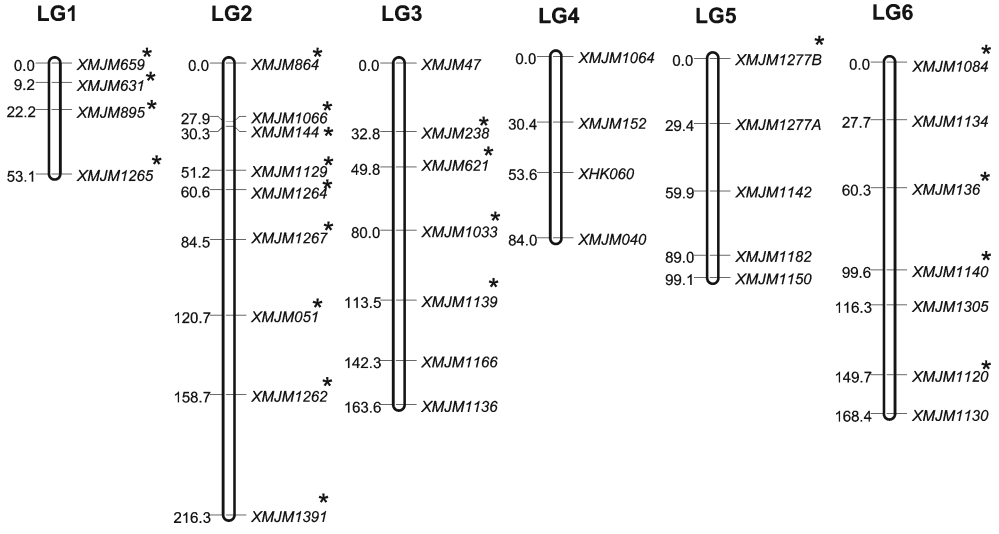

Figure 4. A genetic linkage map of jute (*C. olitorius*) prepared using 120 F_6 RIL derived from the cross JRO 524 × PPO4. The markers are shown on the right of the linkage groups, and map distances between markers are indicated in cM on the left. *Loci showed segregation distortion.

Discussion

The SSR-enriched genomic libraries used in this study proved to be an effective means of developing SSR markers. Our results showed that 56.22% (393 SSR containing sequences out of 699 new sequences) of the cloned sequences in enriched libraries carried SSR. This frequency is lower than the one reported earlier in jute (*C. olitorius*, 67.26%, Mir *et al*. 2009) but corresponds to a 200 to 500-fold enrichment when compared to the 0.1% to 0.3% of SSR identified by screening normalized genomic libraries that were not enriched for SSR (Liu *et al.* 1995; Szewc-McFadden *et al.* 1996; Kubik *et al.* 1999; Saal and Wricke 1999). The frequency of SSR-containing clones in jute was lower when compared with frequencies reported in other plant SSR-enriched libraries such as switch grass (*Panicum virgatum* L, 83.5%, Wang *et al*. 2010), sunflower (*Helianthus annus*, 89.0%, Tang *et al*. 2002), eggplant (*Solanum melongena*, 81.7%, Nunome *et al*. 2009) and Italian ryegrass (67.6%, Hirata *et al*. 2006). However, it was, significantly higher than those reported for onion (*Allium fistulosum*, 34.4%, Tsukazaki *et al*. 2007) and quinoa (*Chenopodium quinoa*, 37%, Jervis *et al*. 2008). The frequency of class-I SSR (111, 58.11%) was higher in libraries enriched with $(AC)_n$ and $(AG)_n$ motifs, whereas that of class-II SSR (286, 68.75%) was higher in libraries enriched with $(AAC)_n$ and $(AAG)_n$ motifs. These results are in agreement with those reported by us earlier (Mir *et al.* 2009).

The overall density of SSR was 1SSR/0.32 kb, which is higher than 1 SSR/0.43 kb reported earlier (Mir *et al.* 2009). As many as 152 (38.67%) sequences contained multiple SSR (366, 60.2%) with a mean density of 1SSR/0.25 kb in comparison to 1 SSR/0.23 kb in our earlier study (Mir *et al.* 2009). Remaining 241 (61.32%) sequences with individual SSR showed a much lower density of 1 SSR/0.48 kb. During the present study, frequencies of SSR with different motifs were as follows: GA (63.10%) and CA (24.6%) among dinucleotide repeats and AAG/TTC (29.38%), AAC/TTG (12.93%), TCT/AGA (10.24%), TGT/ACA (8.9%) among trinucleotide repeats, which are in agreement with those observed in *Arabidopsis thaliana* (Cardle *et al.* 2000). Among dinucleotide repeats, many more $(GA)_n$ SSR are polymorphic followed by $(CA)_n$. These results are also in agreement with the previous results obtained in switch grass (Wang *et al.* 2010), tall fescue (Hirata *et al.* 2006), perennial ryegrass (Jones *et al.* 2001) and other cereal crops.

In the present study, some of the SSR (17.82%) could not be used for designing primers, since flanking sequences within 25 bp of the 5′- and/or 3′- end(s) were truncated. Similar SSR were earlier reported in ryegrass (*Lolium perenne*, 49%, Jones *et al*. 2001) and white clover (*Trifolium repens,* 40%, Kolliker *et al*. 2001).

Out of the 66 polymorphic markers, a majority belonged to class-I SSR (24.25%) having length greater than 20 bp; perhaps in future, one should focus on the identification of SSR with >20 bp only in order to maximize polymorphism. The level of polymorphism (20.62%) observed within the species *C. olitorius* (JRO 524 and PPO4), was lower than what was reported in our earlier study in jute (50%, Mir *et al*. 2009). This may be attributed to the use of both class-I and class-II SSR in the present study, because only class-I SSR were used in the previous study (it is known that the polymorphism shown by class-II SSR is generally low). It may be noticed that at 47% of the polymorphic SSR loci, null alleles were detected during the present study, which is in agreement with earlier studies in jute where 31.1% (Mir *et al.* 2008) to 46% (Mir *et al.* 2009) null alleles were reported. Null alleles have also been reported in many other plant species; for instance, in two separate studies in wheat, null alleles were recorded at 13% (Plaschke *et al.* 1995) and 10% (Prasad *et al.* 2000) SSR loci. Null alleles generally result due to mutations in the primer binding sites (Gupta *et al*. 2003; Fraser *et al*. 2004; Rungis *et al*. 2004).

The average PIC for the SSR with dinucleotide repeats was 0.115, while the average PIC for the SSR with trinucleotide repeats was 0.149. However, there are reports that the polymorphism level in trinucleotide repeats is lower than that in dinucleotide repeats for rice (Blair *et al.* 1999) and ryegrass (Jones *et al.* 2001). The average PIC value (0.135) observed in the present study was lower than the average PIC value (0.24) reported earlier, although the average number of alleles per locus (2.20) observed in the present study was similar to the average number of alleles (2.51) reported in earlier study (Mir *et al.* 2008).

It may be recalled that out of all 374 SSR containing sequences (contigs and singletons), only eight sequences showed significant similarity with eight different EST of cotton. These EST together encode the following proteins: 24-sterol C-methyltransferase, RNA-binding protein AKIP1-like, STYLOSA protein and five other predicted proteins with unknown function. The SSR (*MJM1195*) derived from one of these sequences was polymorphic between the two parental genotypes of the fibre fineness mapping population. The remaining seven SSR may also prove to be polymorphic if used with a diverse collection of jute genotypes. Therefore, the above SSR may be used as gene-based functional markers in future molecular breeding and genetic analysis studies.

Another important observation in the present study was that out of 55 polymorphic loci that were used in the construction of linkage map, 35 loci (63.63%) deviated from the 1:1 expected ratio ($P < 0.05$) showing segregation distortion. While segregation distortion is generally believed to be greater in interspecific crosses, reaching as high as 68.5% (Paterson *et al.* 1988), levels can also be high in intraspecific crosses (Hall and Willis 2005). Deviation from the expected Mendelian segregation ratios was previously reported for several mapping populations in rice also (Harushima *et al.* 1996; Xu *et al.* 1997; for a review, see Lyttle 1991). In our analysis, loci in linkage groups 1, 2, 3, 5 and 6 exhibited segregation distortion. Distorting factors can be deleterious recessive alleles (Berry *et al.* 1995), self-incompatibility alleles (Barzen *et al.* 1995), structural rearrangements

(Quillet *et al.* 1995) or differences in DNA content (Jenczewski *et al.* 1997). In *Arabidopsis*, high level of segregation distortion was attributed to higher frequency of recombination (Liu S. S. *et al.* 1996). Whatever be the cause, segregation distortion should result from unequal transmission of specific chromosomes, or at least parts of chromosomes to the progeny (Thoquet *et al.* 2002).

Our mapping efforts did not give seven linkage groups that were expected on the basis of the haploid chromosome number of jute (i.e., $n = 7$), which indicates that additional markers are needed to cover all of the seven linkage groups and to provide complete coverage of the jute genome. Nevertheless, the map prepared in the present study is a significant step forward. More SSR markers will be used in future with the hope of further enrichment of this genetic map. SNP and DArT markers should also be developed to overcome the scarcity of molecular markers and a lower level of polymorphism in jute for preparing high density molecular map. One should also be careful while developing the mapping population, since selection of parents for developing a mapping population is critical for a successful construction of a genetic (linkage) map. Although, the parents should be genetically diverse enough to exhibit sufficient polymorphism, but they should not be too distant to exhibit either high sterility in the progenies or high levels of segregation distortion during linkage analysis (Semagn *et al.* 2006).

Research on the development and use of genomics resources in jute has lagged behind all other major crops in the world. The identification and use of molecular markers associated with QTL/genes of interest for molecular breeding is yet to be undertaken in this crop. As a first step, to realize the dream of molecular breeding for fibre fineness in jute, additional new SSR markers were developed in the present study to enrich the repertoire of molecular markers already available in jute. The genetic linkage map that was developed for jute in the present study can be enriched and used for QTL mapping of important agronomic traits and for identification of marker–trait associations. The associated markers can then be used for marker-assisted selection to breed superior jute cultivars. Moreover, functional polymorphic markers identified during the present study and those are likely to be identified in future studies will also be useful in genetic dissection of traits leading to successful molecular breeding.

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