Development and Characterization of Large-Scale Simple Sequence Repeats in Jute

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ABSTRACT

Jute is an important crop of the Indian subcontinent and comprises tossa jute (*Corchorus olitorius*) and white jute (*C. capsularis*). The yield and fiber quality of this crop remained stagnant for many years and could not be improved through conventional plant breeding. Also, no effort has been made to develop molecular markers on a scale required for marker-assisted selection (MAS) to supplement conventional plant breeding. As a first step toward deploying MAS for jute improvement, 2469 simple sequence repeats (SSRs) were developed in tossa jute (JRO 524) using four SSR-enriched genomic libraries. A random subset of 100 SSRs (25 SSRs from each library) was used to detect polymorphism between the parental genotypes of each of the two recombinant inbred line (RIL) mapping populations. The RILs are being developed from JRO 524 × PPO4 (for fiber fineness) and JRC 321 × CMU 010 (for lignin content) crosses to prepare molecular maps and conduct quantitative trait loci (QTL) analyses. Both SSR length polymorphism and ± polymorphism (null alleles, i.e., presence and absence of specific SSR) were detected; 50 SSRs detected polymorphism between the two genotypes of tossa jute, whereas 45 SSRs detected polymorphism between the two genotypes of white jute. This SSR allelic polymorphism in jute is higher than that reported in other crops and is adequate for construction of genetic maps for QTL analysis. The large-scale SSRs will also prove useful in studying genetic diversity, population structure, and association mapping.

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Abbreviations: AFLP, amplified fragment length polymorphism; MAS, marker-assisted selection; PCR, polymerase chain reaction; QTL, quantitative trait loci; RAPD, random amplified polymorphic DNA; RIL, recombinant inbred line; SSR, simple sequence repeat; STMS, sequence-tagged microsatellite site.

Jute is a dicotyledonous fiber-yielding crop, belonging to the genus *Corchorus* of the family Tiliaceae. The genus comprises 50 to 60 species, including the two fiber-yielding cultivated species, *C. olitorius* (the tossa jute) and *C. capsularis* (the white jute). While the Indo-Burma region, including South China (Kundu, 1951), is the center of origin for *C. capsularis*, Africa is the center of origin for *C. olitorius* (Roy et al., 2006). Both the cultivated species are diploid ($2n = 14$), with their genomes differing in size: 1350 Mb for *C. olitorius* and 1100 Mb for *C. capsularis* (Samad et al., 1992). These two species constitute an important crop of the South East Asian countries and Brazil, providing environment-friendly (biodegradable and renewable) ligno-cellulose fiber. Jute fiber is second in importance only to cotton and was also identified as an alternative to European hemp (*Cannabis sativa* L.) as early as the end of 18th century (Ghosh, 1983).

In India, jute is grown in the eastern region, covering an area of little more than 0.8 million ha. Its productivity of 22 quintal ha^{-1}

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gives an annual production of 10 million bales (1 bale = 180 kg) of fiber, which is 40% of the world's jute production. In addition to its traditional use as jute fiber (for making sacks, ropes, handmade clothes, wall hangings, etc.), it is now being used for manufacturing the following value-added industrial products: (i) geotextiles (http://www.jute.com/geojute.html, verified 25 June 2009) for reinforcing river embankments and for control of soil erosion, (ii) fiber-reinforced building materials, (iii) packaging materials, and (iv) paper. These additional uses add to the value of jute fiber (Islam et al., 2005).

During the decade 1993 to 2003, the productivity of jute in India improved only marginally, from 19 × 10^2 kg ha^{-1} to 21 × 10^2 kg ha^{-1} (Sinha et al., 2004). This is largely because of inadequate efforts made toward breeding new and superior jute cultivars. This is obvious from the fact that only 12 improved cultivars of white jute and 10 improved cultivars of tossa jute have been developed in India (see Roy et al., 2006). Therefore, there is a need for developing new cultivars of jute with improved fiber yield and fiber quality through intensive breeding efforts. Jute is a self-pollinated crop, but it can have a considerable amount of natural outcrossing—as high as 32% at the minimum isolation distance of 0.5 m (Basak and Paria, 1975; Datta et al., 1982). This necessitates controlled pollination during breeding programs and also during the development of mapping populations through single-seed descent.

It has been recognized that the use of molecular-marker approaches, particularly marker-assisted breeding, may accelerate the pace of achieving the targeted goals in any crop-breeding program. This would be particularly true for quality traits, including fiber fineness and lignin content in jute, because these complex quantitative traits are difficult to score. Molecular markers and quantitative trait loci (QTL) mapping can help in a study of genetic architecture, leading to improvement of these traits through marker-assisted selection (MAS). The random amplified polymorphic DNA (RAPD), chloroplast–simple sequence repeat (SSR), nuclear sequence-tagged microsatellite site (STMS)–SSR, and amplified fragment length polymorphism (AFLP) markers have already been used to assess genetic diversity within the available jute germplasm (Qi et al., 2003a,b; Hossain et al., 2002, 2003; Basu et al., 2004; Roy et al., 2006). The SSRs have become the markers of choice because of several desirable features, including their abundance, multiallelic and codominant nature, high level of reproducibility, and cross-species transferability (for reviews, see Gupta et al., 1996; Gupta and Varshney, 2000). However, no concerted effort to develop SSRs (or markers of any other type) on a large scale has ever been undertaken in jute. Such an effort is necessary for developing molecular maps to be used for QTL interval mapping.

We previously developed 45 genomic SSRs and used them for genetic diversity analyses in the two cultivated species of jute (Mir et al., 2008). This study was extended further to large-scale isolation and characterization of SSRs, leading to the development of 2469 SSR markers for use in the construction of framework linkage maps and QTL interval mapping. This marks the beginning of our long-term plan for identification of marker-trait associations leading to MAS for rapid and precise breeding of superior jute cultivars.

MATERIALS AND METHODS

Plant Material

During the present study, four inbred lines of jute were used. These included two genotypes of *C. olitorius* (JRO 524 and PPO4) and two genotypes of *C. capsularis* (JRC 321 and CMU 010). These four inbreds included three cultivars and one mutant (CMU 010), which are the parents of the two mapping populations (under development), one each for fiber fineness and lignin content—the two important fiber-quality traits. The seed material of these four genotypes was procured from Central Research Institute for Jute and Allied Fibers (CRIJAF), Barrackpore, India. Certain characteristics of these four genotypes are presented in Table 1.

DNA Isolation

Seeds of each of the four genotypes were germinated in the laboratory. DNA was extracted from 10-d-old seedlings following modified CTAB method (Saghai-Maroof et al., 1984). The DNA was purified by RNaseA treatment, followed by phenol:chloroform extraction. The purified DNA was quantified with a UV-spectrophotometer (model UV5704SS, Electronic Corporation of India Limited, Hyderabad, India).

Construction of Simple Sequence Repeat–enriched DNA Libraries

Four SSRs ($[AC]_n$, $[AG]_n$, $[AAC]_n$ and $[AAG]_n$) were selected for the construction of four independent SSR-enriched genomic libraries. This selection was based on the information that these SSRs are most abundant in plant genomes (Gupta and Varshney, 2000), particularly in jute (Mir et al., 2008). The enrichment for SSRs was done separately for each of the four SSRs, using genomic DNA of *C. olitorius* cv. JRO 524. The work for constructing four SSR-enriched genomic libraries was outsourced to Genetic Identification Service (GIS; Chatsworth, CA). The protocol used by GIS is as follows: the genomic DNA was partially digested with a mixture of seven blunt-end restriction endonucleases (*Rsa*I, *Hae*III, *Bsr*B1, *Pvu*II, *Stu*I, *Sca*I, and *Eco*RV). Size-separated DNA fragments, ranging from 300 to 750 bp, were ligated with adapters and separately enriched for each specific SSR motif using biotinylated capture molecules (CPG, Lincoln Park, NJ). The captured fragments were amplified and digested with *Hin*dIII to remove the adaptors, and the fragments were cloned in pUC19 vector. GIS supplied ligation mixtures of all four libraries obtained using this protocol. Using each of the above four ligation mixtures separately, transformation of *Escherichia coli* strain DH10B (Invitrogen) was done by electroporation using MicroPulser (BIO-RAD, Gladesville, New South Wales, Australia). The recombinant clones were used for plasmid isolation and sequencing of cloned inserts.

Plasmid Isolation, Sequence Data Assembly, and Designing of Primers for Simple Sequence Repeats

Plasmid DNA of 4224 recombinant clones (1056 clones from each of the four libraries) was isolated using QIAprep Spin Miniprep kit (QIAGEN GmbH; Hidden, Germany) on a TECAN robotic platform. The cloned inserts were sequenced using ABI 3700 automated DNA analyzer at the University of Delhi South Campus, New Delhi. The sequences were processed through base calling and quality control using PHRED (Ewing et al., 1998) and trimming (for low-quality and vector sequences) by Cross Match (http://www.phrap.org/phrap_documentation.html, verified 24 June 2009). In this manner, 3885 good-quality clone sequences (Phred score >20) became available; these were assembled using PHRAP into 767 contigs (2663 clone sequences) and 1222 singletons. Contigs as well as singletons were used for mining ≥12 nucleotide-long SSRs. Primers were designed for 923 Class I and 725 Class II SSRs using Web-based SSRPrimer software (Jewell et al., 2006).

Identification of Polymorphic Simple Sequence Repeats

A set of 100 SSRs (25 SSRs from each of the four enriched libraries) was screened for polymorphism on each of the two pairs of parental genotypes of two mapping populations (under preparation), one each for fiber fineness (*C. olitorius*) and lignin content (*C. capsularis*). These mapping populations are being developed from the crosses JRO 524 × PPO4 (for fiber fineness) and JRC 321 × CMU 010 (for lignin content) for preparation of molecular maps and subsequent QTL interval mapping. No variation within a genotype was detected when more than one plant from the same genotype was used for the same assay.

Table 1. Summary of characteristics of four jute (*Corchorus*) genotypes used for developing two mapping populations.

Species	Genotype	Characteristics
C. olitorius	(i) JRO 524	Coarse fiber, selection derived from Sudan green × JRO 623
	(ii) PPO 4	Fine fiber, high tensile strength and low lignin content, derived from CRIJAF accession OIJ-154
C. capsularis	(i) JRC 321	High lignin content, selection from indigenous germplasm Hewti
	(ii) CMU 010	Low lignin, mutant

RESULTS AND DISCUSSION

Simple Sequence Repeat–Enriched Genomic DNA Libraries

The results of sequencing and SSR enrichment among 3885 clones belonging to four SSR-enriched libraries are presented in Table 2. As mentioned earlier, these 3885 sequences were assembled into 1989 sequences (767 contigs and 1222 singletons), which were used for SSR-mining. As many as 1338 sequences (67.26%) contained SSRs, a majority (531 or 39.73%) containing two dinucleotide SSRs, namely, $(AC/TG)_n$ (267; ~20%) and $(AG/TC)_n$ (264; 19.73%). The frequencies of sequences containing trinucleotide repeats were relatively low ($[AAC/TTG]_n$ [150; 11.21%] and $[AAG/TTC]_n$ [175; 13.08%]). The remaining 35% (482) of sequences contained tetranucleotide repeats and other higher-order SSR repeats. This suggested that the SSR-enrichment process was more successful with dinucleotide repeats than with trinucleotide repeats.

Characteristics of Simple Sequence Repeat–Containing Clones and the Identification of Simple Sequence Repeats

The above 1338 (67.26%) SSR-containing sequences carried 2469 SSRs. This high proportion of SSRs is desirable for the discovery of SSRs and development of SSR markers. Traditional colony hybridization-based approaches of SSR-enrichment of the genomic libraries were found to give very low frequencies of SSRs (see Mir et al., 2008). A majority (~59%) of the SSR-containing sequences had a solitary SSR, whereas two or more than two SSRs were available in the remaining ~41% of sequences (Table 2). On the basis of the sequences sampled during the present

Table 2. Details of sequencing and simple sequence repeats (SSRs) detected in 1989 sequences (singletons + contigs) belonging to SSR-enriched genomic libraries of *C. olitorius cv. JRO 524.*

Description of clone sequences and SSRs	Library				Total
	$(AC)_n$	$(AG)_n$	$(AAC)_n$	$(AAG)_n$	
Total no. of clones sequenced	987	950	982	966	3885
Total singletons	226	266	360	370	1222
No. of clones assembled in contigs	761	684	622	596	2663
Total contigs	197 (2–35)†	205 (2–24)†	168 (2–34)†	197 (2–35)†	767
Total no. of SSR-containing sequences (singletons + contigs)	322 (544)‡	317 (535)‡	328 (751)‡	371 (639)‡	1338 (2469)‡
Total no. of sequences (singletons + contigs) with single SSR	197	212	147	231	787
Total no. of sequences with >1 SSR	125	105	181	140	551
-Sequences with 2 SSRs each	83	58	77	95	313
-Sequences with 3 SSRs each	23	19	48	20	110
-Sequences with 4 SSRs each	7	11	21	5	44
-Sequences with >4 SSRs each	12	17	35	20	84
Total no. of Class I SSRs (>20 bp)	339	273	219	247	1078
Total no. of Class II SSRs (12–20 bp)	205	262	532	392	1391
Simple SSRs					
Dinucleotide	470	402	33	57	962
Trinucleotide	38	58	666	500	1262
Tetranucleotide	29	44	33	43	149
Pentanucleotide	7	31	19	39	96

†Range of no. of clones per contig.

‡The figures in parentheses are the total no. of SSRs, because a single sequence can have more than one SSR.

study, the overall density of SSRs was 1 SSR/0.43 kb in jute. This density is much higher than the density of 1 SSR/1.70 kb reported in our earlier study in jute (Mir et al., 2008). The observed higher density of SSRs during the present study may be biased upward as a result of the study of genomic sequences sampled through the process of SSR enrichment. Further, a separate analysis of the jute sequences containing multiple SSRs showed a mean density of 1 SSR/0.23 kb, suggesting that ~41% of the SSRs are organized more closely in the form of clusters forming islands of SSRs in the jute genome, whereas the remaining ~59% of individual SSRs with a much lower density (1 SSR/0.56 kb) are separated by larger inter-SSR sequences in the jute genome.

A further examination of the SSR-containing sequences suggested that they contained all four types of SSRs used for the preparation of SSR-enriched libraries. A few other types of di-, tri-, and tetranucleotide repeats ($[AT]_n$, $[TCT]_n$, $[AGA]_n$, $[ACA]_n$, $[AGC]_n$, $[TTTC]_n$, $[AAGAA]_n$, etc.) were also available (Table 3). This occurrence of additional repeat motifs might have resulted because of chance and might indicate their abundance in the jute genome. Of 2469 SSRs detected, 1078 were Class I and the remaining 1391 were Class II SSRs (Table 2). The proportion of Class I SSRs was higher in the libraries enriched with dinucleotide repeats, whereas Class II SSR had a higher proportion in libraries enriched for trinucleotide repeats. This suggested that length of the repeat motif of the SSRs is inversely proportional to the total length of SSRs (Fig. 1). This also means that short microsatellites are more frequent than the long SSRs, which agrees with earlier reports in other plant species (Morgante et al., 2002; Grover et al., 2007).

An analysis of repeat motifs in SSRs shows that nearly half (51%) of the total SSRs discovered during the present study included trinucleotide repeats, followed by di- (39%), tetra- (6%), and pentanucleotide (4%) and other higher order repeats (Table 3). This is in agreement with the findings of our earlier study in jute as well as studies involving other plant species (Morgante et al., 2002; Mir et al., 2008).

Table 3. Frequencies of different simple sequence repeats (SSRs) identified in sequences of representative clones from four SSR enriched libraries (figures in italics represent frequencies of enriched SSRs).

Motif	Library designation			
	$(AC)_n$	$(AG)_n$	$(AAC)_n$	$(AAG)_n$
Dinucleotide				
$(AC)_n/(GT)_n$	*413*	27	21	11
$(AG)_n/(CT)_n$	28	*363*	11	46
Others	29	12	1	–
Trinucleotide				
$(AAC)_n/(TTG)_n$	7	1	*350*	4
$(AAG)_n/(TTC)_n$	–	18	14	*287*
Others	31	39	302	209
Tetranucleotide				
$(AAAG)_n/(TTTC)_n$	2	7	1	10
$(AGAA)_n$	–	12	–	5
$(CTCC)_n$	–	4	1	–
Others	27	21	31	28
Pentanucleotide				
$(AAAAG)_n/(TTTTC)_n$	–	4	–	7
$(CTCTT)_n$	–	9	–	6
Others	7	18	19	26
Total	544	535	751	639

Simple Sequence Repeat Polymorphism

Primers were designed for 1648 SSRs (923 Class I and 725 Class II SSRs) derived from the four SSR-enriched libraries. A random subset of primer pairs for 100 SSRs (25 SSRs × 4 SSR-enriched libraries) was used for detecting polymorphism between and within two pairs (four genotypes) of parental genotypes of the two mapping populations (under preparation), one belonging to *C. olitorius* and the other belonging to *C. capsularis* (Table 4). Ninety-eight of the 100 SSRs were found to be polymorphic. Polymorphism due only to length variation was observed in 52 SSRs, due only to null alleles, in 15 SSRs, and due to both length variation and null alleles, in 31 SSRs (Fig. 2). Polymerase chain reaction (PCR) amplifications were repeated to exclude the possibility of failure of PCR reactions as the cause of recorded null alleles. As many as 59 SSRs exhibited polymorphism both at interspecific and intraspecific levels. The remaining 39 SSRs exhibited polymorphism either at the interspecific level (23 SSRs) only or at the intraspecific level only (9 SSRs in *C. olitorius*; 7 SSRs in *C. capsularis*). Although the percentage of polymorphic SSRs involving both the species was 98% during the present study, at the interspecific level, only 50% of SSRs were polymorphic in *C. olitorius* and 45% in *C. capsularis*. The proportion of SSRs detecting length polymorphism (84.7%) was a little less than double that exhibiting ± polymorphism (46.9%). The high level of polymorphism among as few as four genotypes during the present study is a bit surprising, but is partly because of the use of four genotypes belonging to two different species. In three earlier studies, including our own, SSR polymorphism in jute was found to range from 91.11 to 100%, although the number of genotypes in these earlier studies was higher, ranging from 10 to 81 (Roy et al., 2006; Mir et al., 2008; Akter et al., 2008). This suggested that jute is unique in exhibiting a higher level of SSR polymorphism, particularly when examined at both intraspecific and interspecific levels. In other materials, a fairly wide range of polymorphism has been reported (e.g., *Cicer arietinum* [33%; Sethy et al., 2006], other *Cicer* species [79%; Burstin et al., 2001], *Pisum sativum* [72%; Hüttel et al., 1999], bread wheat [*Triticum aestivum* L.] [90.45%;

Stachel et al., 2000]). The results of the present study also confirmed high transferability of the *C. olitorius* SSRs to *C. capsularis*, suggesting their possible cross-species use and also their use in comparative genomic analysis. The mean number of alleles per SSR locus involving both species was 2.56 (range 1 to 4); in *C. capsularis* it was 1.45 (range 1 to 2), which was slightly lower than a mean number of 1.50 alleles per locus (range 1 to 2) in *C. olitorius*. A relatively higher mean number of alleles in *C. olitorius* suggested a higher level of genetic diversity among *C. olitorius* genotypes than in *C. capsularis* genotypes, which agrees with the results of our earlier studies involving SSR and AFLP analyses in jute (Mir et al., 2008; Das et al., 2008).

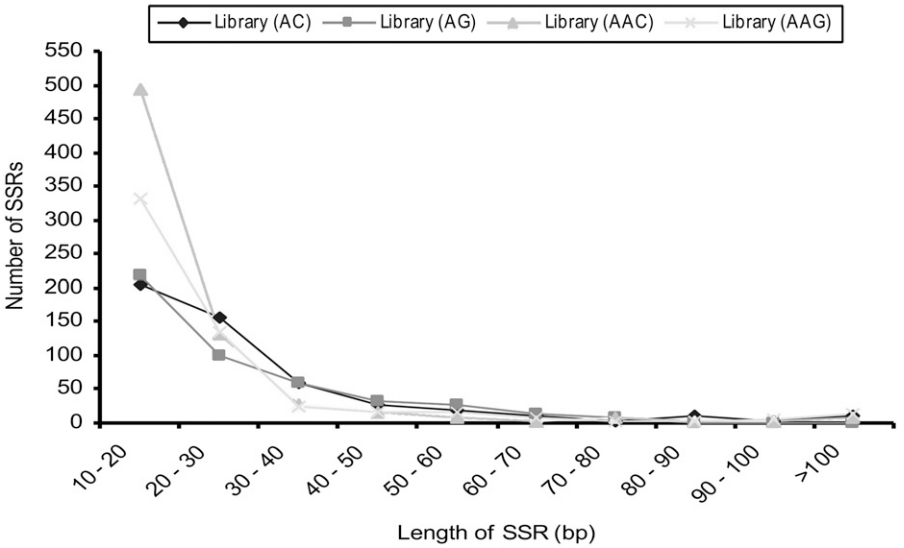

Figure 1. Relationship between the frequency and length of simple sequence repeats (SSRs) containing dinucleotide and trinucleotide repeat motifs in four SSR-enriched genomic libraries of jute (*Corchorus*).

CONCLUSIONS

The large-scale development of SSRs achieved during the present study should prove useful for detecting DNA polymorphism and construction of molecular genetic maps in jute. These maps will be used for QTL interval mapping and a variety of other studies, including map-based cloning of genes. This will facilitate application of MAS, leading to precise breeding in jute in a cost-effective and time-saving manner.

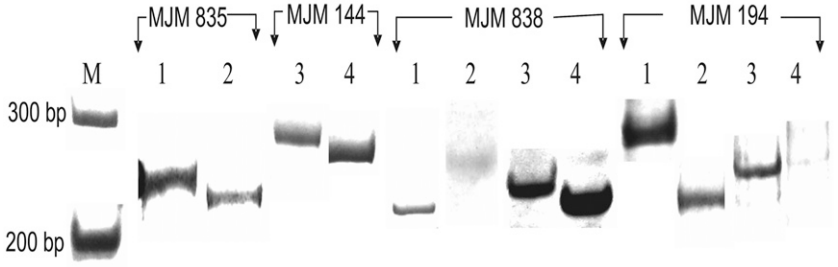

Figure 2. Representative polymerase chain reaction amplification pattern showing length and ± type of polymorphism because of the four simple sequence repeat (SSR) primer pairs (MJM 835, MJM 144, MJM 838, MJM 194) in two genotypes of *C. capsularis* (1 = JRC 321, 2 = CMU 010) and *C. olitorius* (3 = JRO 524, 4 = PP04), which are the parents of two mapping populations under preparation. Length variation is shown by SSR MJM 835 and MJM 194 between genotypes of *C. capsularis*, whereas SSR MJM 144 and MJM 838 show length variation between genotypes belonging to *C. olitorius*. Similarly, ± type of polymorphism was shown by SSR MJM 838 and MJM 194 between genotypes belonging to *C. capsularis* and *C. olitorius*, respectively.

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Table 4. Details of simple sequence repeat primer used for the study of DNA polymorphism.

Serial no.	Primer name	Motif		Primer sequence (5′-3′)	No. of alleles	Product size
						bp
1	MJM† 6	$(tg)_{12}$	F	AATTACAAACTGGAGGTGGTC	2	143
			R	AATGGAATGGAGCTAACATCT		
2	MJM 9	$(gt)_{14}$	F	GTAGACATGTTGTCCATTCGT	2	212
			R	CCAACCTACTTAGTTGTGTGC		
3	MJM 15	$(ac)_{12}$	F	CTTAGATACCTGTGGCTTGTG	2	346
			R	CTTGTCATGGATGGTGTAAGT		
4	MJM 18	$(tg)_{11}$	F	TGTGTTCACTCACTCAGCAGG	2	371
			R	AAAGGCAATAAATACCCATCCA		
5	MJM 22	$(tg)_{14}$	F	TTCCAGATCCTGTTTCACCAC	2	355
			R	ATGAGCCCTACATGGCCTACT		
6	MJM 34	$(tg)_{11}$	F	TCAAGAGCAGACGAAATGCTT	3	392
			R	TTGGACAGATCTCCTGGTCTTT		
7	MJM 37	$(tc)_{18}$	F	ACCACGTGGAGAGTCATCTTG	2	345
			R	CGAACCTGACCTGGTATTGAA		
8	MJM 39	$(tc)_{18}$	F	AGAGTGGCTGGTGCATACCT	3	287
			R	GCCACTGTCTGTGTGGAGTTT		
9	MJM 44	$(ca)_{11}$	F	GTTCCAGGTTCACCTGTTCAA	2	399
			R	TGATTGGTGGACAAGAATCCTA		
10	MJM 47	$(tg)_{13}$	F	ATTCGTTGCCACGTGATATTC	3	195
			R	CAAAGACCTACATTTGACCCA		
11	MJM 52	$(gt)_{14}$	F	ATTACGCCAAGCTTCCAGTTC	3	312
			R	TTTCCACAATCAAGGACGAAG		
12	MJM 59	$(tg)_{11}$	F	TATCTCCCATTGAACCTGCAC	2	112
			R	AGCAACCTTGGGAACATT		
13	MJM 65	$(tg)_{13}$	F	CGCTTGACATGATTACGCC	2	290
			R	TCAAGAAAGCAAAGAGTGGGA		
14	MJM 78	$(ac)_{18}$	F	GCCAAGCTTCAGGCAATTAAA	2	377
			R	CCCACTAGAACTGTCAATAAAGT		
15	MJM 80	$(ac)_{14}$	F	CTTTCCCTGTTTATTGTGCCA	2	186
			R	ACAAAGGACAACCAAGGCTCT		
16	MJM 562	$(ac)_{12}$	F	GATGCACTGATTGTGGGAGAT	3	385
			R	TTAACCCAAACATGTGGTCAAA		
17	MJM 563	$(ca)_{26}$	F	CTTGGTTGTGGTGGTTGAACT	2	318
			R	AAACCCACCATAGTTGTGTGC		
18	MJM 565	$(ac)_{34}$	F	CTCCTTGGTTGTGGTAGTGGA	3	293
			R	GAGTGCATACACGAGTGCAAA		
19	MJM 568	$(ca)_{15}$	F	TTTGCATAACCAATGACACCA	2	243
			R	TGGTATGTGATTATGGCAGGG		
20	MJM 569	$(gt)_{23}$	F	CGCCAGAGAAGCAAATGTAAC	2	386
			R	TAGAGCTCACCAGAGACTGCC		
21	MJM 571	$(ca)_{43}$	F	TGCTATCAGATTCCATTTGGC	3	382
			R	TGCCATTGGTTTGAGTTATGC		
22	MJM 574	$(tg)_{13}$	F	TCCCTAGCTTCATCTCCCAAT	4	336
			R	AAGAGATAGAGGGCAGCCAAC		
23	MJM 575	$(ac)_{28}$	F	TTGGCCCTCTCATATTTGTTG	3	295
			R	TACGTTGAGTGCAAACACGAG		
24	MJM 577	$(gt)_{13}$	F	TGAATTTATGCAGTGGACATGA	3	167
			R	CAGGGCAAGGATCAATAAACA		
25	MJM 579	$(ac)_{14}$	F	CAGGGCAAGGATCAATAAACA	2	169
			R	TGAATTTATGCAGTGGACATGA		
26	MJM 99	$(tc)_{26}$	F	TTCATACTTGGTTGCAGTTTC	2	268
			R	TACCATATCATCCAAGTCGTC		
27	MJM 101	$(ga)_{10}$	F	TATGGTTCTACAGCAAGAAGG	2	143
			R	ACAAGTCCACAAGCATCATT		
28	MJM 104	$(ga)_{18}$	F	CATCTCAGTATCCTTGGTCTG	3	395
			R	CACATGCTAAGCCAGATAAAC		
29	MJM 113	$(ct)_{11}$	F	CAGATCAGACTCAAACTCAGC	3	360
			R	CAACAAGAACCAAACAGAAAG		
30	MJM 117	$(tc)_{13}$	F	CCTATGCATATTCCATCTCTG	3	367
			R	GAGACTGACTACTTGGCACTG		
31	MJM 123	$(ga)_{12}$	F	TGGGCATATGATAGGTTTCTA	3	101
			R	ATCTCACTAAAGAGGATCCGA		
32	MJM 126	$(ct)_{14}$	F	TACTAACACCCAATTTAGCCA	2	360
			R	GTGAGAGGAGAGAGTAGGGAG		
33	MJM 129	$(gt)_{11}$	F	CCTAACTCGTATCCCATTCTT	3	273
			R	CCCAGTCTCTCTCTCTCTCTC		
34	MJM 136	$(ga)_{25}$	F	CATAAGGGCATGTGACTAGAG	3	180
			R	AGATGAGGTATGCTCTTCTGTT		
35	MJM 139	$(ag)_{14}$	F	TGTCAAGAGTTGTCCCTCTAA	2	218
			R	GGGAAAGAAAGGTAAACGATA		
36	MJM 141	$(ag)_{12}$	F	CCCTTGAAATAAGCCTCTAAC	2	360
			R	AATCATACCGAATTCACACAC		
37	MJM 144	$(ga)_{13}$	F	CTAGAAATCCAAACAGTGTGC	3	287
			R	CACTATCGACAATGTTAAGGC		
38	MJM 146	$(ct)_{12}$	F	GCCAAGCTTCTGGTAACTAAT	2	169
			R	CACAGAATCCTGAGTTGAAAG		
39	MJM 151	$(tc)_{17}$	F	GATGGAGGAACTAATACCGAT	2	325
			R	GTAGGGATATTCCTGAAGGG		
40	MJM 143	$(ag)_{12}$	F	TGTAAGAGTTACCAAATCGGA	3	291
			R	TGTTGGCCTGATATAGTCTGT		
41	MJM 600	$(ct)_{16}$	F	GCTAGATTTCCTTCCATTCGG	3	136
			R	CATTCCACGCTCCTTGTTG		
42	MJM 592	$(ag)_{12}$	F	CGAACGTTTCGGCAAATATAA	3	375
			R	CTCGAATTTGATTGGGAGTCA		
43	MJM 595	$(tc)_{21}$	F	TAGCAAGGCGGCTAGGTTAAT	3	271
			R	GGGTGATTCAAGGTTGTCAAA		
44	MJM 598	$(tc)_{25}$	F	TGTCGTTCCGTTTGTCAAAGT	3	285
			R	TGCCCATTTGATCTAACCATC		
45	MJM 599	$(ag)_{26}$	F	GATAGTGATTATGATCCGCCG	3	389
			R	ATTGCTTCAGTTGCAATGCTT		
46	MJM 602	$(tc)_{12}$	F	CTCACTTAATGCCTCCTTCCC	3	207
			R	TCGTTGGAGTTTCTGATGGAG		
47	MJM 607	$(ct)_{18}$	F	ATTAGTGGCGACTCCTCCATT	2	222
			R	TTGGTGTGGACCTTACAGGAG		
48	MJM 669	$(ct)_{23}$	F	GGAAGATGGGTAAGCCTGAAG	3	283
			R	ATTTCATGCATCCAACAGAGG		
49	MJM 689	$(tc)_{14}$	F	GTGGGATCATTCTGTTAGGCA	2	227
			R	CCCTTATTTCGCAGATCAACA		
50	MJM 697	$(ga)_{19}$	F	TGACCAATAAATGCAAGGAGG	2	361
			R	TACATAGGGAGTGGGCAACAC		
51	MJM 168	$(tgt)_{30}$	F	ATGATTACGCCAGCTTCTAC	2	289
			R	GTTCTTCAGTCAGTGCAAGTC		

Table 4. Continued.

Serial no.	Primer name	Motif		Primer sequence (5′-3′)	No. of alleles	Product size
						bp
52	MJM 173	$(caa)_4$ ct $(aca)_7$	F	CCAACCACAACTGAACCTAT	3	152
			R	TGTTGTTGTGATTGTGATGA		
53	MJM 179	$(atc)_7$	F	GTTGATTGTTGTTGTTGTGC	2	268
			R	ATGAAGATGAAGATCCACCA		
54	MJM 184	$(ttg)_7$	F	GATCATTTGGATCAAGCATT	2	196
			R	TCAACAACAGAAACACCAAA		
55	MJM 190	$(aca)_6$	F	AAATCAAATATGTTCGACGG	2	117
			R	GTTTAACAACTTCAGGTTCA		
56	MJM 194	$(aga)_9$	F	CATATGGTCGTGACTTTGATT	4	260
			R	AGTGTTTGTTGACCAAGAGTG		
57	MJM 198	$(caa)_7$	F	CACAACAACATCAACAACAAG	3	360
			R	GGAGATTGAAATATGGAGGTC		
58	MJM 201	$(aac)_6$	F	GCCAGAAGATATGGAGAAGAT	3	343
			R	GAGAATCTCAGTCTTGCTGTG		
59	MJM 209	$(caa)_{10}$	F	ACCAATTACATCTGCTTCAAC	3	362
			R	GACGATTATTAATTGGTGGTG		
60	MJM 212	$(caa)_7$	F	GCAGCATCTTCTACAACAGTC	3	133
			R	ATATAGATGGTCGTGTTGGTG		
61	MJM 218	$(aac)_7$	F	CTCAGAATGTGGGTAACAATC	3	158
			R	AGGATGCTGATAGTGATGATG		
62	MJM 221	$(caa)_{11}$	F	CCAAGCTTAACACCATCATTA	4	369
			R	ATTATGTTGAGATTGTGGCTG		
63	MJM 229	$(tgc)_9$	F	AGAGGAATTAGGGTTAGGGTT	3	252
			R	CAACAACAACATCAACAACAG		
64	MJM 238	$(caa)_{15}$	F	CATCCCAACTCTAAACAACAA	4	218
			R	GAAGTGGATGAGGTTGAGAA		
65	MJM 245	$(aac)_6$	F	TGAAGATTCTCGTCAACCTAA	2	386
			R	TTGTGAAGAGGGAGATTACAC		
66	MJM 754	$(ttg)_9$	F	TTCATGAGCATCAGCATCTTG	3	340
			R	GGCAGAAGGAGAAGAAGAGGA		
67	MJM 757	$(caa)_{10}$	F	TCCTCACCAACAACAACAACA	3	273
			R	TGATTCAAAGCTAAAGCAGCC		
68	MJM 759	$(tgt)_{10}$	F	GTACCAAGGGATGTGACGCTA	4	190
			R	ACCTTGCAGCAAATACAGCAG		
69	MJM 767	$(tgt)_5$	F	TGTCTTCTTCTTTCTTGGGCA	2	386
			R	AGGGAATTGCATCCCAGTAAC		
70	MJM 772	$(ttg)_7$	F	ATGTCTATGCCATTGGAGCTG	2	198
			R	TCACATTACTTCCAGCACACT		
71	MJM 781	$(caa)_9$	F	CTTGTTTGCTATTCTTTGCGG	2	255
			R	TACGAGCTGCTGCTTCTCTTC		
72	MJM 791	$(gtt)_{10}$	F	AGACCATATTCCAGCCGTTCT	3	330
			R	AGCAATGTCATTTCAACCAGG		
73	MJM 795	$(aca)_7$	F	ACAGAGCAGACAACAAACGCT	3	143
			R	CTGCCTAGCGGTAATCTCCTT		
74	MJM 811	$(cac)_8$	F	TCATTAGGATTTAAGTCACCGGA	2	324
			R	AAAGGAAGGAAAGGAGGAAGG		
75	MJM 828	$(tgt)_8$	F	TGTCTTCTTCTTTCTTGGGCA	3	386
			R	AGGGAATTGCATCCCAGTAAC		
76	MJM 267	$(ttc)_8$	F	GGAAATCATATTTACCGCTCT	2	306
			R	TTCTAATGGTTATTGCTTCCC		
77	MJM 273	$(gaa)_7$	F	CTATTGAAGAGGTTGTCATCG	2	381
			R	CCATTAATTCATACCGTGTGT		
78	MJM 278	$(tct)_6$	F	ATCAATCAAGCATCATCAGTC	2	285
			R	AGGAAGGATAGGGAAGAAAGT		
79	MJM 284	$(gaa)_7$	F	CCAAGAGTAAGTAAGCAACCA	2	224
			R	GTTCACTACGGCCTCTTTC		
80	MJM 291	$(ttc)_7$	F	ACTCTCCTCTCATGAGTCACA	2	196
			R	AGTTTATCTGCCACATACCAA		
81	MJM 294	$(aag)_6$	F	CATGCATATTACTTGAAAGGG	2	269
			R	GGGACACAATCTGATATTGAA		
82	MJM 296	$(gaa)_{10}$	F	GAGACCAGACCATGTAGAGAA	2	150
			R	GCAATTTACACGATTAGGATG		
83	MJM 304	$(ctt)_{32}$	F	TTCTCGCTCTCCTCTTCAT	3	350
			R	CTTCAACTACCACCACATCAT		
84	MJM 313	$(gaa)_7$	F	CAAAGAGCCTAGAGGAAGAAG	2	197
			R	CGTGACTTAAACGCACAGTA		
85	MJM 832	$(aga)_{12}$	F	CAAGCTTTAAGATGACAAGTTGC	3	164
			R	TGAGCAGCTAGGTTCACGATT		
86	MJM 835	$(aga)_{13}$ aa $(aga)_2$	F	ATTCGCCAAGCTTGTGAACTT	3	252
			R	GAAGGAAACGCGCTCTTAGAC		
87	MJM 838	$(aag)_9$ aaag $(aga)_8$	F	AGATTTAACCACAGGGATTTC	4	218
			R	ATAAGGCCCTTGTATCATTTC		
88	MJM 841	$(aag)_{31}$	F	AGTGGAGGGACCATTCAGACT	1	200
			R	TTCCCATTGTCTTGGTGATTC		
89	MJM 843	$(tct)_{19}$	F	TAACGTTTCCGCTGTCCTAAA	2	270
			R	CATGCAATCATCAGAAGCAGA		
90	MJM 848	$(ctt)_{13}$	F	TTGTTGGGTATGAACCGTATTC	3	148
			R	AGTGTGCTATGGCACCTCTGT		
91	MJM 898	$(tct)_8$	F	TCTCTCCAATTCCTTTCAGCA	3	368
			R	GAACAATGATGACGAGACGGT		
92	MJM 902	$(ttc)_7$	F	TAAATCCCTCCAAACAATCCC	2	322
			R	GGCTGTGGAGGAGAGAAGAGT		
93	MJM 904	$(ctt)_{36}$	F	TTCCCATTGTCTTGGTGATTC	1	215
			R	AGTGGAGGGACCATTCAGACT		
94	MJM 907	$(aga)_7$	F	GAACAGAAAGCAGAAGAGTCGAG	3	137
			R	ATTTCTTCCGGCCAATCACTA		
95	MJM 910	$(aga)_8$	F	AAGAAGTTGGTTGGAGAGGGA	2	212
			R	ACTCTCATGTTCATGCCTTGG		
96	MJM 912	$(ttc)_{11}$	F	AACGGTCCACAGATCTACACG	3	127
			R	CGAAACTTGAGGTTGACGAAG		
97	MJM 914	$(gaa)_{23}$	F	TGGCTATGCCATTGACACTAA	4	366
			R	CTTATGCTGCTTCGGCTTATG		
98	MJM 916	$(aag)_{16}$	F	ATCTCCATTTCAGGAGGAGGA	2	175
			R	GCTGCATTGCTTTACTCTTGC		
99	MJM 919	$(gaa)_{19}$	F	GGATCGAGGAAGAGGAATTGT	3	231
			R	CACCTGATTATGGTGGGTGAG		
100	MJM 922	$(tct)_{11}$	F	TGTTATCCAAGTCAACCTCCG	2	354
			R	AAACAGTCCAAGGGAACCACT		

†MJM, Meerut jute microsatellites.

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