ONLINE RESOURCES

Development and crosstransferability of functionally relevant microsatellite markers in *Dendrocalamus latiflorus* and related bamboo species

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[Bhandawat A., Sharma V., Sharma H., Sood A. and Sharma R. K. 2014 Development and crosstransferability of functionally relevant microsatellite markers in *Dendrocalamus latiflorus* and related bamboo species. *J. Genet*. **93**, e48–e55. Online only: http://www.ias.ac.in/jgenet/OnlineResources/93/e48.pdf]

Introduction

Bamboo belongs to subfamily Bambusoideae (family Poaceae), which comprises nearly 1200 species worldwide. Of these, 130 species representing 18 genera of Bambusoideae are found in India, making it a major hot spot of bamboo genetic resources (Sharma *et al.* 2008). Bamboo is an important forest resource due to its rapid growth rate, adaptability to harsh environment, high CO_2 fixation rate, and hence, can be extremely important in reclaiming vegetative cover in deforested areas. *Dendrocalamus latiflorus* (Ma Bamboo) is a fast growing sympodial bamboo species that is of major economic importance, particularly in Asia (Lin *et al*. 2007). Bamboo has a remarkable life history with little genetic characterization. Microsatellite markers are used as tools for assessing genetic variation and dissecting complex traits. Nevertheless, such marker resource is severely limited in bamboo (Sharma *et al*. 2009; Lu *et al*. 2009; Ramalakshmi and Piramanayagam 2010) and nonexistent in *D. latiflorus.* Genic microsatellites derived from publicly available expressed sequence tag (EST) database have gained considerable importance because they are inexpensive to develop, ubiquitously dispersed in genome, codominantly inherited and often a putative function can be assigned (Provan *et al*. 2001; Chung *et al*. 2006). As genic simple sequence repeats (SSRs) are developed from expressed part of genome, these markers can be used across a number of related species (Barkley *et al*. 2005; Arya *et al*. 1993). They are proven as an efficient tool for assessing functional genetic diversity, genome wide association studies (GWAS) and linkage mapping (Varshney *et al*. 2005). However, existing SSR markers resource are not enough for various genotyping studies to draw concrete conclusions across the bamboo complex. Therefore, the present study was undertaken to identify functionally relevant microsatellite markers from publicly available ESTs database in *D. latiflorus.* The 64 novel microsatellite markers developed were also tested for crosstransferability and phylogenetic studies in 36 bamboo species and five related crop plants.

Materials and methods

Plant material and DNA extraction

Amplification validation of newly developed genic SSR markers was done in five random accessions of *D. latiflorus.* However, crosstransferability studies were conducted in three random individuals from each of the 36 species of bamboo (maintained at CSIR-IHBT, Palampur, India) and single representatives of rice, wheat, maize, barley and sugarcane (obtained from CSKHPKV, Palampur, India, and Indian Institute of Sugarcane Research, Lucknow, India). Genomic DNA was isolated from the fresh leaf tissue collected from individual genotype using CTAB method as described by Doyle and Doyle (1990) with minor modifications.

Data mining and genic SSR marker development

A total of 9574 EST sequences of *D. latiflorus* were retrieved on 18 November 2011 from the NCBI (NCBI; http://www.ncbi.nlm.nih.gov/entrez) and assembled into potential unigenes using DNASTAR Lasergene ver. 7.1 (DNASTAR Madison, USA). Unigenes were subsequently searched for the presence of SSRs (motif length 2–6) using Repeat Masker (http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker). SSRs with nucleotide length ≥12 bp

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Keywords. crosstransferability; DLUGMS; phylogenetics; SSRs; unigene.

Table 1. Characterization of sixty-four genic microsatellite markers of *Dendrocalamus latiflorus*.

Locus ID	Constituting EST accession details	Primer sequence (5′–3′)	T_a (°C)	Repeat motif	Function	*E* value	No. of alleles	PIC	Expected size (bp)	Obtained size (bp)	SSR location	Location on rice chromosome
DLUGMS1	JK013865.1, JK012007.1, JK015685.1, K016954.1*	F: CGCTCTCTTGGAGAGAAGA R: GCCACCCTTTGACTCCATTA	53	$(GA)_{20}$	adenosylmethionine decarboxylase EC: 4.1.1.50	4.2E-179	8	0.493	226	900–1000	5′UTR	4
DLUGMS2	JK016909.1, JK014902.1, JK011077.1, JK008274.1**	F: TCCCATTTGTCCGTCTCTTC R: CAGCCTCATCACCATCCTCT	55	$(GA)_{14}$	Cysteine synthase EC: 2.5.1.47	2.30E-125	20	0.269	162	100–800	Codon	4
DLUGMS3	JK015749.1, JK011948.1, JK012079.1, JK012868.1, JK013456.1, JK014679.1, JK012720.1, JK016358.1	F: TGCCGGTGCTTTCTTACTCT R: GGAGGAAGGGATGGGAGTAG	53	$(CT)_{11}$	Plastid RNA polymerase sigma factor EC: 2.7.7.6	1.60E-80	8	0.44	175	150–1000	5′UTR	8
DLUGMS4	JK008162.1, JK012960.1, JK011812.1, JK013827.1, JK014118.1	F: GCAGAAAGAAGGGAACGAGA R: CAGGAAGCACGAGATGTCAA	53	$(GA)_{13}$	Uncharacterized protein	1.50E-49	9	0.27	169	130–900	5′UTR	2
DLUGMS5	JK012061.1, JK010089.1, JK015031.1, JK016302.1, JK009944.1, JK012967.1, JK015440.1, JK009434.1, JK017180.1	F: GGGGCACCAGTTTATTCCTT R: GCGAGCTGTGGAGAGAGAAT	53	$(GA)_{10}$	Myb-cc type transfactor	2.10E-30	6	0.334	103	110–1000	5′UTR	9
DLUGMS6	JK011847.1, JK009370.1, JK017069.1	F: AGCGAAAGAGGCTGACAAAG R: CCAAGGAACCAAGGAGACAA	53	$(GA)_{15}$	Exocyst complex subunit	1.80E-36	16	0.384	233	130–1000	Codon	1
DLUGMS7	JK017284.1, JK011792.1, JK016691.1, JK008269.1, JK011652.1, JK010701.1, JK008912.1	F: TCCAGGCACTTGAGGAGACT R: CGGAAGTAGGCAACAAGGTC	55	$(TC)_{11}$	COBRA-like protein	2.20E-102	20	0.403	245	100–1000	Codon	7
DLUGMS8	JK009907.1, JK014207.1	F: CCTGCGTGTGAGAGATTCTG R: TCGAAAGAACCACAGGGACT	55	$(TC)_{20}$	Putative LIM domain containing protein	3.90E-09	10	0.324	226	350–1000	5′UTR	3
DLUGMS9	JK012091.1, JK015583.1, JK011806.1	F: GAACCCAACCGAGAGAGACA R: ATACCCGGTTCAGCTTCCTT	55	$(GA)_{16}$	Uncharacterized protein	1.90E-54	19	0.211	165	150–700	5′UTR	2
DLUGMS10	JK015799.1, JK008754.1, JK008360.1	F: CTCCGCATCGACGAGAAC R: GCGTCCAGTAAATTCCTTGC	53	$(GA)_{14}$	Uncharacterized protein	6.20E-70	11	0.370	176	100–1200	5′UTR	1
DLUGMS11	JK009202.1, JK009087.1	F: GGGGAGAAGAGGCTGGAAG R: CGGCAATGGCAGATATCACT	53	$(TC)_{18}$	Protein kinase-like domain	1.90E-30	15	0.403	160	125–1000	5′UTR	10
DLUGMS12	JK010134.1, JK009399.1	F: CTATCCTCCTCCCCAAATCC R: TTCGCTTCGAGGGTTAAATG	51	$(CGG)_7$	DNA binding protein	1.00E-23	7	0.457	191	175–550	Codon	6
DLUGMS13	JK016240.1. JK009900.1	F: CCTTCCTCGTTTCCCTTTTC R: TTCGCTTCGAGGGTTAAATG	55	$(CGG)_8$	Uncharacterized protein	1.00E-52	5	0.322	221	150–500	5′UTR	1
DLUGMS14	JK012062.1	F: TCCTCCGTGCTTAAACCCTA R: GTGAAACAGATGGGGGAGAA	53	$(GA)_{29}$	Isco large subunit-binding protein subunit beta	2.00E-41	10	0.385	239	100–1000	5′UTR	2
DLUGMS15	JK009740.1, JK012396.1, JK012897.1, JK014592.1	F: GGGGACCATTTGACAACTCA R: CTCTTTGCGAGGAAGTCACA	53	$(TC)_{10}(CA)_5$	Uncharacterized protein	1.20E-43	20	0.264	205	100–1000	5′UTR	–
DLUGMS16	JK015521.1	F: GGGAGATACAGTTCCGTTGG R: TCCTTGATGGAGCGGACTAC	55	$(GA)_{26}$	Protein mo25	5.10E-76	8	0.401	159	125–600	Codon	3
DLUGMS17	JK013403.1	F: CGGTTGGCCTTCTATGAGAG R: CCATCGATGATAGCACAGGA	53	$(TC)_{24}$	NA	–	12	0.364	231	150–400	Codon	12

Table 1 (*contd*)

Locus ID	Constituting EST accession details	Primer sequence (5′–3′)	T_a (°C)	Repeat motif	Function	*E* value	No. of alleles	PIC	Expected size (bp)	Obtained size (bp)	SSR location	Location on rice chromosome
DLUGMS18	JK014127.1	F: TCTCTCCTCCCTTCCAAACA R: ATATACCCTGCGAGCTGGTG	53	$(GA)_{19}$	Nucleotide-sugar transporter family protein	2.00E-87	18	0.257	159	75–900	5′UTR	6
DLUGMS19	JK012424.1	F: GAGATTGGCCAGAAACAAGG R: CCTGTTGATCTTCCCTCCAA	53	$(GA)_{20}$	Cycloartenol-c-24-methyltransferase 1 EC: 2.1.1.41	1.40E-58	19	0.379	202	150–900	5′UTR	7
DLUGMS20	JK007968.1	F: GGGGATACGAGAGCAGAAGA R: ATTGTGTCCCCTACCTGCTG	54	$(TC)_{26}$	Uncharacterized protein	5.50E-62	13	0.225	170	200–1000	5′UTR	1
DLUGMS21	JK008587.1	F: CTCACCAATTCCGCTCTTTC R: TGAGCACAAAGAGCTTCGAC	53	$(GGA)_9$	Protein fam43a	2.00E-40	5	0.102	218	300–1000	5′UTR	3
DLUGMS22	JK015949.1, JK012362.1	F: ACAGAGGCCACAAGTTCCAC R: TGACACAGGAGTCCGAACAG	55	$(CAG)_{13}$	Transcription initiation factor iid subunit a-like protein	5.00E-22	22	0.436	241	225–1000	Codon	1
DLUGMS23	JK008290.1	F: AAGGAAAAAGGGCTGGGTTA R: TCGTCGTCATCACTTTGCTC	51	$(ATG)_{14}$	Nucleolar protein 14-like	1.00E-15	11	0.442	216	175–800	Codon	3
DLUGMS24	JK011685.1, JK016445.1	F: CTCTTCTCGTCCCGAATGTG R: GGGAGCTCCAACACCAAATA	53	$(TCC)_9$	Sec-independent protein translocase protein tata e-like protein	7.00E-52	12	0.404	249	400–1000	Codon	11
DLUGMS25	JK010315.1, JK013562.1, JK013878.1, JK012830.1, JK010547.1	F: GAGGGACTTGATGGATTGGA R: ATGTTATTGCGCTTGTGCTG	51	$(GAA)_8$	Tic62 protein	6.50E-124	21	0.419	246	150–1000	5′UTR	10
DLUGMS26	JK012317.1	F: CACTCGTCCTCCTCTCGTCT R: CATAGCCATGGTGGCCTAAT	53	$(TTC)_{10}$	Probable anion transporter chloroplastic-like	6.60E-12	20	0.329	199	175–1000	5′UTR	12
DLUGMS27	JK007811.1	F: GGGGGTGACTCTTCTTCTTCTT R: CTCCGGTCTTCTTCACCAAG	55	$(CTG)_6$	sf16 protein	2.10E-12	8	0.279	184	150–700	5′UTR	5
DLUGMS28	JK014934.1	F: CCTCTCTCAAGGCAGAGCAG R: GGAAACAAGAACGGGAGGAT	53	$(CAG)_8$	d-xylose-proton symporter-like 2	6.40E-27	20	0.476	244	150–900	5′UTR	10
DLUGMS29	JK011930.1	F: GCAGTTGGCGTCATACAGAG R: TTTACCCAACTGCGGCTAAC	53	$(CTG)_7$	NA	–	13	0.283	232	150–700	5′UTR	–
DLUGMS30	JK008439.1	F: CGGAGGCACTCTACCAGAAG R: AGCGAGGGCTGAATGAGAG	54	$(CAG)_7$	Swi snf complex subunit swi3c-like	9.00E-80	22	0.394	206	100–800	Codon	12
DLUGMS31	JK013016.1	F: GTCCTGGTGCTCTCTTCTGC R: GATTTGAGGGAAAGGCAACA	51	$(TCG)_8$	NA	–	21	0.405	201	150–1000	Codon	12
DLUGMS32	JK008723.1, JK007848.1	F: GCGATCAGCTGCTATCTGTCT R: GAAGCACCTCGAATCGAAAG	53	$(TTC)_{10}$	NA	–	13	0.223	168	150–225	5′UTR	–
DLUGMS33	JK013189.1	F: ACAAGGGACGAGGAGGAAAT R: TCTCTCTTCCTGGGCAGTGT	53	$(TTC)_7$	Beta-mannosyl-glycoprotein 4-beta-n-acetylglucosaminyl transferase-like	7.50E-39	14	0.274	178	200–1000	5′UTR	12
DLUGMS34	JK011665.1	F: GGCTGTGTTCTGTTCCGTTT R: CTGCTCTTGGGGTTCTTGAG	53	$(CCA)_{16}$	hva22-like protein a-like	2.00E-50	19	0.355	167	150–1000	5′UTR	11

Table 1 (*contd*)

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DLUGMS35	JK016414.1	F: GTCCCGCACCTTGAGTCTTA R: GCTCAGATGGTGATGCTGAA	53	$(CAG)_{12}$	Protein time for coffee-like	1.10E-23	12	0.296	220	150–900	3′UTR	7
DLUGMS36	JK015613.1	F: TTGGGACTCTTGGCTACTCG R: GGCATGTGCTCAATCAACAG	53	$(CAA)_9$	NA	–	11	0.314	221	150–800	3′UTR	–
DLUGMS37	JK008693.1	F: CGCAGCTACACTGCACAAGT R: GCAAAGATGTTCCCTGCAAT	51	$(TTC)_9$	Protein ruptured pollen grain	6.10E-41	6	0.333	192	400–500	Codon	1
DLUGMS38	JK013845.1	F: GGGGATTGGAAGAGAAGAGG R: GTTTTGGTGCGAAGGGATT	55	$(CCG)_6$	Nucleoprotein autopeptidase	1.70E-53	15	0.113	205	200–800	5′UTR	12
DLUGMS39	JK009597.1, JK008001.1, JK010687.1, JK015034.1	F: GGGGGAGAAGGAAAGAGAGA R: GCAGGGAATAAGCAAGCAAT	59	$(TC)_{15}$	Associated with hox family expressed	2.00E-31	14	0.16	212	220–440	5′UTR	12
DLUGMS40	JK012105.1, JK009519.1	F: TGGCATCTATTGCACTGCTC R: CCCAAATAAAACACGGCACT	60	$(TC)_{15}$	Uncharecterized protein	2.10E-04	11	0.32	210	180–310	Codon	1
DLUGMS41	JK015335.1	F: TCGTCACAATCCTCAATCTCC R: TAAACGGCGAAGCAATATCC	60	$(TCC)_9$	f-box only protein 9	1.50E-35	10	0.23	178	240–260	5′UTR	7
DLUGMS42	JK016282.1, JK010864.1	F: AATCCAATGGTAGCGTCTGC R: CAAATCCCGATCCAACAACT	60	$(GA)_{17}$	NA	–	17	0.23	236	150–400	5′UTR	4
DLUGMS43	JK015411.1	F: TTTGTTGCCATTGTTTGATGA R: TCTACGAACACCCCAGAACC	60	$(TC)_{37}$	NA	–	16	0.18	218	150–300	Codon	–
DLUGMS44	JK013966.1	F: AGCAACCCATCTCCTCTTCA R: TCCTCCTCCTTGTTTCTCCA	60	$(GATTG)_5$	Autophagy-related protein 8 h	2.80E-17	17	0.17	151	100–500	5′UTR	–
DLUGMS45	JK014353.1	F: CACCGTGTGGTTACCTTTCC R: TGAGGAGGAGCTTGAAGAGG	60	$(TC)_{16}$	Ras-related protein rab-18 EC: 3.1.4.12	8.90E-49	14	0.19	242	150–300	5′UTR	6
DLUGMS46	JK009036.1, JK014339.1	F: TCAGCCTCACTCCCTCTCTC R: CGAGCAAGAGCAAAGAAACC	60	$(TC)_{13}$	Protein kinase	7.80E-126	7	0.21	250	150–220	5′UTR	–
DLUGMS47	JK015407.1	F: GGGGACTCTCCTCTTCGTCT R: GATCTGAGGCTTCTCCATCG	60	$(TC)_{11}$	NA	–	9	0.18	202	220–310	5′UTR	–
DLUGMS48	JK008350.1	F: GGCAGAGGCAAAATTAAGGA R: AACTCTTTGGGACTGCAAGG	59	$(CCAGG)_4$	Methyltransferase pmt20	1.80E-48	12	0.18	233	175–305	5′UTR	9
DLUGMS49	JK016374.1, JK009946.1, JK017036.1, JK008364.1, JK011683.1, JK008230.1	F: AAGCAAAAACAGGGAGGAGA R: CCTTCTCGTTCATGGCTGA	59	$(GA)_{14}$	Probable ADP-ribosylation factor gtpase-activating protein agd5-like	4.60E-146	18	0.19	162	125–200	5′UTR	7
DLUGMS50	JK011637.1	F: AGACTCTCCACTCGTGACTCG RCCGCGAACTCCACAGACTAT	60	$(CTCCG)_6$	Ubiquitin-conjugating enzyme	7.40E-42	18	0.21	223	180–350	5′UTR	5
DLUGMS51	JK011966.1	F: CATTGGCCCATGTAACTTTTC R: CGAGCAAGTGTTGTCCTGAA	59	$(CAG)_8$	NA	–	11	0.26	212	210–300	5′UTR	–
DLUGMS52	JK015049.1, JK012804.1, JK016096.1, JK009743.1,	F: CCATCTCTCGTCTCCTCTCG R: TTGCTCAGAAATGGCAAGAA	60	$(CAT)_9$	Nudix hydrolase 13	1.20E-44	16	0.17	216	210–360	5′UTR	4
DLUGMS53	JK012403.1, JK008176.1, JK015351.1	F: GGCCGTCAACAGAAAGTTGT R: GCGAGACAACAACACAGCAC	60	$(CCCGG)_5$	Protein-l-isoaspartate o-methyltransferase	9.10E-67	5	0.15	157	160–225	5′UTR	8
DLUGMS54	JK011876.1, JK015787.1	F: CACAGGGAGCAACATCAAGA R: CCGATCATAAAACCAACTGAAA	59	$(TTTC)_7$	NA	–	12	0.29	211	225–450	Codon	–
DLUGMS55	JK015578.1	F: GGAAATTGCTGAAGCAAGGA R: TCACCAGCATCAACACCATT	60	$(ATG)_7$	Uncharacterized protein	1.30E-13	7	0.38	213	190–300	Codon	8

Table 1 (*contd*)

Locus ID	Constituting EST accession details	Primer sequence (5′–3′)	T_a (°C)	Repeat motif	Function	*E* value	No. of alleles	PIC	Expected size (bp)	Obtained size (bp)	SSR location	Location on rice chromosome
DLUGMS56	JK014579.1	F: CAATCTCGGAGCCGAACTAC R: ATACCACCAGGCACAAGAGC	60	$(ACCTG)_4$	NA	–	4	0.35	191	230–260	Codon	3
DLUGMS57	JK016762.1 JK016224.1	F: AGCCAGTCCACCATTACCAG R: GGGAGAGTCGACTGAATTGG	60	$(CTCCG)_5$	Isoamyl acetate-hydrolyzing	4.00E-92	8	0.42	160	400–600	5′UTR	11
DLUGMS58	JK015971.1, JK011601.1, JK015146.1	F: GACAGCGCTCGTCAGGAT R: ATCAATCCGGGCATGATAAC	60	$(GGAGA)_7$	Allantoinase	1.10E-165	3	0.45	193	600–700	5′UTR	4
DLUGMS59	JK015226.1	F: GCCCTTCTTCAGGGGATAAC R: TCCATCAACCCTTGTTGGTT	60	$(CAG)_{39}$	NA	–	8	0.39	364	300–400	Codon	8
DLUGMS60	JK008696.1, JK010674.1	F: GGATGTAGCTCCACCATCTGA R: CAGCTGGCTCAGATGTCATT	59	$(CCA)_5$	Hepatoma-derived growth factor-related protein	2.60E-68	16	0.23	242	200–260	Codon	3
DLUGMS61	JK008660.1	F: TTCCTCATCTTGCAGGCTTT R: GCAAAATTTCCGTCGATTGT	59	$(GGAGA)_7$	ATP binding	4.30E-23	14	0.20	194	170–320	5′UTR	6
DLUGMS62	JK010801.1, JK015826.1, JK008740.1, JK012294.1, JK015199.1, JK015589.1, JK014881.1, JK015262.1, JK009405.1, JK008842.1, JK013098.1, JK009009.1, JK012372.1, JK017047.1	F: ATAGCCATGTACGCATGCAC R: GCTTACAGGTTTCACACAACCA	59	$(CGTG)_6$	Serine threonine protein kinase	2.90E-66	17	0.22	183	175–380	3′UTR	2
DLUGMS63	JK009171.1	F: AAGAAGGCGAAAAAGGAAGC R: ATTGTCCCATCACAGCATCA	60	$(ATCG)_5$	Zinc finger protein	4.30E-34	7	0.18	190	400–1000	5′UTR	3
DLUGMS64	JK014199.1	F: CACCACCCTTTCCTCTCCTC R: TTCTGCCCTCGGTTAAATTG	60	$(CCCTG)_4$	Integral membrane	2.60E-12	7	0.21	201	600–700	5′UTR	6

T_a, annealing temperature; PIC, polymorphism information content; EC, enzyme commission; *E* value, expected value.

*Remaining constituting EST accessions for DLUGMS 1: JK009472.1, JK008051.1, JK015896.1, JK009887.1, JK011499.1, JK008115.1, JK008798.1, JK015433.1, JK007776.1, JK011917.1, JK011536.1, JK011924.1, JK008988.1, JK008647.1, JK008942.1, JK008147.1, JK008829.1, JK010855.1, JK010106.1, JK012532.1, JK014637.1, JK010853.1, JK013721.1, JK016625.1, JK014307.1, JK014676.1, JK011786.1, JK015490.1, JK011982.1.

**Remaining constituting EST accessions for DLUGMS 2: JK017099.1, JK009251.1, JK013425.1, JK010490.1, JK014727.1, JK013720.1, JK008669.1, JK015114.1, JK016415.1, JK011240.1, JK009535.1, JK017027.1, JK013822.1, JK008487.1.

were identified, which were classified as class I (≥20 nucleotides) and class II (12–19 nucleotides) type of repeats. ORFs (open reading frames) were identified using web-based nucleotide translation tool available at Expasy (http://web.expasy.org/translate/). Relative distribution of SSRs with respect to longest ORF were identified and categorized as 5′UTR, 3′UTR or codon dwelling. SSR primers were designed using web-based Primer3 ver.0.4.0 (http://frodo.wi.mit.edu/) and prefixed as DLUGMS *Dendrocalamus latiflorus* UniGene-derived microsatellites markers (DLUGMS). Details about marker characteristics are shown in table 1.

Evaluation of amplification potential

PCR amplification was performed in 10 μL reaction volume in I-Cycler (Bio-Rad, Berkeley, USA) consisting 1× PCR buffer (10 mM Tris-pH 9.0, 50 mM KCl, 0.01% gelatin, 1.5 mM $MgCl_2$), 200 μM of each dNTPs, 15 ng each of forward and reverse primers, 0.2 U *Taq* DNA polymerase (Bangalore Genei, Bangalore, India) and 20 ng of template DNA. The PCR protocol consisted of one denaturation cycle at 94°C for 4 min, followed by 35 cycles of 94°C for 1 min, annealing at optimum temperature (T_a) for 1 min, and extension at 72°C for 2 min. The final extension was carried out at 72°C for 7 min.

Genotyping was carried out on denaturing polyacrylamide gels consisting of 7% polyacrylamide (acrylamide : bisacrylamide = 19:1) and 7 M urea in 1× TBE buffer. The PCR reactions were mixed with equal volume of loading dye (98% formamide; 0.8 mM EDTA; 0.025% each of bromophenol blue and xylene cyanol), denatured at 94°C for 5 min and snap cooled on ice. Samples along with 50-bp ladder (Fermentas, Vilnius, Lithuania) were loaded on preheated sequencing gels, at 55 W for 2 h. After the run, amplified fragments were visualized using standard silver staining protocol (Promega, Madison, USA).

Functional categorization

SSR containing unigenes were subjected to Java web-start version of Blast2GO (http://www.blast2go.com/b2ghome) for annotation and functional categorization. SSR-containing sequences were searched against nonredundant data base of NCBI using BLASTX ver. 2.2.26+ with E-value cut-off 0.001. InterPro gene ontologies (GOs) were merged to the obtained annotations. Kyoto encyclopaedia of genes and genomes (KEGG) identifiers were provided and mapped on metabolic pathways using the same tool. *In silico* comparative analysis was done to determine putative chromosomal location using BLASTN (http://www.gramene.org/).

Data analyses

The polymorphism was determined according to the presence (1) or absence (0) of amplification. PIC of each marker across 113 random collections representing 37 bamboo species was calculated according to (Anderson *et al.* 1993). Nei's genetic identity was calculated using PopGene ver. 1.32 (Yeh *et al.* 1997). A UPGMA tree was constructed on the basis of species-wise average genetic similarity among the tested random individuals using SAHN clustering module of NTsys ver. 2.02e (Rohlf 1998).

Results and discussion

A total of 4663 (2.7 Mb) unigenes were predicted from 9574 ESTs of *D. latiflorus*. In total, 106 unigenes containing 111 SSRs (109, class I; 2 class II) with repeat frequency of 1/24.3 Kb unigene sequence were identified. SSR frequency was found to be 40.4% (-di), 39.4% (-tri), 4.6% (-tetra) 14.7% (-penta) and 0.9% (-hexa) types of repeat motifs. Maximum repeats (66%; mostly direpeats) were found in 5′UTR region showing their major role in gene regulation. However, trirepeats were common in 3′UTR (50%) and CDS-dwelling (33.3%) region, respectively, favouring lesser chances of frame-shift mutations.

Ninety primer pairs were identified and initially validated in five random genotypes of *D. latiflorus.* Sixty-four DLUGMS primers detected reliable amplicons in *D. latiflorus* and were subsequently utilized for crosstransferability in 113 genotypes (three each of 36 bamboo species) and five related crop plants as indicated (figure 1). In total, 817 bands ranging from 3–22 (avg. 12.7) per SSR marker were amplified. Sixty-four markers recorded an average of 92.7% and 84.5% interspecies and intergenera crosstransferability, respectively. Due to high degree of crosstransferability at species and genera level, the novel microsatellite marker would have wider applicability in multiple species characterization. Polymorphism information content (PIC) of DLUGMS loci across 37 bamboo species ranged from 0.102–0.493. Eighteen primer pairs (28.1%) produced successful amplification in all the 36 species of bamboo, therefore, can be used as universal conserved orthologous markers for molecular analysis across bamboo complex. Some markers produced only a single band in an individual suggesting polyploidization followed by selective gene loss (Jackson and Chen 2009). Other markers produced multiple amplicons suggesting either locus duplication or mutation in primer binding sites. Such pattern suggests that each species have different reproductive and evolutionary pattern of genome rearrangements. Some amplicons exceeded far beyond the expected size suggesting large intronic insertions in SSR regions. Such complex patterns of allele distribution and polymorphism have been previously reported in maize having a large genome size (Matsuoka *et al.* 2002).

Eighty-two SSR containing sequences showed significant BLAST hits. GOs were obtained for 42 SSR containing sequences, which were subsequently assigned to three functional categories namely biological process (24.5%), cellular components (33.0%) and molecular function (39.6%).

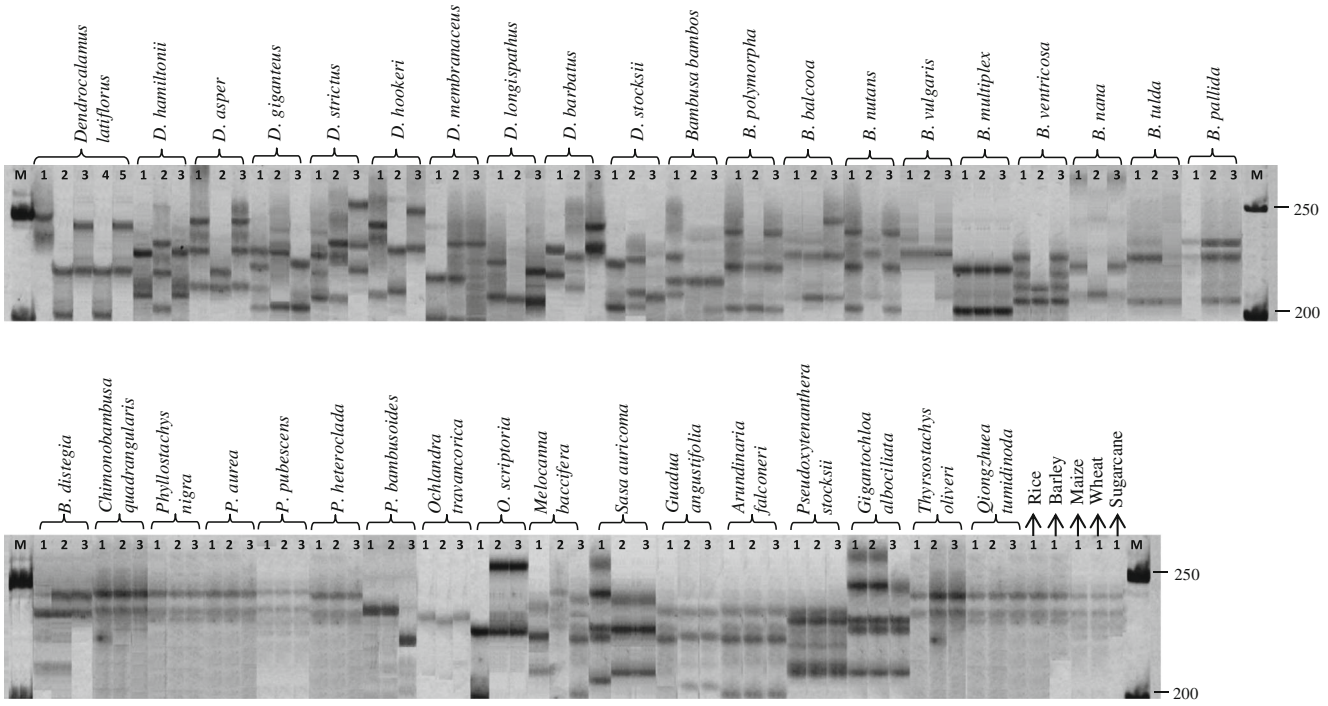

Figure 1. PCR amplification profile generated with primer DLUGMS 60. Lanes 1–5 represent accessions of different bamboo species as indicated. M, 50-bp DNA ladder (MBI Fermentas) as size standards.

Ten unigenes coding for 12 enzymes involved in different molecular pathway were identified. Genomic localization of 54 SSR loci was determined through comparative mapping in rice genome (table 1).

Phylogenetic relationships established among 37 species of bamboo were broadly in accordance with the taxonomic classification proposed by Ohrnberger (1999). Two major groups were revealed with all the 37 bamboo species clustered in a major group and five related crop plants namely rice, barley, maize, wheat and sugarcane remained as outgroup with maximum genetic affinities between wheat and maize. *Dendrocalamus* and most of the *Bambusa* species clustered together in a subgroup, where all the *Dendrocalamus* species precisely remained under same clade. Six species namely *Melocanna baccifera*, *Pseudoxytenanther astocksii*, *Arundinaria falconeri*, *Sasa auricoma*, *Guadua angustifolia* and *Gigantochloa albociliata* irrespective of their subtribe were clustered as an intermediate group. All the five *Phyllostachys* species and two *Ochlandra* species were also clustered in two minor subgroups. Based on the above inferences, these markers proved their wider applicability in future molecular diversity, conservation and phylogenetic studies in bamboo.

Acknowledgements

We thank Council, of Scientific and Industrial Research (CSIR), New Delhi, India, for funding. This is IHBT communication no. 3425.

References

Anderson J. A., Churchill G. A., Autroque J. E., Tanksley S. D. and Sorrells M. E. 1993 Optimising parental selection for genetic linkage maps. *Genome* **36**, 181–186.

Arya L., Verma M., Gupta V. K. and Karihaloo J. L. 2009 Development of EST–SSRs in finger millet (*Eleusine coracana* ssp *coracana*) and their transferability to pearl millet (*Pennisetum glaucum*). *J. Plant Biochem. Biotechnol.* **18**, 97–100.

Barkley N. A., Newman M. L., Wang M. L., Hotchkiss M. W. and Pederson G. A. 2005 Assessment of the genetic diversity and phylogenetic relationships of a temperate bamboo collection by using transferred EST–SSR markers. *Genome* **48**, 731–737.

Chung A. M., Staub J. E. and Chen J. F. 2006 Molecular phylogeny of *Cucumis* species as revealed by consensus chloroplast SSR marker length and sequence variation. *Genome* **49**, 219–229.

Doyle J. J. and Doyle J. E. 1990 Isolation of plant DNA from fresh tissue. *Focus* **12**, 13–15.

Jackson S. and Chen Z. J. 2009 Genomic and expression plasticity of polyploidy. *Curr. Opin. Plant Biol.* **13**, 1–7.

Lin C. S., Liang C. J., Hsaio H. W., Lin M. J. and Chang W. C. 2007 *In vitro* flowering of green and albino *Dendrocalamus latiflorus*. *New. For.* **34**, 177–186.

Lu J., Katsuhiko Y., Fang W. and Tang D. 2009 Identification of the hybrid bamboo F1 by SSR markers. *Sci. Silvae Sin.* **45**, 29–34.

Matsuoka Y., Mitchell S. E., Kresovich S., Goodman M. and Doebley J. 2002 Microsatellites in Zea variability, patterns of mutations and use for evolutionary studies. *Theor. Appl. Genet.* **104**, 436–450.

Ohrnberger D. 1999 *The bamboos of the world*. Elsevier, Amsterdam, The Netherlands. pp. 324–325.

Provan J., Powell W. and Hollingsworth P. M. 2001 Chloroplast microsatellites: new tools for studies in plant ecology and evolution. *Trends Ecol. Evol.* **16**, 142–147.

Ramalakshmi O. I. and Piramanayagam S. 2010 Mining of SSR markers from expressed sequence tags of bamboo species. *Bioinformation* **5**, 240–243.

Rohlf F. J. 1998. NTSYS-pc 2.0e. Exeter Software, Setauket, New York, USA.

Sharma R. K., Gupta P., Sharma V., Sood A., Mohapatra T. and Ahuja P. S. 2008 Evaluation of rice and sugarcane SSR markers for phylogenetic and genetic diversity analyses in bamboo. *Genome* **51**, 91–103.

Sharma V., Bhardwaj P., Kumar R., Sharma R. K., Sood A. and Ahuja P. S. 2009 Identification and cross species amplification of EST derived SSR markers in different bamboo species. *Conserv. Genet.* **10**, 721–724.

Varshney R. K., Graner A. and Sorrells M. E. 2005 Genic microsatellite markers in plants: Features and applications. *Trends Biotechnol.* **23** 1, 48–55.

Yeh F. C., Yang R. C. and Boyle T. 1997 POPGENE, version 1.32 edn. Software Microsoft Window-Based Freeware for Population Genetic Analysis.

Received 20 November 2013, in revised form 27 December 2013; accepted 31 December 2013
Unedited version published online: 4 June 2014
Final version published online: 24 July 2014