https://doi.org/10.56093/ijas.v90i5.104330

Indian Journal of Agricultural Sciences **90** (5): 860–7, May 2020/Article

Characterization of new microsatellite markers from sugarcane (*Saccharum officinarum*) transcriptome

YIJING GAO, HUI ZHOU, JUNXIAN LIU, JINGCHAO LEI, WEIXING DUAN, CUIFANG YANG, SHAN ZHOU, XIANG LI, GEMIN ZHANG, BAOQING ZHANG, HONGWEI TAN, ZEPING WANG and YANGRUI LI*

Guangxi Academy of Agricultural Sciences, Nanning 530 007, China

Received: 06 July 2018; Accepted: 29 August 2019

ABSTRACT

Microsatellites, or simple sequence repeats (SSR), developed by expressed sequence tag (EST) databases is an economical and efficient tools that can be used to perform genetic investigations at a functional level. Here, a new sugarcane database of transcriptome from our variety, GT35, was examined for the presence of SSRs. To test the utility of EST-derived SSR markers, a total of 51 new EST-SSRs were identified for possible use as potential genetic markers from no redundant SSR-positive ESTs, which were unmapped with the sequences available in the NCBI'EST database of sugarcane by BLASTN. Polymorphisms of the identified 51 EST-SSR markers were evaluated in 21 sugarcane genotypes, planted and collected in 2013 at Sugarcane Research Institute of Guangxi Academy of Agricultural Sciences (SRI-GXAAS) in China. High polymorphisms were detected in terms of number of alleles ranging from 5-36 with an average of 16.8 per locus and polymorphism information content values ranging from 0.74 to 0.95 with a mean of 0.92. Average transferability to *Erianthus arundinaceus* and *Narenga porphyrocoma* was 23.9% and 34.4%, respectively. The ability to establish genetic relationship was analyzed by cluster analysis, the result of which revealed that the major grouping was in accordance with taxonomical classification. The development of new EST-SSR markers presented in this work will have important implications for genetic analysis and breeding.

Key words: EST, Microsatellites, Sugarcane, Transcriptome

Sugarcane (*Saccharum* spp.) is a major economical crop because it is the main source of both sugar and alcohol, accounting for two thirds of the world's sugar production (Carson and Botha 2000). In China, sugarcane is the most widely grown crop in tropical regions, with Guangxi Province accounting for more than 65% of the total cane sugar production (Li 2010). Microsatellites or simple sequence repeats (SSRs), short segments of DNA of one to six tandem repeated base pairs, are one of the most suitable markers in plant marker technology due to their co-dominant nature, locus specificity, and high reproducibility.

Over the past few years, EST sequencing for sugarcane projects have been initiated in South Africa (Carson and Botha 2000), Brazil (SUCEST, http://sucest.lad.ic.unicamp.br/en/), Australia (Cordeiro *et al*. 2001), Brazil (http://compbio.dfci.harvard.edu/tgi/) (Silva *et al*. 2012) and China (Huang *et al*. 2016). Cordeiro *et al.* (2001) reported a preliminary analysis on the development of functional SSRs markers from the database in Australia and identified 21 polymorphic EST-SSRs and tested their transferability to closely related genera. Meanwhile, Silva *et al.* (2001) used the SUCEST database to evaluate 20 EST-SSRs and found this database to be a good source for the development of molecular markers. In addition, Souza's group developed 837 sugarcane EST-SSRs in total using the SUCEST database (Pinto *et al*. 2004, Oliveira *et al*. 2007, Oliveira *et al*. 2009, Pinto *et al*. 2010, Marconi *et al*. 2011). Furthermore, Silva *et al.* (2012) characterized 53 highly polymorphic EST-SSR markers from the EST sugarcane database deposited in the Gene Index Project (http://compbio.dfci.harvard.edu/tgi/).

The present study was conducted to characterize the potential of a novel set of EST-SSRs developed from our new sugarcane database of transcriptome as molecular markers for sugarcane breeding (Huang *et al*. 2016). The objectives included the following: (i) identify the polymorphism information content (PIC) of these novel EST-SSRs, (ii) evaluate the transferability of the EST-SSRs to close relative species and genera, and (iii) determine their utility in genetic relationship and phylogenetic analysis.

MATERIALS AND METHODS

Plant material and DNA extraction: Plant materials consisted of 21 accessions, including three genera and five *Saccharum* species, were planted and collected in 2013

*Corresponding author e-mail: 387655593@qq.com

Table 1 Sugarcane genotypes used for marker validation with pedigree relationships and their collection sites

Genotype	Pedigree	Source
GT15[a] (*Saccharum* sp.)	Huanan 56-12 × Neijiang 59-782	SRI-GXAAS
GT18 (*Saccharum* sp.)	CP65-357 × F172	SRI-GXAAS
GT21 (*Saccharum* sp.)	Ganzhe 76-65 × Yacheng 71-374	SRI-GXAAS
GT28 (*Saccharum* sp.)	CP80-1018 × CP89-1475	SRI-GXAAS
GT29 (*Saccharum* sp.)	Yacheng 94-46 × ROC22	SRI-GXAAS
GT32 (*Saccharum* sp.)	Yuetang 91-976 × ROC1	SRI-GXAAS
GT35 (*Saccharum* sp.)	ROC23 × CP84-1198	SRI-GXAAS
GT37 (*Saccharum* sp.)	Zhanzhe 92-126 × CP72-2086	SRI-GXAAS
GT43 (*Saccharum* sp.)	Yuetang 85-177 × GT92-66	SRI-GXAAS
Guifu98-296 (*Saccharum* sp.)	GT91-131 induced by radiation of Co^{γ} ray	SRI-GXAAS
ROC16 (*Saccharum* sp.)	F171×74-575	SRI-GXAAS
ROC25 (*Saccharum* sp.)	79-6048×69-463	SRI-GXAAS
Yunzhe06-281 (*Saccharum* sp.)	Dezhe 93-88 × Yunrui 99-155	SRI-YNAAS
Yunzhe05-250 (*Saccharum* sp.)	Yuenong 73-204 × CP72-1210	SRI-YNAAS
Badila (*S. officinarum*)		SRI-GXAAS
GXS85-30 (*S. spontaneum*)		SRI-GXAAS
57NG208 (*S. robustum*)		SRI-YNAAS
Nagans (*S. bareri*)		SRI-YNAAS
GuangXi bamboo cane (*S. sinense*)		SRI-YNAAS
GXB87-36 (*Erianthus arundinaceus*)		SRI-GXAAS
GXN1 (*Narenga porphyrocoma*)		SRI-GXAAS

[a]GT is the call sign of the clones bred from Sugarcane Research Institute of Guangxi Academy of Agricultural Sciences (SRI-GXAAS), Nanning, China.

at Sugarcane Research Institute of Guangxi Academy of Agricultural Sciences (SRI-GXAAS) (22°85'N, 108°25'E, 61.01 m altitude) in China, with a subtropical monsoon climate (Table 1). Five of the 21 accessions were kindly provided by Sugarcane Research Institute of Yunnan Academy of Agricultural Sciences (SRI-YNAAS) in China (Table 1). Genomic DNA was extracted from young leaves using the SDS method (Huang *et al*. 2010). DNA quantity and concentration were estimated by spectrophotometry (260/280 nm) and agarose gel electrophoresis (1%). DNA samples were diluted to 40 ng/μL in sterile deionized water and stored at -20°C.

Data mining and the development of SSR markers: The NWISRL (http://ssr.nwisrl.ars.usda.gov/) program was used to mine microsatellites in 101,255 unigenes of the sugarcane transcriptome database of variety GT35 deposited in SRI-GXAAS (Huang *et al*. 2016). The search criteria were followed: dinucleotides repeated more than seven times or tri-, tetra- and pentanucleotides repeated more than five times. Primers were designed using Primer primer version 5.0 software and called EST. The main parameters for primers were defined according to the following characteristics: primer length between 18-22 nucleotides, PCR product size between 100-300 bp, and optimum annealing temperature between 50-67°C. The forward primer of each primer pair was labelled at the 5'-end with the fluorochromes FAM. EST-SSR sequences were analyzed in the NCBI non-redundant EST database for likely homology.

DNA amplification and capillary electrophoresis: The amplification reaction mix consisted of 40 ng of genomic DNA, 5×10^{-4} M of each primer pair, 2×10^{-4} M of dNTPs mix, 1 × PCR accompanying buffer (including 2.0×10^{-3} M $MgSO_4$) and one unit of EasyTaq DNA polymerase (Gransgen, China) in a final volume of 20 μL. PCR was performed on a T-gradient thermocycler (Biometra, German). Cycling conditions were 5 min at 95°C followed by 35 cycles of 30 s at 94°C, 30 s at the appropriate annealing temperature, 1 min at 72°C, and a final extension step of 5 min at 72°C. Fragment analysis of amplified PCR products were conducted using capillary electrophoresis (CE) with ABI 3730XL Genetic Analyzer (Applied Biosystems, USA) following the manufacturer's instruction. Each CE sample contained 1 μl diluted post-PCR reaction mixture, 1 μl of ROX-500 size standards, and 10 μl deionized formamide. During the CE process, fluorescence signals from both the size standard and PCR amplified fragments were captured and saved automatically as FSA files.

Data analysis: FSA files were analyzed with GeneMarker software (v2.4.0) to produce capillary electrophoregrams of amplified DNA fragments. Alleles were manually assigned to regular fluorescence peaks and the sizes of alleles were calibrated by the GeneMarker software against the ROX-500 size standards.

The data were scored based on the presence (1) or absence (0) of clear and distinct peaks to form the matrix, for each of the 21 sugarcane genotypes. PIC values were

calculated as:

$$PIC = 1 - \sum_{j=1}^{n} P_{ij}^2 ,$$

where P_{ij} is the frequency of the *j*th pattern for marker i and is summed over n patterns (Anderson *et al*. 1993). The estimate of EST-SSR-based genetic similarity (GS) among the accessions was calculated according to the Jaccard's similarity coefficient (Jaccard P 1901). The corresponding genetic similarity matrix was used to generate a dendrogram based on the Unweighted Pair Group Method with the Arithmetic Average (UPGMA) algorithm. All analyses were carried out using NTSYSpc 1.0.

RESULTS AND DISCUSSION

Development of EST-SSR markers: Of the 101255 unigenes from the sugarcane transcriptome database of variety GT35 deposited in SRI-GXAAS (Huang *et al*. 2016), a total of 31199 sequences were unmapped by BLASTN with sequences of sugarcane available in the NCBI'EST database. To obtain novel EST-SSR markers, 87 EST-SSRs were designed in the 31199 unmapped sequences for possible use as potential genic markers according to the search criteria adopted. Of the 87 markers, 51 EST-SSRs, which gave amplified fragments of foreseen size using GT35 as template, were identified. The details of primer pair sequences and foreseen product size with SSR motifs for the selected markers are described in Supplementary Table 2.

The amplification success rate of the EST-SSRs was 58.6% (51/87) and is similar to those reported in previous studies in sugarcane (60%, Cordeiro *et al*. 2001, 62%, Pinto *et al*. 2010) and barley (64%, Thiel T *et al*. 2003). The remaining (42.0%) primer pairs failed to amplify DNA or resulted in weak DNA amplification. A possible explanation for this result could be that the primer pair encompasses long introns in the genomic DNA, resulting in amplification products either out of the detection range or even not detected. In addition, 2 EST-SSRs (EST1-21 and EST2-10) had a smaller amplified product than expected, which may result from a small deletion within the sequence framed by the two primers (Nicot *et al*. 2004). However, there was no increase in the product size. In fact, compared with genomic SSRs, amplicon size deviated from expectation more frequently (Cordeiro *et al*. 2001, Kota *et al*. 2001, Nicot *et al*. 2004, Thiel *et al*. 2003, Yu *et al*. 2004).

Polymorphism analysis: Fifty-one identified primer pairs flanking SSR regions were used to analyze the polymorphisms in the set of 21 sugarcane genotypes (15 genotypes came from a interspecific hybrid *Saccharum* spp., 5 genotypes from five *Saccharum* species: *S. officinarum*, *S. spontaneum*, *S. robustum*, *S. barberi* and *S. sinense*, and the remaining from 2 different genera *Erianthus Michanx* and *Narenga Bor*). A total of 858 alleles were detected with a range of 5 (EST4-14) to 36 (EST1-18) and the mean of 16.8 alleles per EST-SSR (Fig 1). The PIC values ranged from 0.74 (EST1-7) to 0.95 (EST1-5 etc.) and the average PIC was 0.92 (Table 2).

The average number of alleles and the high PIC values obtained in this study were typical SSR markers, revealing high levels of polymorphism. These results suggest that the isolated markers will be useful in determining sugarcane parentage and making clonal assessments. Furthermore, the markers exhibited a great potential for discrimination and construction of genetic linkage maps, as they will most

Table 2 Details of 51 new polymorphic EST-SSR markers in sugarcane

SSR[a]	Motif	Tm	Foreseen size (bp)	Forward primer (5'-3')	Reverse primer (5'-3')
EST1-2	(AC)7	55	118	ACCCTCCAGCCACCCTT	GCATACACCATCCATCCCT
EST1-5	(AG)11	67	169	AGGCGGGACCGAACAA	GACCTCATCTGCCGGACTG
EST1-6	(GT)12	60	157	ATTAGTGGCTCTGCCGTTAT	CAATGGTAGAGTGAAAGTGGG
EST1-7	(TC)7	52	202	TCTGCCCAAGACGGTAA	GCTGCCTGAGTTGCTGA
EST1-9	(CT)10	58	186	AACCTTCCTTCCGCTCCC	GAATCTTGACCGCCTTTGG
EST1-12	(CT)12	64	186	TGCGAGCTGCGATGGA	GGTTGGGACCTGGGTTG
EST1-14	(AG)10	50	288	TTATTCAGACCTCCGCTAC	ACGACGGCTCAGGAAT
EST1-15	(TA)8	52	237	CCAAACCCAAGGCTCA	ATGCGACTAAAGGTTGAAGA
EST1-16	(GA)7	51	253	GGAATCCAAGCCAACG	CCACGGCTGCTCTTCT
EST1-17	(CT)7	50	152	CCTTCACCTGGAATCGT	CAAGCCCAACTCCTCAA
EST1-18	(TC)8	62	131	CATAACCACTTGGACCACC	CCGTTGACCTGCGAAT
EST1-19	(AG)8	55	269	GAAGTTGATGCCAGATGGG	TCCCTTCCTGCCAACTCT
EST1-21	(TC)8	67	243	CTCGGCGTCACCGTCAT	GAGGGCTTCTCATCATCACTAG
EST1-22	(CGC)5	62	291	AATCCGCCAGCACCTACCC	GATCTCGTCGGGCAGGTCC
EST1-23	(TG)7	59	233	ATTCACTTCACTGCCCAAGC	TAGGGACGGAGGGAGTAGG
EST1-24	(TC)8	63	269	ATGGAGGAGGCAAAGAGA	AATGCGAACAAACAGACG

Cond.

Table 2 (*Concluded*)

SSR[a]	Motif	Tm	Foreseen size (bp)	Forward primer (5'-3')	Reverse primer (5'-3')
EST1-26	(GA)8	54	138	AGGCAAGAACCCCAAGC	ATTCCACAACCAAAACCC
EST2-1	(CTG)11	50	141	AACCGAACCTGAACTCC	AGATGCCGACGACCTG
EST2-2	(GCC)7	56	157	TCATCCAACGCCACCG	ACTGCCCGCACCACATC
EST2-4	(GGC)6	56	128	GCAGCTTTCTCGATTCCC	CTCCACCAAACCCTCCCT
EST2-7	(CTC)7	59	151	CATTGCTACTCGCATTCACC	ACCACCAACAGCCTTCTCAT
EST2-8	(GTG)6	52	242	ATCCGGGACCATGAATC	TTGCCAAACGAACACG
EST2-9	(ACA)8	55	274	GAACCACATCACCCTATACCA	CAGCAGCTTCCAGTCATCAA
EST2-10	(CGC)6	53	181	CAGGACTCGCTTCTCCG	CATCATCTTCTTCGGCAAC
EST2-11	(CCT)7	54	197	TAATCTGCTCTGCGTCTCC	GGTGATCCAGGGCGTGT
EST2-13	(GCG)6	57	249	AAGCATAATCAGGCAAAGG	GCCAACTCCGTCTCCAC
EST2-14	(CAG)8	55	234	GTGGGCGTGGTAGGGAT	CTGCTCGTGCTCTTGCTC
EST2-15	(CGC)6	56	169	ACGAGTAGGAGTAGGACGACG	CCCATAGCCTGCCTGATAG
EST2-18	(ACA)10	50	128	CTGAGAACGAAATGGGTG	CCACCTTGCTTGGGAC
EST2-19	(GTT)6	50	217	CGTGCTGGAACCGTAA	GCCGCAGAAACAATCA
EST2-20	(TTG)7	54	215	ATAAGATCCGTGGTAGGGTAA	AGGGACGAAGGGAGTGC
EST2-21	(CCA)6	63	205	GCGGAGGAGGGAGGACAA	ATGAGTAGCGACTGTGCGAGTG
EST2-22	(CTC)8	64	163	GTCATTTGAAGCATCAGCATTG	TGAGGGTGGGAAAGAGCAG
EST2-23	(CCT)7	59	205	CACCAACCCTAGATCCACCC	ACGGCCCGAGGCAAGT
EST2-27	(GAC)6	62	138	GCCGCAAACCATGCTGAAC	GAGCATCATCCCTCCATTCACA
EST2-28	(ATC)6	54	156	GCCAGCAAATCTCCCAC	AAGGCTGAGTTAATAAGATGCA
EST2-32	(GCG)5	51	260	GCGTAACATCTTCTTGCTG	CGTTGTCGTCCCCACT
EST2-34	(CTC)5	66	261	GCCGCACATCCGTTGG	GAGGAAGAGGAGTTCGAGGG
EST2-35	(TCG)6	57	126	GGTGATGGTGTCGCTTGTG	ACCGTGCTGTCCCTGCT
EST3-3	(GCAG)5	51	117	ACCGAGTGGAGTAGTAGGC	GGGTTGGAAGGAGGAAG
EST4-2	(CCA)5-(CCT)5	63	202	TCCTTCCCCGACTCTTCTCC	GCATGAGCTGGCCACGC
EST4-3	(GTC)4-(GCC)3	57	113	GGCTTCCAGATTTCCTCTACTT	GCAACGCAGCATTTCCA
EST4-5	(AGAA)3-(AAG)3	58	164	GGTCGTTGCTCTTCAGTTGC	CCTCTTCCGATTCTTTCCTTT
EST4-6	(GGC)3-(CTC)4-(CCT)5	52	210	CGAATGGCAGGAACGA	CGCCAGTTGAGGGAGA
EST4-8	(AG)5-(GATT)3	54	250	TGTTCAGCTTGGCTACTGC	TCCTGATTCCTTCCGTTG
EST4-9	(TCC)5-(CAC)3	53	153	CTGGATGACCGCCGTAT	AGAACAAGTGGCAACAAGC
EST4-12	(GCA)3-(GGC)4	60	253	AGTCGTTCTCGGAGCTGTCG	CCCTGGCGGCAGTTGTT
EST4-14	(ATG)3-(GAT)3	50	206	CAGTAGCAGCAACAGTAGTAAC	TGCTACTACCAACCTCGTC
EST4-17	(TCC)5-(TCC)3	58	141	CCCTCGAACTCCTCTTCCTC	AGGGGTTAGAATAGAACCCATG
EST4-19	(TCC)4-(CCT)3	58	210	CAGCCTCTTCTTTCCGTTCA	TCAAACAGGAGCAGCAGAAAG
EST4-21	(GCTG)3-(GCT)3	55	168	CTGGCTAACAAACAAGGGAC	CACCGTCATTGAGACCAGATA

[a]EST1, EST2, EST3 and EST4 are di-, tri-, tetranucleotide and combined nucleotide primer pairs, respectively.

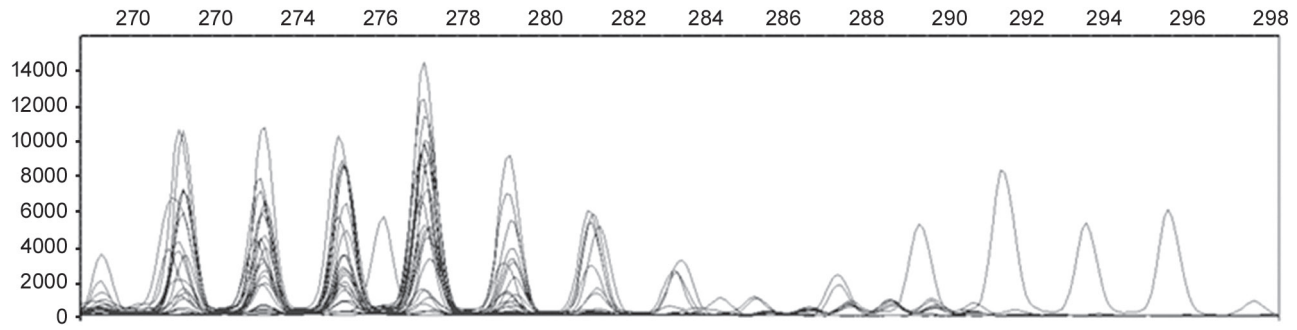

Fig 1 Capillary electrophoregrams showing the relative positions of amplified DNA fingerprints or alleles for 21 genotypes with EST-SSR EST1-24. The X-axis shows the size scale of 269-298 bp from left to right. The Y-axis values show the fluorescence intensity that reflects the quantity of amplified DNA fingerprints.

likely segregate in crosses.

Transferability potential: Of the 51 EST-SSRs evaluated, 45 (88.2%) and 50 (98.0%) could be transferred to *Erianthus* Michanx and *Narenga* Bor, totaling to 213 and 291 alleles with a mean of 4.2 and 5.7 alleles per EST-SSR respectively. Maximum transferability to *Erianthus* Michanx and *Narenga* Bor was 60% (EST1-2) and 63.6% (EST1-6 and EST1-7), respectively, with the average transferability of 23.9% and 34.4% respectively (Table 3).

Most of the 51 EST-SSRs could be transfered successfully to two related genera and the rate of transferability for *Narenga* Bor was higher than that of *Erianthus* Michanx. However, the number of alleles transferred for majority of EST-SSRs indicated low level of polymorphism, indicating the particular allelic region to be highly conserved and allowing no variation in the SSR or its surrounding sequence. Alternatively, the primers might amplify a region of DNA, which had undergone an SSR expansion in one lineage but not in related genera. In addition, since only one accession was used to represent each genus, more genotypes will be evaluated to better determine transferability.

Genetic similarity and cluster analysis: Allelic data from the 51 EST-SSRs were used to construct a dendrogram in the presented sugarcane genotypes, which evaluated their potential of acting as polymorphic markers in genetic studies. Genetic similarities based on the Jaccard coefficient varied from 0.390 between GT32 and GXB87-36 to 0.733 between GT32 and GT35, respectively, with an average of 0.590. The dendrogram showed GXB87-36, belonging to related genus of *Erianthus arundinaceus*, in a separate cluster I, with the others in cluster II. At the 0.47 similarity level, cluster II was divided into two subgroups, GXN1 and the rest genotypes, which were attached to the genus

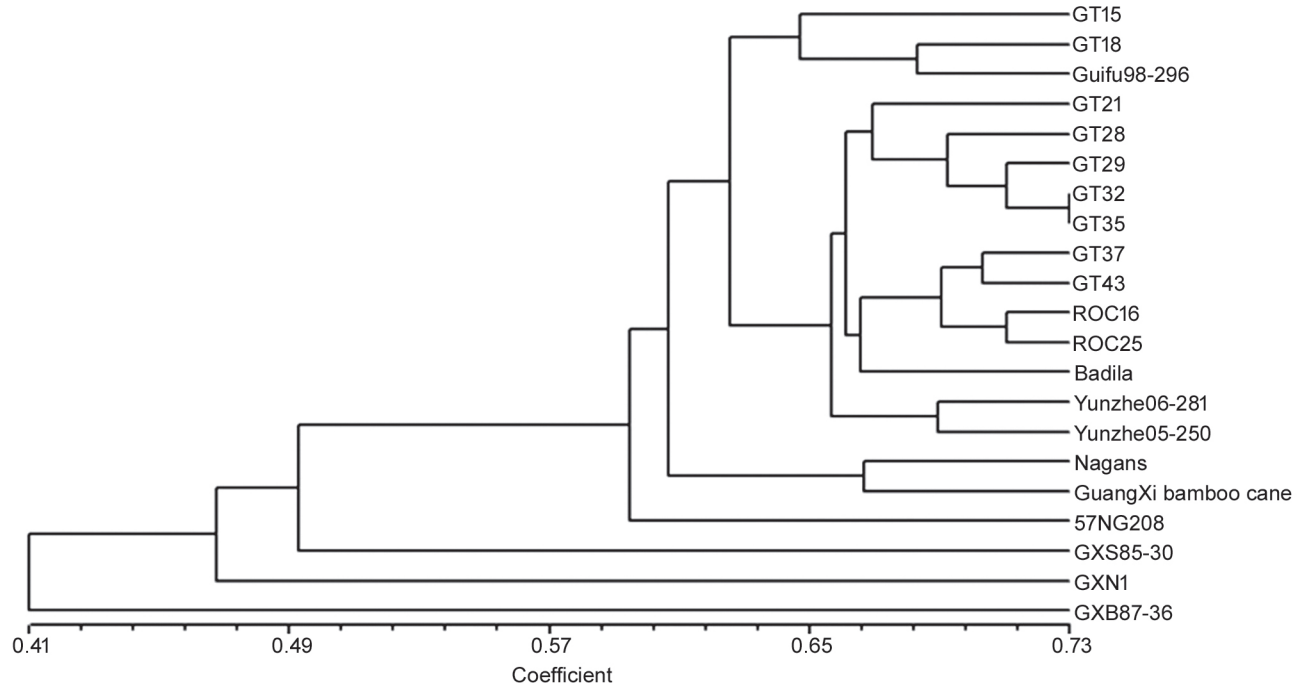

Fig 2 UPGMA dendogram representing the relationship among the 21 genotypes of sugarcane using Jaccard's similarity coefficients.

Table 3 Polymorphism and transferability of 51 new polymorphic EST-SSR markers in sugarcane

SSR	Allele range (bp)	Number of allele	PIC	Primers transferred (*Erianthus Michanx*)		Primers transferred (*Narenga Bor*)	
				No.	%	No.	%
EST1-2	105-328	10	0.91	6	60.0	6	60.0
EST1-5	101-187	13	0.95	0	0.0	1	7.7
EST1-6	138-161	11	0.83	4	36.4	7	63.6
EST1-7	116-239	11	0.74	3	27.3	7	63.6
EST1-9	100-259	27	0.95	12	44.4	10	37.0
EST1-12	112-330	22	0.95	1	4.5	7	31.8
EST1-14	176-323	17	0.94	1	5.9	8	47.1
EST1-15	81-297	17	0.95	1	5.9	7	41.2
EST1-16	110-379	15	0.94	4	26.7	6	40.0
EST1-17	147-196	6	0.82	3	50.0	3	50.0
EST1-18	113-230	36	0.95	12	33.3	17	47.2
EST1-19	152-388	19	0.95	7	36.8	5	26.3
EST1-21	111-223	13	0.94	2	15.4	5	38.5
EST1-22	103-310	9	0.79	2	22.2	0	0.0
EST1-23	107-335	14	0.95	0	0.0	7	50.0
EST1-24	269-298	17	0.90	3	17.6	13	76.5
EST1-26	107-264	15	0.95	5	33.3	4	26.7
EST2-1	125-399	13	0.94	4	30.8	4	30.8
EST2-2	76-189	18	0.90	8	44.4	7	38.9
EST2-4	124-319	18	0.91	2	11.1	1	5.6
EST2-7	87-165	14	0.94	4	28.6	3	21.4
EST2-8	208-243	8	0.93	2	25.0	4	50.0
EST2-9	98-306	25	0.95	4	16.0	5	20.0
EST2-10	100-158	6	0.85	0	0	2	33.3
EST2-11	86-399	16	0.92	4	25.0	5	31.3
EST2-13	91-374	28	0.95	10	35.7	10	35.7
EST2-14	93-380	26	0.95	20	76.9	17	65.4
EST2-15	118-292	22	0.95	6	27.3	8	36.4
EST2-18	120-362	13	0.95	3	23.1	3	23.1
EST2-19	78-364	16	0.90	0	0.0	2	12.5
EST2-20	96-370	17	0.82	1	5.9	4	23.5
EST2-21	127-363	25	0.95	4	16.0	6	24.0
EST2-22	87-185	17	0.95	0	0.0	3	17.6
EST2-23	89-334	18	0.95	5	27.8	4	22.2
EST2-27	125-141	7	0.80	2	28.6	5	71.4
EST2-28	95-232	13	0.95	2	15.4	2	15.4
EST2-32	100-336	23	0.95	1	4.3	9	39.1
EST2-34	233-259	10	0.93	3	30.0	4	40.0
EST2-35	92-381	21	0.95	4	19.0	3	14.3
EST3-3	81-395	28	0.95	7	25.0	10	35.7
EST4-2	76-396	27	0.95	11	40.7	1	3.7
EST4-3	94-341	18	0.95	4	22.2	6	33.3
EST4-5	81-302	10	0.86	0	0.0	1	10.0

Cond.

Table 3 (*Concluded*)

SSR	Allele range (bp)	Number of allele	PIC	Primers transferred (*Erianthus Michanx*)		Primers transferred (*Narenga Bor*)	
				No.	%	No.	%
EST4-6	98-351	35	0.95	7	20.0	12	34.3
EST4-8	122-279	22	0.95	8	36.4	8	36.4
EST4-9	93-313	18	0.95	9	50.0	11	61.1
EST4-12	88-256	12	0.93	1	8.3	2	16.7
EST4-14	148-211	5	0.83	1	20.0	1	20.0
EST4-17	124-155	17	0.95	4	23.5	7	41.2
EST4-19	137-249	9	0.93	4	44.4	5	55.5
EST4-21	103-168	11	0.93	2	18.2	3	27.3
Total		858		213		291	
Mean		16.8	0.92	4.2	23.9	5.7	34.4

of *Narenga porphyrocoma* and *Saccharum*, respectively (Fig 2).

UPGMA cluster analysis of 21 genotypes in the present study produced meaningful groupings based on pedigree of the accessions. The *Erianthus* and *Narenga* clones appeared as two outgroups in the dendrogram, sharing GS values of 0.42 and 0.47 respectively. These data support the classification of *Erianthus* and *Miscanthus* as separate genera, but also indicate some evolutionary relationship to the *Saccharum* genera. By analyzing the dendogram, it was observed that genotypes belonging to *Saccharum* genus (*S. officinarum*, *S. spontaneum*, *S. robustum*, *S. barberi* and *S. sinense*) were grouped together, suggesting that they share a narrow genetic base. There into, *S. officinarum* evolved from *S. robustum*, and *S. barberi* and *S. sinense* introgressed mainly from hybridization between *S. officinarum* and *S. spontaneum* (Chen *et al*. 2011). Therefore, 57NG208 (*S. robustum*), Nagans (*S. bareri*) and GuangXi bamboo cane (*S. sinense*) shared closer relationships with Badila (*S. officinarum*) than with GXS85-30 (*S. spontaneum*). Furthermore, modern sugarcane cultivars are interspecific hybrids derived from crosses between *S. officinarum* and *S. spontaneum* with approximately 80-85% of the genome contributed by *S. officinarum*, 10-15% by *S. spontaneum* (D'Hont *et al*. 1996). This result was representative, which agrees with the possible evolutionary course of sugarcane genotypes.

ACKNOWLEDGMENTS

This research was financially supported by National Key R&D Program of China (2018YFD0201100), National Natural Science Foundation of China (31960450, 31760415, 31901510, 31801422), Guangxi Key Research and Development Program (Gui Ke AB16380126, Gui Ke AB16380157), Guangxi Special Fund for Scientific Base and Talent (Gui Ke AD17195100), Guangxi Sugarcane Innovation Team Project for Modern Agro-industry Technology (gjnytxgxcxtd-03-01), Guangxi financial project: sugarcane new varieties breeding and extension, Team Project for Guangxi Academy of Agriculture Sciences (2015YT04, 2015YT02).

REFERENCES

Anderson J A, Churchill G A, Autrique J E, Tanksley S D and Sorrells M E. 1993. Optimizing parental selection for genetic linkage maps. *Genome* **36**(1): 181-6.

Carson D L and Botha F C. 2000. Preliminary analysis of expressed sequence tags for sugarcane. *Crop Science* **40**(6): 1769-79.

Chen R, Xu L, Lin Y, Deng Z, Zhang M, Luo J, Zhang H, Gao S, Xu J, Xu L, Que Y, Chen P, Yuan Z and Lin G. 2011. *Modern Sugarcane Genetic Breeding,* pp 20-24. China Agriculture Press**,** Beijing, China.

Cordeiro G M, Casu R, McIntyre C L, Manners J M and Henry R J. 2001. Microsatellite markers from sugarcane (*Saccharum* spp.) ESTs cross transferable to erianthus and sorghum. *Plant Science* **160**(6): 1115-23.

D'Hont A, Grivet L, Feldmann P, Rao S, Berding N and Glaszmann J C. 1996. Characterisation of the double genome structure of modern sugarcane cultivars (*Saccharum* spp.) by molecular cytogenetics. *Moletical and Genied Genetics* **250**(4): 405-13.

Huang D L, Qin X L, Liao Q, Gao Y J and Fang F X. 2010. Simple and rapid procedure for isolation of high quality genomic DNA from sugarcane. *Biotechnology Bulletin* **20**(5): 101–6.

Huang D L, Gao Y J, Gui Y Y, Chen Z L, Qin C X, Wang M, Liao Q, Yang L T and Li Y R. 2016. Transcriptome of high-sucrose sugarcane variety GT35. *Sugar Tech* **18**(5): 520–8.

Jaccard P. 1901. Etude de la distribution florale dans une portion des Alpes et du Jura. *Bulletin De La Societe Vaudoise Des Sciences Naturelles* **37**(142): 547-79.

Kota R, Varshney R K, Thiel T, Dehmer K J and Graner A. 2001. Generation and comparison of EST-derived SSRs and SNPs in barley (*Hordeum vulgare* L.). *Hereditas* **135**(2-3): 145-51.

Li Y R. 2010. *Modern Sugarcane Science*, pp 1-4. China Agriculture Press**,** Beijing, China.

Marconi T G, Costa E A, Miranda H R, Mancini M C, Cardoso-Silva C B, Oliveira K M, Pinto L R, Mollinari M, Garcia A A F and Souza A P. 2011. Functional markers for gene mapping and genetic diversity studies in sugarcane. *BMC Research Notes* **4**: 264.

Nicot N, Chiquet V, Gandon B, Amilhat L, Legeai F, Leroy P,

Bernard M and Sourdille P. 2004. Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags (ESTs). *Theoretical and Applied Genetics* **109**(4): 800-5.

Oliveira K M, Pinto L R, Marconi T G, Margarido G R A, Pastina M M, Teixeira L H M, Figueira A V, Ulian E C, Garcia A A F and Souza A P. 2007. Functional integrated genetic linkage map based on EST-markers for a sugarcane (*Saccharum* spp.) commercial cross. *Molecular Breeding* **20**(3): 189-208.

Oliveira K M, Pinto L R, Marconi T G, Mollinari M, Ulian E C, Chabregas S M, Falco M C, Burnquist W, Garcia A A F and Souza A P. 2009. Characterization of new polymorphic functional markers for sugarcane. *Genome* **52**(2): 191-209.

Pinto L R, Oliveira K M, Ulian E C, Garcia A A and Souza A P. 2004. Survey in the sugarcane expressed sequence tag database (SUCEST) for simple sequence repeats. *Genome* **47**(5): 795-804.

Pinto L R, Oliveira K M, Marconi T, Garcia A A F, Ulian E C and Souza A P. 2010. Characterization of novel sugarcane expressed sequence tag microsatellites and their comparison with genomic SSRs. *Plant Breeding* 125(4): 378-84.

Silva J A G D. 2001. Preliminary analysis of microsatellite markers derived from sugarcane expressed sequence tags (ESTs). *Genetics and Molecular Biology* **24**(1-4): 155-9.

Silva D C, Souza M C P D, Filho L S C D, Santos J M D Barbosa G V D S and Almeida C. 2012. New polymorphic EST-SSR markers in sugarcane. *Sugar Tech* **14**(4): 357-63.

Thiel T, Michalek W, Varshney R K and Graner A. 2003. Exploiting EST databases for the development and characterization of gene-derived SSR-markers in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* **106**(3): 411-22.

Yu J K, Dake T M, Singh S, Benscher D, Li W, Gill B and Sorrells M E.2004. Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. *Genome* **47**(5): 805-18.