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Identification of elite Indian sugarcane varieties through DNA fingerprinting using genic microsatellite markers

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Abstract

Sugarcane is one of the important crops for sustainable economy and global energy security with high phenotypic polymorphism. Precise identification and characterization of plant genetic resources is one of the most essential aspects for breeding programs. Sugarcane popular hybrid varieties and genetic stocks routinely being used as a parent in hybridization program were included in the study. Molecular cataloguing was accomplished using a set of 70 primer pairs, of which ~ 39% were found to be robust polymorphic and unique in all the genotypes used. The number of DNA bands amplified by expressed sequenced tags derived simple sequence repeat (EST-SSR) primers ranged from 1 to 10 with an average of 5 bands per primer pair and their fragment size ranged between 50 and 1690 bp. Polymorphism information content (PIC) values were ranged from 0.17 to 1.00 with an average of 0.58. Unique DNA fingerprints of thirteen elite sugarcane varieties were generated with 27 polymorphic sugarcane specific EST-SSR markers. Analyses of DNA profiles produced by microsatellite markers showed the unique bands specific for individual variety. Generated DNA profiles would facilitate accurate variety identification in the perspective of protection of the breeder's intellectual property rights, farmer's rights and their purity testing. In addition, developed reliable DNA fingerprinting system will be able to track a potent parent genotype for breeding programs. The identified genotype specific and unique EST-SSR markers will be valuable for conservation and management of sugarcane genetic resources.

Keywords DNA fingerprinting · EST-SSR markers · Morpho-agronomical descriptors · Variety identification · Germplasm conservation and management · Purity testing

Introduction

Sugarcane (*Saccharum* sp. hybrid L.) is an important agro-industrial crop which contributes greatly to national gross domestic product (GDP) and provides employment opportunities to farmers, agriculture labourers, transporters and factory workers in India as well as in many other countries (Hoang et al. 2015). Sugarcane crop provides feedstock to industry for sugar and jaggery production and industrial cellulosic biomass reprocessed to electricity production and second-generation bioethanol production (De Souza et al. 2013). Ever-increasing number of sugarcane varieties being developed by the different public or/and private institutions in India and many other sub-tropical and tropical countries of the world. Variety diversification is the most important to improve farmers economic status and survival of the sugar industry. This is not only the desirableness of variety protection as well as the exigency of monitoring the genetic diversity in breeding programs. Therefore variety identification and characterization is extremely important for all agricultural practices (Anonymous 2012). Precise identification of the newly developed crop varieties is also essential for their registration, seed multiplication, trade and inspection, and germplasm conservation and management. Since genotype identification and characterization is the primary step in any crop improvement program, quick recognition of cultivars

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would hence provide value-aided information for crop breeding (Korir et al. 2012).

The traditional approach to germplasm identification is based on set of morpho-agronomical descriptors which involves observation and recording of gross morphological characters (Artschwager and Brandes 1958; LaBorde et al. 2008). In sugarcane there are the some major characteristics used to assess the distinctiveness, uniformity and stability (DUS) which includes; leaf sheath trichomes, shape of ligule, dewlap colour, leaf blade curvature, leaf blade width, internode colour, internode diameter, shape of internode, rind surface appearance, stalk waxiness, shape and size of bud, groove of bud, bud tip in relation to growth ring, number of millable canes (NMC) per stool, cane height, and others (Anonymous 2006). Phenotypic traits are often multigenic, not available at all growth stages and considerably influenced by prevailing environment (viz; light, ambience temperature, relative humidity, rainfall, atmospheric pressure, soil health and others) as well as geographical factors (latitude, altitude, tropical and sub-tropical) (Govindaraj et al. 2012; Singh et al. 2018). Thus, in present scenario a strong base is extremely required for the cultivar identification, protection of the breeder's intellectual property rights, germplasm conservation and paternity testing of the released varieties (Hemaprabha et al. 2010) and early assessment of plantlets in sugarcane nursery (Silva et al. 2012).

With the devise of several molecular marker techniques, morpho-agronomical descriptors have supplemented with DNA markers in genotype identification and characterization (Singh et al. 2019a). Uniquely, DNA-based markers are not influenced by environmental fluctuations and detectable in all tissues regardless of plant age, growth and developmental stages, thus allowing precise germplasm identification, conservation and management across different environmental conditions (Singh et al. 2013, 2014a, 2015). Thus, molecular markers have emerged as powerful tools to identify the plant varieties. Several molecular marker techniques have been devised for DNA fingerprinting based genotype identification (Singh et al. 2011, 2017). Microsatellites or simple sequence repeat (SSR) marker technique preferred as a potent molecular tool owing to their desirable genetic attributes of reproducibility, relative abundance, multiallelic nature, co-dominant inheritance and amenable to high throughput genotyping in sugarcane and allied grasses (Cordeiro et al. 2001; Varshney et al. 2002; Singh et al. 2014b, 2019b).

However cost-effective DNA marker systems (SSRs) have been developed by several research groups over the world, but a huge number of varieties have been developed and used in breeding which makes fingerprinting job expensive in terms of laboratory resources, labor and time expenditure (Zhu et al. 2012; Singh et al. 2019a). In spite of that, it is inevitable to assemble the fingerprinting database of the elite commercial sugarcane varieties for frequent and unambiguous identification with the other developed similar varieties (Hemaprabha et al. 2010; Singh et al. 2017). In present study, EST-derived sugarcane microsatellites (EST-SSR) markers developed by our team (Singh et al. 2019a) in previous investigation were used to identify and characterize 13 popular Indian sugarcane varieties through DNA fingerprinting. Most of the selected varieties are under cultivation at medium to large scale in sub-tropical areas of India. Used varieties represent important genetic stock and frequently being used as parents to establish a cross for breeding high yielding variety. The variety specific molecular cataloging was carried out for each individual accession which maintained as a reference dataset at Sugarcane Research Institute (UPCSR), Shahjahanpur, (U.P.) India.

Materials and methods

Sugarcane varieties

A set of 13 promising sub-tropical CoS, (Coimbatore–Shahjahanpur) CoSe (Coimbatore–Seorahi) and UP (Uttar Pradesh) sugarcane varieties developed at Sugarcane Research Institute (UP Council of Sugarcane Research), Shahjahanpur, (UP), and its regional center Genda Singh Sugarcane Breeding & Research Institute (GSSBRI), Seorahi, (UP), India were selected. Although used all the 13 varieties are not frequently under cultivation at farmer's fields but, they have ruled over the entire sugarcane growing areas of the northern India and are still being cultivated in many of the sugar factories of Uttar Pradesh. Since they are having some untapped useful alleles in their genome, owing to this reason these varieties are frequently used as proven parents in sugarcane genetic improvement programs to breed promising varieties for subtropical India. Genetically pure material of these genotypes was collected from experimental farms of the institute. Variety name, their parentage, maturity group, year of release, and originating center are detailed in Table 1.

Extraction of genomic DNA and PCR amplification

Genomic DNA was extracted from disease free immature fresh leaves of all the 13 sugarcane varieties using CTAB protocol described by Hoisington (1992) with minor modifications (Singh et al. 2011). To remove out the RNA content, raw DNA was treated with RNase enzyme for 1 h at 37 °C. The DNA was diluted to a final concentration of ~25 ng/µl as determined by agarose-gel electrophoresis using known concentration of uncut λ DNA as standard. Total 361 functionally informative microsatellite primers were designed from conserved nucleotide sequences SSR motifs of the

Table 1 Description of the 13 elite sugarcane varieties identified with informative microsatellite (EST-SSR) markers

S. no.	Sugarcane varieties	Parentage	Maturity group	Year of release and notification	Originating centre
1.	CoS 767	Co 419 × Co 313	Mid-late	1982	UPCSR, Shahajahanpur
2.	CoS 99259	CoS 0767CG	Mid-late	2004	UPCSR, Shahajahanpur
3.	CoS 96268	Co 1158 × Co 62198	Early	2007	UPCSR, Shahajahanpur
4.	CoS 8432	MS 68/47 × Co 1148	Mid-late	1987	UPCSR, Shahajahanpur
5.	UP 0097	Se1444/91 × Se1854/91	Mid-late	2003	SRS, Gorakhpur
6.	CoS 95255	Co 1148 × Co 62198	Early	2004	UPCSR, Shahajahanpur
7.	CoS 96269	BO 108 × Co 1148	Mid-late	2004	UPCSR, Shahajahanpur
8.	CoSe 05451	CoPant 90223 GC	Early	2012	GSSBRI, Seorahi
9.	CoS 8436	MS 68/47 × Co 1148	Early	1987	UPCSR, Shahajahanpur
10.	CoS 08279	CoLK8102 × Co89003	Mid-late	2012	UPCSR, Shahajahanpur
11.	CoSe 92423	BO 91 × Co 453	Mid-late	2001	GSSBRI, Seorahi
12.	CoS 03251	Co 1158 × C0 62198	Early	2011	UPCSR, Shahajahanpur
13.	CoSe 03234	BO 91 PCGC	Early	2008	GSSBRI, Seorahi

non-redundant EST-database of sugarcane under our previous study (Singh et al. 2019a). To check the genetic uniformity of the SSR primers, ten individual DNA samples of each sugarcane variety were assayed with five sugarcane specific EST-SSR primers. Only monomorphic DNA profiles were observed between individuals within a genotype and thus establish confirmation to marker's genetic uniformity. A set of seventy sugarcane EST-SSR markers were employed in genetic characterization assay, among them 27 primer pairs (Table 2) that generated unambiguous and reproducible profiles were used to construct DNA fingerprints of the 13 sugarcane varieties under study. Conserved genetic regions of the SSR motifs were PCR amplified with polymorphic EST-SSR primers in 10 µl reaction volume. PCR mixture were containing 25 ng of template DNA, 0.02 mM of both forward and reverse primers, 10 mM of each dNTPs (dATP, dTTP, dGTP, and dCTP), 0.5 U of Taq DNA polymerase (Bangalore Genei™, India) and 1.0 µl of 10× PCR buffer with 2.5 mM of $MgCl_2$. The PCR reactions were performed in a thermo cycler (M J Research PTC 100) using the following thermal conditions as; initial denaturation at 94 °C for 5 min, followed by 30 cycles consisting of 94 °C for 1 min, primer annealing at optimised (50–60 °C) temperature for each primer pair for 1 min, and primer elongation at 72 °C for 2 min followed by a final primer extension step at 72 °C for 5 min. Three times PCR reactions were repeated for each primer pair to ensure the reproducibility of EST-SSRs.

Analysis of microsatellite (EST-SSR) data

The PCR products were resolved on a 7.5% native polyacrylamide gel electrophoresis (PAGE) in vertical gel electrophoresis unit (Bangalore Genei™) using 0.5× TBE buffer. Visualization of bands was carried out by 0.5 µg/ml ethidium bromide (EtBr) and images were captured under UV light using gel documentation system (Alpha Innotech). Thirteen genotypes were compared with each other using their PCR amplified DNA profiles generated with specific markers. Only unambiguous, clear-cut, robust, and reproducible bands were scored and unclear and diffused bands ignored.

Results and discussion

DNA fingerprints and molecular cataloging of identified varieties

The elementary DNA fingerprints of the 13 popular sugarcane varieties were generated successfully with the 27 robust polymorphic EST-SSR markers. A set of 70 primer pairs were used to amplified, of which ~39% were found to be robust polymorphic. The number of DNA bands amplified by EST-SSR primers was ranged from 1 to 10 with an average of 5 bands per primer and their fragment size ranged between 50 and 1690 bp. Polymorphism information content (PIC) value was ranged from 0.17 to 1.00 with an average of 0.58 (Table 2). For instance, DNA profiles of the sugarcane variety CoS 767 was generated using a set of 22 EST-SSR markers (Fig. 1). The developed microsatellite markers were found to be potentially suitable for DNA fingerprinting based genotype identification and characterization. The molecular profiles and genotype specific unique DNA bands produced by different EST-SSR markers have maintained as a molecular catalogues of each identified variety (Table 3).

The results of the DNA fingerprinting in promising sugarcane varieties have revealed the authenticity of EST-SSR marker system for the identification and characterization of

Table 2 Details of microsatellite (SSR) primers like; primer Id (SuMS; sugarcane microsatellite), primer sequence, repeat motifs, Ta, number and size of fragments amplified, and their PIC value

S. no.	Primer ID	Primer sequence (5′→3′)	Repeat motif	Annealing temp (T_a °C)	No. of band amplified	Band size range (bp)	PIC value
1.	SuMS1	F-GTCTCTTTTGGGTTTCATCTC R-AGATCTTTGCCTCAGTTTCAT	(ACC)4	50	1–6	50–556	0.77
2.	SuMS2	F-GCATATGCAATTATGACTTCAC R-CCAATGAATGTGTGTGTCTG	(GTCCT)5	52	2–5	106–1354	0.77
3.	SuMS3	F-AACAGAGATAGCGTTCAGGTT R-TAGACTATGACACGGACCAGT	(AC)11	50	1–6	50–1125	0.25
4.	SuMS4	F-CAAAGCAAAAGAAGAAGAAGA R-CCAGAAACTGCAAAAGATGT	(TCCT)4	52	1–3	103–940	0.72
5.	SuMS5	F-AATTGGAGAAATCAGGAAGAC R-AGAGTTTGCATGCCTGAC	(AGG)4	55	1–9	146–1560	1.00
6.	SuMS6	F-TGATTGTCTAGTTGTGTGTGC R-ATTTTCATCAACTGGCAGAG	(AAG)4	52	1–4	50–940	0.20
7.	SuMS7	F-AACAAAGCAAAAGAGAAGAGG R-AGAAGTCTACGCCAGCACT	(GA)12	53	3–8	206–1606	0.34
8.	SuMS8	F-GAGTATATAAGGAGGGGCAAG R-TACAGGTTTAGGTCGTCGTTA	(GAG)4	53	1–10	110–1248	0.17
9.	SuMS9	F-ATCCAATCCAAGTTCCAAG R-CACGGACTGGATCGTTAC	(GTT)5	53	1–7	50–1280	0.50
10.	SuMS10	F-TGAAGCTTGCTTTAATCCATA R-CTATCCTGTTTTGTTGCATGT	(CCG)5	53	1–7	58–1310	0.50
11.	SuMS11	F-TTAATCTTGTTGAGTGGCTGA R-ATACTGTCACGATGGTGCTT	(GAGGA)3	53	1–4	50–1150	0.50
12.	SuMS12	F-AGAGAAGGTGATTGTTCTTGG R-GTTAGAATAACAAACGGCGTA	(AT)10	52	1–6	348–1182	0.50
13.	SuMS13	F-CCATTAAGGTGGTTCTCCA R-TTTTTGAGCATCCTGCTACT	(GCG)4	52	1–7	50–1495	0.47
14.	SuMS14	F-TAATATCGCGATGCTCTAGTT R-ATACTGTCACGACGGTGCT	(GGA)4	52	2–10	50–1205	0.60
15.	SuMS15	F-AGGTATTCGCGATGATCTAGT R-ATTTTCATCAACTGGCAGAG	(GGT)5	53	1–7	120–1595	0.86
16.	SuMS16	F-GCAAGCTTGAGGCTAGAAT R-CTTCAATTTGGAAGAAACTTG	(GGA)4	53	1–7	130–1690	0.70
17.	SuMS17	F-GTACGCGGGAGAGTCAAG R-GTTGCAGAAACAAATGAAAGA	(CGC)6	53	1–8	420–1321	0.67
18.	SuMS18	F-CTCCATGGGAAATCTTCAT R-GTGAGGGCGATAAATTAGG	(TGT)7	53	0–6	75–1203	0.60
19.	SuMS19	F-AGGATAAAGAGGGGAAGAAAG R-TTTCTCATCATCCTTCCTTTT	(TCA)4	53	1–5	150–1132	0.86
20.	SuMS20	F-GAAAGGATAAAGAGGGGAAG R-TTTTTCATTTTCTGGCTCTC	(AC)11	50	0–4	345–1132	0.25
21.	SuMS21	F-CGTTTATCATGTGGGTGTAGT R-ATTAATAGAGGCAAGCAAAGC	(AGCT)3	50	1–8	210–1200	0.50
22.	SuMS22	F-CTCGCTCCTCTTCGAGAT R-GCATCATCAAACAATCCTG	(ATCA)3	50	1–4	236–781	0.60
23.	SuMS23	F-CCTGCCTCCTCAGTCTCT R-GCGGAAAAAGGGTAGAGT	(GGTT)3	50	1–6	76–1666	0.20
24.	SuMS24	F-GTTCCCCTTTCTCTCGTC R-AGTTCTTCAAATCAAGCCTCT	(ATTG)3	50	2–4	701–1245	1.00
25.	SuMS25	F-AAGTTGAGCATCCATCCAT R-GTAGGCTTTCTTCTCTTCGTC	(AAG)6	53	1–8	74–943	0.56
26.	SuMS26	F-ATGCTCTTCTTCATAGTGTCC R-CAGCCTGATCCTCGTCTC	(ACTAT)3	53	3–5	81–650	1.00

Table 2 (continued)

S. no.	Primer ID	Primer sequence (5′→3′)	Repeat motif	Annealing temp (T_a °C)	No. of band amplified	Band size range (bp)	PIC value
27.	SuMS27	F-CTTCTCCTACCCCTCAGGT R-CTAACCCTCAACCATAGAAGG	(GGTT)4	53	1–5	50–1045	0.60
	Average			52.2	5.0	–	0.58

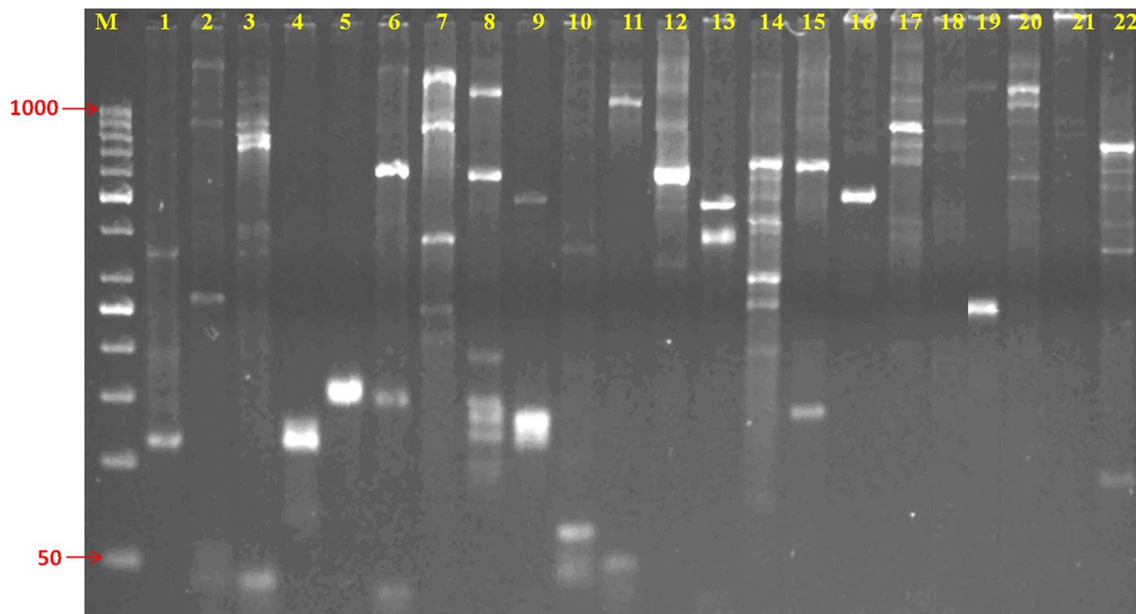

Fig. 1 DNA profiling of sugarcane variety CoS 767 generated by 22 sugarcane microsatellites (SMS) markers. Note: M—50 bp ladder, lane 1—SMS1, 2—SMS3, 3—SMS4, 4—SMS9, 5—SMS10, 6—SMS11, 7—SMS14, 8—SMS15, 9—SMS17, 10—SMS18, 11—SMS23, 12—SMS24 13—SMS25, 14—SMS27, 15—SMS28, 16—SMS29, 17—SMS30, 18—SMS33, 19—MS37, 20—SMS41, 21—SMS42, 22—SMS43

genotypes (Jannoo et al. 2001; Piperidis 2003). DNA fingerprinting in sugarcane is relatively simple than the in seed propagated crops and found to be more uniform and stable fingerprints once generated (Singh et al. 2011). Thus, DNA profiles generated by these functional markers produced using genomic DNA create a unique identity of the analyzed genotype and hold promise the ultimate tool for biological individualization of sugarcane varieties (Singh et al. 2013).

Hence, DNA fingerprinting would provide accurate and stable varietal recognition in regards of plant variety protection, authenticity testing and germplasm conservation and utilization in addition to quality assurance for release of new varieties to the country (Singh et al. 2013, 2015). Molecular characterization to assign identity to the cultivars could also be used in DUS testing, variety registration, and in resolution of dispute (Hemaprabha et al. 2010). Moreover, to develop a DNA profile of plants has great importance in breeding, owing to facilitating for the identification of duplicates in the germplasm collection and the organization of crosses leads to an improved breeding outcome (Ruiz et al. 2011). The identification of varieties based on molecular markers is important to set up distinctness, uniformity and stability of protected cultivars (Hemaprabha et al. 2010; Swapna et al. 2011). Polymorphic microsatellites were used to generate unique molecular profiles for the identification of individual genotypes that can be used in breeding program to assist the protection of new sugarcane varieties (Silva et al. 2012). Accordingly, DNA fingerprinting is a valuable tool to serve in the protection of newly developing sugarcane varieties by the different breeding programs.

Table 3 Details of molecular cataloguing (DNA profiling) of the 13 popular sugarcane varieties using sugarcane EST-derived SSR markers

Marker Id	Size (bp) of variety specific DNA bands amplified with different SSR markers within 13 sugarcane varieties												
	CoS 767	CoS 99259	CoS 96268	CoS 8432	UP 0097	CoS 95255	CoS 96269	CoSe 05451	CoS 8436	CoS 08279	CoS 92423	CoS 03251	CoS 03234
SuMS1	146, 215, 370	102, 140	101, 111, 137, 248, 365, 535	50	50	102, 138, 146, 158, 197, 386	104, 133	139	101, 137, 152	140, 270, 556	175	*	*
SuMS2	271, 910, 1025, 1250	319, 456, 563, 1255	265, 858, 1192, 1345	381, 486	330, 435, 496	106, 188, 267, 292, 572	45, 265	*	*	145, 395	*	*	*
SuMS3	370, 421, 768, 893, 1030, 1125	347	50, 341, 760	357, 767, 951, 1031	64, 311	50, 244, 365, 676, 818	50, 340	340	341	522	440	*	*
SuMS4	103	108	108, 200	110	110	114, 228	108, 120, 200	117	113, 260, 440	156, 305, 940	138	*	*
SuMS5	145	150	146	155	135, 155, 215, 258, 590, 993, 1157, 1359, 1560	157	142	155	149	60, 220, 350, 415	185, 280, 300	160, 180, 210	186
SuMS6	142, 637, 1216	150, 626	50, 440, 625	50, 150, 635	61, 138, 623	154, 640	142, 176	152	144, 633	54, 210, 670, 940	52	150, 225, 610	180
SuMS7	211, 255, 395, 818, 1140	258, 402, 550, 806, 1082, 1203	206, 243, 385, 848, 916, 1232	262, 414, 602, 798, 865, 1017	228, 363, 787, 871, 924, 1024, 1030, 1606	427, 572, 612, 800, 1000	258, 390, 610, 800, 865, 1096	252, 394, 815, 895, 1205	212, 394, 593, 739, 834, 911	415, 670, 1360	320, 503, 740, 967, 1016, 1087, 1307	*	*
SuMS8	110, 122, 139, 190, 611, 1050,	135	114, 126, 183, 591, 1092	135, 618, 1017	128, 145, 588	149, 205, 255, 395, 618, 818, 936, 1058	135, 590	141	135	182	145, 175, 237, 262, 575, 625, 700, 727, 1016, 1248	125, 146, 174, 225, 432, 810, 1100	170, 217
SuMS9	122, 517	50, 130, 210, 248, 325, 318, 1280	105, 124, 501	125, 525	121, 131, 483	115, 133, 213, 350, 540, 605, 1120	125, 510	125	121, 132	51, 182, 670, 781	160, 636	123, 210, 380, 472, 643, 815	153, 170
SuMS10	58, 370	62	59, 357	59	77, 335, 500, 680, 780, 1060, 1310	60, 408	60, 145	55, 73, 860	*	88, 150, 223, 574	77, 127, 187, 470	60, 140, 225, 315	80, 136, 182, 207, 233, 372

Table 3 (continued)

Marker Id	Size (bp) of variety specific DNA bands amplified with different SSR markers within 13 sugarcane varieties												
	CoS 767	CoS 99259	CoS 96268	CoS 8432	UP 0097	CoS 95255	CoS 96269	CoSe 05451	CoS 8436	CoS 08279	CoS 92423	CoS 03251	CoS 03234
SuMS11	50, 1008, 1095	50, 975, 1060	978	46, 926	1150	905	48, 938	60, 928	1017	1383	1069, 1162	40, 510, 710, 1150	45, 55, 145, 1000
SuMS12	348, 598	613, 741, 858, 1060	597, 858	586,	472, 500, 590	625, 827, 980, 1095	354, 606, 820	346, 484, 593, 799, 1037, 1182	*	898, 1280	740, 1016, 1264	*	*
SuMS13	853, 1073	420	248, 381, 474, 584, 1360	37, 269, 408, 499	40, 238, 472, 592, 1818	418, 535	270, 408, 430, 498, 598, 748, 1155	50, 502	50, 274, 502, 606	630, 780	50, 337, 503, 615, 1495	*	*
SuMS14	402, 506	50, 210, 270, 326, 428, 653	153, 187, 254, 296, 407, 430,584, 638, 1068, 1205	72, 272, 316, 402, 442, 466, 661	60, 180, 238, 280, 308, 391, 501, 651, 764, 846	290, 334, 452, 670, 946	76, 175, 272, 302, 322, 455, 598, 647, 987, 1082	315, 346, 450, 650	323, 475, 677	300, 415, 475, 522, 649, 985	210, 337, 385, 566, 728, 805	275, 330, 465, 530, 770, 889	*
SuMS15	194, 265, 314, 457, 528, 598, 664	140, 653	122, 610,1259	132, 636	127, 632, 1125, 1380, 1595	134, 655	630	139, 640	140, 655, 1006	198, 955	790	120, 180, 265, 425, 485, 710	173, 636
SuMS16	130, 664	529, 1016, 1200	512, 1153	534, 1003	494, 520, 572, 1350, 1515, 2043	647, 990	524, 789, 987, 1111	325	1098, 1298	476, 780, 830, 1203, 1404, 1540, 1690	636	*	*
SuMS17	540, 402, 456, 664, 768, 835, 1008, 1142	456, 666, 806, 975, 1130, 1255	420, 638, 720, 812, 989	448, 644, 777, 938	865, 904	467, 585, 670, 723, 791, 1035, 1132	455, 545, 748, 720, 789, 911, 1171	449, 651, 728, 815, 963, 1057, 1139, 1321	460, 755, 843, 1563	*	*	240, 415, 457, 580, 690, 770	486
SuMS18	*	185, 340, 600, 840, 1016, 1203	*	*	*	*	*	*	201, 345, 555, 647, 747, 1187	*	*	75, 128, 160, 178, 274, 462, 511, 663	335, 399, 470, 590, 795, 1085
SuMS19	871, 1073	725, 825, 895, 1281	696, 930, 1045	*	*	345, 827, 1058, 1132	*	*	*	*	*	150, 250, 280, 515, 535	204
SuMS20	*	*	*	*	*	591, 692	*	*	*	*	*	*	*

Table 3 (continued)

Marker Id	Size (bp) of variety specific DNA bands amplified with different SSR markers within 13 sugarcane varieties												
	CoS 767	CoS 99259	CoS 96268	CoS 8432	UP 0097	CoS 95255	CoS 96269	CoSe 05451	CoS 8436	CoS 08279	CoS 92423	CoS 03251	CoS 03234
SuMS21	255, 1095	264, 293	232, 1104	255	210, 223	270, 731, 895, 980	254, 290, 710, 900, 710, 900, 1000, 1200	261	268, 1148	402, 564	285, 330	*	*
SuMS22	*	*	*	*	*	*	*	*	*	781	*	*	236, 255, 346
SuMS23	611, 753, 871, 967, 1073, 1268	626	430, 578, 728, 957, 1080	990, 1117	555, 592, 1200, 1282, 1494, 1666	92, 106, 490, 605, 856, 968, 1132	454, 590, 1000, 1082, 1171, 1235	981, 1057	98, 508, 764, 891, 1028, 1123	1571	*	76, 90, 122, 145, 315, 422, 515, 642	131, 482, 534, 636, 1025, 1095
SuMS24	701, 871	*	*	*	781, 897, 1245	*	*	*	*	*	*	*	*
SuMS25	80, 370, 438, 574, 637, 737	741	345, 411, 459, 530, 704	81, 366, 430, 548, 610, 653, 698, 900	88, 319, 508, 523, 600, 726, 792, 1116	90, 400, 467, 572, 684, 723, 905	85, 138, 382, 442, 560, 730, 778, 936	742	780	120, 573, 745, 843, 1080	875	74, 136, 285, 510, 615	104, 165, 350, 412, 490, 580, 716, 943
SuMS26	*	*	*	*	*	*	*	*	*	*	*	81, 135, 256, 535, 650	215, 230, 407
SuMS27	*	*	*	*	472, 504, 737, 917, 1045	*	*	511	*	*	*	50, 290, 318, 512, 697	*

*Marker not used for the identification of particular variety

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Author contributions RBS conceived and designed the experiment, performed the experiments, and wrote the paper. BS was involved in acquisition and interpretation of data. RKS revised the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest directly or indirectly and informed consent to publish this study and that the manuscript complies with the ethical standards of the journal.

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