RESEARCH ARTICLE

NEW GENIC SSR MARKERS IDENTIFIED FOR THREE MAJOR ENZYMES ASSOCIATED WITH SUCROSE SYNTHESIS IN SUGARCANE

R.M. Shanthi*, G. Hemaprabha, R. Vigneshwari, T.S. Sarath Padmanabhan and K. Mohanraj

Abstract

Sucrose content of juice is an important component in terms of sugar recovery percent in sugarcane. The pattern of sucrose accumulation in high and low sucrose genotypes in relation to sucrose synthesis, transport and sucrose accumulation has not been fully understood. Earlier investigations in sugarcane have shown that the enzymes invertases, sucrose phosphate synthase (SPS) and sucrose synthase (SS) are collectively responsible for the synthesis and breakdown of sucrose in the various cellular compartments. Development of new EST-SSR markers associated with major sucrose metabolic pathway enzymes represents an important tool for genetic analysis in sugarcane. In the present study, 17 high sucrose Co canes and four low sucrose sugarcane clones were selected for the survey of polymorphic SSR alleles using 12 primers. Non-redundant and locus specific SSR motifs located in the coding region of the sugarcane genome were used for designing primers for three major sucrose metabolic pathway enzymes. The primer AF050129 designed for the cell wall invertase enzyme amplified six SSR alleles ranging from 153 bp to 372 bp. Polymorphism for the SSR allele of 224 bp size was distinctly observed by its presence in all the 17 high sucrose clones and absence in the four low sugar clones. For the enzyme neutral invertase (NI), the primer TA31929_4547 amplified two SSR alleles of 151 and 167 bp size in the high sucrose genotypes while it was not seen in the low sugar representatives. Two SSR alleles of 466 bp and 525 bp size amplified by the primer TA 32256_4547 for the enzyme phosphofructokinase (PFP) were found in high sugar clones but absent in low sugar clones. The SSR marker TA31929_4547 for Nl enzyme was found to be more informative with the highest PIC value of 0.5989 and with a high estimate of genetic diversity (0.6585). The presence of NI enzyme specific SSR alleles of 151 and 167 bp size in eight *Saccharum officinarum* clones that commonly appear in the pedigree of a majority of Indian Co canes indicated the introgression pattern of sugar specific alleles in the ancestral species clones. The new genic SSR markers have enabled the identification of genic regions associated with the enzymes of sucrose metabolism in *S. officinarum* clones, retained through selection in the elite parental breeding stocks, and this will assist in developing an informative marker database for genotyping applications in sugarcane.

Key words : Sugarcane, genic SSRs, cell wall invertase, neutral invertase, phosphofructokinase

Introduction

Commercial sugarcane cultivars are advanced generation hybrids between two polyploid ancestors, namely *Saccharum officinarum* (2n=80) and *Saccharum spontaneum* (2n=40-128). Large genome size (10 Gb) and aneuploidy render genome based breeding efforts a challenging task in sugarcane (Carson and Botha 2002; Lakshmanan et al. 2005). It is expected that both the transgenic and marker–assisted routes for sugarcane improvement will contribute to enhanced sugar, stress tolerance and high yield. Considerable numbers of microsatellites have been characterised in the protein coding sequences (CDs) and non-coding untranslated regions (UTRs) for several plant species. In

R.M. Shanthi*, G. Hemaprabha, R. Vigneshwari, T.S. Sarath Padmanabhan, K. Mohanraj
ICAR- Sugarcane Breeding Institute, Coimbatore 641 007, Tamil Nadu, India
*Email: RM.Shanthi@icar.gov.in

sugarcane, Cordeiro et al. (2000) and Parida et al. (2009) developed a large number of microsatellite markers from the genomic sequences. Oliveira et al. (2009) designed SSR markers from the sugarcane ESTs and these were used to construct a functional genetic linkage map of *Saccharum* spp. EST-SSRs have unique advantages over other genomic DNA-based markers such as detecting the variation in the expressed portion of the genome, giving a perfect marker–trait association (Gupta et al. 2003). However, information on only a limited number of genic microsatellite markers is available in the public domain. Sucrose being a polygenic trait, molecular markers will serve as a valuable tool for the selection of promising clones in their early stages of development for use in sugarcane breeding programmes. Markers linked to sucrose accumulation have been identified in a mapping population derived from a cross between a commercial sugarcane cultivar (Q 165) and an *S. officinarum* clone (IJ76-514), and 37 marker associations have been reported for brix and sucrose content (Aitken et al. 2006). However, the markers identified in the mapping studies so far have been neither applied in breeding programmes nor tested in commercial germplasm. This study aims to develop and analyze genic SSR markers from the expressed sequences derived from the TIGR database for three major enzymes involved in the sucrose metabolic pathway, viz. neutral invertase (NI), cell wall invertase (CWI), phosphofructokinase (PFP). An attempt was also made to identify the genomic regions of sugar specific alleles that are introgressed from the ancestral species clones to the present day commercial hybrids.

Materials and methods

Mining of microsatellites and designing of primers

For the development of gene based microsatellites markers, unigene data sets such as the gene indices from the Institute of Genomic Research (TIGR: http://www.tigr.org/tdb/tgi) were used. Microsatellite search was carried out on a high performance computer cluster using a version developed with the Perl script available as SSRIT (Simple Sequence Repeat Identification Tool) at Cotton Microsatellite Database (CMD) (http://www.cottonssr.org). This program was operated with the parameters set to detect di to hexanucleotides of specified lengths. To further examine the location of SSRs in the sequences in relation to the putative coding region, the SSR server used the FLIP program (Brossard 1997) which is available through the Organelle Genome Megasequencing Project (OGMP). FLIP is a UNIX C program that finds/translates open reading frames (ORFs) in sequences. Using the FLIP output, the longest ORF is identified and the relative SSR location is reported. Using the CMD SSR tool, we uploaded a batch of 157190 sequences in FASTA format and selected the motif type and repeat length to search. Primers were designed using Primer3 (Rozen and Skaletsky1999) with the following specifications: optimum primer length of 20 nucleotides (nt) (18-26 nt), optimum melting temperature of 50°C (45-55°C), optimum product size of 125 base-pairs (100-350 bp) and an optimum G/C content of 50% (45-55%). Twelve primer pairs were used for studying the SSR alleles specific to sucrose metabolising enzymes in 21 clones representing high and low sugar genotypes.

Genomic DNA extraction and PCR

Twenty one sugarcane clones were used for the study on the polymorphism of SSR alleles associated with three key enzymes in sucrose metabolism. Seventeen Co canes with more than 18.0% juice sucrose were considered high sucrose types and the clones Co 618, ISH 176, GU 04(28) E02, GU12-49 represented the low sucrose genotypes in this study (Table1). Genomic DNA was isolated from shoot apical tissue following the CTAB method (Murray and Thompson 1980). The concentration of extracted DNA was quantified using spectrophotometer (ND-1000) at absorbance 260/280 nm and diluted to 25 ng/µl. PCR reactions were performed in final volume of 10µL reaction mixture containing 50ng of genomic DNA, 0.33 Mm dNTPs, 2.5 mM MgCl, 0.15 µM of each primer, 0.5 U of Taq polymerase. PCR reaction conditions were setup as follows: 95°C for 2 min followed by 35 cycles of 94°C for 1 min, primer

Table 1. Sugarcane clones used for the polymorphism survey of sugar specific SSR alleles

Clone	Parentage	Juice sucrose (%)
	(i) High sugar clones	
Co 775	PoJ 2878 x Co 371	18.84
Co 1148	P 4383 x Co 301	18.67
Co 1158	Co 421 GC	18.42
Co 62198	CP 34-120 x Co 775	19.85
Co 7314	Co 1287 self	19.46
Co 8353	Co 419 x Co 1148	19.54
Co 86011	Co 7314 x Co 775	19.91
Co 86032	Co 62198 x CoC 671	21.06
Co 86249	CoJ 64 x CoA 7601	19.02
Co 88025	CoC 671 somaclone	20.42
Co 89003	CoC 671 x Co 6806	19.88
Co 94008	Co 7201 x Co 775	19.51
Co 94012	CoC 671 somaclone	21.46
Co 94019	Co 7201 x C79518	19.40
Co 96002	CoC 671 polycross	21.20
Co 06022	GU 92275 x Co 86249	20.80
CoC 671	Q 63 x Co 775	21.40
	(ii) Low sugar clones	
Co 618	POJ 2878 x Co 285	16.46
ISH 176	Co 6806 x *S. sinense* (Khakai)	14.01
GU 04(28) E02	[*E. procerus* (IND 90-776) x *S. officinarum* (PIO-96-436)]	8.20
GU 12-49	[*E. procerus* (IND 90-776) x *S. officinarum* (PIO-96-436)] x Co 775	14.59

melting temperature for 40 sec, 72°C for 40 sec followed by 72°C for 7 min. PCR products were resolved on 8% polyacrylamide gel and stained with silver nitrate. The gels were visualized in UV in a gel documentation system (Alpha Innotech).

Data analysis

The resulting band fragments were scored as alleles and their size determined using the 100 bp ladder (GeNeI), presence denoted by allelic size and absence as 0. For scoring, stutters were avoided and discernible bands were scored as alleles. Allele sizes were estimated for the survey panel and mapping gels based on comparison with 100 bp molecular weight ladder. Repeats of SSRs were scored as haplotypes with each locus representing the genetic information of each SSR marker. SSR allele diversity at each microsatellite locus was measured in terms of the number of alleles (N_A) and major allele frequency (M_{AF}). Weir's gene diversity (GD) and its polymorphism information content (PIC) were calculated using Power Marker v3.25 (Liu and Muse 2005) to indicate the ability of each marker to detect polymorphisms in the sugarcane clones taken for this study. The PIC value was calculated using the formula, $PIC=1-\sum P_{ij}^2$ (Anderson et al. 1993), where P_{ij} is the frequency of the jth allele for the ith locus summed across all alleles for the locus.

Results and discussion

The differential ability of sugarcane genotypes to accumulate sucrose in their stems is the net result of sucrose synthesis and breakdown. Several investigations in the past reported that the primary sucrose metabolism is governed by enzymes such as invertase, sucrose synthase and sucrose phosphate synthase (Quick and Schaffer 1996). Recent studies have suggested that targeted modification of these enzymes involved in sucrose metabolism enhances sucrose yield in sugarcane (Chandra et al. 2012). The results on SSR polymorphism and its informativeness pertaining to three major enzymes, namely cell wall invertase, neutral invertase and pyrophosphate dependant phosphofructokinase involved in sucrose metabolism are discussed in this paper.

Amplification efficiency and polymorphic potential of the genic SSR markers

All the 12 SSR primer pairs were found to produce amplification in the 21 sugarcane clones used in this study. However, polymorphic amplifications were produced by only six primers (Table 2). Invertase is the key enzyme involved in sucrose metabolism that hydrolyzes sucrose into glucose and fructose for cell growth and other metabolic processes (Sturm and Tang 1999; Roitsch and Gonzalez 2004). Three types of invertase isoenzymes (vacuolar invertase, cell wall invertase and alkaline/neutral invertase) have been reported in higher plants, which are distinguished based on their sub cellular localization, solubility, optimum pH and isoelectric point.

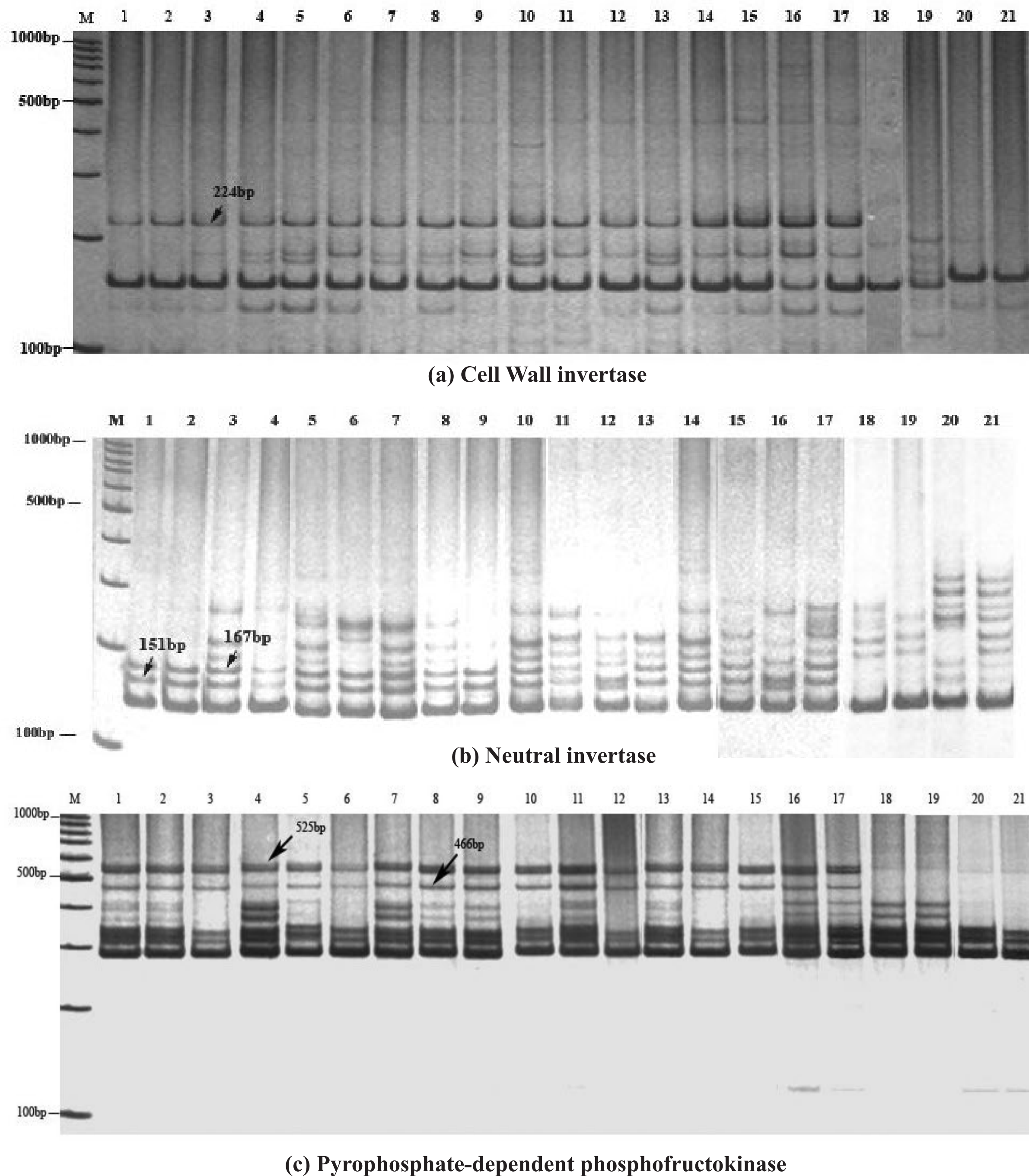
The cell wall invertases (CWI) have an acidic pH optimum and are believed to regulate the sucrose metabolism in both source and sink organs in higher plants (Yuan Yao et al. 2014). The primer AF050129 designed for the cell wall invertase enzyme amplified six SSR alleles ranging from 153 bp to 372 bp (Fig. 1a). Of these, the SSR allele size of 153 bp was monomorphic. Distinct polymorphism was observed for the SSR amplicon of 224 bp size by its presence in all the 17 high sucrose clones and absence in low sucrose clones (Co 618, ISH 176, GU 04(28) E02, GU 12-49).

Table 2. Details of the microsatellites specific to key enzymes involved in sucrose synthesis in sugarcane

Marker	Enzyme	Repeat motif	Forward and Reverse primer sequences (5' to 3')	Ta (°C)	Size range (bp)
TA31929_4547	Neutral invertase (NI)	$(GGA)_5$	AAGTTTGGTGAGACCCAAGG GCGTGAGCTCTAAGTCGTCC	53	139 - 303
AF050129	Cell wall Invertase (CWI)	$(TTC)_5$	CCGTCTTCTTCAGGGTGTTC CGTAGAGGTGAGCGTCCTTC	51	153 - 224
TA32931_4547	Pyrophosphate-dependent phosphofruc-to-1-kinase (PFP)	$(AT)_5$	AACTGCAATGGAGCAGTGTG CTCTACAACGACAGCACCCA	60	127 - 187
TA32256_4547	Pyrophosphate-dependent phosphofruc-to-1-kinase (PFP)	$(AGG)_6$	CTGAGTTCTCCAATGGCGTC GTATCCGTAGTCGCCCTCCT	58	296 - 525
CA139117	Pyrophosphate-dependent phosphofruc-to-1-kinase (PFP)	$(GCC)_6$	CAAGAGAGATGAACGCCGAC GACTGCCTCCCAGAGAACAC	56	287-572
CA172223	Pyrophosphate-dependent phosphofruc-to-1-kinase (PFP)	$(AGG)_6$	AGTTCTCCAATGGCATCGTC GTATCCGTAGTCGCCCTCCT	56	296-630

Expression of cell wall acid invertase genes (Cwin 1) was reported to be higher in the developing internodes of a high sucrose genotype Muntok Java than in a low sucrose genotype PIN 84-1. The internodes of the high sucrose-storing genotype appear to be metabolically more active than those of the low-sucrose genotype indicating that the cell-wall invertase gene may be a good candidate for improving sucrose accumulation in sugarcane (Lingle and Dyer 2004).

Transcripts of neutral invertase (NI) have been detected in all sugarcane tissues in 60, 120 and 240 kDa forms and a strong correlation between hexose levels and NI in younger culm tissue has been reported (Vorster and Botha 1998) thus implicating the role of NI enzyme in growth and



(c) Pyrophosphate-dependent phosphofructokinase

Fig. 1. Allelic diversity of three enzymes involved in sucrose metabolism in high sucrose and low sucrose sugarcane clones. Polymorphism for enzyme specific SSR alleles for (a) cell wall invertase (b) neutral invertase and (c) pyrophosphate-dependent phosphofructokinase. Lanes: M - 100 bp ladder; 1- Co 775; 2 - CoC 671; 3 - Co 1148; 4 - Co 1158; 5 - Co 7314; 6 - Co 8353; 7 - Co 62198; 8 - Co 86011; 9 - Co 86032; 10 - Co 86249; 11 - Co 88025; 12 - Co 89003; 13 - Co 94008; 14 - Co 94012; 15 - Co 94019; 16 - Co 96002; 17 - Co 06022; 18 - Co 618; 19 - ISH 176; 20 - GU 04(28) EO2; 21 - GU 12-49

metabolism. The primer TA31929_4547 designed for NI amplified SSR alleles ranging from 139 bp to 303 bp (Fig. 1b). Two SSR alleles of 151 and 167 bp size were observed in the 17 high sucrose genotypes while it was not amplified in the four low sugar representatives. Two SSR amplicons of 280 bp and 303 bp size were observed only in the two low sugar clones derived from *Erianthus* (GU 04(28) EO2, GU12-49).

Pyrophosphate-dependent phosphofructokinase (PFP) is another key enzyme involved in the phosphorylation of fructose directing the sucrose utilization process and regulating the activity of this enzyme has been claimed as a successful strategy to increase sucrose yield (Botha and Groenewald 2001). A negative correlation was reported between PFP activity and sucrose levels in both commercial varieties as well as in a segregating F1 sugarcane population (Whittaker and Botha 1999). Further, it was reported that transgenic sugarcane clones with reduced PFP activity had a 50% increase in sucrose levels in immature internodal tissues (Groenewald and Botha 2008). The primer TA 32256_4547 designed for PFP enzyme exhibited polymorphism by the presence of two SSR alleles of 466 bp and 525 bp size in all high sucrose genotypes and it was absent in the four low sucrose clones (Fig. 1c).

Informativeness of the genic SSR markers

A total of 32 alleles were detected with these primer sets at four SSR loci with an average of six alleles per loci. The amplicon size ranged from 127 to 525 bp. The major allele frequency per locus varied from 0.4333 (TA31929_4547) to 0.7778 (AF050129) with an average of 0.5606. GD ranged from 0.3575 (AF050129) to 0.6585 (TA31929_4547). PIC values varied from 0.3119 (AF050129) to 0.5989 (TA31929_4547). PIC depends on the number of detectable alleles and the frequency of SSR alleles in the population examined. The SSR marker TA31929_4547 for NI enzyme was found to be more informative with the highest PIC value of 0.5989 and a high estimate of GD (0.6585) (Table 3).

Table 3. Total number of alleles amplified and allelic diversity estimates for the SSR loci among high sugar and low sugar genotypes of sugarcane

Marker	N_A	M_{AF}	GD	PIC
TA31929_4547	9	0.4333	0.6585	0.5989
AF050129	4	0.7778	0.3575	0.3119
TA32931_4547	4	0.5778	0.5519	0.4936
TA32256_4547	7	0.5741	0.5563	0.4863
CA139117	4	0.6300	0.4660	0.3574
CA172223	4	0.5880	0.4864	0.3681

N_A = Number of detectable alleles; M_{AF} = Major allele frequency; GD=Gene diversity;
PIC = Polymorphic information content

Polymorphism pattern of sugar specific alleles in the ancestral species clones

Polymorphism in the sugar specific alleles of popular commercial hybrids is likely to provide vital information on the transcript sequences specific to sucrose accumulation. Ever since Barber made crosses between *S. officinarum* (Vellai) and *S. spontaneum* (Coimbatore form) successfully (Barber 1916), the attempt was repeated several times using different clones in the process of evolving superior varieties. However, only 20 clones of *S. officinarum* and two clones of *S. spontaneum* have been utilized so far in the development of present day varieties (Natarajan 2006). As a part of the present studies, an attempt was made to understand the allelic diversity in the ancestral species clones appearing in the pedigree of high sucrose Indian Co canes. Eight *S. officinarum* clones that frequently appear in the pedigree of popular Co canes were taken for this study. The SSR amplicons of 151 and 167 bp size specific to NI enzyme that were present in the high sucrose genotypes could be observed in these eight *S. officinarum* clones that appear in the pedigree of the Co canes (Fig. 2). Earlier, Reffay et al. (2005) adopted a mapping approach with pedigree analysis to identify the genomic regions associated with sucrose and fibre content from the *S. spontaneum* clone 'Mandalay' that appears in the pedigree of many cultivars in the Australian breeding programme. Out of 352 markers generated, 86 could be identified as of Mandalay descent and 23 marker–trait associations were identified for pol, brix and CCS across two seasons. The pedigree records of the Indian Co canes traces back to a common set of 7-8 *S. officinarum* clones. The SSR markers for the NI observed in these *S. officinarum* clones suggest that these sugar specific

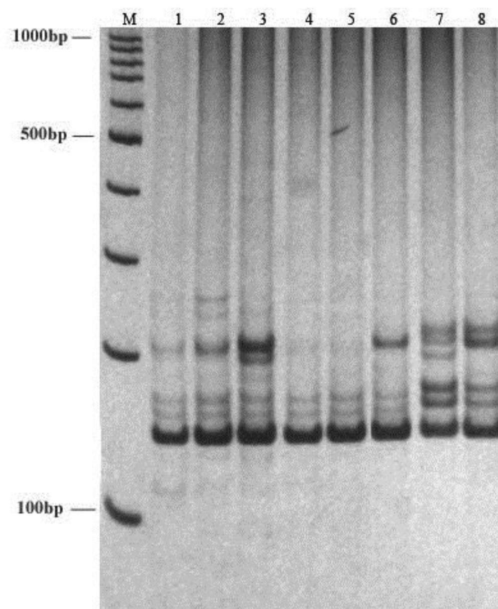


Fig. 2. SSR alleles for the enzyme neutral invertase in *Saccharum officinarum* clones that appear in the pedigree of Co canes used in the study. Lanes: M - 100 bp ladder; 1 - Ashy Mauritius; 2 - Black cheribon; 3 - Striped Mauritius; 4 - Green sport; 5 - BM Hitam; 6 - Badila; 7 - Vellai; 8 -Uahi-e-pele

alleles probably got retained through selection in the present day commercial germplasm of our breeding programme. This finding suggests that combining the pedigree analysis and marker information allows the identification of genomic regions associated with sugar specific alleles.

The present study identified microsatellites for three major sucrose metabolizing enzymes in a set of high sucrose Co canes that are commonly used as parents in our breeding programmes. The results on SSR allelic diversity indicated that the primer TA31929_4547 for NI enzyme was able to differentiate high and low sucrose clones at its locus. Also, this primer enabled the identification of genomic regions of NI specific alleles in the *S. officinarum* clones that commonly appeared

in the pedigree of a majority of Indian Co canes. Thus, genic SSRs with high polymorphic potential would enable us to construct a smaller and informative microsatellite database comprising markers located in the coding regions of the sugarcane genome. Further, these transcript SSRs are expected to improve the detection of marker–trait associations of the QTLs influencing sucrose content because they are part of the transcribed domain of the sugarcane genome.

References

Aitken JS, Jackson PA, McIntyre CL (2006) Quantitative trait loci identified for sugar related traits in a sugarcane cultivar (*Saccharum* spp.) x *S. officinarum* population. Theor Appl Genet 12:1306–1317.

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME (1993) Optimizing parental selection for genetic linkage maps. Genome 36:181-186.

Barber CA. (1916) Studies in Indian sugarcanes, No.2. Mem Agric India, Bot Ser 8:103–199.

Botha FC and Gronewald JH (2001) Manipulating sucrose metabolism with a single enzyme: pyrophosphate-dependant phosphofructokinase (PFP) in sugarcane. Proc Int Soc Sugar Cane Technol 24:592-594

Brossard N (1997) FLIP:a Unix Program used to find/translate orfs. bionet software. http://www.bch.umontreal.ca/ogmp/manlinks/flip.txt

Carson DL, Botha FC (2002) Genes expressed in sugarcane maturing internodal tissue. Plant Cell Rep. 20:1075-1081.

Chandra A, Jain R, Solomon S (2012) Complexities of invertases controlling sucrose accumulation and retention in sugarcane. Curr Sci 102:857-866.

Cordeiro GM, Casu R, McIntyre CL, Manners, JM, Henry, RJ (2000) Microsatellite markers from sugarcane (*Saccharum spp*.), a highly polyploidy species. Plant Sci 155:161-168.

Groenewald JH and Botha FC (2008) Down-regulation of pyrophosphate: fructose 6-phosphate 1- phosphotransferase (PFP) activity in sugarcane enhances sucrose accumulation in immature internodes. Transgenic Res 17:85-92.

Gupta PK, Rustogi S, Sharma S, Singh R, Kumar N, Balyan HS (2003) Transferable EST-SSR markers for the study of polymorphism and diversity in bread wheat. Mol Genet Genom 270:315-323.

Lakshmanan P, Geijskes RJ, Aitken KS, Grof CPL, Bonnet GD, Smith GR (2005) Sugarcane Biotechnology: the challenges and opportunities. In Vitro Cell Dev Biol Plant 41:345-363.

Lingle SE, Dyer JM (2004) Polymorphism in the promoter region of the sucrose synthase-2 gene of *Saccharum* genotypes. J Am Soc Sugarcane Technol 24:241-249.

Liu K, Muse SV (2005) Power marker: Integrated analysis environment for genetic marker data. Bioinformatics 21(9): 2128 – 2129.

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res 8:4321-4325.

Natarajan US (2006) Nobilisation - a pivotal breeding procedure in sugarcane. In: Winter School on Current Strategies on Sugarcane Breeding and Genetics. pp. 48-56. Sugarcane Breeding Institute, Coimbatore, India.

Oliveira KM, Pinto LR, Marconi, TG, Mollinan M, Ulian EC, Chabregas SM, Falco MC, Burnquist W, Garcia AAF, de Souza AP (2009) Characterisation of new polymorphic functional markers for sugarcane. Genome 52:191-209.

Parida SK, Kalia SK, Kaul S, Dalal V, Hemaprabha G, Selvi A, Parida A, Singh A, Gaikwad K, Sharma TR, Srivastava, PS, Singh NK, Mohapatra T (2009) Informative genomic microsatellite markers for efficient genotyping applications in sugarcane. Theor Appl Genet 118:327-338

Quick WP, Schaffer AA (1996) Sucrose metabolism in sources and sinks. In: Photoassimilate Distribution in Plants and Crops: Source-Sink Relationships (Zamski E, Schaffer AA, eds) pp. 115-156. Marcel Dekker, New York.

Reffay N, Jackson PA, Aitken KS, Hoarau JY, Honts AD, Besse P, McIntyre CL (2005) Characterisation of genome regions incorporated from an important wild relative into Australian sugarcane. Mol Breed 15:367-381.

Roitsch T, Gonzalez MC (2004) Function and regulation of plant invertase: sweet sensations. Trends Plant Sci 9(12): 606-613.

Rozen S, Skaletsky H (1999) Primer3 on the WWW for General Users and Biologist Programmers. In: Bioinformatics Methods and Protocols in the series: Methods in Molecular Biology, (eds. Krawetz S and Misener S), 132: 365-386. Humana Press, Totowa, NJ.

Sturm A and Tang GQ (1999) The sucrose cleaving enzymes of plants are crucial for development growth, and partitioning. Trends Plant Sci 4: 401-407.

Vorster DJ, Botha FC (1998) Partial purification and characterisation of sugarcane neutral invertase. Phytochemistry 49(3):651-655.

Whittaker A, Botha FC (1999) Pyrophosphate: D-fructose-6-phosphate 1-phosphotransferase activity patterns in relation to sucrose storage across sugarcane varieties. Physiol Plant 107:379-386.

Yuan Yao, Meng-Ting Geng, Xiao-Hui Wu, Jiao Liu, Rui-Mei Li, Xin-Wen Hu, Jian-Chun Guo (2014) Genome-wide identification, 3D modeling, expression and enzymatic activity analysis of cell wall invertase gene family from Cassava (*Manihot esculenta* Crantz). Int J Mol Sci 15:7313-7331.

Received : 23 July 2016; Revised & Accepted : 15 November 2016