Electronic Journal of Plant Breeding, 1(4):420-425 (July 2010)

Research Article

DNA fingerprinting for identification and protection of elite sugarcane (*Saccharum* spp.) varieties

Hemaprabha G., Arun Krishna, , J. Vincy, P. Priji. P, Swapna Simon and P. Govindaraj

Abstract

As sugarcane is vegetatively propagated through stem cuttings, there is uniformity and stability of the DNA fingerprints once generated. This has made DNA fingerprinting a reliable method of varietal identification in this crop. One of the major ways sugarcane industries would benefit from molecular markers is in the use of SSRs for cultivars identification. In this study, 16 elite clones of tropical and sub-tropical India *viz* Co 86032, Co 99004, Co 0118, Co 0209, Co 0218, Co 0237, Co 0238, Co 0241, Co 0314, Co 0403, Co 0409 ,Co 2001-09, Co 2001-13, Co 2001-15, Co 0239 and , Co 0240 were analyzed with twenty sugarcane specific STMS primers with high polymorphism information content. Analyses of the profiles generated on silver stained 8 % polyacrylamide gels led to the identification of unique bands for specific clones. The bands specific to each clone listed. For instance, Co 0118 a fast spreading variety in subtropical India could be identified by the presence of the bands viz. $NKS25_{228}$, $NKS57_{805}$ and $NKS\ 3_{340}$. The material used in this study are of recent origin and their DNA fingerprinting would provide proper varietal identification in the context of Plant Variety Protection, legitimacy testing and germplasm conservation and utilization in addition to quality assurance for delivery of new cultivars to the industry. Moreover, these clones being a gene pool for high yield, juice quality and resistance to various abiotic and biotic stresses, the fingerprints enable identification of more diverse clones for systematic utilization in hybridization programme to benefit development of genotypes with economic worthiness and tolerance to stress conditions.

Key words :

Sugarcane, elite genotypes, fingerprinting, STMS markers, PVP and DUS

Introduction

Identification of sugarcane varieties is done by a set of morphological descriptors based on gross cane morphology. Though characterization based on morphological characters is easy and economical, availability of a large number of varieties invite constant attention in assuring varietal purity. The more useful markers to generate unambiguous fingerprints were generated through DNA technology. Plant DNA fingerprinting relies on the application of molecular marker techniques to identify cultivars. Several molecular marker systems are available for the identification of plant breeder's materials. Results of microsatellite analysis in sugarcane have indicated the power of this marker system in characterizing and identifying cane varieties in several countries (Cordeiro and Henry, 1999; Piperidis, 2003, Jannoo *et al*., 2001). Molecular characterization for assigning identification to cultivars can be used in DUS tests, variety registration and dispute settlement. In this study, fingerprinting was carried out in 24 elite genotypes commercial value using a set of 24 sugarcane specific STMS markers.

Division of crop improvement, Sugarcane Breeding Institute, Coimbatore-641007

Material and Methods

Recent and important tropical and sub-tropical Co canes, sixteen in number bred at Sugarcane Breeding Institute, Coimbatore and its regional center at Karnal *viz*. Co 86032, Co 99004, Co 0118, Co 0209, Co 0218, Co 0237, Co 0238, Co 0241, Co 0314, Co 0403, Co 0409, Co 2001-09, Co 2001-13, Co 2001-15, Co 0239 and , Co 0240 formed the material for the study. These were analysed using 20 sugarcane specific STMS

(Sequence Tagged Micro Satellites) primers with high discriminative power. The primers used and their sequence are given in Table 1.

PCR Amplification and electrophoresis

DNA from young leaves of the above parental clones was isolated using CTAB method (Murray and Thomson 1980). PCR was performed in a thermal cycler (Gene Amp PCR System 9700, ABI) using a 10 µl reaction mix consisting of final concentration of 20ng template DNA, 1pmol each of forward and reverse primers, 0.5 units Taq polymerase, 1x Taq DNA polymerase buffer, 2mM dNTPs and 1.75mM $MgCl_2$. The basic cycling profile was 5 min at 94°C followed by 30 cycles of 30 seconds at 94°C, 30 seconds at the annealing temperature standardized for each primer (ranging from 51°C to 59°C) and 50 seconds extension at 72°C and a final extension of 5 minutes at 72°C. PCR products were resolved through polyacrylamide gel electrophoresis (PAGE) on 7.5% in non-denaturing polyacrylamide gels with 0.8% cross linker using 0.5x TBE buffer in a vertical electrophoresis apparatus (Hoefer SE 300 Ruby) and silver stained. The gels were documented in a gel documentation system (Flurochem™ 5500, Alpha Innotech) to detect polymorphism and polymorphic bands quantified. .

Results and discussion

DNA Fingerprinting

DNA fingerprinting in sugarcane is less complicated in comparison with seed propagated crop as the traits are fixed by vegetative propagation through stem cuttings, thereby facilitating uniformity and stability of the fingerprints once generated. Twenty STMS primers generated polymorphic profiles in the materials studied (Fig. 1). The specific fragment unique to each genotype is given in Table 2. Thus DNA fragment patterns generated by these multilocus markers generated from the genomic DNA fragments make up a unique feature of the analysed genotype and hold promise the ultimate tool for biological individualization of sugarcane clones. The markers showed stability and reproducibility, making STMS ideal for fingerprinting the genotypes studied.

Microsatellites have been used to distinguish sugarcane cultivars. Work in Australia was pioneering in these lines. Cordeiro and Henry (1999) used over 100 micro satellite sequences on a set of five lines. Fingerprinting sugarcane through use of random decamer microsatellite and telomere sequences indicated that specific micro satellite and telomere sequences could be used to identify cane varieties (Harvey and Botha, 1995). More loci per primer with a greater proportion of polymorphism indicated that specific micro satellite and telomeric sequences were useful to identify cane varieties. Microsatellites were found to be valuable not only for their rapidity to generate markers, but also for their ability to discriminate 96 cultivars of Mauritius with just two primer pairs (Jannoo *et al*. 2001). Using maize microsatllites, Selvi *et al*. (2003) could differentiate sugarcane clones. Hemaprabha *et al*. (2006) studied 45 Genbank derived STMS primers on 25 commercial hybrids of diverse origin. Twenty primers could distinguish each hybrid, while the remaining primers were effective in combination of two or three. Based on polymorphism information content, twenty five most polymorphic STMS primers were identified for testing on genetically more similar genotypes including six somaclones and five induced mutants derived from a sugar rich variety viz. CoC 671. Seventeen primers detected genetic differences among the mutants, in contrast to only three among the somaclones and the best primers that could differentiate commercial hybrids as well as the mutants and somaclones identified for routine fingerprinting of sugarcane hybrids. The results obtained are thus in the line of earlier studies and generated reference markers to identify a specific cultivar.

Varietal distinction using molecular markers could be feasible only if the tests are straightforward, inexpensive, reliable, reproducible and capable of unambiguous analysis. Though DNA profiling has not been adopted by UPOV as an essential character, this may be included as supplementary character information. In the revised UPOV (1991) convention the enactment of PBR may be facilitated by DNA profiling technique, which will help in extending the PBR in essentially derived varieties. STMS is found to be a very useful molecular marker system for plant variety characterization (UPOV-BMT, 2002). As India is emerging a leader in the biotechnology sector, plant variety protection provides several advantages including safeguarding the interest of plant breeders. ,.International recognition of the National Plant Variety protection system, provides protection of own breeders against appropriation of their varieties by genetic engineers and helps in securing position in WTO

(Chowdhury, 2005). The present study provided sufficient number of STMS markers to distinguish sugarcane varieties which are/ will be in cultivation in the country. The results also highlighted that STMS markers largely fulfill the feasibility for fingerprinting. The cost could be further lowered by virtue of their suitability to multiplexing.

References

Choudhury, R.K. 2005. Development of DUS system in India and future needs. In ICAR-IPA National Conference on IPR and Management of Agricultural Research held at New Delhi from August 27-29.

Cordeiro, G.M., G.O. Taylor and R.J. Henry. 2000. Characterization of microsatellite markers from sugarcane (*Saccharum* sp) a highly polyploid species. Plant Sci. 155: 161-168.

Harvey M. and Botha, F.C. 1996. Use of PCR-based methodologies for determination of DNA diversity between *Saccharum* varieties. Euphytica. 89; 257-265.

Hemaprabha,G., P.Govindaraj and N.K. Singh. 2006. STMS markers for fingerprinting of varieties and genotypes of sugarcane (*Saccharum* spp.). Indian Journal of Genetics & Plant Breeding 66(2): 95-99

Jannoo. N. Forget. L. and Dookun. A. 2001. Contribution of microsatellites in sugarcane breeding in Mauritius.

Murray, M.G and Thompson, W.F. 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acid Research. 8: 4321-4325.

Piperidis, G. 2003. Progress towards evaluation of SSR as a tool for sugarcane variety identification. ISSCT IV Mol. Biol. Workshop, Montpellier, France.

Selvi A, Nair N.V, Balasundaram N and Mohapatra T. 2003. Evaluation of maize microsatellite markers for genetic diversity analysis and fingerprinting in sugarcane. Genome 46: 394 - 403.

UPOV--BMT. 2002. BMT/36/10. Progress report of the 36th session of the technical committee, the technical working parties and working group on biochemical and molecular techniques and DNA profiling in particular. Geneva.

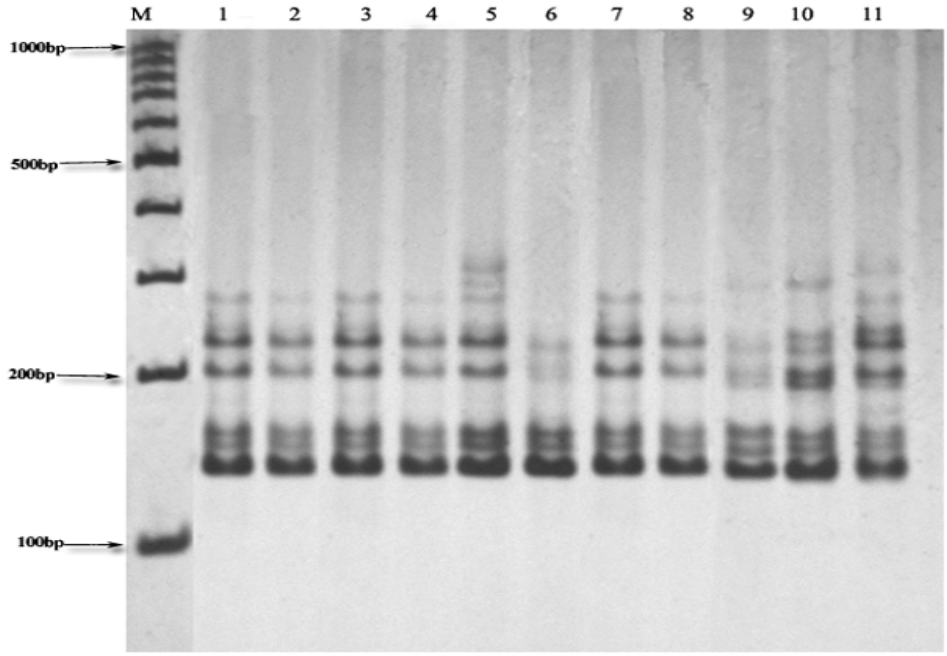
1. **Table1. STMS primers used in fingerprinting elite genoptypes of sugarcane**

Sl.No	STMS Primer	Primer sequence	
		Forward(F)	**Reverse (R)**
1	NKS 1	tggcatgtgtcatagccaat	ccccaactgggacttttaca
2	NKS 2	gctgtcccgttccaagttac	gcgaccggattatgatgatt
3	NKS 7	ttacagcctggagctcgttt	cgaagcctctcctctcctc
4	NKS 8	gtgacagcggcttgttcag	ttaaacacgcagccattcag
5	NKS 11	caccactcacatccacttgc	tatggagagatgctgctgct
6	NKS 16	gacagaatatgccatggataacaa	cgttctctggtcctattgagc
7	NKS 17	gctcgccatgaatagaaagg	accgaggtaggagggagtgt
8	NKS 20	cagccaagggtgagaaaaag	tttactatgcaccaagatacacg
9	NKS 23	taaacccccgaaaaagaacc	tccggaggtagatccatttg
10	NKS 24	tatatggcgaggacagatgc	gggttcagaattagagcaatcg
11	NKS 31	aaccaccactcatcgtcctc	caccgagttcccattgttct
12	NKS 33	ctccttctccttcgcatcct	cacctttctggagcacgtta
13	NKS 34	cgtcttgtggattggattgg	tggattgctcaggtgtttca
14	NKS 38	tgaactcggcaacagttttt	cccaccaagtcgttctgaat
15	NKS 40	gatggaggctttgcaatgat	gcatgtcccactgaactgaa
16	NKS 42	accgattgttcagtgggaag	acctagcaatttacaagagaattaga
17	NKS 43	ctgatgggaggttgaaggaa	ataagcaccaaaagcgtggt
18	NKS 48	acaataaccccgcagacatc	taatgcgtcatttggagcag
19	NKS 49	ctcacgtcctgttggtgcta	tacatgggacacatgcttgc
20	NKS 53	agctcacgtgtgtgtgtgtg	gtgcagtgtcaggggaccta

Table 2 .Recent varieties/ elite genotypes and the specific STMS primers

Genotype	STMS primer	Presence of band (bp)
Co 0118	NKS 25	228
	NKS 57	805
	NKS 3	340
Co 0218	NKS 16	255
	NKS 8	185, 263, 283, 298
Co 0237	NKS 2	567
	NKS 17	381
Co 0238	NKS 34	318
	NKS 42	508
	NKS 23	330
Co 0239	NKS 48	306
	NKS 34	318
	NKS 33	900
	NKS 49	503, 794
Co 0240	NKS 20	736
Co 0241	NKS 53	547
	NKS 31	228
Co 0314	NKS 8	188
	NKS 23	576
	NKS 31	290
	NKS 43	177
	NKS 53	209
Co 2001-09	NKS 43	920
	NKS 42	330
Co 2001-13	NKS 8	185, 263, 283, 298
	NKS 42	330
	NKS 53	209
	NKS 49	135, 370, 433
	NKS 7	207
Co 2001-15	NKS 49	500
	NKS 38	754
	NKS 23	465
Co 0403	NKS 24	420
Co 0409	NKS 43	476
Co 99004	NKS 43	167, 484, 575
	NKS 7	207
Co 86032	NKS 53	209
	NKS 23	341
Co 0209	NKS 11	304,327
	NKS 42	434
	NKS 23	206

Fig. 1. Molecular profiling of elite genotypes of sugarcane (*Saccharum* spp.)



M - 100bp ladder

1-Co 86032	**4 - Co 2001-15**	**7- Co 0314**	**10 - Co 0238**
2-Co 99004	**5 - Co 0209**	**8 - Co 0409**	**11 - Co 0239**
3-Co 2001-13	**6 - Co0218**	**9 - Co 0118**	