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Characterization of Induced Sugarcane Somaclones and their Sources Varieties Using Random Amplified Polymorphic DNA

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Abstract

Sugarcane somaclones and their sources varieties were analyzed by RAPD molecular markers to check the variation at molecular level based on 1.4% agarose gel electrophoresis (AGE). Six RAPD primers generated 237 bands with average 39.5 varied from 15 to 63 with size ranging 145 - 1000 bp among the four sugarcane varieties and their 12 somaclones. Genetic diversity or polymorphism information content (PIC) value ranged from 0.39 to 0.50 for all loci across the 4 varieties and their 12 somaclones based on RAPD markers. Dendrogram based on linkage distance using unweighted pair group method of arithmetic means (UPGMA) based on 6 RAPD primers indicated segregation of the 4 sugarcane varieties and their somaclones into two main clusters at linkage distance 36. Variety Isd 39 was observed in main cluster C_1 while its (Isd 39) somaclones and other varieties (Isd 37, Isd 38 and Isd 40) and also their somaclones were found in main cluster C_2 having different sub-clusters. Theirfore, it may be concluded that RAPD markers can be used for identification of somaclonal variation and the relationship between sources varieties and their somaclones.

Introduction

Sugarcane is globally a major source of raw material for the production of commercial sugar and has multipurpose crop providing not only sugar, but also a series of value added products and by-products such as energy, chemicals, and

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single cell protein, ethanol, bio-gas, fertilizer, fibre board and paper, polishers, cosmetics and candles. Although many countries are producers, only six of them account for 65% of the world's entire sugarcane production. Among these Brazil is the largest one (Viera 2002). It is very urgent to increase cane productivity without further area expansion to meet the future need of sugar and gur. The chemical composition of a matured, sound and normal sugarcane stalk of the species *Saccharum officinarum* are water 74.96%, sugar 13.40%, fibre 10.04%, ash 0.64%, N_2 bodies 0.58%, fat and wax 0.38%. Sugarcane is propagated vegetatively for commercial planting by stem cuttings. Tissue culture offers an opportunity to mass produce disease free planting material and is now used to supplement commercial sugarcane propagation in many countries including Brazil, the United States, India and Cuba (Lakshmanan et al. 2006). Somaclones may show variation for different parameters like yield, sugar recovery, disease resistance, drought and salt tolerance, maturity etc. It is not controversial that tissue culture tools are playing their part in sugarcane improvement and at the same time for a plant breeder assessment of genetic diversity.

Genetic markers have contributed much to understanding plant genetic diversity. Molecular markers are extensively being used to measure the variability present at genetic level, within and among the genotypes. The most commonly applied molecular markers used to study polymorphism are random amplified polymorphic DNA (RAPD).

The objectives have been planned for four explant sources varieties *viz*., Isd 37, Isd 38, Isd 39 and Isd 40 in order to develop somaclones as well as to calculate the genetic variability in the somaclones compared to their sources varieties by the use of DNA markers such as RAPD. These informations certainly will facilitate the supplementation of the breeding programme prevailing in Bangladesh.

Materials and Method

The experiment was conducted at the Biotechnology Laboratory, Bangladesh Sugarcane Research Institute (BSRI), Ishurdi, Pabna, Bangladesh during 2010 to 2011 to obtain *in vitro* plant regeneration potentiality of BSRI released varieties Isd 37, Isd 38, Isd 39 and Isd 40. The leaf sheath explants were collected from 8 - 10 months old field grown sugarcane from BSRI experimental field. At first MS supplemented with green coconut water (10%) containing 3 mg/l of 2, 4-D was prepared for callus induction. After five weeks of explantation, the calli were inoculated for shooting on MS supplemented with concentration BAP (2 mg/l) + Kn (1 mg/l) and maintained by sub-culturing every two weeks and then regenerated shoots were inoculated for rooting by sub-culturing every two

weeks on MS supplemented with 5 mg/l NAA. Rooted plantlets were acclimatized and transplanted to polybag and then field, respectively.

For molecular studies, young meristem cylinder from 12 somaclones and their donor parents were taken from R_0 regeneration and grinded using extraction buffer solution and amount of chemicals were important considerations for DNA isolation. DNA was extracted from sugarcane using the method modified and combined from the methods of Aljanabi et al. (1999) and mini-prep method adopted from Hossain et al. (2006) and Shahnawaz (2006). The DNA concentration was determined by Nano drop Spectrophotometer (2000/2000c, Thermo Scientific, USA) and was diluted to a concentration of 50 ng/µl. Samples were stored at –20^0C for further use. Polymorphism was studied using RAPD as illustrated by Mondal et al. (2009) which supported by Williams et al. (1990). Six RAPD primers (Operon Technologies, Inc., Alameda, California, USA) were used. List of polymorphic primers and their sequences are subsequently given.

The reaction mixtures 10 µl was amplified for each DNA sample in a Thermal Cycler (Genius, Techne, Cambridge Ltd.). Agarose gel (1.4%, w/v) was used for RAPD electrophoresis. The ethidium bromide at 10 mg/ml was added in gel for detection. Bands were viewed under ultraviolet trans-illuminator and documentation (FluorChem FC2, Cell Biosciences, USA) and also analysis system was used to make photographs. Besides, it was printed and saved on CD for lateral use.

The presence and absence of a DNA fragment was considered as basis of polymorphism. DNA loci if present were scored as '1' and if not were scored as '0'. The number of alleles per locus was determined and the polymorphic information content (PIC) values were calculated using the formula (Mondal et al. 2009). A dendrogram was constructed by using uweighted pair group method of arithmetic means (UPGMA) algorithm (Sneath and Sokal 1973) provided in the software (Statistica computer package).

Results and Discussion

In this study, six RAPD markers were used to measure the variability present in the 12 somaclones along with their parents generated through somaclonal variation. Although, nowadays, the different types of molecular markers are increasingly being used to study the distribution and patterns of genetic diversity in populations.

A total number of 175 DNA fragments were polymorphic indicating 74.67% polymorphism while 128 were unique bands. The average number of DNA polymorphic fragments produced by each primer was 29.17. The size of

amplification products ranged from 145 to 1000 bp (Table 1). The maximum number of polymorphic bands (45) was produced by the primer OPA-04, while the minimum number (12) was produced by primer OPA-01. Primer OPA-07 depicted maximum (80.56%) polymorphism followed by primers OPA-01 (80.00%) and OPB-06 (74.13%). Primer OPB-05 was found to be least polymorphic with 68.57% polymorphism.

Table 1. Performance of bands and PIC values as influenced by various RAPD primers among sixteen sugarcane genotype.

Primer codes	Size ranges (bp)	No. of polymorphic bands	No. of unique bands	Polymor-phism (%)	Number of bands/genotype	PIC value
OPA - 01	357 - 711	12	10	80.00	0.93	0.39
OPA - 02	278 - 988	22	17	73.33	1.88	0.46
OPA - 04	165 - 1000	45	29	71.43	3.94	0.50
OPB - 05	365 - 955	24	16	68.57	2.19	0.49
OPB - 06	145 - 944	43	30	74.13	3.63	0.49
OPA - 07	258 - 1000	29	26	80.56	2.25	0.50
Total	-	175	128	448.02	14.82	2.83
Average	-	29.17	21.33	74.67	2.47	0.47

The PIC indicates the effective number of alleles that can be detected per marker in a set of individuals. It is the the discriminatory power of the marker. Data depict that the PIC value ranges from 0.39 to 0.50. Maximum PIC value 0.50 was detected by primers OPA-04 and OPA-07 followed by primer OPB-05 and OPB-06 with 0.49 PIC value.

Detection of somaclonal variation through RAPD markers have been applied in sugarcane by many workers (Saini et al. 2004, Jain et al. 2005, Suprasanna et al. 2006, 2007, Devarunmath et al. 2007). Lal et al. (2008) investigated variability in sugarcane genotypes using 16 RAPD primers and amplified a total of 110 scorable fragments. Nair et al. (2002) used 25 RAPD primers to detect variability in 28 elite Indian sugarcane varieties and they observed 63.74% polymorphism.

Genetic diversity is commonly measured by genetic distance of genetic similarity, which signifies that there are either differences or similarities present at the genetic level (Weir 1990). The greatest linkage distance (44) was recorded between somaclone CC-40-6 and variety Isd 39 followed by linkage distance (43) between somaclones CC-40-2 and CC-40-6. Variety Isd 37 had the least linkage distance (14) with somaclone CC-40-3. Besides, Isd 37 had linkage distance 19, 28 and 24 with other varieties Isd 38, Isd 39 and Isd 40, respectively. Somaclones CC-37-1, CC-37-4 and CC-37-5 had linkage distance 22, 25 and 26 with their

parent variety Isd 37. Somaclones CC-38-1, CC-38-2 and CC-38-5 had linkage distance 23, 21 and 25 with their parent variety Isd 38. Parent variety Isd 39 exhibited linkage distance 31, 36 and 33 with CC-39-3, CC-39-5 and CC-39-6, respectively. Furthermore, parent variety Isd 40 showed linkage distances 35, 24 and 36 with its somaclones CC-40-2, CC-40-3 and CC-40-6, respectively. The least linkage distance between parent and somaclone indicates that maximum changes occurred at the chromosomal or gene level (Table 2).

Table 2. Summary of linkage distances (based on statistica) values for different genotype pairs of sugarcane.

Varieties/ somaclones	Isd 37	CC–37–1	CC–37–4	CC–37–5	Isd 38	CC–38–1	CC–38–2	CC–38–5	Isd 39	CC–39–3	CC–39–5	CC–39–6	Isd 40	CC–40–2	CC–40–3	CC–40–6
Isd 37	0	22	25	26	19	24	16	22	28	17	22	19	24	27	14	30
CC–37–1	22	0	31	30	23	30	20	28	38	25	24	21	32	33	20	36
CC–37–4	25	31	0	35	28	27	23	29	35	28	31	28	35	38	23	37
CC–37–5	26	30	35	0	29	34	26	32	42	29	32	29	36	37	24	36
Isd 38	19	23	28	29	0	23	21	25	35	22	25	22	29	30	17	33
CC–38–1	24	30	27	34	23	0	26	28	34	27	30	27	34	37	22	38
CC–38–2	16	20	23	26	21	26	0	24	34	19	24	21	26	31	16	32
CC–38–5	22	28	29	32	25	28	24	0	30	25	30	27	34	35	22	36
Isd 39	28	38	35	42	35	34	34	30	0	31	36	33	42	43	28	44
CC–39–3	17	25	28	29	22	27	19	25	31	0	23	22	27	28	17	29
CC–39–5	22	24	31	32	25	30	24	30	36	23	0	23	32	35	20	32
CC–39–6	19	21	28	29	22	27	21	27	33	22	23	0	29	32	15	33
Isd 40	24	32	35	36	29	34	26	34	42	27	32	29	0	35	24	36
CC–40–2	27	33	38	37	30	37	31	35	43	28	35	32	35	0	27	43
CC–40–3	14	20	23	24	17	22	16	22	28	17	20	15	24	27	0	26
CC–40–6	30	36	37	36	33	38	32	36	44	29	32	33	36	43	26	0

Genetic relationship among the 12 somaclones and their 4 parents (Isd 37, Isd 38, Isd 39 and Isd 40) generated some different cluster at the linkage distance range from 36 to 14 by all of the 6 primers. Dendrogram based on linkage distance using indicated segregation of the 4 sugarcane varieties and their somaclones into two main clusters C_1 (Isd 39) and C_2 at the linkage distance of 36 (Fig. 1). Besides, C_2 produced different sub clusters. Formation of clustering and sub clustering in the tree diagram confirmed the presence of variability at DNA level among somaclones with respect to their parents. Nevertheless, it is also suggested that to get more precise results, the number of primers should be increased. Very limited reports on the use of DNA markers for the estimation of

somaclonal variation are available. Hence, this study can be used as a point reference for further studies.

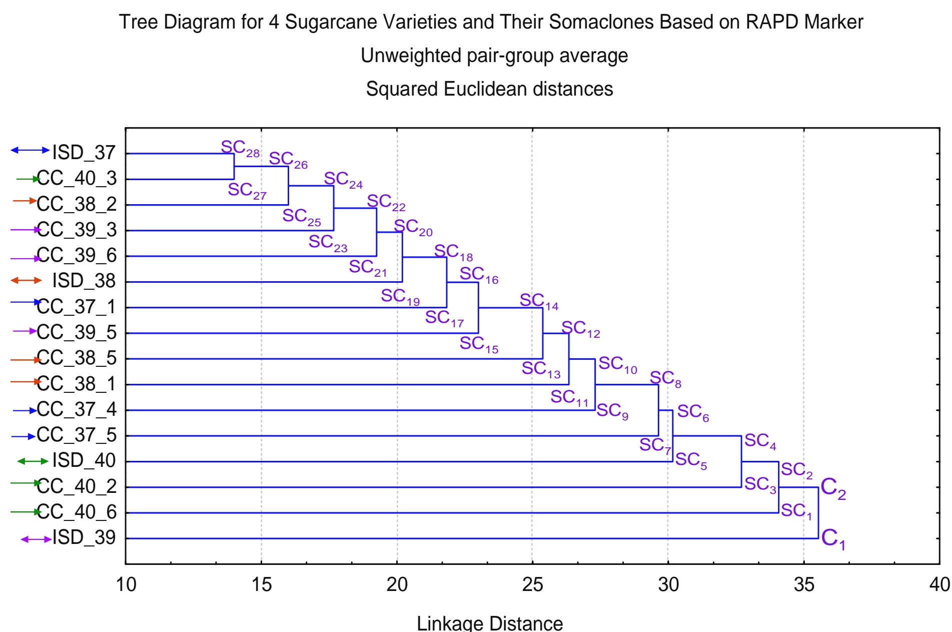

Fig. 1. Cluster analysis by UPGMA means of four sugarcane varieties and their somaclones based on 6 RAPD markers. (C = Cluster, SC = Sub-cluster).

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