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Field performance and RAPD analysis for assessment of genetic variation in sugarcane somaclones

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Abstract

Sugarcane somaclones derived by callus culture of the sugarcane variety CoC 671 were evaluated for their quantitative attributes and assessed for the genetic variation by RAPD analysis. Assessment at 12th month, VSI 2179 gave higher cane yield compared to somaclones and the parent CoC 671. Somaclone variants, VSI 1748 and VSI 2179 gave significantly higher sugar yield over the check CoC 671. VSI 1748, VSI 2003 and VSI 2179 were significantly superior for brix, sucrose and CCS percentage over their parent. RAPD profiling of somaclones, VSI 1733 with 21 primers (13.8%), VSI 1748 and VSI 1855 with four primers (3.0%) and VSI 2003 and VSI 2179 with one primer (0.3%) revealed polymorphism as compared to CoC 671. These promising clones are being evaluated in different agro-climatic zones of Maharashtra.

Key words: Sugarcane, somaclonal variation, field performance, RAPD, genetic variation, CoC 671

Introduction

Enhancement in yield potential and sugar recovery of sugarcane through conventional breeding methods is a slow process and time consuming. Plant tissue culture seems to be a good choice for sugarcane improvement since high variability can induce genetic variations through *in vitro* callus culture [1]. Somaclonal variation is cheaper than other methods of genetic manipulation, it has been most successful in crops with limited genetic systems or narrow genetic base [2]. The potential benefit of somaclonal variation for improved cane yield and sugar recovery along with increased resistance against the diseases like Fiji disease and sugarcane mosaic has been well documented [3, 4]. Further morphological variation in stooling and erectness among sugarcane somaclones has been reported [5]. Several strategies can be used to assess the genetic variation of tissue culture raised clones such as morphological descriptions, physiological observations, cytological studies [6] and molecular studies [7, 8]. Random Amplified Polymorphic DNA (RAPD) analysis has been used to assess the somaclonal variability [9]. The present research was focused on the development of somaclones from the sugarcane variety CoC 671 through callus culture and assessment of their field performance and genetic variation.

Materials and methods

Induction and isolation of somaclones

Healthy tops from 3-4 months old plants of sugarcane variety CoC 671 were collected from Breeder Seed plot, Vasantdada Sugar Institute, Pune. The spindle segments (5-6 cm) containing growing tips and young leaves were dissected out and washed under running tap water for half an hour and the explants were sterilized with 70 % alcohol for 30 seconds followed by 0.1% (w/v) HgCl2 for 3-4 min. Washed 5-6 times with distilled water under aseptic conditions. The spindles were then dissected to collect the leaf segments containing the apical meristem (2mm). The explants were inoculated on MS medium [10] supplemented with 2,4-D (3mg/l), thiamine HCl (1mg/l), inositol (20mg/l), sucrose (25g/l) and coconut water (100ml/l). All the cultures were incubated under 3500-4200 Lux light at 23±2ºC temperature and 65-70% RH. After 5-6 sub-culture, the callus was transferred to MS medium augmented with naphthalene acetic acid (0.5mg/l), kinetin (0.5mg/l), inositol (20mg/l), thiamine HCl (1mg/l), casein hydrolysate (400mg/l), sucrose (20g/l) and coconut water (100ml/l). Regenerated plants were transferred

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to fresh medium every 25 days, to achieve rapid shoot growth. The regenerated shoots were separated after 40-45 days and transferred to rooting medium, containing half concentration of basal MS medium supplemented with indole-3-butyric acid (IBA 3mg/l), indole acetic acid (IAA 0.1mg/l) and 4 % sucrose. A total of 993 well-rooted plantlets were transferred to polybags containing a mixture of sand, soil and organic compost in the proportion of 1:1:1(W/W). These plantlets were hardened for six weeks in the glasshouse and further subjected to secondary hardening for six weeks outside.

Clonal selection

A total of 626 somaclones were planted in the Institute's farm during the year 2002-03, as the somaclone 1(S1) generation along with setts of the parental variety CoC 671 after every ten rows comparison. Observations on morphological and quality parameters were recorded at regular intervals. Thirty promising variants (5%) out of 626 somaclones were selected on the basis of quality traits and advanced to second generation (S2) as a Clonal trial-I in an augmented design, in the year 2003-04 with the national checks, CoC 671 and Co 86032. On the basis of the yield and quality traits, five somaclones (0.8%) were advanced to third generation (S3) as a Clonal trial-II in the year 2004-05 and planted along with the checks CoC 671 and Co 86032 in a completely randomized block design. Observations were taken on qualitative traits, and the five soma clones were further advanced to S4 generation.

Field trial

During the 2005-06 planting season, selected five somaclonal variants *viz*., VSI 1733, VSI 1748, VSI 1855, VSI 2003 and VSI 2179 were evaluated along with the parental variety CoC 671 and the national check Co 86032 in a randomized block design with three replications at the institute's farm. The plot size was 24 sq m. having four rows per treatment. The row length was 6m and distance between two rows was 1m. Two eye bud setts were planted end to end. The crop was fertilized @250 +125+125 kg of N, P205 and K20 respectively/ha. Periodical observations were recorded on quantitative traits, (percent germination, tillering number, number of internodes per cane, millable height of cane, single cane weight, cane girth, number of millable canes per plot and cane yield per plot) as well as qualitative traits (brix, sucrose , purity and commercial cane sugar (CCS) % and t/ha at 8, 9, 10, 11 and 12th month. Standard analytical method described by Meade and Chen [11] was followed. The data were statistically analyzed as per the methods described by Panse and Sukhatme [12].

DNA isolation

Total genomic DNA was isolated from the fresh leaves of randomly selected three plants from each somaclone and the parental variety CoC 671 by modified Doyle and Doyle method [13]. DNA samples were quantified and diluted in TE buffer.

RAPD fingerprinting

A total of 29 arbitrary decamer primers from Kit A, AB, C, G and K (Operon Technologies, USA), were used for the PCR amplification of the genome of CoC 671 and five somaclones. Amplification was carried out in 25µl of reaction mixture containing 50ng template DNA, 2.5µl of 10x PCR buffer, 2.5 mM $MgCl_2$, 2 mM dNTPs, 15ng of primer and 1 Unit of Taq Polymerase (Bangalore Genei). DNA amplification was performed in BIO-RAD icycler programmed for 45 cycles as follows: one cycle of 4.30 min at 92°C, 1 min at 35°C, 2 min at 72°C; followed by 44 cycles each of 1 min at 92°C, 1 min at 35°C, 2 min at 72°C followed one final extension cycle of 15 min at 72°C. The amplification products were size-separated by gel electrophoresis in 1.4% agarose (Sigma) gel with 1x TBE and stained with ethidium bromide. All the reactions were repeated at least twice, and consistently reproducible bands were used in the analysis.

Results and discussion

The effect of auxin (2, 4-D) and coconut water for the development of callus was found good for the growth of callus. A whitish yellow and compact callus growth was found in the medium having 3mg of 2, 4-D, 1mg of thiamine HCl, 20mg of inositol, 25g of sucrose and 100ml of coconut water per liter. The basal MS medium supplemented with 0.5mg of NAA, 0.5mg of Kinetin, 20mg of inositol, 1mg of thiamine HCl, 400mg of casein hydrolysate, 20gm of sucrose and 100ml of coconut water per liter showed the best shoot multiplication and vigour. Profuse roots were observed in the medium containing half MS medium supplemented with 3mg of IBA, 0.1mg of IAA and 4% sucrose per liter. The callus induced through plant regeneration of CoC 671 yielded 626 somaclones. Several reports are available on regeneration of plants via callus in sugarcane. However, the *in vitro* response seems to be cultivar dependent, media composition, the level of ploidy and genetic mosaicism are capable of inducing *in vitro* variability [14]. Evidence suggests that growth regulators induce

somaclonal variation during the various phases of cell culture seem to exert their effects on cell division [15], disorganized growth and selective proliferation of specific cell types [16]. Such variations have been reported in callus cultures of sugarcane [17] and were attributed to numerical variation in chromosome seen in sugarcane varieties and also due to the asynchronous division of cells. Such events will also lead to variation in chromosome number differ in phenotypic expression (dwarfs, lack of vigour) from normal plants.

Among the 626 somaclones transplanted in the field for clonal selection, some clones reported variations in cane diameter, stalk length, weight of cane, number of millable canes per stool, shape and size of internodes, presence of wax on internodes, eye bud, colour of cane etc. Similar results have been documented by earlier workers and the variations are quantified by morphological, cytogenetic and isozymes studies [17, 18].

Field evaluation studies (Tables 2 & 3) reveal that somaclone VSI 1748 (28.94 t/ha) and VSI 2179 (27.90 t/ha) were significantly superior to the check CoC 671 (20.93 t/ha) at 12th month for sugar yield. At 8, 9, 10, 11 and 12th month, the Brix, Sucrose and CCS and Purity % of VSI 1748, VSI 2179 and VSI 2003 was found to be superior over their parent. At 10th month, VSI 2179 was significantly superior for Brix % (24.19), Sucrose % (23.00) and CCS % (16.66) than the parental variety CoC 671 (23.19 % Brix, 21.45 % Sucrose and 15.36 % CCS). Similarly, VSI 2003, VSI 1855 and VSI 1748 recorded higher Brix % (23.51, 23.55 & 23.40), Sucrose % (22.04, 21.83 and 21.68), and CCS % (15.87, 15.64 and 15.53) than the parent. At the time of harvest (12th month), VSI 1748, VSI 2003 and VSI 2179 were found to be significantly superior for Brix % (25.34, 24.68 & 24.66), Sucrose % (24.97, 24.15 & 23.71) and CCS % (18.36, 17.70 & 17.26) over the parent, CoC 671 check (23.92 % Brix, 22.18 % Sucrose and 15.89 % CCS). Similarly, VSI 1748 and VSI 2003 were significantly superior for purity % (98.54 and 98.28) than the parental line (92.72 %). In an another study, Jalaja *et al.* [19] reported that the somaclonal variant Co 94012 of CoC 671, recorded increased values in respect of cane yield (t/ha), CCS (yield t/ha), Sucrose % and CCS%.

The data on cane yield and their contributing characters in the present study revealed that none of the somaclones was found to be significantly superior to the parent CoC 671 (Table 1). However, three variants *viz.*, VSI 2179, VSI 1748 and VSI 2003 gave higher cane yield as compared to CoC 671. VSI 2179 gave higher cane yield (161.0 t/ha) as compared to the rest of the somaclones and also CoC 671 (132.0t/ha) at the harvest (12th month). VSI 1855 recorded the highest tillering ratio (7.69) at 90 days compared to CoC 671 (6.60). VSI 2003 had comparatively higher single cane weight (1.51Kg) as compared CoC 671 (1.29 Kg) at 12th months. VSI 2179 (9.55 cm), VSI 2003 (9.49 cm) and VSI 1748 (9.22 cm) recorded higher cane girth than CoC 671 (9.12cm). Nagai *et al.* [20] reported significant differences in the subclonal populations of somaclones for stalk number, stalk length, stalk diameter, stalk volume, leaf width, leaf

Table 1. Data on cane yield, quality and their contributing characters in somaclonal variants of sugarcane genotype CoC 671

Variant/ variety	Cane yield (t/ha)	Commercial cane sugar (t/ha)	No. of millable canes (lakh/ha)	Cane girth (cm)	Single cane wt. (kg)	Millable height of cane (cm)	No. of Inter-nodes per cane	Tillering ratio at 90 days	Germination % at 45 days
VSI 1733	120.00	18.04	0.89	8.99	1.34	245.10	21.56	7.32	53.13
VSI 1748	157.67	28.94*	1.16	9.22	1.35	253.33	22.56	7.02	57.30
VSI 1855	138.00	23.25	1.01	9.09	1.35	268.80	22.10	7.69	51.47
VSI 2003	147.33	26.10	0.97	9.49	1.51	265.00	21.96	6.78	64.40
VSI 2179	161.00	27.90*	1.13	9.55	1.41	266.90	22.66	7.19	64.36
CoC 671	132.00	20.93	1.02	9.12	1.29	246.67	22.80	6.60	58.86
Co 86032	158.67	23.68	1.26	8.65	1.26	226.77	21.66	7.09	60.00
SE ±	9.98	1.77	0.053	0.28	0.088	8.90	5.61	5.03	3.75
CD at 5%	30.70	5.45	0.163	0.86	0.270	27.40	1.72	1.54	11.56
CV %	11.92	12.73	8.60	5.33	11.30	6.09	4.38	12.28	11.18

*Significantly superior at 5%

Table 2. Periodical observation on quality traits in somaclonal variants of sugarcane genotype CoC 671

Variant/variety	Brix %					Sucrose %				
	8 Month	9 Month	10 Month	11 Month	12 Month	8 Month	9 Month	10 Month	11 Month	12 Month
VSI 1733	17.68	20.08	21.07	22.94	23.49	15.14	17.29	19.19	21.07	21.22
VSI 1748	18.20	22.25	23.40	25.30	25.34*	16.52	20.04	21.68	25.18	24.97*
VSI 1855	18.40	21.59	23.55	24.69	24.45*	16.16	19.49	21.83	23.72	23.29
VSI 2003	18.45	21.12	23.51	24.59	24.68*	16.70	19.28	22.04	23.54	24.15*
VSI 2179	18.61	21.80	24.19*	24.51	24.66*	16.41	19.16	23.00*	23.91	23.71*
CoC 671	17.92	21.21	23.19	24.45	23.92	16.29	19.17	21.45	23.34	22.18
Co 86032	17.02	19.88	21.09	21.77	22.77	16.27	17.60	19.04	20.30	20.91
SE ±	0.48	0.35	0.80	0.38	0.16	0.42	0.35	0.27	0.69	0.43
CD at 5%	1.49	1.10	0.86	1.18	0.51	1.31	1.09	1.00	2.14	1.32
CV %	4.66	2.94	1.12	2.78	1.20	4.56	3.26	2.67	5.24	3.24

*Significantly superior at 5%

Table 3. Periodical observation on quality characters in somaclonal variants of sugarcane genotype CoC 671

Variant/variety	CCS %					Purity %				
	8 Month	9 Month	10 Month	11 Month	12 Month	8 Month	9 Month	10 Month	11 Month	12 Month
VSI 1733	10.45	11.96	13.64	15.04	15.03	85.58	86.13	91.11	91.82	90.35
VSI 1748	11.72	14.17	15.53	18.52	18.36*	90.84	90.07	92.62	99.06	98.54*
VSI 1855	11.29	13.81	15.64	17.24	16.89	89.70	90.27	92.68	95.86	95.26
VSI 2003	11.84	13.72	15.87	17.10	17.70*	90.57	91.32	93.73	95.73	98.28*
VSI 2179	11.49	13.39	16.66*	17.48	17.26*	88.09	88.08	94.07*	97.30	96.06
CoC 671	11.57	13.57	15.36	16.94	15.89	90.95	90.35	91.06	95.46	92.72
Co 86032	11.77	12.34	13.47	15.24	14.92	95.46	88.50	90.25	93.21	91.82
SE ±	0.34	0.33	0.26	0.71	0.39	1.97	1.61	1.00	1.40	1.20
CD at 5%	1.04	1.03	0.82	2.19	1.22	6.09	4.96	3.10	4.31	3.70
CV %	5.15	4.37	3.04	7.34	4.17	3.80	3.13	1.89	2.54	2.20

*Significantly superior at 5%

area, brix and pollen production capability. Doule [21] observed that somaclone TC 435, TC 434 and TC 045 had significantly higher millable height of cane, number of internodes and tillering ratio at 120 days, respectively as compared to the donor parent variety CoC 671. Liu and Chen [22] found significant variations amongst sugarcane somaclones from 8 varieties in characters such as cane yield, sugar yield, stalk number, length, diameter, volume, density and weight. They found that somaclone 70-6132 had higher cane yield, sugar yield and stalk number (by 32%, 34% and 6% respectively) than the parental variety.

RAPD analysis reveals DNA polymorphism as differences in amplification patterns, using primers of random sequences that search for complementarities in the genome. *In vitro* stress leads to changes at preferential sites, such as repetitive DNA, thereby activating transposable elements as well as DNA methylation pattern. Change in the RAPD profile may thus results from loss/gain of a primer annealing, caused by point mutations or by the insertion or deletion of odd sequences or transposition elements [6, 23]. In the present study, RAPD markers were used to assess the occurrence of genetic variation in five different somaclones identified on the phenotypic basis. The

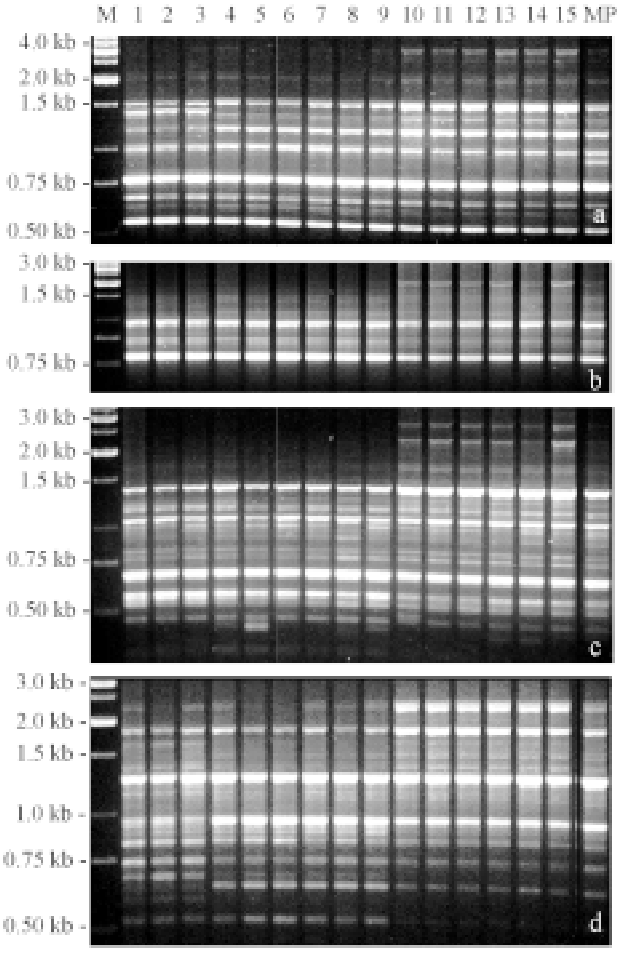

Fig. 1(a-d): Polymorphic RAPD profiles of sugarcane variants; a. OPA 17, b. OPAB 03, c. OPAB o5 and d. OPAB 14. Lane M, marker-1 kb ladder, Lane 1-3: VSI 1733, Lane 4-6: VSI 1748, Lane 7-9: VSI 1855, Lane 10-12: VSI 2003, Lane 13-15: VSI 2179 and Lane MP: mother parent CoC 671

banding pattern of the PCR amplified products from the donor parent was compared with the five somaclonal variants. Out of the forty-five different decamers tested, twenty-nine resulted in reproducible amplification. A total of 304, 285, 285, 287 and 287 bands were produced, out of which 262, 278, 278, 286 and 286 are monomorphic bands and 42, 07, 07, 01 and 01 are polymorphic to clone VSI 1733(A), VSI 1748(B), VSI 1855(C), VSI 2003(D) and VSI 2179(E) respectively. The clones show different polymorphic primers, in clones A with twenty-one primers (13.8%), clones B and C with four primers (OPAB 03, OPAB 05, OPAB 07 and OPAB 09 with 3.0%) and clones D and E with one primer (OPA 19 with 0.3%) (Fig. 1) and can be used for individual somaclone identification. Genomic variations as revealed by RAPD analysis are apparent in individual somaclone, which albeit need to be considered for the cultivar authentication purpose [24]. The more number of monomorphic bands is the consequence of single parent CoC 671 as base material for the development of these somaclones and further variation analysis the most advanced marker systems can be employed.

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