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CHARACTERIZATION OF SOMACLONAL VARIANTS OF SUGARCANE ON THE BASIS OF QUANTITATIVE, QUALITATIVE, AND GENETIC ATTRIBUTES

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Abstract

Somaclonal variation is an important tool for creating genetic diversity in sugarcane. Somaclones of NIA-1198 were developed through callus culture and subjected to field trials under randomized complete block design with three replications over two consecutive years (2013-14 and 2014-15). Crop was harvested after 12 months of planting each year and the quantitative and qualitative parameters were determined at harvesting stage. Quantitative traits included plant height, girth, tillers, internode length, number of internodes, stool weight, and cane yield, whereas qualitative parameters included CCS%, brix %, sucrose %, fiber %, purity, sugar recovery, and sugar yield. The data of both cropping seasons were pooled and subjected to statistical analysis and other tests. Statistically significant differences were observed for all the characteristics among somaclonal population. Somaclone SC8 was observed to have highest cane yield of 77.87 t/ha against the 64.87 t/ha of the parent. While, somaclone SC30 exhibited highest sugar yield of 11.58 t/ha as compared to the parent's 7.86 t/ha. Sugar recovery was also observed to be maximum for the SC30 (14.42%). SC12 somaclone presented highest cane height of 372 cm whereas maximum number of tillers were harvested in SC13, and SC30 (9.0). On comparing the somaclones for percent variation, highest range of variation was recorded for sugar yield which increased as much as 47.5 % in some of the somaclones whereas it decreased up to -55.7 % in others. Cluster analysis of the parameters classified the genotypes into five major clusters. Only 4 somaclones were observed to appear with the parent (NIA-1198) in the same cluster. Cluster 1 was distinguished by highest quantitative traits, cluster 2 was characterized by maximum qualitative parameters, and cluster 5 recorded highest fiber contents. Pearson's correlation analysis showed strong correlation of cane yield with the cane girth (0.536), and tillers per plant (0.607). Whereas, sugar yield was observed to have highest correlation with cane yield (0.814), CCS % (0.604) and sugar recovery (0.596). On principal component analysis (PCA) of the pooled data, parameters under study were observed to divide into five principal components (PCs) which contributed for up to 93.18% variability. Many of the somaclones were observed to be placed on high distances from each other on the score plot of PCA. Genetic parameters of the somaclonal population showed that the characteristics under study were highly heritable, and possessed low environmental variance. The heritability values for all the characters were estimated to be more than 93% at least. It can be concluded from the study that somaclonal variations can create highly diverse populations of sugarcane for evaluation in cane breeding programs.

Key words: Cluster analysis, Principal component analysis, Somaclonal variations, Sugarcane, Tissue culture.

Introduction

Sugarcane (*Saccharum officinarum* L.) is world's largest crop by production with an annual production of 1899.9 million tonnes (Food and Agriculture Organization of UN (FAO) statistics, 2014). It is grown in 70 countries around the world (Chatenet *et al.*, 2001). Sugarcane is one of the major cash crops of Pakistan as it serves as the sole source of sugar in the country. Its value addition in the GDP is 0.7% whereas it accounts for 3.4% of the GDP in agriculture sector of the Pakistan (Pakistan Agriculture Research, 2015). Pakistan ranks at fifth position with respect to area under sugarcane cultivation however unfortunately, the position of the country is 51st in the world for per hectare yield of the crop with an average yield of 57 t/ha (Pakistan Bureau of Statistics, 2015). Although yield per hectare in the country has been improving over the time, Pakistan harvests one of the lowest cane and sugar yield among the sugarcane cultivating countries (Naqvi, 2005).

Therefore, it is necessary to develop new and better sugarcane varieties to benefit the farming community as well as the sugar industry and to explore the full potential of this crop. It has also been noticed that the yield of sugarcane varieties in particular areas of cultivation is decreasing with the passage of time, making it even indispensable to develop new sugarcane genotypes (Khan *et al.*, 2009). However, sugarcane has an incredibly complex and one of the most intricate genome among the plants, which makes its breeding complicated. Furthermore, sugarcane breeding is limited by several other factors as well e.g. polyploid nature of genome, late maturity, high water requirements, and low resistance to insect pests and fungal diseases (Van den Bulk, 1991; Leal *et al.*, 1996; Bairu *et al.*, 2011; De Setta *et al.*, 2014;). Moreover, in subtropical countries like Pakistan, sugarcane varietal development is also constrained by absence of flowering and seed viability (Khan *et al.*, 2008).

Creation of genetic variability is the very first requisite towards any crop breeding program. In many of the crops, it is obtained through manipulation of natural hybridization i.e. by making selective crosses and artificial selection. This, however, is not posssible for sugarcane breeding in Pakistan because of absence of flowering, and pollen sterility (Khan *et al.*, 2008). Hence, alternative approaches are needed to be exploited for developing sugarcane varieties optimum for agroclimatic conditions of the country. Different approaches for the improvement of sugarcane crop include mutation breeding, genetic engineering, and somaclonal variations (Rajeswari *et al.*, 2009; Raza *et al.*, 2014). Somaclonal variations have practically served the purpose of sugarcane improvement since long, by providing the scientists with an excellent option to obtain genetic diversity in plant progenies

whereas genetic engineering needs to be matured; and safety of the genetically engineered crops is yet to be addressed (Shahid *et al.*, 2011).

Somaclonal variations are generated in *In vitro* conditions by supplying higher concerntrations of auxins in the culture medium (Leal *et al.*, 1996; Heinz & Mee, 1971). Plant tissues, when grown in such medium, grow into a deformed mass of cells known as callus which is developed in to plantlets by the shifting this mass into shooting and rooting medium afterwards. The plantlets passing through a callus stage, are genetically dissimilar from the parent as a result of mutations which occur during the process of callus formation (Silvarolla, 1992). Although, the source of the explants, and ploidy level of the donor plant are important factors in establishing these variations; epigenetic factors i.e. growth medium for plants, is regarded as the major determinant of the extent of tissue culture variations (Karp, 1992). Hence, somaclonal variations equip us with the ability to change characteristics of the concerned genotypes, and select promising plants (Ahloowalia, 1995). The phenomenon of somaclonal variations have already assisted the development of high yielding, early maturing, disease resistant, and drought tolerant varieties having various superior characteristics over the mother plants (Snyman *et al.*, 2011; Khan & Khan, 2010; Singh *et al.*, 2008). Thus, tissue culture technology is extremely important especially for sugarcane breeding considering the constrains in cane breeding discussed earlier (Hoy *et al.*, 2003).

Precise assessment of genetic diversity has paramount importance in cane breeding. It helps in assisting the desirable genotypes' selection and identifying the segregating populations for further amendment, and evaluation of divers genotype. Exploitation of genetic remoteness among numerous genotypes is vital for crop improvement programs (Malik *et al*., 2010). Multivariate analysis techniques like Cluster Analysis and Principal Component Analysis (PCA) can be employed for assessing the genetic divergence of the sugarcane accessions under study. Whereas, correlation analysis finds its applications in determination of interaction of several crop parameters among each other.

Present study was aimed at development of elite sugarcane clones from NIA-1198 genotype. Tissue culture variants were developed, acclimatized, and evaluated for qualitative and quantitative parameters in the field trial over two years. Moreover, the study also targeted the evaluation of potential of the somaclonal population in terms of diversity of traits and association between each pair of the characteristics. The data engendered from this investigation will be employed in developing novel breeding strategies and selection measures to develop sugarcane genotypes having higher cane and sugar yields.

Materials and Methods

Explants were taken from NIA-1198 genotype. Apical meristematic tissues were collected from eight months old plants grown at experimental field of Nuclear Institute of Agriculture (NIA), Tando Jam. Slices from the donor plant were subjected to sterilization using ethanol and sodium hypochlorite. Sterilized growth media (pH 5.7) was used *for In vitro* development of plantlets. Plant tissues were cultured on MS medium containing 3-5 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D), following an incubation in dark so that callus induction could be facilitated (Murashige & Skoog, 1962). Shoot multiplication was done by transferring the embryogenic calli with induced shoot into MS medium containing 2mg/L BAP (6-benzylaminopurine) concentration, whereas root multiplication was conducted using recommended concentrations of indole-3-butyric acid (IBA) as per protocols of Khan *et al.* (2004).

Plantlets exhibiting excellent development were subjected to field trials once acclimatized in the plastic pots and then in green house of the institute. A total set of 50 somaclones, including the parent as check, were planted at the experimental field of NIA, Tando Jam by following Randomized Complete Block Design (RCBD). Sowing was done in the month of October i.e. autumn season for two consecutive years (2013-14 and 2014-15). Double sets of sugarcane, each with two buds were used as sowing material. Each somaclone was planted in three replications and a row to row distance of one meter was maintained among the somaclones. The recommended dose of fertilizer was used (N, P, and K at 150,100, and 100 Kg/ha of Urea, DAP and SOP). Somaclones were grown until maturity adopting agronomic practices (Khan *et al.*, 2008).

Quantitative and qualitative characters of the plants were determined in the month of November at each harvest. Fourteen quantitative and qualitative parameters were determined for the somaclonal population. Quantitative characteristics included height, girth, number of internodes, length of internodes, tillering, stool weight, and yield, while, the list of investigated qualitative parameters comprised of brix %, CCS %, recovery %, purity %, sucrose %, fiber %, and sugar yield. Biochemical traits of the crop were studied at Sugarcane Biotechnology Laboratory, NIA, following the protocols of the sugarcane laboratory manual for Queensland sugar mills (Anon., 1970; Khan *et al.*, 2015). Plant height, girth, tillers, number of internode, internode length, stool weight and yield were determined by randomly harvesting five plants of each plot.

The recorded data of both years were pooled and subjected to various heritability and statistical tests. The statistical differences among pool data were determined using Statistix software version 8.1 on Windows operated system using Tuckey's multiple range test at $\alpha = 0.05$ level of significance (Statistix 8.1, Tallahassee, Florida, USA). One-way analysis of variance (ANOVA) was done for all the studied parameters. Same statistical package was utilized to determine Pearson's correlation to have an insight into the effects of determined qualitative and quantitative characteristics on each other. Origin v. 2016, was used for hierarchical cluster analysis employing Ward's Linkage Cluster Analysis method (Kumar *et al.*, 2009). Squared Euclidean distance was used for the cluster matrix,the data were normalized to z-score before subjecting to this analysis. Dendrogram was developed for visualizing the results and interrelation of the somaclonal population. Finally, the mean values of the characteristics under study were calculated for each cluster using Microsoft Office Excel version 2016.

Moreover, principle component analysis (PCA) of the somaclonal population and the parent was also done through same version of Origin software. Mean values of the variables were standardized prior to PCA in order to evade the effects of difference in scales. Number of principal components were assessed from the scree plot determining the elbow of the graph for identifying the contributors towards the maximum variance. Score plot was exploited to determine the positioning of the genotypes under study into the principle components, whereas loading plot was utilized to have an insight into placement of parameters in the subspaceof principal components.

Genetic parameters like heritability percentage in broad sense, coefficients of variation, and genetic advance at 5%, 10%, 15%, 20% and 25% selection intensities were also used to evaluate somaclonal variants. Method of Brewbaker (1964) was employed for calculation of phenotypic variance, genotypic variance, and environmental variance, calculated as per the formula given below:

$$GCV = \delta^2g/X \times 100; PCV = \delta^2p/X \times 100,$$

δ^2g, and δ^2p represent standard deviation of the genotypic and phenotypic variances, and X represents grand mean of the samples.

Broad sense heritability was determined using method of Mahmud *et al.* (1951) whereas the method of Allard (1999) was followed for estimation of expected genetic advance.

Results

Number of significant variants in somaclonal population: Significant variations were observed in several traits in all the evaluated somaclones (Tables 1 and 2). The somaclones also performed differently when compared against the parent. Total 686 observations of the population were analyzed in the study and it was found that 342 observations differed from the parent non-significantly ($p<0.05$), 322 records decreased significantly, whereas 22 records increased significantly. Maximum number of observations of augmented parameters were counted for qualitative parameters like sucrose %, and brix % (four each). Plant girth, height, purity, recovery, CCS%, number of tillers per plant and cane yield were also found to be significantly higher in some of the somaclones (Fig. 1). Whereas, sugar yield was observed to increase significantly in two somaclones that were subjected to the field trials. Highest number of decreased observations were recorded for number of internodes, internode length, and cane height, for which the mentioned characteristics were significantly reduced in 48, 46, and 41 plants respectively. Least number of variants were observed for cane girth, and purity %. It was also seen that *In vitro* mutagenesis caused the parameters of the somaclones to decrease in most of the cases when compared against the parent (Fig. 1), whereas the number of improved observations were less. Sugar yield of the somaclones showed highest range of variations when compared to the parent as it demonstrated maximum increase of up to 47.4% in some of the somaclones, and a decrease of as much as -55.6% in others. Plant height also showed similar trends with a range of percent variation from -68% to +20% (Fig. 2).

Quantitative and qualitative outcomes of somaclonal variations: The evaluation of comparative performance of somaclones made it evident that somaclones exhibited highly significant differences from the parent. Pooled data about means and comparisons of the studied parameters against the parent is presented in Tables 3 and 4. Somaclonal variations caused both increase and decrease of the concerned parameters in the progeny plants.

Tallest plants having the mean millable cane height of 372cm were observed in somaclone SC12 against the parent with a height of 310 cm. Plant height was also observed to increase in SC8 and SC7 (364, and 332 cm respectively). SC42 somaclone showed least height of 100 cm. Girth of the parent was recorded to be 2.6 cm whereas one of the somaclones viz. SC11 showed a girth of 3.4 cm. In another clone, SC12 3.3 cm girth was recorded. Least cane thickness was seen in SC2 (2.1 cm), as presented in Table 3. Number of tillers per plant were found to be at par with the parent in most of the somaclones, however, two somaclone i.e. SC 13, and SC30recorded 9.0 tillers per plant against the parent (8.0). Thirty somaclones showed statistically similar observations for the number of tillers against the parent, whereas the tillers were statistically decreased in 19 somaclones with least count for SC14 for which mean tillers per plant were observed to be only five.

Table 1. Pooled analysis of variance (mean squares) for different quantitative characters in somaclones.

Somaclones	DF	Height (cm)	Girth (cm)	No. of internode per stool	Internodes length (cm)	Tillers per plant	Cane weight (kg)	Cane yield (t/ha)
Rep	2	1364.2	0.18807	3.0867	1.0323	4.38000	0.29179	29.179
Somaclones	49	13009.8**	0.20804**	36.0414**	19.7059**	2.68027**	3.53994**	353.994**
Error	98	224.2	0.01126	0.7397	0.6758	0.30517	0.10544	10.544
Total	149							
CV		7.42	4.35	4.08	7.92	8.12	6.41	6.41

Table 2. Pooled analysis of variance (mean squares) for different qualitative characters in somaclones.

Somaclones	DF	CCS %	Brix %	Fiber %	Sucrose %	Purity %	Sugar recovery %	Sugar yield (t/ha)
Rep	2	0.02472	0.0766	0.8416	0.0586	0.0881	0.02183	0.47994
Somaclone	49	8.45910**	11.7770**	16.1524**	11.7770**	10.3322**	7.38378**	8.23265**
Error	98	0.26426	0.4421	1.1239	0.4433	0.4932	0.25856	0.27538
Total	149							
CV		4.22	3.09	7.46	3.83	0.87	4.45	8.48

Table 3. Assessment of quantitative traits of sugarcane somaclones and the parent under Tando Jam agroclimatic conditions.

Somaclones	Height (cm)	Girth (cm)	Number of internodes per stool	Internodes length (cm)	Tillers per plant	Cane weight	Cane yield (t/ha)
NIA-1198	310 b-c	2.6 b-f	31.33 a	16.74 ab	8.00 a-c	6.49 b-d	64.87 b-d
SC2	204 g-l	2.1 h	22.67 c-g	12.91 c-e	8.00 a-c	4.79 f-i	47.87 f-i
SC6	283 b-d	2.3 d-h	20.33 f-l	14.24 b-d	7.67 a-d	5.82 d-f	58.20 d-f
SC7	332 a-b	2.8 b	25.00 bc	10.24 e-k	6.00 d-f	6.45 c-e	64.53 c-e
SC8	364 a	2.7 bc	31.00 a	12.24 d-f	6.67 b-f	7.79 a	77.87 a
SC11	228 e-i	3.4 a	23.33 c-e	10.24 e-k	8.00 a-c	7.18 a-c	71.80 a-c
SC12	372 a	3.3 a	26.33 b	17.57 a	8.33 ab	6.39 ab	63.87 c-e
SC13	249 d-g	2.6 b-e	18.00 k-m	10.24 e-k	9.00 a	7.32 a-c	73.20 a-c
SC14	263 c-f	2.5 b-g	22.00 d-h	12.91 c-e	5.00 f	3.43 j	34.33 j
SC23	231 e-i	2.6 b-f	21.00 e-j	11.91 d-g	6.00 d-f	4.42 g-j	44.20 g-j
SC24	278 c-e	2.4 c-h	20.33 f-l	15.24 a-c	7.67 a-d	4.79 f-i	47.87 f-i
SC27	155 l-o	3.2 a	19.33 h-m	9.91 f-k	7.67 a-d	6.42 c-e	64.20 c-e
SC30	249 d-g	2.7 b-d	22.67 c-g	10.44 e-j	9.00 a	7.55 c-e	75.53 ab
SC35	154 l-o	2.7 b-d	12.00 p	9.71 f-k	8.00 a-c	4.75 f-i	47.53 f-i
SC38	249 d-g	2.4 c-h	22.00 d-h	12.24 d-f	6.00 d-f	4.42 g-j	44.20 g-j
SC41	263 c-f	2.3 e-h	22.00 d-h	12.91 c-e	6.67 b-f	5.42 d-g	54.20 d-g
SC42	100 r	2.4 c-h	12.67 op	8.91 h-l	8.00 a-c	4.79 f-i	47.87 f-i
SC48	102 qr	2.4 c-h	15.00 no	7.57 k-m	6.00 d-f	5.08 f-h	50.77 f-h
SC49	108 p-r	2.3 e-h	17.33 mn	6.91 lm	5.67 ef	4.41 g-j	44.11 g-j
SC50	151 m-q	2.3 d-h	21.67 d-i	7.57 k-m	6.67 b-f	5.39 e-g	53.87 e-g
SC52	163 l-o	2.3 d-h	18.67 j-m	9.57 f-l	6.00 d-f	3.79 ij	37.87 ij
SC54	159 l-o	2.3 d-h	25.00 bc	6.91 lm	7.00 b-e	4.39 g-j	43.87 g-j
SC57	243 d-g	2.5 b-g	22.67 c-g	11.57 d-h	7.00 b-e	5.45 d-g	54.53 d-g
SC58	126 o-r	2.3 e-h	20.00 g-m	6.91 lm	6.00 d-f	4.45 g-j	44.53 g-j
SC61	263 c-f	2.5 b-g	22.00 d-h	12.91 c-e	5.00 f	3.39 j	33.87 j
SC63	231 e-i	2.6 b-f	21.00 e-j	11.91 d-g	6.00 d-f	4.39 g-j	43.87 g-j
SC70	169 l-o	2.3 e-h	20.67 e-k	8.91 h-l	7.00 b-e	4.39 g-j	43.87 g-j
SC71	196 h-m	2.5 c-h	18.00 k-m	11.91 d-g	5.00 f	4.39 g-j	43.87 g-j
SC72	233 d-g	2.6 b-f	23.00 c-f	10.91 e-i	6.00 d-f	4.79 f-i	47.87 f-i
SC73	139 n-r	2.3 d-h	22.00 d-h	6.91 lm	7.00 b-e	6.39 c-e	63.87 c-e
SC75	203 g-l	2.6 b-f	23.00 c-f	9.57 f-l	7.67 a-d	5.75 d-f	57.53 d-f
SC78	102 qr	2.3 e-h	19.00 i-m	5.91 m	6.00 d-f	3.79 ij	37.87 ij
SC80	249 d-g	2.4 c-h	22.00 d-h	12.24 d-f	6.00 d-f	4.45 g-j	44.53 g-j
SC81	102 qr	2.3 e-h	19.00 i-m	5.91 m	6.00 d-f	3.79ij	37.87 ij
SC83	163 l-o	2.2 gh	20.00 g-m	8.91 h-l	6.67 b-f	3.79 ij	37.87 ij
SC84	154 l-o	2.3 e-h	21.33 d-j	7.91 j-m	7.00 b-e	3.45 j	34.53 j
SC85	172 k-o	2.3 e-h	19.00 i-m	9.91 f-k	8.00 a-c	5.39 e-g	53.87 e-g
SC88	179 j-n	2.3 d-h	18.00 k-m	10.91 e-i	6.00 d-f	4.62 g-i	46.20 g-i
SC90	126 o-r	2.3 e-h	17.67 l-n	7.91 j-m	6.00 d-f	5.45 d-g	54.53 d-g
SC91	178 j-n	2.2 e-h	21.67 d-i	8.91 h-l	8.00 a-c	4.79 f-i	47.87 f-i
SC92	263 c-f	2.3 d-h	22.00 d-h	12.91 c-e	7.00 b-e	5.79 d-f	57.87 d-f
SC95	233 d-g	2.4 c-h	23.00 c-f	10.91 e-i	7.00 b-e	4.79 f-i	47.87 f-i
SC96	166 l-o	2.4 c-h	19.00 i-m	9.57 f-l	6.00 d-f	4.79 f-i	47.87 f-i
SC98	201 g-m	2.2 gh	20.00 g-m	10.91 e-i	8.00 a-c	5.39 e-g	53.87 e-g
SC100	243 d-g	2.6 b-f	22.00 d-h	11.91 d-g	7.00 b-e	5.45 d-g	54.53 d-g
SC102	221 f-k	2.3 e-h	24.00 b-e	9.91 f-k	6.67 b-f	4.45 g-j	44.53 g-j
SC105	181 i-n	2.3 e-h	22.00 d-h	8.91h-l	7.00 b-e	5.79 d-f	57.87 d-f
SC112	165 l-o	2.2 f-h	21.00 e-j	8.57 i-m	6.00 d-f	3.79 ij	37.87 ij
SC116	152 m-q	2.2 gh	18.67 j-m	8.91 h-l	6.33 c-f	4.05 h-j	40.53 h-j
SC121	200 g-m	2.3 e-h	23.33 c-e	9.24g-l	7.00 b-e	4.79 f-i	47.87 f-i
Tuckey's HSD value	50.39	0.35	2.89	2.76	1.85	1.09	10.93
S.E.	224.2	0.01126	0.7397	0.6758	0.30517	0.10544	10.544

Table 4. Assessment of qualitative traits of sugarcane somaclones and the parent under Tando Jam agroclimatic conditions.

Somaclones	CCS %	Brix %	Fiber %	Sucrose %	Purity %	Sugar recovery	Sugar yield
NIA-1198	13.11 b-i	21.95 e-j	16.62 a-e	17.80 e-j	81.02 c-k	11.38 b-h	7.86 c-f
SC2	14.83 ab	25.32 ab	16.03 a-g	21.17 ab	83.60 ab	13.94 ab	7.11 d-i
SC6	12.75 c-j	22.35 d -i	14.54 c-i	18.20 d-i	81.43 a-i	11.99 c-i	7.42 d-h
SC7	12.18 e-l	22.02 e -j	16.49 a-e	17.87 e-j	81.15 c-j	11.45 d-k	7.86 c-e
SC8	8.59 r	17.45 o	16.89 a-d	13.30 o	75.98 o	8.07 q	6.71 e-k
SC11	12.09 e-l	22.32 d -i	18.52 ab	18.17 d-i	81.38 b-i	11.36 e-k	8.66 cd
SC12	13.33 b-i	22.85 c-h	13.45 d-k	18.70 c-h	81.84 a-h	13.64 ab	8.51 cd
SC13	14.49 ab	24.42 a -d	14.02 c-j	20.27 a-d	83.00 a-c	14.29 a	10.60 ab
SC14	13.47 b-h	22.45 d- i	10.61 jk	18.30 d-i	81.51 a-h	12.66 b-g	4.63 n-s
SC23	11.00 k-p	19.79 j -n	12.44 h-k	15.64 j-n	79.01 j-n	10.34 j-o	4.88 l-s
SC24	13.79 a-f	23.29 b- g	12.80 f-k	19.14 b-g	82.18 a-g	12.96 a-e	6.60 e-l
SC27	14.53 ab	24.72 a-c	15.09 b-h	20.57 a-c	83.20 a-c	13.66 ab	9.32 bc
SC30	15.34 a	25.59 a	14.45 c-i	21.44 a	83.78 a-f	14.42 a	11.58 a
SC35	10.56 l-q	19.89 j -n	16.25 a-f	15.74 j-n	79.13 i-n	9.93 k-p	5.02 k-s
SC38	11.85 g-m	20.95 h-l	13.11 e-k	16.80 h-l	80.19 e-l	11.14 g-l	5.24 j-s
SC41	12.11 e-l	20.79 h- m	10.44 k	16.64 h-m	80.02 f-m	11.38 d-k	6.57 e-l
SC42	12.73 d-j	21.79 f-j	11.79 i-k	17.64 f-j	80.94 c-k	11.97 c-i	6.08g-p
SC48	13.74 a-f	22.79 c- h	10.61 jk	18.64 c-h	81.78 a-h	12.92 a-f	6.98 d-j
SC49	14.48 a-c	24.12 a- e	12.69 f-k	19.97 a-e	82.79 a-d	13.61 ab	6.38 e-n
SC50	12.45 d-k	21.79 f-j	13.59 d-k	17.64 f-j	80.94 c-k	11.70 c-j	6.70 e-k
SC52	11.83 g-m	21.79 f-j	17.55 a-c	17.64 f-j	80.94 c-k	11.12 g-l	4.49 p-s
SC54	8.72 r	17.95 no	19.13 a	13.80 no	76.88 no	8.20 q	3.83 rs
SC57	9.43 p-r	18.62 m -o	17.36 a-c	14.47 m-o	77.70 m-o	8.87 o-q	5.15 k-s
SC58	8.97 qr	17.29 o	12.41 h-k	13.14 o	75.99 o	8.43 pq	3.98 q-s
SC61	13.47 b-h	22.45 d-i	10.61 jk	18.30 d-i	81.51 a-h	12.66 b-g	4.56 o-s
SC63	11.00 k-p	19.79 j-n	12.44 h-k	15.64 j-n	79.01 j-n	10.34 j-o	4.84 l-s
SC70	11.65 i-n	20.79 h -m	13.53 d-k	16.64 h-m	80.02 f-m	10.95 h-m	5.12 k-s
SC71	10.14 m-r	18.79 l -o	12.69 f-k	14.64 l-o	77.90 l-o	9.53 l-q	4.44 p-s
SC72	11.75 h-n	20.79 h-m	12.89 f-k	16.64 h-m	80.02 f-m	11.05 h-m	5.61 i-q
SC73	9.80 o-r	18.79 l-o	15.47 b-h	14.64 l-o	77.90 l-o	9.21 n-q	6.27 e-o
SC75	13.49 b-g	23.52 a-g	15.65 a-h	19.37 a-g	82.35 a-f	12.68 b-g	7.76 c-g
SC78	13.26 b-i	22.79 c- h	13.53 d-k	18.64 c-h	81.78 a-h	12.47 b-h	6.27 e-n
SC80	11.85 g-m	20.95 h- l	13.11 e-k	16.80 h-l	80.19 e-l	11.14 g-l	5.28 j-r
SC81	13.26 b-i	22.79 c -h	13.53 d-k	18.64 c-h	81.78 a-h	12.47 b-h	5.02 k-s
SC83	10.56 l-q	20.29 i-m	18.47 ab	16.14 i-m	79.54 h-m	9.93 k-p	4.00 q-s
SC84	10.08 n-r	19.45 k-o	17.36 a-c	15.30 k-o	78.66 k-n	9.47 m-q	3.49 s
SC85	11.94 g-l	20.62 h-m	10.61 jk	16.47 h-m	79.86 g-m	11.23 g-k	6.42 e-m
SC88	12.06 f-l	21.79 f-j	16.09 a-g	17.64 f-j	80.94 c-k	11.34 f-k	5.57 i-r
SC90	14.10 a-d	23.43 a-g	11.66 i-k	19.28 a-g	82.28 a-f	13.26 a-c	7.69 c-g
SC91	10.98 k-p	20.29 i -m	15.50 b-h	16.14 i-m	79.54 h-m	10.32 j-o	5.26 j-r
SC92	11.25 j-o	20.29 i-m	13.53 d-k	16.14 i-m	79.54 h-m	10.58 i-n	6.52 e-m
SC95	12.21 e-l	21.35 g-k	12.89 f-k	17.20 g-k	80.56 d-k	11.47 d-k	5.84 h-p
SC96	12.16 e-l	21.29 g-k	12.88 f-k	17.14 g-k	80.40 e-k	11.43 d-k	5.84 h-p
SC98	13.34 b-i	23.35 a-g	15.75 a-h	19.20 a-g	82.23 a-f	12.54 b-h	7.19 d-i
SC100	10.68 l-q	20.45 i- m	18.47 ab	16.30 i-m	79.70 h-m	10.04 k-o	5.83 h-p
SC102	10.71 l-p	19.45 k - o	12.59 g-k	15.30 k-o	78.66 k-n	10.06 k-o	4.77 m-s
SC105	10.55 l-q	19.79 j - n	15.76 a-h	15.64 j-n	79.02 j-n	9.92 k-p	6.10 f-p
SC112	13.99 a-d	23.45 a - g	12.45 h- k	19.30 a-g	82.30 a - f	13.15 a-c	5.29 j-r
SC116	13.82 a-e	23.29 b - g	12.65 g-k	19.14 b-g	82.18 a-g	12.99 a-d	5.59 i-r
SC121	14.04 a-d	23.75 a - f	13.57 d-k	19.60 a-f	82.52 a-e	13.19 a-c	6.72 e-k
Tuckey's HSD value	1.73	2.23	3.56	2.24	2.36	1.61	1.77
S.E.	0.26426	0.4421	1.1239	0.4433	0.4932	0.25856	0.4285

The table presets the pooled data for several quantitative characteristics of somaclonal population against the parent recorded over the cropping seasons of two consecutive years viz., 2013-2014, and 2014-2015. Pooled data were subjected to statistical analysis to determine the significant differences. Different letters in the same column represent that the difference is significant at $p<0.05$ (Table 3).

The table presets the pooled data for several qualitative characteristics of somaclonal population against the parent recorded over the cropping seasons of two consecutive years viz., 2013-2014, and 2014-2015. Pooled data were subjected to statistical analysis to determine the significant differences. Different letters in the same column represent that the difference is significant at $p<0.05$ (Table 4).

Parent was seen to be superior to all the somaclones in terms of number of internodes. None of the derived plants had significantly higher count for this characteristic, however SC8 showed statistically equivalent potential for the trait. Mean number of internodes was found to be 31.33 for the parent, and 31.0 for the mentioned progeny. SC12 also showed good potential regarding number of internodes per stool (26 internodes). Minimum potential for internode production was seen in SC35 i.e., 12 internodes per plant. SC12 showed maximum length of the internodes which was observed to be 17.57 cm whereas the mother plant had internode length of 16.74 cm. Least observation for internodes length was recorded for SC81 which had mean internode length of 5.91 cm (Table 3).

Cane yield, one of the major parameter of concern for sugarcane varietal development program, was also increased significantly in one somaclone. SC8 showed 20.04% increase in the yield potential by producing 77.87 t/ha of cane vs. 64.87 t/ha of the donor plant. Two more somaclones, viz.SC30 (75.53 t/ha), and SC13 (73.20) also showed higher yields against the mother plant, however, the difference was not statistically significant. Lowest cane yield was produced by SC61 for which the trait was recorded to be 33.87 (t/ha).

Similar variations were observed in quality traits as well. The progeny plants differed from the parent in several qualitative characters. Although the mutations caused decrease in most of the quality traits, significant and encouraging observations were also recorded in many cases. Almost all the qualitative parameters surpassed the parent in one somaclone or the other, except fiber %. Commercial cane sugar (CCS%) was enhanced in many callus derived plants however these differences were at par with the parent, except SC30, which possessed highest CCS contents (15.34%), statistically significant against the parent (13.11). Positive changes in somaclones were observed considering brix, sucrose, and purity as well. SC30 presented the maximum brix % values of 25.59 when compared to the donor's 21.95% brix. Various other somaclones also had significantly higher, or closer brix % when compared with NIA-1198.

Sucrose % of the somaclones demonstrated same trends when compared to the check. Sucrose was statistically increased in four somaclones. SC30 (21.44), SC2 (21.17), and SC27 (20.57) were observed to be the superior clones in terms of sucrose as they produced excellent sucrose contents than the check (17.80). SC58 showed least sucrose of 13.14%. Moreover, purity was augmented in several clones whereas SC30 showed the highest purity. However, we noticed statistically similar fiber production capacity in many of the somaclones, but none of the progeny plants could statistically surpass the parent in fiber potentialin spite of the fact that the reads of the parameter were higher in twenty clones, when compared with the parent (non-significant differences).

Sugar recovery, and sugar yield, are the two most important qualitative characteristics of cane crop. Both of these traits are the major contributors towards the possible maximum sugar production from the crop. Both of these characters were enhanced in many of the clones in somaclonal population. SC30, SC13, and SC2 presented maximum sugar recovery of 14.42, 14.29, 13.94% respectively, while the parent's sugar recovery was observed to be 11.38%. Thus, as much as 3.04% improvement was observed in sugar recovery of SC30 which can perform as a promising clone in the future trials. Furthermore, SC30 was observed to produce maximum sugar yield of 11.58 t/ha followed by SC13 with a sugar yield of 10.60. SC27 also recorded excellent sugar yield of 9.32 t/ha against the parent's 7.86 t/ha. SC84 was observed to have the lowest sugar yield of 3.49 t/ha (Table 4).

Pearson's correlation analysis: Correlation analysis of the data showed that most of the quantitative parameters correlated strongly with other quantitative parameters, whereas highly positive correlation was observed for qualitative traits with other qualitative parameters, however, association of the quantitative parameters with qualitative parameters was mostly low, or negative (Table 5).

Cane yield showed highly significant correlation with stool weight, tillers per plant, and cane girth. Correlation values of these parameters with the yield were 1.0, 0.607, and 0.536 respectively. Cane yield was also observed to have positive correlation (non-significant) with cane height and number of internodes. It was also seen that cane yield did not have significant correlation with any of the qualitative traits. Sugar yield, on the other hand, was observed to have highly positive correlation with many of the quantitative and qualitative characters. It possessed strong positive correlation with every qualitative trait we analyzed in the study except fiber content. It was significantly correlated to cane yield (0.814), CCS % (0.604), sugar recovery (0.596), sucrose % (0.594), brix % (0.594), purity (0.569), tillers per plant (0.529), cane girth (0.487), and internodes length (0.175). Plant height was noticed to be highly linked to girth (0.421), number of internodes (0.703), and internodes length (0.841). All of these associations were significant at $p<0.01$. Number of internodes were significantly correlated with length of internodes (0.413), whereas negative correlation of cane internodes was observed with CCS %, brix %, sucrose %, purity, and sugar recovery. Internodes length also showed negative correlation with fiber % (-0.068).

Table 5. Pearson's correlation coefficients among various parameters in sugarcane somaclones.

	Height	Girth	Tillers per plant	Number of internodes	Internodes length	Stool weight	Cane yield	CCS %	Brix %	Sucrose %	Fiber %	Purity	Sugar recovery
Girth	0.421**												
Tillers per plant	0.058	0.243											
Number of internodes	0.703**	0.237	0.0154										
Internodes length	0.841**	0.257	0.0809	0.413**									
Stool weight	0.248	0.536**	0.607**	0.234	0.146								
Cane yield	0.248	0.536**	0.607**	0.234	0.146	1.0**							
CCS %	0.017	0.091	0.062	-0.104	0.156	0.057	0.057						
Brix %	0.108	0.201	0.165	-0.024	0.177	0.088	0.088	0.946**					
Sucrose %	0.108	0.201	0.165	-0.024	0.177	0.088	0.088	0.946**	1.0**				
Fiber %	0.067	0.194	0.365*	0.257	-0.068	0.246	0.246	-0.403**	-0.203	-0.203			
Purity	0.111	0.177	0.133	-0.045	0.189	0.050	0.050	0.951**	0.993**	0.993**	-0.224		
Sugar recovery	0.013	0.086	0.073	-0.118	0.155	0.053	0.053	0.996**	0.955**	0.955**	-0.402**	0.959**	
Sugar yield	0.182	0.487**	0.529**	0.119	0.175*	0.814**	0.814**	0.604**	0.594**	0.594**	-0.012	0.569**	0.596**

**Correlation is significant at $p<0.01$ level
*Correlation is significant at $p<0.05$ level

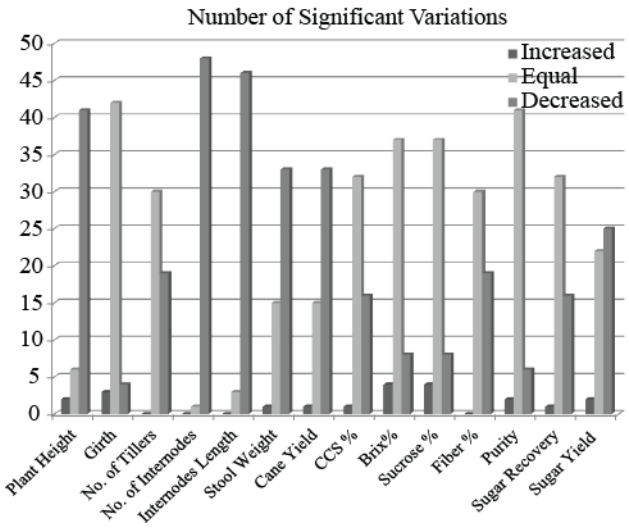

Fig. 1. The figure denotes the number of significant variations in quantitative and qualitative characteristics of somaclonal population against the parent recorded over the cropping seasons of two consecutive years viz., 2013-2014, and 2014-2015. Pooled data were subjected to statistical analysis to determine the significant differences.

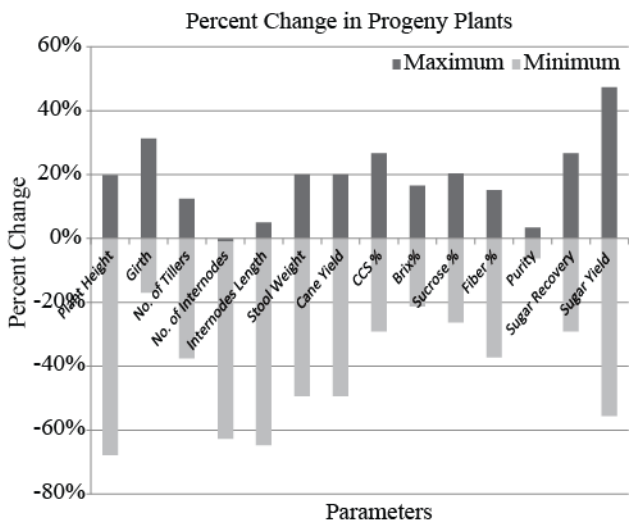

Fig. 2. The figure denotes the maximum percent change of quantitative and qualitative characteristics of somaclonal population against the parent.

Number of significant variations ($p<0.05$) observed for different quantitative and qualitative traits of somaclones as compared to the parent (control). Maximum percent change of characteristics in somaclones against the parent (Figs. 1 and 2). Most of the quality parameters, demonstrated highly significant correlation among each other. CSS % depicted highly positive correlation with brix % (0.946), sucrose % (0.946), purity % (0.951), sugar recovery (0.996), and sugar yield (0.604). All of these associations were significant at significance level of 0.01. Brix %, sucrose %, and purity also showed similar correlation with all the qualitative parameters except fiber contents. Furthermore, correlation of another major parameter of the cane genotypes i.e. sugar recovery was also high and significant at significance of 0.01 with other qualitative characters concerned. Sugar recovery is one of the most important parameters under consideration in any cane improvement program. It was seen to have significant positive correlation with CCS % (0.996), brix % (0.955), sucrose % (0.955), purity (0.959) and sugar yield (0.596) Sugar recovery showed negative correlation with fiber %, which was further correlated negatively with every other quality trait.

Hierarchical cluster analysis: Cluster analysis of the sugarcane somaclones along with the parent resulted in five major clusters (Fig. 3). Interestingly, only four somaclones grouped with the parent in same cluster. It depicted that somaclonal mutations caused huge variability in the progeny plants which thus appeared in other clusters majorly distant from the parent. Five major clusters were identified, each of which further divided into two sub-clusters at least. Every cluster represented certain distinguishing unique features of the genotypes they comprised of (Table 6).

Cluster 1 comprised of five genotypes. This cluster in fact showed most promising characters in terms of quantitative traits. Parent, along with four somaclones, emerging in this group included SC8, SC7, SC11, and SC12. It was observed to have accessions with distinguishing features of high quantitative traits. The mean values of the parameters under study showed that the cluster had high cane height, girth, internodes length, number of internode, and cane yield along with good sugar yield. Qualitative parameters of the group were seen to be low against other clusters. Moreover, somaclones having excellent qualitative traits were seen to group into cluster 2. The group embraced SC2, SC98, SC6, SC121, SC24, SC13, SC27, and SC30. The group was distinguished by high qualitative characters along with average quantitative traits. The clones possessed excellent, and highest CCS %, sucrose %, brix %, and sugar recovery, purity and sugar yield values.

Cluster 3 embraced highest number of somaclones, and could be subdivided into various sub-clusters. Total number of accessions in this cluster was 21 (Table 6). This group had low qualitative and quantitative parameters. Cluster 4, on the other hand, represented the somaclones having least quantitative characters along with good CCS and sugar recovery, and average brix %, sucrose and purity values. Whereas cluster 5, represented the group having highest fiber % in combination with least CCS %, brix %, and sucrose %.

Table 7 represents the D values among different clusters. Distance among the clusters can help in determining the possible promising crosses for targeting the improvement of concerned parameters, or for the purpose of obtaining the desired genetic diversity in the progeny plants. It was observed that cluster 5 and cluster 3 had least distance. Cluster 3 and cluster 4 were also recognized to be quite close. Whereas most distant clusters were cluster 4 and cluster 1. Cluster 1 (the cluster comprising of the parent genotype) also had huge cluster distance from the cluster 5 (Table 7).

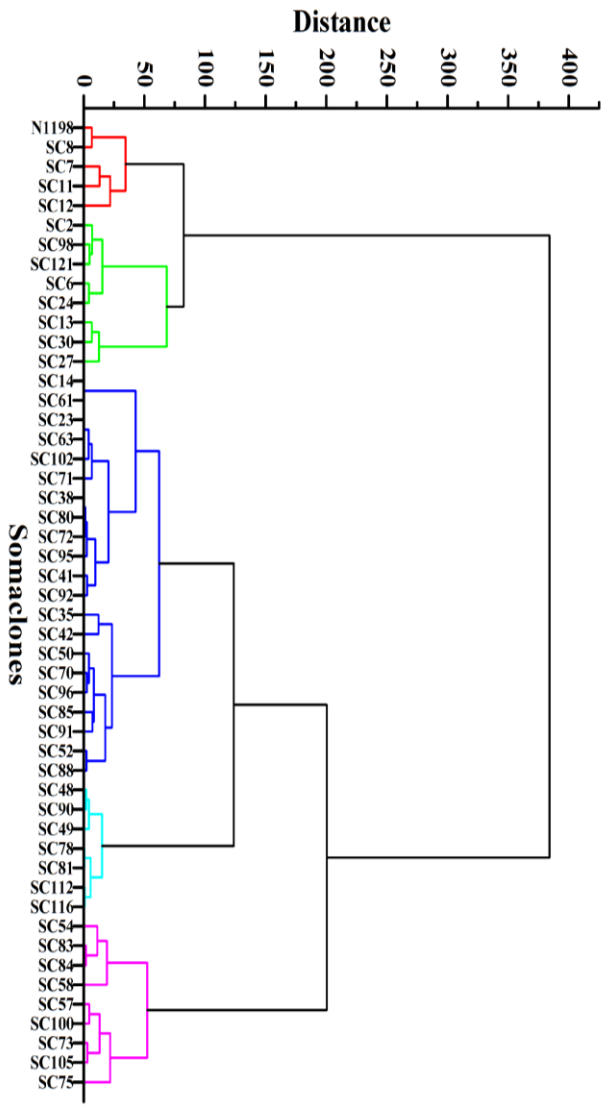

Fig. 3. Hierarchical cluster analysis of somaclones and the parent developed through Ward's linkage method.

Principal component analysis: Principle component analysis of the data reduced the variables under study to five principle components (PCs) contributing towards major variance of the data. Eigen vectors of the matrix depicted that these five PCs contributed for 93.18% variability among genotypes for traits under study and therefore were given importance for further exploration. Variability accounted by every principal component was represented by scree plot. It was observed that the PC1 showed 41.04% variance with eigen value of 5.74, followed by principle component 2 which depicted the variability of 26.50%, and an eigen value of 3.71. The variability and eigen values decreased gradually towards PC 5 with an accumulative variability of these PCs up to overall 93%. Elbow of the scree plot was observed at 5th PC and it was seen tending to be straight after 5th PC, representing that the contribution of the following PCs will not be very effective (Fig. 4).

The most contributing traits in first component viz. PC1 were: brix, CCS, sucrose, purity, sugar recovery and sugar yield i.e. qualitative traits of the sugarcane. Thus, PC1 was seen to be mostly related to quality traits while PC2 to cane yield, and stool weight. Other quantitative traits viz. plant height, girth, tillers, and number of internodes, also had some contribution towards this PC. In third component (PC3), number of internodes was an effective trait however, cane height, and internode length had greatest influence. Moreover, for PC4, it was seen that fiber % had the maximum contribution towards variability of the PC. Whereas, PC5 was mostly related to tillers of plants (Table 8).

The character loading was employed for determining the somaclonal component scores for score plot as given in Fig. 5. Score plot presents a visual representation of the closeness of somaclones over the subspace based on the similarity determined through all the variables under study. Somaclones SC23, 63, 91, 35, 102; SC96, 88; SC80, 38; and SC14, 61 were very close to each other. Whereas SC75, and SC49; SC11 and 84; and SC54 and SC2 were observed to be positioned at opposite axis to each other. The score plot made it evident that the somaclonal population was very diverse in the parameters analyzed. Parameters of the somaclones were projected on the loading plot on PC1 and PC2 subspace. It was observed that cane yield, sugar yield, brix, purity, CCS, sucrose and recovery had long vectors, while minimum length of the vector related to internode length. Fiber % and qualitative characters were at opposite axis in the plot's subspace (Fig. 6).

Variance components, heritability, and genetic advance: Variance components, genetic advances at different intensities (5, 10, 15, 20, and 25%) and broad sense heritability are presented in Tables 9, and 10. One of the major observations from the data were the comparison of genotypic and environmental variances. It was seen that the environmental variance was very low as compared to the genotypic variance. Highest values of genotypic variance were found for height and cane yield among the quantitative traits; and for purity among the qualitative characters. Lowest values were observed for girth and recovery among mentioned groups respectively. Genetic gain of the crop was analyzed through genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV). Cane height showed highest genotypic and phenotypic coefficients of variation (56.56, and 57.04 respectively), followed by internodes length (GCV: 42.78, PCV: 43.51) among the quantitative traits. Similarly, for quality characters, sugar yield (GCV: 46.35, PCV: 47.12) had the highest values followed by fiber contents (GCV: 28.28, PCV: 29.25). Brix % showed the least values for these two parameters (GCV: 15.95, PCV: 16.24). Furthermore, heritability values of the population under study were also determined. Heritability values were found to be very high for all the characters under study. The range of the heritability % was from 93% to 98%. Highest heritability was found for height and number of internodes (98%), while minimum was estimated for fiber % (93%). Cane yield, sugar recovery, and sugar yield, all showed heritability of 97%.

Genetic advance (G.A), is also an important parameter which can be applied along with heritability and variance estimates to assess the expected genetic gain. This helps in predicting better genotypes for selection. Genetic advance was analyzed at five different

selection intensities viz., 5%, 10%, 15%, 20%, and 25%. It was seen that the genetic advance values declined with every increment in selection intensity. It was also observed that the genetic advance of any trait at selection intensity of 5% was approximately 70% higher than its G.A at 25% intensity. Maximum genetic advance was estimated for height (115.69) followed by sugar yield (94.07), at 5 % level of intensity. Genetic advance of the same parameters at 25% level of intensity were observed to decrease to 71.27, and 57.95 respectively. Internodes length and cane yield were the other characters to demonstrate high G.A of 86.78 and 75.53 respectively. Conversely, least genetic advances at all levels of intensity were shown by purity % values.

Table 6. Classification of somaclonal genotypes into clusters based on Euclidean distance.

Cluster	Genotypes	Characters
Cluster 1	NIA-1198 (Parent), SC7, SC8, SC11, SC12	Excellent Quantitative Characters- Cane height, girth, Internodes, cane yield
Cluster 2	SC2, SC98, SC6, SC121, SC24, SC13, SC27, SC30	Excellent Qualitative Characters- CCS, Brix, Sucrose, Purity, Sugar recovery, Sugar yield, and Tillers
Cluster 3	SC14, SC61, SC23, SC63, SC102, SC71, SC38, SC72, SC80, SC95, SC41, SC92, SC35, SC42, SC50, SC70, C96, SC85, SC91, SC52, SC88	Low qualitative and quantitative characters, can be exploited for obtaining genetic diversity in crosses
Cluster 4	SC90, SC48, SC49, SC78, SC112, SC116, SC81	Good sugar recovery, CCS, Purity
Cluster 5	SC54, SC57, SC58, SC83, SC84, SC100, SC73, SC75, SC105	Highest fiber %

Table 7. Cluster distances (D values) among the dendrogram's clusters based on Euclidean distance.

Cluster	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cluster 1	0	13.39735	28.25945	43.02234	41.77101
Cluster 2	13.39735	0	18.33589	16.00075	40.3874
Cluster 3	28.25945	18.33589	0	11.79163	10.78433
Cluster 4	43.02234	16.00075	11.79163	0	35.32472
Cluster 5	41.77101	40.3874	10.78433	35.32472	0

Table 8. Principal components of 50 sugarcane genotypes for 14 characters.

Variable	PC1	PC2	PC3	PC4	PC5
Plant height	0.12075	0.28135	0.53474	-0.05304	0.01981
Girth	0.17715	0.27678	0.03641	-0.05598	-0.80048
Tillers per plant	0.14838	0.27873	-0.29926	0.27431	0.48227
Number of internodes	0.0599	0.29448	0.41238	0.17291	0.0732
Internode length	0.14288	0.19972	0.50815	-0.10676	0.29527
Stool weight	0.17925	0.40563	-0.25739	-0.23216	0.03835
Cane yield	0.17925	0.40563	-0.25739	-0.23216	0.03835
CCS%	0.37444	-0.21258	0.0142	-0.03551	0.06158
Brix%	0.38503	-0.17001	0.02326	0.18098	-0.04988
Sucrose %	0.38503	-0.17001	0.02326	0.18098	-0.04988
Fiber %	-0.06385	0.28675	-0.1016	0.79768	-0.13071
Purity %	0.38036	-0.18561	0.04182	0.17334	-0.03909
Sugar recovery	0.3717	-0.22854	-0.00135	-0.03218	0.04651
Sugar yield	0.34662	0.19739	-0.22146	-0.18858	0.03499

Table 9. Estimation of genotypic variance, phenotypic variance, heritability (h^2%) and genetic advance (G.A%) for quantitative traits of somaclones.

Parameters	Height	Girth	Tillers per plant	Number of internodes	Internodes length	Stool weight	Cane yield
Genotypic coefficient of variation (GCV)	56.56	18.70	24.08	28.46	42.78	37.15	37.15
Phenotypic coefficient of variation (PCV)	57.04	19.20	25.41	28.75	43.51	37.70	37.70
Genotypic variance (σ^2_g)	13009	0.21	2.68	36.04	19.71	3.54	353.99
Phenotypic variance (σ^2_p)	13234	0.22	2.99	36.78	20.38	3.65	364.54
Environmental variance (σ^2_e)	224.20	0.01	0.31	0.74	0.68	0.11	10.54
Heritability (H)%	98%	95%	90%	98%	97%	97%	97%
Genetic advance (G.A) at 5%	115.69	37.57	47.06	58.12	86.78	75.53	75.53
Genetic advance (G.A) at 10%	98.41	31.96	40.04	49.45	73.82	64.25	64.25
Genetic advance (G.A) at 15%	87.14	28.30	35.45	43.78	65.37	56.89	56.89
Genetic advance (G.A) at 20%	78.51	25.50	31.94	39.44	58.89	51.25	51.25
Genetic advance (G.A) at 25%	71.27	23.15	28.99	35.81	53.46	46.53	46.53

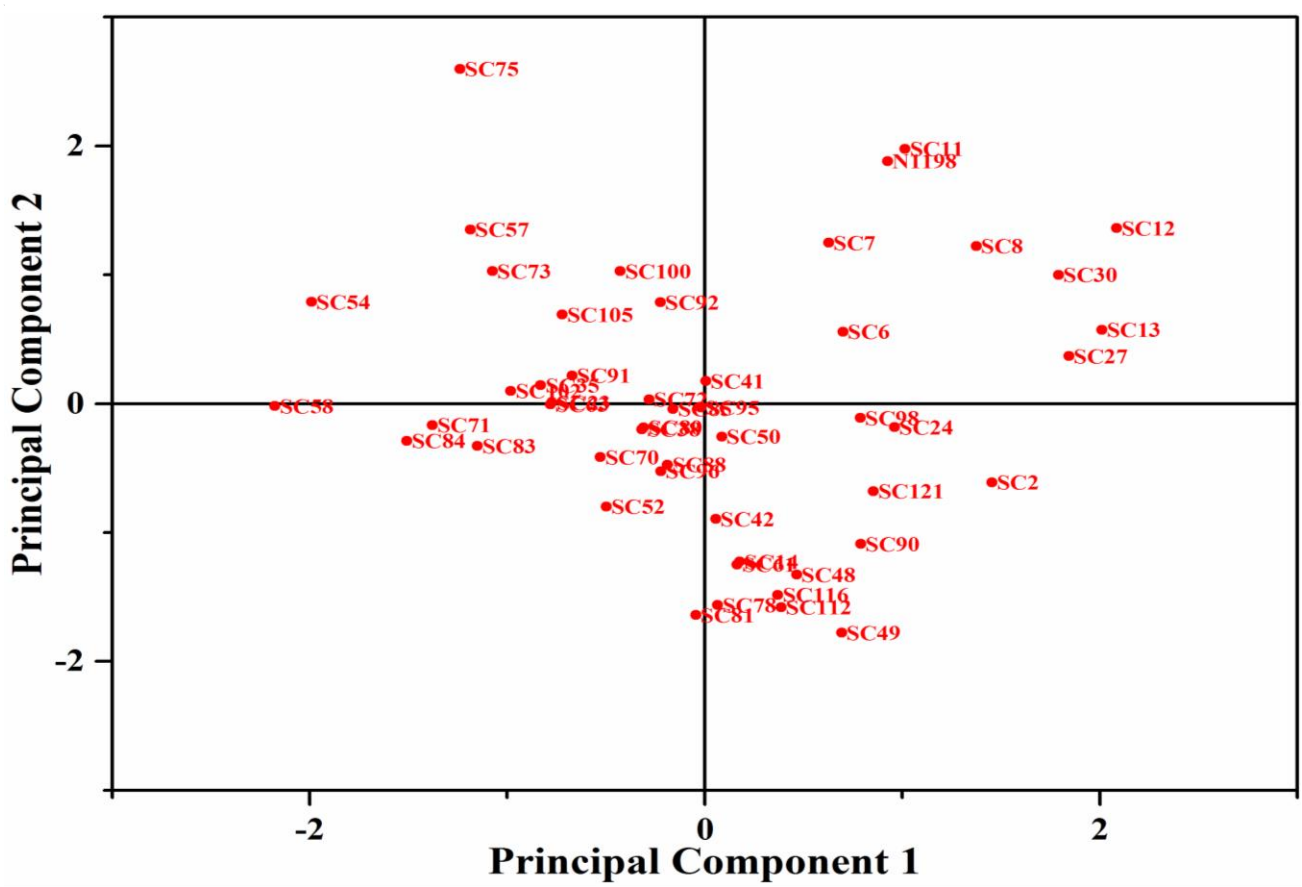

Fig. 5. Scores plot analysis using principal component axis I and II for 50 genotypes of sugarcane.

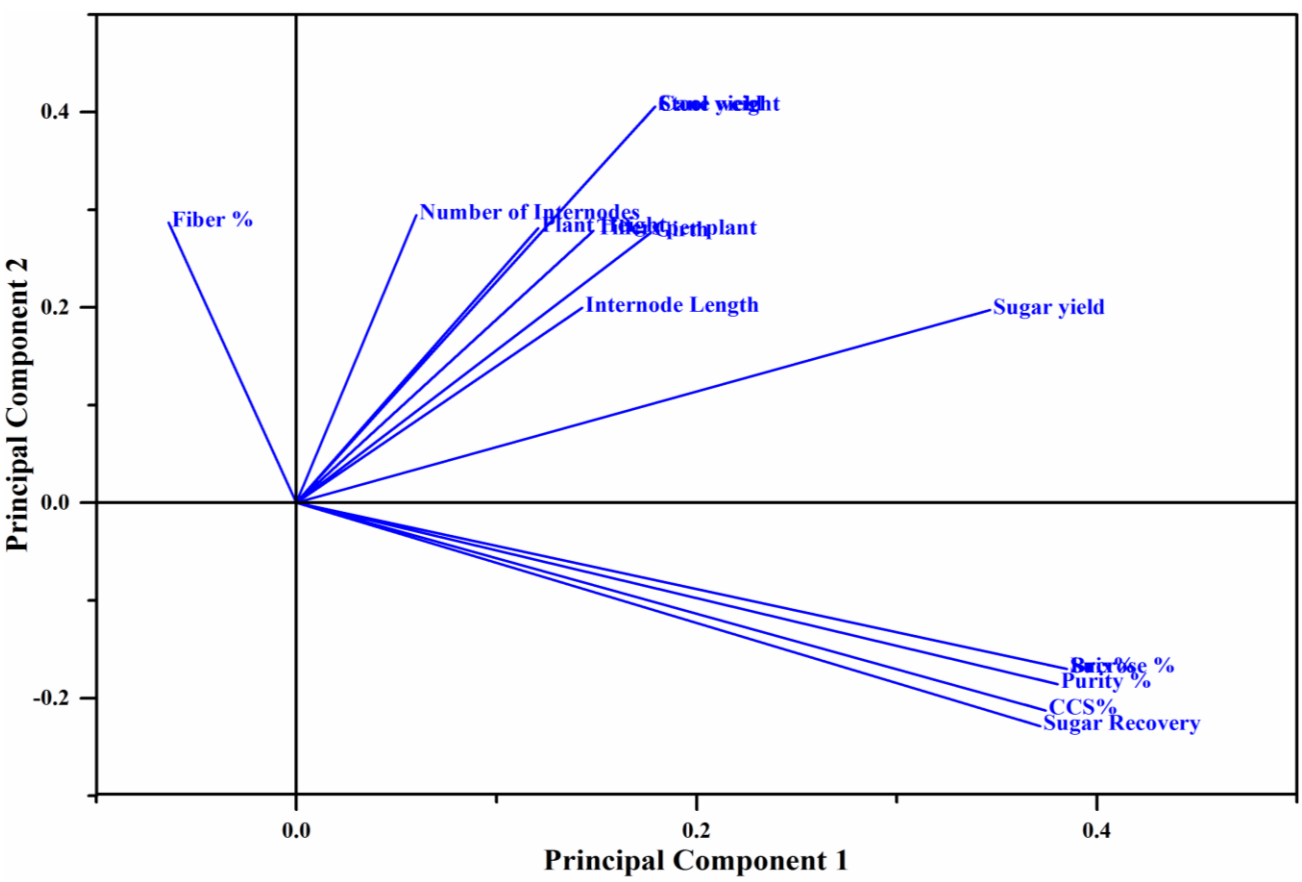

Fig. 6. Loading plot of 50 genotypes of sugarcane on Principal Component axis I and II.

Table 10. Estimation of genotypic variance, phenotypic variance, heritability (h^2%) and genetic advance (G.A%) for qualitative traits of somaclones

Parameters	CCS	Brix	Sucrose	Fiber	Purity	Sugar recovery	Sugar yield
Genotypic coefficient of variation (GCV)	23.90	15.95	19.75	28.28	3.99	23.80	46.35
Phenotypic coefficient of variation (PCV)	24.27	16.24	20.12	29.25	4.09	24.21	47.12
Genotypic variance (σ^2g)	8.46	11.78	11.77	16.15	10.33	7.38	8.23
Phenotypic variance (σ^2p)	8.72	12.22	12.21	17.28	10.83	7.64	8.51
Environmental variance (σ^2e)	0.264	0.442	0.443	1.124	0.493	0.259	0.275
Heritability (H)%	97%	96%	96%	93%	95%	97%	97%
Genetic advance (G.A) at 5%	48.55	32.30	40.00	56.41	8.04	48.25	94.07
Genetic advance (G.A) at 10%	41.30	27.47	34.03	47.99	6.84	41.05	80.02
Genetic advance (G.A) at 15%	36.57	24.33	30.13	42.49	6.06	36.35	70.86
Genetic advance (G.A) at 20%	32.94	21.92	27.14	38.28	5.46	32.75	63.84
Genetic advance (G.A) at 25%	29.91	19.90	24.64	34.75	4.96	29.73	57.95

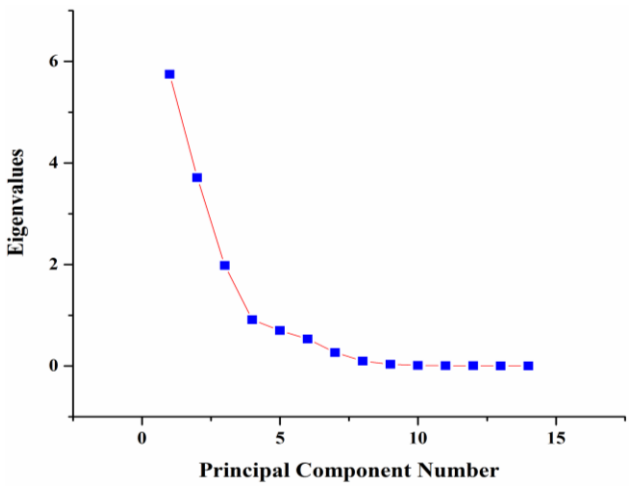

Fig. 4. Scree plot analysis between eigen values and the number of principal components using principal component analysis.

Discussion

Somaclonal variation is an excellent tool for varietal development programs of vegetatively propagated crops. It has been established that somaclones from the tissue culture technology are not replicas of the parent and they develop mutations while passing through callus stage because of genetic as well as epigenetic factors (Siddiqui *et al.*, 1994; Dalvi *et al.*, 2012). Somaclonal variations can lead to changes in various qualitative and quantitative parameters. The phenomenon is random and some of the somaclones can improve in certain characteristics while others could accumulate unwanted changes (Silvarolla & de Aguiar-Perecin, 1994; Raza *et al.*, 2014). This can be attributed as the major reason we obtained a diversity of somaclones with distinct characteristics in our study.

Significant variations were observed among the parent and the somaclonal population. The differences were observed to maintain among the progeny plants over two years of the study. Cane height in many of the tissue culture derived plants was different from NIA-1198 (parent). Few of the progeny plants were at par with the parent regarding height and most of them possessed significantly smaller cane height values. However, two somaclones were observed to be significantly taller than the parent. The observed changes in cane height were in agreement to the work of Sood *et al.* (2006) who reported a tissue culture derived variety with better cane height and sugar recovery than the parent. Furthermore, cane girth was also improved in some of the somaclonal progeny plants. Hoy *et al.* (2003), also published the production of sugarcane clones with higher girth through tissue culture technology. Moreover, tillers, stool weight and cane yield were increased significantly in some of the somaclones though the characters declined in most of the progeny genotypes. Our observations are in parallel to the study of Sood *et al.* (2006). Similar observations about the phenomenon has also been reported by other researchers (Doule, 2006).

Similarly, somaclones exhibited wide range of variations in qualitative parameters as well. Generally, higher number of positive variations were observed for quality characteristics against the quantitative traits. CCS, brix, purity, sugar recovery, and sucrose % were all increased in some of the somaclones significantly. Siddiqui *et al.* (1994), also reported rise in qualitative characters in most of the progeny plants after callus culture. Moreover, variability in qualitative traits was also reported by Khan *et al.* (2004). The somaclonal population presented increased cane as well as sugar yield, thus producing highly promising clones which had higher sugar recovery, cane yield and sugar yield. SC30 recorded excellent sugar yield (11.58 t/ha),and cane yield (75.53 t/ha) along with highest sugar recovery of 14.42%. Although the highest cane yield (77.87 t/ha) was observed for SC8 however, its sugar yield was low which could have been a result of declined sugar recovery; because of the negative association of quantitative and qualitative parameters of the cane (6.71 t/ha). Up to 47% increase in sugar yield was observed in some of somaclones, whereas increase in cane yield was as high as 20%, which was very promising indeed. The somaclone showing good combination of cane and sugar yield can be employed in evaluation and selection for ultimate development of super clones harvesting good cane as well as sugar yield, which is the major aim of sugarcane improvement programs. Doule (2006) published a similar report and described the production of somaclones with higher sugar yield in their study. This also lied parallel with the observations of Khan *et al.* (2004), Sood *et al.* (2006), and Khan *et al.* (2015).

Most of the sugarcane traits are affected by various genotypic, phenotypic, and environmental factors. Thus, correlation studies are important to develop improved sugarcane clones with desired characteristics (Chaudhary

& Joshi, 2005). We observed that many of the sugarcane traits were highly associated with each other genotypically when analyzed through Person's correlation analysis. The major confirmation we obtained from this analysis was a clear representation of highly positive and significant correlation of quantitative traits with other quantitative traits; and qualitative characters with the other qualitative traits. However, we observed very low, or negative correlation among the quantitative and qualitative traits. A low, and negative correlation of these two classes of parameters is a major hurdle in cane improvement for sugarcane breeders. Cane yield was observed to be highly correlated with girth and tillers of the genotypes, whereas sugar yield was significantly determined by CCS, sugar recovery, and cane yield. Our observation regarding correlation matrix are in agreement with Raza *et al.* (2014), and Ahmed *et al.* (2010). Skinner (1971) also mentioned that cane girth and tillering capacity was a major contributing factor towards the cane yield. High values of qualitative traits correlation among each other was also supported by the reports of Khan *et al.* (2009) and Ahmed *et al.* (2010). It is evident from the data that correlation studies are extremely important in varietal development programs and such associations can help the breeders in selection of parameters.

Cluster analysis of the pool data through ward's linkage analysis made it evident that somaclonal variants exhibited huge variability against the parent. Presence of just four progeny genotypes along with the parent in first cluster showed that remaining population viz. 45 clones were highly distant from the parent. Cluster analysis can be used to predict promising crosses for achieving desired combinations of characteristics in the progeny plants, and for getting the genetic diversity in the descendants. Yadav and Singh (2010) reported similar results when they had subjected the crop data to cluster analysis; same has been reported by Vivekananda and Subramanian too earlier in 1993. From the cluster data, we concluded that cluster 1 could be crossed with the populations of cluster 2 to achieve a good combination of high quality and quantity traits in progenitors, whereas, fiber % could be increased by crosses with cluster 5. Also, it would be interesting to cross cluster 4 with cluster 1 as these two groups were most distant from each other. Our results also lined parallel to the study of Tahir *et al*. (2013), and Shahzad *et al.* (2016). Furthermore, Luo *et al*. (2005), and You *et al.* (2013) have also reported similar findings for the sugarcane data. The formation of cluster groups is vital in progenitor choice in crop breeding, since the new hybrid populations must be selected based on the magnitude of their dissimilarities in order to increase the potential of the progenitors in future crosses.

The applications of multivariate statistical analysis like PCA has great potential to predict the relationships among various variables and this tool can find applications in understanding the nature of parameters under study (Al-Sayed *et al.*, 2012). We conducted a PCA analysis of normalized scores of the data and it was observed that the 14 characters under study were reduced to five primary PCs. PC1 embraced highest variance by the qualitative traits majorly. This PC was responsible for the highest variability in the data. Moreover, PC2 comprised of highest eigen values of cane yield and other quantitative characters related to the yield. Scores plot and loading plot of the principal components confirmed the variability, and the greatest variability contributors. Tahir *et al.* (2013) has reported similar findings classifying the characters under study in to certain PCs. Our results were also supported by the study of Shahzad *et al.* (2016). Moreover, our results agreed to Deepak *et al.* (2012) who reported the presence of quality traits in first two principal components. Furthermore, Al-Sayed *et al.* (2012) also found yield related characters within initial PCs just like our results. However, contrary to the observations of Shahzad *et al.* (2016) we obtained 93% variability within first five PCs whereas they reported 72% variation in first 7 PCs. Contrary to some of the other reports, we observed small angle among different vectors in the loading plot (Gulnaz *et al.*, 2012). Somaclones were observed to be placed on high distances on the score plot which depicted high variability. Such observations were in agreement to the reports of Gulnaz *et al.* (2012), Shahzad *et al.* (2016), and Tena *et al.* (2016).

Finally, genetic parameters were also determined in order to get an insight into the potential of the clones to transfer their characteristics to further generations. The estimated parameters included heritability, variance, and genetic advance. Our major findings of the analysis agreed to numerous earlier reports. High genotypic variance was computed as compared to the environmental variance which was parallel to the report of Tyagi & Singh (1998). High heritability of all the analysed parameters was also supported by Khan *et al.* (2009). Higher heritability values depicted that the selection based on the studied parameters could be promising, as if the heritability values are low, environmental effects can result in unwanted selections. Higher genetic advance (G.A.) values of height, and sugar yield suggested that it could be possible to select better cane varieties based on these parameters. Both of these parameters are directly related to, and determined by cane weight and sugar recovery. Thus, these traits could also help selecting the better genotypes. Similar results have also been reported by Chaudhary (2001) and Khan *et al.* (2009) previously.

Sugarcane improvement is tricky because of the fact any successful variety of cane crop needs to have acceptable combination of quantitative and qualitative traits. Genetic and climatic requirements of the cane hybridization in Pakistan, make it indispensable to explore other paths to get genetic diversity in order to obtain better cane genotypes. Somaclonal variations as a source of genetic diversity, give rise to progeny plants which are different from the donor (Roy *et al.*, 2010). Such variants, when screened for desired characteristics, can be a source of creating diversity, and obtaining the ideal cane cultivars (Siddiqui *et al.*, 1994). It was evident from our study that the somaclones were morphologically, agronomically, and qualitatively very distinct from the parent (Nickell & Maretzki, 1969; Krishnamurthi & Tlaskal, 1974). Our results elucidate that somaclonal variation can excellently serve the purpose of getting genetic diversity in the cane to develop elite high yielding, and early maturing varieties, which is the ultimate goal of sugarcane varietal development programs.

We achieved promising results in terms of quantitative as well as qualitative characters, and many of the somaclones showed excellently improved sugar yield (SC30), cane yield (SC8), and cane height (SC12). Subjecting these somaclones in further evaluation, and crosses between the promising genotypes, can ultimately lead to development of elite sugarcane cultivars.

Conclusions

It can be concluded from the study that the tissue culture technology can serve to obtain the genetic diversity in sugarcane. Somaclonal variations can ultimately lead to production of superior cultivars having better sugar and cane yield. Genetic dissection of the somaclones showed that the plants were highly diverse in nature. Somaclones possessing excellent qualitative and quantitative characters will be subjected to further evaluation for ultimately serving the agricultural sector, and sugar industry of the country.

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