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SCREENING OF SUGARCANE SOMACLONES OF VARIETY BL4 FOR AGRONOMIC CHARACTERISTICS

SABOOHI RAZA*[1,2], SYEDA QAMARUNNISA[1], ISHRAT JAMIL[1], BEENA NAQVI[3], ABID AZHAR[1] AND JAVED A. QURESHI[1]

[1]*The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Pakistan.*
[2]*Nuclear Institute of Agriculture (NIA), TandoJam, Pakistan.*
[3]*Pakistan Council of Scientific and Industrial Research (PCSIR), Karachi, Pakistan*
**Correspondence e-mail: razasaboohi@yahoo.com; Tel # 092213013559827*

Abstract

Considering the environmental conditions of Pakistan where sugarcane breeding is constrained due to non viable fuzz (seeds) production. Somaclonal variation could prove to be a useful tool to overcome the difficulties in cane breeding. In the present study, 324 sugarcane somaclones regenerated from immature leaf roll callus of sugarcane variety BL4 were evaluated for their yield and yield contributing characters and the quality traits of cane. The field trial of somaclones showed variation in 160 somaclones from the mother plant in at least one character observed. Most of the somaclones showed variation in weight of stalks per plant; however, only twenty four out of 89 clones showed increase in the weight of the stalks per clump. The second highly variable trait was the number of stalks, 88 plants showed either increase or decrease in the number of stalks. It is noteworthy that the sucrose accumulation was not increased in any of the somaclones. Twenty one somaclones were selected for their increased yield potential. The comparative performance of these selected clones revealed that clones 'K-250, K-265, K-251, K-109, K-106, K-300 and K-315 gave better sugar yield /plant as compared to BL4. Maximum sugar yield/plant was observed in Clone 'K-250' (2.5 Kg) followed by K-265 (2.44 Kg), whereas the average sugar yield of BL4 was 1.2 Kg/plant.

Introduction

Sugarcane crop improvement in different countries relies on conventional breeding, mutation breeding, somaclonal variation and genetic engineering (Dalvi *et al.,* 2012*;* Rajeswari *et al*., 2009). Sugarcane improvement through conventional methods is time-consuming (Cox *et al*., 2000), and is strictly dependent on the nature of flowering, viability of pollen, seed (Moore & Nuss, 1987; Khan *et al.*, 2008) and the genomic complexity of sugarcane crop (Ingelbrecht *et al*., 1999). Considering the environmental conditions of Pakistan where sugarcane breeding is limited due to non viable fuzz (seeds), somaclonal variation presents an alternative solution to overcome many difficulties in cane breeding (Shahid *et al*., 2011).

An array of variations has been observed using tissue culture techniques in different crops (Nawaz *et al*., 2013). This variation is termed 'somaclonal variation'. Although somaclonal variation is undesirable for clonal propagation and genetic transformation efforts (Gao *et al.*, 2011, Pandey *et al.*, 2012), it may serve as a useful tool in some crop improvement programmes (Evans *et al*., 1984; Brown & Thorpe, 1995; Tiwari *et al.*, 2010). Thus far, for sugarcane, only a few improved variants have been released as cultivars after extensive efforts in different laboratories (Larkin & Scowcroft, 1983; Krishnamurthi & Tlaskal, 1974). As most of the agronomic important traits are quantitatively inherited in sugarcane, the frequency of positive mutation in terms of high yield and increased sucrose content is very limited. Moreover, such variations are often unstable, which limits the ability of this phenomenon to be used as a tool for crop improvement in sugarcane. (Kresovich *et al.*, 1986; Irvine *et al.*, 1991; Hoy *et al.*, 2003; Matsuoka & Giglioti, 2005). However, the frequency of phenotypic variation and the type of variation mostly depends on the genotype, explants used and the culture conditions. Pre-existing variability among the cells may play a major role in the frequency of somaclonal variation (Brown & Thorpe, 1995; Hoy *et al.*, 2003). Present study was conducted to evaluate the field performance of regenerated somaclones for yield and yield contributing characters and the quality traits of cane in varity BL4.

Materials and Methods

R1 generation of 324 somaclones regenerated from immature leaf roll callus of sugarcane variety BL4 were evaluated for their yield and yield contributing characters and the quality traits of cane in the experimental field of Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE) University of Karachi using randomized complete block design (RCBD) with three replications. Plant height, cane diameter, number of internodes, length of internodes, leaf length, number of stalks/ stool and weight of stool were measured. Quality of the cane was estimated by measuring brix% (using hand refractometer, Alla France) and pol% (using polarimeter, ATAGO, Japan) from extracted juice of cane. Fiber % was calculated as described by Thangavelu & Rao (1982). Commercial cane sugar percentage (CCS %) was calculated using Australian Commercial Cane Sugar (CCS) formula given by Meade & Chen (1977). Cane sugar recovery percent (CSR %) was calculated by the following formula:

$$\text{CSR \%} = \text{Commercial Cane Sugar (CCS) \%} \times 0.94$$

where, CCS is commercial cane sugar, and 0.94 is net titre (sugar losses) (Ghaffar *et al.*, 2011).

One way analysis of variance (one way Anova) was done using SPSS version 17.0 and pair wise comparison of means of phenotypic traits of all somaclones with means of mother plant BL4 was done by calculating fisher's least significant difference (LSD) at $p < 0.05$. Pearson's correlation between all phenotypic traits and Duncan's multiple range test (DMRT) of the selected clones were done by SPSS version 17.0.

Result and Discussion

Out of all well grown somaclones in three replicates, 160 somaclones exhibited variations over its mother plant in at least one character observed in this study. Maximum variation was observed in weight of stalks per plant, 89 somaclones showed variation in this trait. Twenty four out of 89 clones showed increase in the weight of stalks per clump (Fig. 1). Reduced weight of stalk/plant was observed in 65 somaclones ranging from 21-97% decrease (Fig. 1). The second highly variable trait was the number of stalks, 88 plants showed either increase or decrease in number of stalks (Fig. 1). In 32 somaclones, substantial increase in the number of stalks was observed, and 56 clones showed decreased number of stalks. After weight of stalks/ stool and number of stalks/ stool, maximum variations were observed in brix% where 44 somaclones showed increase in the length of internodes and 43 somaclones showed decrease in the brix% (Fig. 1). Decrease in the brix% observed was upto 35%. Khan *et al*., (2004) and Roy *et al*., (2010) also observed similar decrease in the brix% which was contradictory to the observation by Siddique *et al*., (1994), where increased brix% was observed in some of the clones. Changes in the cane diameter were observed in 31 somaclones, where a minor (15-20%) increase in the diameter was observed in only 5 clones (K-26, K80, K178, K152 and K156) ranging from 3.34-3.5cm; where cane diameter of BL4 was 2.9±0.089 cm. Hoy *et al.*, (2003) also observed smaller cane diameter and increased number of stalks in the plants regenerated from callus culture of immature leaf rolls. Higher number of internodes, greater length of internodes and smaller diameter was also reported by Sood *et al.,* (2006). Twenty eight clones showed variation in cane height and twenty somaclones showed variation in the number of internodes respectively (Fig. 1).

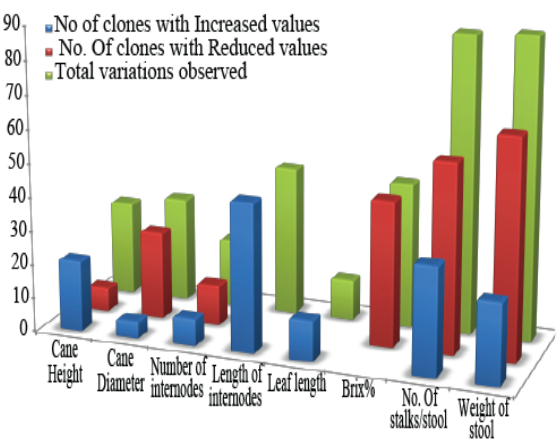

Fig. 1. Number of significant variations ($p<0.05$) observed in eight different characters.

Cane yield and cane quality are major parameters for evaluating the commercial sugarcane hybrids in sugarcane improvement programmes. The yield of sugarcane is a quantitative character dependent upon various traits (Ahmed *et al*., 2010). Correlation studies in sugarcane are helpful in selecting for improved clones (Kadian *et al*., 2006). Correlation studies reveal that the cane yield / plant was positively correlated with number of stalks per plant, cane diameter, cane height and numbers of internodes at $p<0.01$ (Table 1). Numbers of stalks/stool seems to be an independent factor that greatly influenced the weight/stool (Table 1); while Sood *et al*., (2006) found a strong negative relationship between the number of stalks with its diameter in the somaclones of sugarcane var. CoJ 64, Roy *et al*., (2010) reported that the thinner canes were more prominent in the first generation (R_0) which turned to thicker canes in subsequent generations. In this study, cane diameter was positively correlated with all traits studied except for the number of stalks. However, the cane height, and number of internodes had a positive contribution in the cane yield but had a negative impact over the sugar yield. Brix% was only positively correlated with cane diameter at $p<0.01$ (Table 1), which in turn correlated with all other yield contributing factors except for number of stalks.

As no significant improvement in the brix% was observed, the criterion for selecting superior clone compared to the mother plant was to find the clones that had the high yield potential but not at the cost of brix. It was found that the two traits, the number of stalks/stool and the cane diameter were two of the major yield contributory factors that did not negatively correlate with the brix% (Table 1). That is the reason why all the clones showing substantially greater number of stalks were selected except for clones 'K-17, K-27, K-88, K-121, K-198, K-226, K-241, K-253, K-255, K-262, K-269, K285, K-296, where most of them showed no significant improvement either in the yield or the brix% , while clone 'K-27, K-241 and K-253 had significant decrease in the Brix% and clone 'K-226 and K-285 had overall significant decrease in the yield i-e weight /stool. Significant increase in the cane diameter was observed only in five clones as mentioned above out of which clone 'K-80, K-178 and K-152' had no significant difference in the weight/stool and in the brix% (Data not shown). For this reason these clones were not selected, while clone'K-293 had more promising yield potential due to increased height and slight increase in the cane diameter with no significant loss in Brix% was included in the selected clones.

The evaluation of comparative performance of the selected clones through Duncan's multiple range test (DMRT) is given in Table 2. The biochemical assessment for the Commercial Cane Sugar and sugar recovery % by analysing pol % and fiber % reveals that K-106, K- 250, K- 251, K- 265, K-287,K- 293, K-300 and K-315 is comparable with the CCS % of BL4 (Table 2). Howerver, the clones 'K-250, K-265, K-251, K-109, K-106, K-300 and K315 gave better sugar yield /plant as compared to BL4. Maximum sugar yield/plant was observed in Clone 'K-250' (2.5 Kg) followed by K-265 (2.44 Kg). The average sugar yield of BL4 was 1.2 Kg.

Table 2. Comparative performance of the selected somaclones.

S.#	Clone	Cane diameter (cm)	Cane height (cm)	No. of internodes	Stalks/stool	Weight/stool (kg)	Brix %	Pol %	Fiber %	CCS %	Sugar recovery	Sugar yield/ plant (Kg)
1.	BL4	2.90abc	190.8ab	23.0bc	8.3h	9.5j	20.1^{ns}	17.3a	7.11b	13.7a	12.9a	1.21fghij
2.	K26	3.47a	194.0ab	16.5c	15.0b	19.5c	18.3^{ns}	12ef	11ef	7.2fg	6.8fg	1.33defgh
3.	K103	2.94abc	212.0ab	22.7bc	11.0def	15.25ef	20.0^{ns}	14.2bcde	10.8ef	9.3cdefg	8.7cdefg	1.31defgh
4.	K106	2.55c	199.0ab	21.5bc	15.0b	17.5d	22.0^{ns}	16.4abc	8.5bcd	11.4abcd	10.7abcd	1.90cd
5.	K107	2.55c	237.3ab	24.0bc	10.5efg	13ghi	17.7^{ns}	11.9ef	9.8cde	7.4efg	7.0efg	.92hij
6.	K109	2.92abc	214.3ab	24.0bc	13.0bcd	22.25b	19.0^{ns}	13.6cdef	8.5bcd	9.2cdefg	8.6cdefg	1.93bcd
7.	K129	2.97abc	204.0ab	21.3bc	13.0bcd	17.5d	19.3^{ns}	12.1def	13.9g	6.7g	6.3g	1.11ghij
8.	K150	2.73bc	178.0ab	16.0c	10.0fgh	13ghi	19.2^{ns}	13.1def	4.3a	9.0defg	8.4def	1.04ghij
9.	K156	3.34ab	302.0a	36.0a	8.5gh	13ghi	22.0^{ns}	16.6ab	20.9h	10.0bcde	9.4bcdef	1.23fghij
10.	K193	2.71bc	242.5ab	31.5b	11.0def	11.5i	19.0^{ns}	14.0bcde	9.0bcd	9.7cdefg	9.1cdefg	1.05ghij
11.	K197	2.90abc	219.0ab	25.5bc	13.5bcd	16.25de	19.1^{ns}	13.5cdef	8.0bcd	9.1cdefg	8.5cdefg	1.39cdefg
12.	K250	2.92abc	254.3ab	24.3bc	18.0a	25.50a	18.7^{ns}	14.8abcd	12.0fg	10.4abcd	9.8abcde	2.51a
13.	K251	2.65bc	210.0ab	24.0bc	14.5bc	20.5c	19.5^{ns}	15.0abcd	8.0bcd	10.9abcd	10.2abcde	2.10abc
14.	K260	2.81abc	216.7ab	22.0bc	12.0cde	12.75gh	20.5^{ns}	11.8ef	7.7bc	6.3g	6.0g	0.77j
15.	K265	3.03abc	190.0ab	24.5bc	11.0def	24a	19.0^{ns}	15.0abcd	10cde	10.8abcd	10.2abcde	2.45ab
16.	K276	2.60c	175.7ab	19.7c	11.0def	19.5c	18.0^{ns}	11.5f	12.0fg	6.6g	6.2g	1.22fghij
17.	K278	2.88abc	235.7ab	26.7abc	10.5efg	14.0fgh	18.7^{ns}	12.9def	14.4g	7.9defg	7.4defg	1.04ghij
18.	K287	2.81	166.7b	20.0c	11.5efg	12.2fgh	19.3^{ns}	15.0abcd	7.0b	11.2abcd	10.4abcde	1.46defgh
19.	K293	3.18abc	294.3a	31.0b	9.0gh	14fgh	19.6^{ns}	17.1a	21.0h	11.3abcd	10.7abcd	1.52defg
20.	K300	2.92abc	165.7b	18.7c	10.5efg	14.5fg	19.7^{ns}	16.4abc	6.8b	12.8abc	12.0abc	1.75cdef
21.	K308	2.92abc	165.0b	17.3c	12.5bcd	12.5hi	18.7^{ns}	12.0ef	10.0cd	7.1fg	6.7g	0.84ij
22.	K315	2.76abc	232.0ab	22.7bc	11.0def	14.00fg	18.3^{ns}	17.0a	10.6de	13.6ab	12.8ab	1.80cde
	LSD0.01	0.61	106.18	9	2.26	1.53	4.0	2.54	2.34	3.19	3.0	0.50

DMR test (0.01): Different letters show significant differences at $p \leq 0.01$

Table 1. Pearson correlation coefficients between the different agronomical traits of somaclones grown in the experimental field of KIBGE

		Weight of stalks/stool	Length of internodes	Cane Height	Number of internodes	Brix%	Number of stalks/stool	Cane diameter
Weight of stalks/stool	Pearson Correlation	1						
	Sig.							
Length of internodes	Pearson Correlation	0.189**	1					
	Sig.	0.000						
Cane height	Pearson Correlation	0.198**	0.359**	1				
	Sig.	0.000	0.000					
Number of internodes	Pearson Correlation	0.120**	0.134**	0.685**	1			
	Sig.	0.005	.001	0.000				
Brix%	Pearson Correlation	-0.019	-0.156**	-0.104*	-0.027	1		
	Sig.	0.660	0.000	0.014	0.520			
Number of stalks/stool	Pearson Correlation	0.775**	0.062	0.069	-0.026	-0.004	1	
	Sig.	0.000	0.148	0.109	0.542	0.926		
Cane diameter	Pearson Correlation	0.163**	0.117**	0.258**	0.247**	0.263**	0.071	1
	Sig.	0.000	0.005	0.000	0.000	0.000	0.098	

** Correlation is significant at 0.01 level
* Correlation is significant at 0.05 level

As discussed earlier, a number of phenotypic variations were observed in *in vitro* regenerated plants for important agronomic traits an observation shared by other scientists (Heinz & Mee, 1969; Liu *et al*., 1983; Chen, 1986) and has been utilized in the sugarcane crop improvement programmes (Nickell & Maretzki, 1969; Heinz, 1973; Krishnamurthi & Tlaskal, 1974). It is noteworthy that sugar recovery is the major output of the sugarcane, which was not improved in the experiments conducted, but some clones showed sugar recovery comparable to BL4 with increased yield potential through which the sugar yield/ hectare could be improved. Some of the clones (clones 'K-250, K-265, K-251, K-109, K-106, K-300 and K315) produced during this study are worth pursuing in successive generations as they may hold the potential for enhanced yield.

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