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EVALUATION OF SUGARCANE (*SACCHARUM* SPP. COMPLEX) MUTANTS FOR YIELD, YIELD CONTRIBUTING TRAITS AND QUALITY PARAMETERS

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ABSTRACT
In the present investigation, field grown mutants were characterized for yield and quality contributing traits in comparison to parent variety Co 99004 and Co 94012. The MS + 2, 4-D (4.0 mg/l) + sucrose (20 g/l) were used for the callus induction. For *in vitro* mutagenesis, EMS (0.5%) 2 hours, while, NaCl 100 mM concentration and PEG 2 % treatment was found lethal dose (LD_{50}) for *in vitro* selection of salt and drought tolerant mutants. The mutants, on field evaluation, exhibited significant variability for characters *viz.*, tiller numbers at 120 DAP, number of shoots at 240 DAP, number of millable canes, stalk length, stalk diameter, number of internodes per stalk, stalk weight and cane yield at time of harvest and also for Brix, sucrose %, juice purity, CCS % and fibre % at harvest. The mutants developed from treatment T_1 and T_2 *i.e.*, EMS (LD_{50}) and EMS (LD_{50}) + NaCl (LD_{50}) was found significantly superior over parent variety in tiller numbers at 120 days, number of shoots at 240 DAP, number of millable canes, stalk length, number of internodes per stalk, stalk weight, cane yield, juice brix, juice purity %, sucrose % of juice, CCS %, pol % of cane and CCS (t/ha) in both the genotypes. The present study reports beneficial traits of the mutants derived from Co 99004 and Co 94012 in their field performance. The selected mutants may be further evaluated as new genotypes and may also be utilized for further breeding programme as genetic stalk to improve yield and qualitative traits in sugarcane.

KEY WORDS: Sugarcane, *in vitro*, mutagenesis, salt, drought, tolerance.

INTRODUCTION

Sugarcane, a major commercial sugar crop grown in India, plays a significant role in the socio-economic empowerment and prosperity of rural population. Sugarcane (*Saccharum* spp. Complex) yields are declining due to varietal degeneration and susceptibility to biotic and abiotic stresses, a major concern in breeding programs for enhancing productivity. Since sugarcane is a long duration crop, there is a need for development of early maturing, high yielding and high sugared stress tolerant varieties to meet the demand. Complexity and size of the sugarcane genome is a major limitation in its genetic improvement. Development of superior sugarcane varieties through conventional hybridization program is time consuming and has the problem of transfer of undesirable characters/ traits into the newly developed hybrids/variety. Sundaram *et al.* (2010) has recently reported about boarding the genetic base of sugarcane through introgression of resistant genes by intergeneric hybridization and the identification with molecular analysis. Since conventional plant breeding methods are found to be slow to create substantial improvement, hence attempts were made to introduce genetic variability in sugarcane by *in vitro* culture techniques and mutation breeding (Krishnamurthi and Tlaskal, 1974; Larkin and Scowcroft, 1981; Jain *et al.*, 1997;Sreenivasan and Jalaja, 1998; Wagih *et al.*, 2004; Borras *et al.*, 2005; Patade *et al.*, 2006, 2008; Muthusamy *et al.*, 2007;

Kenganal *et al.*, 2008; Khan *et al.*, 2009; Dalvi *et al.*, 2012 and Koch *et al.*, 2012). Attempts to improve Co 99004 and Co 94012 for salt and drought tolerance through conventional breeding have not yielded fruitful results. Present study describes the development of mutants from cv. Co 99004 and Co 94012 with salt and drought tolerance and other agronomic traits *viz.* yield and high sugar content in addition to its phenotypic characters.

MATERIALS & METHODS

This study was carried out in the Tissue Culture Laboratory and farm of Main Sugarcane Research Station (MSRS), Navsari Agricultural University, Navsari. Healthy young leaf explants including apical meristems were obtained from the shoot of commercial sugarcane variety Co 99004 and Co 94012. These sections were washed thoroughly under running tap water for 20 min followed by washing with soap water (2 drops of Labolin into 250 ml of water) for about 5 to 6 minutes in a sterile 1 liter conical flask followed by cleaning the explants with distilled water. The shoots were rinsed in 5 per cent sodium hypochlorite for 10 minutes. Then shoots were thoroughly rinsed in 70 per cent ethanol for 30 seconds followed by sterilize double distilled water

for 4-5 times till ethanol was completely washed out from the surface of explant. Surface sterilization was performed by using 0.1 per cent mercuric chloride solution. Shoots were shaken vigorously for 5 minutes. Finally shoots were rinsed 3 to 4 times with sterile double distilled water to remove all traces of chemicals. The second innermost and inner most whorls of developing leaf was cut in to small pieces of approximately one centimeter length with the help of a sterile sharp blade and utilized as explant for callus induction on MS medium supplemented with 4mg/l 2,4-D + 20g/l sucrose.

***In vitro* performance**

Calli were established from the smaller pieces of explants and callus induction made on Murashige and Skoog (1962) medium, supplemented with 20 g/l sucrose, 7.5 g/l agar and 4 mg/l 2, 4-D. The medium was adjusted to pH 5.8 with NaOH (0.1 N), autoclaved at 120°C and 15 lbs psi pressure for 15 min. After 4 weeks, embryogenic *calli* were separated from the explants and treated with different treatment of EMS (0.5%) *i.e.*, 0, 1, 2, 3 and 4 hours (Kenganal *et al.*, 2008). Lethal dose (LD_{50}) of EMS was decided on the basis of survival per cent of callus. After deciding LD_{50} of EMS the callus was transferred to MS media supplemented with different levels of NaCl (0, 50, 100, 150 and 200 mM). Lethal dose (LD_{50}) of NaCl was decided on the basis of survival per cent of callus. After deciding LD_{50} of EMS, the callus was transferred to MS media supplemented with different treatments of PEG (0, 1, 2, 3 and 4%). Lethal dose (LD_{50}) of PEG was decided on the basis of survival percent of callus.

Salt tolerant plantlets were regenerated from callus treated with EMS (LD_{50}) on MS medium supplemented with the NaCl (LD_{50}). Similarly drought tolerant plants were regenerated from callus treated with EMS (LD_{50}) on MS medium supplemented with the PEG (LD_{50}). Plantlets were also regenerated from callus treated with EMS (LD_{50}) on MS medium supplemented with the NaCl (LD_{50}) + PEG (LD_{50}). Cultures were grown in sterilized plastic petri-plates closed with paraffin wax strip. Plantlets were regenerated and then rooted after 3 to 4 weeks of transfer of high healthy callus on regeneration and root medium, *i.e.*, MS medium of the same composition as earlier mentioned, but with special hormones (Patel, 2007) and incubated in a growth chamber under long-day conditions (16/8 hours light/dark cycle) at a temperature of 25 ± 2°C and relative humidity of 55 ± 5 %. Light was provided by white fluorescent tubes (40 W) with approximately 2000 lux/m light intensity. The healthy plantlets were selected as tolerant mutants for further evaluations.

Field performance

After root formation plantlets were grown in polyhouse for primary hardening then shed net house for secondary hardening for one month each. Mutants rose from different treatment *i.e.*, T_1 (EMS 0.5% LD_{50}), T_2 (EMS 0.5% LD_{50} + NaCl LD_{50}), T_3 (EMS 0.5% LD_{50} + PEG LD_{50}), T_4 (EMS 0.5% LD_{50} + NaCl LD_{50} + PEG LD_{50}), T_5 (untreated single budded raised plants) and T_6 Control (untreated normal set planting) of each variety Co 99004 and Co 94012 were evaluated for yield, yield contributing traits and quality parameters under field condition. They were planted at 90cm X 40cm distant in three replications in Factorial Randomized Block Design (FRBD) at M.S.R.S., Navsari in plot number G-16 during 2012-2013 for comparative study. The gross plot area was 6.0m x 0.9m with three rows. All recommended practices including plant protection measures were followed.

Morphological and biochemical analysis

Under *in* vitro culture number of shoots was counted after 30 days of callus induction on regeneration medium. Length of regenerated shoots from callus developed on different treatment media, measured in centimeter. Root length of *in vitro* plantlets was measured in centimeter and individual roots were counted. These observations were recorded 30 days after inoculation on rooting media. At the time of harvest 5 somaclones were randomly selected from each treatment of each replication and observations were recorded of selected somaclones *i.e.*, survival % at 45 days after planting (DAP), tillers at 120 DAP, shoots at 240 DAP, stalk length at 360 DAP, stalk diameter at 360 DAP, internodes per stalk at 360 DAP, number of millable canes, single cane weight, cane yield at harvest, commercial cane sugar (CCS) t/ha, juice brix percent at 360 DAP, sucrose percent juice 360 DAP, juice purity per cent at 360 DAP, CCS per cent at 360 DAP, pol percent of cane at 360 DAP and fiber percent of cane at 360 DAP.

Statistical analysis:

The data generated from the various *in vitro* experiments were subjected to statistical analysis in Factorial Completely Randomized Design (FCRD) whereas; field experiments were subjected to statistical analysis in FRBD as prescribed by Panse and Sukhatme (1985). Transformation of data was carried out prior to statistical analysis as suggested by Steel and Torrie (1981).

RESULTS & DISCUSSION

***In vitro* performance**

The results on survival per cent of callus to different EMS (0.5%) treatments are given in Table 1. Culture exposed to EMS (0.5%) for 2 hours treatment gave almost 50% survival response compared to control (non-treated culture) in both the genotypes. Our results are in line with Kenganal *et al.* (2008) in sugarcane. Koch *et al.* (2009) exposed callus to 8mM and 16mM EMS for development of tolerant cell. The response of callus to EMS was different for different genotypes because of different genetic constitution and methods of its application. The results on survival percent of callus to different NaCl treatments are given in Table 2. Culture exposed to 100 mM treatment recorded almost 50% survival as compared to control (non-treated culture) in both the genotypes. Similar results were obtained by Patade and Suprasanna (2009) for 171.1 mM and Munir and Aftab (2009) for 120mM NaCl. These results are in close agreement with those reported by Gandonou *et al.* (2005b), Kenganal *et al.* (2008) and Shomeili *et al.* (2011). The results on survival per cent of callus to different PEG treatments are given in Table 3.

TABLE1: Survival per cent of callus after 30 days to different EMS (0.5%) treatments to determine lethal dose (LD_{50}) of EMS

Genotype	EMS 0.5% (Hour)	% Callus survive					
		1	2	3	4	5	Mean
	1	68	70	69	68	71	69.20
	2	48	47	49	48	47	47.80
Co 99004	3	12	11	13	11	12	11.80
	4	2	3	2	3	2	2.40
	0 (control)	100	100	100	100	100	100.00
	1	69	70	70	69	70	69.60
	2	49	48	49	48	49	48.60
Co 94012	3	12	12	13	13	13	12.60
	4	3	3	2	3	3	2.80
	0 (control)	100	100	100	100	100	100.00

TABLE 2: Survival per cent of callus after 30 days to different treatments of NaCl to determine lethal dose (LD_{50}) of NaCl

Genotype	Conc. of NaCl (mM)	% Callus survive					
		1	2	3	4	5	Mean
	50	86	87	88	87	86	86.80
	100	49	50	49	50	49	49.40
Co 99004	150	23	22	22	23	22	22.40
	200	5	4	5	4	4	4.40
	0 (control)	100	100	100	100	100	100.00
	50	88	87	88	89	88	88.00
	100	51	50	49	50	51	50.20
Co 94012	150	24	23	22	24	23	23.20
	200	3	4	3	4	3	3.40
	0 (control)	100	100	100	100	100	100.00

TABLE 3: Survival per cent of callus after 30 days to different treatments of PEG (8000) to determine lethal dose (LD_{50}) of PEG

Genotype	PEG Conc. %	% Callus survive					
		1	2	3	4	5	Mean
	0.5	85	86	84	85	84	84.80
	1	65	66	67	65	66	65.80
Co 99004	2	46	48	47	48	47	47.20
	3	20	19	21	20	21	20.20
	0 (control)	100	100	100	100	100	100.00
	0.5	86	87	86	87	86	86.40
	1	68	67	67	67	68	67.40
Co 94012	2	49	49	48	47	49	48.40
	3	21	21	20	21	20	20.60
	0 (control)	100	100	100	100	100	100.00

Culture exposed to 2% treatment resulted in almost 50% survivals as compared to control (non-treated culture) in both the genotypes. These results are very close with those reported by Islam *et al.* (2009), Munir and Aftab (2009), Bidabadi *et al.* (2012) and Dalvi *et al.* (2012).

The results on the different *in vitro* screening treatments on root shoot development are given in Table 4. The significantly highest number of shoots (33.0) were obtained in treatment T_1 followed by treatment T_5 (25.1) and T_2 (23.0) in cv. Co 99004 (Fig. 1). Significantly the highest number of shoots (28.0) was obtained in treatment T_1 in cv. Co 94012 (Fig. 2). The treatment T_1 gave significantly the highest shoot length (7.52 cm) followed by treatment T_5 whereas, lowest shoot length (6.19 cm) was observed in treatment T_4 in cv. Co 99004 (Fig. 3). Treatment T_1 gave significantly highest shoot length (7.01 cm) followed by treatment T_5 (6.43 cm) and T_2 (6.0 cm) in cv. Co 94012 (Fig. 4). Significantly the maximum root length was recorded in treatment T_1 *i.e.* 2.48 cm in cv. Co 99004 followed by treatment T_5 (2.41 cm). Significantly the maximum root length was recorded in treatment T_1 *i.e.* 2.17 cm followed by treatment T_5 (2.14 cm) and T_2 (2.02 cm) in cv. Co 94012 (Table 4).

The number of shoots obtained from 1 gram of callus, shoot length and root length were observed maximum in un-treated control followed by EMS 0.5% (LD_{50}) and EMS 0.5% (LD_{50}) + NaCl (LD_{50}) in both genotypes. The reason is that the cells grown under any kind of stress may have to spend more metabolic energy than those grown in the absence of stress (Croughan *et al*., 1981). This extra energy most probably is used up in regulating osmotic adjustment thus decreasing the growth of the cells. An interesting observation during the present study was that all the NaCl and PEG treated sugarcane callus cultures had lower number of shoots per culture bottlel when shifted to the regeneration medium as compared to the control. A decrease in shoot number per culture unit, however, is well documented by Naik and Babu (1988), Gandonou *et al.* (2005b), Patade and Suprasanna (2009) and Shomeili *et al.* (2011) in sugarcane. Working with rice callus cultures, Lutts *et al.* (1999) observed a decreased number of shoots produced per callus in rice at all salt levels.

TABLE 4: Effect of different selection pressure on shoot regeneration, development and root formation after 30 days in sugarcane cv. Co 99004 and Co 94012

Variety	Treatments		Number of shoots per gram of	Shoot length (cm)	Root length (cm)
	T_1	Control	33.0	7.52	2.48
	T_2	EMS 0.5% (LD_{50}) + NaCl (LD_{50})	23.0	7.11	2.30
Co 99004	T_3	EMS 0.5% (LD_{50}) + PEG (LD_{50})	16.0	7.13	2.18
	T_4	EMS 0.5% (LD_{50}) + NaCl (LD_{50}) + PEG (LD_{50})	11.9	6.19	2.15
	T_5	EMS 0.5% (LD_{50})	25.1	7.29	2.41
	T_1	Control	28.0	7.01	2.17
	T_2	EMS 0.5% (LD_{50}) + NaCl (LD_{50})	21.0	6.00	2.02
Co 94012	T_3	EMS 0.5% (LD_{50}) + PEG (LD_{50})	12.9	5.61	2.00
	T_4	EMS 0.5% (LD_{50}) + NaCl (LD_{50}) + PEG (LD_{50})	10.8	4.86	1.98
	T_5	EMS 0.5% (LD_{50})	21.1	6.43	2.14
		V	0.141	0.014	0.013
SEM ±		T	0.223	0.022	0.021
		V X T	0.315	0.031	0.030
		V	0.418	0.041	0.040
CD at 5%		T	0.661	0.065	0.063
		V X T	0.935	0.092	NS
CV %			4.909	1.507	4.327

It was also observed during this study that the plants regenerated from salt and PEG treated callus had less shoot length. The literature is not available on this aspect in sugarcane. However, Winicov (1991) have also selected salt-tolerant cell lines from *Medicago sativa*, by a single-step selection process on tissue culture medium containing 1% NaCl. In another similar study in potatoes, Marconi *et al*., (2001) made a comparative growth analysis among salt-tolerant plants of potato obtained by *in vitro* recurrent selection methods. Results of the present work also indicated that root formation per shoot affected by salt and PEG treatments. Salt and water stress significantly influenced the root length. In contrast, an increase in root length was recorded in plants regenerated from NaCl-treated callus cultures as compared to the corresponding controls by Munir and Aftab (2009). Rodriguez *et al*. (1997) reported a rapid but transient reduction in growth rates of plant roots under salt stress.

Field performance

The results on various characters of field evaluation of mutants obtained in present study are given in Table 5. The mutants morphologically resemble to their donor parent to large extent. However, some mutants showed morphological variation in top, stalk diameter, internode length and eye-bud. Moreover, some somaclones retained earliness and high sucrose content of donor parent (Khan *et al.* 2004; Jalaja *et al.*, 2006; Patel, 2007; Singh *et al.*, 2008; Doule *et al.*, 2008; Khan *et al.*, 2009; Roy *et al.*, 2010; Dalvi *et al.*, 2012). Since most sugarcane cultivar are chromosomal mosaics, the extent of variation in somaclonal population partly depend on genotypes used (Patel, 2007; Patade *et al.*, 2006).

Survival percent at 45 DAP was significantly highest in treatment T_3 (96.67%) in cv. Co 99004. Similarly, in case of cv. Co 94012 survival was significantly higher in treatment T_4 (94.44%). In cv. Co 99004, number of tillers at 120 DAP was significantly higher in treatment T_1 (169.8) than control T_6 (142.5). Similarly, in cv. Co 94012 it was significantly higher in treatment T_1 (175.8) than control T_6 (156.7). In present study, number of shoots at 240 DAP was significantly higher in treatment T_1 (135.8) followed by treatment T_2 as compared to control T_6 in cv. Co 99004. Likewise, in case of cv. Co 94012 number of shoots was significantly higher in treatment T_1 (140.6) (Table 5). Jalaja *et al.* (2006) and Singh *et al.* (2008) reported results were in line with the present findings.

TABLE: 5 Average performances of sugarcane mutants for yield, yield contributing and quality attribute (cv. Co 99004 and Co 94012)

Characters		Survival % at 45 DAP	Tillers 120 DAP (000/ha)	Shoots at 240 DAP ('000/ ha)	NMC at 360 DAP (000/ha)	Stalk length (cm) at 360 DAP	Stalk diameter (cm) at 360 DAP	Internode/stalk at 360 DAP	Stalk weight (kg) at 360 DAP	Juice brix at 360 DAP	Sucrose % of juice 360 DAP	Juice purity % at 360 DAP	CCS % at 360 DAP	Fiber % cane at 360 DAP	Scrose/Pol % cane at 360 DAP	CCS (t/ha) 360 DAP	Cane yield (t/ha)
Co 99004	T_1	94.44	169.8	135.8	108.7	286.5	2.47	25.7	1.235	22.30	21.33	95.64	15.49	13.10	16.40	19.78	127.68
	T_2	94.44	161.9	129.5	103.6	284.7	2.46	24.5	1.248	22.13	20.90	94.42	15.10	13.29	16.03	18.56	122.86
	T_3	96.67	155.2	124.2	99.3	265.6	2.33	23.0	1.063	20.10	18.83	93.67	13.56	14.48	14.22	13.43	99.12
	T_4	91.11	151.8	121.1	97.1	269.3	2.30	22.7	1.056	20.07	18.54	92.38	13.27	14.52	14.00	12.46	93.96
	T_5	94.44	161.0	127.3	102.7	283.7	2.51	24.0	1.244	22.10	20.83	94.23	15.03	13.30	15.97	18.05	119.99
	T_6	58.35*	142.5	114.0	91.2	275.9	2.56	23.3	1.170	20.07	18.88	94.08	13.62	13.89	14.37	13.87	101.93
Co 94012	T_1	92.22	175.8	140.6	110.1	273.0	2.74	25.7	1.247	22.43	21.10	94.04	15.22	13.12	16.22	20.31	133.70
	T_2	93.33	170.9	136.7	107.3	272.4	2.56	25.7	1.245	21.70	20.23	93.23	14.53	13.32	15.51	18.63	128.14
	T_3	92.22	168.2	134.6	106.0	255.2	2.53	24.2	1.053	20.14	18.63	92.49	13.33	14.32	14.10	14.45	108.23
	T_4	94.44	161.9	129.5	103.6	241.6	2.43	24.8	1.048	20.11	18.55	92.23	13.27	14.33	14.04	14.06	105.73
	T_5	92.22	170.4	134.3	106.7	270.0	2.61	25.5	1.243	21.70	20.22	93.22	14.53	13.32	15.51	18.49	127.20
	T_6	55.19*	156.7	125.3	100.3	252.9	2.82	24.8	1.186	20.60	19.29	93.64	13.89	14.11	14.64	15.89	114.61
SEM ±	V	0.662	2.265	1.671	1.432	2.256	0.024	0.173	0.008	0.133	0.144	0.301	0.115	0.090	0.109	0.220	1.455
	T	1.146	3.930	2.895	2.480	3.907	0.042	0.299	0.013	0.231	0.250	0.522	0.199	0.155	0.188	0.381	2.520
	V X T	1.621	5.447	4.094	3.507	5.526	0.059	0.422	0.019	0.327	0.354	0.738	0.281	0.220	0.266	0.539	3.564
CD	V	NS	6.642	4.902	4.199	6.617	0.070	0.506	NS	NS	NS	0.884	NS	NS	NS	0.645	4.268
	T	3.363	11.505	8.491	7.274	11.460	0.122	0.876	0.038	0.678	0.733	NS	0.583	0.455	0.552	1.117	7.392
	V X T	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV %		3.21	5.92	5.48	5.90	3.55	4.02	2.99	2.74	2.68	3.10	1.37	3.42	2.76	3.06	5.66	5.36

T_1 = EMS, T_2 = EMS + NaCl, T_3 = EMS + PEG, T_4 = EMS + NaCl + PEG, T_5 = untreated single budded raised plant, T_6 = Control (untreated sett planting),
* = Germination percent.

Fig. 1 Effect of different selection pressure on shoot regeneration from callus of sugarcane cv. Co 99004 94012 (A = EMS 0.5% LD_{50}, B = EMS 0.5% LD_{50} + NaCl LD_{50}, C = EMS 0.5% LD_{50} + PEG LD_{50}, D = EMS 0.5% LD_{50} + NaCl LD_{50} + PEG LD_{50}, and E = Control)

Fig. 2 Effect of different selection pressure on shoot regeneration from callus of sugarcane cv. Co 94012 94012 (A = EMS 0.5% LD_{50}, B = EMS 0.5% LD_{50} + NaCl LD_{50}, C = EMS 0.5% LD_{50} + PEG LD_{50}, D = EMS 0.5% LD_{50} + NaCl LD_{50} + PEG LD_{50}, and E = Control)

Fig. 3 Effect of different selection pressure on shoot and root development from callus of sugarcane cv. Co 99004 & Co 94012 (A = EMS 0.5% LD_{50}, B = EMS 0.5% LD_{50} + NaCl LD_{50}, C = EMS 0.5% LD_{50} + PEG LD_{50}, D = EMS 0.5% LD_{50} + NaCl LD_{50} + PEG LD_{50}, and E = Control)

The number of millable canes at harvest were significantly highest in treatment T_1 (108.7) followed by treatment T_2 in cv. Co 99004. Similarly, in cv. Co 94012 NMC was significantly higher in treatment T_1 (110.1). The significantly maximum length of stalk (286.5 cm) was observed in treatment T_1 followed by treatment T_2 as compared to control T_6 in cv. Co 99004. In case of cv. Co 94012, it was significantly highest in treatment T_1 (273.0 cm) followed by treatment T_2 as compared to control T_6. In present finding it was observed that, diameter of stalk was higher in treatment T_6 (2.56 cm) followed by treatment T_5 in cv. Co 99004. Similarly in cv. Co 94012 (Table 5), it was highest in treatment T_6 (2.82 cm). Similar results were obtained by Patel *et al.* (2004), Doule *et al.* (2008) and Dalvi *et al.* (2012) in field evaluation of mutants of CoC 671 for characters number of millable cane, stalk length and stalk diameter.

Internode per stalk at 240 DAP was significantly higher in treatment T_1 (25.7) followed by treatment T_2 in cv. Co 99004. In case of cv. Co 94012, internodes per stalk were numerically higher in treatment T_1 and T_2 (25.7). The mean data pertaining to stalk weight (kg) at 360 DAP showed significant variation in treatments (Table 5). In cv. Co 99004, it was significantly higher in treatment T_2 (1.248 kg) followed by treatment T_5 (1.244 kg) as compared to control T_6 (1.170 kg). Similarly, in cv. Co 94012 stalk weight was significantly higher in treatment T_1 (1.247 kg). Present findings are in agreement with the Patel *et al.* (2004), Patel (2007), Jalaja *et al.* (2006), Singh *et al.* (2008), Doule *et al.* (2008) and Dalvi *et al.* (2012).

In the present investigation it was observed that, the brix per cent was significantly highest in treatment T_1 (22.30%) followed by treatment T_2 (22.13%) in cv. Co 99004. Similarly, in cv. Co 94012 juice brix percent was significantly higher in treatment T_1 (22.43%) as compared to control T_6 (20.60%). The mean data pertaining to sucrose per cent of juice at 360 DAP showed significant variation in treatments (Table 5). In cv. Co 99004, it was significantly higher in treatment T_1 (21.33%) followed by treatment T_2 (20.90%) and T_5 (20.83%). Similarly, in cv. Co 94012 sucrose per cent was significantly higher in treatment T_1 (21.10%) as compared to control T_6 (19.29%).

The juice purity percent at 360 DAP in cv. Co 99004, was numerically higher in treatment T_1 (95.64%) followed by treatment T_2 (94.42%) as compared to control T_6 (94.08%). Similarly, in cv. Co 94012 juice purity was higher in treatment T_1 (94.04%). In present study it is reported that, CCS percent at 360 DAP was significantly higher in treatment T_1 (15.49%) followed by treatment T_2 (15.10%) as compared to control T_6 (13.62%) in cv. Co 99004. In case of cv. Co 94012, it was significantly higher in treatment T_1 (15.22%) followed by T_2 compared to control T_6 (13.89%). Fiber percent at 360 DAP was significantly lower in treatment T_1 (13.10%) in cv. Co 99004. In case of cv. Co 94012 (Table 5), it was significantly lower in treatment T_1 (13.12%) followed by T_2 (14.53%) than control T_6 (14.11%). Patel (2007), Singh *et al.* (2008), Doule *et al.* (2008) and Dalvi *et al.* (2012) reported that somaclone perform superior in juice purity and CCS per cent over their parent source.

In cv. Co 99004, pol percent of cane at 360 DAP was significantly higher in treatment T_1 (16.40%) followed by treatment T_2 (16.03%) as compared to control T_6 (14.37%). Similarly, in cv. Co 94012 it was significantly higher in treatment T_1 (16.22%) followed by treatment T_2 (15.51%) (as compared to control T_6 (14.64%). Present finding showed that, CCS t/ha was 19.78 t/ha in treatment T_1 was significantly highest followed by treatment T_2 (18.56 t/ha) as compared to control T_6 (13.87 t/ha) in cv. Co 99004. Similarly, in cv. Co 94012 significantly highest CCS t/ha was 20.31 t/ha in treatment T_1 followed by treatment T_2 (18.63 t/ha) as compared to control T_6 (15.89 t/ha). The significantly highest cane yield of 127.68 t/ha was achieved in treatment T_1 followed by treatment T_2 (122.86 t/ha). Likewise, in cv. Co 94012 cane yield was significantly highest in treatment T_1 (133.70 t/ha) as compared to control T_6 (114.61 t/ha) (Table 5). The present findings are in agreement with the findings of previous workers *i.e.*, Patel (2007), Singh *et al.* (2008), Doule *et al.* (2008) and Dalvi *et al.* (2012).

Patel (2007) studied different phenotypic variation in the somaclones of sugarcane. The phenotypic and agronomical variations such as increased internode length, bifurcation of cane, zigzag arrangement, splitting of leaf sheath, large eye buds, internode cracks, white parallel strips and profuse tillers per plant were also recorded in somaclones of CoN 95132. Dalvi *et al.* (2012) observed that somaclone TC 922 was superior in yield and its contributing characters over its parent CoC 671. Doule *et al.* (2008) noticed that somaclone VSI 2179 gave significantly higher cane yield, sugar yield, brix, sucrose % and CCS % over parent CoC 671.

Mutants of sugarcane cv. Co 99004 and Co 94012 exhibited potential variation in field. On the overall basis it was observed that mutants obtained from EMS treatment and salt stress treatments were found to be best considering yield and quality parameters over their respective parents in both genotypes. The selected mutants from these treatments may be further evaluated as new genotypes and may also be utilized for further breeding programme as genetic stalk to improve yield and qualitative traits in sugarcane.

CONCLUSION

These identified mutants for salt and drought tolerant may be utilized as a parent in hybridization programme for the development of tolerant genetic stock. The present study suggests that *in vitro* mutagenesis can be exploited to develop and improve agronomical traits, increase resistance to biotic and abiotic stress in crops like sugarcane wherein the conventional breeding programs take longer time and labour intensive.

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