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Genetic variability analysis in sugarcane (*Saccharum spp. complex*) through mutagenesis

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Abstract
Two sugarcane varieties CoN-05071 and CoN-07072 was used for induction of genetic variability through *in vitro* mutagenesis. In this study 25-30 days old white globular Callus tissue is considered as suitable explants material. To undergo mutagenic treatments with two levels of EMS 0.3% and 0.2% for 30min and 60min and resulted very good callus appearance in both the varieties. Maximum callus survival percent was found in callus treated with EMS 0.3%+30min (90.77%) in CoN-05071 whereas, EMS 0.2%+30min callus treated was showed maximum survival percent (78.63%) in CoN-07072. Maximum regeneration percent was found in explants treated with EMS 0.3%+30min (78.77%), and SA 0.2%+30min (82.45%) in CoN-05071 and CoN-07072 respectively. In the present study EMS 0.2% and 0.3% and SA 0.2%, 0.3% treatments were most effective mutagenic treatments. The effectiveness decreased with increasing time intervals.

Keywords: genetic variability, sugarcane (*Saccharum spp. complex*)

1. Introduction
Sugarcane (*Saccharum* spp., 2n=40 to 128) belongs to the family Poaceae (Graminae) and tropical sugarcane originated from Oceania (New Guinea) and Indian cane (*Saccharum spontaneum* L.) originated from North Eastern India.

It is the main sugar producing crop that contributes more than 75% to the total sugar pool at the global level. Globally, it occupies about 20 M ha land, a little about 2 % of total cropped area, producing 1350 MT of cane (FAO, 2004). In World area 20.42 million ha, production 1333.2 million tones and productivity 65.20 t/ha. In India area 5.341 million ha, production 345.6 million tones and productivity 64.70 t/ha. In Gujarat area 0.182 million ha, production 13.3 million tones and productivity 71 t/ha. (Anon. 2015).

A basic requirement for the success of crop improvement through breeding is availability of genetic variability. In the absence of variability, the kind of assemblies that a breeder to create is not possible. The next phase of variability creation is to extend the phenomenon of spontaneous mutation to experimental development of genetic changes through mutagenesis. The breeding potential of a crop species depends on the exploitation of the existing variability through selection or the variability generated through hybridization or mutation. In sugarcane, hybridization cum selection practice is a lengthy.

Mutation is the process, in which genes are permanently alternated under environment conditions while being transferred between generations. As also to these alternations in nature, developing science also have provided a chance for mankind to create artificial mutations by using multi techniques. Chemical or physical factors that lead to mutation are called mutagens and living creatures that have had permanent hereditary changes are called mutants.

2. Materials and methods
The present investigation was undertaken to at the Sugarcane Tissue culture Laboratory, Main Sugarcane Research Station, Navsari Agricultural University, Navsari, Gujarat during 2015-2016. Material used were sugarcane variety CoN-05071 and CoN-07072. Callus induced after 25 days of inoculation was cut in to small pieces, weighted and treated with EMS (Ethyl methane Sulphonet), SA (Sodium Azide) and MMS (Methyl Methane Sulphonet) of 0.2% and 0.3% solution (prepared in sterilized distilled water and membrane filtered) for 30 minute and 60 minute. Mutated callus was cultured back on MS medium consisting 4 mg/l 2-4, D + 2% sucrose. Factorial complete randomized design used in this experiment with three replication. Survival per cent, regeneration per cent, quality of callus.

3. Results and discussion

Quality of callus

Quality of callus is differentiated as good callus, moderate callus, very good callus and poor callus on the basis of characters of callus such as appearance, compactness and colour. Untreated callus was found very good, where as in variety CoN-05071 Poor callusing was observed in treatments T_7, T_{10} (SA 0.3% + 30 min, (MMS 0.2% + 60 min). In two treatments T_3 and T_5 very good callus was found in cultivar CoN-05071. In treatments (T_2, T_8, T_{11}, T_{12}) good callus was found in cultivar CoN-05071.

In the variety CoN-07072, two treatments (T_{13}, T_{19}) resulted in very good callus out of twelve treatments, where as five treatments (T_{14}, T_{15}, T_{17}, T_{21}, T_{23}) showed good callus and five treatments (T_{16}, T_{18}, T_{20}, T_{22}, T_{24}) showed poor callus quality. The response of both the varieties to various concentrations of chemical mutagenic agents was found different at higher concentrations and longer treatment duration. The variety CoN-05071 yielded good and moderate callus, whereas at higher concentrations (0.3%) and high treatment time periods (60 min) the variety CoN-07072 yielded poor callus.

Both the varieties responded differently to the various doses of mutagens and time periods at higher concentrations and high treatment time periods. Similar results were obtain by Rutherford *et al.* (2013) [1].

Callus survival per cent

Genotype CoN-05071 supplemented with MS + 4 mg/l, 2,4-D, T_3 (EMS 0.3% + 30 min) registered maximum Survival percent (90.77%) followed by T_5 (SA 0.2% + 30 min), on the other hand, callus treated with (MMS 0.2% + 60 min) registered minimum survival per cent (36.53%).

Genotype CoN-07072 supplemented with MS + 4 mg/l, 2,4-D T_{13} (EMS 0.2% + 30 min) registered maximum survival per cent (78.63%) followed by T_{19} (SA 0.03% + 30 min) and T_{23} (MMS 0.3% + 30 min). On the other hand callus treated with (MMS 0.3% + 60 min) registered minimum survival per cent (34.53%).

Overall the callus treated with (EMS 0.3% + 30 min) registered maximum Survival per cent (90.77%) in CoN-05071 and in CoN-07072 (78.63%) (EMS 0.2% + 30 min). Untreated callus showed 100% survival in comparison with treated callus by various chemical mutagenic agents. In both the genotypes MMS at (60 min) treatment showed poor survival per cent irrespective to the concentrations. Similar results were noticed by Kanganal *et al.* (2008) [5], Koch *et al.* (2009) [6] and Gadak (2014) [7]. Callus survival per cent varied at different levels of EMS, SA and MMS in both the genotypes (Talebi *et al.* 2012, Bashir *et al.* 2013, Soeronto 2003) [2, 3, 4].

Callus Regeneration per cent

Significantly highest regeneration per cent (78.77%) was observed in treatment T_3 (EMS 0.3% + 30 min) followed by treatment T_5, 74.25% (SA 0.2% + 30 min), whereas minimum regeneration per cent was noticed in treatment T_{10}, 34.20% (MMS 0.2% + 60 min) in Cultivar CoN-05071. Significantly highest regeneration per cent (82.45%) was observed in treatment T_{17} (SA 0.2% + 30 min) followed by treatment T_{15}, 72.30% (EMS 0.3% + 30 min), whereas minimum regeneration per cent was noticed in treatment T_{24}, 44.47% (MMS 0.3% + 60 min) in Cultivar CoN-07072.

Maximum regeneration per cent was observed in cultivar CoN-07072 in the treatment concentration (SA 0.2%+30 min) followed by treatment combination T_3, where callus was treated with (EMS 0.3%+30 min) in cultivar CoN-05071. Similar results were observed by Ather *et al.* (2009) [8]. Immersion time in EMS, SA and MMS was more influential to callus regeneration than the concentration. Increase in concentration resulted in reduction in regeneration per cent. Regeneration potential was directly proportional to the mutagen treatment given to callus in both the cultivar. Similar results were observed by Patel *et al.* (2004) [9], Kanganal *et al.* (2008) [5] and Koch *et al.* (2009) [6].

Table 1: Effect of different chemical mutagenic agents on quality of callus

Sr. No.	Variety (G)	Treatment (C + P)	Quality
T1	CoN-05071 (G_1)	EMS 0.2% + 30 min	++
T2		EMS 0.2% + 60 min	+
T3		EMS 0.3% + 30 min	+++
T4		EMS 0.3% + 60 min	++
T5		SA 0.2% + 30 min	+++
T6		SA 0.2% + 60 min	++
T7		SA 0.3% + 30 min	- -
T8		SA 0.3% + 60 min	+
T9		MMS 0.2% + 30 min	++
T10		MMS 0.2% + 60 min	- -
T11		MMS 0.3% + 30 min	+
T12		MMS 0.3% + 60 min	+
T13	CoN-07072 (G_2)	EMS 0.2% + 30 min	+++
T14		EMS 0.2% + 60 min	+
T15		EMS 0.3% + 30 min	+
T16		EMS 0.3% + 60 min	- -
T17		SA 0.2% + 30 min	+
T18		SA 0.2% + 60 min	- -
T19		SA 0.3% + 30 min	+++
T20		SA 0.3% + 60 min	- -
T21		MMS 0.2% + 30 min	+
T22		MMS 0.2% + 60 min	- -
T23		MMS 0.3% + 30 min	+
T24		MMS 0.3% + 60 min	- -

+ Good Callus (White Globular)
++ Moderate Callus (Yellowish)
+++ Very Good Callus (Whitish yellow)
- - Poor Callus (Brown)

Plate 1: Quality of callus

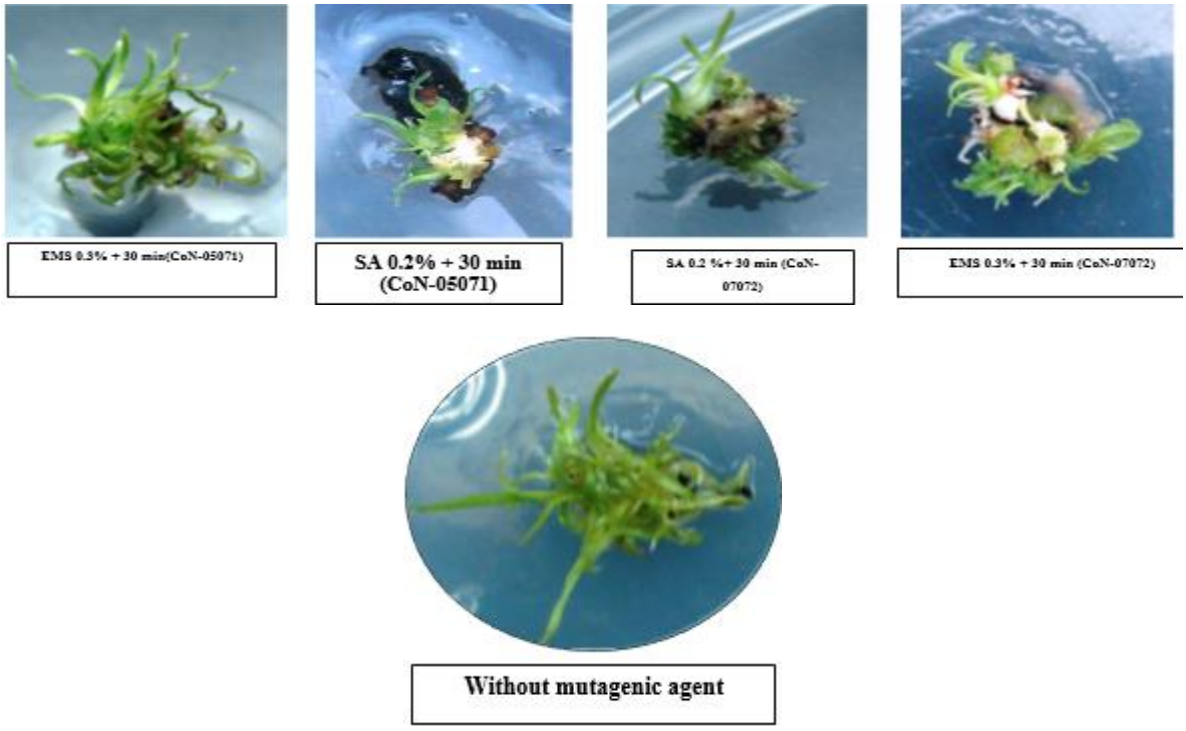

Plate 2: Regeneration of callus

Table 2: Effect of different chemical mutagenic agents on survival per cent

S. No.	Variety (G)	Treatment (C + P)	Mean
T1	CoN-05071 (G_1)	EMS 0.2% + 30 min	72.10
T2		EMS 0.2% + 60 min	68.50
T3		EMS 0.3% + 30 min	90.77
T4		EMS 0.3% + 60 min	74.93
T5		SA 0.2% + 30 min	86.97
T6		SA 0.2% + 60 min	78.13
T7		SA 0.3% + 30 min	38.23
T8		SA 0.3% + 60 min	72.57
T9		MMS 0.2% + 30 min	74.33
T10		MMS 0.2% + 60 min	36.53
T11		MMS 0.3% + 30 min	70.33
T12		MMS 0.3% + 60 min	74.30
T13	CoN-07072 (G_2)	EMS 0.2% + 30 min	78.63
T14		EMS 0.2% + 60 min	70.43
T15		EMS 0.3% + 30 min	67.00
T16		EMS 0.3% + 60 min	40.33
T17		SA 0.2% + 30 min	72.47
T18		SA 0.2% + 60 min	44.33
T19		SA 0.3% + 30 min	72.53
T20		SA 0.3% + 60 min	60.60
T21		MMS 0.2% + 30 min	68.40
T22		MMS 0.2% + 60 min	41.43
T23		MMS 0.3% + 30 min	70.50
T24		MMS 0.3% + 60 min	34.53

SEM ±	G	0.068
	C	0.119
	P	0.068
	G x C	0.168
	C x P	0.168
	G x P	0.097
	G X C X P	0.237
CD at 5 (%)	G	0.195
	C	0.337
	P	0.195
	G x C	0.477
	C x P	0.477
	G x P	0.275
	G X C X P	0.674
CV(%)		0.63
	Without mutagenic agent (G_1)	100
	Without mutagenic agent (G_2)	100

G-Genotypes, C-Chemical and P-Time period

Table 3: Effect of different chemical mutagenic agents on regeneration per cent

S. No.	Variety (G)	Treatment (C + P)	Mean
T1	CoN-05071 (G_1)	EMS 0.2% + 30 min	62.45
T2		EMS 0.2% + 60 min	58.33
T3		EMS 0.3% + 30 min	78.77
T4		EMS 0.3% + 60 min	68.53
T5		SA 0.2% + 30 min	74.25
T6		SA 0.2% + 60 min	66.57
T7		SA 0.3% + 30 min	42.53
T8		SA 0.3% + 60 min	51.24
T9		MMS 0.2% + 30 min	65.80
T10		MMS 0.2% + 60 min	34.20
T11		MMS 0.3% + 30 min	68.17
T12		MMS 0.3% + 60 min	62.32
T13	CoN-07072 (G_2)	EMS 0.2% + 30 min	70.24
T14		EMS 0.2% + 60 min	60.33
T15		EMS 0.3% + 30 min	72.30
T16		EMS 0.3% + 60 min	46.50
T17		SA 0.2% + 30 min	82.45
T18		SA 0.2% + 60 min	51.40
T19		SA 0.3% + 30 min	68.32
T20		SA 0.3% + 60 min	54.69
T21		MMS 0.2% + 30 min	60.85
T22		MMS 0.2% + 60 min	46.30
T23		MMS 0.3% + 30 min	67.55
T24		MMS 0.3% + 60 min	44.47
SEM ±	G		0.040
	C		0.070
	P		0.040
	G x C		0.099
	C x P		0.099
	G x P		0.057
	G X C X P		0.140
CD at 5 (%)	G		0.115
	C		0.199
	P		0.115
	G x C		0.281
	C x P		0.281
	G x P		0.162
	G X C X P		0.398
CV (%)			0.40
	Without mutagenic agent (G_1)		78.00
	Without mutagenic agent (G_2)		72.00

G-Genotypes, C-Chemical and P-Time period

4. Conclusion

Experimental material (callus tissue) of two sugarcane varieties CoN-05071 and CoN-07072 was exposed two different levels of chemical mutagenic agents for different time periods. Callus tissue of 25-30 days old white globular form is consider as suitable explants material to undergo mutagenic treatments. On the basis of visual observation such as colour, firmness, appearance quality of callus is differiated

in to four groups after treatments. Two levels of EMS 0.3% and 0.2% for 30 min and 60 min resulted very good callus appearance in both the varieties. Maximum callus survival percent was found in callus treated with EMS 0.3%+30min (90.77%) in CoN-05071 whereas, EMS 0.2%+30min callus treated was showed maximum survival percent (78.63%) in CoN-07072. Maximum regeneration percent was found in explants treated with EMS 0.3%+30min (78.77%), SA0.2%+30min (82.45%) in CoN-05071 and CoN-07072.

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