

Original Research Article

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Effects of Different Mutagenic Chemicals on Callogenesis in Sugarcane (*Saccharum officinarum*) Clones 2008T42 and 2009T5

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ABSTRACT

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Unexpanded leaf rolls were used as explants for callus induction with different concentrations of mutagenic chemicals (sodium nitrite and ethylmethane sulfonate). The effect of these mutagenic chemicals on callusing, shooting and rooting was studied. Callus induction was observed in different concentrations of sodium nitrite (3 mg l^{-1} , 5 mg l^{-1} and 7 mg l^{-1}) and EMS ($0.6 \text{ }\mu\text{M}$, $0.8 \text{ }\mu\text{M}$, $1.0 \text{ }\mu\text{M}$) along with controls. Among all the treatments 3 mg l^{-1} (T_4) SN and $0.6 \text{ }\mu\text{M}$ EMS (T_{10}) in 2009T5 were found to be the best for callus induction traits. The treatments responded with minimum number of days for callus initiation, maximum number of explants induced callus with high callus induction frequency and high callus size.

Introduction

Sugarcane (*Saccharum officinarum* L.) is one of the economically important crops widely cultivated in the tropics to subtropics and annually provides around 60 to 70% of the world's sugar (Shah *et al.*, 2009). Unfortunately, the production of this crop retained by several biotic and abiotic stresses such as bacterial and fungal diseases, drought, salinity, freezing etc. The improvement of sugarcane plant resistance to these stresses is of great importance. Genetic potential of a variety plays an important role in determining the stress resistance, yield and quality of it. Genetic variability is the key factor in any breeding method. The genetic variability

created through conventional breeding techniques is slow and depend on recombination (Mascarenhas, 1991).

In conventional breeding method, development of elite sugarcane cultivars with high sugar yield and disease resistance are often defeated by tight linkage between cane quality, cane yield and disease resistance etc. The developments in plant tissue culture have opened up new possibilities in creating genetic variability. The use of tissue culture for creation of somaclonal variation can be used to increase the speed of efficiency of the breeding process to improve the accessibility of existing germplasm of sugarcane and create new variation for crop improvement.

Mutagenesis refers to the artificial induction of genetic variation via the use of physical or chemical mutagens (Drake and Koch, 1976). It was first carried out using X-rays in the fruit fly, *Drosophila* spp., by Muller in 1927 (Van Harten, 1998). In plants, various methods which include heat treatment, centrifugation and ageing of seeds, were initially carried out in an attempt to induce mutations (Van Harten, 1998). Ionizing radiation, X-rays, gamma rays and thermal neutrons were later used, but the first attempts resulted in low mutation frequencies and lethal effects on the plants, which were resolved by improving treatment conditions (Novak and Brunner, 1992 and Brunner, 1995). The mechanisms that result in mutations during induced mutagenesis are similar to those that result in spontaneous mutations during *in vitro* culture (Jain *et al.*, 1998). However, the frequency of mutagen-induced mutations is higher than that of spontaneous mutations in *in vitro* culture (Novak and Brunner, 1992). Obtaining desired mutations through the use of mutagens is based on chance and may also result in lethal effects that can disrupt normal plant development (Roane, 1973).

Materials and Methods

Plant Material

Pre-release sugarcane clones, 2008T42 (mid late) and 2009T5 (early) were chosen for the present investigation (fig no 1). The plants were raised and maintained under field conditions as per the recommended agronomic practices. These plants served as the source of explants for all the *in vitro* studies conducted during the course of investigation.

Culture conditions

The cultures were incubated in a culture room maintained at a temperature of $25\pm 2^{\circ}\text{C}$, relative humidity of 70 per cent and 16 hours

of photoperiod with light intensity of 2500 lux. Subcultures were done for every 2-3 weeks according to the need of the experiment.

Somaclonal Induction

Callus culture was initiated from the innermost leaves and leaf sheaths surrounding the apical meristem of 6 months old field grown 2008T42 and 2009T5. The young leaf bits were inoculated with MS basal medium (Murashige and Skoog, 1962) supplemented with 4 mg l^{-1} 2, 4-Dichlorophenoxyacetic acid (2, 4-D) along with different levels of concentrations of mutagenic chemicals (Sodium nitrite and EMS) on induction of somaclones (Table 1). The medium was solidified with 0.8% agar (Hi media). The pH was subjected to 5.8. For the induction of callus the cultures were inoculated in dark. The callus was subcultured three to four times at 15 days interval.

In order to study the effects of different levels of concentrations of mutagenic chemicals on induction of callus different treatments were developed as follows.

Results and Discussion

Treatmental differences were significant for number of days for callus initiation. Time taken for callus initiation ranged from 12.2 to 16.5 days when the medium was treated with different concentrations of sodium nitrite and EMS (Table 4.2). The no. of days for callus initiation in controls are 9.7 (2008T42) and 10.2 (2009T5). Maximum number of days (16.5) taken for callus initiation was recorded with 7 mg l^{-1} (T_3) followed by 16.1 days with 5 mg l^{-1} (T_2) in SN treatments (Plate no.2). Whereas minimum number of days (12.2) for callus initiation was recorded with $0.6\text{ }\mu\text{M}$ (T_7) followed by 12.3 days with $0.8\text{ }\mu\text{M}$ (T_8) in EMS treatments (T_8). With respect to

sodium nitrate maximum number of days taken for callus initiation was observed with 7 mg l⁻¹ in 2008T42 (T₃) followed by 5 mg l⁻¹ (T₂) in 2008T42 and minimum with 3 mg l⁻¹ in 2009T5 (T₄) followed by 5 mg l⁻¹ in (T₅) in 2009T5 (Plate no.2). With respect to EMS, 1.0 μM in 2009T5 (T₁₂) recorded maximum number of days for callus initiation followed by 0.8 μM in 2009T5 (T₁₁) and minimum with 0.6 μM in 2008T42 (T₇) followed by 0.8 μM in 2008T42 (T₈) (Plate.no. 1). Both the clones took minimum number of days for callus initiation with 3 mg l⁻¹ SN and 0.6 μ M BMS. Increase in the concentration of two mutagens delayed the time taken for callus initiation.

The mean number of explants inducing callus for different concentrations of sodium nitrite and EMS ranged from 3.67 to 5.67 (Table 1) as against 6.00 in the both controls, 2008T42 (C₁) and 2005T9 (C₂). Significant differences among treatments were observed.

Maximum mean number of explants induced callus (5.67) was recorded with 3 mg l⁻¹ of sodium nitrate (T₄) followed by 5.33 with 0.6 μM of EMS (T₁₀, T₇) and 3 mg l⁻¹ of sodium nitrate (T₁) and minimum was recorded as 3.67 with 7 mg l⁻¹ of sodium nitrate (T₆) followed by 4.00 with 7 mg l⁻¹ of sodium nitrate (T₃) in both sugarcane clones of 2008T42 and 2005T5.

With respect to sodium nitrate for both clones, 2009T5 recorded maximum mean number of explants induced callus (5.67) at 3 mg l⁻¹ (T₄) followed by 2008T42 (5.33) at 3 mg l⁻¹ (T₁) concentration and minimum mean number of explants induced callus was observed in 2009T5 (3.67) with 7 mg l⁻¹ (T₆) followed by 2008T42 (4.00) at 7 mg l⁻¹ (T₃) (Plate no.2). With respect to EMS, both clones recorded maximum mean number of explants induced callus (5.33) at 0.6 μM (T₇, T₁₀) followed by 2009T5 (5.00) with 0.8 μM (T₁₁) and minimum was observed with 1.0 μM (T₉) in

2008T42 (4.00) followed by 2008T42 (4.33) and 2009T5 (4.33) with 0.8 μM (T₈) and 1.0 μM (T₁₂) concentrations respectively (Plate no.1).

The treatments differed significantly for callus induction frequency. Callus induction frequency ranged from 63.44 to 91.59% for different concentrations of sodium nitrite and EMS in both the clones. It was 100% in both the controls. Maximum callus induction frequency (91.59%) was recorded with 3 mg l⁻¹ of sodium nitrate (T₄) followed by 88.52% with 3 mg l⁻¹ of sodium nitrate (T₁) and the minimum was recorded as 63.44% with 7 mg l⁻¹ of sodium nitrate (T₆) followed by 67.64% with 1.0 μM of EMS (T₉). With respect to sodium nitrate, 2009T5 recorded maximum callus induction frequency (91.59%) at 3 mg l⁻¹ (T₄) followed by 2008T42 (88.52%) at 3 mg l⁻¹ (T₁) concentration and minimum callus induction frequency was observed in 2009T5 (63.44%) with 7 mg l⁻¹ (T₆) followed by 2008T42 (67.72%) at 7 mg l⁻¹ (T₃) (Plate.no.2). In case of EMS, the genotype, 2009T5 recorded maximum callus induction frequency (87.03%) at 0.6 μM (T₁₀) followed by 2008T42 (85.63%) with 0.6 μM (T₇) and minimum callus induction frequency was observed with 1.0 μM (T₉) in 2008T42 (67.64%) followed by 2008T42 (71.06%) with 0.8 μM (T₈) (Plate.no.1).

The mean callus size was estimated as 2.8 and 3.0 cm in controls, 2008T42 (C₁) and 2005T9 (C₂), respectively. The mean callus size ranged from 1.1 - 2.3 cm for different concentrations of sodium nitrite and EMS (Table 1). Differences among treatments were significant. Maximum mean callus size (2.3 cm) was recorded with 0.6 μM of EMS (T₁₀) followed by 2.1 cm with 0.6 μM of EMS (T₇) and the minimum was recorded as 1.1 cm with 7 mg l⁻¹ of sodium nitrate (T₆) followed by 1.2 cm with 1.0 μM of EMS (T₉) and 7 mg l⁻¹ of sodium nitrate (T₃).

Table.1a

Treatments	Variety	MS media + 4mg l ⁻¹ 2, 4-D + Sodium nitrite (mg l ⁻¹)	MS media + 4mg l ⁻¹ 2, 4-D +EMS (μM)
T ₁	2008T42	3	0
T ₂	2008T42	5	0
T ₃	2008T42	7	0
T ₄	2009T5	3	0
T ₅	2009T5	5	0
T ₆	2009T5	7	0
T ₇	2008T42	0	0.6
T ₈	2008T42	0	0.8
T ₉	2008T42	0	1.0
T ₁₀	2009T5	0	0.6
T ₁₁	2009T5	0	0.8
T ₁₂	2009T5	0	1.0
C ₁	2008T42	0	0
C ₂	2009T5	0	0

Table.1b Effect of different concentrations of mutagenic chemicals on callusing in two sugarcane clones

Treatments	Mean no. of days for callus initiation	Mean no. of explants induced callus	Callus induction frequency (%)	Mean callus size (cm)
SN 3 mg l ⁻¹ 2008T42 (T ₁)	15.7	5.33	88.52 (70.17)	1.9
SN 5 mg l ⁻¹ 2008T42 (T ₂)	16.1	5.00	83.21 (65.78)	1.4
SN 7 mg l ⁻¹ 2008T42 (T ₃)	16.5	4.00	67.72 (55.35)	1.2
SN 3 mg l ⁻¹ 2009T5 (T ₄)	13.6	5.67	91.59 (73.14)	2.0
SN 5 mg l ⁻¹ 2009T5 (T ₅)	14.0	5.00	81.23 (64.32)	1.5
SN 7 mg l ⁻¹ 2009T5 (T ₆)	15.2	3.67	63.44 (52.77)	1.1
EMS 0.6 μM l ⁻¹ 2008T42 (T ₇)	12.2	5.33	85.63 (67.76)	2.1
EMS 0.8 μM l ⁻¹ 2008T42 (T ₈)	12.3	4.33	71.06 (57.36)	1.6
EMS 1.0 μM l ⁻¹ 2008T42 (T ₉)	12.7	4.00	67.64 (55.30)	1.2
EMS 0.6 μM l ⁻¹ 2009T5 (T ₁₀)	13.3	5.33	87.03 (68.89)	2.3
EMS 0.8 μM l ⁻¹ 2009T5 (T ₁₁)	13.5	5.00	81.88 (64.71)	1.9
EMS 1.0 μM l ⁻¹ 2009T5 (T ₁₂)	13.7	4.33	71.11 (57.46)	1.6
2008T42 (Control) (C ₁)	9.7	6.00	100.0 (90.00)	2.8
2009T5 (Control) (C ₂)	10.2	6.00	100.0 (90.00)	3.0
C.D at 5%	0.187	0.343	2.119	0.091
(±) SE(m)	0.064	0.118	0.728	0.031

Plate.1 Callus induction in different concentrations of EMS with controls in sugarcane clone



C₁(2008T42)



C₂(2009T5)



T₇(EMS 0.6 μ M 2008T42)



T₁₀(EMS 0.6 μ M 2009T5)

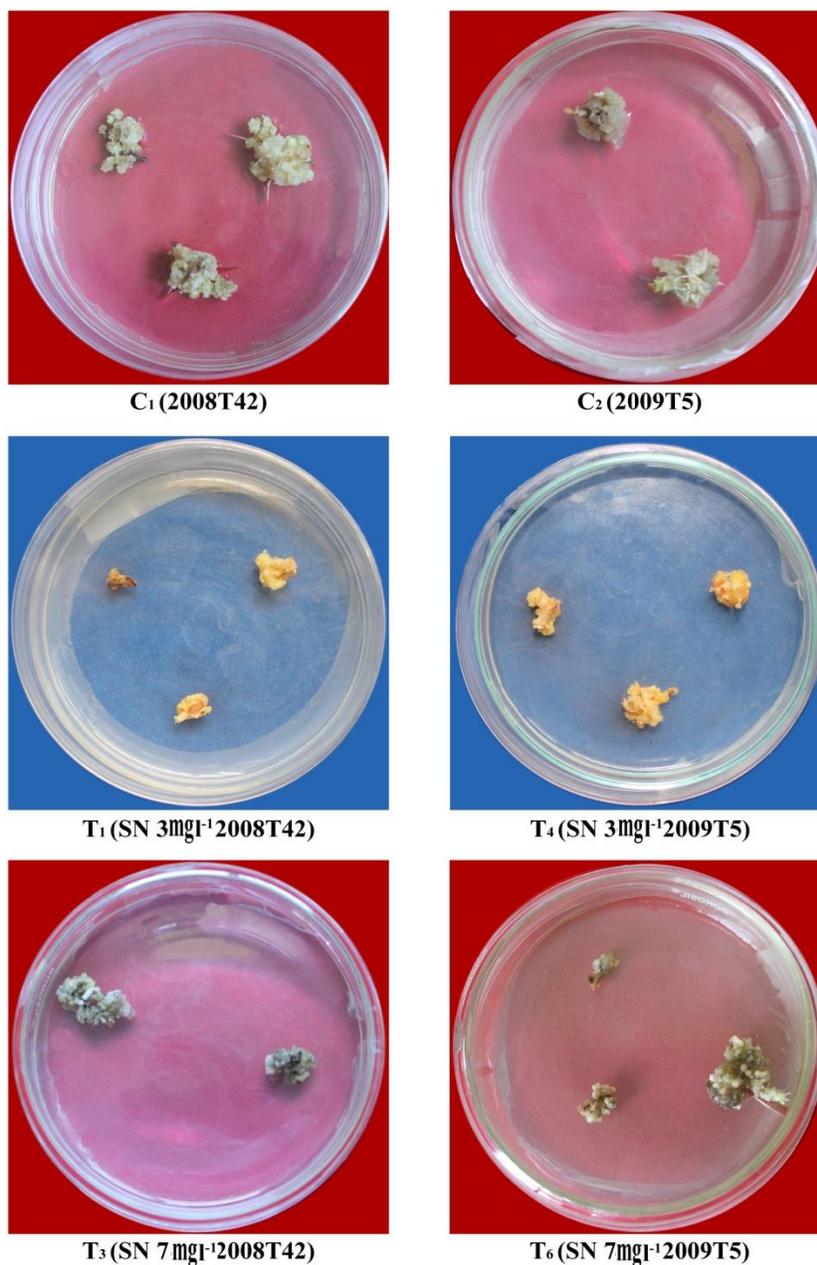


T₉(EMS 1.0 μ M 2008T42)



T₁₂(EMS 1.0 μ M 2009T5)

Plate.2 Callus induction in different concentrations of sodium nitrite with controls in sugarcane clone



With respect to sodium nitrate, 2009T5 recorded maximum mean callus size (2.0 cm) at 3 mg l⁻¹ (T₄) followed by 2008T42 (1.9 cm) at 3 mg l⁻¹ (T₁) concentration and minimum mean callus size was observed in 2009T5 (1.1 cm) with 7 mg l⁻¹ (T₆) followed by 2008T42 (1.2 cm) at 7 mg l⁻¹ (T₃) (Plate.no.2). In case

of EMS, 2009T5 recorded maximum mean callus size (2.3 cm) at 0.6 μM (T₁₀) followed by 2008T42 (2.1 cm) with 0.6 μM (T₇) and minimum mean callus size was observed with 1.0 μM (T₉) in 2008T42 (1.2 cm) followed by 2008T42 (1.6 cm) and 2009T5 (1.6 cm) with 0.8 μM (T₈) and 1.0 μM (T₁₂) concentrations,

respectively (Plate.no.1). Maximum callus production was observed in the controls and callus production decreased with increase in concentration of mutagenic chemicals. Yasmeen, *et al.*, (2013) and Khan, *et al.*, (2009) reported stimulation in callus growth at lower doses and poor stimulation in high doses of mutagenic chemicals. Bajaj *et al.*, (1970) and Siddiqui & Javed (1982) also reported stimulation in callus growth at lower doses of gamma irradiation. Callus induction was reported to be cent per cent in controls without EMS and sodium nitrite. Similar kind of results were also reported by Begum *et al.*, (2011). With the increase in the concentration of the mutagen there was an increase in number of days required for the callus initiation. Gahukar and Jambhule (2000) also found similar type of decrease in callus obtained with increased dose of gamma rays and EMS in sugarcane. With the increased level of these chemicals the percentage of callus initiation decreased. This was also supported by Munsamy *et al.*, (2013) and Sung (1976) who had reported a decrease in survival of soybean cell suspension cultures with increasing concentrations of EMS. The callus size also decreased with an increase in concentration of sodium nitrite and EMS in both clones. Similar decrease in callus size was observed by Reddy *et al.*, (1987) in castor bean and by Singh and Singh (1993) in sugarcane with the increase in the dose of gamma irradiation. The present results agree with those reported by Ather *et al.*, (2009), Taghian (1998) and Raza *et al.*, (2010) for sugarcane.

Maximum callus production was observed in the controls and callus production decreased with increase in concentration of mutagenic chemicals. There was an increase in number of days required for callus initiation, a decrease in callus induction frequency and also a decrease in callus size with an increase in concentration of sodium nitrite and EMS in

both clones. EMS was proved to produce better response than sodium nitrite in both clones. The results revealed that EMS rather than sodium nitrite was found to have better response for callus induction in both 2008T42 and 2009T5 sugarcane clones. Because EMS showed callus induction with lowest mean number days for callus initiation, maximum number of explants induced callus with highest callus induction frequency and good callus size. In both clones, 2009T5 showed better response in both mutagenic chemicals when compared with 2008T42 callus induction.

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