

Original Research Article

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Regeneration of Sugarcane (*Saccharum officinarum* L.) Clones from Mutagenic Treated Calli

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ABSTRACT

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An experiment was carried out at the Agricultural Research Station, Perumallapalle for the regeneration of somaclones. Two sugarcane clones, 2008T42 (susceptible to red rot) and 2009T5 (susceptible to smut) were used as plant material. Calli from the controls and different concentrations of mutagenic chemicals (sodium nitrite and EMS) were transferred on to MS media with 3 mg l⁻¹ BAP, 2 mg l⁻¹ IAA and 2 mg l⁻¹ kinetin for evaluating the effect on shooting. In various treatments, 0.6 μM EMS in 2009T5 (T₁₀) exhibited good response for shoot induction as evident by minimum time taken for shoot initiation, more shoot number, frequency and shoot length. Full grown shoots were transferred on to rooting medium with 3 mg l⁻¹ NAA for initiation and elongation of roots to study the effect of mutagenic chemicals on root induction. 0.6 μM EMS in 2009T5 (T₁₀) was found to be better for root induction among all the treatments. It has produced more number of roots with higher length of root in less time.

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).

For most grasses like sugarcane, callus induction and plant regeneration from the induced callus is not only time consuming but can cause somaclonal variation (Spangenberg *et al.*, 1998; Choi *et al.*, 2000 and Goldman *et al.*, 2004). As discussed by Lakshmanan *et al.*, (2006), the first report of large variations in both chromosome number and morphological traits in sugarcane plants regenerated from callus was by Heinz and Mee (1969). Since then, there have been frequent reports of genetic variability in tissue-cultured sugarcane (Lourens and Martin, 1987; Burner and Grisham, 1995; Taylor *et al.*, 1995 and Hoy *et al.*, 2003), and numerous studies to assess the extent of variability arising from *in vitro* regeneration and its transmission into successive generations *via* vegetative

propagation (Lourens and Martin, 1987 and Burner and Grisham, 1995). These investigations demonstrated that large amounts of somaclonal variability occur in *in vitro* derived propagules, irrespective of the method of regeneration.

Materials and Methods

Pre-release sugarcane clones, 2008T42 (mid late) and 2009T5 (early) were chosen for the present investigation (Plate 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. 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.

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.

Calli cultured on MS medium supplemented with different levels of mutagenic chemicals (sodium nitrite and EMS) were transferred on to MS medium supplemented with BAP (3 mg

l^{-1}) + IAA (2 mg l^{-1}) + Kinetin (2 mg l^{-1}). Full grown shoots were transferred on to half-strength MS medium supplemented with NAA (3 mg l^{-1}). In order to study the effects of callus derived from different levels of concentrations of mutagenic chemicals on regeneration (Table 1).

Results and Discussion

Calli from the controls and different concentrations of mutagenic chemicals (sodium nitrite and EMS) were transferred on to MS media with 3 mg l^{-1} BAP, 2 mg l^{-1} IAA and 2 mg l^{-1} kinetin for evaluating their effect on shooting. 2009T5 was found to be early in response to sodium nitrite treatments (T_4) whereas in case of EMS, 2008T42 (T_7) showed better response when compared with 2009T5 in recording lower number of days for shoot initiation (15.4 days). Shooting frequency of 85.40 per cent and 85.23 per cent was found to be high for 2009T5 in sodium nitrite (T_4) and EMS (T_{10}), respectively when compared with 2008T42. EMS was observed to be better than SN in recording high shooting frequency in both the clones. EMS (T_{10}) recorded lower number of days (59.8 days) for complete shooting when compared with sodium nitrite. In both clones, 2009T5 exhibited earlier shooting than 2008T42 in sodium nitrite and EMS. The data revealed significant differences among the treatments for all the parameters studied under shooting (Plate No. 2 & 3).

It was observed that gradation in concentration of sodium nitrite and EMS treatments had a profound effect and gradual reduction in survival, growth and regeneration capacity of two sugarcane clones. Regeneration potential was inversely proportional to the concentration of mutagenic treatments (Alain *et al.*, 2002). For mean number of days for shoot initiation and for complete shooting an increase with increased level of concentration

of both sodium nitrite and EMS was recorded. These results are in agreement with the previous findings in sugarcane, banana, chrysanthemum, petunia and soybean (Siddiqui and Javeed, 1982; Omar *et al.*, 1989; Bhagwat and Duncan, 1997; Latado *et al.*, 2004 and Berenschot *et al.*, 2008). Khawale *et al.*, (2007) also reported a decline in the regeneration capacity of shoots in grapevine as the dose of mutagenic agent increased. Data were recorded on number of shoots produced per explants and length of shoots at 35 days and 45 days in two sugarcane clones whose explants were treated with different concentrations of SN and EMS. Maximum number of shoots per explant (16.67 and 14.37) and more length of shoot (4.0 and 7.4 cm) were observed in EMS treatments (T_{10}) when compared with sodium nitrite treatments at both 35 days and 45 days. Among two clones, 2008T42 showed better response with sodium nitrite (T_1), whereas, 2009T5 exhibited better response for number shoot per explants and shoot length with EMS (T_{10}) (Plate No. 2 & 3).

The results revealed reduction in the number of regenerated shoots and shoot length with increasing concentrations of sodium nitrite and EMS. Similar result was reported by Omar *et al.*, (1989). Using radiation induced variation in Chrysanthemum, Misra and Datta (2007) reported a reduction number of shoots per explant with the increase in radiation dose. The maximum number of plantlets regeneration was observed in the control treatments. This result is in agreement with Yasmeen, *et al.*, (2013). The reasons could be attributed to the presence of non-viable cells in the mutagenic treated callus, which later on affected the formation of shoots. This is in agreement with the findings of Khan *et al.*, (2007). The effect of sodium nitrite and EMS on number of shoot buds per culture was similar to that of days required for shoot differentiation.

Table.1

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.2 Effect of mutagenic chemicals on shooting in two sugarcane clones

Treatments	Mean no. of days for shoot initiation	Shoot regeneration frequency (%)	Mean no. of days taken for completing shooting
SN 3 mg l ⁻¹ 2008T42 (T ₁)	20.2	80.80 (63.98)	62.9
SN 5 mg l ⁻¹ 2008T42 (T ₂)	20.6	64.00 (52.81)	76.1
SN 7 mg l ⁻¹ 2008T42 (T ₃)	21.3	63.05 (53.72)	82.0
SN 3 mg l ⁻¹ 2009T5 (T ₄)	17.5	85.40 (67.51)	62.6
SN 5 mg l ⁻¹ 2009T5 (T ₅)	18.4	70.44 (57.04)	73.2
SN 7 mg l ⁻¹ 2009T5 (T ₆)	19.7	65.17 (53.80)	75.4
EMS 0.6 μM l ⁻¹ 2008T42 (T ₇)	15.4	83.13 (66.72)	61.7
EMS 0.8 μM l ⁻¹ 2008T42 (T ₈)	16.1	71.33 (57.60)	68.5
EMS 1.0 μM l ⁻¹ 2008T42 (T ₉)	17.6	65.16 (53.80)	71.6
EMS 0.6 μM l ⁻¹ 2009T5 (T ₁₀)	17.6	85.23 (67.37)	59.8
EMS 0.8 μM l ⁻¹ 2009T5 (T ₁₁)	19.2	72.75 (58.51)	62.6
EMS 1.0 μM l ⁻¹ 2009T5 (T ₁₂)	19.3	66.33 (54.50)	66.1
2008T42 (Control) (C ₁)	13.0	96.22 (78.85)	58.1
2009T5 (Control) (C ₂)	15.0	98.97 (84.73)	53.0
C.D at 5%	1.508	1.997	3.432
(±) SE(m)	0.518	0.686	1.179

Values in parentheses represent arc sine transformed values

SN - Sodium nitrite,

EMS-Ethyl methane sulfonate

Table.3 Effect of mutagenic chemicals on mean no. of shoots per explants and mean length of shoot in two sugarcane clones

Treatments	Mean no. of shoots per explant at 35 days	Mean length of shoot (cm) at 35 days	Mean no. of shoots per explant at 45 days	Mean length of shoot (cm) at 45 days
SN 3 mg l ⁻¹ 2008T42 (T ₁)	14.89	3.6	12.68	6.6
SN 5 mg l ⁻¹ 2008T42 (T ₂)	9.89	2.3	7.00	5.3
SN 7 mg l ⁻¹ 2008T42 (T ₃)	7.56	1.6	5.61	4.6
SN 3 mg l ⁻¹ 2009T5 (T ₄)	11.22	3.5	9.87	6.5
SN 5 mg l ⁻¹ 2009T5 (T ₅)	8.00	2.2	6.96	5.2
SN 7 mg l ⁻¹ 2009T5 (T ₆)	7.22	1.5	5.37	4.5
EMS 0.6 μM l ⁻¹ 2008T42 (T ₇)	11.56	3.8	10.09	6.7
EMS 0.8 μM l ⁻¹ 2008T42 (T ₈)	10.11	2.4	7.23	5.3
EMS 1.0 μM l ⁻¹ 2008T42 (T ₉)	8.22	1.9	6.73	4.8
EMS 0.6 μM l ⁻¹ 2009T5 (T ₁₀)	16.67	4.0	14.37	7.4
EMS 0.8 μM l ⁻¹ 2009T5 (T ₁₁)	12.22	2.7	10.83	5.4
EMS 1.0 μM l ⁻¹ 2009T5 (T ₁₂)	9.44	2.0	7.67	4.8
2008T42 (Control) (C ₁)	21.44	4.1	20.94	8.4
2009T5 (Control) (C ₂)	30.00	4.5	27.91	8.5
C.D at 5%	1.008	0.168	0.376	0.354
(±) SE(m)	0.346	0.058	0.129	0.122

SN - Sodium nitrite; EMS-Ethyl methane sulfonate

Table.4 Effect of mutagenic chemicals on rooting in two sugarcane clones

Treatments	Mean no. of days taken for root initiation	Mean no. of roots per shoot	Rooting frequency (%)	Mean length of root (cm)
SN 3 mg l ⁻¹ 2008T42 (T ₁)	14.0	20.37	76.30 (60.87)	3.3
SN 5 mg l ⁻¹ 2008T42 (T ₂)	17.5	17.03	67.63 (55.30)	2.5
SN 7 mg l ⁻¹ 2008T42 (T ₃)	21.3	14.00	63.01 (52.52)	1.7
SN 3 mg l ⁻¹ 2009T5 (T ₄)	14.3	19.67	74.16 (59.40)	3.2
SN 5 mg l ⁻¹ 2009T5 (T ₅)	17.7	16.85	67.33 (55.12)	2.3
SN 7 mg l ⁻¹ 2009T5 (T ₆)	21.7	13.46	62.30 (52.10)	1.4
EMS 0.6 μM l ⁻¹ 2008T42 (T ₇)	13.2	21.00	80.60 (63.93)	3.6
EMS 0.8 μM l ⁻¹ 2008T42 (T ₈)	15.7	19.00	70.28 (56.87)	3.0
EMS 1.0 μM l ⁻¹ 2008T42 (T ₉)	17.6	15.00	68.58 (55.82)	2.5
EMS 0.6 μM l ⁻¹ 2009T5 (T ₁₀)	10.7	24.17	83.07 (65.79)	3.8
EMS 0.8 μM l ⁻¹ 2009T5 (T ₁₁)	12.7	18.37	70.42 (57.02)	3.1
EMS 1.0 μM l ⁻¹ 2009T5 (T ₁₂)	15.7	16.67	68.72 (55.97)	2.5
2008T42 (Control) (C ₁)	8.7	27.67	89.43 (71.36)	4.0
2009T5 (Control) (C ₂)	7.4	29.03	90.27 (72.40)	4.3
C.D at 5%	0.815	1.474	4.625	0.187
(±) SE(m)	0.280	0.506	1.588	0.064

Values in parentheses represent arc sine transformed values; SN - Sodium nitrite; EMS-Ethyl methane sulfonate

Plate.1 Sugarcane clones of 2008T42 and 2009T5



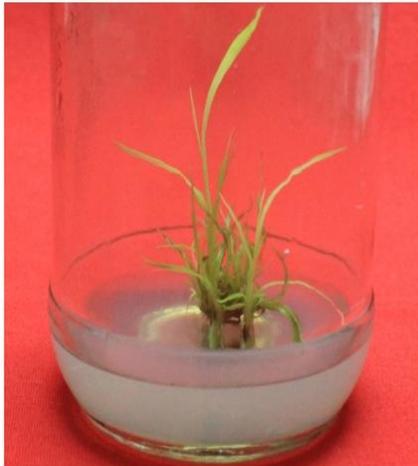
Plate.2 Shoot induction in different concentrations of EMS with control in sugarcane clones



C₁(2008T42)



C₂(2009T5)



T₇(EMS 0.6 μM 2008T42)



T₁₀(EMS 0.6 μM 2009T5)



T₉(EMS 1.0 μM 2008T42)



T₁₂(EMS 1.0 μM 2009T5)

Plate.3 Shoot induction in different concentrations of sodium nitrite with control in sugarcane clones



C₁(2008T42)



C₂(2009T5)



T₁(SN 3 mg l⁻¹ 2008T42)



T₄(SN 3 mg l⁻¹ 2009T5)



T₃(SN 7 mg l⁻¹ 2008T42)



T₆(SN 7 mg l⁻¹ 2009T5)

Plate.4 Root induction in different concentrations of EMS with control in sugarcane clones



C₁ (2008T42)



C₂ (2009T5)



T₇ (EMS 0.6 μM 2008T42)



T₁₀ (EMS 0.6 μM 2009T5)

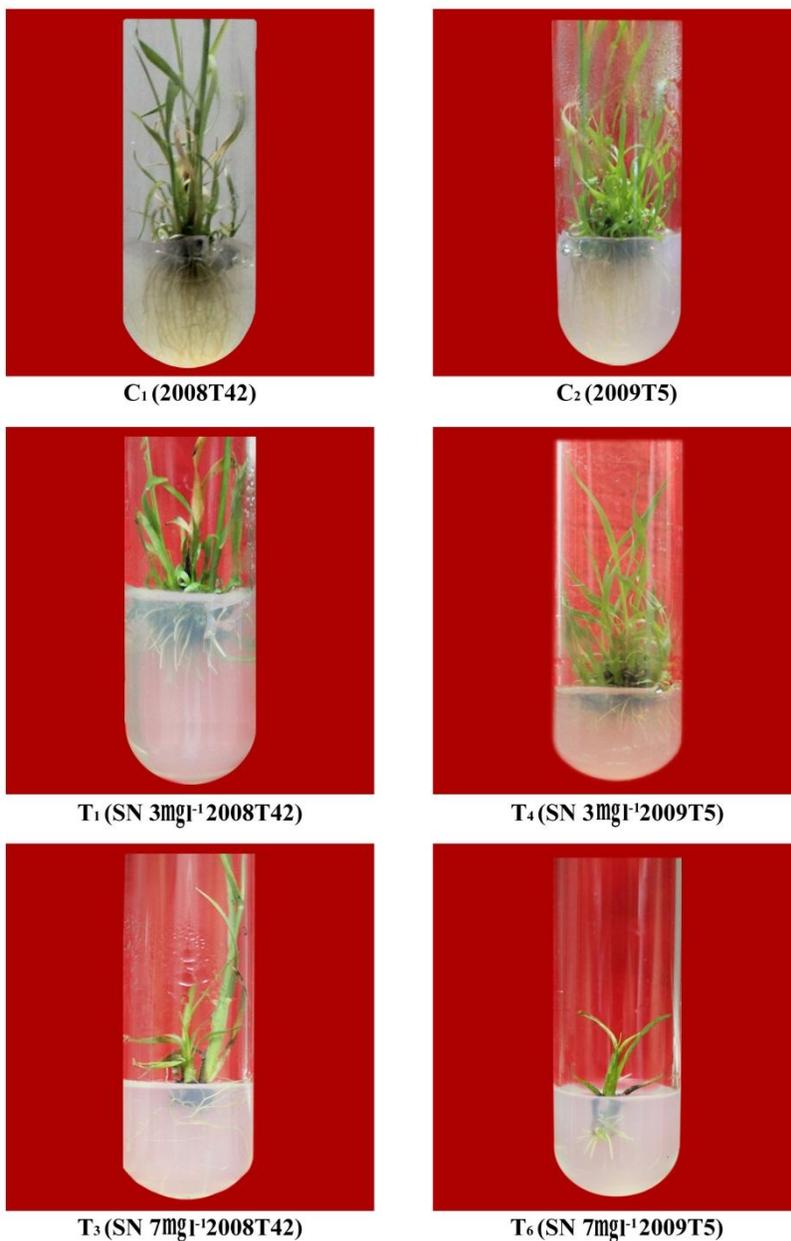


T₉ (EMS 1.0 μM 2008T42)



T₁₂ (EMS 1.0 μM 2009T5)

Plate.5 Root induction in different concentrations of sodium nitrite with control in sugarcane clones



Full grown shoots were transferred on to rooting medium for initiation and elongation of roots and the effect of mutagenic chemicals on root induction was studied. Among both the clones, 2008T42 (T₁) was found to have taken less number days (14.0 days) for root initiation than 2009T5 in sodium nitrite treatments. Whereas in case of EMS

treatments, 2009T5 (T₁₀) was found to be early (10.7 days) than 2008T42 (T₇ with 13.2 days). Among the two mutagenic chemicals, EMS (T₁₀) was better in response than sodium nitrite with maximum mean number of roots per shoot (24.17) and better rooting frequency (83.07). 2008T42 (20.37) showed better response in sodium nitrite (T₁) than 2009T5

while in EMS, 2009T5 (T₁₀) was better than 2008T42 with respect to mean number of roots per shoot (24.17) and rooting frequency (83.07 per cent). EMS showed the maximum mean length of roots (3.8 cm) in 2009T5 (T₁₀) and 200T42 than SN. The clone, 2008T42 exhibited maximum mean length of roots (3.3 cm) in sodium nitrite (T₁) than EMS whereas 2009T5 (T₁₀) showed good response in EMS than sodium nitrite with respect to root length (3.8 cm) (Plate No. 4 & 5). Significant difference was observed between these treatments. These results are in agreement with results obtained previously, (Shomeili, 2011 and Bhatnagar-Mathur *et al.*, 2008).

Increase in concentrations of sodium nitrite and EMS had a gradual reduction in survival, growth and regeneration capacity of two sugarcane clones. Regeneration potential was inversely proportional to the concentration of mutagenic chemicals. EMS was found to produce good response on shooting than sodium nitrite. 2009T5 exhibited good response in shooting than 2008T42 in both sodium nitrite and EMS.

At 35 days and 45 days in two sugarcane clones culture time had detrimental effect on mean number of shoots per explants even though the mean length of shoot increased. The effect of mutagenic chemicals on shoot number was similar to the effect on days required for shoot differentiation. In general, there was a reduction in the number of regenerated shoots and shoot length with increasing concentrations of SN and EMS. The maximum number of plantlets regeneration was observed in the control treatments. The reasons could be attributed to the presence of non-viable callus in the mutagenic treated callus which later on affected the formation of shoots.

Shoot obtained from sodium nitrite and EMS treated calli showed less response for root

differentiation and took more number of days to differentiate roots as compared with the shoots obtained from calli without treatment. Both root length and mean number of rooted shoots decreased with increase in concentration of mutagenic chemicals. Among different treatments evaluated, EMS (T₁₀) was regarded as the best in all treatments of 2009T5 and 2008T42 for the different parameters; mean number of days taken for root initiation, mean number of roots per shoot, rooting frequency and mean length of roots.

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