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# Effect of 2, 4 D and EMS On *in vitro* Regeneration in Sugarcane Cultivar, Co86032

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**A B S T R A C T**

The present studies were carried out at Agricultural Research station, Perumallapalle to establish an efficient system for *in vitro* regeneration of sugarcane cultivar, Co86032 using chemical mutagens; Ethyl methane sulphonate (EMS) and 2, 4-dichlorophenoxy acetic acid (2, 4 D). Young leaf rolls were used as explants for callus induction on MS medium containing different concentrations of EMS (0.6 µM $l^{-1}$, 0.8 µM $l^{-1}$ and 1.0 µM $l^{-1}$) and 2, 4-D (4mg $l^{-1}$, 5mg $l^{-1}$ and 6 mg $l^{-1}$). Among the different concentrations, Co86032 had taken minimum number of days (11.89) for callus induction with 2, 4 D @ 4mg $l^{-1}$, recording maximum mean callus size of 2.19 cm with high callus induction frequency (96.30%) and maximum mean number of explants inducing callus (2.78) in addition with superior response in shooting, rooting and hardening characters than EMS. EMS and 2, 4 Dat higher concentration (1.0 µM $l^{-1}$ and 6mg $l^{-1}$, respectively) had inhibitory effect on callusing, shooting and rooting and produced morphological traits variation which can be exploited in breeding programs.

## Introduction

Sugarcane is an important commercial crop in many developing/developed countries. Considering its importance in the agricultural industry, concerted efforts are being made for its improvement using conventional and biotechnological techniques. There are many reports on tissue culture and plant regeneration methods in sugarcane. Initial attempts to regenerate plants through *in vitro* technique were made on sugarcane by Nickell (1964) and Heinz *et al.*, (1969). Genetic variability apart from conventional breeding can be easily discernible in *in vitro* regenerated somaclonal variants. Tissue culture techniques through mutation induction can be used to increase the speed or efficiency of breeding programs to get a new diversity of germplasm (Jain 2010). Mutation induction can be done by using

chemical mutagens and also with use of growth regulators which have high activity, such as 2, 4 D (Smiullah *et al.,* 2012; Abdullah *et al.,* 2013). Chemicals induce mainly point mutations, and are thus ideal for producing missense and nonsense mutations, which would provide a series of change-of-function mutations (Talebi *et al.,* 2012).

The occurrence of somaclonal variation in tissue culture derived plants is an alternative method to sort out many barriers of traditional breeding program. In addition to rejuvenation of sugarcane clones different chemical mutagens can be applied to increase the frequency of variations. Somaclones were developed eliminating certain defects like leaf spines (Co7717), leaf drying (Co7704) apart from resistance to biotic and abiotic stresses. The sugarcane variety, Co86032 is a popular variety extensively cultivated in India. Creating useful variation in the popular variety in terms of its internode traits as well as quality traits will be worth full as it was used in many breeding programmes. Hence an attempt was made to standardised the production protocol for somaclonal variation using a growth hormone 2, 4 D and EMS chemical mutagen. Therefore, the present investigation has been undertaken to establish plant regeneration protocol through young leaf roll culture under different chemical mutagenic concentrations in sugarcane.

## Materials and Methods

The experimental work was carried out at Agricultural Research station, Perumallapalle with young leaf rolls of popular sugarcane variety, Co86032. Surface sterilization of sugarcane spindle was carried out after excision and removing of young leaves using liquid detergent water containing a few drops of Tween-20 (20 ml $l^{-1}$) for 5-10 minutes. After these treatments, explants were washed again with sterile deionising water for 3-5 washings, each for 3-5 minutes. After these initial washings, explants were kept in an aqueous mixture solution of bavistin (10 g $l^{-1}$) and streptomycin (1 g $l^{-1}$) for 10-15 minutes and then washed thoroughly in sterile deionising water for 3-5 washings, each for 3-5 minutes. Sterilization with sodium hypochlorite (3.0%) was done for 10 minutes in laminar flow hood followed by sterilization with ethyl alcohol (70%) for 30 seconds and washed with sterile distilled water for 2-3 times.

The effect of different concentrations of mutagenic chemicals, 2, 4 D (4mg $l^{-1}$, 5mg $l^{-1}$ and 6 mg $l^{-1}$) and EMS (0.6 µM $l^{-1}$, 0.8 µM $l^{-1}$ and 1.0 µM $l^{-1}$) on *in vitro* regeneration from leaf roll explants was studied using M.S media supplemented with mutagens. Transverse sections (1-2 cm) were made from young, innermost tightly furled leaves to obtain smart setts (leaves discs) and were placed in the petriplate containing callus induction media with different concentrations of chemical mutagens. Calli cultured on M.S medium supplemented with different levels of mutagenic chemicals were transferred on to M.S medium supplemented with BAP (3 mg $l^{-1}$) + IAA (2 mg $l^{-1}$) + Kinetin (2 mg $l^{-1}$) for shoot initiation and growth. Full grown shoots were transferred on to half-strength M.S medium supplemented with NAA (3 mg $l^{-1}$) for root initiation and growth. Tissue culture (TC) seedlings raised *in vitro* with well developed roots and shoots were carefully transferred to polythene covers containing coconut peat: soil: sand in the ratio of 1:1:1 and kept in a shade net house for 15-20 days and relative humidity was maintained. Data on callusing, shooting, rooting and hardening was recorded.

## Results and Discussion

Treatment differences were significant for number of days taken for callus induction.

Time taken for callus induction ranged from 11.89 to 15.33 days when the medium was treated with different concentrations of two chemicals (Table 1). The number of days taken for callus induction in control was 10.22 days. Minimum number of days (11.89) taken for callus induction was recorded with 2, 4-dichlorophenoxy acetic acid (2, 4 D) @ 4 $mgl^{-1}$and EMS @ 0.6 µM $l^{-1}$ followed by 13.3 days with 2, 4 D @ 5 mg $l^{-1}$ and EMS @ 0.8 µM $l^{-1}$ whereas maximum number of days (15.33) taken for callus induction was observed with EMS @ 1.0 µM $l^{-1}$ followed by 14.11days with 2, 4 D @ 6 mg $l^{-1}$ (Fig. 1). The mean number of explants inducing callus ranged from 1.88 to 2.78 (Table 1) as against 3.00 in the control. Maximum mean number of explants inducing callus (2.78) with 2, 4 D @ 4 mg $l^{-1}$and EMS @ 0.6 µM $l^{-1}$ followed by 2.59 with EMS @ 0.6 µM $l^{-1}$ was recorded and minimum mean number of explants inducing callus was observed as 1.88 with EMS @ 1.0 µM $l^{-1}$ followed by 2.00 with 2,4 D @ 6 mg $l^{-1}$ concentrations. Callus induction frequency ranged from 59.30 to 96.30 % for different concentrations of chemicals. 2, 4 D @ 4 mg $l^{-1}$recorded maximum callus induction frequency (96.30%) followed by 88.85 % with EMS @ 0.6 µM $l^{-1}$ concentration and minimum callus induction frequency of 59.30 % was observed with EMS @ 1.0 µM $l^{-1}$followed by 70.37 % with 2,4 D @ 6 mg $l^{-1}$ (Fig. 1). The mean callus size ranged from 1.24 cm - 2.19 cm with different concentrations of chemicals (Table 1). 2, 4 D @ 4 mg $l^{-1}$ recorded maximum mean callus size of 2.19 cm followed by 1.8 cm with 2, 4 D @ 5 mg $l^{-1}$ concentration and minimum mean callus size (1.24 cm) was observed with EMS @ 1.0 µM $l^{-1}$ followed by 1.6 cm with 2, 4 D @ 6 mg $l^{-1}$ (Fig. 1). EMS and 2, 4 D at higher concentrations inhibited callus growth and development. Khan *et al.,* (2009) reported stimulation in callus growth at lower doses and poor stimulation in high doses of mutagenic chemicals. With the increase in the concentration of the mutagen there was an increase in number of days required for the callus initiation. Kona *et al.,* (2018) also reported the same with 2003V46 genotype culture using EMS and 2,4 D where decrease in callus induction frequency, callus size was observed with increase in concentration of mutagenic chemicals. 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 concentration of chemicals the percentage of callus initiation was decreased. This was also supported by the results of 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 chemicals 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.

Significant difference among all the treatments for number of days for shoot initiation was observed. Number of days taken for shoot initiation was ranged from 14.22 to 18.22 in treatments (Table 1). EMS @ 1.0 µM $l^{-1}$had recorded more number of days taken for shoot initiation (18.22) followed by EMS @ 0.8 µM $l^{-1}$ with 17.56 and minimum number of days (14.22) was recorded with 2,4 D @ 4 mg $l^{-1}$ followed by 2, 4 D @ 5 mg $l^{-1}$ with 15.44 days (Fig. 2). Among all treatments, shoot induction frequency ranged from 57.78– 89.44 % with significant difference. Maximum shooting frequency (89.44 %) was recorded with 2, 4 D @ 4 mg $l^{-1}$ followed by EMS @ 0.6 µM $l^{-1}$ with 81.67% and minimum shooting frequency of 57.78 % was recorded with EMS @ 1.0 µM $l^{-1}$ concentration followed by 2, 4 D @ 6 mg $l^{-1}$ with 66.11 % (Fig. 2). The range among mutagenic treatments varied from 7.33 to 19.44 for

number of shoots per explant. The chemical 2, 4 D @ 4 mg $l^{-1}$ had recorded the maximum number of shoots per explant (19.44) followed by 2, 4 D @ 5 mg $l^{-1}$ (16.56). The chemical EMS @ 1.0 µM $l^{-1}$ followed by EMS @ 0.8 µM $l^{-1}$ recorded minimum number of shoots per explant (7.33 and 9.78, respectively).The mean shoot length ranged from 1.44cm to 3.48 cm with mutagenic treatments. 2, 4 D @ 4 mg $l^{-1}$ recorded maximum shoot length (3.48 cm) followed by EMS @ 0.6 µM $l^{-1}$ (3.11 cm) while minimum shoot length (1.44 cm) was recorded with EMS @ 1.0 µM $l^{-1}$ followed by 2, 4 D @ 6 mg $l^{-1}$ (1.64 cm) (Table 1) (Fig. 2). Effect of higher concentrations of EMS and 2, 4 D was inhibitory on shoot development also.

An increase with increased level of concentration of chemicals was recorded for mean number of days for shoot initiation. Shoot induction frequency showed decreased trend with increase in concentration of mutagens. These results are in agreement with the previous findings in sugarcane, rice, chrysanthemum (Bhagwat and Duncan, 1997; Latado *et al.,* 2004 and Berenschot *et al.,* 2008; Kona *et al.,* 2018). The results revealed reduction in the number of regenerated shoots and shoot length with increasing concentrations of chemical mutagens. Using radiation induced variation in wheat, Misra and Datta (2007) reported a reduction in number of shoots per explant with the increase in radiation dose.

Mean response of root initiation ranged from 8.22 days to 17.89 days. Minimum number of days (8.22) taken for root initiation was recorded with 2, 4 D @ 4 mg $l^{-1}$ followed by 2, 4 D @ 5 mg $l^{-1}$ with 11.11 days and maximum number of days (17.89) was recorded with EMS @ 1.0 µM $l^{-1}$ followed by EMS @ 0.8 µM $l^{-1}$ with 15.56 days (Table 1 and Fig. 3). The number of roots per shoot was ranged from 13.44 to 21.89.2, 4 D @ 4 mg $l^{-1}$ recorded maximum mean number of roots per shoot (21.89) followed by 2, 4 D @ 5 mg $l^{-1}$ (19.11), whereas EMS @ 1.0 µM $l^{-1}$recorded minimum number of roots per shoot (13.44) followed by EMS @ 0.8 µM $l^{-1}$with 15.67 (Table 1). The range for rooting frequency was from 61.67 to 86.67 % in treatments.

EMS @ 1.0 µM $l^{-1}$recorded the lowest rooting frequency of 61.67 % followed by EMS @ 0.8 µM $l^{-1}$ with 65.56 % and the highest frequency (86.67 %) was recorded with 2, 4 D @ 4 mg $l^{-1}$ followed by 2, 4 D @ 5 mg $l^{-1}$ with 78.33 % (Table 1) (Fig. 3). With respect to root length, control had recorded maximum root length (4.06). Average root length ranged from 1.57 to 3.32 cm in all treatments. The clone Co86032 showed maximum mean root length of 3.32 cm with 2, 4 D @ 4 mg $l^{-1}$followed by EMS @ 0.6 µM $l^{-1}$ with 3.28 cm whereas EMS @ 1.0 µM $l^{-1}$ recorded minimum mean root length (1.57 cm) followed 2, 4 D @ 6 mg $l^{-1}$ with 1.8 cm (Fig. 3). The results revealed that the shoots obtained from mutagenic chemicals treated callus, showed less response to root differentiation and took more number of days to differentiate roots as compared to the shoots obtained from callus without treatment. The maximum root length was observed in control when compared with mutagenic treatments. This was supported by Yasmin *et al.,* (2011). Both root length and mean number of rooted shoots decreased with increase in concentration of four chemicals. These results are in agreement with results obtained previously (Bhatnagar-Mathur *et al.,* 2008, Shomeili *et al.,* 2011 and Kona *et al.,* 2018).

2, 4 D @ 4 mg $l^{-1}$recorded the lowest time taken for hardening (9.44 days) followed by EMS @ 0.6 µM $l^{-1}$ (11.33) and 2, 4 D @ 5 mg $l^{-1}$ (11.56) whereas EMS @ 1.0 µM $l^{-1}$ recorded the highest time taken for hardening (15.89) followed by 2, 4 D @ 6 mg $l^{-1}$ (13.44) (Table 1). Significant difference was observed between treatments for hardening percentage. 2, 4 D @ 4mg $l^{-1}$ recorded maximum

hardening percentage (82.22 %) followed by 2, 4 D @ 5 mg $l^{-1}$with 77.45 %; whereas, EMS @ 1.0 µM $l^{-1}$ recorded minimum hardening percentage (55.33 %) followed by 60.78 % with 2, 4 D @ 6 mg $l^{-1}$. Time taken for hardening and hardening percentage depends on the type of mutagenic chemical employed and the nature of genotype.

Hoque and Morshad (2014) reported that after the mutagenic chemicals [Ethyl methane sulphonate (EMS), Methyl methane sulphonate (MMS), 5-Bromo Uracil (BU) and 2, 4 D] treatment of callus survival rates of newly created potato somaclones were only 17.18% in plastic tray and 37.16% in field condition. Similar results were obtained by Uzma *et al.,* (2012) where they had reported tremendous (98%) hardening efficiency of in vitro regenerated sugarcane plants on sandy clay loam soil under glasshouse.

Gill *et al.,* (2006) and Behera and Sahoo (2009)reported 95% and 85% hardening percentage, respectively of sugarcane tissue culture derived plantlets under glass house conditions. Seedlings obtained after hardening were evaluated for various morphological characters.

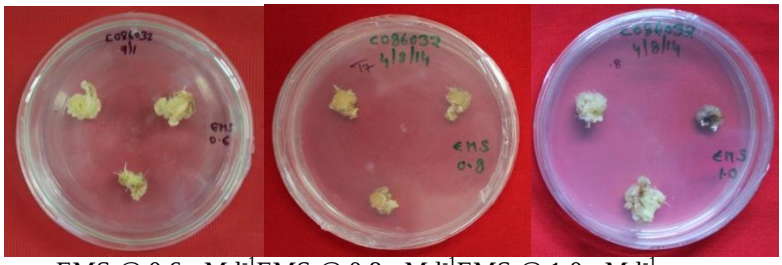
**Table.1** Effect of different concentrations of chemical mutagens on callusing, shooting, rooting and hardening in Co86032

| Characters | EMS 0.6 µM $l^{-1}$ | EMS 0.8 µM $l^{-1}$ | EMS 1.0 µM $l^{-1}$ | 2,4 D 4 mg $l^{-1}$ | 2,4 D 5 mg $l^{-1}$ | 2,4 D 6 mg $l^{-1}$ | Co86032 (Control) | C.D |
|---|---|---|---|---|---|---|---|---|
| **No. of days for callus induction** | 11.89 | 13.33 | 15.33 | 11.89 | 13.33 | 14.11 | 10.00 | 0.674 |
| **Mean No. of explants induced callus** | 2.78 | 2.59 | 1.88 | 2.78 | 2.33 | 2.00 | 3.00 | 0.15 |
| **Callus induction frequency (%)** | 88.85 (70.47) | 74.28 (59.50) | 59.30 (50.34) | 96.30 (78.88) | 77.78 (61.85) | 70.37 (57.00) | 100.00 (90.00) | 0.617 |
| **Callus size (cm)** | 1.73 | 1.69 | 1.24 | 2.19 | 1.8 | 1.6 | 2.81 | 0.124 |
| **No. of days for shoot initiation** | 16.33 | 17.56 | 18.22 | 14.22 | 15.44 | 16.44 | 13 | 0.565 |
| **Shoot regeneration frequency** | 81.67 (64.64) | 72.22 (58.17) | 57.78 (49.45) | 89.44 (71.02) | 78.33 (62.24) | 66.11 (54.38) | 91.67 (73.24) | 1.855 |
| **No. of shoots per explant** | 11.56 | 9.78 | 7.33 | 19.44 | 16.56 | 12.22 | 24.44 | 0.493 |
| **Average shoot length (cm)** | 3.11 | 2.26 | 1.44 | 3.48 | 2.61 | 1.64 | 4.12 | 0.053 |
| **No. of days for root initiation** | 12.44 | 15.56 | 17.89 | 8.22 | 11.11 | 12.89 | 7.56 | 0.389 |
| **No. of roots per shoot** | 18.78 | 15.67 | 13.44 | 21.89 | 19.11 | 16.00 | 26.33 | 0.447 |
| **Rooting frequency** | 77.22 (61.47) | 65.56 (54.05) | 61.67 (51.73) | 86.67 (68.58) | 78.33 (62.24) | 66.11 (54.38) | 89.44 (71.02) | 1.612 |
| **Average root length (cm)** | 3.28 | 2.4 | 1.57 | 3.32 | 2.54 | 1.8 | 4.06 | 0.047 |
| **Time taken for Hardening** | 11.33 | 13.22 | 15.89 | 9.44 | 11.56 | 13.44 | 8.22 | 0.436 |
| **Hardening percentage** | 77.00 (61.32) | 68.22 (55.66) | 55.33 (48.04) | 82.22 (65.03) | 77.45 (61.62) | 60.78 (51.20) | 86.00 (68.00) | 0.355 |

**Table.2** Frequency of somaclones for various characters in Co86032

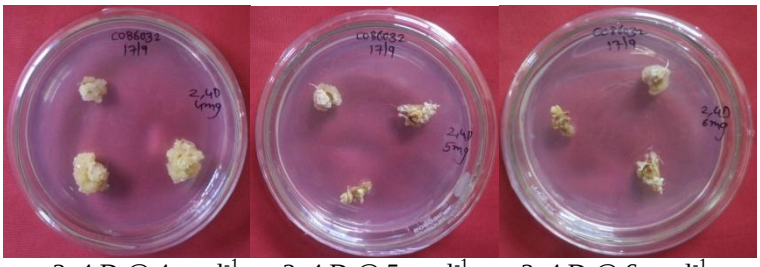
| S. No. | Characteristics | EMS (0.8 µM $l^{-1}$ and 1.0 µM $l^{-1}$) | 2, 4 D (5 mg $l^{-1}$ and 6 mg $l^{-1}$) |
|---|---|---|---|
| **1.** | Dense leaf sheath hairiness | 2 | 2 |
| **2.** | Deltoid shape of ligule | 4 | 3 |
| **3.** | Deltoid and incipient shapes of inner auricle | 4 | 3 |
| **4.** | Yellow Internode colour (Unexposed) | 1 | 0 |
| **5.** | Greenish yellow Internode colour (Exposed) | 1 | 0 |
| **6.** | Cylindrical Internode shape | 1 | 0 |
| **7.** | Oval shape of bud | 2 | 3 |
| **8.** | Small size of bud | 2 | 1 |
| **9.** | Presence of bud groove | 2 | 1 |
| **10.** | More number of internodes compared to parent (>37) | 1 | 0 |
| **11.** | Two root eye rows | 3 | 0 |

**Fig.1** Callus induction in different concentrations of chemical mutagens with control in Co86032



EMS @ 0.6 µM $l^{-1}$ EMS @ 0.8 µM $l^{-1}$ EMS @ 1.0 µM $l^{-1}$

Control



2, 4 D @ 4 mg $l^{-1}$ 2, 4 D @ 5 mg $l^{-1}$ 2, 4 D @ 6 mg $l^{-1}$

**Fig.2** Shoot induction in different concentrations of chemical mutagens with control in Co86032

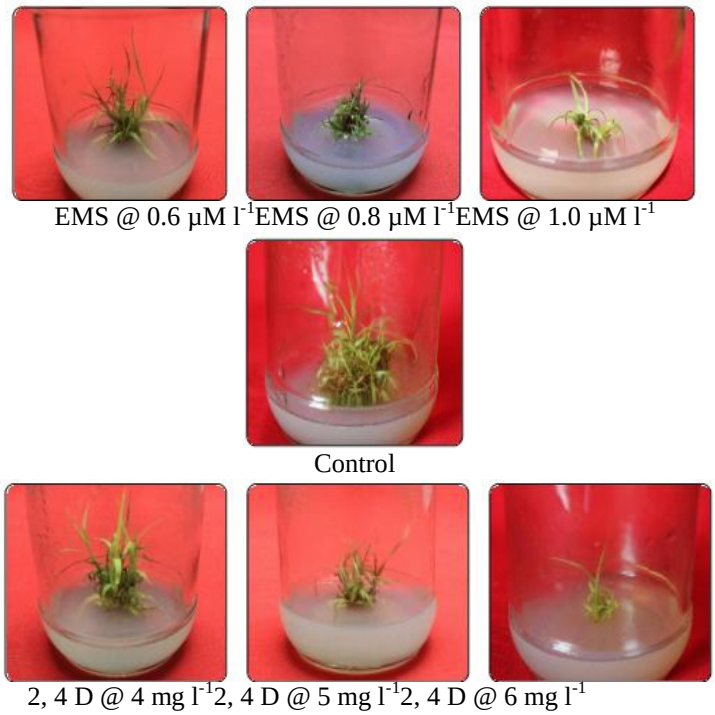
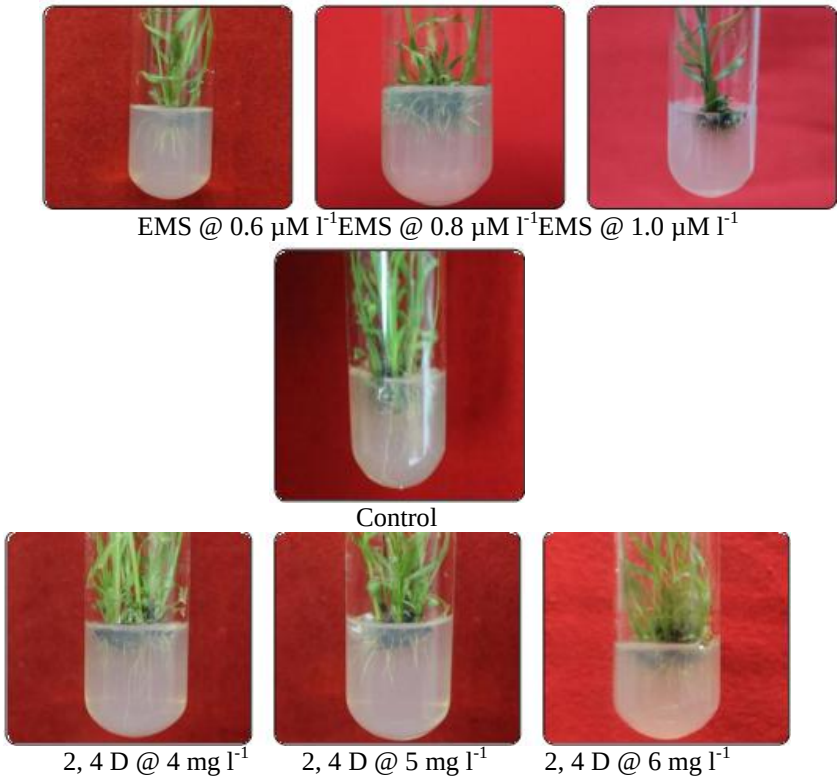



**Fig.3** Root induction in different concentrations of chemical mutagens with control in Co86032



Some plants obtained with EMS @ 1.0 µM $l^{-1}$and 2, 4 D @ 6 mg $l^{-1}$ showed dense leaf sheath hairiness, deltoid shape of ligule, deltoid and incipient shapes of inner auricle, yellow internode colour (unexposed), greenish yellow internode colour (exposed), thin internode diameter, cylindrical shape of internodes, corky patches, both medium and heavy wax on internode, oval shape of bud, small bud size, presence of bud groove, round internode cross section, more number of internodes in contrast to parent Co86032 (Table 2). Similar type of variations for various morphological characters such as leas sheath hairiness, bud size, shape, and number of root eye rows on the node were observed by Kona *et al.,* (2018) and Sreenivasan and Jalaja (1984).

In conclusion, the growth hormone, 2, 4 D @ 4 mg $l^{-1}$was found to be best among different treatments. It had shown good performance in all the stages of culture *viz*., callusing, shooting and rooting. Whereas EMS @ 1.0 µM $l^{-1}$ showed hindering effect in all the stages of culture. Though a growth hormone, 2, 4 D at higher concentration (6 mg $l^{-1}$) had inhibitory effect on callusing, shooting and rooting similar to that of the mutagen, EMS. It can be concluded that EMS @ 1.0 µM $l^{-1}$ along with 2, 4 D @ 6 mg $l^{-1}$can be used to produce variation inCo86032. Somaclone with more number of internodes produced from mutagens in Co86032 can be used for commercial cultivation after evaluating them for yield and quality.

## References


Abdullah, Smiullah, Farooq Ahmad Khan, RameezIftikhar, Muzaffar Raza, M., Rasheda Aslam, GhazanfarHammad, AmbreenIjaz, Wasif Zafar, M and Usman Ijaz. 2013. Detection of Somaclonal Variation in Micropropagated Plants of Sugarcane and SCMV Screening through ELISA. *Journal of Agricultural Science.* Vol. (5): 199-208.

Behera, K. K and Sahoo, S. 2009. Rapid *in vitro* micropropagation of sugarcane through callus culture. *Nature and Science.* 7(4): 1-10.

Berenschot, P.T.H., Hull, P and Kartha, S.F. 2008. The spectrum of molecular changes associated with somaclonal variation. IntAssoc Plant Tissue Cult Newslett. 66:14-25.

Bhagwat, O and Duncan, J. 1997. Identification of sugarcane genes induced in disease resistant somaclones upon inoculation with *Ustilagoscitaminea* or *Bipolaris sacchari*. *Plant Physiology and Biochemistry*. 43:1115-1121.

Bhatnagar-Mathur, P., Vadez, V and Sharma, K.K. 2008. Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. *Plant Cell Rep*. 27: 411-424.

Gahukar, A and Jambhule, P.K. 2000.*In vitro* selection and characterization of *Vignaradiata* cell line resistant to PEG induced drought stress. *Acta Physiologiae Plantarum*. 16(1): 53-60.

Gill, R., Malhotra, P.K and Gosal, S. 2006. Direct plant generation from cultured young leaf segments of sugarcane. *Plant Cell Tissue and Organ Culture*. 84: 227-231.

Heinz, D.J., Mee, G.W.P and Nickell, L.G. 1969. Chromosome number of *Saccharum* species hybrids and their cell suspension cultures. *American Journal of Botany*. 56: 450-456.

Hoque, M. E and Morshad, M. N. 2014.Somaclonal Variation in Potato (*Solanum tuberosum* L.) Using Chemical Mutagens. *The Agriculturists.* 12(1): 15-25.

Jain, S.M. 2010. Mutagenesis in crop improvement under the climate change. *Romanian Biotechnol. Lett*. 15 (2): 88–106.

Khan, S.A., Rashid, H., Chaudhary, M.F., Chaudhry, Z., Fatima, Z., Siddiqui, S.U and Zia, M. 2009. Effect of cytokinins on shoot multiplication in three elite sugarcane varieties. *Pakistan J. Bot*. 41: 1651-165.

Kona P., Hemanth Kumar M., Reddy K.H.P., Reddy D.M., Eswar Reddy N.P., Latha P and Balaji M.S. 2018. Effect of 2,4 D & EMS on *in vitro* regeneration in sugarcane cultivar, 2003V46. *International Journal of Microbiology Research.* 10(3):1090-1093.

Latado, H.M., Maheshwaran, M and Rangasamy, S.R.S. 2004. Influence of genotypes and culture media on callus induction and plant regeneration in *Oryza* spp. *J. Genet. Plant Breed*. 43: 99-106.

Misra, S.W and Datta, J.T. 2007.Field assessment of somaclonal variation in wheat. *Journal of Experimental Botany*. 37: 1114-1128.

Munsamy, A., Rutherford, R., Snyman, S and Watt, M. 2013. 5-Azacytidine as a tool to induce somaclonal variants with useful traits in sugarcane (*Saccharum* spp.). *Plant Biotechnology Reports*. 7(4): 489.

Nickell, L.G. 1964. Tissue and cell culture of sugarcane another research tool. *Hawaii Plant Research*. 57:223-229.

Reddy, K. R. K., Rao, G. P., Malathi, N and Bahadur, B. 1987. Effect of gamma irritation on growth and phenolic production in castor bean cultures. *Curr. Sci*., 56: 23, 1234-1235.

Shomeili, M., Nabipour, M., Meskarbashee, M and Memari, H.R. 2011. Evaluation of sugarcane (*Saccharum officinarum* L.) somaclonal variants tolerance to salinity *in vitro* and *in vivo* cultures. *African Journal of Biotechnology*. 10(46): 9337-9343.

Singh, S. K and Singh, S. B. 1993. Effect of gamma rays on callus growth and plant regeneration in sugarcane CV Co 687. *Indian Sugar*, 43: 3, 181-182.

Smiullah, Farooq, A.K, Abdullah, Aqeel, A., Muhammad, A.J., Zafar, I., Rameez, I and Javed, I.W. 2012.*In vitro* regeneration, detection of somaclonal variation and screening for SCMV in sugarcane (*Saccharum* spp.) somaclones. *Afr. J. Biotech*. 11(48): 10841-10850.

Sreenivasan, T.V and Jalaja, N.C. 1984.*Tissue culture in sugarcane*. Paper presented at the 9th tissue culture conf. Coimbatore.

Sung, N. 1976. Smut disease assessment by PCR and microscopy in inoculated tissue cultured sugarcane cultivars. *Plant Science*. 167:987-994.

Talebi, A.B., Shahrokhifar, B and Talebi, A.B. 2012. Ethyl methane sulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose determination. *Am. J. Plant. Sci*. 3:1661–1665.

Uzma, M.R., Khan, A., Muhammad, I., Hussain, S.H., Shah, T., Kumar, S., Inam, M., Zubair, H.U., Rehman, A., Sher, N., Rehman, S., Ahmed and Ali, G.M. 2012. Rapid *in vitro* multiplication of sugarcane elite genotypes and detection of sugarcane mosaic virus through two steps RT-PCR. *Int. J. Agric. Biol*. 14: 870–878.

Yasmin, S., Khan, A.K., Khatri, A., Seema, N., Siddiqui, M.A. and Bibi, S. 2011. Plant regeneration from irradiated embryogenic callus of sugarcane. *Pak. J. Bot.* 43(5): 2423-2426.

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