Mutation induction has become an established tool in crop improvement to supplement the existing germplasm and to improve characters of outstanding cultivar without altering the remaining genetic constitution. By taking the advantages of the capacity of callus cell that undergo genetic changes in culture; mutation induction in vegetatively propagated plants are the ability to change one or a few existing characters of the plants. Callus is a suitable material to undergo mutation induction (Rahimi, et al., 2013) because cells are actively dividing so that the chance of differentiation and divide continuously (Jain, 2000 and Mattjik, 2005) [10, 16]. Callus was very sensitive to mutagens because the cells are actively dividing so that the chance of mutation was very large (Patade and Suprasanna 2008) [20].

Although conventional breeding has contributed to the development of agronomically improved cultivars, limitations such as narrow gene pool, complex genome, poor fertility and the long breeding and selection cycle make it difficult to undertake further improvement. In addition, to sustain sugarcane production and to improve productivity, tolerance to biotic and abiotic stresses, nutrient management and improved sugar recovery are some of the concerns. Mutation induction has become an established tool in crop improvement to supplement the existing germplasm and to improve cultivars in certain specific traits. The main advantages of mutation induction in vegetatively propagated plants are the ability to change one or a few characters of outstanding cultivar without altering the remaining genetic constitution. By taking the advantages of the capacity of callus cell that undergo genetic changes in culture; many genomically desirable callus derived plants have been obtained (Amin et al., 2013) [2]. This is referred to as somaclonal variation and has been of great interest in obtaining useful agronomic clones (Jain, 2000, Rahimi et al., 2013) [10, 25]. With the advent of in vitro technique, interest in the use of in vitro plant material for mutation has begun. Mutation induction can be empowered by in vitro technique. Many examples of different vegetatively propagated species show that the combination of in vitro and mutagenesis is relatively inexpensive, simple and efficient (Rahimi et al., 2013) [25]. The present study was, therefore, undertaken. The chances of mutation depend on the number, age and growth stages of plants which are used as explant material (explants). Use of callus as explants material for mutation induction is very effective because the callus is population of unorganized mass of cells that have not under gone differentiation and divide continuously (Jain, 2000 and Mattjik, 2005) [10, 16]. Callus was very sensitive to mutagens because the cells are actively dividing so that the chance of mutation was very large (Patade and Suprasanna 2008) [20].

**Abstract**

The present investigation on was carried out in south Gujarat heavy rainfall region of India. Callus derived from sugarcane variety CoN – 13073 subjected for mutagenic treatment varying in different concentration as well as time period. Callus culture of 25 - 30 days old white globular form is considered a suitable material to undergo mutagenic treatments. Among the mutagenic treated calli, maximum survival per cent was observed in EMS 0.2% + 60 min treatment. Maximum regeneration per cent (78.2%) was found in explants treated with treatment MMS 0.3% + 30 min. In case of the number of days for shoot formation, callus treated with EMS 0.2% + 60 min registered a maximum of 15.20 days. The maximum numbers of multiple shoots were observed in MMS 0.2% + 60 min (30.4). Time factor found to be highly significant with a wide range of mean performance. The effect of the mutagenic agent was found to be succeeding to create variability at a lower concentration for lower time intervals for most of the characters under study. In the present study EMS, 0.2% in addition to 0.3%, treatments were most effective. And the effectiveness decrease with the increase in the time interval.

**Keywords:** Genetic, variability, sugarcane, chemical, culture 

**Introduction**

Sugarcane (Saccharum spp. complex, 2n = 40 to 128) belongs to the family Poaceae. Tropical sugarcane originated from Oceania (Papua New Guinea islands) and Indian diploid cane (Saccharum spontaneum L.) originated from North Eastern India. It is the main sugar producing crop that contributes more than 77 per cent to the total sugar pool at the global level. Globally, it occupies a little about 2 per cent of the total cropped area. In World area 20 million ha, production 1333.2 million tones and productivity 65.20 t/ha (Anon, 2017) [1]. Although conventional breeding has contributed to the development of agronomically improved cultivars, limitations such as narrow gene pool, complex genome, poor fertility and the long breeding and selection cycle make it difficult to undertake further improvement. In addition, to sustain sugarcane production and to improve productivity, tolerance to biotic and abiotic stresses, nutrient management and improved sugar recovery are some of the concerns. Mutation induction has become an established tool in crop improvement to supplement the existing germplasm and to improve cultivars in certain specific traits. The main advantages of mutation induction in vegetatively propagated plants are the ability to change one or a few characters of outstanding cultivar without altering the remaining genetic constitution. By taking the advantages of the capacity of callus cell that undergo genetic changes in culture; many genomically desirable callus derived plants have been obtained (Amin et al., 2013) [2]. This is referred to as somaclonal variation and has been of great interest in obtaining useful agronomic clones (Jain, 2000, Rahimi et al., 2013) [10, 25]. With the advent of in vitro technique, interest in the use of in vitro plant material for mutation has begun. Mutation induction can be empowered by in vitro technique. Many examples of different vegetatively propagated species show that the combination of in vitro and mutagenesis is relatively inexpensive, simple and efficient (Rahimi et al., 2013) [25]. The present study was, therefore, undertaken. The chances of mutation depend on the number, age and growth stages of plants which are used as explant material (explants). Use of callus as explants material for mutation induction is very effective because the callus is population of unorganized mass of cells that have not under gone differentiation and divide continuously (Jain, 2000 and Mattjik, 2005) [10, 16]. Callus was very sensitive to mutagens because the cells are actively dividing so that the chance of mutation was very large (Patade and Suprasanna 2008) [20].
Variation and this variation can be improved by chemical mutagen. The use of mutagen often causes cell damage and thus affects the ability of putative mutant cell regeneration. The ability of each cell to regenerate shoots depends on level of sensitivity of each cell and dosage compensation.

2. Materials and Methodologies
This investigation was carried out at the sugarcane tissue culture laboratory, Main Sugarcane Research Station, Navsari Agricultural University, Navsari, Gujarat, during the year 2017-2018.

### Table 1: The compositions of Murashige and Skoog (1962) medium Constituents amounts (mg/l)

| Sr. No. | Stock solution | Constituent salt                     | Quantity/litre medium (stock) | Needs in one litter of media |
|--------|----------------|--------------------------------------|------------------------------|-----------------------------|
| 1      | Stock-A        | Sodium Nitrate (NaNO₃)               | 82.00 g                      | 20 ml                       |
| 2      | Stock-B        | Potassium Nitrate (KNO₃)             | 95.00 g                      | 20 ml                       |
| 3      | Stock-C        | Calcium chloride dehydrate (CaCl₂.2H₂O) | 88.00 g                      | 05 ml                       |
| 4      | Stock-D        | I. Boric acid (H₃BO₃)                | 1.240 g                      | 05 ml                       |
|        |                | II. Potassium dihydrogen phosphate (KH₂PO₄) | 34.00 g                      |                              |
|        |                | III. Sodium molybdate (Na₂MoO₄.2H₂O)  | 0.050 g                      |                              |
|        |                | IV. Cobalt chloride (CoCl₂.6H₂O)      | 0.005 g                      |                              |
|        |                | V. Potassium Iodide (KI)             | 0.166 g                      |                              |
| 5      | Stock-E        | I. Magnesium sulphate (MgSO₄.7H₂O)   | 74.00 g                      | 05 ml                       |
|        |                | II. Zinc sulphate (MgSO₄.7H₂O)       | 0.172 g                      |                              |
|        |                | III. Cupric sulphate (CuSO₄.5H₂O)    | 0.005 g                      |                              |
|        |                | IV. Manganese sulphate (MnSO₄.5H₂O)  | 4.500 g                      |                              |
| 6      | Stock-F*       | I. Ferrous sulphate (FeSO₄.7H₂O)     | 5.560 g                      | 05 ml                       |
|        |                | II. Sodium EDTA (Na₂EDTA.2H₂O)       | 7.460 g                      |                              |
| 7      |                | Meso inositol                         | 10.00 g                      | 10 ml                       |
| 8      |                | Glycine                               | 200.0 mg                     | 02 ml                       |
| 9      |                | Nicotinic acid                        | 200.0 mg                     | 2.50 ml                     |
| 10     |                | Pyrodoxine acid                       | 200.0 mg                     | 2.55 ml                     |
| 11     |                | Thiamine HCl                          | 1.000 g                      | 0.1 ml                      |
| 12     |                | 2,4-Dichlorophenoxy acetic acid       | 200.0 mg                     | 20ml                        |
| 13     |                | Sucrose                               | 20.00 mg                     | 20 g                        |
| 14     |                | Coconut water                         | 10%                          | 100 ml                      |
| 15     |                | Agar agar                             | 8gm                          | 8 g                         |

Addendum: Sucrose 30 g/l, Myoinositol 100 g/l and Agar 8 g/l.

*The FeSO₄.7H₂O was dissolved in approximately 40 ml of distilled water and heated. The Na₂EDTA.2H₂O was 50 gm taken and dissolved in approximately 40 ml of distilled water separately and mixed while heating (under continuous stirring) with FeSO₄.7H₂O solution. After cooling, the volume was adjusted to 100 ml. heating and stirring resulted in a more stable FeNa₂EDTA complex.

2.2 Preparation of explants and regeneration of callus from leaf whorl meristem

Disease - free, genetically true-to-type and actively growing cane tops were selected from 5 to 7 months old sugarcane crop. Cane tops with the growing apices were cut approximately 10 cm long and washed thoroughly in running tap water for 30 minutes. Outer sheaths of cane tops were removed by wiping the sheath with rectified spirit. The shoots were then washed with soapy water (2 drops of Labonin into 250 ml of water) for about 5 to 6 minutes in a sterile 1-litre conical flask, followed by cleaning the materials 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 sterilizing double distilled water for 4-5 times till ethanol was completely washed out from the surface of the material. Surface sterilization was performed using 0.1 per cent mercuric chloride solution. Shoots were shaken vigorously for 5 minutes. Then the container was taken to the laminar clean air station. They were rinsed 3 to 4 times with sterile double distilled water to remove all traces of chemicals. The isolation of shoot apex was done by carefully removing the 2 - 3 outer whorls of the developing leaves with the help of a sterile sharp blade. Than explants were cut such that it forms circular disc by cutting perpendicular to the central axis with a surgical blade and inoculated on medium supplemented with 2,4-D.

Establishment of callus cultures and regeneration of sugarcane was reported by Nickell, (1964) [19] and Barba and Nickell (1964) [18]. Callus culture of sugarcane have also been successfully established using young shoot and young leaves as explants on MS medium containing 2,4-D and coconut milk (Nadar et al. 1977) [18], Liu and Chen (1984) [13] as well as, Bhansali and Singh (1984) [5]. Similar response was also reported by Barba et al. (1977) [4] and Manan and Amin (1999) [14]. Where in they successfully established callus culture by manipulating 2,4-D concentration in medium. In India, good amount of studies were carried out on micro propagation of sugarcane to study somaclonal variations. The result obtained from the present investigation was discussed in this chapter.

2.3 Multiplication and maintenance of callus

The callus masses obtained in various concentrations of 2, 4-D were multiplied and maintained by sub culturing. Every ten days the healthy looking and fast growing tissue fragments were subcultured.
from the callus mass were sub cultured on MS medium supplemented with NAA (0.5 mg/l) and BAP (1.0 mg/l). The growth of callus at the weekly interval was measured in terms of fresh weight. Callus masses so obtained were used as the experimental material in the present investigation.

2.3.1 Standardization of regeneration medium for shoot differentiation from established callus cultures
The investigation of shoot regeneration from callus was carried out on MS medium. The numbers of shoots obtained from the program of regeneration from calli were counted after 30 days of callus cultured on regeneration medium whereas, length of shoots (cm) was measured before transferred into rooting medium. The medium details for shoot regeneration are as under: (MS + 0.5 mg/l NAA + 0.5 mg/l BAP).

2.4 In vitro mutagenesis
The friable callus induced after 25 days of inoculation was cut into small pieces, weighted and treated with EMS (Ethyl methane Sulphonate), SA (Sodium Azide) and MMS (Methyl Methane Sulphonate) of 0.2% and 0.3% solution (prepared in sterilized distilled water and membrane filtered) for 30 minute and 60 minute separately and inoculated on MS medium consisting 4 mg/l 2,4-D + 2% sucrose. Detailes of treatment combinations and experimental design are narrated in Table 2, observation recorded on survived. The LD$_{50}$ of EMS, SA and MMS treatments were determined for further screening of mutants.

| Statistical Design | Completely Randomized Design with factorial concept suggested by Panse and Sukhatme (1985). |
|--------------------|----------------------------------------------------------------------------------|
| Repetitions        | 3                                                                                 |
| Explant used       | callus tissues                                                                     |
| Treatments details | J (six different chemical mutagenic agents)                                       |
| C$_1$              | EMS (0.2%)                                                                        |
| C$_2$              | EMS (0.3%)                                                                        |
| C$_3$              | SA (0.2%)                                                                         |
| C$_4$              | SA (0.3%)                                                                         |
| C$_5$              | MMS (0.2%)                                                                        |
| C$_6$              | MMS (0.3%)                                                                        |
| Factor 2, P (Two different emersion time period) |
| P$_1$              | 30 min                                                                            |
| P$_2$              | 60 min                                                                            |

Table 2: Details of treatment combinations for in vitro mutagenesis

2.5 In vitro selection
Somaclones were raised from treated callus on MS medium allowed to regenerate and the following observations were recorded.

2.6 Observations recorded
2.6.1 Survival per cent
Survival per cent recorded after the application of chemical mutagens considering LD$_{50}$ (lethal dose).

2.6.2 Regeneration per cent
Regeneration per cent recorded on the basis of no of treated cultures undergone regeneration process for shoot formation.

2.6.3 Number of days for shoot formation
The number of days required for shoot formation of callus on MS medium supplemented with different cytokinins and enzymes.

2.6.4 Number of multiple shoots
The numbers of shoots were counted after 30 days of callus inoculation on regeneration medium.

3. Results and discussion
3.1 Quality of the callus
On the basis of visual observations considered such as appearance, compactness and color, quality of callus is differentiated as good callus, moderate callus, very good callus and poor callus. Moderate callus formation was observed in the explants used in treatments. Leaf whorl exhibited moderate to good callus formation in most of the treatments except treatment EMS (0.2%) with both the time periods. Similar results were observed by Rutherford et al. (2013)\textsuperscript{11}.

Table 3: Effects of different chemical mutagenic agents on callus survival per cent

| C (Concentrations of mutagens) | P (Time period for emersion) | P$_1$(30 min) | P$_2$(60 min) |
|--------------------------------|----------------------------|---------------|---------------|
| C$_1$ (EMS - 0.2%)             | +++                        | +             | +++           |
| C$_2$ (EMS - 0.3%)             | +                         | +             | --            |
| C$_3$ (SA – 0.2%)              | -                         | +             | -             |
| C$_4$ (SA – 0.3%)              | -                         | -             | -             |
| C$_5$ (MMS – 0.2%)             | +                         | +             | +             |
| C$_6$ (MMS – 0.3%)             | -                         | -             | -             |

+ Good Callus (White Globular)
++ Moderate Callus (Yellowish)
+++ Very Good Callus (Whitish yellow)
-- Poor Callus (Brown)
3.2 Effects of different chemical mutagenic agents on callus survival per cent

Both the concentrations, 0.2% and 0.3% of EMS and MMS registered optimum survival per cent at both the treatment intervals. Whereas, higher and lower concentration of SA registered poor callus survival per cent (as disposed in Figure 1 to 4). Overall, lower concentration of EMS and MMS at 30 min treatment period registered similar survival per cent compared to untreated callus (see Plate. No. 1). As indicated in Table. 4, interaction effect found to be significant. Highest calli survival per cent (81.70%) registered in C₁P₂ (EMS 0.2% + 60 min), statistically at par with 80.40% in C₆P₁ (MMS 0.3% + 30 min), followed by 77.60% in C₁P₁ (EMS 0.2% + 30 min). On the other hand, callus treated with C₆P₄ (SA 0.3% + 60 min) registered lowest survival per cent (56.4%). These findings are in accordance with Chaudhari (2017) [6]. Maximum survival per cent (81.7%) was observed in treatment combination (EMS 0.2% + 60 min) followed by 80.4% in callus treated with (MMS 0.3% + 30 min). Similar results were observed by Purnamanishingh and Hutami (2016) [24]. Higher concentration of SA (0.3%) at 60 min depicted poor survival per cent. Similar results were observed by Kanganal et al. (2008) [11], Koach et al. (2009) [12], Gadakh (2014) [8] and Chaudhari (2017) [6].

Table 4: Effects of different chemical mutagenic agents on callus survival per cent

| C (Concentrations of mutagens) | P (Time period for emersion) | P₁(30 min) | P₂(60 min) | Mean C |
|--------------------------------|-----------------------------|------------|------------|--------|
| C₁                             | (EMS - 0.2%)                | 77.60      | 81.70      | 79.65  |
| C₂                             | (EMS - 0.3%)                | 74.20      | 67.50      | 70.85  |
| C₃                             | (SA – 0.2%)                 | 70.40      | 62.70      | 66.55  |
| C₄                             | (SA – 0.3%)                 | 60.50      | 56.40      | 58.45  |
| C₅                             | (MMS – 0.2%)                | 70.60      | 71.07      | 70.83  |
| C₆                             | (MMS – 0.3%)                | 80.40      | 76.20      | 78.30  |
| Mean P                         |                             | 72.28      | 69.26      |        |
| Effect                         |                             | S.Em.+     | C.D. @ 5%  | CV%    |
| C                              |                             | 0.81       | 2.37       |        |
| P                              |                             | 0.47       | 1.37       | 2.81   |
| C x P                          |                             | 1.15       | 3.35       |        |

Plate 1: Quality of the callus

Plate 2: Without mutagenic agent

Plate 3: Survival per cent
3.3 Effect of different chemical mutagenic agents on regeneration per cent

Immersion time in EMS, SA and MMS were more influential to callus regeneration than the concentration. Increased concentration resulted in a reduction in regeneration per cent. The interaction effect of the different mutagenic agents with different time periods was found to be significant for regeneration per cent, that indicates soma clones regenerated under different mutagenic treatment influenced at the cellular level. As shown in Table 5, interaction effect was found to be a significant one. Higher regeneration per cent (78.2%) was observed in treatment C₆ P₁ (MMS 0.3% + 30 min) which is at par with 76.7% in treatment C₁ P₂ (EMS 0.2% + 60 min) numerically followed by 74.5% in treatment C₂ P₂ and 70.4% in treatment C₃ P₂ (MMS 0.2% + 60 min), whereas, minimum regeneration per cent (52.5%) was notice in treatment C₄ P₁ (SA 0.3% + 30 min). Increase in the concentration along with increase in time period resulted into reduction in regeneration per cent. Regeneration potential was directly influenced by higher concentration of mutagenic agent and immersion time. Similar results were observed by Patel et al. (2004) [22], Kanganal et al. (2008) [11], Koch et al. (2009) [12] and Gadakh (2014) [8].

Table 5: Effects of different chemical mutagenic agents on callus regeneration per cent

| C (Concentrations of mutagens) | P (Time period for emersion) | P₁(30 min) | P₂(60 min) | Mean C |
|-------------------------------|-----------------------------|------------|------------|--------|
| C₁ (EMS - 0.2%)               |                             | 68.40      | 76.70      | 72.55  |
| C₂ (EMS - 0.3%)               |                             | 70.30      | 74.50      | 72.40  |
| C₃ (SA – 0.2%)                |                             | 62.30      | 56.70      | 59.50  |
| C₄ (SA – 0.3%)                |                             | 52.50      | 54.40      | 53.45  |
| C₅ (MMS – 0.2%)               |                             | 62.60      | 70.40      | 66.50  |
| C₆ (MMS – 0.3%)               |                             | 78.20      | 67.40      | 72.80  |
| Mean P                        |                             | 65.72      | 66.68      |        |
| Effect                        |                             | 5.Em. +    | C.D. @ 5%  | CV%    |
| C                            |                             | 0.62       | 1.79       |        |
| P                            |                             | 0.35       | 1.04       |        |
| C x P                         |                             | 0.87       | 2.54       |        |

Plate 4: Regeneration per cent from callus
3.4 Effect of different chemical mutagenic agents on the number of days for shoot formation

Among twelve treatment combinations, eight treatment combinations registered a lower number of days for a shoot formation and four treatments showed the more days of shoot formation as compared to control. Among the three mutagenic agents, EMS and MMS registered minimum numbers of days for shoot formation. Higher concentration of SA (0.3%) and increased treatment time period (60 min) have more influence on the number of days for shoot formation. As represented in Table 6, interaction effect of both the factors displayed significant, in which calli treated with C1P2 (EMS 0.2% + 60 min) registered minimum number of days of shoot formation, 15.20 days statistically at par with treatment C6P1 (16.40 days, MMS 0.3% + 30 min) and treatment C5P2 (16.8 days, EMS 0.3% + 60 min). Whereas, the maximum number of shoot formation (32.4 days) was registered in treatment C4P2 (SA 0.3% + 60 min). Application of higher concentration of mutagenic agents resulted into more number of days for shoot formation i.e. 18 to 32 days. Inhibitory effects were observed among the higher concentration of mutagenic treatments. Similar results were reported by Delvi et al. (2012) [7], Melion and Barba (1980) [15], and Rutherford et al. (2013) [26]. Genetic nature of CoN - 13073 did not respond well to mutagenic treatments for this character.

Table 6: Effects of different chemical mutagenic agents on the number of days for shoot formation

| C (Concentrations of mutagens) | P (Time period for emersion) | P1(30 min) | P2(60 min) | Mean C |
|-------------------------------|-----------------------------|------------|------------|--------|
| C1 (EMS - 0.2%)               |                             | 20.70      | 15.20      | 17.95  |
| C2 (EMS - 0.3%)               |                             | 26.60      | 16.80      | 21.70  |
| C3 (SA – 0.2%)                |                             | 18.20      | 20.40      | 19.30  |
| C4 (SA – 0.3%)                |                             | 28.70      | 32.40      | 30.55  |
| C5 (MMS – 0.2%)               |                             | 26.40      | 20.60      | 23.50  |
| C6 (MMS – 0.3%)               |                             | 16.40      | 18.20      | 17.30  |
| Mean P                        |                             | 22.83      | 20.60      |        |

| Effect | S. Em. + | C.D. @ 5% | CV% |
|--------|----------|-----------|-----|
| C      | 0.41     | 1.19      |     |
| P      | 0.24     | 0.69      |     |
| C x P  | 0.58     | 1.69      |     |

Fig 2: Show the regeneration per cent

Fig 3: Show the number of days for shoot formation
3.5 Effect of different chemical mutagenic agents on the number of multiple shoots

Vast differences were observed among the treatments. Among twelve treatments, eight treatments showed a higher number of multiple shoots and four treatments showed the lower number of multiple shoots. Lower and higher concentrations of EMS and MMS influenced the number of multiple shoots, overall among the three mutagenic agents, Sodium Azide responded poorly to the number of multiple shoots as compared to control. As illustrated in table 7, significantly higher number of multiple shoots, 30.4 was observed in treatment C_5P_2 (MMS 0.2% + 60 min) followed by 28.6 in treatment C_2P_2 (EMS 0.3% + 60 min), 27.8 in treatment C_4P_1 (MMS 0.3% + 30 min) and 27.4 in treatment C_3P_1 (EMS 0.3% + 30 min), whereas, minimum number of multiple shoots, 8.6 were observed in treatment C_4P_2 (SA 0.3% + 60 min). Multiple shoot ratio in different sugarcane genotypes were worked out concurrently by various researchers like Hendre et al. (1983) [9], Patel et al. (2001) [21], Patel (2012) [12] and Chaudhari et al. (2017) [6].

Table 7: Effect of different chemical mutagenic agents on the number of multiple shoots

| C (Concentrations of mutagens) | P (Time period for emersion) | P_1 (30 min) | P_2 (60 min) | Mean C |
|-------------------------------|-----------------------------|-------------|-------------|--------|
| C_1 (EMS - 0.2%)              |                             | 10.80       | 14.60       | 12.70  |
| C_2 (EMS - 0.3%)              |                             | 27.40       | 28.60       | 28.00  |
| C_3 (SA – 0.2%)               |                             | 10.20       | 12.40       | 11.30  |
| C_4 (SA – 0.3%)               |                             | 14.60       | 8.60        | 11.60  |
| C_5 (MMS – 0.2%)              |                             | 18.70       | 30.40       | 24.55  |
| C_6 (MMS – 0.3%)              |                             | 27.80       | 16.80       | 22.30  |
| Mean P                        |                             | 18.25       | 18.57       |        |
| Effect                        |                             | S.Em. +     | C.D. @ 5%   | CV%    |
| C                             |                             | 0.41        | 1.19        |        |
| P                             |                             | 0.24        | 0.67        | 5.430  |
| C x P                         |                             | 0.58        | 1.69        |        |

Plate 5: Multiple shoots from regenerated calli

**Fig 4:** Number of multiple shoots
of days for shoot formation, callus treated with treatment (EMS 0.2% + 60 min) registered a minimum number of days for shoot formation (15.2 days). Among three mutagenic treatments, EMS and MMS registered a minimum number of days for shoot formation. Highest number of multiple shoots, 30.4 was observed at (MMS 0.2% + 60 min), whereas, minimum number of multiple shoot formation was obtained for (SA 0.3% + 60 min). Sixteen per cent treatments depicted a greater number of multiple shoots, while forty per cent showed lower number of multiple shoots in comparison with the untreated callus shoots. Genetic variability having importance in any crop improvement program. Creation of genetic variability in existing gene pool leads to development of wide range of characterization. Identification and evaluation of variable characterization followed by utilization involves standard proven methodology and different techniques. In vitro induced mutagenesis in the present investigation resulted wide range of variability for various characters. The amount and frequency of mutagenic agents played important role in generation of variability among sugarcane verities studied. Imposing mutagens at cellular/tissue (callus) level would be more effective and authenticated in the present experiment for various characters studied. Further evaluation needs to be required for assuming the in vitro generated variability consistency and its longevity by heritability and genetic advance study or molecular distinction and mapping program.

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