www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(12): 14-21

www.thepharmajournal.com
Received: 14-10-2021
Accepted: 20-11-2021

Joshi AJ
Department of Genetics and Plant Breeding, S. D. Agricultural University, Gujarat, India

Parmar VL
Navsari Agricultutural University, Navsari, Gujarat, India

Parthsinh M Rehevar
Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

Chaudhari SD
Navsari Agricultutural University, Navsari, Gujarat, India

Corresponding Author:
Joshi AJ
Department of Genetics and Plant Breeding, S. D. Agricultural University, Gujarat, India

Deciphering *in vitro* mutated sugarcane clones for growth parameters

Joshi AJ, Parmar VL, Parthsinh M Rehevar and Chaudhari SD

DOI: https://doi.org/10.22271/tpi.2021.v10.i12a.9626

Abstract
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 shoot length (26.6 cm) was observed in the treatment, EMS 0.2% + 30 min. Maximum root length (12.8 cm) was observed in treatment MMS 0.3% + 30 min. A higher concentration of all mutagenic agents registered higher root length with the maximum of 1.14 g was observed in treatment EMS 0.3% + 60 min. Maximum leaf area (44.7 cm^2/plant) was observed in treatment MMS 0.2% + 30 min. Irrespective to concentration SA and EMS found significant for this character. Maximum chlorophyll content index (4.87) was observed in treatment EMS 0.2% + 30 min. In case of this character higher to lower concentration as well as time period influenced in both higher and lower directions. Among the treatments, the maximum multiplication rate (12.45%) was observed in treatment EMS 0.2% + 30 min. Both factors showed different effects on this character. 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 decreases with the increase in the time interval.

Keywords: Deciphering, mutated, sugarcane, clones, parameters

1. Introduction
The *Saccharum* complex includes the agronomically and industrially important sugarcane species obtained from *S. officinarum*. *S. spontaneum and S. robustum* crosses. Varied ploidy levels with the total chromosome number ranging from 40 to 128 and a genome size range is 3.36 to 12.64 Gb (Panje and Babu, 1960 [19]; Daniels and Roach, 1987 [7]; Sreenivasan *et al*., 1987 [24]; Da Silva *et al*., 1995 [6]; D'Hont *et al*., 1998 [5]; Ha *et al*., 1999 [8]; Zhang *et al*., 2012 [28]. etc.). Modern cultivars have a variable chromosome number (2n = 40 to 128). Sugarcane is a typical glycophyte and hence exhibits stunted growth or no growth under salinity, with its yield falling to 50 per cent or less than its true potential. By taking the advantages of the capacity of callus cell that undergo genetic changes in culture; many agronomically desirable callus derived plants have been obtained (Amin *et al*., 2013) [1]. This is referred to as somaclonal variation and has been of great interest in obtaining useful agronomic clones (Jain, 2000, Rahimi *et al*., 2013) [9, 21]. Few of the most effective chemical mutagens that has been used on various stages of plants are EMS, SA and MMS (Talebi *et al*., (2012), Bashir *et al*. (2013) and Soeranto, 2003) [26, 2, 23]. These chemical mutagenic agents cause point mutation and a little damage to the chromosomes and are favorable for breeding activities among the selected mutagenic agents EMS is often used because it is easy to get and does not leave toxins after hydrolyzate (Van Harten, 1998 [27] and Medina *et al*. (2005) [14]. According to Svetleva and Crino (2005) [25], induction of mutation combined with *in vitro* culture is a favorable method because it can increase the frequencies of formation of new variations. *In vitro* culture techniques can produce somaclonal.

2. Materials and Methods
The commercial cultivar of sugarcane CoN – 13073 (2008 N 210) grown in Gujarat was used as the source of explants in this experiment developed from inter varietal hybridization of CoN - 91132 x CoC - 671, followed by clonal selection. CoN - 91132 is a mid-late maturing variety, higher yielder, good ratooner, drooping leaves shows splitting and resistance to major

diseases and pests which is a female parent of an experimental variety. CoC - 671 is a male parent of an experimental variety which is wonder cane having a high yield, high sucrose content but susceptible to red rot and wilt. It is non - flowering, non - lodging with good ratoonability. It has a broad, green leaves, and sparse spine. It is showing resistance toward major diseases like wilt, red rot and whip smut as well as resistance toward major pests like early shoot borer, top borer, root borer, internode borer, scale insect and mealy bug etc.

Table 1: Treatment details

Twelve Treatment combinations		
C_1P_1	:-	EMS 0.2% + 30 min
C_1P_2	:-	EMS 0.2% + 60 min
C_2P_1	:-	EMS 0.3% + 30 min
C_2P_2	:-	EMS 0.3% + 60 min
C_3P_1	:-	SA 0.2% + 30 min
C_3P_2	:-	SA 0.2% + 60 min
C_4P_1	:-	SA 0.3% + 30 min
C_4P_2	:-	SA 0.3% + 60 min
C_5P_1	:-	MMS 0.2% + 30 min
C_5P_2	:-	MMS 0.2% + 60 min
C_6P_1	:-	MMS 0.3% + 30 min
C_6P_2	:-	MMS 0.3% + 60 min

2.1 Observations

2.1.1 Shoot length (cm)

Five plantlets were randomly selected from each treatment on 60 days after planting and the length of the shoot was measured from the collar region to the tip of a topmost leaf. The average value of shoot length for each treatment was computed and recorded.

2.1.2 Root length (cm)

Five plants were randomly selected from each treatment on 60 days after planting. The length of the root was measured from the collar region down to the tip of the longest root. The average value of root length for each treatment was computed and recorded.

2.1.3 Biomass content (g)

Biomass content of each plantlet was recorded on the basis of fresh weight; Fresh weight of callus was determined by weighing plantlets immediately after washing with distilled water to remove the adherings.

2.1.4 Leaf area (cm^2 $plant^{-1}$)

The leaves from plants selected from each treatment were used for the estimation of leaf area after 60 days of planting. Leaf area was measured by leaf area meter (Model LI3000, LI-COR, USA) and expressed as cm^2.

2.1.5 Chlorophyll Content Index (CCI)

The chlorophyll content index was recorded with the help of chlorophyll content meter (CCM - 200 Plus manufactured by Apogee Instrument). It measures the absorbance of both wavelengths and calculates a Chlorophyll Concentration Index (CCI) value that is proportional to the amount of chlorophyll in the sample of each treatment.

2.1.6 Multiplication rate (%)

After two cycles of sub culturing in the solid media, spindles were transferred to combinations of liquid multiplication media and compared the multiplication rates. Multiplication of shoot cultures was carried out by culturing mother clusters developed *in vitro*.

2.2 Statistical analysis

The data generated from the various *in vitro* mutagenic treatments were subjected to statistical analysis in completely randomized design with factorial concept (FCRD) technique with three repeats as suggested by Panse and Sukhatme (1985) [20].

3. Results and Discussion

3.1 Effect of different mutagenic agents on shoot length (cm)

Among 12 treatments, eight treatments showed a desirable effect on shoot length. Individual mutagens, time period factors found to be significant. The lower and higher concentration of mutagenic agents EMS, MMS registered differences for shoot length. Overall, both higher and lower concentration of sodium azide resulted in poor shoot length. Interaction effect found to be significant on shoot length (refer Table 2). Significantly higer length registered by C_1P_1 (26.60 cm) followed by C_2P_2 (25.20 cm). Significantly higher shoot length was registered in C_2 (21.80 cm) followed by C_1 (20.60 cm). In P_1 significantly higher shoot length was recorded. Similar results were noticed by Kangna *et al*. (2008) [10], Koach *et al*. (2009) [13]. and Chaudhari *et al*. (2018) [4].

Table 2: Effect of different mutagenic agents on shoot length (cm)

C (Concentrations of mutagens)/P (Time period for emersion)		P_1(30 min)	P_2(60 min)	Mean C
C_1	(EMS - 0.2%)	26.60	14.60	20.60
C_2	(EMS - 0.3%)	18.40	25.20	21.80
C_3	(SA – 0.2%)	9.70	14.40	12.05
C_4	(SA – 0.3%)	12.20	8.60	10.40
C_5	(MMS – 0.2%)	24.30	14.20	19.25
C_6	(MMS – 0.3%)	18.60	10.40	14.50
Mean P		18.30	14.57	
Effect		S.Em.+	C.D. @ 5%	CV%
C		0.09	0.28	1.41
P		0.05	0.16	
C x P		0.13	0.39	

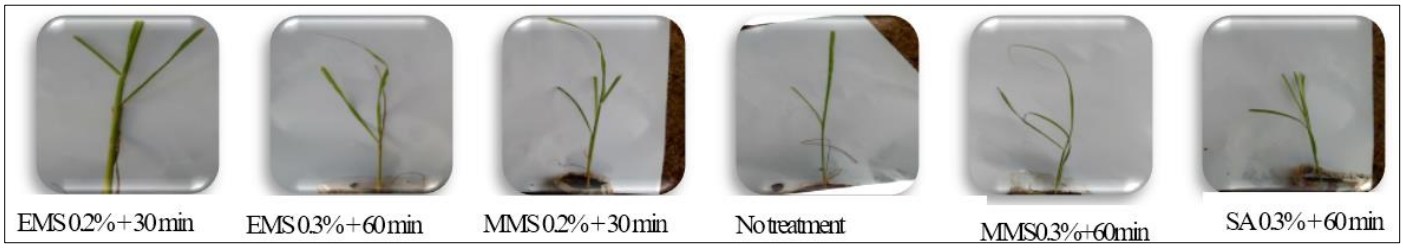

Plate 1: Shoot length

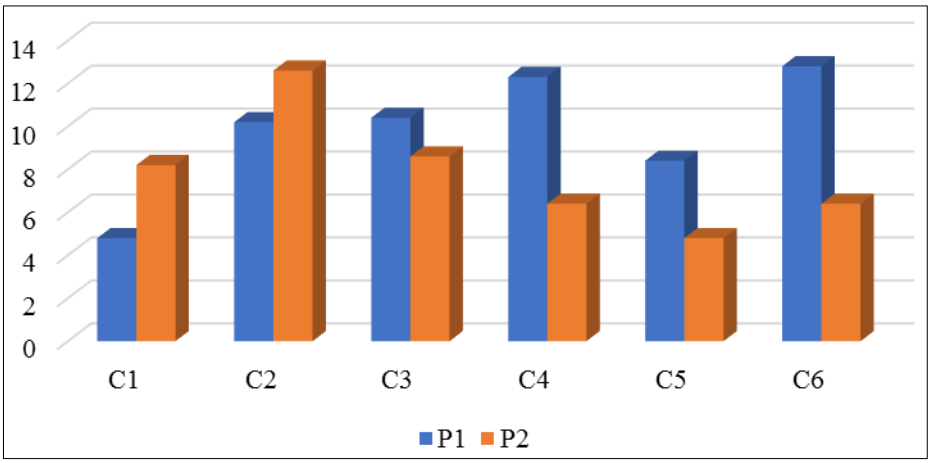

Fig 1: Effect of different mutagenic agents on shoot length (cm)

3.2 Effect of different chemical mutagenic agents on root length (cm)

Among the twelve treatments, nine treatments showed the good results, whereas, three treatments resulted declining direction. The overall higher concentration was of three mutagenic agents irrespective of treatment period exhibited maximum root length. Wide range on mean performance was observed for this character. After 25 days of transplanting in the polyethene bag, root length was recorded. As put in Table 3, effect of interaction was found to be significant for root length. 12.8 cm was observed in treatment C_6P_1 (MMS 0.3% + 30 min) statistically at par with 12.6 cm in treatment C_2P_2 (EMS 0.3% + 60 min), 12.3 cm in treatment C_4P_1 (SA 0.3% + 30 min) numerically followed by 10.4 cm in treatment C_3P_1 (SA 0.2% + 30 min), whereas minimum root length, 4.8 cm was observed in treatment C_1P_1 (EMS 0.2% + 30 min). Significantly higher root length was registered in C_2 (11.40 cm) followed by C_6 (9.60 cm). In P_1 significantly higher root length was recorded. Inhibitory effect of higher treatment time period was observed for root length. Similar results were observed by Muttuswami *et al.* (2001) [16]. and Khan *et al.* (2008) [12].

Table 3: Effect of different mutagenic agents on root length (cm)

C (Concentrations of mutagens)/P (Time period for emersion)		P_1(30 min)	P_2(60 min)	Mean C
C_1	(EMS - 0.2%)	4.80	8.20	6.50
C_2	(EMS - 0.3%)	10.20	12.60	11.40
C_3	(SA – 0.2%)	10.40	8.60	9.50
C_4	(SA – 0.3%)	12.30	6.40	9.35
C_5	(MMS – 0.2%)	8.40	4.80	6.60
C_6	(MMS – 0.3%)	12.80	6.40	9.60
Mean P		9.82	7.83	
Effect		S.Em.+	C.D. @ 5%	CV%
C		0.15	0.43	4.120
P		0.09	0.25	
C x P		0.21	0.61	

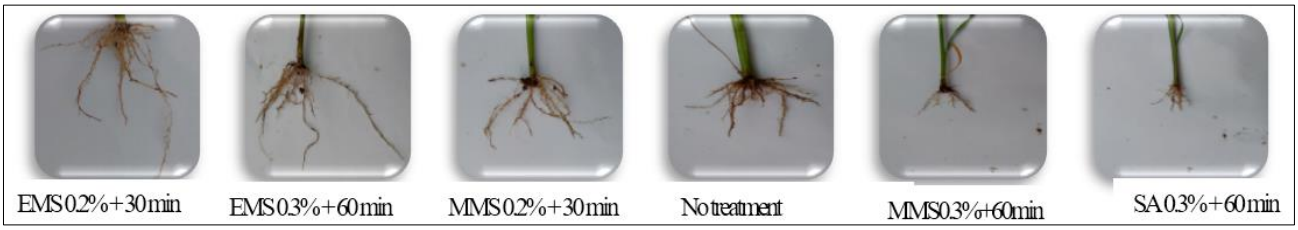

Plate 2: Root length

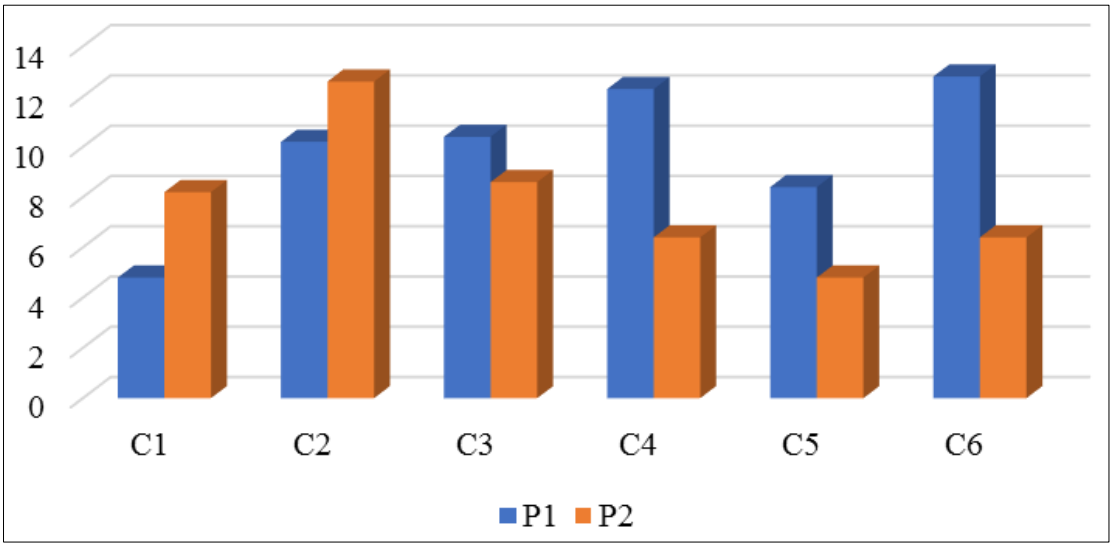

Fig 2: Effect of different mutagenic agents on root length (cm)

3.3 Effect of different chemical mutagenic agents on biomass content (g) after transplanting

Wide range of variability in terms of mean performance was observed from 0.54 g to 1.14 g. Individual mutagenic concentration, time period and interaction effect was found significant for this character. Data furnished in Table 4. indicated that after 25 days of transplanting at primary hardening significantly higher biomass content (1.14 g) was recorded in treatment C_2P_2 (EMS 0.3% + 60 min) followed by 1.12 g in treatment C_6P_1 (MMS 0.3% + 30 min), 1.08 g in treatment C_5P_1 (MMS 0.2% + 30 min) and 0.97 g in treatment C_1P_1 (EMS 0.2% + 30 min). Whereas, minimum biomass content (0.54 g) was observed in treatment C_5P_2 (MMS 0.2% + 60 min). Significantly higher biomass content was registered in C_6 (0.94 g) numerically followed by C_2 (0.91 g). In P_1 significantly higher biomass content was recorded. Evaluation of biomass content under *in vitro* condition provides pre-predicted amount of ion accumulation and synthesis of energy by utilization of photo period. Similar results were obtained by bengus Yu. V. (1999) [3].

Table 4: Effect of different mutagenic agents on biomass content (g)

C (Concentrations of mutagens)/P (Time period for emersion)		P_1(30 min)	P_2(60 min)	Mean C
C_1	(EMS - 0.2%)	0.97	0.58	0.78
C_2	(EMS - 0.3%)	0.67	1.14	0.91
C_3	(SA – 0.2%)	0.56	0.87	0.72
C_4	(SA – 0.3%)	0.94	0.62	0.78
C_5	(MMS – 0.2%)	1.08	0.54	0.81
C_6	(MMS – 0.3%)	1.12	0.76	0.94
Mean P		0.89	0.75	
Effect		S.Em.+	C.D. @ 5%	CV%
C		0.004	0.012	1.22
P		0.002	0.007	
C x P		0.006	0.017	

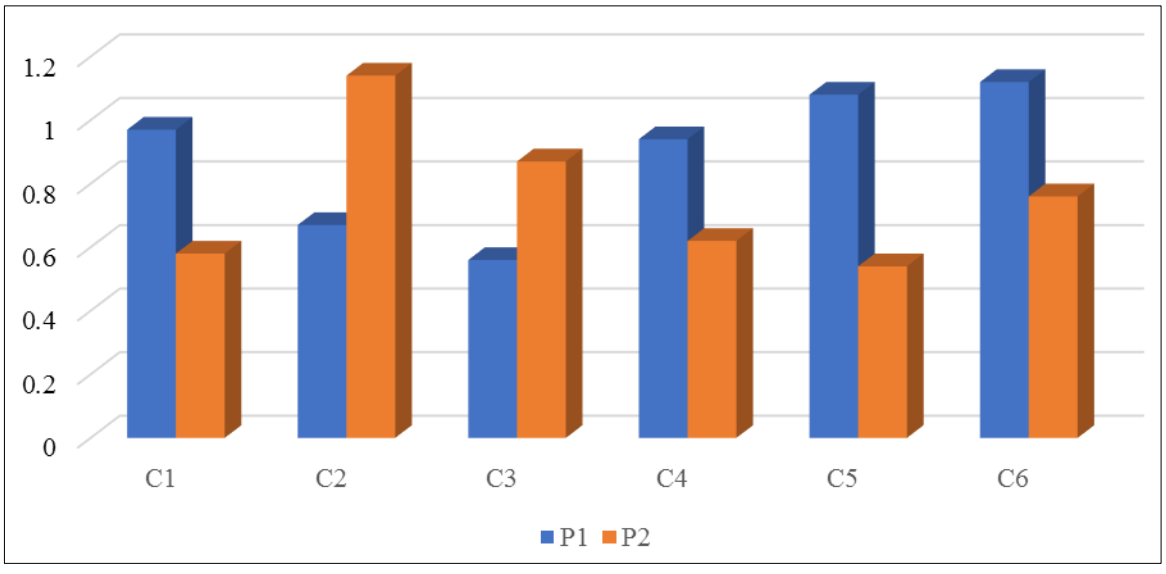

Fig 3: Effect of different mutagenic agents on biomass content (g)

3.4 Effect of different chemical mutagenic agents on leaf area (cm^2/plant)

Among the three mutagenic agents, SA and MMS resulted desirably in leaf area irrespective of concentration, whereas, mutagenic agent EMS was found to be poor for leaf area. Significanlty higher leaf area, 44.7 cm^2/plant was observed (as per the data emblished in Table 5) in treatment C_5P_1 (MMS 0.2% + 30 min) followed by, 42.6 cm^2/plant in treatment C_6P_1 (MMS 0.3% + 60 min), 42.2 cm^2/plant in treatment C_3P_1, (SA 0.2% + 30 min) and, 41.6 cm^2/plnat in treatment C_4P_1 (SA 0.3% + 30 min), whereas, minimum leaf area, 32.5 cm^2/plant was observed in treatment C_2P_2 (EMS 0.2% + 60 min). Effect of mutagenic concentration found to be significant on leaf area (cm^2/plant). Higher leaf area was registered in C_5 (40.55 cm^2/plant), statistically at par with C_3 (39.45 cm^2/plant). In P_1 significantly higher biomass content was recorded. Treatment time played important role rather than mutagenic concentration for leaf area. Similar results were reported by Mohmood someili *et al.* (2011) [22] and K.R.E Padmathilake *et al.* (2007) [18].

Table 5: Effect of different mutagenic agents on leaf area (cm^2/plant)

C (Concentrations of mutagens)/P (Time period for emersion)		P$_1$ (30 min)	P$_2$ (60 min)	Mean C
C_1	(EMS - 0.2%)	40.20	32.50	36.35
C_2	(EMS - 0.3%)	36.40	38.60	37.50
C_3	(SA – 0.2%)	42.20	36.70	39.45
C_4	(SA – 0.3%)	41.60	34.20	37.90
C_5	(MMS – 0.2%)	44.70	36.40	40.55
C_6	(MMS – 0.3%)	42.60	32.60	37.60
Mean P		41.28	35.17	
Effect		S.Em.+	C.D. @ 5%	CV%
C		0.41	1.19	2.62
P		0.24	0.69	
C x P		0.58	1.69	

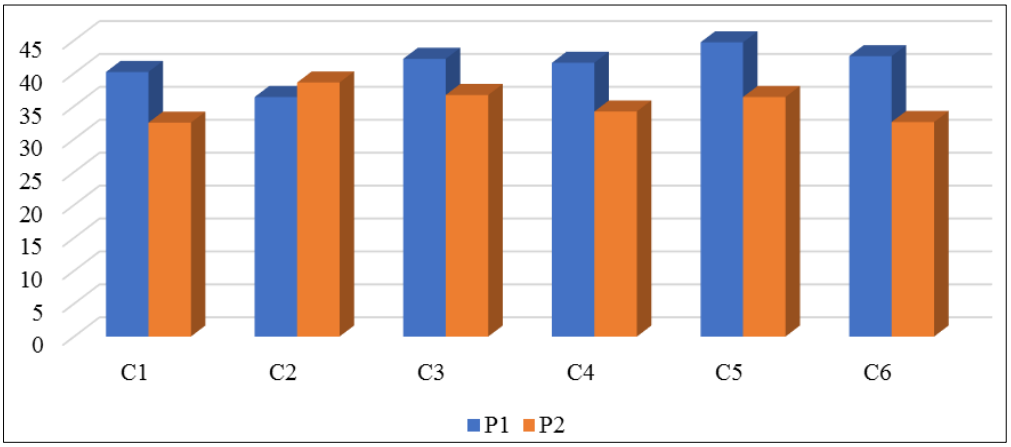

Fig 4: Effect of different mutagenic agents on leaf area (cm^2/plant)

3.5 Effect of different chemical mutagenic agents on chlorophyll content index

Significantly higher chlorophyll content index (as disclosed in Table 6), 4.87 was observed in treatment C_1P_1 (EMS 0.2% + 30 min) numerically followed by 4.64 in treatment C_4P_2 (SA 0.3% + 60 min), 4.56 in treatment C_2P_1 (EMS 0.3% + 30 min) and 4.47 in treatment C_5P_1 (MMS 0.2% + 30 min), whereas minimum chlorophyll content, 3.52 was observed in treatment C_5P_2 (MMS 0.2% + 60 min). Effect of mutagen found to be significant on chlorophyll content index. Higher chlorophyll content index was registered in C_1 (4.29 g) which is at par with C_2 (4.22 g) as well as C_4 (4.24 g), followed by 4 g in C_5. In P_1, the significantly higher chlorophyll content index was recorded. Similar results were observed by Michael *et al.* (1997) [15] and Shoemeli (2011) [22]. Cholophyll content can be used as sensitive indicator of callus and metabolic activity.

Table 6: Effect of different mutagenic agents on leaf area (cm^2/plant)

C (Concentrations of mutagens)/P (Time period for emersion)		P$_1$ (30 min)	P$_2$ (60 min)	Mean C
C_1	(EMS - 0.2%)	4.87	3.70	4.29
C_2	(EMS - 0.3%)	4.56	3.87	4.22
C_3	(SA – 0.2%)	4.16	3.62	3.89
C_4	(SA – 0.3%)	3.84	4.64	4.24
C_5	(MMS – 0.2%)	4.47	3.52	4.00
C_6	(MMS – 0.3%)	4.27	3.67	3.97
Mean P		4.36	3.84	
Effect		S.Em.+	C.D. @ 5%	CV%
C		0.04	0.12	2.44
P		0.02	0.07	
C x P		0.06	0.17	

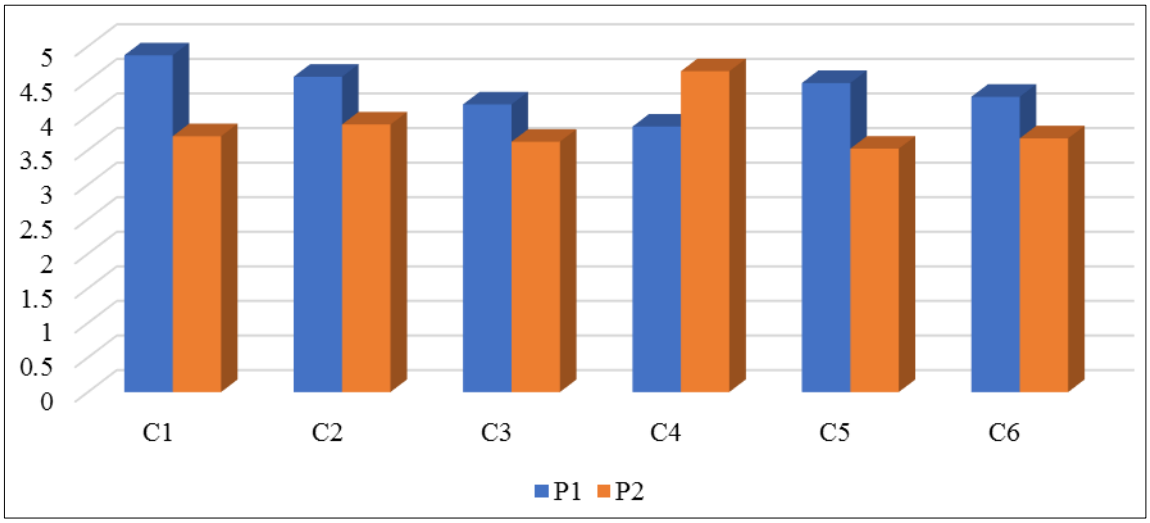

Fig 5: Effect of different mutagenic agents on leaf area (cm^2/plant)

3.6 effect of different chemical mutagenic agents on multiplication rate per cent

Individual mutagenic agents and time periods interacted significantly. Interaction effect of a mutagenic agent with time periods was also found to be significant. Among the treatments (kit out Table 7), significantly higher multiplication rate of 12.45% was observed in treatment C_1P_1 (EMS 0.2% + 30 min) numerically followed by 12.26% in treatment C_6P_1 (MMS 0.3% + 30 min), 11.82% in treatment C_5P_1 (MMS 0.8% + 30 min) and, 10.67% in treatment C_2P_2 (EMS 0.3% + 60 min). Whereas, minimum multiplication rate (4.57%) was observed in treatment C_3P_2 (SA 0.2% + 60 min). Significantly higher multiplication rate was registered in C_6 (10.5%), followed by 10.4% in C_1. In P_1 significantly higher multiplication rate was recorded. Similar results were reported by Imtihaz Khan *et al.* (2009) [11]. and Naik *et al.* (2012) [17]. Higher and lower concentrations of mutagenic agents responded desirabley at lower time exposure.

Table 7: Effect of different mutagenic agents on leaf area (cm^2/plant)

C (Concentrations of mutagens)/P (Time period for emersion)		P_1 (30 min)	P_2 (60 min)	Mean C
C_1	(EMS - 0.2%)	12.45	8.34	10.40
C_2	(EMS - 0.3%)	5.42	10.67	8.05
C_3	(SA – 0.2%)	6.42	4.57	5.50
C_4	(SA – 0.3%)	7.63	8.16	7.90
C_5	(MMS – 0.2%)	11.82	6.51	9.17
C_6	(MMS – 0.3%)	12.26	8.74	10.50
Mean P		9.33	7.83	
Effect		S.Em.+	C.D. @ 5%	CV%
C		0.013	0.037	0.360
P		0.007	0.021	
C x P		0.018	0.052	

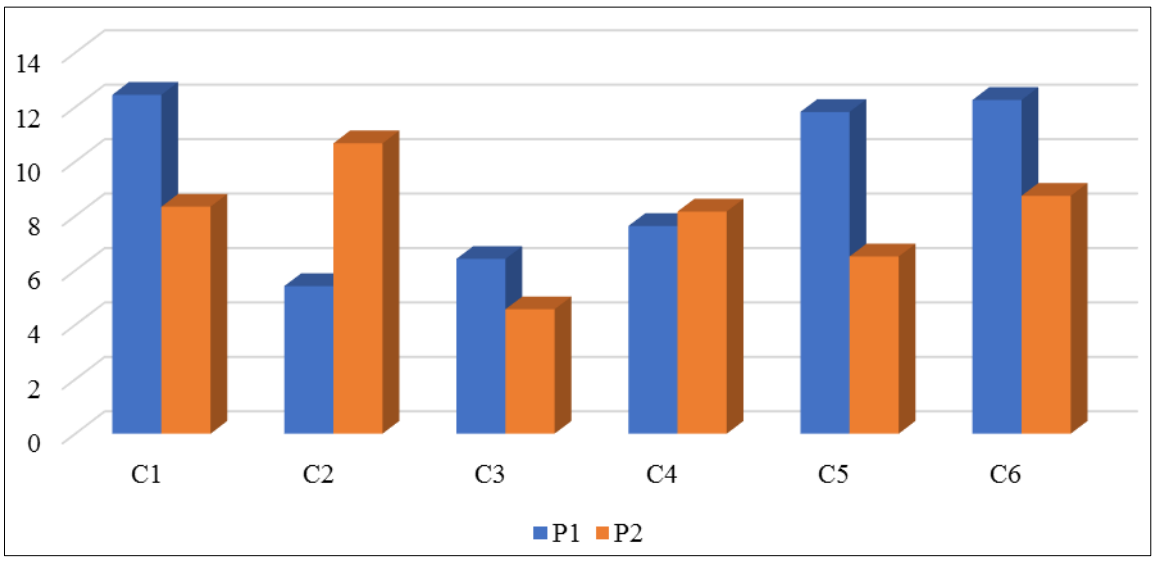

Fig 6: Effect of different mutagenic agents on leaf area (cm^2/plant)

4. Summery and conclusion

Maximum shoot length (26.6 cm) was observed in treatment (EMS 0.2% + 30 min). Individual mutagen as well as time period factor was found to be significant for this character. At the same time, an interaction effect of both the factors was found highly significant. The lower and higher concentration of mutagenic agents EMS and MMS registered significant differences for shoot length. Overall both higher and lower concentration of SA registered poor shoot length. Maximum root length (12.8 cm) was observed in treatment (MMS 0.3% + 30 min). The higher concentration was of three mutagenic agents irrespective of treatment period exhibited maximum root length. Wide range on mean performance was observed for this character. Maximum biomass yield (1.14 g) was observed in treatment (EMS 0.3% + 60 min). Wide range of variation was observed for mean performance from 0.54g to 1.14 g. Individual mutagenic concentration, time period and interaction effect were found highly significant for this character. Maximum leaf area (44.7 cm^2/plant) was observed in treatment (MMS 0.2% + 30 min). Among the three mutagenic agents, SA and MMS registered positive significant difference for leaf area irrespective of concentration, whereas, mutagenic agent EMS was found poor for leaf area parameter. Maximum chlorophyll content index (4.87) was observed in treatment (EMS 0.2% + 30 min). Among the twelve treatments, five treatments showed a significant difference in the positive direction and seven treatments showed significant difference in negative direction as compared to control. Both higher and lower concentration as well as time period influenced chlorophyll content index. Most of the treatments exhibited significance for multiplication rate in positive direction except treatment C_3P_2. Individual mutagenic agents and time periods showed significant differences and the interaction effect of mutagenic agents with time periods was also found highly significant. Among the treatments maximum multiplication rate (12.45) was observed in treatment (EMS 0.2% + 30 min). Upon comparison, poor multiplication rate was observed in lower concentration with high treatment time period of Sodium Azide. For this character mutagenic agents EMS and MMS registered in desirable direction. The mutagenic effect was higher at lower concentration of mutagen and low time intervals, for most of the characters under study. In the present study, EMS levels (0.2% and 0.3%) were most effective mutagenic treatments. The effectiveness decreases with increase in time interval.

5. References

1. Amin S, Kaloo ZA, Singh S. Comparative efficiency of different explants for *in vitro* callus production in *Inula royleana* DC, a threatened medicinal plant growing in Kashmir Himalaya. International Journal of Advanced Research 2013;1(7):617-623.
2. Bashir S, Wani AA, Nawchoo IA. Mutagenic sensitivity of gamma rays, EMS and sodium azide in *Trigonella foenumgraecum* L. Sci Res Report 2013;3(1):20-6.
3. Bengus Y. Decorative periclinal chimeras of *Weigela praecox* (Lemoine) Bailey created by chemical mutagenesis 1999.
4. Chaudhari SD. Genetic variability analysis in sugarcane (*Saccharum* spp. complex) through mutagenesis, an unpublished M.Sc. thesis; submitted to Navsari Agricultural University, Navsari, Guajrat, India 2018.
5. D'Hont A. Unraveling the genome structure of polyploids using FISH and GISH, examples of sugarcane and banana. Cytogenetic and genome research 2005;109(1-3):27-33.
6. Da Silva J, Honeycutt RJ, Burnquist W, Al-Janabi SM, Sorrells ME, Tanksley SD, *et al*. *Saccharum spontaneum* L. 'SES 208' genetic linkage map combining RFLP-and PCR-based markers. Molecular Breeding 1995;1(2):165-179.
7. Daniels J, Roach BT. Taxonomy and evolution. Sugarcane improvement through breeding 1987;1:7-84
8. Ha S, Moore PH, Heinz D, Kato S, Ohmido N, Fukui K. Quantitative chromosome map of the polyploid *Saccharum spontaneum* by multicolour fluorescence in situ hybridization and imaging methods. Plant molecular biology 1999;39(6):1165-1173.
9. Jain SM. Tissue culture-derived variation in crop improvement. Euphytica 2000, 1-4.
10. Kangna M, Hanchinal R, Nadaf, HL. Ethyl methane sulphonate (EMS) induced mutation and select ion for salt tolerance in sugarcane *in vitro*. Indain J. Pl. Physiol 2008;13(4):405410.
11. Khan IA, Qurainy Fahad Al, Anwar Firoz. Sodium azide a chemical mutagen for enhancement agronomic traits in crop pants. Environment and We: An International Journal of Science & Technology 2009;4(1-21):0975-7120.
12. Khan SA, Rashid H, Chaudhary MF, Chaudhry Z, Afroz A. Rapid micropropagation of three elite sugarcane (*Saccharum officinarum* L.) varieties by shoot tip culture. African Journal of Biotechnology 2008;7(13).
13. Koch AC, Ramgareeb S, Snyman SJ, Watt MP, Rutherford RS. Pursuing herbicide tolerance in sugarcane: screening germplasm and induction through mutagenesis. In Proceedings of the Annual Congress-South African Sugar Technologists' Association 2009;82:629-632.
14. Medina FIS, Amano E, Tano S. Mutation breeding manual. Forum for Nuclear Cooperation in Asia 2005, 1 - 177.
15. Michael R, Anne-Alineor V, Dale S, Mansfield TA. How can stomata contribute to salt tolerance? Ann. Bot 1997;80:387-393.
16. Muthusamy A, Vasanth K, Sivasankari D, Chandrasekar BR, Jayabalan N. Effects of mutagens on somatic embryogenesis and plant regeneration in groundnut. Biologia Plantarum 2007;51(3),430-435.
17. Naik KH. Dissertation on Regeneration and plantlet development from somatic tissue in sugarcane variety CoC 671 (*Saccharum* spp. complex) an unpublished M.Sc. thesis; submitted to Bhagavan Mahaveer college of M. Sc. biotechnology, Surat 2012.
18. Padmathilake KRE, Wickramaarachchi VN, Anver MAMS, Bandara DC. Biological and economic fasiability of growing mint (*Mentha sylvestris* L.), Mustard (*Brassica integrifolia* l.) and Asamodagma (*Trachyspermum involucratum* L.) under hydro phonics. Trophical Agricultural Research 2007;19:193-201.
19. Panje R, Babu C. Studies in *Saccharum spontaneum* distribution and geographical association of chromosome numbers. Cytologia 1960;25:152-172.
20. Panse VG, Sukhatme PV. Statistical Methods for Agricultural Workers, I.C.A.R., New Delhi 1985.
21. Rahimi M, Daneshvar MH, Heidari M, Yari F. *In vitro* micropropagation of *Fritillaria imperialis* L. through

induction of indirect organogenesis. International Journal of Agronomy and Plant Production 2013;4(3):418-424.
22. Shomeili M, Nabipour M, Meskarbashee M, Memari HR. Evaluation of sugarcane (*Saccharin officinarum* L.) somaclonal variants tolerance to salinity *in vitro* and *in vivo* cultures. African Journal of Biotechnology 2011;10(46):9337-9343.
23. Soeranto. Peran Iptek Nuklirdalam Pemul Iaan Tanaman Untuk Mendukung Industri Pertanian. Jakarta: Puslitbang Teknilogi Isotop dan Radiasi. Badan Tenaga Nuklir Nasional 2003.
24. Sreenivasan TV, Ahloowalia BS, Heinz DJ. Chapter 5-cytogenetics, ined. Don J. H., editor. Pp. 211–253. Sugarcane callus tissue. Crop Sci. 1987;17:814-816.
25. Svetleva DL, Crino P. Effect of ethyl methanesulfonate (EMS) and N-nitrose-Nethyl Urea (ENU) on callus growth of common bean. J Cent Eur Agric 2005;6(1):59-64.
26. Talebi AB, Talebi AB, Shahrokhifar B. Ethyl methane sulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose determination 2012.
27. Van Harten AM. Mutation breeding: theory and practical applications. Cambridge University Press 1998.
28. Zhang J, Nagai C, Yu Q, Pan YB, Ayala-Silva T, Schnell RJ *et al*. Genome size variation in three *Saccharum* species. Euphytica 2012;185(3):511-519.