Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [2] January 2021 : 127-132

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

EMS induced mutagenic effects on meiotic behaviour and photosynthetic pigment content in *Setaria italica* (L.) Beauv.

[1]Girjesh Kumar and [1]Indresh Kumar Pandey*
[1] Department of Botany, University of Allahabad, Prayagraj, UP, India
*Email: pandey197@gmail.com

ABSTRACT
This study is aimed at mutagenic impacts of Ethyl Methane Sulfonate (EMS) to find out potential dose to be effective in mutation breeding of climate resilient grass, Setaria italica (L.) Beauv., commonly known as Foxtail millet. Pure inbred seeds were treated with different doses of EMS to analyse the effects on cytology and photosynthetic pigment content. Sensitivity of plant for different doses of chemical mutagen was studied. It was found that meiotic abnormalities increases with increase in mutagen dose. At lower doses EMS has positive impact on photosynthetic pigment content which increased significantly but at higher doses pigment content was decreased. This confirms the suitability of lower doses of EMS for creating genetic variation by mutation breeding.
Keywords: *Ethyl Methane Sulfonate (EMS), Mutagenesis, Chromosomes, Photosynthetic pigment*

Received 12.12.2020 Revised 01.01..2021 Accepted 11.01. 2021

INTRODUCTION

Millets are climate resilient small seeded plants belonging to Poaceae family. They are considered as one of the oldest cereal grown in drought prone geographical areas. Since time immemorial millets had been an important component of diet in India. But in last sixty years wheat and rice has replaced millets as main dietary components so they are also called as Forgotten crops or Orphan crops. With the advent of lifestyle diseases millets are regaining importance due to their nutritional value. Moreover, millets are advantageous over other crops as they can be grown in dry weather and nutrient-poor soil, produce stable yield due to climate resilience properties and also their grains may be stored for long time securing food supply[6]. *Setaria italica* (L.) Beauv., commonly known as foxtail millet, is a versatile small millet crop known for its climate resilience properties. It is a diploid self-pollinated C_4 grass grown as relict crop in several parts of Asia and Europe [12]. Meiosis has been considered as a paradoxical process which is a harmonious combination of universality and uniqueness. Any cytogenetic event is regulated by certain specific genes. Mutations in these genes results into abnormalities which physiologically may confer beneficial traits in plants. Meiotic abnormalities are cause for morphological and genetic variations which leads to intraspecific reproductive barriers and evolution. This fundamental principle is the basis for all mutation breeding experiments.

Mutation breeding is a popular efficient technique for genetic improvement of crops. Mutation breeding is a popular technique for crop improvement. Improvement of any cultivar or a variety depends on the changes in the mode of gene action and induction of genetic variability. Induced mutagenesis is a potent tool for creating genetic variability in crops. Physical and chemical mutagens can create variability in quantitative and qualitative traits of crops. Various mutagens induce favourable mutations in plants. Mutagenesis causes a broad range variation in morphological as well as in cytological parameters. In foxtail millet, mutation breeding is easier and popular tool than artificial hybridization because of small florets causing difficulties in emasculation and pollination.

Ethyl Methane Sulfoate (EMS) is a chemical mutagen which is used to induce mutation in seeds. EMS has affinity for guanine rich areas resulting into breaks or damage in chromosomes. It acts as an alkylating agent and induces random point mutations, single base substitution, at a very high frequency which may create novel stop codons in the genes [2, 21]. EMS has been a common mutagen in mutation breeding programmes which is also used to develop a large number of mutants utilized in development of TILLING populations [16]. EMS has given successful results in many plants which includes generation of morphological diversity, improvement of desirable traits, male sterility and disease resistance. Some

common recent examples include resistance to Potyvirus in tomato [18] and increased phenolic content in egg plant [23]. Cytological analysis of meiotic behaviour in response to certain specific doses of a mutagen is one of the most important index to estimate the potency of mutagen against various genotypes [17]. Although some mutagenic studies have been done in the plant but less attention has been given on cytological studies.

MATERIAL AND METHODS

Seed Procurement Inbred seeds of *Setaria italica* (L.) Beauv. were collected from NBPGR, Akola centre, Maharashtra, India.

Seed treatment Healthy seeds were pre-soaked in distilled water for overnight so that seed coat becomes permeable for chemical mutagen. Three different concentrations of EMS solutions *viz.* 0.1 %, 0.3 % and 0.5% were prepared in phosphate buffer solution at pH 7.0. Pre-soaked seeds were treated with these three freshly prepared EMS solutions for 5 hours. Some pre-soaked seeds were kept in distilled water to be used as control set. After completion of 5 hours, treated seeds were thoroughly washed with water to remove the excess of mutagen which remained over the seeds. EMS treated seeds along with control set were sown in field in replicates of three for each treatment to raise plants for the study of different cytological and biochemical parameters.

Fixation and meiotic study At flowering stage inflorescence with appropriate size were fixed in carnoy's fixative (3:1, absolute alcohol: glacial acetic acid) for 24 hours. Thereafter, inflorescence were transferred to 70 % alcohol to preserve it at 4 ^{0}C for further use. For meiotic studies slides were prepared by anther squash technique using 2 % acetocarmine stain.

Statistical analysis Data obtained from cytological and biochemical studies were statistically analysed using SPSS 16.0 software. Observations for each treatment were recorded in triplicates and one independent variable was used. One way analysis of variance (ANOVA) was done for further analysis. For calculation of mean Duncan's multiple range test (DMRT, $P<0.05$) was performed and graphs were prepared using Sigma plot 10.0 software. For biochemical study similar statistical analysis of photosynthetic pigment content was done.

Photosynthetic pigment content analysis Lichtenthaler's method was used for estimation of photosynthetic pigment content [15]. In 5 ml of 80% acetone, 20 mg of fresh leaves from each treatment were crushed. To reduce the probability of errors experiment was conducted in triplicates.

RESULTS AND DISCUSSION

In control plants meiosis was found normal with nine bivalents (2n=18). In control plants metaphase-1 and anaphase-1 was normal without any anomaly in chromosomal configuration. In treated sets stickiness was the most common abnormality. Scattering at metaphase, unorientation, asynchronous division are most common meiotic abnormalities which were observed in Pollen Mother Cells of all treated plants. Among metaphasic abnormalities, magnitude of precocious movement, unorientation, multivalent formation and asynchronous division uniformly increased with increasing dose of mutagen. Cytomixis with channels is a significant finding among other abnormalities. Asynchronous division and unorientation were more frequent in stages of meiosis-I in comparison to stages of meiosis- II.

Meiosis is the most significant process in the life cycle of an organism which consists of physiological, biochemical, cytogenetic and phenotypic events in a highly coordinated manner leading to genetic recombination, reduction in chromosome number and formation of gamete [11]. Mutation in genes associated with meiotic process may cause disrupted meiosis and chromosomal abnormalities. In some cases it may lead to gametic sterility[13]. Study of cytological abnormalities and estimation of its magnitude is an important parameter to assess the sensitivity of any plant to specific physical and chemical mutagen. Meiotic studies showed that although similar types of chromosomal aberrations were formed in all treated plants but frequency and magnitude varied from dose to dose. In present experiment frequency of different abnormalities consistently increased with the increasing dose of mutagen. Chromosomal abnormalities included Scattering, Precocious movement of chromosomes, Stickiness, Unorientation, Multivalent, Secondary associations of bivalents, Asynchronous division, Forward movement and Laggards. Along with it cytomixis with transfer of genetic material through channels was also observed. (Figure 1,2).

The recorded cytological data are shown (Table 1) which infers that magnitude of meiotic abnormalities were increased with the increasing concentration of mutagen. Different types of chromosomal abnormalities were observed which have been reported in other plants by some workers. Most of the plants showed a general pattern of increase in abnormality percentage with increase in dose of mutagens. But in response to mutagen plants show different kinds of cytological abnormalities depending upon the

basic nature of chromosomes and action of mutagen on genes. In present study significant observations include stickiness as the most prevalent chromosomal abnormality at all doses of EMS and both metaphase as well as anaphase. Most common cause for stickiness is nucleic acid depolymerisation or nucleoprotein dissociation and changes in their organisation [4, 22]. In some cases, stickiness in chromosomes may be a final outcome of chromosomal breakage [8] or improper folding of DNA [7]. EMS, being a potent alkylating agent, must have caused condensation of chromosomes by direct action on target proteins involved in meiosis resulting into disturbances in chromosomes leading to stickiness. Main reasons for precocious movement of chromosomes are early terminalisation, stickiness and abnormal movement of some chromosomes during anaphase [19]. These effects might have been caused due to abnormality in spindle activity or deformed chromosomal pairing [1] leading to haphazard chromosomal movement resulting into precocious movement of chromosomes. Multivalent formation under the influence of mutagens are considered to be a result of irregular pairing and breakage [3]. Formation of multivalents in many plants have been attributed to breakage and reunion of chromosomes through translocation [14]. Irregular pairing and breakage of chromosomes may be a result of translocations and inversions. Formation of laggards may be attributed to delayed terminalisation due to chromosomal stickiness or in some cases due to slow chromosomal movement [10].

Treatment	No. of PMC's observed	Metaphasic Abnormalities (%)							Anaphasic Abnormalities (%)				Telophasic Abnormalities (%)	Oth (%)	T.Ab. (%)
		Sc	Pm	St	Un	Mv	Sa	Asy	Fm	Lg	Un	St			
CONTROL	285	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.1%	287	0.23±0.11	-	0.81±0.12	0.46±0.11	-	-	0.57±0.22	-	0.35±0.20	0.58±0.10	0.93±0.12	0.34±0.04	0.34±0.19	4.99±0.40
0.3%	277	0.71±0.19	0.23±0.11	1.07±0.19	0.48±0.12	0.48±0.13	-	0.71±0.19	0.47±0.11	0.96±0.32	0.95±0.30	1.32±0.13	1.15±0.28	0.36±0.21	8.97±0.28
0.5%	280	0.58±0.30	0.59±0.12	0.71±0.01	0.59±0.12	1.18±0.10	0.58±0.22	1.07±0.22	0.59±0.12	0.59±0.12	1.91±0.34	2.38±0.12	1.19±0.25	0.47±0.12	12.51±0.75

Table 1: Effect of Ethyl Methane Sulfonate (EMS) on meiotic behaviour and chromosomal morphology in *Setaria italica* (L.) Beauv.

Abbreviations: Sc- Scattering**; Pm-** Precocious at metaphase**; St-** Stickiness**; Un-** Unorientation**; Mv-** Multivalent**: Sa-** Secondary association**: Asy-** Asynchronous: **Fm-** Forward movement**; Lg-** Laggard**; Oth-** Others**; TAB–**Total abnormality percentage**.**

***Different letters in the superscript denote significant differences means ($p < 0.05$) by DMRT (one way ANOVA)**

Cytomixis is due to action of chemical mutagen. It causes aneuploidy in plants with unique morphological features and gametes without reduction in chromosome number [20]. This phenomenon is also responsible for reduced pollen fertility.

Photosynthetic pigment content was calculated in unit µg (mg fresh weight)$^{-1}$. In comparison to leaves of control plant content of chlorophyll a, chlorophyll b and carotenoids increased in both 0.1% and 0.3% EMS treatment. But at highest dose of 0.5 % EMS treatment content of all three pigments were decreased in comparison to control. Maximum effect was seen in case of carotenoid content and chlorophyll a was least affected. At lower doses along with significant cytogenetic changes there was an increment in photosynthetic pigment content. Photosynthetic pigment content is directly correlated with photosynthetic efficiency of plants. Chlorophyll a and chlorophyll b are responsible for photosynthetic process and carotenoids protect chlorophyll from photo-destruction. Due to mutagenic effects enzyme system in plants are considered to be activated leading to increased photosynthetic pigment content [5, 9]. But at 0.5 % EMS concentration both chlorophyll and carotenoids are reduced. This result infers that at lower doses EMS cause positive effects on photosynthetic pigment content.

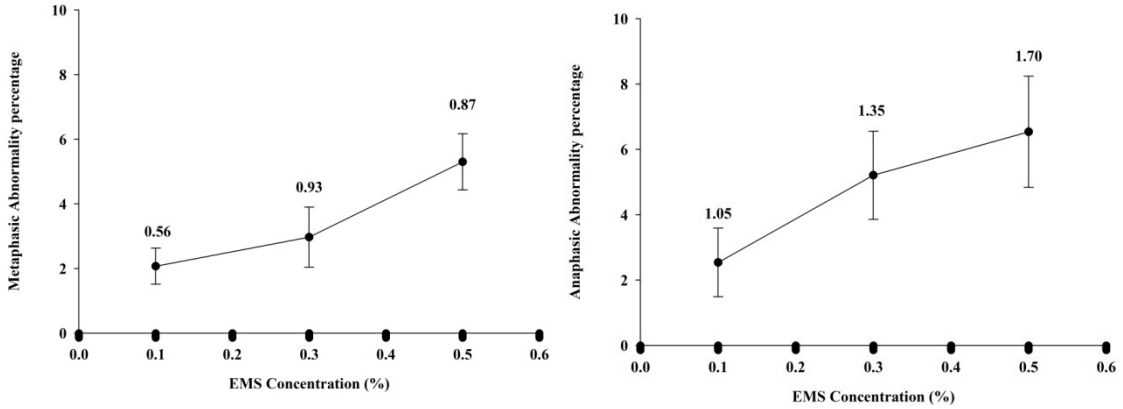

Figure 1: Graph showing effect of EMS on metaphasic and anaphasic abnormality percentage in *Setaria italica* (L.) Beauv

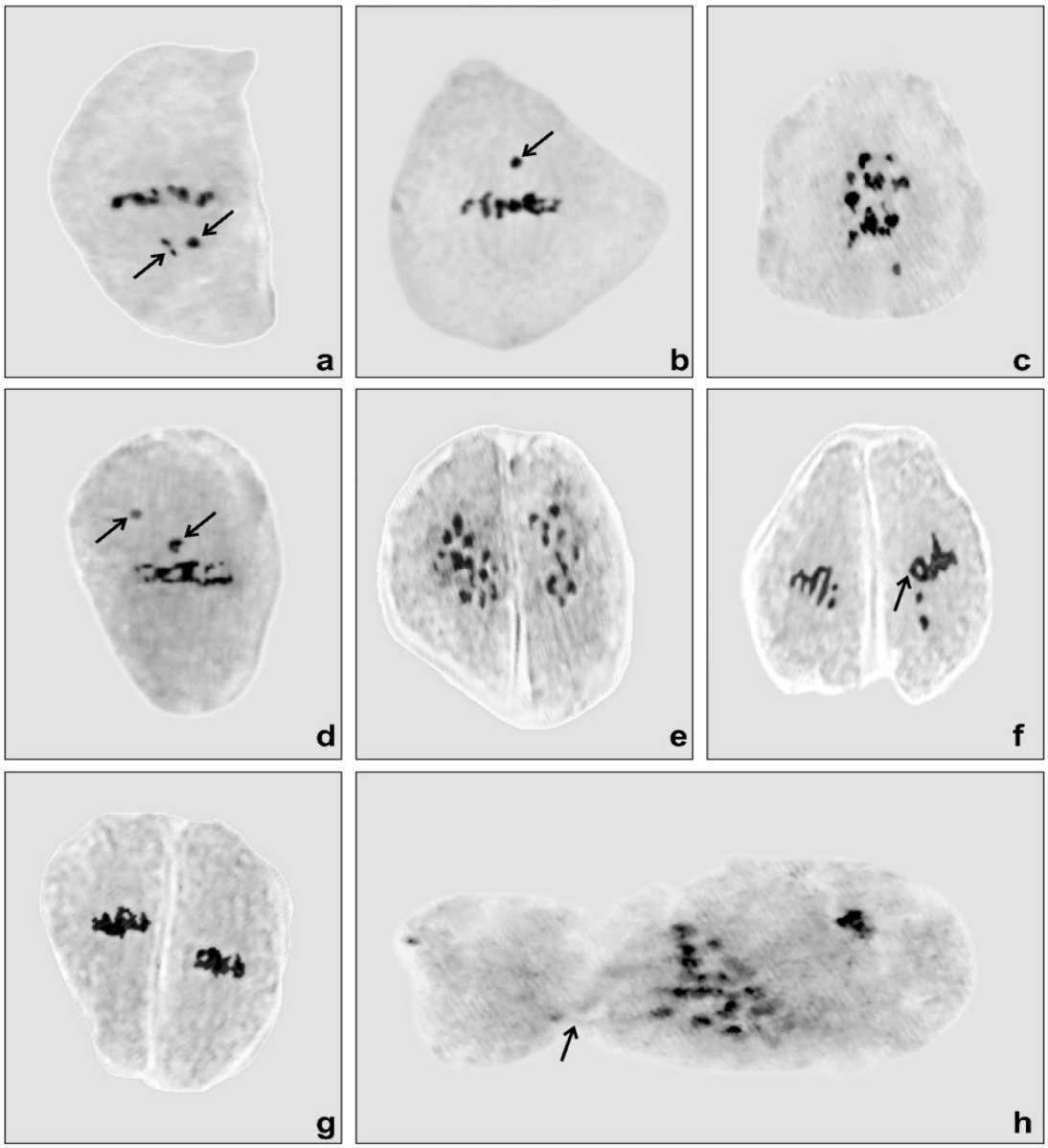

Figure 2: Cytological plate

Legend of figures- **a:** Two precocious at Metaphase-I; **b:** Stickiness and precocious at Metaphase-I; **c:** Scattering at Metaphase-I; **d**: Precocious Metaphase-I; **e**: Scattering at Metaphase-II; f: Asynchronous and loop formation at Metaphase-II; **g**: Sticky at Metaphase –II; **h:** Cytomixis with two channels

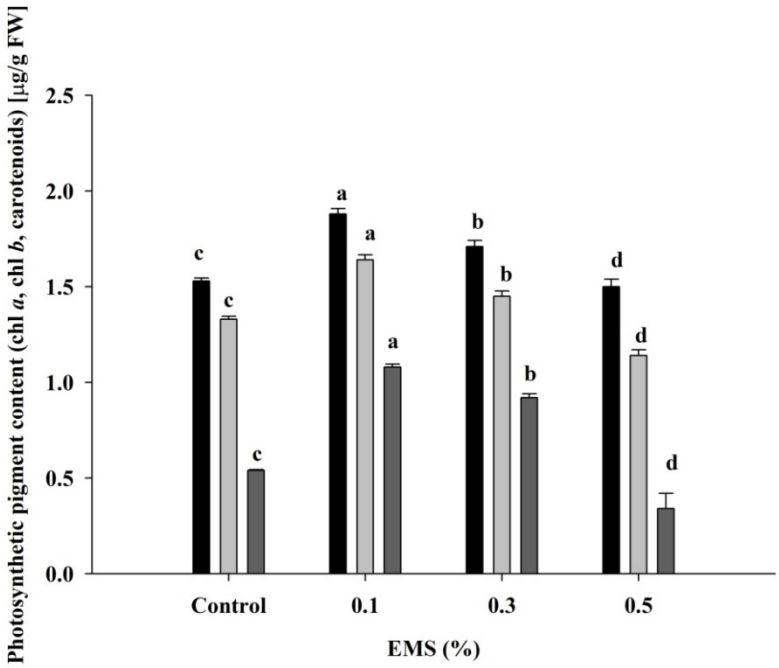

Figure 3: Graph showing effect of Ethyl Methane Sulfonate (EMS) on photosynthetic pigments in *Setaria italica* (L.) Beauv

CONCLUSION

Analysis of reductional division is an important dependable index in measurement of mutagen potency. Hence, studies on meiotic disturbances indicate that genetic load resulting from mutation is an important integral part of mutagenesis experiments. It also helps to determine the suitability of type and dose of mutagen for a plant to achieve maximum output. At lower doses of EMS change in genetic variability was observed with increment in photosynthetic pigment content. This infers that lower doses of EMS may successfully induce beneficial characters in foxtail millet.

DECLARATION OF INTEREST

The authors report no conflict of interest.

REFERENCES

1. Agarwal, R. & Ansari, M.Y.K. (2001). The effect of aniline on root tip cells of *Vicia faba* L. J Cytol Genet, 2:129–134
2. Arisha, M. H., Liang, B.-K., Shah, S. N. M., Gong, Z.-H. & Li., D.-W. (2014). Kill curve analysis and response of first generation *Capsicum annuum* L. B12 cultivar to ethyl methane sulfonate. *Genet. Mol. Res.,* 13, 10049–10061
3. Bhat, T.A., Parveen, S. & Khan, A.H. (2007a). Meiotic studies in two varieties of *Vicia faba* L. (Fabaceae) after EMS treatment. Asian J Plant Sci, 6:51–55
4. Evans HJ (1962) Chromosome aberrations induced by ionizing radiations. Int Rev Cytol 13:221–321
5. Ferreira-Castro F, *et al*. (2007). Effects of gamma radiation on maize samples contaminated with *Fusarium verticillioides*. Appl Radiat Isot; 65(8):927–933.
6. Gale, M.D., Devos, K.M. & Moore, G. (1996). Rice as the pivotal genome in the new era of grass comparative genetics. Rice Genetics III, 77-84
7. Gaulden, M.E. (1987). Hypothesis: some mutagens directly alter specific chromosomal proteins to produce chromosome stickiness. *Mutagenesis,* 2:357–365.
8. Jabee, F. & Ansari, M.Y.K. (2005). Mutagenic effectiveness and efficiency of hydrazine sulphate (HS) in inducing cytomorphological mutations in *Cicer arietin*um L. var. K-850. J Cytol Genet, 6:161–166
9. Junaid, A., Mujib, A. & Sharma, M.P. (2008). Effect of growth regulators and ethylmethane sulphonate on growth, and chlorophyll, sugar and proline contents in *Dracaena sanderiana* cultured in vitro**.** Biol. Plant., 52 (3) 569-572.
10. Jayabalan, N. & Rao, G.R. (1987). Gamma radiation induced cytological abnormalities in *Lycopersicon esculentum* Mill. Var. Pusa Ruby. Cytologia, 52, 1–4.
11. Kau,l M.L.H. & Singh, R.B. (1991). Male sterility in barley. 5. Gene action and microsporogenesis. Cytobios, 66: 71-85.
12. Kawase, M. & Sakamoto, S. (1982). Geographical distribution and genetic analysis of phenol colour reaction in foxtail millet, *Setaria italica* (L.) Beauv. Theor. Appl. Genet., 63, 117-119.

13. Khan, S. & Goyal, S.(2009). Improvement of mungbean varieties through induced mutations. Afr. J. Plant Sci., 3: 174-180.
14. Kumar, G. & Gupta, P. (2009). Induced karyomorphological variations in three phenodeviants of *Capsicum annuum* L. Turk J Biol, 33:123–128.
15. Lichtenthaler HK. (1987). Chlorophylls and carotenoids, the pigments of photosynthetic biomembranes. In: Douce, R. and Packer, L. (eds.), Methods Enzymol; 148, 350-382.
16. Pathirana, R. (2012). "Plant mutation breeding in agriculture," in Plant Sciences Reviews 2011. *CAB Rev.* 107.
17. Patil, B.C. & Bhat, G.I. (1992). A comparative study of MH and EMS in the induction of chromosomal aberrations on lateral root meristem in Clitoria ternatea L. Cytologia. 57: 259-264.
18. Piron F, Nicolaï M, Minoïa S, Piednoir E, Moretti A, Salgues A, *et al.* (2010). An Induced Mutation in Tomato eIF4E Leads to Immunity to Two Potyviruses. PLoS ONE 5(6): e11313.
19. Permjit, K. & Grover, I.S. (1985). Cytological effect of some organophosphorus pesticides. II Mitotic effect. Cytologia, 50:199–210.
20. Sheidai, M., Riahi, H. & Hakim, M. (1993). Cytomixis in *Asparagus* L. Nucleus, 36:59–62.
21. Talebi, A., Talebi, A. & Shahrokhifar, B. (2012). Ethyl Methane Sulphonate (EMS) Induced Mutagenesis in Malaysian Rice (cv. MR219) for Lethal Dose Determination. *American Journal of Plant Sciences*, Vol. 3 No. 12, pp. 1661-1665.
22. Tarar, J.L. & Dnyansagar, V.R. (1980). Comparison of ethyl methane sulphonate and radiation induced meiotic abnormalities in *Turnera ulmifolia* Linn. Var. Angustifolia Wild. Cytologia, 45:221–231.
23. Xi-ou X, Wenqiu L, Wei L, Xiaomin G, Lingling L, Feiyue M & Yuge L (2017) The Analysis of Physiological Variations in M_2 Generation of *Solanum melongena* L. Mutagenized by Ethyl Methane Sulfonate. *Front. Plant Sci.* 8:17.

CITATION OF THIS ARTICLE

G Kumar and I K Pandey. EMS induced mutagenic effects on meiotic behaviour and photosynthetic pigment content in *Setaria italica* (L.) Beauv. Bull. Env. Pharmacol. Life Sci., Vol 10[2] January 2021 : 127-132