Bangladesh J. Nuclear Agric, 37(2): 19-27, 2023 *ISSN 0258-7130*

DOI: https://doi.org/10.3329/bjnag.v37i2.71777

MUTAGENIC EFFECT OF ETHYL METHANE SULFONATE ON SOME FIBER SPECIES

N.B. Atiq*, M.S. Haque, M.M.A. Noor, S.A. Sammy, M. Perves and S. Khanam

Abstract

Jute, kenaf and mesta are prominent fibrous plants with significant industrial and nutritional value for optimization of their cultivation process can have substantial agricultural and economic implications. The study was conducted on exploring the impact of ethyl methane sulfonate (EMS) presoaking treatments on the germination and the sensitivity of fiber crops to mutagens. This research aimed to evaluate the germination potential of jute, kenaf and mesta variety using various concentrations of EMS presoaking treatments, and analyze the sensitivity and induced variability in the M_1 generation. Seeds were presoaked in four different concentrations of EMS. The subsequent parameters, including germination, root length, and shoot length, were measured at three crucial growth stages: 12, 24, and 36 days after sowing (DAS). A probit analysis was conducted to determine the LD_{50} value, which signifies the concentration at which 50% of the population exhibits an adverse effect. Moreover, significant variances were detected in germination rates, root elongation, and shoot growth across the different presoaking treatments. The findings emphasize the sensitivity of these fibrous plants to EMS concentrations, with increasing concentrations having a detrimental effect on growth parameters. This information can be instrumental for breeders and agriculturists looking to optimize germination in these crops.

Key words: Mutation, Mutagen, Probit analysis, LD_{50}

Introduction

Jute, a significant bast fiber in the world of fibrous crops, stands out not just for its economic value but also for its commendable eco-friendly attributes. Often hailed as the green counterpart to cotton, it occupies a coveted position in terms of usage, global consumption, production and availability (Ahmed and Nizam, 2008). The adaptability of jute is evident in its manifold applications spanning textiles, construction, packaging, agriculture, and beyond. As a natural fiber, it possesses superior tensile strength, alongside a low extensibility factor, making it an ideal choice for crafting a multitude of everyday items including ropes, twines, hessian fabrics, carpets, and rugs (Mir *et al*., 2008). Its eco-centric attributes, like recyclability and 100% biodegradability, further endorse its environmental credentials.

Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture, BAU Campus, Mymensingh-2202

*Corresponding authors' email: nusratamia@gmail.com

However, the glory days of jute, especially during the 1970-80s when it covered a sprawling 9 lakh hectares of Bangladesh's cultivable land, have witnessed a decline. By 2017-22, these numbers plummeted to a mere 6-7 lakh hectares, producing roughly 77-84 lakh bales of fiber (BBS, 2022). This reduction is partially attributed to the pressing need to allocate land for food crops, catering to the escalating demands of a booming population. But the challenge doesn't end here. Jute's progress is hampered by intrinsic biological constraints such as its high sensitivity to photoperiodism and cross-breeding issues between cultivated and wild species. These hurdles underline the urgency to innovate and develop adaptable jute genotypes, which not only have a shorter life span but are also versatile in varied cropping patterns (Islam *et al*., 2017; Sarker *et al*., 2007).

In light of these challenges, there emerges a beacon of hope: chemical mutagenesis, with a special focus on Ethyl Methane Sulfonate (EMS) treatments. This method promises an efficient alternative, as mutation breeding offers plant breeders a swift mechanism to enhance crop qualities ranging from yield and quality to resilience (Oladosu *et al*. 2016; Bolbhat and Dhumal, 2009). The introduction of genetic variability via mutation breeding can pave the way for unveiling mutant lines enriched with sought-after traits (Mei *et al*., 2007).With these insights, our study takes the helm, aiming to scrutinize the germination potential of not just jute, but also kenaf and mesta, when subjected to the chemical mutagen, EMS.

Materials and Methods

Seed Selection and Preparation

For this study, we sourced high-quality seeds from distinct fibrous plants: jute varieties (O-9897, O-795, O-72 and BJRI deshi patshak), kenaf (HC-95) and Mestapat-1. Careful scrutiny ensured only the healthiest seeds were retained for experimentation, ensuring consistency in our observations.

Seed Treatment Procedure

A batch of two hundred seeds for each specified treatment underwent a thorough cleansing process. This involved washing the seeds thrice using a 0.1% (v/v) Tween-20 solution prepared in distilled water. This step ensured removal of any contaminants from the seed surface.

With precision, seeds were subsequently exposed to EMS concentrations of 0.5%, 0.75%, 1.0%, and 1.5%. Each concentration was applied for specific durations: 2, 3, 4, and 6 hours, respectively. To ensure uniform EMS penetration, the seeds were subjected to continuous agitation in an electric shaker set at 80 rpm.

Control seeds were incubated in distilled water maintaining the same incubation condition.To ensure no residual EMS remained, the treated seeds underwent eight consecutive washes using distilled water.

Post-cleansing, seeds were air-dried under ambient room conditions. Once adequately dried, seeds were sown under two distinct settings: standard Petri dishes and field conditions. All field-based experiments were meticulously executed at the Bangladesh Institute of Nuclear Agriculture, located in Mymensingh, Bangladesh.

Data Collection and Analysis

LD_{50} value for EMS was calculated based on probit analysis (Finney, 1978). Probit analysis was carried out in excel by following procedure

Mortality percentage of seeds was calculated for all the doses and the value was rounded to the nearest whole number. Corrected mortality percentage was calculated using Abbott's formula.

Corrected mortality=$\frac{M\ observed - M\ control}{100 - M\ control} \times 100$

All the corrected values were rounded to the nearest whole number. Probit value was worked for the corresponding corrected mortality percentage value. Probit graph was drawn using probit values on Y-axis against treatment concentration on X-axis. EMS dose at corresponding probit 5 values was estimated as LD_{50} for the mutagen.

Results and Discussion

Effects of EMS on germination in fibrous plants

As displayed in *Fig. 1*, the data elucidates the sensitivity of different varieties of fibrous plants to varying EMS levels. At control stage (0% EMS) presented an impressive germination rate for kenaf (HC-95) and jute (O-9897) with a full 100% germination. As we introduce EMS, we observe a predictable reduction in germination rates. The most profound dip in germination was witnessed in Mestapat-1 at 1.5% EMS, settling at just 30%. This decremental trend with escalating EMS doses echoes the patterns noted in prior studies on crops like rice, reinforcing the established knowledge on mutation breeding (Mohamad *et al*., 2006; Manneh *et al*., 2007).

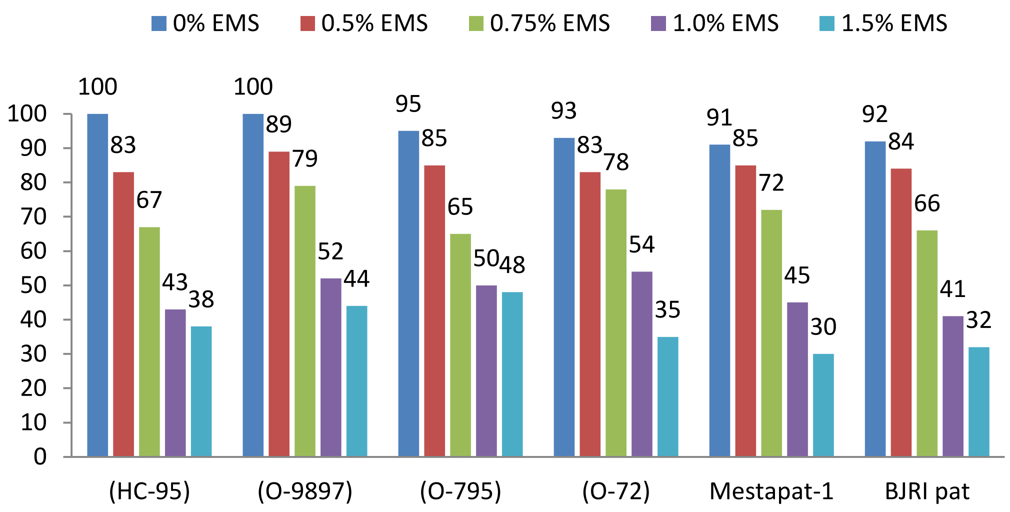

Fig1. Effect of different doses of EMS on germination% of different varieties of jute, kenaf and mesta.

LD$_{50}$ values from probit analysis

These values essentially capture the EMS concentration at which half the sampled population of seeds failed to germinate. Moreover, different varieties exhibited varied sensitivities. HC-95 had the most resistance with the lowest LD$_{50}$ value of 1.09%, whereas both O-795 and O-72 showed heightened sensitivity with LD$_{50}$ values peaking at 1.17%. Such differential responses across varieties resonate with observations by Ramchander, *et al*., 2015 and Rajarajan, *et al*., 2016, emphasizing that even under uniform treatment conditions, the genetic makeup of the plant significantly influences the outcomes.

Table1. Probit Analysis for Calculating LD_{50} Doses

Varieties	Mutagenic doses (%)	Observed mortality (%)	Corrected mortality (%)	Probit table value	LD_{50} value (%)
(HC-95)	Control	0	0	0	1.09
	0.5	17	17	4.05	
	0.75	33	33	4.56	
	1.0	57	57	5.18	
	1.5	62	62	5.28	
(O-9897)	Control	0	0	0	1.16
	0.5	11	11	3.77	
	0.75	21	21	4.19	
	1.0	48	48	4.95	
	1.5	56	56	5.15	
(O-795)	Control	5	0	0	1.17
	0.5	15	11	3.77	
	0.75	35	32	4.53	
	1.0	50	47	4.92	
	1.5	52	49	4.97	
(O-72)	Control	7	0	0	1.17
	0.5	17	11	3.77	
	0.75	22	16	4.01	
	1.0	46	42	4.8	
	1.5	65	62	5.28	
Mestapat-1	Control	9	0	0	1.13
	0.5	15	7	3.52	
	0.75	28	21	4.19	
	1.0	55	51	5.03	
	1.5	70	67	5.44	
BJRI deshi patshak	Control	8	0	0	1.12
	0.5	16	9	3.66	
	0.75	34	28	4.42	
	1.0	59	55	5.15	
	1.5	68	65	5.3	

Based on mutagenic doses, observed mortality (%), corrected mortality (%) and probit values were calculated (Table1) and also the LD_{50} was determined for different varieties (Fig.2)

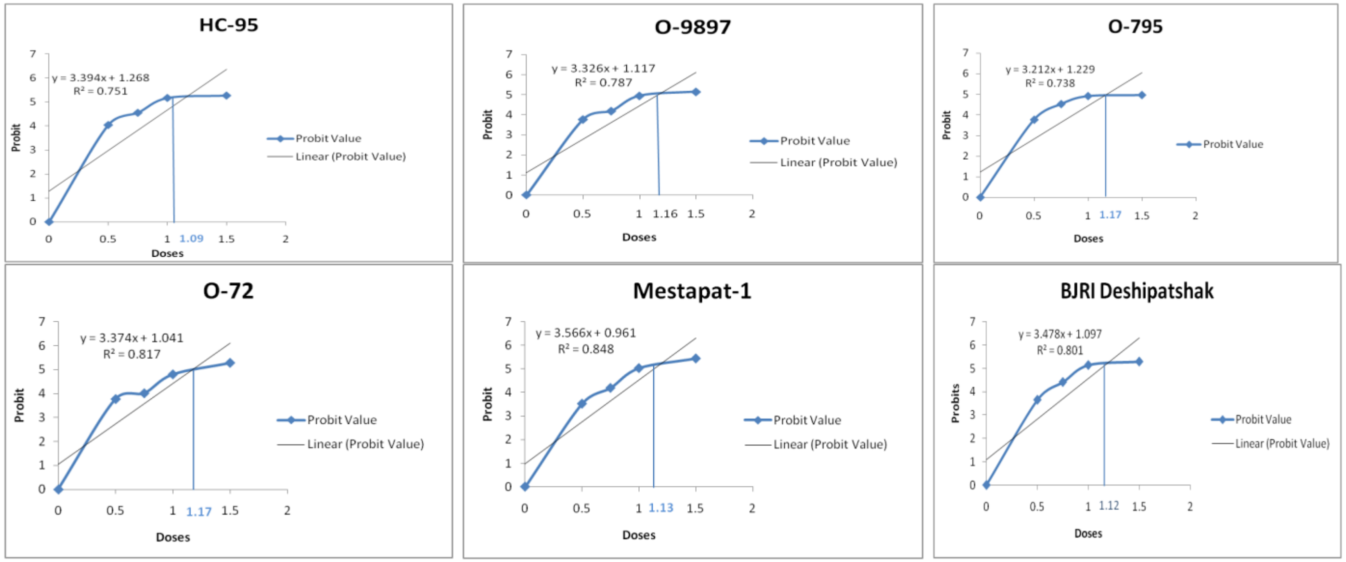

Fig. 2. Plots of mutagenic doses vs. probits for calculation of LD_{50} of EMS.

Root growth patterns in response to EMS doses

As we navigate through Table 2, a noteworthy trend surfaces. The root length in Mestapat-1 (36 DAS) stood out, reaching a substantial 14 cm in the control group. On the contrary, Jute (O-9897) recorded a mere 6.2 cm at 36 DAS with a 0.75% EMS dosage. Such disparities underscore the variable resilience and responses of different varieties to EMS treatments. Moreover, certain varieties, like the BJRI deshi patshak, exhibited notable fluctuations in root length across various EMS doses, highlighting the intricate ways in which EMS interacts with the genetic makeup of each variety.

Table 2. Effect of different doses of EMS on Root length of different varieties of jute, kenaf and mesta

Treatments	Kenaf			Jute												Mestapat-1		
EMS level	(HC-95)			(O-9897)			(O-795)			(O-72)			BJRI deshi patshak					
	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS
0% EMS	7.7	10.3	10.75	2.88	5.4	7.8	3.2	6.0	7.75	3.2	7.0	7.25	3.7	7.08	7.0	2.42	9.0	14.0
0.5% EMS	7.3	10.1	11.6	2.1	5.9	8.0	4.4	6.3	8.5	4.6	6.2	8.2	5.0	6.5	9.75	6.38	9.0	10.0
0.75% EMS	9.1	9.9	11.0	3.5	4.5	6.2	2.5	7.0	8.5	4.0	6.4	8.4	3.6	8.2	8.8	5.7	10.0	10.0
1.0% EMS	7.4	8.8	10.2	3.2	5.1	7.0	4.0	8.3	8.4	3.5	6.0	7.25	4.6	7.9	8.0	5.5	9.0	12.0
1.5% EMS	5.3	8.7	10.3	2.2	5.2	6.4	3.2	5.8	7.0	2.6	5.6	6.5	2.9	4.5	6.6	6.75	7.6	8.0
Mean	7.36	9.56	10.77	2.78	5.22	7.08	3.46	6.68	8.03	3.58	6.24	7.52	3.96	6.84	8.03	5.35	8.92	10.8
SE	0.61	0.34	0.25	0.27	0.23	0.36	0.33	0.45	0.29	0.34	0.23	0.35	0.37	0.66	0.58	0.77	0.38	1.02

Shoot growth trends in the wake of EMS treatments

Table 3. Effect of different doses of EMS on shoot length of different varieties of jute, kenaf and mesta

Treatments	Kenaf			Jute												Mestapat-1		
EMS level	(HC-95)			(O-9897)			(O-795)			(O-72)			BJRI deshi patshak					
	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS	12 DAS	12 DAS	36 DAS	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS	12 DAS	24 DAS	36 DAS
0% EMS	22.7	25.6	44.3	6.13	13.3	28	7.7	15.6	27.3	6.4	17.8	24.5	7.6	16.2	34.3	13.6	19.0	33.0
0.5% EMS	20.9	24.9	45.4	5.5	16.4	26.5	8.0	18.6	27.0	9.9	16.0	22.6	10.0	13.0	29.5	15.0	19.0	32.0
0.75% EMS	22.3	24.8	41.3	5.2	13.5	26.4	7.0	17.3	23.8	4.8	18.4	26.4	6.6	18.7	25.0	18.1	22.5	31.0
1.0% EMS	18.5	23.1	40.0	4.0	15.3	25.6	6.3	17.6	21.2	5.9	16.6	23.0	7.5	19.8	25.6	16.8	22.7	28.2
1.5% EMS	15.1	22.4	11.5	3.3	12.8	22.6	3.2	15.3	20.2	4.4	12.8	17.5	7.1	14.4	24.6	7.5	20.0	26.0
Mean	19.9	24.16	36.5	4.82	14.26	25.82	6.44	16.88	23.9	6.28	16.32	22.8	7.76	16.42	27.8	14.2	20.64	30.04
SE	1.4	0.6	6.32	0.51	0.68	0.89	0.86	0.62	1.45	0.97	0.97	1.48	0.58	1.27	1.84	1.84	0.82	1.28

As portrayed in Table 3, shoot growth, much like root growth, exhibited a decremental trend with increasing EMS doses. The kenaf (HC-95) variety towered over others with a striking shoot length of 44.3 cm at 36 DAS in the control setup. Yet, as we introduce EMS, this length experiences a significant reduction. For instance, in the presence of a 1.5% EMS dose, the shoot length of the same variety plunged to a mere 11.5 cm at 36 DAS. Such observations spotlight the profound influence of EMS on plant growth, emphasizing the need for judicious utilization of such mutagenic agents in breeding programs.

Conclusion

The meticulous examination of the influence of Ethyl Methane Sulfonate (EMS) on fibrous plants such as jute, kenaf and mesta has yielded noteworthy insights. The data clearly underscores the decremental impact of EMS on germination and growth rates across the studied plant varieties. At optimal conditions without EMS interference, these plants exhibit their maximum potential in both germination and growth. However, as the EMS concentration increases, there's a pronounced reduction in germination percentage, root length, and shoot length. Some plants showcase heightened resilience to EMS, while others are more susceptible. This emphasizes the importance of understanding each plant's mutagenic effects and doses before introducing mutagenic agents in breeding programs. Based on the results of the study, the LD_{50} value of EMS treated kenaf (HC-95) was found to be 1.09%, jute varieties (O-9897, O-795, O-72 and BJRI deshi patshak) were 1.16%, 1.17%, 1.17% and 1.12%, respectively and mesta was 1.13%. By this, we can easily determine the mutation doses for fibrous crops. The study’s findings are crucial for plant breeders, geneticists and agriculturists. Since there is very less literature of lethal doses in fibrous crop, determination of optimum mutagen doses could be useful while formulating fiber crop mutation breeding programme for improvement of specific traits in fibrous crops.

Acknowledgement

This research was supported by the project entitled “Development of climate resilient crop varieties and profitable crop ….in Haor, Charland, Saline and Hilly areas.” under the Ministry of Climate Change and Environment. The authors are grateful to the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh for providing laboratory and field facility throughout the experimental work.

References

Ahmed, Z. and Nizam, S.A. 2008. Jute - Microbiological and biochemical reserach. Plant Tissue Cult. & Biotechnol. 18: 197-220.

BBS. 2015. Hand book of Agricultural Statistics, December, 2015. Bangladesh Bureau of Statistics (BBS).Ministry of Planning, Govt. People's Repub. Bangladesh.

Bolbhat, S.N. and Dhumal, K.N. 2009. Induced macromutations in horsegram [*Macrotyloma uniflorum* (Lam.) Verdc]. Legume Res. 32(4): 278–281.

Finney, D.J. 1978. Statistical Method in Biological Assay. Charles Griffin Co.

Islam, M.S., Saito, J.A., Emdad, E.M., Ahmed, B., Islam, M.M. 2017. Comparative genomics of two jute species and insight into fibre biogenesis. Nat. Plants. 3:16223.

Manneh, B., Stam, P., Oliver, S. and Struic, P.C. 2007. QTL-base analysis of genotype by environmental interaction for grain yield of rice in stress and non- stress environment. Euphytica. 156: 213-226.

Mei, S., Wu, Y., Zhang, Y., Liu, B.M., Jiang, J.Y., Xu, X. and Yu, Z.L. 2007. Mutation of rice (*Oryza sativa* L.) LOX-1/2 near-isogenic lines with ion beam implantation and study of their storability. Nuclear Instruments and Methods in Physics Research Section B: Beam Interaction and Materials and Atoms. 265: 495-500.

Mir, R.R., Rustgi, S., Sharma, S., Singh, R., Goyal, A., Kumar, J., Tyagi, A.K., Khan, H., Sinha, M.K., Balyan, H.S. and Gupta, P.K. 2008. A preliminary genetic analysis of fibre traits and the use of new genomic SSRs for genetic diversity in jute. Euphytica. 161: 413-427.

Mohamad, O., Nazir, M., Alias, B., Azlan, I., Rahim, S.A., Abdullah, H., Othman, M.Z., Hadzim, O., Saad, K., Habibuddin, H.A. and Golam, F. 2006. Development of improved rice varieties through the use of induced mutations in Malaysia. Plant Mutation Reports. 1: 27-34.

Oladosu, Y., Rafi, M.Y., Abdullah, N., Hussin, G. and Ramli, A. 2016. Principle and application of plant mutagenesis in crop improvement: a review. Biotechnol. Biotech. Eq. 30:1–16.

Rajarajan, D., Saraswathi, R. and Sassikumar, D. 2016. Determination of lethal dose and effect of gamma ray on germination percentage and seedling parameters in ADT (R) 47 rice. Int. J. Adv. Biotechnol. Res. 6(2): 328-332.

Ramchander, S., Ushakumari, R. and Arumugam P.M. 2015. Lethal dose fixation and sensitivity of rice varieties to gamma radiation. Indian J. Agric. Res. 49 (1): 24-31.

Sarker, R.H., Amin, A., Hossain, G. and Hoque, M. 2007. In vitro regeneration in three varieties of white jute (*Corchorus capsularis* L). Plant Tissue Cult. Biotechnol. 17:11–18.

Scott, S., Jones, R. and Williams, W. 1984. Review of data analysis methods for seed germination. Crop Sci. 24: 1192–1199.