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RESEARCH ARTICLE

Dose Optimization, Mutagenic Effectiveness and Efficiency of EMS in Proso Millet (*Panicum miliaceum* L.)

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ABSTRACT

Mutation breeding is an important approach to crop improvement. Identifying beneficial mutagen and its optimum dose are the prerequisites for any successful mutation breeding program. The present study aimed to identify the optimum dose, mutagenic effectiveness, and efficiency of various concentrations of EMS mutagen in proso millet variety ATL 1. Ten treatment concentrations 10 to 100 mM and control were evaluated using germination paper, tray, and field method to estimate the effect of the mutagen on seedling growth and survival in M_1 generation. Based on viable mutation frequency in M_2 generation, the mutagenic effectiveness and efficiency of the mutagen were determined. The mean lethal concentration (LD_{50}) and mean concentration for 50 per cent reduction in growth (GR_{50}) doses were determined to be 41 mM and 45 mM, respectively based on survival per cent and shoot length reduction over control. The mutagenic effectiveness was the highest at 50 mM and mutagenic efficiency was the highest at 40 mM. Broad-spectrum of viable mutants was identified in M_2 generation that could be utilized to develop improved cultivars in the crop.

Keywords: *Proso millet; Mutation; EMS; LD50; Mutagenic effectiveness; Efficiency*

INTRODUCTION

Millets are hardy crops that can develop well in various climatic and soil conditions. Small-seeded millets are commonly called small millets. In India, the commonly grown small millets are finger millet, foxtail millet, kodo millet, little millet, proso millet, and barnyard millet (De Wet, 1986). They were part of the traditional agriculture system in India but were replaced by the high-yielding varieties of rice, wheat and maize during the 1960s. Unlike common cereals like rice, wheat, etc., research in millets, particularly small millets, remained neglected for many years. However, the changing climate, increased drought, high surface temperature, and unpredictable rainfall patterns have revived the importance of these hardy crops. Among millets, Proso millet (*Panicum miliaceum* L.) is an important small millet crop grown for food and feed globally. It is a self-pollinated tetraploid (2n=4x=36) with short growing seasons spanning 60 and 90 days (Hunt *et al*., 2014). It has high water and nutrient use efficiencies and grows well in arid and semi-arid regions. It is also rich in protein, fiber, vitamins, minerals and essential amino acids. Though the crop has good potential as a climate-resilient nutritional food crop, the advances in crop improvement are far behind. One major hindrance in developing improved cultivars in the crop is the difficulty in employing artificial hybridization (Gupta *et al*., 2015), as the florets are small and remain open only for a short period (Rajasekaran and Francis, 2020).

Mutation breeding can be utilized to overcome this hurdle and widen the genetic variability in the crop. Also, it is a very successful breeding method for crop improvement of many self-pollinated crops and used to develop more than 3200 varieties in various crops (IAEA, 2021). Among the chemical mutagens used for mutation breeding, alkylating agents are the most important class of mutagens. It has contributed to more than 80 % of mutant varieties (Oladosu *et al*., 2016). Ethyl methane sulphonate (EMS) is the most used mutagenic agent. However, there are very few mutation breeding efforts using chemical mutagenesis in proso millet (Maluszynski *et al*., 2000). There is a lack of literature on the optimum dose of EMS mutagen and its effectiveness

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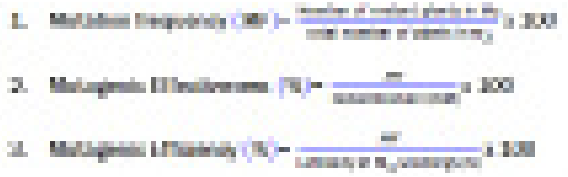
in mutation induction in the crop. The optimum dose at which seeds are treated to develop the mutant population largely influences the frequency of beneficial mutants identified in the advanced generations. The mutation frequency represented in terms of the respective dose, and biological damage further gives insight into the efficiency and effectiveness of various concentrations of mutagen. Hence, the main objective of the study was to determine the mean lethal concentration (LD_{50}), mean concentration for 50 per cent reduction in growth (GR_{50}), the most effective and efficient treatments of EMS in Proso millet variety ATL 1 and document the frequency and types of mutations generated in M_2 generation.

MATERIAL AND METHODS

The seeds of variety ATL 1 were used for the present study. The seeds were procured from the Centre of Excellence in Millets, Tamil Nadu Agricultural University, Athiyandal. The chemical mutagen used to induce mutagenic population was Ethyl Methane Sulphonate (EMS) (chemical formula-$CH_3SO_3CH_2CH_3$, Molecular weight- 124.16, Density-1.206 g/mL, Sigma-Aldrich®). The experiment was carried out at the Department of Millets, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University during 2019-2020. Dried seeds of the variety (moisture content 12 %) were presoaked in distilled water for 8 hours. The seeds were then dried between blotting paper. The treatment concentrations were imposed by immersing the seeds (M_0) for 6 hours in 0 (control), 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mM solution of EMS. One thousand five hundred seeds were used per treatment concentration and the solution with seeds was shaken intermittently to ensure uniform exposure of the mutagen. The treated (M_1) seeds were thoroughly washed under running tap water for 4-5 times to remove the remnants of the chemical. The seeds were then dried using blotting paper and used for the experiment. The effect of mutagen on seed germination, growth and seedling survival was studied using three methods, i.e., germination paper, tray and field method and replicated thrice. In the germination paper trial, 25 seeds were placed in rows on moistened germination paper and replicated thrice. For tray trial, plastic trays filled with planting mixture (field soil: compost in 1:1 ratio) were used and 50 seeds were sown per treatment in rows and replicated thrice. For field trial, line sowing of 500 seeds per treatment were done in rows during August-October 2019. Germination percent (7 DAS), shoot length (14 DAS) and survival percentage (21 DAS) were recorded from all three methods. Treatments closer to LD_{50} and GR_{50} concentration were forwarded to M_2 generation to record the mutation frequency, mutagenic effectiveness and efficiency. Seeds from 200 single M_1 plants from 40 mM, 50 mM, 60 mM and 70 mM treatments from field method were forwarded to M_2 generation as ear to row progenies to constitute 200 M_2 families (4 rows per family). The M_2 generation was raised during Feb-May 2020 and 11,111 plants were maintained. All the recommended agronomic practices were followed during the crop period.

The observations on germination, shoot length, and survival precent from the ten treatments (10 to 100 mM) were used to study the effect of EMS mutagenesis on seedling growth in M_1 generation. Probit analysis was used to determine the concentrations of EMS at which we expect maximum beneficial mutations with minimum lethality (LD_{50}) or growth reduction (GR_{50}). LD_{50} is the concentration at which we expect maximum mutation frequency with minimum lethality (fifty percent). The GR_{50} is the concentration producing 50 per cent reduction in growth calculated based on seedling parameters like reduction in shoot length. Probit analysis is the statistical procedure used in determining these LD_{50} and GR_{50} doses. It is a tailored regression model for determining the dose-response relationship. It transforms the sigmoid curve into a straight line giving a linear equation with the dependent (Y) and independent variable (X) (Finney, 1952). The relationship shows the sensitivity of the species to various doses of the mutagen.

No chlorophyll mutants were recovered in M_1 generation and hence the chlorophyll mutation scoring was repeated in M_2 generation. The chlorophyll mutants observed in M_2 population were identified and scored (Gustafsson, 1940). Viable mutations in M_2 population were scored and used for the mutation frequency, mutagenic effectiveness and efficiency calculations. All the calculations and graphs were generated using Microsoft® Excel®. The formulas used are (Konzak, 1965):



RESULTS AND DISCUSSION

Effect of EMS mutagen on seedling growth and survival in M_1 generation

Germination of seeds, shoot length, and survival of the M_1 seedlings were decreased with an increase in the concentration of the mutagen. The observations are represented as percentage over control values for easy comparison and are presented in Table 1. In the germination paper trial, seeds did not germinate at higher concentrations

viz., 90 mM and 100 mM. Germination percentage ranged between 40 per cent (70 mM) to 100 (10 mM) per cent but did not show a distinct dose-response reduction. Shoot length reduced from 87 (10 mM) to 18 (80 mM) per cent. Survival of the seedlings reduced from 100 (10 mM) to 16 (80 mM) per cent. In tray method, no germination was observed at 90 mM and 100 mM concentrations, and germination per cent ranged from 10 (80 mM) to 100 (10 mM) per cent. Shoot length in tray decreased from 96 (10 mM) to 26 (60 mM) per cent. Though 10 per cent germination was observed at 80 mM, no seedlings survived till 21 days. Survival percentage declined from 97 (10 mM) to 12 (70 mM) per cent. In the field trial, germination percentage reduced from 96 (10 mM) to 12 (80 mM) per cent and shoot length declined from 92 (10 mM) to 32 (70 mM) per cent. Survival percentage ranged from 5 (60 mM) to 96 (10 mM) per cent. Similar to the observations from tray method, no seedlings survived at 80 mM treatment.

Table 1. Effect of EMS mutagenesis on germination, seedling growth and survival of proso millet in M_1 generation

	Germination paper			Tray			Field		
Dose (mM)	G %	SL (%OC)	S%	G %	SL (%OC)	S %	G %	SL (%OC)	S %
Control	100	100	100	100	100	100	100	100	100
10	100	87	100	100	96	97	96	92	96
20	100	79	97	98	95	95	98	72	93
30	100	72	90	100	72	89	87	67	87
40	96	67	86	92	66	80	86	58	82
50	100	64	70	78	42	68	80	48	60
60	60	64	59	73	26	23	76	38	5
70	40	31	38	30	39	12	55	32	6
80	70	18	16	10	-	-	12	-	-

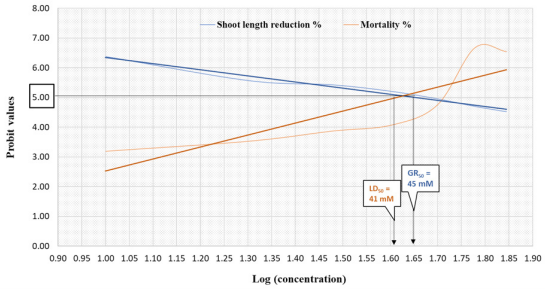
G %: Germination per cent, SL: Shoot length, S %: Survival per cent, % OC: per cent over control,

Multiple gene copies in polyploids compensate for the mutagenesis induced chromosome and cellular level damages to a greater extent in the early stages of growth (Swaminathan, 1957). This could be attributed to significantly high germination observed even at higher concentrations. However, survival percentage showed drastic decline beyond 50 mM, particularly in the field trial. The similar declining trend with an increase in treatment concentrations of EMS have been reported in rice, rapeseed etc. (Talebi *et al*., 2012; Yadav *et al*., 2016). The results from the three methods, i.e., germination paper, tray and field trial, revealed that the field method followed by tray method was more reliable than the germination paper method. The effect on seedlings was more severe in the field followed by the tray and the results showed closer correspondence to the field experiment. Out of the seedling traits recorded, mortality percentage and shoot length reduction showed a distinct dose-response relationship. Hence, mortality percentage of seedlings in tray and field methods were used to calculate LD_{50} concentration and reduction in shoot length over control was used to determine the GR_{50} concentration. LD_{50} and GR_{50} from tray method were 50 mM and 48 mM respectively. The LD_{50} and GR_{50} concentrations were estimated from the field method as 41 mM and 45 mM respectively (Figure 1). In a similar study in barnyard millet, the LD_{50} value was fixed as 70 mM based on shoot length reduction (Ramesh *et al*., 2019). Muduli and Misra (2007) reported 0.30 % and 0.45 % EMS as beneficial treatments for obtaining high frequency of beneficial mutants in finger millet. In sorghum, Wanga *et al*., reported optimum doses between 0.36 % to 0.6 % for various genotypes (Wanga *et al*., 2020).

Table 2. Mutagenic effectiveness and efficiency of EMS mutagen in proso millet in M_2 generation

Concentration (mM)	Total number of plants	Number of viable mutants in M_2	Viable mutation frequency (%)	Mutagenic Effectiveness (%)	Mutagenic Efficiency (%)
30	2500	448	17.9	59.73	138.49
40	3561	1078	30.3	75.68	171.51
50	2800	1386	49.5	99.00	123.75
60	1450	302	20.8	34.71	21.86
70	800	78	9.8	13.93	10.39

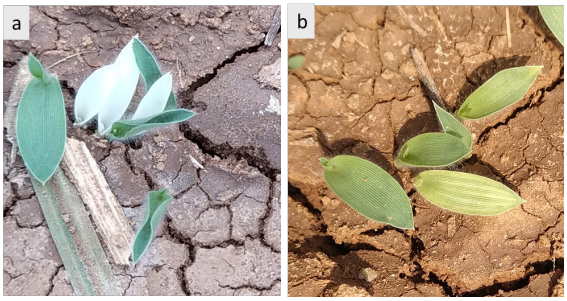
Fig 1. Probit analysis to determine the LD_{50} and GR_{50} concentration of EMS in proso millet variety ATL 1



Mutagenic effectiveness and efficiency in M_2 generation

Chlorophyll mutants are generally regarded as indices for understanding the mutability of a cultivar. In the present study, chlorophyll mutants were only detected at 50 mM, 60 mM and 70 mM in M_2 generation. Number of chlorophyll mutants increased with increase in the concentration of EMS. The chlorophyll mutants observed were *albino* and *striata* mutants (Figure 2).

Fig 2. Chlorophyll mutants identified in M_2 generation; a) albino b) striata



chlorophyll mutations were lethal and these mutants did not survive to maturity. Viable mutants observed in each treatment in M2 were scored and the mutation frequency was estimated (Table 2). Maximum and minimum frequencies of viable mutants were recorded at 50 mM (49.5 %) and 70 mM (9.8 %), respectively. Mutagenic effectiveness represents the cultivars response or mutation rate of cultivar to the increase in concentration of the mutagen. Mutagenic effectiveness was the highest at 50 mM followed by 40 mM, and the lowest at 70 mM. Mutagenic efficiency considers the biological damage for the mutations detected. The highest mutagenic efficiency was estimated at 40 mM followed by 30 mM, and the lowest at 70 mM. Though mutation frequency was the highest at 50 mM, the higher lethality of seedlings over 40 mM, has reduced the efficiency value. In finger millet, the efficacy of EMS mutagen treatments was determined based on the ability to produce superior mutants in advanced generations (Muduli and Misra, 2007).

Table 3. Phenotypic mutant spectrum in M_2 generation

Trait	Mutant type	Frequency (%)
Plant height	Short	1.93
	Tall	2.41
Plant habit	Prostrate	0.37
Panicle Shape	Diffused	3.99
	Spike	0.02
Panicle compactness	Open	7.50
Days to flowering	Early	5.51
	Late	0.66
Lodging	Non-Lodging	0.25
Tillering	High	0.01
Apiculus color	Absent	0.69
Leaf mutants	Narrow leaf	1.66
	Broad leaf	2.77
	Long leaf	0.04
	Crinkled leaf	0.02
Panicle and peduncle length	Long Panicle	0.83
	Long Peduncle	0.41
Sheath pigmentation	High	0.02
Total		29.63

In the present study, a wide spectrum of mutations was detected in the M_2 population, including mutants for plant growth and habit, leaf morphology, panicle types, tillering habit, days to flowering, and pigmentation of sheath and apiculus. The various types of visible mutants recorded and their frequency are presented in table 3. The viable phenotypic mutations accounted for a total of 29.63 per cent of the total M_2 population. The most common mutation observed was for panicle compactness. The panicles in control were semi-compact ,while in many mutants open type panicle was common (7.5 %). A rare panicle type i.e., 'spike' was detected as a single plant mutation in M_2 generation (Figure 3).

Fig 3. Representative panicle type mutant identified in M_2 generation: a) wild type panicle b) 'spike' type panicle

A similar finding for panicle variations has been reported in foxtail millet (Gupta, 1975). Other beneficial mutants detected were early flowering and late-flowering types, broad and narrow-leaved

mutants, long panicle mutants, etc. These could be used as parental material for crop improvement. In foxtail millet, a mutant library was developed using EMS mutagenesis, which is being utilized for functional genomics (Sun *et al*., 2019).

CONCLUSION

Mutation breeding is a powerful tool utilized for crop improvement program in many crops. Proso millet being a self-pollinated crop with limited variability, mutation induction can be used to widen the genetic variability. From the present study, it can be concluded that the optimum treatment of EMS in ATL 1 variety of proso millet is 40 mM. The most efficient and effective mutagenic EMS concentration is 40 mM and 50 mM, respectively. The high frequency of useful mutants observed in M_2 generation will be evaluated in advanced generations and utilized for varietal development. Hence, the study proves the applicability of EMS mutagenesis in proso millet for crop improvement.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

The authors assure that the research work submitted here is original and not subjected to any plagiarized content.

Consent for publication

All the authors agreed to publish the content

Competing interests

There were no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail: chithuragul@gmail.com

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