Effects of Ethyl Methane Sulphonate Mutagen and Determination of Lethal Dose (LD_{50}) in Rice Varieties

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Abstract:- Ethyl Methane Sulphonate (EMS) is a chemical mutagen widely used to improve rice characters by mutation breeding. The experiment was conducted to find out the optimum lethal dose (LD_{50}) of EMS on four rice varieties viz., Aye Yar Min, Rice Berry, Jasmine 105 and Paw San (Black) for creating genetic variation. Dry seeds of four rice varieties were directly treated with different doses of EMS 0.00%, 0.25%, 0.50%, 0.75%, 1.00% and 1.25% for 22 hours. The effects of different EMS doses and varieties and their interaction showed for the traits like germination %, shoot length and root length were significant differences. The lethal dose (LD_{50}) values were detected by regression analysis for different varieties. The lethal dose (LD_{50}) values for germination %, shoot length and root length were observed at Aye Yar Min (0.52 to 0.55%), Rice Berry (0.55 to 0.68%), Jasmine 105 (0.57 to 0.60%) and Paw San (Black) (0.62 to 0.66%). The three studied traits were decreased with increase in dose of EMS. The sensitivity of EMS appeared to be related to the type of variety. The lethal dose (LD_{50}) values determined for the different rice varieties could be useful while formulating future rice varietal improvement breeding programmes.

Keywords:- *Rice Varieties, Ethyl Methane Sulphonate (EMS), Lethal Dose (LD_{50})* .

I. INTRODUCTION

In Myanmar, rice is the most important dominating crop, and the total rice growing area was 7.22 million hectares and production reached 28.01 million MT, with an average yield of 3.92 MT/ha (Department of Planning [DoP], 2019). Rice is usually grown during the monsoon and summer seasons in which most of the varieties were high yielding varieties or hybrids. Some of cultivated varieties have pleasant aroma and great quality and thus, it has great market demand. However, long age, photoperiod sensitivity and high plant crown are some of the weaknesses of those cultivars. In order to improve the characters of plant, plant breeding is often conducted.

Mutation may produce raw material which can be used for the genetic improvement of crops (Chowdhury & Bhuyan, 2021). Genetic mutations are a deliberate way of changing one or more desired plant traits. The induced mutagenesis serves as an important tool for correcting a particular defect or creating usable genetic variation (Rajarajan, Saraswathi, Sassikumar & Ganesh, 2014). When a high yielding variety has a defect, the defect can be also being changed without much alteration in the genetic background. Artificial induction of mutations is done through the use of physical or chemical mutagens.

Chemical mutagens have extensively been applied to make genetic changes in crop plants for breeding investigation as well as genetic studies. EMS alkylates guanine bases and ultimately results in mispairing-alkylated G pairs with T instead of C, resulting in primarily G/C to A/T transitions which would provide a series of change of function mutations (Bhat et al., 2007). EMS is frequently used for seed mutation because it is effective and induces high-frequency point mutations, some of which lead to a novel stop codon for different genes (Chen et al., 2013; Talebi, Talebi & Shahrokhifar, 2012). Jain (2010) stated that the determination of lethal dose - LD_{50} at the beginning is a prime step to initiate the EMS induction. Mba, Afza, Bado and Jain (2010) reported that LD_{50} refers to the mutation dose that result in 50% reduction in seed germination percentage after seed exposure for a prescribed time period under specific conditions. Moreover, the mutagenic efficiency of a chemical mutagen depends not only on the properties of the chemical, but also on the genotype. The success of mutation breeding greatly depends on the rate of mutation, the number of screened plants and the mutation efficiency. To avoid extreme loss of actual experimental materials, sensitivity tests must be conducted to determine lethal doses (LD_{50}) before massive treatment of similar materials are accepted. Therefore, the study was conducted to investigate optimum lethal dose (LD_{50}) from the effects of different EMS doses in different rice varieties.

II. MATERIALS AND METHODS

Laboratory experiment was carried out on October, 2021 for lethal dose (LD_{50}) determination which is used as an optimum dose for mutation induction at Experimental and Lecture Building-2, Department of Plant Breeding, Physiology & Ecology, Yezin Agricultural University. The experimental materials comprised of four rice varieties, namely Aye Yar Min, Rice Berry, Jasmine 105 and Paw San (Black).

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Dry, well filled seeds of four rice varieties were directly treated with ethyl methane sulphonate (EMS) 100 ml of 0.00%, 0.25%, 0.50%, 0.75%, 1.00% and 1.25% were prepared in distilled water. About 1800 seeds of each tested variety were soaked with EMS different concentrations for 22 hours. After completion of the treated time, the EMS solutions were drained and the seeds were thoroughly rinsed under running tap water for 4 hours to remove the residual of mutagen sticking to the seed coat. After that the seeds from each dose were placed onto the filter paper in three petridishes and then incubated for 7 days at room temperature. Each petridish contained 100 treated seeds and considered as a replication. The number of seeds that germinated under that condition was recorded every day. The seedling height and root length of the plants were measured for seedling growth. The germination percentage was computed with the following equation.

$$\text{Germination percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

An experimental investigation was organized based on 4 × 6 factorial arrangement in completely randomized design with three replications and the random block included six levels of EMS concentration (including non-treated control). Mean values of treated and non-treated populations were compared to investigate studied traits differences by using least significant difference (LSD) test at 0.05 probability level. The lethal dose (LD_{50}) was estimated by using the regression correlation to find the effective dose of different varieties (Awais, Nualsri & Soonsuwon, 2019).

III. RESULTS AND DISCUSSION

A. Analysis of variance for seed germination, shoot length and root length

The present research was an effort to induce mutation in four rice varieties viz. Aye Yar Min, Rice Berry, Jasmine 105 and Paw San (Black). Analysis of variance of the studied traits like germination %, shoot length and root length showed significant differences ($P < 0.01$) (Table 1). The effect of different doses of EMS on different varieties revealed the highly significant differences for all tested traits. The results showed that EMS dose, variety and EMS dose × variety interaction were highly significant for all the traits (germination %, shoot length and root length) indicating that differences exist within varieties regarding sensitivity to mutagenic treatments. Genotype wise analysis also revealed the highly significant differences between different mutagen treatments in each genotype in Table 1. Differences in varietal response to different EMS doses have also been reported in various chickpea genotypes by Shah, Mirza, Haq & Atta (2008) where seed germination linearly decreased with increasing concentration of EMS.

Table 1. Mean squares for the effect of different of EMS doses on germination %, shoot length and root length in four rice varieties

Source of Variation	d.f	EMS 22 hrs		
		Germination %	**Shoot Length**	**Root Length**
EMS Dose	5	15148.00**	118.43**	90.17**
Variety	3	4003.70**	5.03**	16.16**
EMS Dose × Variety	15	519.00**	0.87**	2.79**
Error	48	11.20	0.19	0.13
CV %		**9.11**	**13.66**	**12.74**

*, ** and ns indicate significant at LSD 5% level, significant at LSD 1% level and non- significant, respectively.

B. Effect of various doses of EMS on germination % and decision of lethal dose (LD_{50})

In the present study, biological effects of the mutagenic treatments were determined the rate of germination in the M_1 generation. Germination percentages of Aye Yar Min, Rice Berry, Jasmine 105 and Paw San (Black) under different doses were calculated based on the germinated seeds after EMS has been applied and compared with control (0.00%).

Germination started in the second day after sowing however it was delayed by one day at 0.75%, 1.00% and 1.25% dose of EMS. Paw San (Black) exhibited the highest overall seed germination percentage of 98.67%, as compared to 83.33%, 82% and 44% of Rice Berry, Jasmine 105 and Aye Yar Min, respectively. It was also more resistant to higher dose (1.25%) of EMS than other three varieties. Largeseeded variety Paw San (Black) was more resistant to chemical mutagen than the small-seeded variety Aye Yar Min. Kumar and Sinha (2003) who observed the small-seeded genotype (Pat L 96-7) in lentil and it was to be more sensitive to physical mutagen than the large-seeded genotype (Precoz), and bold-seeded type in chickpea (C44) appeared more radio and chemo resistant than introgression genotype (CH40/91) (Shah et al., 2008). No germination was noticed at 0.75%, 1.00% and 1.25% EMS for Aye Yar Min, 1.00% and 1.25% EMS for Jasmine 105. Although the dose of 0.75% EMS, Rice Berry and Paw San (Black) were germinated 6.33% and 46.33% respectively. When Rice Berry and Paw San (Black) were exposed to 1.25% of EMS, they germinated 0.67% and 1.00% respectively. Effective doses of mutagen varied from genotype to genotype (Wani et al., 2014). According to the results, EMS mutagen caused significant influence on germination. This was manifested as significantly decline ($P < 0.01$) in seed germination as the EMS concentration was increased. This reduction may be attributed due to disturbances at cellular level (caused either at physiological level or at physical level) including chromosomal damages or due to the combined effect of both (Khan & Tyagi, 2010). The same relationship was also reported by Talebi et al. (2012) in rice and Pavadai, Girija and Dhanavel (2010) in soybean. Unan, Deligoz, Al-Khatib and Mennan (2021) reported in rice that an attendant decrease in germination occurred with applied increase in the

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concentration of EMS. The results in this study showed that a diminution in seed germination observed with a corresponding increase in EMS dose. All varieties differed in the extent of reduction in seed germination. The median lethal dose (LD_{50}) is usually used as a critical parameter for chemically induced mutagenesis. Arisha, Liang, Shah, Gong and Li (2014) reported the EMS concentration that yields 50% seed lethality is used as an indicator of high mutation frequency. The lethal dose (LD_{50}) values for mutagenesis of four varieties with different EMS doses for each treatment were estimated through linear regression based on lethal rate. Based on the germination lethal rate of four varieties, LD_{50} values were 0.52% for Aye Yar Min, 0.55% for Rice Berry, 0.59% for Jasmine 105 and 0.66% for Paw San (Black) (Table 2 and Figure 1). EMS concentration approaching to 1.00% is considered to be deadly for germination and successful survival of the plant.

Among the exposure doses, the concentration of 0.50% was found to be the optimum for effective mutagenesis and might be preferred for maximum germination of mutant seeds. In addition, there were high coefficients of determination for each of the linear functions suggesting that there was a notable association between the reduction in seed germination and the concentration of the mutagen. Seed sensitivity to absorption dose is a genetically controlled character of a cultivar. Different cultivars can have different lethal dose (LD_{50}) values.

Table 2. Lethal dose (LD_{50}) calculation based on the germination lethal rate with different EMS doses

EMS doses (%)	Germination (%)			
	Aye Yar Min	Rice Berry	Jasmine 105	Paw San (Black)
0.00	44.00	83.33	82.00	98.67
0.25	41.33	79.33	76.33	94.33
0.50	22.00	46.00	63.67	84.67
0.75	0	6.33	7.33	46.33
1.00	0	2.00	0	1.00
1.25	0	0.67	0	1.00
Lethal Dose (LD_{50})	**0.52%**	**0.55%**	**0.59%**	**0.66%**

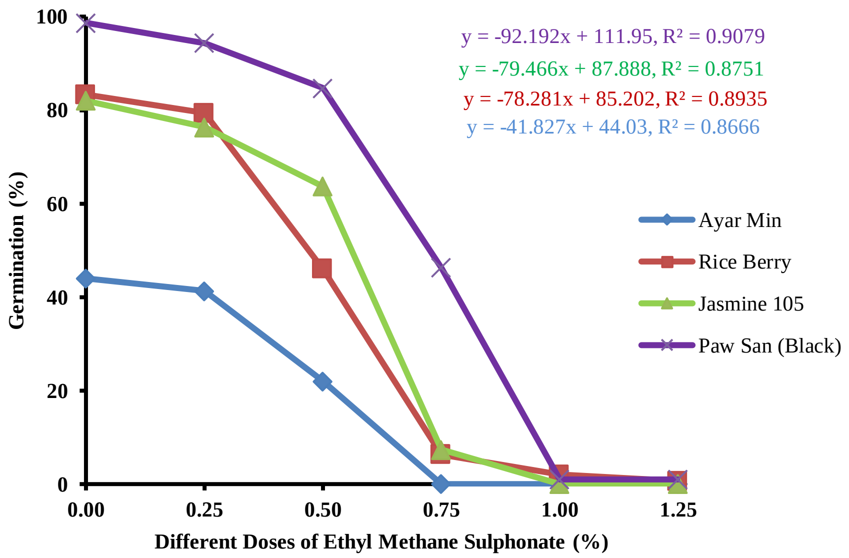

Fig 1. Effects of different ethyl methane sulphonate doses on germination (%) in four rice varieties

C. Effect of various doses of EMS on shoot length and root length and decision of lethal dose (LD_{50})

Shoot length is widely used as an index to determine the biological effects of various physical and chemical mutagens in M_1 (Rashid, Shamshad & Jankuloski, 2021). It is typically used as an indicator of genotype response to a mutagen and various methods can be devised depending on the species. Shoot length and root length can be used as study characters to investigate the optimum doses for gamma rays and EMS for a treatment on a wide scale in a breeding programme (Shah et al., 2008). They were measured as reduction in shoot length and root length of seedlings on 7 days after germination. Shoot growth and the dosage of physical or chemical mutagens have been showed to have a linear relationship. According to the results, shoot length exhibited a decreasing trend in all varieties with increase in the doses of EMS in treatment. Effect of various doses of EMS on shoot lengths and decision of LD_{50} EMS mutagenesis induced a significant impact ($P < 0.01$) on four tested varieties. The reduction in the shoot length may be attributed to the damage to the process of cell division and cell elongation that generally result after mutagenic treatment (Iqbal, 1969; Walther, 1969). The maximum shoot lengths were found in control for Aye Yar Min as 6.14 cm, Rice Berry as 6.59 cm, Jasmine 105 as 6.9 cm and Paw San (Black) as 7.43 cm which gradually show a fall off to below 3.81 cm at 0.50% EMS, 1.47 cm and 2 cm at 0.75% EMS (Table 3 and Figure 2). Shoot length at 0.25% concentration being the highest among the EMS doses for all varieties, while the least shoot length has been recorded when 0.75% of EMS concentration has been applied. A significant decrease was observed over 50% seed lethality when EMS dose was 0.50% and higher. Oldach (2011) reported that LD_{50} is quite arbitrary and might lead to a high number of (mostly deleterious) mutations in every plant. LD_{50} values were observed on the shoot length of four varieties, for 0.55% Aye Yar Min, 0.59% Rice Berry, 0.6% Jasmine and 0.63% Paw San (Black). The highest mean root lengths were measured at 4.71 cm Aye Yar Min, 4.21 cm Rice Berry, 5.67 cm Jasmine 105 and 8.30 cm Paw San (Black) for the 0.00% EMS dose. (Table 3 and Figure 3) show that the root length decreased after increasing the concentration of EMS as compared to non-treatment control ($P < 0.01$). Minimum reduction in root length was observed after mutagenesis was induced with 0.25% concentration of EMS. The result of the seedling experiment indicated that increasing EMS doses caused a significant decrease in seedling root development. The least reduction of root lengths was observed at 0.50% for Aye Yar Min and at 0.75% for Rice Berry, Jasmine 105 and Paw San (Black). LD_{50} values for root length estimated by the linear model for Aye Yar Min, Rice Berry, Jasmine 105 and Paw San (Black) were 0.55%, 0.68%, 0.57% and 0.62% respectively. A significant decrease was observed over 50% seed lethality when EMS doses have been applied above 0.50%. The highest mutation frequency is expected to occur in the treatment that kills 50% of the treated material (Jain, 2010).

Table 3. Lethal dose (LD_{50}) calculation based on the shoot length and root length in different EMS doses

	Shoot Length (cm)			
EMS doses (%)	**Aye Yar Min**	**Rice Berry**	**Jasmine 105**	**Paw San (Black)**
0.00	6.14	6.59	6.90	7.43
0.25	5.83	5.61	6.33	7.31
0.50	3.81	4.99	5.33	6.04
0.75	0	1.47	1.47	2.00
1.00	0	0	0	0
1.25	0	0	0	0
Lethal Dose (LD_{50})	0.55%	0.59%	0.60%	0.63%
	Root Length (cm)			
EMS doses (%)	**Aye Yar Min**	**Rice Berry**	**Jasmine 105**	**Paw San (Black)**
0.00	4.71	4.21	5.67	8.30
0.25	4.53	4.19	4.81	8.20
0.50	2.81	4.12	4.41	6.74
0.75	0	1.47	0.67	1.62
1.00	0	0	0	0
1.25	0	0	0	0
Lethal Dose (LD_{50})	0.55%	0.68%	0.57%	0.62%

In addition, there were high coefficients of determination (R^2 value) for each of the linear function suggesting that there was a notable association between the reduction in shoot length and root length and the concentration of the mutagen. This clearly indicated that the mutagens caused significant influence on shoot length and root length that displayed a dose dependent negative linear relationship between dose and shoot length and root length. No readings were observable for seed germination at 1.00% and 1.25%, therefore, there were no consideration for shoot length and root length in this treatment. Similar results have also been reported for MR 219 (Talebi et al., 2012), Basmati Rice (Rashid et al., 2021) when treated with EMS.

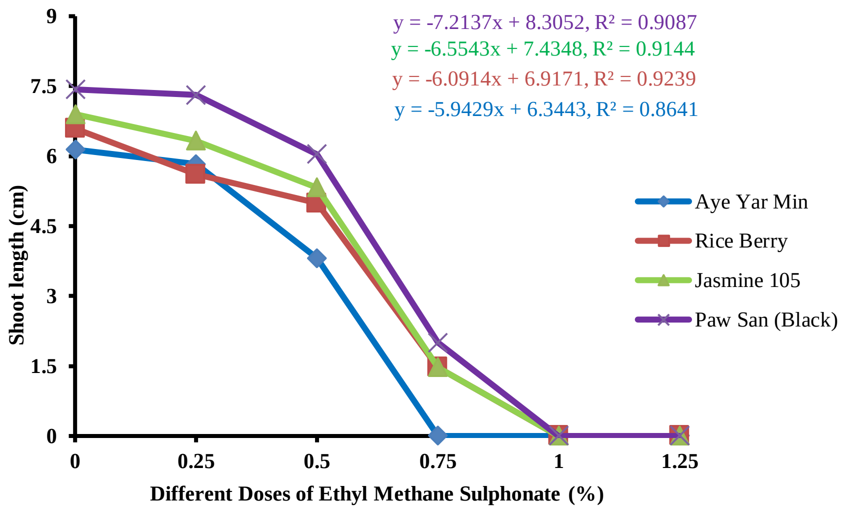

Fig 2. Effects of different ethyl methane sulphonate doses on shoot length in four rice varieties

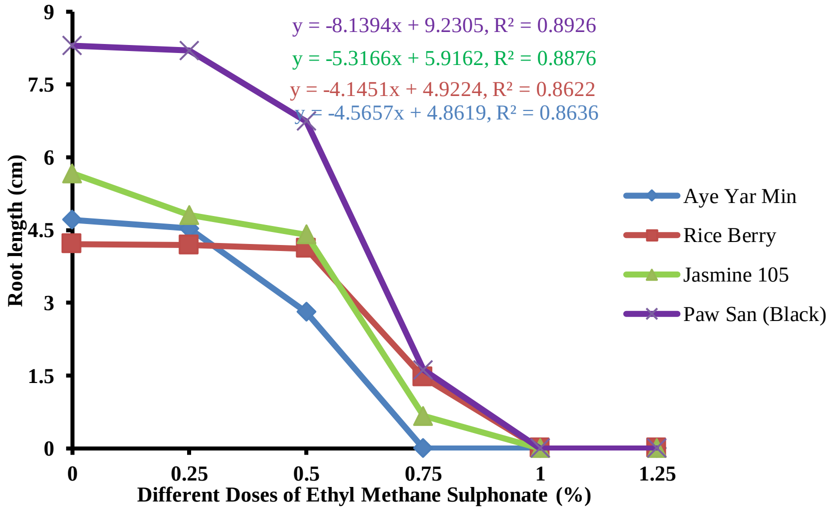

Fig 3. Effects of different ethyl methane sulphonate doses on root length in four rice varieties

IV. CONCLUSIONS

In the present investigation, seed germination, shoot length, root length were evaluated to assess the lethal dose (LD_{50}). The analysis of variance showed highly significant differences germination %, shoot length and root length ($P < 0.01$). This studied traits uncovered highly significant differences among EMS doses, varieties and their interaction showing that differences exist within varieties regarding sensitivity to mutagenic treatment. From the results it can be inferred that germination %, shoot length and root length can be used with equal reliability for estimating the suitable doses of EMS for treatment on a large scale in a breeding program. The LD_{50} values for germination %, shoot length and root length were observed at Aye Yar Min (0.52 to 0.55%), Rice Berry (0.55 to 0.68%), Jasmine 105 (0.57 to 0.60%) and Paw San (Black) (0.62 to 0.66%) of EMS dose to create maximum variability with minimum numbers of undesirable mutants. These optimum mutagen doses determined for four different rice varieties could be useful while formulating rice mutation breeding programme for improvement of specific traits in rice. Mutagenic response is more or less linear with the dose for all tested varieties. The trend of EMS resistance on basis of three traits in four varieties was in the order i.e., Paw San (Black) > Jasmine 105 > Rice Berry > Aye Yar Min in the present study.

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