Bangladesh J. Bot. **53**(4): 1077-1080, 2024 (December) **- Short communication**
DOI: https://doi.org/10.3329/bjb.v53i4.78129

EMS DOSE OPTIMIZATION TO DEVELOP LARGE-SCALE MUTANTS IN RAPESEED (*BRASSICA* L.)

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Keywords: Oilseed, Rapeseed, Mutation breeding, EMS dose, LD50, Survivability

Abstract

Mutation breeding is a great way to create variation in plant genomes, and Ethyl Methane Sulphonate (EMS) is commonly used for this purpose. To determine the optimum dose of EMS for developing large-scale mutants, *Brassica napus* and *B. rapa* (BARI Sarisha-18 and BARI Sarisha-20, respectively) were treated using six different concentrations (T0:0%, T1:0.1%, T2:0.2%, T3:0.3%, T4:0.4%, and T5:0.5%) of EMS. For *B. napus,* 100% germination was observed in T0, T1, and T2, while for *B. rapa,* it was in T0 and T1 treatment. The highest survival rates, 70% for *B. napus* and 60% for *B. rapa*, were recorded at T1 treatment. Morphological traits such as leaf number, shoot and root length, and fresh and dry weights were highest in T1 treatment for both species. The LD50 was calculated at 0.16% for *B. napus* and 0.18% for *B. rapa,* indicating the ideal dose for generating a large number of mutants.

Brassica sp. (Rapeseed) is the leading and widely grown oilseed crop in Bangladesh as they contribute nearly 73 and 71% to its total area coverage and production, respectively (BBS 2023). Since 2010, although the area covered by rapeseed has begun to expand more rapidly, the average yield is much lower than the world average. Therefore, more high-yielding cultivars to further increase and stabilize productivity are needed. Conventional breeding, different biotechnological methods as well as mutation breeding are commonly used to create genetic variability for achieving desired cultivars. The main advantage of mutation breeding over other techniques is the possibility of improvement of one or a few characters without changing the whole genotype. Chemical mutagen, Ethyl Methane Sulphonate (EMS) produces random mutations in the plant genome which is potent and effective for inducing variability (Channaoui *et al*. 2019). The dosage of mutagen is the most important factor as an overdose might kill too many treated individuals and a lower dose will produce fewer mutations. In Bangladesh, till now no rapeseed variety has been developed using EMS-induced mutation breeding. Therefore, keeping the above points in view the present study was conducted to determine the optimum dose of EMS to develop large-scale mutants in different varieties of rapeseed.

The experiment was carried out at the Molecular Biology Laboratory of Oilseed Research Centre, Bangladesh Agricultural Research Institute, Joydebpur. The healthy and disease-free 20 seeds of BARI Sarisha-18 and BARI Sarisha-20 from *Brassica napus* and *B. rapa*, respectively were pre-soaked in distilled water at room temperature for 10 hours. EMS solution of five different concentrations (T1 : 0.1%, T2 : 0.2%, T3 : 0.3%, T4 : 0.4%, and T5 : 0.5%) was prepared in 0.1 M phosphate buffer at pH 7 (Fowler and Stefansson 1972). After removing excess water, seeds were treated with the EMS solution with orbital shaking at 120 rpm for 2 hrs. Subsequently, the seeds were thoroughly washed with running tap water for another 2 hrs. Along with, an equal number of seeds were also exposed to the same condition without EMS treatment and were

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considered as non-treated control (T0 : 0%). Both the treated and non-treated seeds were plated separately in a Petri dish (20 seeds/Petri dishes) to emerge radical and plumule. After seven days of planting, data on germination percentage was recorded from the Petri dishes and calculated. Another batch of treated and non-treated seeds (20 seeds/pot) was sown in plastic pot. The survivability % was used to determine the LD50 dose for each variety. The statistical analysis was done using R packages (R Core Team 2022) and Microsoft Excel 2016.

The germination percentage was not affected up to 0.2 and 0.1% in *B. napus* and *B. rapa*, respectively but, a decreasing trend was noticed with the increasing dose of EMS except treatment T3 (10%) in *B. rapa* (Fig. 1). Maximum seedling survival of 60 and 70% was found in T1 (0.1%) treatment for *B. napus* and *B. rapa*, respectively. However, no plant was found to survive at T5 treatment in *B. napus* and T4 and T5 treatments in *B. rapa* (Fig. 2). Mutagens cause injuries to the genetic material which eventually leads to a reduction in the rate of respiration and energy production and finally decrease in seed germination (Bolbhat *et al*. 2012)).

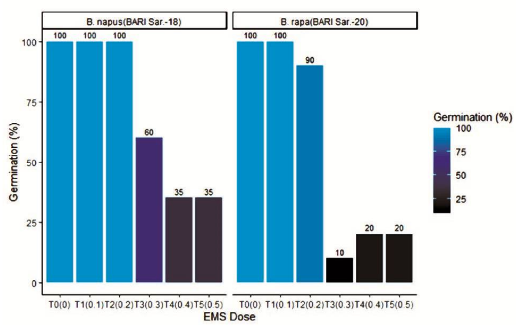

Fig. 1. Effects of different doses of EMS on the percentage of germination of B. *napus* and *B. rapa*.

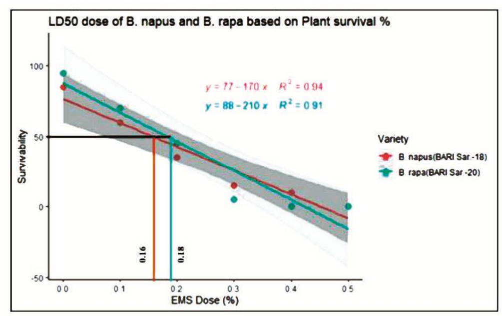

Fig. 2. LD50 dose of *B. napus* and *B. rapa* based on percent plant survival.

An optimum dose is a dose at which adequate genetic variation can be obtained with the lowest plant lethality. The best way to determine the optimum mutagenic dose is the calculation of LD_{50} and ignoring LD_{50}, the mutagenic dose would be misleading (Habib *et al.* 2021). In the present study, to calculate the EMS dose of LD_{50}, survivability percentage data at day 28 was used. A regression line was drawn for each species of rapeseed (Fig. 2) at which approximately 50% of seedlings were found to survive and the optimum dose (LD_{50}) was found at 0.16 and 0.18% for *B. napus* and *B. rapa*, respectively. The dose of LD_{50} may vary from mutagen to mutagen, species to species, and one genotype to another. A 0.8% EMS concentration optimum (LD_{50}) for *B. napus* var. RGS003 was reported by Emrani *et al.* (2011) while LD_{50} doses of 0.42 and 0.73% EMS was reported for two different *B. juncea* variety (Yadav *et. al*. 2016). The optimum EMS concentration for *B. rapa* has not been found in any literature yet. Therefore, the optimum EMS dose of 0.16% and 0.18% for *B. napus* (BARI Sharisa-18) and *B. rapa* (BARI Sharisa-20) found in this research could be used to develop large-scale mutants for rapeseed.

The morphological attributes from this experiment revealed that the different treatments considerably influence the number of leaves, shoot and root length, shoot and root fresh weight and dry weight of both rapeseed species (Table 1). The maximum number of leaves per seedlings at T1 treatment for both species and the minimum at T3 treatment in *B. napus* and T2 in *B. rapa* was found. Longer shoot and root, higher fresh and dry weight of root and shoot were observed in T1 and these attributes were lower in T3 treatment for *B. napus.* On the other hand, for *B. rapa* the lowest root and shoot length along with their fresh and dry weight were found in T2 and were highest in the T1 treatment. The negative influence of higher concentration on morphological attributes as compared to the control might be due to induced physiological damage in the seeds and seedlings and reduced cell division rates as well as activation of growth hormones such as auxin (Kumari *et al*. 2016).

Table 1. Effect of EMS on different characters of *Brassica napus* (BARI Sarisha-18) and *B. rapa* (BARI Sarisha-20) at 28 DAS.

Treatment	No. of plants	No. of leaves	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)
B. napus								
T0:Control	17	6.667	9.667	4.833	0.740	0.021	0.085	0.004
T1:0.1	12	8.600*	13.23*	6.56*	1.24*	0.032*	0.138*	0.013*
T2:0.2	7	5.000	9.200	3.06*	0.450	0.009	0.040	0.002
T3:0.3	3	4.000*	5.630*	2.36*	0.18*	0.0036*	0.015*	0.001
T4:0.4	2	5.667	9.567	4.567	0.520	0.009	0.044	0.002
Mean		5.987	9.859	4.275	0.626	0.015	0.064	0.004
CV		29.30	29.15	38.36	63.45	83.76	75.68	113.87
SE		0.716	1.173	0.670	0.162	0.005	0.020	0.002
LSD		1.841	2.820	1.721	0.417	0.013	0.051	0.005
Value of b		-0.660	-0.780	-0.470	-0.150	-0.005	-0.021	-0.002
B. rapa								
T0:Control	19	8.000	10.850	5.950	5.599	0.235	0.795	0.084
T1:0.1	14	10.500	10.900	6.250	6.699	0.395	0.126	0.110
T2:0.2	9	7.500	4.950	2.300	2.324	0.075	0.329	0.028
Mean		8.667	8.767	4.833	4.874	0.235	0.417	0.074
CV		18.54	37.75	45.49	46.69	68.22	82.23	56.53
SE		0.928	1.911	1.270	1.314	0.093	0.198	0.024
LSD		3.990	8.218	5.460	5.651	0.398	0.851	0.104
Value of b		-0.250	-2.950	-1.825	-1.630	-0.080	-0.230	-0.027

*SL= Shoot length, *RL= Root length, *SFW= Shoot fresh weight, *RFW= Root fresh weight, *SDW= Shoot dry weight, *RDW= Root dry weight.

The percentage of seed germination and seedlings' survival percentage was highly reduced with increasing doses of EMS. At the lowest dose of EMS, all the studied parameters showed positive responses over control in both species while responses were negative with an increasing concentration of EMS for morphological characters. Therefore, the lowest concentration of the chemicals was the most effective in causing mutations. However, the LD_{50} dose based on the seedling survival percentage was found 0.16% and 0.18% for *B. napus* and *B. rapa*, respectively. These doses could be useful in rapeseed-mustard mutation breeding programs to improve specific traits. Again, this finding is not adequate as the population is few. Therefore, it is essential to conduct a field experiment with a large number of populations using this optimized dose of EMS to obtain the overall variations in mutants.

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(Manuscript received on 30 September, 2024; revised on 30 November, 2024)