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Optimization of EMS mutagen dose for short day onion

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ABSTRACT

The present experiment was conducted to investigate the effect of chemical mutagen on biological parameters such as seed germination, shoot and root length and to fix the lethal dose (LD50) of EMS in short-day Indian onion cultivar Bhima Dark Red. Presoaked onion seeds were treated with different doses (0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2 % v/v) of Ethyl Methane Sulfonate. The optimal mutagenic doses were calculated using Probit Analysis based on germination percentage, which was considered helpful in developing an onion-mutagenized population to create genetic variability for various qualitative and quantitative traits. Seed germination was recorded after 7 and 12 days of sowing, while seedling parameters such as seedling height, dry and fresh weight of seedlings were taken after 12 days. Data revealed that the mutagenic agent significantly reduced seed germination and seedling growth parameters regarding shoot and root length compared to the control. Furthermore, a significant reduction in the seed germination per cent with the enhanced concentration of EMS dose was exhibited.

Keywords: *Allium cepa* L., EMS mutagen, Mutation, Lethal dose, Plant survival

INTRODUCTION

Onion (*Allium cepa* L., 2n=2x=16), belonging to family *Amaryllidaceae*, has originated in Central Asia and is grown throughout the world. This is possible due to the development of new cultivars adapted to varied climatic conditions through continuous breeding and genetic improvement (Khar and Singh, 7; Khar *et al*., 8). Onion is considered as the second most important vegetable crop after tomato. Although India is the leader (second rank) in area and production of onion after China, yet the productivity and yield potential is quite low comparing to other onion producing countries such as US, China, Japan and Netherland (Khar and Singh, 7). This crop is one of the key horticultural crops which is earning foreign exchange for Indian farmers. In India, onion is found in every kitchen and it is being used for most of the food preparations.

Despite its popularity and importance, not much systematic work on its genetic improvement is done in India. The basic and foremost reasons are the biennial habit, longer time required for breeding work, maintaining genetic uniformity due to highly heterogenous nature, highly cross-pollinating nature and high inbreeding depression (Lawande *et al*., 11). Due to its diverse uses and health beneficial properties as cooked, raw and processed form, the demand for onion has enhanced enormously in last decade worldwide. There is a great gap between demand and supply and to bridge this gap, there is a need to enhance the genetic variability in onion. Exploitation of already available genetic diversity through hybridization is difficult due to flower morphology, highly cross pollination nature etc. Therefore, induction of mutation could be a better option to create heritable variability in the form of desirable mutants having particular trait of interest.

A few induced mutations studies in onion have been conducted (Amjad and Anjum,1; Joshi *et al*., 4; Kato *et al*., 6). Induced mutations have already been used in many crop species to improve agronomical and quality traits affecting plant size, flowering time and fruit ripening, fruit color, self-compatibility, self-thinning, and resistance to pests. Until now, six varieties from Bangladesh, Netherland and Russia have been registered with the International Atomic Energy Agency, FAO. Recently, two cultivars of onion namely *Bina Piaz 1* and *2* have been developed by Bangladesh Institute of Nuclear Agriculture (IAEA, 3). Both the cultivars have been developed through induced mutations. The most effective method for mutation induction is the use of chemicals especially Ethyl Methane Sulfonate (EMS). Globally, EMS is considered to be the best chemical mutagen to create point mutations in plants. Effective utilization of chemical mutagen depends on dose and duration of mutagen and its efficiency to generate desirable genetic changes with minimum unwanted changes. The current study was initiated to create a basic information, which is still lacking, regarding proper dose of chemical mutagen needed for mutation induction in short day Indian onion improvement.

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MATERIALS AND METHODS

One commercial variety Bhima Dark Red (BDR) having red-coloured bulbs and recommended for *Kharif* season cultivation was selected for this study. Dry and uniform seeds were taken and before chemical mutagen treatment, seeds were pre-soaked in double distilled water for 9 hours at room temperature (24±2℃). Seeds were manually shaken after every two hours to ensure the uniform soaking. For EMS treatment, pre-soaked seeds were treated with 0 (control), 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 percent freshly prepared aqueous solution in double distilled water for 6 hours in flask and kept at 25°C temperature in an incubator shaker. After mutagen treatment, seeds were washed thoroughly five times for 3 minutes with water to wipe out mutagenic chemical completely.

After thorough washing, seeds were put in the petri-plate having Whatman filter paper at the base to soak adhering water with seeds. Immediately, seeds were sown in the plastic petri-plates having germinating filter paper at base. For each treatment, 20 seeds were used to grow M_1 seedlings to study effectiveness and efficiency of mutagenesis. The petri-plates were placed in the incubator room where temperature was maintained at 25±1℃. Seed germination in the form of emergence of radicle (about 2 mm size) was recorded after 7 and 12 days by counting the germinated seeds in every treatment and replication wise. Then final seed germination was calculated percentage wise for every treatment. Destructive parameters such as seedling height, fresh and dry weight of randomly selected five seedlings were assessed after 12 days of sowing. This study was conducted in a Completely Randomized Block Design with three replications having 20 seeds of each treatment. LD_{50} was calculated for the seedling parameters of the treatment using the regression analysis (equation of Y (damage) on X (dose) as Y = a + bX) and Probit analysis (Finney, 2). The data was analyzed using Crop Stat (IRRI, Philippines) and treatment means were separated with least significance difference (LSD) at 0.05 level of probability.

RESULTS AND DISCUSSION

Before starting any mutation breeding program in any crop, calculation of mutagen dose and its effectiveness in terms of seed germination and seedling growth is essential (Makeen and Babu, 12). In this study, we observed percent seed germination, seedling height, fresh & dry weight of seedlings affected by various EMS doses. Analysis of variance (mean squares) of data on effect of different EMS doses on seed germination, after 7 &12 days, and seedling growth parameters (length, fresh and dry weight) are presented in Table 1. Data revealed that EMS mutagen treatment had highly significant influence on all the recorded parameters. Similarly, Joshi *et al.,* (4) observed the same trend in onion cultivars using EMS and Sodium Azide (SA) as chemical mutagens.

Seed germination after 7 days

Determination of percent seed germination is a critical and significant parameter to assess the impact and effectiveness of any mutagen in plants in terms of germination inhibition and seedling growth (Nizamani *et al*., 13). Percent seed germination recorded after 7 days of treatment in onion cv. Bhima Dark Red showed significant difference among various treatments (Table 2). Maximum percentage of seed germination was recorded in the control (no EMS treatment) as predicted when compared to the EMS treated seeds. With the increase in EMS dose, percent seed germination elicited declining trend. The data revealed that there was no significant difference in seed germination percentage for the control and lowest dose (0.2 %) as well as 0.4 and 0.6 % EMS treatments. The range of percent seed germination of onion varied from 0.0 (1.2 % EMS) to 63.33 % (control). Correspondingly, Joshi *et al*., (4) observed that there is both stimulating and inhibiting effect of lower dose of chemical mutagen (0.1 % EMS as well as SA) on percent seed germination in onion cultivars (it can be concluded that its genotypic specific) and further enhancement in chemical dose led to significant decline in seed germination. In the present investigation, we have taken lowest 0.2 %

Table 1. Analysis of variance (mean squares) on effect of different EMS doses on onion cv. BDR

Source of variation	df	Germination after 7 days (%)		Germination after 12 days (%)		Shoot length in (cm)		Dry weight (mg)		Fresh weigh (mg)	
		Mean Square	F-value	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value	Mean Square	F-value
EMS	6	1694.05	58.08**	2105.56	139.63**	47.75	668.35**	1.07	872.96**	331.41**	557.47**
Replication	2	8.33	0.29	1.19	0.08	0.17	2.44	0.22	1.75	1.23	2.07
Error	12	29.17		15.08		0.71		0.12		0.59	

Table 2. Effect of EMS concentrations on percent seed germination and seedling growth parameters of onion cv. BDR

EMS Concentration (%)	Seed germination after 7 days (%)	Seed germination after 12 days (%)	Seedling length (cm)	Fresh seedling weight (mg)	Dry seedling weight (mg)
0.0 (Control)	63.33[a]	73.33[a]	11.07[a]	29.09[a]	1.82[a]
0.2	56.67[a]	66.67[a]	10.34[b]	28.07[a]	1.70[ab]
0.4	40.00[b]	46.67[b]	5.49[c]	17.45[b]	1.48[bc]
0.6	36.67[b]	38.33[c]	4.30[d]	12.44[c]	1.26[cd]
0.8	25.00[c]	28.33[d]	3.59[e]	10.31[d]	1.19[cd]
1.0	6.67[d]	15.00[e]	3.11[f]	9.14[d]	1.11[cd]
1.2	0.00[e]	0.00[f]	-	-	-
LSD (0.05)	9.61	6.91	0.47	1.37	0.32

EMS which is found to be at par with the control. EMS dose beyond 1.0 % completely inhibited seed germination. Previous studies have also reported that mutagen dose is inversely proportional to seed germination which means that germination decreased with increased dose of the mutagen. It could be due to the adverse effect at physiological or cellular/molecular level of mutagen at the growing tissues of seed i.e., meristematic tissues (Joshi et al., 4).

Seed germination after 12 days

After 12 days, as expected, the maximum percent seed germination was recorded in non-treated seeds (control) as compared to treated seeds which exhibited that EMS treatment suppressed the germination after 7 and 12 days of sowing. The trend of the seed germination was almost same as recorded after 7 days after treatment (Table 2). Percent seed germination ranged from 77.33 % (control) to 0.0 (1.2 % EMS). Except control and lowest dose of EMS (0.2 %) treatment, all other treatments exhibited significant differences in percent seed germination. Seeds of cultivar BDR presented high sensitivity to higher EMS mutagen doses. The range of percent seed germination varied from 0.0 (1.2% EMS) to 73.33% (control) after 12 days of sowing under controlled conditions. Lowest dose of EMS (0.2 %) showed no significant difference as compared to control. On the other hand, EMS dose above 0.2% significantly reduced and delayed germination in onion. Similar observations have been documented in sesame (Kumari *et al*., 10), pepper (Siddique *et al*., 17) and carnation (Roychowdhury and Tah, 16). It may be due to the modification of various physiological or biological processes in terms of malfunctioning of enzymes, hormonal imbalance, mitotic activity or lethality of the meristematic tissues or cells responsible for seed germination (Khursheed *et al*., 9; Nizamani *et al*., 13). Furthermore, Yadav *et al*., (18) documented that the EMS concentration more than 1% has been immensely lethal and poisonous regardless of genotype and species of plants.

Seedling parameters

Analysis of variance of seedling length and seedling fresh and dry weight revealed that chemical mutagen EMS created significant effect on the seedling parameters (Table 1). EMS treatment had highly significant effect on seedling length and fresh weight but less effect in case of dry weight with enhancing concentration.

Seedling Length

The effect of EMS mutagen on seedling length was recorded by taking the length of 12 days old seedlings (Table 2). Seedling length was noted to show significant differences among all treatments of EMS concentrations over control. Seedling length displayed a broad range of variation from 3.11 (1.0 % EMS) to 11.07 cm (Control). Seedling length was 3.11 cm at maximum dose of EMS (1.0 %) whereas it was 10.34 cm at lowest EMS dose (0.2 %). Highly significant difference was recorded among 0.4 and 0.6% EMS treatments i.e., 10.34 and 5.49 cm, respectively. Highest reduction in seedling length was noted with 0.4% EMS concentration. Similar reports have been reported in mungbean (Kamini and Akhaury, 5). To detect the biological effects of chemical mutagen, seedling length is aptly exploited as an index and a linear relationship exists between length of seedling and the concentration of mutagen. Less doses of EMS mutagen concentration elicited higher shoot length (Kumari *et al*., 10). The main reason could be because of uneven damage or injury to the meristematic cells as a result of genetic injury. The desperately injured cells would yield only a few cell progeny and growth will recur from those cells which are genetically least damaged.

Fresh and dry weight of seedlings

In the present experiment, as with the other seedling parameters, fresh and dry seedling weight also followed the similar trend. Reduction in fresh weight was found to be highly significant in EMS treatments over the control (Table 2). A range of 1.11 to 1.82 mg and 9.14 to 29.09 mg were recorded for dry and fresh weight of seedlings, respectively. In case of fresh weight, no significant differences were exhibited among control & 0.2% and 0.8 & 1.0 % EMS treatment. Seeds treated with 0.4% EMS revealed drastic significant reduction in fresh weight. On the other hand, dry seedling weight elicited significant differences but not in higher ranges. The significant decline in seedling biological growth owing to higher concentration of EMS have been elucidated by various researcher groups in a different way (Roychowdhury and Tah, 16). According to them, it could be because of unexpected enhancement in seed metabolic status at specific level of mutagen dose; upsurging of growth inhibitors; reduction in the auxin hormone and deterioration of assimilation mechanism.

Determination of LD_{50} (Lethal Dose) Value

The lethal or LD_{50} EMS dose for short day Indian onion cv. Bhima Dark Red was calculated through Probit Analysis on the basis of seed germination recorded after 12 days of sowing. The dose response curve based on Probit units were drawn and presented in Fig. 1. Calculated LD_{50} mutagen dose and Probit units based on mortality percentage of mutated plant population is presented in Table 3 and 4. Determination of lethal dose is very important for any mutation breeding program in any crop. It is the minimum concentration of EMS mutagen that allows minimum 50% survival of mutated seeds or seedlings. So, optimum dose is the dose that causes maximum mutation with minimum damage to the plant. This value depends on genotype, hardiness, maturity level, nature of treatment, moisture content and treatment conditions (Parthasarathi *et al.,* 14). Based on the results, the regression equation of relationship between EMS mutagen dose and mortality of onion seed or seedlings was

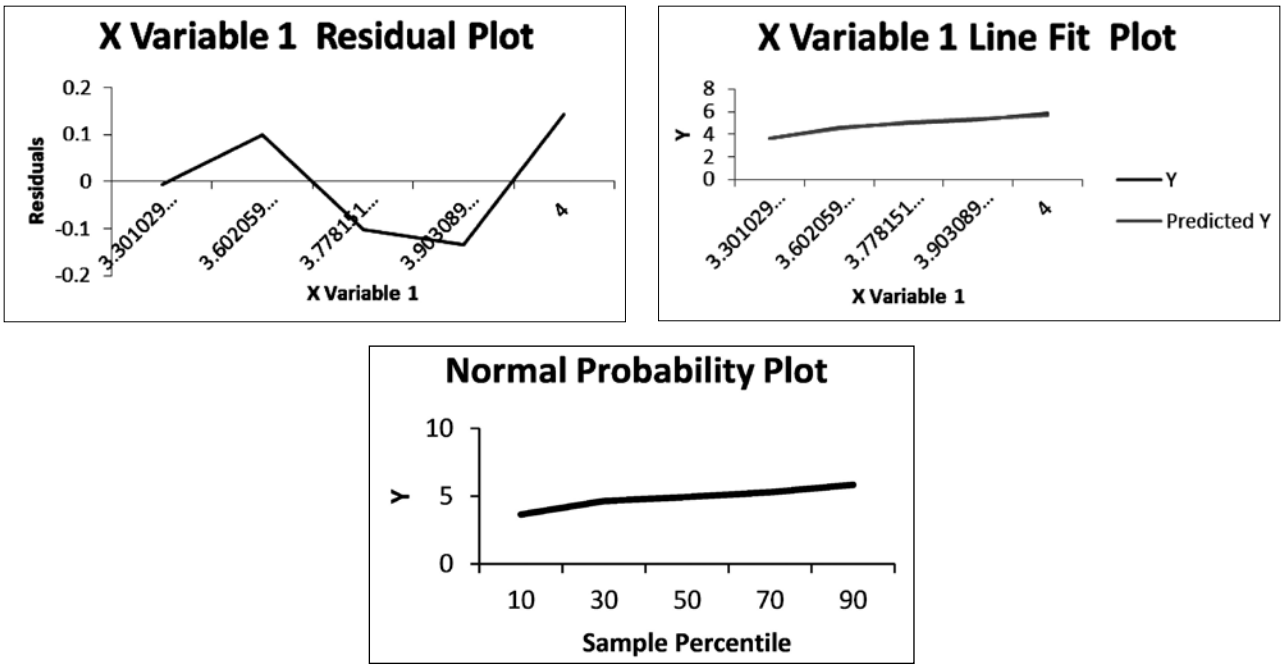

Fig. 1. The relation curve of EMS dose and mortality of onion seed/seedlings of cv. BDR (Y=2.91X -5.93) through Probit Analysis

Table 3. Analysis of variance of Probit Analysis

Source of variation	df	SS	MS	F	Significance F
Regression	1	2.57316	2.57316	130.4851	0.00144
Residual	3	0.05916	0.01972		
Total	4	2.63232			

Table 4. Probit Analysis for LD_{50} concentration of EMS on onion cv. BDR

EMS concentration (%)	EMS concentration in PPM	log10 concentration	Mortality % over control	Probit	LD_{50}
0.2	2000	3.301	9	3.66	5888.4 PPM (0.6 %)
0.4	4000	3.602	36	4.64	
0.6	6000	3.778	48	4.95	
0.8	8000	3.903	61	5.28	
1.0	10000	4.000	80	5.84	

Table 5. Results of Probit analysis to study the effect of EMS on short day onion cv. BDR

Parameter	Coefficients
Intercept	5.927
X Variable 1	2.906

Equation (y=2.91x-5.93), X=3.77, LD_{50}= 5888.437 =6000 ppm or 0.6 %

Y=2.91X -5.93. As per our expectations, enhancing concentration of EMS led in reduction of seed germination significantly. Regression equation also has the meaning that although EMS is a mutagen but it also has a poisonous effect on plant cells as the concentration increases. The results of this experiment exhibited LD_{50} dose value around 6000 ppm (0.6 %) EMS concentration (Table 5). Thus, this is the optimum dose for the cv. BDR for chemically inducing mutations to create viable mutants and maintenance of mutant population for further breeding program. Similar reports have been documented in blackgram (Ramya *et al.,* 15).

To conclude, it can be said that 0.6 per cent EMS is the optimum dose for onion cultivar Bhima Dark Red, which can be used by onion breeders for development of onion mutants to create new breeding material for screening against various biotic and abiotic stresses under rapid changing climatic conditions.

AUTHORS' CONTRIBUTION

Conceptualization of research (AK, HS); Designing of the experiments (AK, SKL, HS); Execution of field/lab experiments and data collection (HS, PV); Analysis of data and interpretation (AK, SKL, HS); Preparation of manuscript (AK, HS).

DECLARATION

The authors declare no conflict of interest

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