SHORT COMMUNICATION

Effects of Ethyl Methane Sulphonate on the Growth and Yield of *Vigna radiata* L. var. PDM-54 Plants

Kanchan Awasthi, Vikash Verma, Neetesh Kumar, Madhu Prakash Srivastava*

DOI: 10.18811/ijpen.v6i02.11

Abstract

Mutagenesis is a potential tool to be employed in crop improvement and may offer greater opportunities for generating suitable genetic variability. Ethyl methane sulphonate (EMS) treatments were quite effective in bringing a reduction in different parameters. In this experiment, the seeds of *Vigna radiata* PDM-54 were used as the test material. The presoaked seeds were treated with 1% EMS for 4, 6, and 8 hours. After treatment seeds were washed in running water and sown along with control in three replicates. Data were recorded on different morphological and cytological parameters in order to assess the mutagenic effect of EMS. The germination percentage of treated seeds displayed a sharp decrease with the increase in treatment duration. The germination was very poor at 6 and 8-hour treatment. The survival percentage was also found to be inversely proportional to the duration of treatment. The yield per plant displayed a gradual decrease with the increase in treatment duration. It indicates that EMS can be used as an effective mutagen to generate enough variability to be subsequently exploited in any crop improvement program.

Keywords: Ethyl methane sulphonate, Growth and Yield, Mitotic index, *Vigna radiata*.

International Journal of Plant and Environment (2020); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

Introduction

V. radiata belonging to the family Leguminoceae is a nativity India, where it has been cultivated since ancient times. The crop is also grown in some parts of South East Asia, Africa, the West Indies, and America. In India, it is widely cultivated in MH, UP, Gujarat, Tamil Nadu, Andhra Pradesh, and Bihar. *V. radiata* is an important rainy season pulse crop and it is grown in more than three million hectares in India. This pulse is an excellent source of protein. Its seed contains about 23% protein, 58% carbohydrate along with minerals, and fats. Mung bean is a small herbaceous, erect, deeply rooted annual, and attaining a height of 45 to 120 cm. It is cultivated mainly as a Kharif crop but in the south, it is grown as a Rabi crop. It is sown from March to April and July to August and takes about three months for maturation (Gnanamurthy & Dhanve, 2009; Rana & Solanki, 2015).

Genetic variability is the most essential requirement for any crop improvement program. The certain and management of genetic variability become the central base to crop breeding. Greater are the chances of producing the desired type. Genetic variability may occur spontaneously, as well as, can be induced artificially. Artificially induced genetic variation is being used effectively to supplement or complement genetic sources of natural origin for practical plant breeding. Useful genetic variation can be induced in modern cultivar, helping to shorten the breeding time or the characteristics of existing genetic resources can be improved to make them more useful for breeding (Arullbalachandran & Mullainathan, 2009a).

By mutagenesis, new previously unknown inactive alleles can be induced in crop plant species to broaden the base of variation. Mutagenesis can be used to induce variability in plant architectural characters. Mutagen is induction is important to provide the genetic variability require for the evolutionary adaptation of species to environmental change. It is the ultimate source of all genetic variation; it provides the raw material for evolution. The genetic variability could be conveniently induced with a wide range of physical and chemical mutagens. The use of chemical mutagen has been advantageous for improvement in some of the crops (Bahar & Akkaya, 2009). Now a whole range of chemical mutagen is available, *viz*., mustard gas, alkylating agents, epoxides, aziridine, nitrosamine, etc. Among these, alkylating agents are the most effective. They react with DNA by alkylating the phosphate group, sugar, as well as, purine and pyrimidine bases (Gupta *et al.,* 2018).

EMS was found to be one of the potent alkylating agents as it generates tremendous variability. Its action is modified by the duration of temperature, pH, temperature, and concentration, etc. It provides more genetic changes, consequently produces the greatest inhibition in vegetative and reproductive growth, and the highest frequency of lethal. Natural pH has been observed to be better for the mutagen treatment. The maximum effectiveness of the mutagen is also observed at 30°C (Javed *et al*., 2016). The pulses are an important source of protein. The induced mutation may be used as an efficient tool for mutagenesis in pulses. Pulses are regarded as indispensable items of diet particularly for vegetarians. The pulses figure prominently in crop rotation and mixed cropping. It is necessary to create novel genetic variability through induced mutations, which has been proved to be a valuable approach in generating variability in pulses (Arullbalachandran & Mullainathan, 2009b).

Department of Botany, Maharishi University of Information Technology, Lucknow-226013, Uttar Pradesh, India

***Corresponding author:** Dr. Madhu Prakash Srivastava, Department of Botany, Maharishi University of Information Technology, Lucknow-226013, Uttar Pradesh, India, Mobile: +91-7905154448, Email: rivastava2010@gmail.com

How to cite this article: Awasthi, K., Verma, V., Kumar, N., & Srivastava, M. P. (2020). Effects of ethyl methane sulphonate on the growth and yield of *Vigna radiata* L. var. PDM-54 plants. International Journal of Plant and Environment, 6(2): 162-164.

Source of support: Nil

Conflict of interest: None

Submitted: 12/12/2019 **Accepted:** 25/04/2020 **Published:** 30/04/2020

Seeds of *Capsicum annuum* L. var. G4 were subjected to different concentrations of methyl methanesulphonate (MMS) and diethyl sulfate (DES). The effects of different mutagenic treatments on meiosis, chiasma frequency, and pollen fertility have been studied in M1 generation. Various types of meiotic aberrations, such as, univalent, multivalent, stickiness, bridge, laggards, cytomixis, etc., were observed in all the treatments. The MMS treatments proved to be more effective in inducing meiotic aberrations as compared to DES (Gulfishan *et al.*, 2012; More & Malode, 2016).

Chemical mutagenesis was used to induce mutations in the wheat variety NN-Gandum-1. This cultivar is moderately resistant to leaf and yellow rust. The aim of mutagenesis was to improve resistance to the disease, as well as, to study the function of genes conferring resistance to the disease. 0.8% EMS dose was found optimum for supporting 45 to 55% germination of NN-Gandum-1. A total of 3,634 M2 fertile plants were produced from each of the M1 plants. Out of these, 33 (0.91%) and 20 plants (0.55%) showed absolute resistance to leaf and yellow rust, respectively. While 126 (3.46%) and 127 plants (3.49%) exhibited high susceptibility to the leaf and yellow rust, respectively (Hussain *et al.*, 2018).

Materials and Methods

The seeds of *V. radiata* var. PDM-54 were used as a test material. The healthy and bold seeds were selected and were used for treatment with EMS. For the experiment, presoaked seeds (for 6 hours) of *V. radiata* var. PDM-54 were treated with 1% EMS for 4, 6, and 8 hours. After treatment, the seeds were washed in running tap water for 1-hour. These treated seeds along with the control were sown in the plot in three replicates, at the rate of 25 seed per row in July, to raise M1 germination. Data were recorded on different morphological and cytological parameters, like plant height, number of branches, number of nodes, number of leaves, leaf length, leaf width, number of pods, pods per cluster, number of seeds per pod, and pod length yield per plant in order to assess the mutagenic effect of EMS.

At the time of flowering, anthers from the flowers were collected and were taken aside, and their squash preparation was made in a drop of acetocarmine. The slide was observed in a microscope under low and high power. Numbers of fertile and sterile pollen were counted in each field. Pollen fertility percent was calculated by using the following formula:

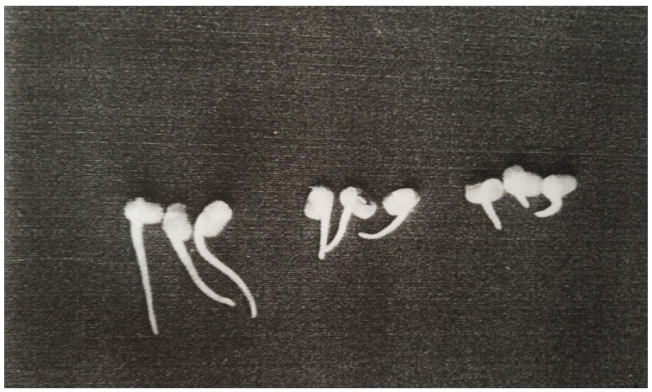


Fig. 1: Germination and root growth of control and EMS exposed *V. radiata*

Pollen fertility % = Number of fertile pollen/Total number of pollen × 100

Data were recorded on different morphological and cytological parameters, like height, number of branches, number of nodes, stem perimeter, number of leaves, leaf length, leaf width, number of pod clusters, pod per clusters, pod length, number of seed per pod, and yield per plant order to assess the mutagenic effect of EMS. The mean value was calculated for different quantitative parameters. The graph was plotted based on a percent of control for each parameter to study the effect of treatment duration. The cytological studies were carried out on the root tips fixed in 1:3 acetic alcohol, from the seed treated with 0.5, 1% EMS. These root tips were stored in 90% alcohol and were used for preparing squashes.

Mitotic Squash Preparation

- Root tips were kept out from 90% alcohol and were washed with tap water.
- They were kept in 5N HCl for hydrolysis for about 15 to 20 minutes at room temperature.
- When the root tips become hydrolyzed, they were taken out of HCl and were washed thoroughly with tap water for 30 minutes.
- Now the root tips are transferred in iron alum solution for half an hour.
- Root tips were again washed and finally, tips were transferred to hematoxylin stain in a staining tube covered with black paper for two to three hours.
- Thereafter the squashes were prepared by taking the tip portion in a drop of 45% acetic acid on the slide and then coverslip was kept over it and gently tapped with the help of a blunt head of a pencil.
- Slides were pressed by keeping them between and folds of blotting paper. Then, the slide was observed in a microscope under low and high power.
- The number of dividing and non-dividing cells and abnormal cells was noted.

Mitotic index and abnormality percent were calculated by using the following formulas:

Mitotic index = Total number of dividing cells/Total number of cells studied × 100

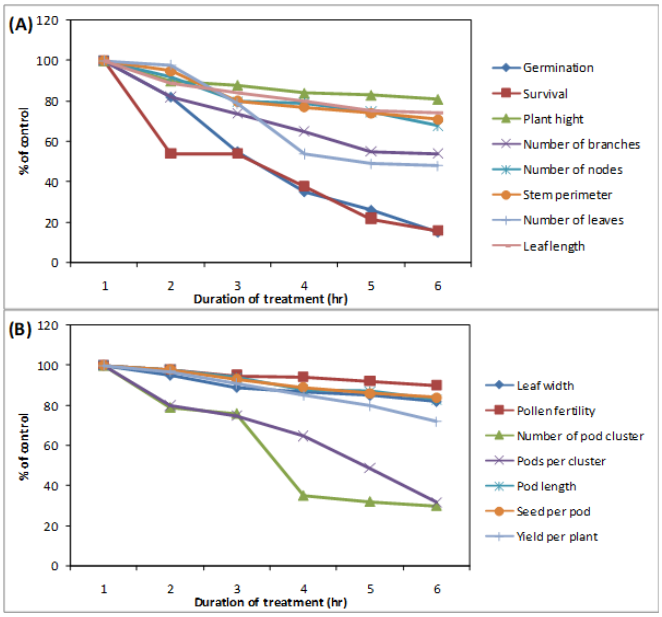
Abnormality % = Total number of abnormal cells/Total number of dividing cells

Results and Discussion

The germination percentage of treated seeds displayed a sharp decrease with the increase in treatment duration. The germination was very poor at 6 and 8-hour treatment (Table 1; Fig. 1). The survival percentage was also found to be inversely proportional to the duration of treatment. All the germination seedlings did not survive, and thus, the survival was lower than germination. Plant height showed a gradual decrease with the increase in treatment duration. The number of branches and numbers of nodes also decreased almost proportionately with the increase in treatment duration. Stem perimeter was also decreased with an increase in treatment duration. The numbers of leaves were also found to be gradually decreased. A slight decrease in leaf length and width were observed with the

Table 1: Mitotic index of V. radiata after different doses of EMS

S. No.	Treatment	Number of dividing cells	Number of non-dividing cells	Total no. of cells studied	Number of abnormal cells	Mitotic index	Abnormality present
1	Control	23	105	128	-	14.11	-
2	0.5%	15	105	120	4	12.5	26.6%
3	1%	10	100	110	5	10	45.4%



Figs. 2A and B: Effect of treatment duration of EMS on the different morphological parameters in *V. radiata* var. PDM-54

increase in treatment duration. Some leaf variants particularly having the variation in the number of the leaflet were observed in the treated plants.

There was a slight decrease in pollen fertility with an increase in treatment duration. The decrease in the number of pod clusters per plant was noticed with an increase in treatment duration, which was very drastic at 8-hour treatment. Similarly, pods per cluster decreased at dose, which was very drastic at 8-hour treatment. Pod length and seeds per pod also showed a decreasing trend with the increase in treatment duration (Figs. 2A and B). The yield per plant displayed a gradual decrease with the increase in treatment duration, which was lowest at 8-hour treatment. The treated seed show retardation in root growth. The root length was decreased as the EMS concentration was increased that the negative correlation was found between the root growth and EMS concentration. The mitotic studies showed that mutagen exerted a strong effect on the root tip cells of *V. radiata* (Gustafsson, 1941; Swaminathan, 1969). The number of dividing cells was decreased with the increase in EMS concentration. Some abnormalities in the dividing cells were observed. The fragment of chromosomes was observed at prophase and metaphase. Clumping and condensation of the chromosome were observed at metaphase. Laggards and bridges were also observed. Mutagenesis is a potential tool to be employed in crop improvement and may offer greater opportunities for generating suitable genetic variability. Enhancement of the frequency of mutation in a predictable manner, and thereby, achieving desired plant characteristics through mutagenesis is an important goal of mutation research.

Conclusion

The germination percentage of treated seeds displayed a sharp decrease with the increase in treatment duration. At 6 and 8 hour treatment duration, the germination and survival percentage was very poor. Yield per plant was decreasing with the increase in treatment duration. So, from the present investigation it was inferred that EMS can be used as an effective mutagen to generate variability in many crop improvement programmes.

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