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Determination of Lethal Dose 50 for Induced Mutagenesis in Soybean [*Glycine max* (L.) Merril] cv. Gepak Kuning Through Ethyl Methane Sulfonate Mutagen

Nilahayati[1], Rd. Selvy Handayani[1], Nazimah[1],
Muhammad Sani Alfikri Harahap[1] Gusti Irawan[1], Rosmaina[2]

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ABSTRACT

Background: The improvement of the genetic and agronomic properties of cultivated plants can be carried out by various plant breeding methods, including mutation breeding. Mutations in nature are very few and rare. Ethyl Methane Sulfonate (EMS) is a chemical mutagen that can cause random mutations resulting in changes in genetic material. This study aims to determine the effect of EMS on the germination of soybean (cv. Gepak Kuning) seeds.

Methods: The research was conducted at the Faculty of Agriculture Laboratory, Universitas Malikussaleh, Indonesia, in January 2023. The concentrations of EMS used were 0%, 0.025%, 0.05%, 0.1%, 0.25%, 0.5% and 1% with a soaking time of 4 hours. EMS concentration of 0% was used as a control treatment. The observed variables were made as percentage of germination (%) and seedling height at 7th day and 14th day after planting.

Result: The results showed that an increase in EMS concentration affected the percentage of germination of soybean (cv. Gepak Kuning) sprouts. A decrease in the percentage of germination was found at an EMS concentration of 0.5%, while a decrease in seedling height had begun to be seen at an EMS concentration of 0.25%. The results of the analysis with CurveExpert 1.4 software showed that the LD_{50} value of the seeds of soybean (cv. Gepak Kuning) treated with EMS mutagen was at a concentration of 0.63%, the LD_{20} dan LD_{30} value was at a concentration of 0.25% and 0.41%, respectively.

Key words: Ethyl Methane Sulfonate, Gepak Kuning Soybean, LD_{50}.

INTRODUCTION

Increasing genetic diversity is a crucial step in the effort to develop new superior varieties of cultivated plants. The mutation breeding method is believed to be very effective for obtaining genetic diversity in a short time and a fast way to produce variations to develop new varieties. Van Harten (1998) states that mutation breeding can increase the desired traits that are not present in the germplasm of a species and can also cause diversity in existing varieties.

Mutation induction can be applied to obtain new variations to improve the genetic properties of plants. Improvement of the genetic properties of a plant can be done by conventional or artificial mutation. Artificial mutations usually use a mutagen. There are two types of mutagens, namely physical and chemical mutagens. The commonly used physical mutagens are gamma rays which have been successful in inducing genetic changes in various cultivated plants (Nilahayati *et al.,* 2019; Nilahayati *et al.,* 2022; Nilahayati *et al.,* 2022; Hanafiah *et al.,* 2016; Horn *et al.,* 2016; Geetha *et al.,* 2021). The chemical mutagens commonly used to induce plant genetic diversity are ethyl methane sulfonate (EMS), diethyl sulfate (DES), methyl methane sulfonate (MMS), hydroxylamine, sodium azide and so on. These compounds cause point mutations. Other compounds such as colchicine, oryzalin and caffeine cause chromosomal mutations, namely an increase in chromosome sets.

[1]Department of Agroecotechnology, Faculty of Agriculture, Universitas Malikussaleh, Jl. Cot Tgk. Ni Reuleut Timu, Aceh Utara, 24355, Indonesia.
[2]Department of Agrotechnology, Faculty of Agriculture and Animal Sciences, Universitas Islam Negeri Sultan Syarif Kasim Riau. Jl. Soebrantas No. 155, Simpangbaru, Panam, Pekanbaru 28295, Riau, Indonesia.

Corresponding Author: Nilahayati, Department of Agroecotechnology, Faculty of Agriculture, Universitas Malikussaleh, Jl. Cot Tgk. Ni Reuleut Timu, Aceh Utara, 24355, Indonesia.
Email: nilahayati@unimal.ac.id

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EMS mutagens have been believed and proven to be effective in causing point mutations in various plants. In addition, the price is also cheap and easy to obtain when compared with other chemical compounds. According to Russel (1992), Ethyl methane sulfonate is a chemical compound that can cause mutations at the DNA level by changing DNA bases. EMS has the chemical formula

$C_3H_8SO_3$. Sambrook and Russel (2001) added that the EMS mutagen is one of the chemicals mutagens included in the group of alkylating agents that can cause point mutations. Point mutations occur in a base which can be insertions, deletions, transversions, or base transitions. Insertions and deletions of one or more bases can cause a change in the reading order thereby changing the order of the amino acids. Transitions and transversions lead to changes in the expression of amino acids.

EMS alters the DNA structure directly by alkylating guanine bases (G), causing mispairing with thiamine (T) instead of cytosine (C), resulting in transition from G/C to A/T. The ease of application to seeds and its detoxification through hydrolysis for disposal make EMS a recommendable mutagen for improving genetic diversity in crop (Serrat *et al.,* 2014). In addition, EMS increase point mutations compared to physical mutagen such as gamma radiation (Van Harten, 1998).

EMS is the most widely used chemical mutagen to induce genetic diversity in cultivated plants. The use of EMS to induce mutations in plants has been carried out by many previous researchers including in barley (Sharamo *et al.,* 2021), chili (Arisha *et al.,* 2015), marigold (Lenawaty *et al.,* 2022), tepary bean (Thangwana *et al.,* 2021) and soybean (Sagel *et al.,* 2017; (Espina *et al.,* 2018). Their results showed that the genetic variability induced by mutagenesis using EMS provides more opportunities to improve desired traits in plants.

LD_{50} determination is a very important step in inducing mutations. LD50 indicates the dose of mutation that results in 50% decrease in seed germination after the seed is treated with EMS for a certain time (Mba *et al.,* 2010). Determination of LD_{50} value for any mutagen is essential to produce maximum viable mutants with minimum damage to the plant. Mba *et al.* (2010) stated, concentration of mutagen is the most critical factor with the results of assays depending to a great extent on the use of optimal concentrations of the mutagen. As a rule, an increase in the concentration of EMS, for instance, normally results in more mutation events, but these are accompanied by a corresponding greater amount of injury to seedlings and lethality.

Each plant genotype has a specific LD_{50} value as a result of EMS mutagen treatment. Previous studies reported that LD_{50} of barley was 0.64% v/v (Sharamo *et al.,* 2021), LD_{50} of chickpeas was EMS 30 mM (Umavathi and Mullainathan, 2015), fenugreek was 0.4% of EMS (Kavina *et al.,* 2020) and the LD_{50} for soybeans (cv. CO1 and CO2) were 26.40 mM and 25.70 mM respectively (Rajendran and Laksmi, 2017).

Mutation induction of soybean (cv. Gepak Kuning) has been carried out by previous researchers using gamma-ray irradiation. However, the radiosensitivity is the lowest or the least sensitive to gamma-ray irradiation compared to other soybean varieties (Indriani *et al.*, 2012). Mutation breeding using EMS on soybean (cv. Gepak Kuning) has never been done by previous researchers. Therefore, this early-stage research was carried out to determine the effect of several concentrations of EMS on the germination of soybean (cv. Gepak Kuning). The LD_{50} value found at this research will be used as a reference for determining EMS concentrations which will be used for induction of genetic diversity in the M_1 generation.

MATERIALS AND METHODS

The dry and dormant seeds of soybean (cv. Gepak Kuning) were treated with EMS treatments and were used in the present study. The study was carried out in Agriculture Faculty Laboratory, Universitas Malikussaleh, Indonesia, in January 2023. The chemical-mutagens used were Ethyl Methane Sulfonate [EMS (CH_3OSO_2 C_2H_5]. The chemical was obtained from HI-MEDIA laboratories, Mumbai, having a half-life period of 30 hours with a molecular weight of 124.16 and density of 1.20. EMS concentrations used were 0%, 0.025%, 0.05%, 0.1%, 0.3%, 0.5% and 1% with 4 hours of immersion time. EMS 0% concentration was used as a control treatment. Each treatment consisted of 30 of soybean (cv. Gepak Kuning) seeds, so that the total number of seeds was 210 seeds.

The germination planting medium is soil and manure with a ratio of 1:1. The seeds that have been planted are watered every day to keep the soil moist. Seeds were planted in germination medium and the percentage of viability was evaluated two weeks after planting. The observed variables were made as percentage of germination (%) at 7th day and 14th day after planting. The seedling height (cm) is measured from the base of the stem to the tip of the seed. Seedling height measurements were carried out at 7th day and 14th day after planting.

Percentage of germination data is used to determine the lethal Dose 50 (LD_{50}) value which is analyzed using the Curve-fit Analysis program. This program is a program to determine the best equation model for the percentage of germination of a population. The lethal dose (LD_{50}) was estimated using a linear regression model as follows:

$$y = a + b$$

y = Dependent variable (*i.e.*, germination percentage).
x = Independent variable (EMS concentration).
a and b = Constant and slope, respectively.

RESULTS AND DISCUSSION

The results showed that there was a decrease in the percentage of germination of soybean (cv. Gepak Kuning) seeds due to the treatment of various EMS mutagen concentrations. The percentage of germination was seen decrease at a concentration of 0.5%, but at the highest concentration, namely 1% EMS, it did not show any growth at all. The average germination of soybean seeds of the soybean (cv. Gepak Kuning) due to various EMS concentrations can be seen in Table 1.

The reduction in germination of seeds due to EMS mutagen treatment has been widely reported by previous researchers, including pepper (Arisha *et al.,* 2015), peanut (Chen *et al.,* 2020), cowpea (Opoku Gyamfi *et al.,* 2022), rice (Talebi *et al.,* 2012) and soybean (Sagel *et al.,* 2017), (Kalpande *et al.,* 2020). The reduction in germination may be due to the seeds absorbing the mutagen, which subsequently reaches the meristem region and affects the germ cell (Serrat *et al.,* 2014). Also, a reduction in germination may be because of the damage of cell constituents (Kumar and Pandey, 2019), alteration of enzyme activity or delay or inhibition of physiological and biological processes (Talebi *et al.,* 2012). A reduced germination percentage could be attributed to disturbances of seed meristematic tissue at cellular level resulting in chromosome damage, disrupting growth promoters due to increased accumulation of growth inhibitors (Jayakumar and Selvaraj, 2003).

EMS mutagen treatment on soybean (cv. Gepak Kuning) seeds also caused a decrease in seedling height. Fig 1 shows that at an EMS concentration of 0.05% there was stimulation of the seedling height, but starting at a concentration of 0.25% there was suppression of the seedling height of soybean (cv. Gepak Kuning). The response of soybean (cv. Gepak Kuning) to EMS concentrations of 0.01% to 1% showed a quadratic response. The graph between EMS concentration and the seedling height of soybean (cv. Gepak Kuning) can be seen in Fig 2.

Table 1: The percentage of germination due to various consentration of EMS in soybean (cv. Gepak Kuning) seed.

EMS concentration (%)	Percentage of germination (7th day after planting)	Percentage of germination (14th day after planting)
0	93.33	86.66
0.05	83.33	83.33
0.075	83.33	83.33
0.1	90	90
0.25	90	83.33
0.5	60	60
1	0	0

In previous research, (Hossain *et al.,* 2021) also found that rice plant height was significantly increased at lower concentration of EMS (1 and 2%) with a 6-12 hours treatment duration compared to the control plant. But when this concentration was increased, plant height was decreased compared to control as well as low concentration treated plant. Talebi *et al.* (2012) added, EMS-induced mutagenesis imposed significant impact on the seedling height of rice cv. MR219. The maximum reduction in seedling height was observed when rice MR219 was treated with a concentration of 0.25%.

For artificially induced mutations either with physical or chemical mutagens, LD_{50} is considered to be an ideal level to achieving high frequency of mutations (Anbarasan *et al.*, 2013). The LD_{50} of the mutagen is useful for determining an optimal dose for mutation induction. The LD_{50} was calculated using seed germination percentage at different doses of EMS. The best curve for soybean (cv. Gepak Kuning) is describe through a quadratic curve based on the results of the analysis using Curve Expert 1.4 (Fig 3).

This study showed that the LD_{50} of soybean (cv. Gepak Kuning) treated with EMS mutagen was found at a concentration of 0.63%, the LD_{20} value was at a concentration of 0.25% and the LD_{30} value was at 0.41%. The regression equation obtained is:

$$Y(x)= 86.87-80.22x-79.36x^2$$

Y(x) = Logarithm of the overall germination growth rate.
x = Concentration of EMS.
a, b, c = Regression parameters.

The LD_{50} differs for each type of plant depending on the stage of plant growth and development and the plant parts treated with EMS mutagen. The EMS concentration

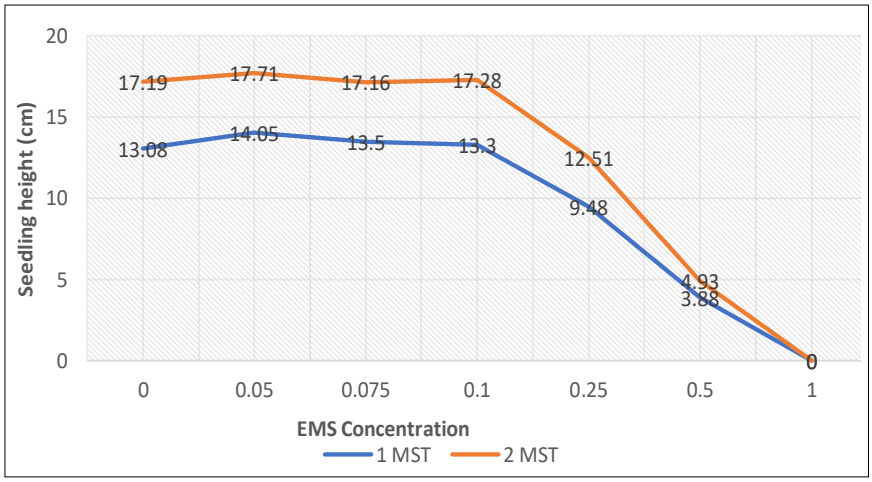

Fig 1: The seedling height of soybean (cv. Gepak Kuning) due to EMS treatment at 7th day and 14th day after planting.

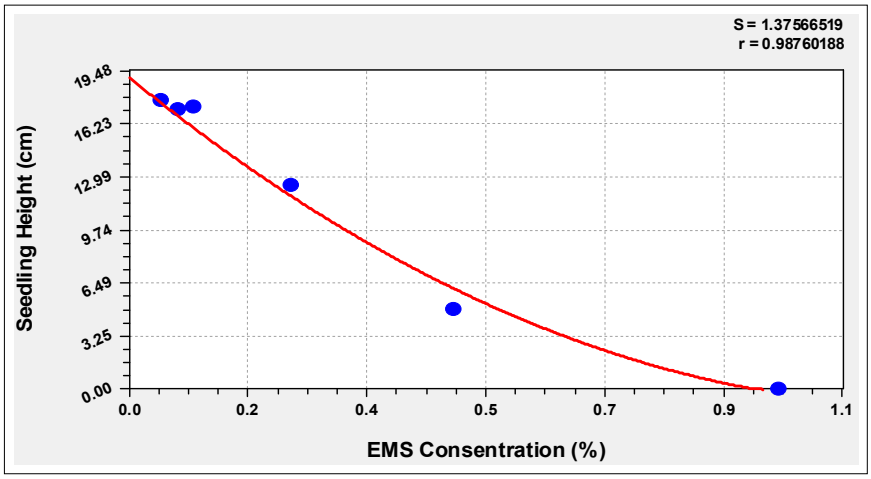

Fig 2: Graph of EMS concentration (%) on the seedling height (cm) of soybean (cv. Gepak Kuning).

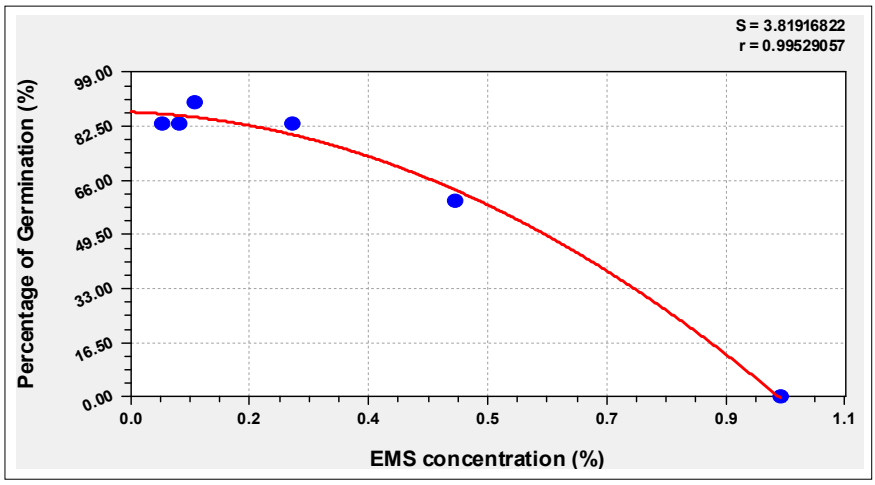

Fig 3: The effect ethyl methane sulphonate on percent germination and fitted straight lines to estimate the LD_{50} in soybean (cv. Gepak Kuning) that were treated with varying concentration of ethyl methane sulphonate.

range below LD_{50} was used to induce plant diversity and the desired character in future studies.

The LD_{50} dose range is useful for estimating the appropriate dose or concentration to induce mutations. The EMS mutagen treatment was carried out at a dose range of 50% with the consideration that the physiological damage was balanced by the genetic changes obtained. Selvaraj *et al.* (2014) stated, optimum dose is the dose that causes maximum mutation with minimum damage to the plant. Furthermore, Van Harten, (1998) added, the EMS concentration range used to induce variability was below the LD_{50} dose, ranging between LD_{20} and LD_{30}. In this study, we found that LD_{20} dose was obtained at a concentration of 0.25% and LD_{30} was found at a concentration of 0.41%.

Kavina *et al.* (2020) have reported the results of a study on the determination of the LD_{50} of the EMS mutagen in Fenugreek. Seeds without EMS treatment showed a germination percentage of 100%. Germination percentage decreased with increasing EMS concentration. EMS-treated Fenugreek showed reduced germination percentage at higher concentrations. The lowest germination percentage (2.1%) was found at a concentration of 1% at 7th days germination observation. Based on the germination data, the LD_{50} value of Fenugreek was obtained at a concentration of 0.4% EMS.

The LD_{50} value in the results of this study is almost the same as the results of previous studies. Arisha *et al.* (2015) studied LD_{50} some pepper cultivar and found that 0.6% (v/v) EMS was the concentration that produced about 50% lethality. Sharamo *et al.* (2021) also found a decreasing trend due to increasing doses and soaking time with EMS on barley. The LD_{50} value of 0.64% (v/v) EMS dosage was identified as an optimal dose for large-scale mutagenesis protocol to select barley mutants with high biomass yield.

The LD_{50} in the present study is much lower than LD_{50} of tepary bean. (Thangwana *et al.,* 2021) reported LD_{50} tepary bean of 3.37%, 2.68% and 2. 26% v/v EMS for genotype 3, 4 and 6, respectively. In addition, there were high coefficients of determination for each of the linear functions (>75%) suggesting that there was a notable

association between the reduction in seed germination and the concentration of the mutagen. These values are much higher than reported in the present study attributed to differences in genotypes and crop species used during mutagenesis.

The mutagenesis protocol will be useful to develop recessive and point mutations to aid selection of best individuals involving the M_2-M_5 mutant families with high yield production in soybean (cv. Gepak Kuning). The present study determined the optimum treatment condition for inducing genetic variation in soybean cv. Gepak Kuning. These results revealed that EMS dosage of 0.63% v/v of EMS can be used to increase genetic variability for key traits in soybean (cv. Gepak Kuning).

CONCLUSION

The increase in EMS concentration affected the percentage of germination and seedling height of soybean (cv. Gepak Kuning). Increasing the EMS concentration from 0.25% to 1% affected and reduced the seedling growth of soybean (cv. Gepak Kuning). The result of EMS mutagen sensitivity test on soybean (cv. Gepak Kuning) obtained an LD_{50} value at an EMS concentration of 0.63%.

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Conflict of interest

All authors declare that they have no conflict of interest.

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