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Conference Paper

Induction of EMS (*Ethyl Methanesulfonate*) Chemical Mutations on Hemp Seed Germination (*Boehmeria nivea* L.)

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ABSTRACT

A Hemp plants are plants that have not been much research and exploration. Propagation of flax plants can be carried out by propagation of rhizomes. Other propagation can use cuttings of rootstocks, middle stems, and shoots, although such propagation is not yet optimal and has a presentation of about 60% on its failure. Genetic diversity in hemp plants aims to increase germplasm. Genetic diversity in multiplying germplasm means to increase the collection of hemp plants. Environmental changes in plant populations can be overcome by genetic diversity. This study aims to identify the germination and success of EMS chemical mutations in the results of hemp plant seed mutations (*Boehmeria nivea* L.). Observations were made on several hemp seed accessions, namely Kumamoto (KM), Bandung A (BDA), Lembang A (LA), Indochina (IDC) and Seiki Selskin (SS). Observational measurements were carried out when the seeds were 7, 14, and 21 DATT with parameters of percentage of germinated seeds and percentage of mutation results. From the observations, the highest percentage was obtained in 2 accessions, namely Bandung A (BDA) and Lembang A (LA) with a percentage of 41.3%. The lowest percentage was found in Indochina's accession with a presentation of 29.3%. The percentage of inhibited germination was highest in EMS treatment of 0.25% with a soaking period of 6 hours. When compared again with treatment control which is almost close to the percentage of germination is at a soaking duration of 3 hours.

Keywords: Induction of mutations, Ethyl Methalsulfonate, germination, and Hemp Seeds

Introduction

The hemp plant is a plant that belongs to the category of plants that produce natural fibers. This plant with the Latin name Boehmeria nivea is usually used as a substitute material in the manufacture of textiles. Hemp plants do not only have potential as a fiber material, but hemp plants also have potential in other uses such as animal feed ingredients, raw materials for composting, and can be used as phytoremediation of soils polluted by heavy metals such as copper. The hemp plant is a plant that has not been carried out much research and exploration. Hemp plant propagation can be done by rhizome propagation (Lestari & Pratama, 2020).

Mutation breeding is a technique that aims to create changes that occur in the genetic material, both at the gene level and at the chromosomal level. Mutations are divided into two, namely natural mutations and artificial mutations. Natural mutations, also known as spontaneous mutations, are mutations that occur randomly in nature. These mutations occur without knowing the exact cause of their occurrence. Artificial mutations, namely mutations originating from external factors with events that are deliberately carried out or planned by humans to achieve a goal (Roini et al., 2021). Improving only a few properties

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by not changing most of the characteristics of the original plant is one of the advantages of mutation breeding and its implementation requires a relatively shorter time (Budi et al., 2019)

Ethyl Methanesulfonate (EMS) is a chemical-based mutagen that is often used in plant breeding techniques to expand genetic diversity. The EMS induction treatment in various species has been shown to be successful in increasing the genetic diversity of various species (Alfikri et al., 2022). Genetic diversity in hemp plants aims to increase germplasm. Therefore, plant breeding activities on hemp plants were carried out to support genetic diversity in hemp plants themselves. Genetic diversity in increasing germplasm means increasing the collection of hemp plants (Govindaraj et al., 2015).

Based on the background of this problem, it is necessary to conduct research to study the mutation induction of the chemical Ethyl Methanesulfonate (EMS) in hemp seed germination.

Material and Methods

The research was carried out from 21 December 2021 to 31 January at the Integrated Laboratory and Greenhouse Research Institute for Sweeteners and Fibers on Jalan Raya Karangploso, Kepuharjo, Kec. Karang Ploso, Malang Regency. This study used tools and materials in the form of hemp seeds 5 Accessions (Kumamoto (KM), Bandung A (BDA), Lembang A (LA), Indochina (IDC) and Seiki Selskin (SS)), petri dishes, straw paper, Ethyl chemicals Methanesulfonate (EMS), cuvert, tissue, filter, and measuring pipette. Data collection was carried out using the direct observation method from initial germination without chemicals to germination with mutation induction. Mutation treatment with 2 levels, namely the concentration of the EMS solution and the soaking time of the seeds. The concentrations used were 0.25%, 0.5%, 0.75% and 1.00% with a long immersion time of 3 and 6 hours. Observations were made when the hemp were 7, 14, and 21 DAT (Day After Transplanting) with the parameters of the percentage of germinated seeds and the percentage of mutations. Calculation of the percentage of germination with the formula as an example can be seen in Formula 1

$$p = \frac{\text{number of sprouts (JK)}}{\text{total number of seeds (JSB)}}\ x\ 100 \quad (1)$$

Where:

p : presentase(%)
JK : number of sprouts
JSB : total number of seeds

Results and Discussion

Percentage of germination power

The results of observing the percentage of germination in the induction of chemical mutations of Ethyl Methanesulfonate (EMS) can be seen in Table 1. The data is the average of the observed data from 15 plant samples at various ages of observation.

Table 1. Hemp seed germination percentage

Percentage of Germination At Various Ages of Observation (%)				
Accession Name	Test			Average
	I	II	III	
Kumamoto	36	32	26	31,33
Bandung A	42	36	46	35,33
Lembang A	46	28	50	41.33
Indochina	28	34	36	29,33
Seiki Selskin	34	38	34	41.33

Based on the results of tests that have been carried out at the Balittas Integrated Laboratory, the germination percentage results are obtained in Table 1. above. The method used in testing the

germination power is using a petri dish and straw paper. Observations were made for 14 results with the highest percentage found in 2 accessions namely Bandung A (BDA) and Lembang A (LA) with a percentage of 41.3%. The lowest percentage is in the accession of Indochina with a presentation of 29.3%.

Seed size has a significant influence on the growth and percentage of seed germination. Large and medium seed sizes provide better seed growth compared to small seed sizes. Seeds that are large and heaviest have a higher percentage of germination than seeds that are medium and light in size (Azahra & Suharto, 2022). Rami has a small and light seed size so the percentage of germination is relatively small.

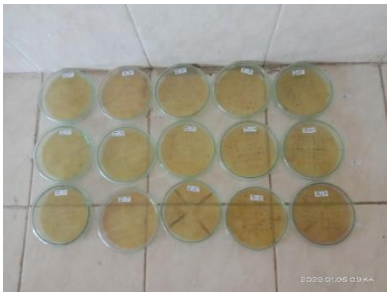

Figure 1. Maintenance of flax seeds

Percentage of germination results of mutations

Results of observation of the percentage of germination power mutation result in the induction of chemical mutations Ethyl Methanesulfonate (EMS) can be seen in Table 2. The data is the average of the observed data from 45 plant samples at various ages of observation.

Table 2. Hemp seed germination percentage

Percentage of Germination Results of Mutations in Various Treatments (%)									
Accession Name	Control	3 hours				6 hours			
		0.25	0.50	0.75	1.00	0.25	0.50	0.75	1.00
Kumamoto	2.88	2.64	0.96	1.20	2.40	0.48	0.72	0.48	1.68
Seiki Selskin	1.92	0.96	1.20	0.96	0.96	0.72	0.72	1.92	1.20
Lembang A	1.44	0.96	0.96	1.20	0.72	0.24	1.20	0.92	0.24
Bandung A	27,34	20,14	22,54	20,67	17,27	14,87	14,63	23,98	9,83
Indochina	23,98	15,83	19,90	21,58	22,30	10,07	14,87	20,14	14,39

The results obtained in the observations are based on Table 2. These include the Kumamoto (KM) accession. It was found that the highest percentage was in control or without treatment. While the EMS treatment experienced a low percentage. The lowest percentage was in the EMS treatment at 0.25% and 0.50% with a long immersion time of 6 hours. Bandung accession A (BDA) showed that the highest percentage was in control or without treatment and EMS treatment was 0.75% with 6 hours of immersion time. While the EMS treatment experienced a low percentage. The lowest percentage in the EMS treatment was in the 0.25% treatment with a long immersion time of 6 hours.

The mutagens that are often used in plant breeding are chemical mutagens and physical mutagens. The frequency and spectrum of mutations depend on the type of mutagen and the dose used (Budi et al., 2019). Gene mutations occur as a result of changes in the genes. Genes that change due to mutations are called mutants (Halimursyadah et al., 2022). In general, the mutation process can cause changes in the genetic properties of plants, both in a positive and negative direction and it is possible that the mutations that occur can return to normal (Putra & Purwani, 2017). The EMS treatment with a concentration of 1% and soaked for 6 hours was no different from the control treatment. The longer the seed soaking with

1% EMS concentration, the lower the plant height and the difference from the control (Suteja et al., 2019).

According to Suteja et al. (2019) stated that chromosomes damaged by mutagens and the relationship between damaged chromosomes and undamaged chromosomes have an important correlation with decreased growth. Germination speed slows down with decreased germination power and longer storage time due to decreased food reserves in seeds including water content as an ingredient in metabolic processes. Germination speed has a close relationship with germination value. The germination rate only shows the average germination days, while the germination value shows the number of seeds germinating in percent per day until the end of the test which is a reflection of the seed's growing power (Pangastuti et al, 2019).

(a) (b) (c)

Figure 2. Germination Observation of Hemp Result of Mutation
(a) 7 DAT, (b) 14 DAT, (c) 21 DAT

Figure 2. (a) shows the observation of germination of mutated hemp in the first week. The first week's observations showed that there were still many plants that had not grown, but some had grown with a small percentage. Figure 2. (b) shows the hemp germination observations resulting from mutations in the second week of observation. This second week of observation shows that the plants have started to grow a lot. Whereas in Figure 2. (c) the observations of hemp germination resulting from mutations in the third week of observation are presented. The third week of observation showed that the plants had grown almost 100%.

Conclusion

The results showed that the highest percentage was found in 2 accessions, namely Bandung A (BDA) and Lembang A (LA) with a percentage of 41.3%. The lowest percentage was found in the Indochina accession with a presentation of 29.3%. The highest percentage of stunted germination was in the 0.25% EMS treatment with 6 hours of soaking time. When compared again with the control treatment which was almost close to the percentage of germination, the soaking time was 3 hours.

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