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GENETIC MUTATION INDUCTION OF *Monstera adansonii* ON VARIOUS MUTAGENS BY DRIPS APPLICATION

Induksi Mutasi Genetik *Monstera adansonii* pada Berbagai Mutagen dengan Aplikasi Tetes

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ABSTRACT
Monstera adansonii *has wide leaves with a split pattern on its strands (split leaf), smooth and shiny to create a basic visualization (background filler) in flower arrangements. This study aims to obtain genetic changes using chemical mutagens. Chemical mutagens used were 250 ppm EMS, 400 and 800 ppm streptomycin, 400 and 800 ppm GA_3. The observations were performed after treatments on the parameter of amount of chlorophyll, stomata and leaf color changes. The results showed that both streptomycin treatments significantly reduced the plant height and the number of leaves. The application of streptomycin in high concentration (800 ppm) decreased the total amount of chlorophyll content in the leaves and changed the color as well as the shape of the Monstera leaves. All chemical mutagen treatments had no effect on stomatal density and stomatal area. The changes in leaf color and shape occurred in the high concentration of 800 ppm streptomycin treatment, could not detected by SSR marker.*

Keywords: *EMS, GA_3, genetics mutation,* Monstera adansonii*, streptomycin*

ABSTRAK
Monstera adansonii memiliki daun yang lebar dengan pola belahan pada helaian (*split leaf*), halus dan mengkilap dapat membuat visualisasi dasar (*background filler*) pada rangkaian bunga. Penelitian ini bertujuan untuk memperoleh perubahan genetik dengan berbagai mutagen kimia. Mutagen kimia digunakan yaitu EMS 250 ppm, streptomisin 400 dan 800 ppm, serta GA_3 400 dan 800 ppm. Pengamatan dilakukan setelah perlakuan, yaitu jumlah klorofil, stomata dan perubahan warna daun. Hasil penelitian menunjukkan bahwa perlakuan streptomycin secara nyata mengurangi tinggi tanaman dan jumlah daun. Sedangkan perlakuan streptomycin dengan konsentrasi tinggi (800 ppm) berhasil menurunkan jumlah klorofil total, serta merubah warna dan bentuk daun *Monstera*. Semua perlakuan mutagen kimia tidak berpengaruh terhadap kerapatan stomata dan luas bukaan stomata. Perubahan warna dan bentuk daun yang terjadi pada perlakuan streptomisin 800 ppm, tidak dapat dideteksi dengan marka SSR.

Kata Kunci: EMS, GA_3, *Monstera adansonii*, mutasi genetik, streptomycin

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INTRODUCTION

Indonesia is a country with the second highest biodiversity in the world after Brazil (von Rintelen et al. 2017). Indonesia has natural resources with diverse flora and fauna throughout the archipelago. This diversity should be protected and preserved so that it can be utilized and become an economic value by the community in the future. Indonesia has more than 134,000 species of biodiversity (KLHK 2014). Ornamental plants are plants that have aesthetic value in terms of shape, leaf color, crown and flowers, often used to decorate the yard, public garden, municipal park and so on. Ornamental plants is beneficial as a source of income for farmers and traders, as well as expanding job opportunities.

Ornamental plant commodities are the commodities that have been hardest hit due to the COVID-19 pandemic. The decline in commodity production in 2020 was very significant when compared to 2019 reaching 25%. This is due to the declining demand for ornamental plants from major events in the community such as weddings, annual exhibitions, and various routine festivals as well as decreased demand from the tourism sector, especially hotel and accommodation, which has been hardest hit by the COVID-19 pandemic (Dirjen Hortikultura 2021). The stagnation of the domestic market was cured by more hobbyists involved in foliage ornamental plant business. Some types of ornamental leaf plants increase their prices due to increased trade among hobbyists. Moreover, the increase in ornamental plant exports was caused by the sluggish domestic market which had an impact on shifting their marketing to the export market. The export value in 2019 was US$13.53 million and increased significantly to US$19.98 million in 2020.

Foliage ornamental plants are plants that have attractive leaf shapes, colors, and structures. These ornamental plants are favored because of beautiful shape and color attracts attention, with the smoothness, firmness and the compactness of leaf composition and good for adding to beauty yard environment as well as for room decoration (Siregar et al. 2018). One kind of foliage ornamental plant that is currently favored in great demand because it has its own aesthetic value is *Monstera*. Indonesia's tropical climate conditions are very suitable for the growth of Monstera plants which have the potential to multiply the types of Monstera plants to prevent extinction. *Monstera* is belong to the Araceae family which has approximately 25 species (de Andrade et al. 2013, Mayo and Andrade 2013). One type of Monstera that is in great demand and sought after by the public is *Monstera adansonii*. *M. adansonii* has wide leaves with a split pattern on the strands (splitleaf), smooth and shiny leaf surface, which can create a background visualization (background filler) in flower arrangements (Mufida 2020). *M. adansonii* is popular because of the beauty and uniqueness of its leaves, and native to Central and South America.

The variegation in Monstera is an important trait which currently becomes popular, because of their rarity and high price. Variegated *Monstera* showed unique patterns of more than one color, such as white or even albino, yellow and sometimes lighter shade of green. According to Marcotrigiano (1997), variegation generated by differential expression of genes, chimeras, leaf blister or virus that resulted in differences composition of the green pigment chlorophyll, as well as anthocyanin and carotenoids. Plant variegation can be induced by artificial mutations treatment, one of which is by chemical induction using chemical mutagens such as ethyl methane sulfonate (EMS), streptomycin, gibberellins, cytokinin and light intensity (Kim et al. 2012, Sandra 2019, Di Benedetto 2020). Mutation induction is an alternative to obtain new variants in *Monstera* plants in a relatively shorter times. Mutations can cause changes in color, motif, and leaf shape (Handayati 2013).

Many mutagenesis techniques are available, including physical (gamma, radiation, X-rays, fast neutrons) and chemical treatment (Gallone et al. 2012, Minisi et al. 2013, Dhakshanamoorthy et al. 2015, Zakir 2018). Currently there are no reports of mutations on *M. deliciosa* and *M. adansonii*.

This study aimed to increase the diversity of *M. adansonii* plants through the chemical mutagens EMS, streptomycin and gibberellins (GA_3). The application of chemical mutagens is expected to have a significant effect on *M. adansonii* plants.

MATERIALS AND METHODS

Location and time

This research was conducted during January - June 2021. The application of mutaion and analysis were accomplished in the Biotechnology and Biosciences Laboratory at Department of Agrotechnology, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi.

Materials

The tools used in this study were 1000 mL beaker, 100 mL measuring cup, 1000 mL Erlenmeyer flask, scales, micropipette, ruler, flower pot, 10 mL dropper, knife, scissors, 2b pencil, and notebook and the materials used in this study were the mother plant of Monstera, 70% alcohol, aquades, tissue, masks, gloves, label paper, EMS chemicals, gibberellins (GA_3), and streptomycin.

Experimental design

This study was arranged in a randomized group design, the treatments were p0: control, p1: 250 ppm EMS, p2: 400 ppm streptomycin, p3: 800 ppm streptomycin, p4: 400 ppm GA_3, p5: 800 ppm GA_3 (Figure 1). There were 6 treatments and repeated four times, each treatment consisted of three plant pots as experimental units and the result that there were 72 experimental units in total. Each pot is planted with 1 plant. The data that obtained from the observations and measurements were analyzed using statistical analysis of variance and if the results showed a significant effect, then further contrast tests will be carried out.

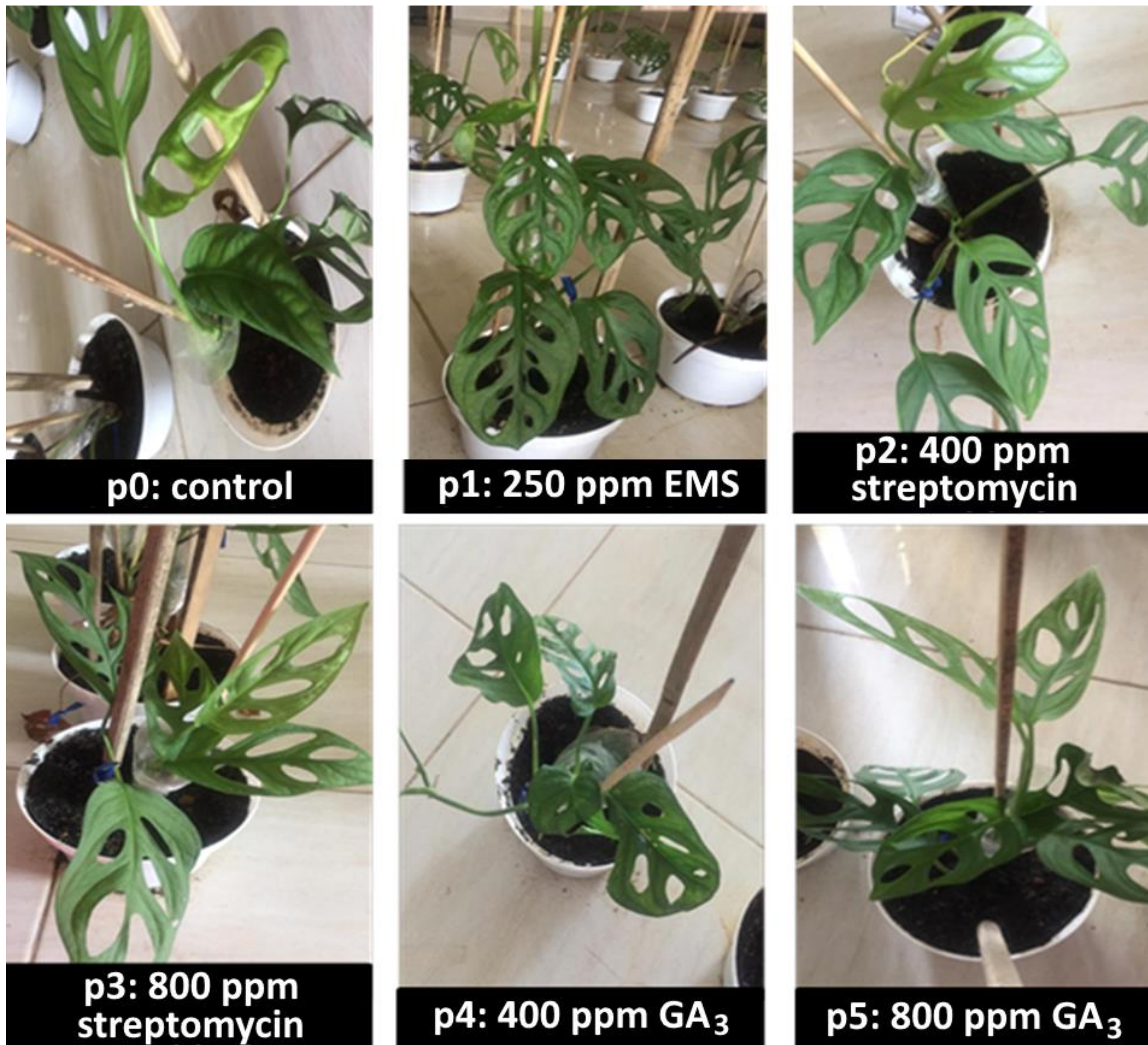

Figure 1. The treatments application on Monstera plants

Tabel 1. SSR Primer sequences and annealing temperature optimization

Locus	Primer forward and reverse	TA (ºC)
Primer 1	TGG GGA TAG AGG GAC TTG AA	54.8 ºC
	TTG CAT TGC GTT GGT AGC	54.1 ºC
Primer 2	TTG GGG ATA GAG GGA CTT GA	54.8 ºC
	GCA TTG CGT TGG TAG CTG	54.7 ºC
Primer 3	TGG GGA TAG AGG GAC TTG AA	54.8 ºC
	GCA TTG CGT TGG TAG CTG	54.7 ºC
Primer 4	TTG GGG ATA GAG GGA CTT GA	54.8 ºC
	TTG CGT TGG TAG CTG GAA	54.5 ºC

Implementation

Growing media that used are manure, husk, and soil. Preparation of growing media is done by mixing manure, husk, and soil in a ratio of 1: 1: 1. Plant propagation is done by stem cuttings. Cut every stems that have hanging roots using a knife that has been sterilized with alcohol. The application of fertilizer was carried out one week before the experimental period, the fertilizer used in this experiment was NPK Mutiara (16: 16: 16) fertilizer. Prepare chemical mutagens with concentrations of EMS 250 ppm, streptomycin 400 and 800 ppm, gibberellins 400 and 800 ppm. The application of chemical mutagens was done by dripping on the growing point of the plant as much as 4 mL pot-1 which was carried out 2 times a day until the end of the experiment.

DNA isolation

DNA was isolated from the mutated leaves tissue that changed in color and shape. Cut of 0,05 g of fresh or frozen plant tissue, and freeze the sample with liquid nitrogen, following by grinding the sample to a fine power then transfer it to a 1.5 ml microcentrifuge tube. After that add 400 µL of GP1 Buffer and 5 µL of RNase A into the sample tube and mix by vortex. Incubate at 60 ºC for 10 minutes. During incubation, invert the tube every 5 minutes. At this time, pre-heat the required elution buffer, 200 µL per sample, to 60 ºC (for step DNA Elution). Add 100 µL of GP2 buffer and mix by vortex, and then incubate on ice for 3 minutes. Place a filter column in a 2 mL collection tube, then transfer the micture to the filter column, After centrifugation for 1 minute at 1.000 ×g then discard the filter column. Carefully transfer the supernatant from 2 mL collection tube to a new 1,5 mL microcentrifuge tube. Add a 1,5 volume of GP_3 buffer (make sure isopropanol was added) then vortex immediately for 5 seconds, add 750 µL of GP_3 buffer to 500 µL of lysate.

GD Column was placed in a 2 mL collection tube. Then transferring 700 µL of mixture (and any remaining precipitate) to the GD column, followed with centrifugation at 14–16.000 ×g for 2 minutes. After discarding the flow-through then place the GD column back in the 2 mL collection tube. The remaining mixture was added into the GD column then centrifuge at 14–16.000 ×g for 2 minutes. Discard the flow-through then place the GD column back in the 2 mL collection tube. W1 buffer of 400 µL was added to the GD column then centrifuge at 14–16.000 ×g for 30 seconds. Discard the flow-through then place the GD column back in the 2 mL collection tube. Washing buffer of 600 µL was added (make sure ethanol was added) to the GD column. Centrifuge at 14–16.000 ×g for 30 seconds. The flow-through was discarding, then place the GD column back in the 2 mL collection tube. Finally, centrifugation for 3 minutes at 14-16.000 ×g was carried out to dry the column matrix.

Residual pigment removal step

When the pigment remain on the column, this optional step should be performed: after washing buffer addition, absolute ethanol of 400 µL was added to the GD column. Centrifugation was performed at 14–16.000 ×g for 30 sec. The flow-through was then discarded and placed the GD column back in the 2 mL collection tube, followed by centrifugation for 3 minutes at 14–16.000 ×g to dry the column matrix. The dried GD column then tranferred to a clean 1.5 mL microcentrifuge tube, and pre-heated elution buffer or TE of 100 µL was added to the

center of the column filter. After keeping for 3–5 minutes to ensure the TE is completely absorbed, followed by centrifugation at 14–16.000 ×g for 30 seconds to elute the purified DNA.

Primer design
LEFT PRIMER 169 18 58.97 55.56 5.00 2.00 GCATTGCGTTGGTAGCTG
RIGHT PRIMER 566 20 59.48 50.00 2.00 1.00 TTGGGGATAGAGGGACTTGA
PRODUCT SIZE: 398, PAIR ANY COMPL: 2.00, PAIR 3' COMPL: 0.00

SSR amplification

The amplification reaction was performed according to the protocol described by Santoso et al. (2016). Use 20 ng of template DNA, 20 ng template DNA, 1× KAPA2G Fast Ready Mix (Biosystem), 0.25 M universal sequence-coupled forward primer (M13), 0.5 μM reverse primer, 0, 5 μM fluorescent label, 20 ng template DNA, 3 L reaction mixture% DMSO and ddH_2O. The primers detailed were shown on Table 1.

Observation parameters

The increase plant height (cm), it measured before treatment and after treatment which was carried out once every two weeks for two month. Using a ruler meter, it was measured from the base of the stem to the tip of the growing point of the plant. Observations on the number of variegata plants were calculated by observing the color changes of the leaves on the plants. Observations were made at the end of the experiment. The observations on the number of non variegated plants that were calculated by observing the leaves of plants that which has no color change, observations were made at the end of the experiment. Stomata area (mm2) = π × ½ length × ½ width of stomata area. Observation of leaf chlorophyll components was observed using Content Chlorophyll Meter (CCM 200+) on young leaves, mature leaves and old leaves.

RESULTS AND DISCUSSION

Plant height and leaves number

Analysis of variance showed that chemical mutagen treatment had a significant effect on plant height and number of leaves of *M. adansonii* at 8 weeks after treatment. The streptomycin treatments, p2 and p3 (Figure 2) significantly reduced the plant height (16.40

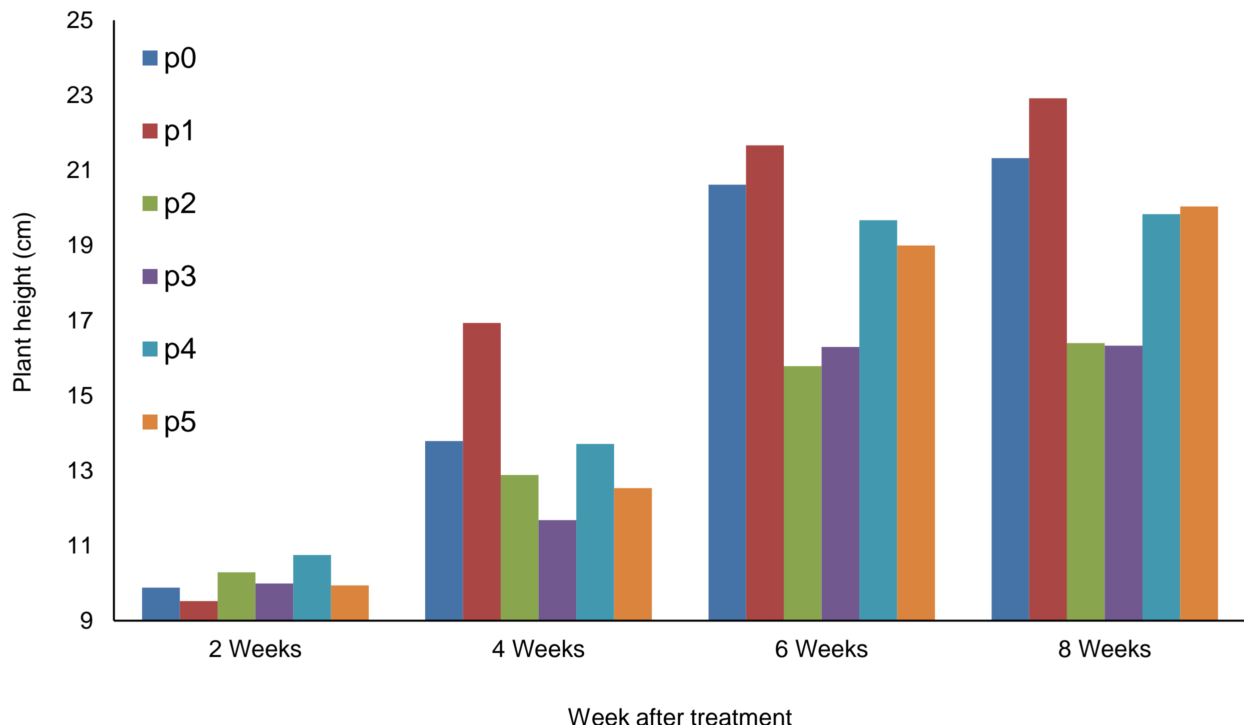

Figure 2. Plant height (cm) increase from various treatments up to 8 weeks (p0: control; p1: 250 ppm EMS; p2: 400 ppm streptomycin; p3: 800 ppm streptomycin; p4: 400 ppm GA_3; p5: 800 ppm GA_3)

and 16.33 cm, respectively) compared to other treatments. This is presumably because streptomycin is strong mutagen compared to other chemical mutagens for *Monstera*. In line with the plant height, the lowest numbers of leaves were obtained in the streptomycin treatments, p2 and p3 (Table 2). The reduction of height and leaves number might be associated with the mutation that stimulated by the mutagens. Potapova and Gorbsky (2017) claimed that cell division was inhibited due to the mutation or chromosomes multiplication. In these conditions, intensifying the level of difficulty in the chromosomes pairing process (Syukur et al. 2019).

The treatments of GA_3 slightly reduce the plant height compared to control and p1 treatment (Figure 2). The application of EMS (p1) had no effect on the plant height reduction. On the contrary, it increases the plant height. This is in accordance with the result of Baghery and Kazemitabar (2014) who reported that the application of EMS by soaking okra seeds for 18 hours at a concentration of 0.515% can improve agronomic characters such as plant height and stem thickness.

Table 2. Number of leaves

Treatment	2 MST	4 MST	6 MST	8 MST
p0	4.08	4.75	5.38	5.79
p1	3.92	4.83	6.29	6.79
p2	4.33	4.75	5.08	5.42*
p3	3.58	4.00	4.50	5.33*
p4	3.67	3.92	6.42	7.08
p5	3.58	4.33	5.08	5.58*

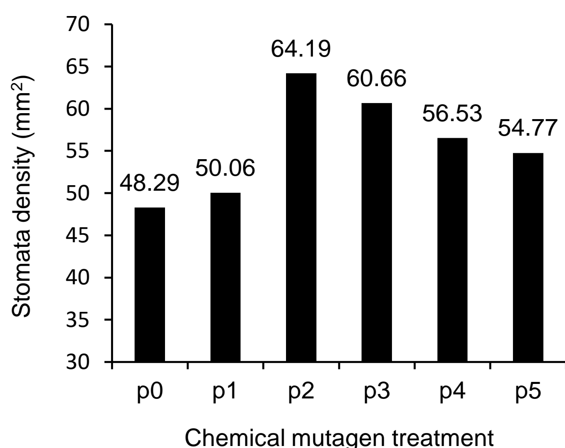

Figure 3. Average stomatal density (mm^2) in the control treatment and various chemical mutagens (p0: control; p1: 250 ppm EMS; p2: 400 ppm streptomycin; p3: 800 ppm streptomycin; p4: 400 ppm GA_3; p5: 800 ppm GA_3)

In Table 2, it shows that GA_3 treatment at low concentration (400 ppm) was likely to increase the leaves number (7.08). This is presumably because gibberellins can stimulate cell elongation and accelerate cell division. This is supported by Coelho et al. (2018) that gibberellin stimulates an increase in plant height and leaves number due to the addition of hormone content around shoot meristem cells. However, in high concentration (800 ppm), gibberellin tended to decrease the number of leaves of *Monstera*. The same phenomenon was observed on the fruit tree, *Spondias tuberosa* (Matos et al. 2020). The use of gibberellin accelerated the vegetative growth of *S. tuberosa* plant, including the leaves number, up to the concentration of 415 ppm. Above this concentration, it reduced the leaves number, as well as other parameter of the plant growth.

Stomatal density and stomatal area

Increasing stomata in the leaf has advantage that more carbon dioxides can be taken up, and the more water can be released. So, higher stomatal density can improve the potential of carbon dioxides uptake and reducing the rate of water loss. The results of variance showed that chemical mutagen treatments had no significant effect on stomatal density, as well as stomatal area. Figure 3 showed that the streptomycin treatments increased the stomatal density upto 64.2 mm^2, compared to the control plant. These phenomena are coincided with Yasmeen et al. (2020) results on sugarcane. They found that stomatal density increased at lower concentration, whereas, it reduced at higher concentration. It seems that the concentration of mutagens in our treatments on *Monstera* plant were not high enough to affect the stomatal density in the leaves. On the other hand, Yan et al. (2021) stated that the mutated poplar (*Populus euroamericana*) had characteristics of larger in the leaf area, with the wider size of stomata and more number of chloroplasts, however, the lower stomatal area on leaf surfaces.

Total chlorophyll content

The results of variance showed that the treatment of EMS, GA_3, Streptomycin had no significant effect on the total chlorophyll content. In Figure 4, GA_3 400 ppm (p4) showed the highest total chlorophyll (294.0 μmol.m^{-2}) and streptomycin 800 ppm (p3) showed the lowest total chlorophyll content (224.6 μmol.m^{-2}). These results indicated that the application of streptomycin in high concentration reduced the total amount of chlorophyll in the leaves. It seems that a raise in the concentration of streptomycin up to 800 ppm damaged the chloroplast organelles causing them to reduce the chlorophyll content. These findings are corresponding to Cunha Neto et al. (2020) results on the legume. Adding the GA_3 at the concentration of 400 and 800 ppm resulted in a higher average of chlorophyll content index compared to streptomycin, though not widely different.

EMS is one of the chemical mutagens used for inducing the mutations in plants. This is in line with Kangarasu (2014) that EMS chemical mutagens can cause point mutations, because they are alkaline so they can cause changes in nitrogen base pairs. GA_3 plays a role in cell division and stimulates plant growth. In addition, GA_3 also stimulates the production of auxin and cytokinin hormones that function in the elongation process of plant roots. This is in line with the research of Yasmin et al. (2014) that the application of GA_3 concentrations can stimulate plant growth, resulting in an increase in plant height and leaf area. Streptomycin is an aminoglycoside antibiotic, obtained from *Streptomyces griseus* and other *Streptomyces* sp. Streptomycin is used to control diseases caused by bacteria and fungi in certain fruits, vegetables, grains, and ornamental plants. The mechanism of action of streptomycin is to inhibit the process of protein synthesis (Demirci et al. 2013).

Variegated plants

Based on the morphological observation, the high concentration of streptomycin treatment showed the change in the color and shape of the *Monstera* leaves (Figure 5 and 6).

In Figure 5, the treatment of 800 ppm streptomycin showed alteration in leaf color and shape. In contrast, the other mutagens treatment did not change in leaf color and shape, the same as control leaves. It is assumed that high concentration of

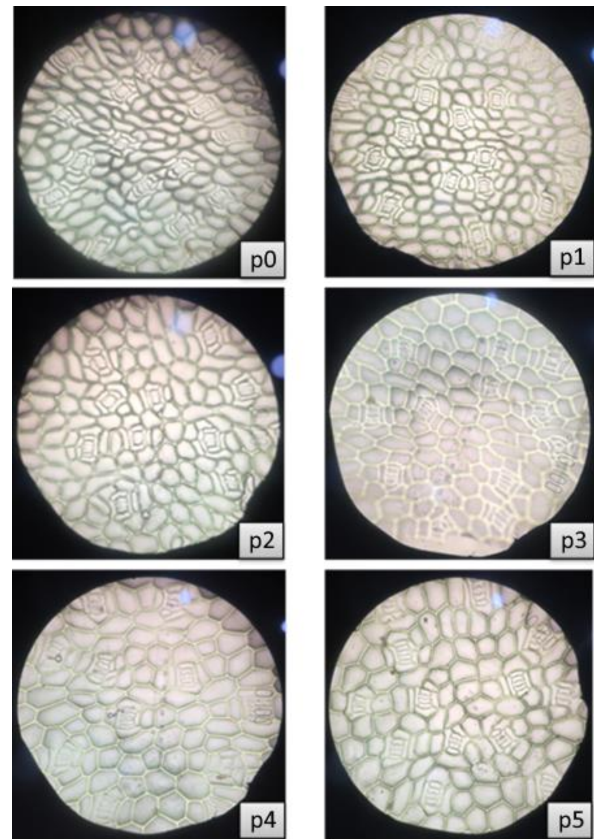

Figure 3. Visualizations of stomatal density under light microscope on various treatments with magnification 40× (p0: control; p1: 250 ppm EMS; p2: 400 ppm streptomycin; p3: 800 ppm streptomycin; p4: 400 ppm GA_3; p5: 800 ppm GA_3)

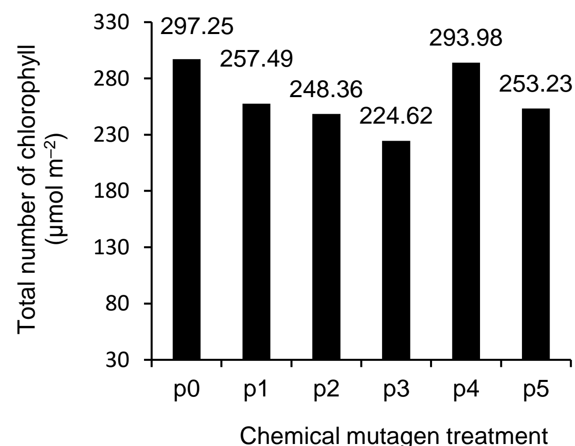

Figure 4. Average total chlorophyll (μmol m^{-2}) in the control treatment and various chemical mutagens (p0: control; p1: 250 ppm EMS; p2: 400 ppm streptomycin; p3: 800 ppm streptomycin; p4: 400 ppm GA_3; p5: 800 ppm GA_3)

streptomycin as an antibiotic protein synthesis resistor, hindering the protein synthesis process resulting in S deficiency in plants. It can be seen that there was a decrease in chlorophyll content in the leaves. Pandey et al. (2014) indicated that to increase the protein content in plants, the addition of S element is needed.

It showed that streptomycin was more effective compared to EMS and GA_3 in

Figure 5. Morphological observation on Monstera leaves, 2 weeks after treatment (p0: control; p1: 250 ppm EMS; p2: 400 ppm streptomycin; p3: 800 ppm streptomycin; p4: 400 ppm GA_3; p5: 800 ppm GA_3)

Figure 6. The treatment of high concentration streptomycin showing change in color and shape of the leaves. Note: left, 2 weeks after treatment; right, 3 weeks after treatment

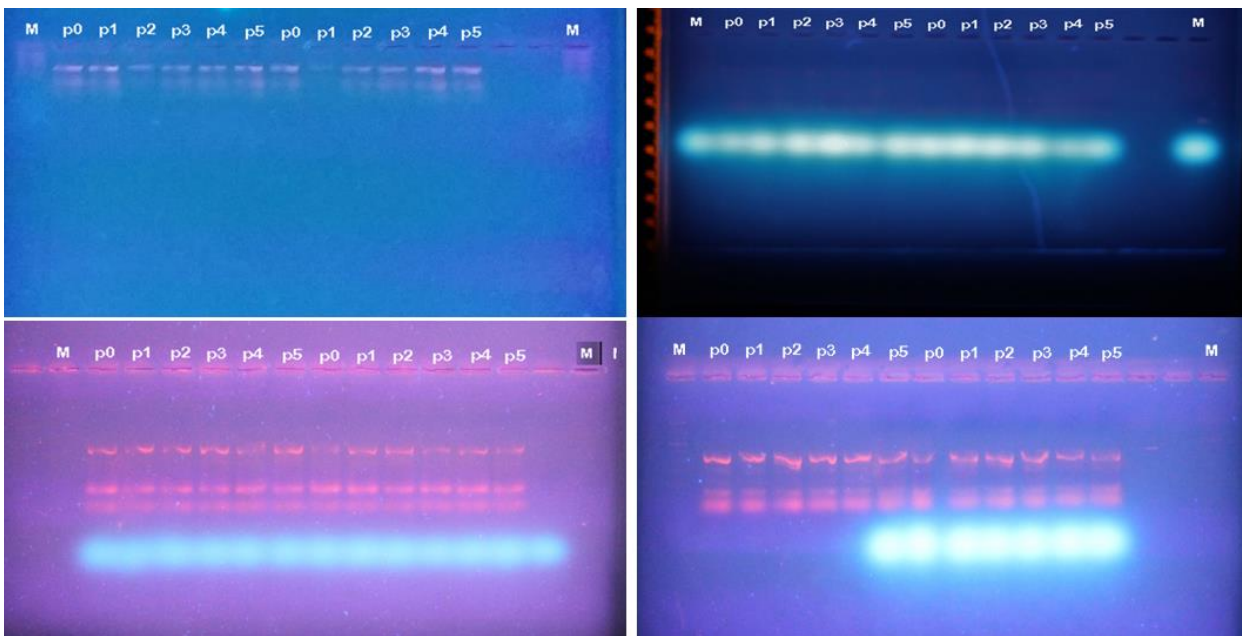

Figure 7. Band pattern resulted from SSR marker assessment on gel electrophoresis of all DNA samples of *M. adansonii* using 4 primers (p0: control; p1: 250 ppm EMS; p2: 400 ppm streptomycin; p3: 800 ppm streptomycin; p4: 400 ppm GA_3; p5: 800 ppm GA_3)

generating mutation on *Monstera*. It is believed that the method of dripping the mutagens solution on the growing point of the plant directly impacts the mutagens entering into plant cells through the mechanism of absorption in the mitotic phase of the cell cycle. According to Syukur et al. (2019) the most effective application of mutagen is at the metaphase stage of mitotic cells which affected the mutation in plant.

DNA analysis on the mutated leaves

For identification and confirmation of induced mutation, SSR markers were assessed by four primers of *M. adansonii* using DNA from 6 plants, which were the control plant, and 5 plants treated with mutagen. Figure 7 displays the electrophoresis results of SSR marker assessment. Unfortunately, the bands pattern did not show any polymorphism on all DNA samples tested. The reasons of this phenomenon might be because the point mutations were not integrated in the DNA sequences during isolation, even though it was obtained from the mutated tissue. Moreover, the primers used in our study might not anneal to the sequences mutated. It needs more primers to cover the sequences nearby or around the mutation areas. In their experiment, Asadi et al. (2020) confirmed the mutant lines of soybean induced by gamma ray using the SSR markers.

Variations in the genetic material of a species will lead to genetic diversity, thereby affecting the biochemical, physiological and morphological differences of the population (Govindaraj et al. 2015, Muraille 2018). A series of evolutionary processes affected by migration, geographic isolation, and natural selection, as well as induced mutation, help increase the genetic variation of population (Hassan et al. 2014). Most mutagens are physical, chemical or biological materials that are highly penetrating and could reach the genetic material in the nucleus (Oladosu et al. 2016, Mullins et al. 2021).

CONCLUSION

The streptomycin treatments of 400 and 800 ppm, reduced significantly the plant height to 16.40 and 16.33 cm, as well as the leaves number down to 5.42 and 5.33. All chemical mutagen treatments had no significant effect on stomatal density in the leaves of *Monstera*. The application of streptomycin in high concentration (800 ppm) reduced the total amount of chlorophyll in the leaves and also changes in the color and shape of the *Monstera* leaves. However DNA analysis results did not confirm these changes as polymorphic bands on the electrophoresis gel using SSR markers. More primers are recommended to assess the induced mutation of *Monstera* leaves resulted from a high streptomycin treatment.

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