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Induction of Variegata Red Majesty Using EMS Mutagen and Gibberellins in Vitro

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Abstract

This study aims to determine how the interaction occurs between the best concentration of EMS with gibberellin in inducing aglaonema variegata and regeneration of aglaonema Red Majesty in vitro. The research was conducted at the Tissue Culture Laboratory of the Faculty of Agriculture, Hasanuddin University and took place from January to June 2022. The study used the method of Separate Plots Design in a Completely Randomized Design as the environmental design. The results showed that the best mutant genetic diversity and in vitro regeneration at 0.3 percent EMS concentration with 15 ppm gibberellin (variegata), and 45 ppm (chimera) and 0.6 percent EMS concentration with 0 ppm gibberellin (variegata) had different characters compared to the control treatment and other treatments

Keywords: Red Majesty, EMS, gibberellins, in vitro, variegata

A. Introduction

Aglaonema is a plant that has an appeal, especially in the beauty of its leaves and is used as an ornamental plant. Aglaonema has around 30 species which have different leaf patterns and colors for each species (Akbar M.R., B.S. Purwoko, I.S. Dewi, W.B. Suwarno, Sugiyanta & M.F. Anshori, 2021). This ornamental plant nationally only has a production of 816,468 trees, while the demand for both export and domestic markets is very large (Indonesian Ministry of Agriculture, 2019). Aglaonema that is highly valued in the market and is on the rise is a mutated Aglaonema such as variegata whose price can reach IDR 1.5 million per leaf (Lakamisi, 2010). According to Kaviani B., Sedaghathoor, S., Motlagh, M. & Rouhi, S. research (2018), the more unique the color of the aglaonema leaves, the higher the demand and selling price. However, the obstacle to obtaining

naturally occurring mutations and variegata aglaonema is very rare because the normal frequency and mutation ratio is between 1:1,000,000.

Artificial mutations are a solution to increase the prospective value of aglaonema. Artificial mutation is a change that occurs in a gene due to the administration of a mutagen (Suteja H.N., N. Rostini. & S. Amien , 2019). Artificial mutations can be done through the multiplication of tissue culture (in vitro). Tissue Culture is a technique that is used to propagate a plant usinbpg isolated plant parts (explants) in an optimal, controlled and aseptic environment in glass bottles (Ziraluo and Piter, 2021). Mutation induction through tissue culture propagation is considered very effective in accelerating the desired in vitro selection and increasing plant diversity in a short time commercially without changing the characteristics of the original cultivar (Maluszynski M., Ahloowalia, B.S. & Sigurbjörnsson, B. 1995).

The most widely used chemical mutagen in plants is the chemical compound Ethyl Methane Sulfonate (EMS) because it has been shown to be effective in causing changes in N bases in DNA or RNA or point mutations so as to produce wide genetic diversity (Wijiono, 2016). This is supported by the research of Putra and Purwani (2017), which states that the use of the EMS mutagen can cause mutants by producing high genetic diversity so that they are used in making mutant plants. In an effort to maximize the formation of mutations, the addition of gibberellic acid (GA3) is useful for in vitro shoot regeneration, growth, biomass and fiber elongation in xylem and is able to play a role in replacing auxin (Chakraborty D., Mandal & Datta, S.K., 2000). The successful use of the gibberellin mutagen in inducing variegata plants is also supported by Dewi's research (2015) which states that the gibberellin hormone can increase the length of epidermal stem cells, plant height, density of stomata on the underside of leaves, and total chlorophyll content.

To obtain optimal results, it is necessary to have a combination of the two mutagen compounds combined with tissue culture techniques (in vitro). This is based on Yoosumran's research (2018) using EMS with several concentrations could produce some different characteristic mutations on Dendranthemum grandiflora plants in vitro and Rajagukguk, S., Dwiyani, R. & Astawa (2018), which used GA3 with a concentration of 20 ppm on grape shoots in vitro capable of having the highest effect on the percentage of sprouting and leafy.

B. Methodology

The research was conducted at the Tissue Culture Laboratory, Hasanuddin University, Makassar, South Sulawesi, Faculty of Agriculture, Hasanuddin University. The study was conducted using a Split Plot Design with Ethyl Methane Sulfonate (EMS) as the main plot and gibberellin (GA3) as a subplot in a completely randomized design (CRD). The EMS levels used were 0% (e0), 0.3% (e1), 0.6% (e2) 0.9% (e3). While the Gibberellin treatment used 4 levels, namely 0 ppm (g0), 15 ppm (g1), 30 ppm (g2), 45 ppm (g3). Thus the treatment consisted of 16 treatment combinations with each treatment being repeated 3 times and each repetition using 3 bottles of culture so that there were 144 experimental units

1. Research procedures

Preparation of control media (without gibberellin) begins by dissolving 30 gram/L sucrose into 500 ml of water, adding MS according to the dosage for 1000 ml solution, 1.5 mg/L IBA, 2 mg/L BAP, and making it up to 1000 ml by adding distilled water . To make the treatment media almost the same as the control media, the difference is only given the addition of Gibberellin (GA3) according to the predetermined concentration and sufficient media to reach 1000 ml by adding distilled water. Then adjust the pH to 5.8 by adding NaOH or HCl. All media added 2 ppm BAP and 1 ppm IBA

The soaking process using EMS was carried out in a sterile Laminar Air Flow. Soaking was carried out for 1 hour 45 minutes, by immersing the shoots in EMS solutions of various concentrations. Planting is done by sticking the explants into the media so that there is perfect contact between the two. Each media that has been planted is stored in the incubation room for 2-3 months. The explants used were from aglaonema Red Majesty plantlets which had been soaked by EMS.

2. Data Analysis

Data analysis used analysis of variance (ANOVA) and continued with the Least Significant Difference Test (LSD) 5% (0.05) using STAR Software to determine the relationship between characters and the amount of genetic diversity formed.

C. Result and Discussion

Plants treated with 0.3% EMS concentration and 15 ppm Gibberellin produced leaves with dominant white spots (variegata) (table 1 and Fig. 1). Plants treated with 0.3% EMS concentration with 45 ppm Gibberellins produced leaves with a dominant dark green color and a few yellow spots (chimera). The treatment of 0% EMS with 0 ppm Gibberellins produced plants with good growth and normal leaf length and width. While the EMS immersion treatment of 0.3%, 0.6% and 0.9% with 0 ppm Gibberellin resulted in variations in color, leaf shape and leaf width which were smaller than the control, the higher the EMS concentration resulted, the leaf length and leaf width became smaller and the growth was slower. slow. In Haswin's opinion (2021), stated that treatment with mutagens at high concentrations resulted in an effect or change on the morphology of the plant. Moreover, using EMS in high concentrations can reduce plant survival rates (Nasri, F., Zakizadeh, H., Vafaee, Y. & Mozafari, A.A., 2021). This is because EMS is a compound that can be toxic so that it can inhibit growth in plants by inhibiting plant chlorophyll production.

Treatment of 0% EMS concentration with 15 ppm, 30 ppm and 45 ppm Gibberellins, produced plants with good growth and larger length and width of leaves than the control. The higher the concentration of Gibberellin, the greater the plant height and leaf area. This is supported by the results of Purwoko B.S., Sulistiyani, D.S. & Gunawan, L.W. (1997), exogenous addition of Gibberellins (GA3) to anthurium plants at concentrations (10, 20, and 30 mg) can cause an increase in leaf area. While the EMS treatment of 0.3% and 0.6% with 15 ppm, 30 ppm and 45 ppm Gibberellins produced variations in leaf color, leaf shape and leaf width which were different from the control. The higher EMS concentration resulted in shorter plant height and at a dose of 0.9% at 0 ppm, 30 ppm and 45 ppm Gibberellins, resulted in browning of leaves and stems until there was death. According to Bhagwat and Duncan (1998), in general, the frequency of mutases can increase with an increase in the concentration of the given mutagen. This is supported by Harten (1998), low concentrations can stimulate changes in plant physiology. In addition, the results of Qosim's research (2012) suggested that EMS treatment was able to encourage cell division in plants, whereas higher EMS concentrations had the potential to cause cell death in plants.

Table 1. Comparison of Aglaonema Red Majesty Plants Control with Treatment on Scoring Evaluation

Treatment	Character			
	Leaf Shape	Leaf Edge	Leaf Color	Dominant Spot Color
E0G0	Oval	Pointed	Pink	Light Green
E0G1	Oval	Pointed	Pink	Dark Green
E0G2	Ellipse	Pointed	Pink	Dark Green
E0G3	Scroll Ellipse*	Pointed	Dark Green*	Light Green
E1G0	Ellipse	Blunt*	Green*	Light Green
E1G1	Ellipse *	Blunt *	Light Green*	Pink and White *
E1G2	Ellipse*	Blunt *	Indian Red*	Light Green*
E1G3	Ellipse*	Blunt *	Dark Green*	Yellow*
E2G0	Oval	Pointed	Pink	Green
E2G1	Ellipse*	Pointed	Dark Green*	Broken White*
E2G2	Ellipse*	Pointed	Green*	Pink and White*
E2G3	Scroll Ellipse*	Tapering*	Green*	Yellowish Green*
E3G0	Ellipse*	Pointed	Broken White*	Pink*
E3G1	Oval	Pointed	Green*	Brownish Yellow*
E3G2	Ellipse*	Blunt*	Pink	Dark Green
E3G3	Oval	Pointed	Reddish Brown*	Light Green

Note: *= In contract to control

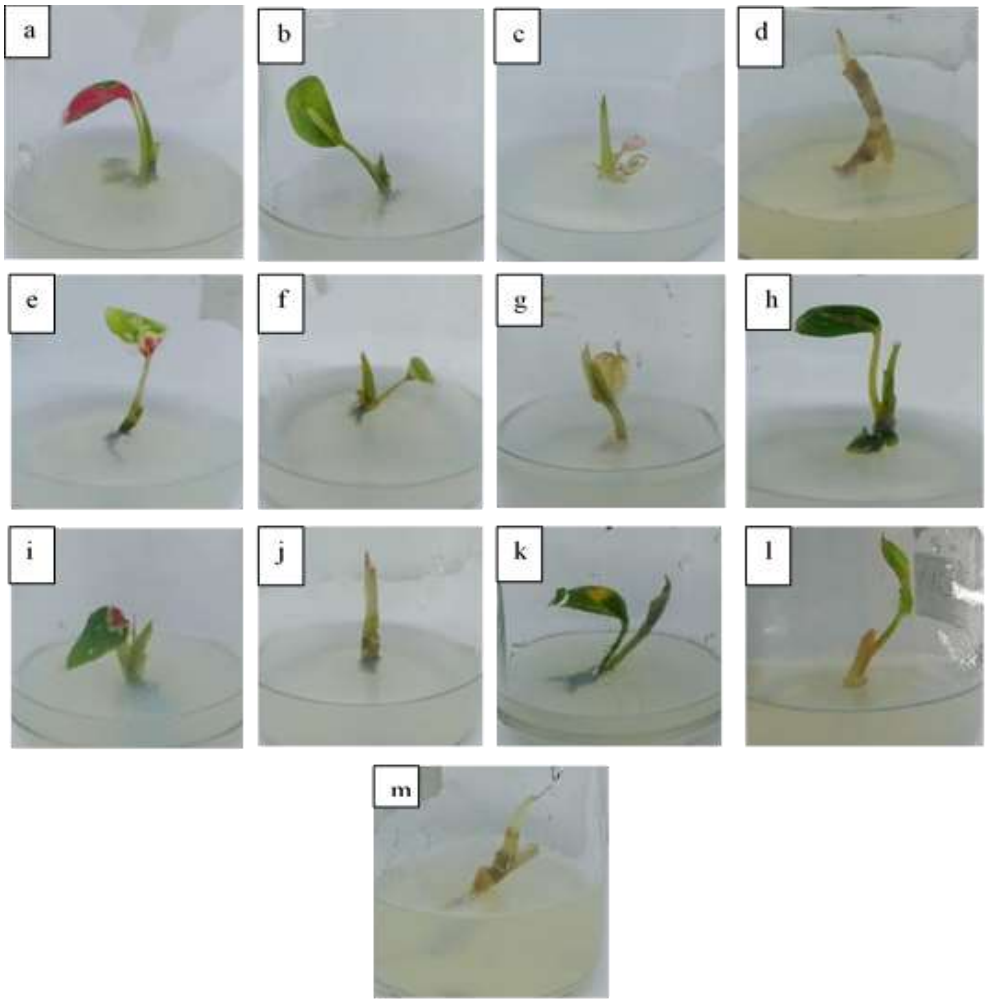

Figure 1. The visual appearance of the leaves based on the results of the leaf morphological analysis scoring. EMS concentration 0 % with 0 ppm Gibberellin (a), EMS 0.3 % with 0 ppm Gibberellin (b), EMS 0.6 % with 0 ppm Gibberellin (c), EMS 0.9 % with 0 ppm Gibberellin (d), EMS 0.3 % with 15 ppm Gibberellin (e), EMS 0.6 % with 15 ppm Gibberellin (f), EMS 0.9 % with 15 ppm Gibberellin (g), EMS 0.3 % with 25 ppm Gibberellin (h), EMS 0.6 % with 25 ppm Gibberellin (i), EMS 0.9 % with 25 ppm Gibberellin (j), EMS 0.3 % with 45 ppm Gibberellin (k), EMS 0.6 % with 45ppm Gibberellin (l), EMS 0.9 % with 45 ppm Gibberellin (m).

The results showed that the fastest germination rate occurred at concentrations without EMS with gibberellin concentrations of 45 ppm giving the fastest average sprouting speed (8.78 days). The longest germination rate (23 days) was in the 0.9% treatment with a concentration of 0 ppm gibberellins. The administration of higher concentrations of EMS and gibberellin resulted in a longer germination speed (Table 2). This is in accordance with the opinion of Priyono & Susilo (2002), the high concentration of EMS given causes more EMS absorption so that the toxicity of EMS will increase and cause growth inhibition. According to Qosim W.A., Yuwariyah W., Y. Hamdani, J.S. Rachmadi M. & Perdani, S.M. (2015), the timing of the emergence of shoots can be caused by a decrease in cell potential in the explant tissue. The meristematic cells in the explants have the potential to form new sprouts, but the administration of mutagens can induce physiological changes in these cells. This change depends on the susceptibility of the meristematic cells that form the explants, causing changes in potential and triggering the growth of cells in the explant tissue which causes differences in the response of the explants. In addition, according to Barani . Akbari, N., & Ahmadi, H. (2013), states that the speed of sprouting is faster with increasing concentrations of Gibberellin (GA3) because GA3 treatment generally responds to cell division.

Table 2. Average Germination Speed (Days) Interaction Treatment of EMS and Gibberellin Concentrations

Gibberellin	EMS				NPG BNT
	e0 (0 %)	e1 (0.3 %)	e2 (0.6 %)	e3 (0.9 %)	
g0 (0 ppm)	18.00^{a}_{r}	18.77^{a}_{r}	20.33^{a}_{q}	23.00^{a}_{p}	1.32
g1 (15 ppm)	16.00^{b}_{s}	18.00^{a}_{r}	19.44^{ab}_{q}	21.89^{ab}_{p}	
g2 (30 ppm)	10.33^{c}_{r}	17.67^{a}_{q}	18.44^{bc}_{q}	21.67^{bc}_{p}	
g3 (45 ppm)	8.78^{d}_{s}	14.77^{b}_{r}	17.89^{c}_{q}	20.55^{c}_{p}	
NPE BNT	1.35				

Note: The same letters in the column (abcd) and in the row (pqrs) indicate no significant difference in the BNT Test level α = 0.05

On the speed of rooting and the speed of forming plantlets, the treatment of EMS and gibberellin concentrations on aglaonema plants had a very significant effect at the 0.05 level, so that it could have a positive effect on the observed variables. Based on the analysis of the mean test (Table 3) for the speed of rooting and the speed of forming plantlets, the fastest average was obtained (31.66 days) at 0.3% EMS concentration with 45 ppm Gibberellin concentration. Rooting speed and plantlet formation were the longest (84.22 days) at 0.9% EMS concentration with 0 ppm gibberellin medium. Giving EMS and gibberellin concentrations affected the speed of rooting of aglaonema explants and the speed of forming plantlets. The results of Pratiwi and Ermavitalini's research (2019), the lower the EMS concentration used, the EMS can function as auxin which helps accelerate root formation and vice versa if the EMS concentration is high it will inhibit growth and can even cause death. In addition, George and Sherrington (1984), administration of Gibberellin (GA3) aims to stimulate the formation of the hormone auxin and can induce explants so as to assist in inducing root formation.

Table 3. Average Speed of Rooting (Days) and Speed of Forming Planlets (Days) in Interaction Treatment of EMS and Gibberellin Concentrations

Gibberellin	EMS				NPG BNT
	e0 (0 %)	e1 (0.3 %)	e2 (0.6 %)	e3 (0.9 %)	
g0 (0 ppm)	36.67^{a}_{r}	33.55^{a}_{r}	54.44^{a}_{q}	84.22^{a}_{p}	5.16
g1 (15 ppm)	35.77^{a}_{r}	32.33^{a}_{r}	50.44^{a}_{q}	78.00^{b}_{p}	
g2 (30 ppm)	34.89^{a}_{qr}	32.11^{a}_{r}	39.11^{b}_{q}	66.89^{c}_{p}	
g3 (45 ppm)	33.66^{a}_{qr}	31.66^{a}_{r}	37.89^{b}_{q}	68.53^{c}_{p}	
NPE BNT	5.12				

Note: The same letters in the column (abcd) and in the row (pqrs) indicate no significant difference in the BNT Test level α = 0.05

In observing the number of shoots, the treatment of various EMS concentrations had a significant effect and the gibberellin treatment had no significant effect so that there was no interaction that had a positive effect on the observed variables. Based on the analysis of the mean test (Table 4), the highest average number of shoots (1.32) was obtained at the concentration without EMS and the lowest number of shoots (1.08) was obtained at the EMS concentration of 0.9%. The use of GA3 had no significant effect because the EMS concentration exerted a stronger suppression on the increase in the number of shoots and only accelerated the growth of aglaonema shoots. Poerba Y.S., Imelda, M., Wulansari, A & Martanti, D. (2009) argued that EMS is a mutated substance that is toxic in in vitro culture, whereas the concentration of EMS given increases, it can reduce growth power, number of shoots, shoot height, and the percentage of rooted cultures.

Table 4. Average Number of Shoots at Various EMS Concentrations

Gibberellin	EMS				Average
	e0 (0 %)	e1 (0.3 %)	e2 (0.6 %)	e3 (0.9 %)	
g0 (0 ppm)	1.11	1.11	1.11	1.11	1.11
g1 (15 ppm)	1.22	1.11	1.00	1.00	1.08
g2 (30 ppm)	1.44	1.11	1.11	1.11	1.19
g3 (45 ppm)	1.50	1.22	1.11	1.11	1.11
Average	1.32 a	1.14 b	1.08 b	1.08 b	
NP (E) BNT α = 0.05			0.16		

Note: The same letters in the column (abcd) indicate no significant difference in the BNT Test level α = 0.05

On the observation of shoot height, the EMS concentration treatment with Gibberellins (GA3) on aglaonema plants had a very significant effect, so that it could have a positive effect on the observed variables. Based on the analysis of the mean test (Table 5), the highest average shoot height (4.50 cm) was obtained at a concentration without EMS with 45 ppm and the lowest average shoot height (2.52 cm) was obtained at an EMS concentration of 0.3% with 0 ppm Gibberellin. This happens because Gibberellin has a good response. According to research by Chakraborty et al., (2000), GA3 has a conducive ability to regenerate shoots in vitro on growth promotion, biomass production and xylem fiber length. In addition, the addition of EMS treatment at high concentrations causes inhibition of plant growth, including the increase in shoot height. According to Harahap M.S.A., Nilahayati., Handayani, R.S., Nazimah, &Hafifah. (2022), growth inhibition in M1 generation plants occurs due to damage to plant physiology as a result of mutagen action, where the high doses of mutagen substances given will also increase the chances of growth inhibition in M1 generation plants. The impact of this is causing a decrease in plant height, leaf size, number of leaves and plant weight.

Table 5. Average Shoot Height (cm) in EMS and Gibberellin Immersion Concentration

Gibberellin	EMS				NPG BNT
	e0 (0 %)	e1 (0.3 %)	e2 (0.6 %)	e3 (0.9 %)	
g0 (0 ppm)	3.78^{b}_{p}	3.41^{c}_{q}	2.64^{c}_{r}	2.52^{c}_{r}	0.19
g1 (15 ppm)	3.90^{b}_{p}	3.55^{bc}_{q}	2.82^{bc}_{r}	2.83^{b}_{r}	
g2 (30 ppm)	3.97^{b}_{p}	3.74^{ab}_{q}	2.93^{b}_{r}	3.02^{b}_{r}	
g3 (45 ppm)	4.50^{a}_{p}	3.90^{a}_{q}	3.62^{a}_{r}	3.53^{a}_{r}	
NPE BNT			0.22		

Note: The same letters in the column (abcd) and in the row (pqrs) indicate no significant difference in the BNT Test level α = 0.05

In observing the number of leaves, the treatment of EMS and Gibberellin concentrations had a very significant effect so that there was an interaction that had a positive effect on the observed variables. Based on the analysis of the mean test (Table 6), the treatment that gave the highest average growth of the number of leaves (1.22 leaves) was obtained from the concentration without EMS with 15 ppm Gibberellin. Meanwhile, the treatment that gave the lowest average number of leaves (not forming leaves) was obtained from the 0.9% EMS treatment with 0 ppm gibberellins, 30 ppm and 45. According to Pratiwi et al. (2013) the application of mutagen substances can result in increased activity of several enzymes due to stimulation of amino acid biosynthesis such as polyphenol oxidase, catalase and pyroxidase which can inhibit growth in leaves. This is supported by Listiani L., Lestari, A., Widyodaru, N., & Sandra, E. (2021), the results of the study showed that EMS administration to Anthurium jenmanii lemon had no impact on the number of leaves formed.

Table 6. Average Number of Leaves (strands) in the Interaction Treatment of EMS and Gibberellin Concentrations

Gibberellin	EMS				NPG BNT
	e0 (0 %)	e1 (0.3 %)	e2 (0.6 %)	e3 (0.9 %)	
g0 (0 ppm)	1.11^{a}_{p}	1.00^{a}_{p}	1.11^{a}_{p}	0.00^{b}_{q}	0.28
g1 (15 ppm)	1.22^{a}_{p}	1.11^{a}_{p}	1.11^{a}_{p}	1.00^{a}_{p}	
g2 (30 ppm)	1.11^{a}_{p}	1.11^{a}_{p}	1.11^{b}_{p}	0.00^{b}_{q}	
g3 (45 ppm)	1.00^{a}_{p}	1.11^{a}_{p}	1.11^{c}_{p}	0.00^{b}_{q}	
NPE BNT	0.25				

Note: The same letters in the column (abcd) and in the row (pqrs) indicate no significant difference in the BNT Test level α = 0.05

The treatment of EMS concentration on the parameter number of roots had a very significant effect while gibberellins did not have a significant effect so that there was no interaction that could have a positive effect on the observed variables. Based on the analysis of the mean test (Table 7) the number of roots, which gave the highest average (3.31 strands), namely the EMS concentration of 0.3% and the EMS concentration which gave the lowest average number of roots (2.58 strands), namely and the EMS concentration of 0.9%. Giving EMS concentration affects the number of aglaonema roots. This is in line with the research of Qosim (2012), that low concentrations in the application of EMS will have properties as an auxin substance in plants and a decrease in plant quantitative, such as the number of roots occurs as a result of random mutations. EMS can cause point mutations at DNA junctions due to changes in nucleotide base pairs that cause changes in amino acids.

Table 7. Average Number of Roots (strands) at Various EMS Concentrations

Gibberellin	EMS				Average
	e0 (0 %)	e1 (0.3 %)	e2 (0.6 %)	e3 (0.9 %)	
g0 (0 ppm)	2.66	3.11	3.00	2.33	2.77
g1 (15 ppm)	2.50	3.55	2.77	2.44	2.81
g2 (30 ppm)	2.33	3.11	2.89	2.77	2.77
g3 (45 ppm)	3.00	3.44	3.11	2.77	3.08
Average	2.62 b	3.31 a	2.94 ab	2.58 b	
NP (E) BNT α = 0.05	0.48				

Note: The same letters in the column (abcd) indicate no significant difference in the BNT Test level α = 0.05

In observing root length, the EMS concentration treatment with Gibberellins on aglaonema plants had a very significant effect so that it could have a positive effect on the observed variables. Based on the analysis of the mean test (Table 8) of root length, which gave the longest average (1.45 cm), namely 0.3% EMS concentration with 0 ppm Gibberellin and the treatment that gave the least root length (0.4 cm), namely 0.9% EMS concentration with 15 ppm Gibberellin–. Giving the right concentration of EMS and Gibberellins can affect the length of aglaonema roots. This is in line with Qosim's research (2012), that low concentrations in the application of EMS will have properties as an auxin substance in plants and vice versa if the dose used is high it will cause growth inhibition and even the plant can become lethal. Furthermore, according to Sekioka and Tanaka (1981), stated that GA3 has a function as an auxin in shoot induction and has a cytokinin ratio of GA3 which plays an important role in determining differentiation in plant tissue.

Table 8. Average Root Length (cm) in Interaction Treatment of EMS and Gibberellin Concentrations

Gibberellin	EMS				NPG BNT
	e0 (0 %)	e1 (0.3 %)	e2 (0.6 %)	e3 (0.9 %)	
g0 (0 ppm)	0.80^{a}_{q}	1.45^{a}_{p}	0.44^{a}_{r}	0.50^{a}_{r}	0.14
g1 (15 ppm)	0.60^{b}_{q}	1.10^{b}_{p}	0.43^{a}_{r}	0.41^{a}_{r}	
g2 (30 ppm)	0.56^{b}_{q}	1.00^{b}_{p}	0.52^{a}_{q}	0.45^{b}_{q}	
g3 (45 ppm)	0.55^{b}_{q}	0.83^{c}_{p}	0.53^{a}_{q}	0.56^{a}_{q}	
NPE BNT	0.13				

Note: The same letters in the column (abcd) and in the row (pqrs) indicate no significant difference in the BNT Test level α = 0.05

D. Conclusion

The results showed the effectiveness of EMS and Gibberellin in inducing aglaonema variegata of the Red Majesty type. The best mutant genetic diversity and in vitro regeneration at 0.3% EMS concentration with 15 ppm gibberellin (variegata), 45 ppm EMS (chimera) and 0.6% with 0 ppm gibberellins (variegata) differed from the control and other treatments. The accuracy in EMS and Gibberellin concentrations has a significant effect on the observed parameters.

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