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Induction of Protocorm-Like Bodies (PLBs) Phalaenopsis spp. Hybrids Mutation through Ultraviolet Irradiation (UV_{254}) and Ethyl Methane Sulfonate (EMS)

Induksi Mutasi Protocorm Like Bodies (PLBs) Phalaenopsis spp. Hybrids melalui Iradiasi Sinar Ultraviolet (UV_{254}) dan Ethyl Methane Sulfonate (EMS)

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ABSTRACT

Phalaenopsis sp. is the most-produced orchid species in Indonesia. Compared to conventional breeding, mutation induction by using mutagens, such as Ultraviolet Light-C (λ = 254 nm) (UV_{254}) and Ethyl Methane Sulfonate (EMS), could probably result in new superior orchid variants. This research aims to get some mutants with phenotypes that have visual differences in the Phalaenopsis spp. hybrids wild type. There were 4 durations of UV_{254} irradiation: 5' on, 85' off; 10' on, 80' off (1 day and 7 days for each treatment); 4 concentrations of EMS used in this research: 0.05%; 0.06%; 0.07%; 0.08% for 6 hours of immersion; selected UV_{254} irradiation (5' on, 85' off (7 days)) combined with these concentrations. UV_{254} irradiation treatment (5' on, 85' off (1 day and 7 days); 10' on, 80' off (7 days)) resulted in some mutants with leaf phenotypes that were visually different from the wild type; 0.05% EMS treatment resulted in PLBs mutant with a visually larger size than the wild type; 0.08% EMS treatment and combination treatments (for EMS 0.05% and 0.08% for each treatment) resulted in non-growing albino PLBs. Hence, mutation induction using UV_{254} and EMS in this research produced several most likely mutants having visual differences that may be more desirable than the wild type.

Keywords:

EMS,

Phenotype,

Mutation,

Combination Treatmen,

UV_{254}.

ABSTRAK

Phalaenopsis sp. merupakan spesies anggrek terbanyak yang diproduksi di Indonesia. Dibandingkan dengan pemuliaan secara konvensional, induksi mutasi dengan menggunakan mutagen, seperti sinar ultraviolet-C (λ = 254 nm) (UV_{254}) dan Ethyl Methane Sulfonate (EMS), dapat meningkatkan probabilitas didapatkannya varian anggrek unggul baru. Penelitian ini bertujuan untuk mendapatkan mutan yang secara fenotipe memiliki kenampakan visual yang berbeda dari Phalaenopsis spp. hybrids wild type. Terdapat 4 durasi iradiasi UV_{254} yang digunakan: 5' menyala, 85' mati; 10' menyala, 80' mati (masing-masing selama 1 hari dan 7 hari); 4 konsentrasi EMS yang digunakan: 0,05%; 0,06%; 0,07%; 0,08% dengan durasi perendaman selama 6 jam; durasi iradiasi UV_{254} terpilih (5' menyala, 85' mati selama 7 hari) dikombinasikan dengan 4 konsentrasi EMS. Perlakuan iradiasi UV_{254} (5' menyala, 85' mati (1 hari dan 7 hari); 10' menyala, 80' mati (7 hari)) menghasilkan beberapa mutan dengan fenotipe daun yang secara visual berbeda dari wild type; perlakuan 0,05% EMS menghasilkan PLBs mutan yang secara visual berukuran lebih besar dari wild type; perlakuan 0,08% EMS dan perlakuan kombinasi (pada EMS dengan konsentrasi 0,05% dan 0,08%) menghasilkan PLBs albino yang tidak mampu tumbuh. Dalam penelitian ini, induksi mutasi menggunakan UV_{254} dan EMS menghasilkan planlet yang kemungkinan besar merupakan mutan dengan perbedaan visual yang mungkin lebih diinginkan daripada wild type

Kata Kunci:

EMS,

Fenotipe,

Mutasi,

Perlakuan Kombinasi,

UV254.

INTRODUCTION

According to the Indonesian Central Bureau of Statistics, orchids are the most-produced floriculture crop in 2020 when compared to other ornamental plants. *Phalaenopsis* sp., known as the moth orchid, is the most popular species and sales reach 75% of the various types of orchids traded in Indonesia, which have unique characteristics, including appealing blossoms with a butterfly shape, huge flowers that can endure up to two months before dropping off, and flowers with a variety of colors and shapes that allow for future development (Sulistianingsih & Purwantoro, 2012; Zahara, 2017). The cross-breeding method can be used to create a new hybrid of the characteristics of the parents to increase the variety of orchids. However, this approach has a low chance of producing crosses with the desired characteristics and is difficult to transmit consistently (Romiyadi et al., 2018). In vitro plant breeding and propagation is one approach that can be used to modify the nature of orchids, from their appearance to their growing capacity. Using in vitro mutation treatment, both chemical and physical mutagens can be used to develop new, superior varieties of orchids, which can relatively accelerate the formation of mutants while expanding the possibilities for variations in orchids (Qosim et al., 2016). The resulting mutants are expected to increase the variety of orchids as well as their selling price.

UV_{254} light constitutes one of the physical mutagens which can induce mutations because it triggers spontaneous changes and the production of new genetic variations, such as causing color variations in flowers (Li et al., 2021). The physical mutagen commonly used for orchids is gamma rays because they can increase vegetative genetic variation in plants (Damayanti, 2021). However, these mutagens have enormous energy and cannot be used carelessly. Magdalita et al. (2019) said that germinating embryos of *Phalaenopsis aphrodite* irradiated with 15 Gy gamma rays showed a faster flowering response than the wild type. Irradiating gamma rays on *Phalaenopsis amabilis* plantlets that are ready to be acclimatized and getting a radiation dose of 25 Gy (Widiarsih & Dwimahyani, 2013). Surya et al. (2016) said that gamma rays above 300 Gy cause a reduction in the percentage of germination in the M1 generation of *Rubus lineatus* and *Rubus chrysophytes*. Radiation with UV light of 200–280 nm produces carnation plants that flower quickly and are shorter (Afza & Iriawati, 2016). Research by Castronuovo et al. (2017) showed a drastic decrease in chlorophyll content due to exposure to UV-C light after 30; 60; and 120 minutes of exposure to *Echinacea purpurea* and *Taraxacum officinale* flowers.

EMS is of chemical mutagen which can trigger alkylation and is most likely to cause gene mutations (Talebi et al., 2012). According to Li et al. (2021), there have been no studies related to mutations caused by chemical mutagens belonging to alkylating agents that cause high heterozygosity in orchids. Mutation induction, which has been proven successful in several studies, utilizes the polyploidization breeding method, using colchicine and nitric oxide (NO) mutagens in *Cymbidium*, *Dendrobium*, *Oncidium*, and *Phalaenopsis* orchids (Li et al., 2021). Romiyadi et al. (2018) induced mutations by using EMS concentrations shown indicated interactions with three *Phalaenopsis* spp. hybrid species on root variables. Qosim et al. (2016) showed that soaking EMS for 12 hours with a concentration of 0.025% and 0.05% affected the formation of shoots from *Phalaenopsis* spp. hybrids. In other crops, such as sugarcane, EMS is used to improve genetics so as to produce high yields (Avivi et al., 2022).

This research aims to study and determine the effect of mutation induction treatment with UV_{254} irradiation and

immersion in EMS with several concentrations on measurable and visible morphological differences. The orchids whose PLBs were used as explants were hybrid orchids from a cross between *Doritaenopsis* sp. × *Phalaenopsis elegant* 'Karin Aloha'. Through mutation treatment with UV irradiation and immersion in EMS in vitro on PLBs from *Phalaenopsis* spp. hybrids, it is expected to obtain new superior orchid variants.

METHODOLOGY

This research was carried out at the University of Surabaya. Some tools were used in this research, such as Laminar Air Flow (LAF) Cabinet, petri dish, UV box incubator, tweezers, analytical scale, culture bottles, and Bunsen burner, whereas the material used in this research were PLBs from *Phalaenopsis* spp. hybrids from a cross between *Doritaenopsis* sp. × *Phalaenopsis elegant* 'Karin Aloha', UV-C light (λ = 254 nm), EMS (Sigma), DMSO, MS medium, 6-Benzyl Amino Purine (BAP), Naphthalene Acetic Acid (NAA), sucrose, gel agent, and 96% alcohol.

PLBs were grown in vitro condition using an MS medium with the addition of BAP and NAA hormones. Then the PLBs were exposed to four different UV_{254} exposure durations: 5' on, 85' off; 10' on, and 80' off (1 day and 7 days for each treatment). Each treatment was repeated five times with twenty clumps per petri dish. Parameters measured included: the average increase in the number of PLBs, PLBs color change, the percentage of regeneration, the survival rate of PLBs, the average percentage of albino PLBs, and the percentage of mutants. Based on these parameters, explants from one duration with the most optimum results were selected to be immersed in EMS as a combination treatment.

By calculating the LC_{50} value using the probit analysis method and getting an LC_{50} value of 0.05% after 6 hours of soaking, the treatment was started. Then, with a 6-hour immersion period, four concentrations were chosen: 0.05%, 0.06%, 0.07%, and 0.08%. According to the following procedure, the EMS reagent was made by dissolving EMS in distilled water and 2% DMSO (Hadebe et al., 2017).

$$\text{Required EMS rate (mL)} = \frac{EMS\ concentration \times (aquades + DMSO2\%)}{100}$$

Example: 0.02% EMS

$$= \frac{0.02 \times (39.2 + 0.8)}{100}$$

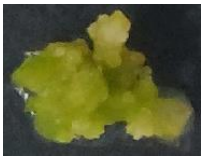
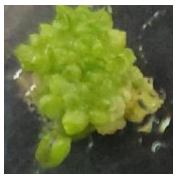
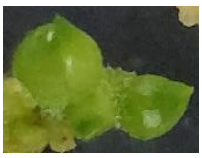
= 0,016 mL EMS

PLBs were immersed for 6 hours in EMS reagent with a concentration of 0.05%; 0.06%; 0.07%; and 0.08%. After that, the PLBs were rinsed and drained, then planted on *in vitro* medium. Each treatment was repeated five times with twenty clumps per petri dish. The parameters measured were the same as the UV_{254} irradiation treatment.

Combination treatment was carried out by immersing the PLBs that had been irradiated with the optimum duration of UV_{254} (5' on, 85' off (7 days)) in EMS with concentrations of: 0.05%; 0.06%; 0.07%; and 0.08%. Then the PLBs were rinsed and drained, then planted on *in vitro* medium. Each treatment was repeated five times with twenty clumps per petri dish. The parameters measured were the same as the UV_{254} irradiation treatment.

Kruskal-Wallis and Mann-Whitney test (α=5%) for identifying the differences between treatments were used in data analysis using the SPSS program for the parameter of the average increase in the number of PLBs and PLBs color change. The PLBs color reference scale uses the standards that have been made (Table 1).

Table 1. Color scale reference for PLBs
Tabel 1. Referensi skala warna untuk PLBs

Score *Skor*	Color *Warna*	Color Reference *Referensi Warna*	Score *Skor*	Color *Warna*	Color Reference *Referensi Warna*
1	Black		6	Chartreuse Green	
2	Grey		7	Lime Green	
3	Brown		8	Pear Green	
4	White		9	Shamrock Green	
5	Yellow		10	Emerald Green	

Other parameters (percentage of regeneration, average percentage of live PLBs, and average percentage of albino PLBs) were obtained by averaging the results per treatment. The percentage of mutants was obtained by calculating the number of mutants with the same character compared to all surviving mutants per treatment. Explants that have visually different phenotypes to the wild type are referred to as mutants and are analyzed descriptively.

RESULT AND DISCUSSION

UV_{254} Irradiation Treatment

At various durations, there was a decrease in measured phenotype parameters (mean number of PLBs, PLBs color, PLBs regenerated, survival rate, and percentage of albino) compared to the wild type (Table 2). UV_{254} irradiation in 5' on, 85' off for 7 days treatment had a decrease that was not significantly different from the wild type and had the highest average survival rate compared to other durations. This can be due to PLB mutations which occur randomly and form pyrimidine dimers which have an impact on increasing their multiplicative ability although not as well as the wild type. Radiation with UV-C light (λ=100-280 nm) causes chromosome damage due to the wavelength energy absorbed by purines and pyrimidines which results in transitions between guanine-cytosine and adenine-thymine nitrogen base pairs (Yulianto et al., 2019).

Damage that occurs due to UV_{254} radiation causes 10 minutes to be more severe as seen from the presence of browning. The mutations that occur cause

Table 2. Effect of duration of exposure to UV_{254} on measured parameters in 7 weeks after planting (WAP) explants

Tabel 2. Pengaruh durasi paparan UV_{254} pada parameter terukur pada eksplan berusia 7 minggu setelah tanam (MST)

Treatment *Perlakuan*	Average Increase in Number of PLBs[1] *Rerata Peningkatan Jumlah PLB*[1]	Color[1,2] *Warna*[1,2]	Regeneration (%) *Regenerasi (%)*	Survival Rate (%) *Tingkat Survival (%)*	Albino (%) *Albino (%)*
Wild type	14 ± 6.10[a]	6[a]	3	70 ± 14.22	2 ± 1.5
5' on, 85' off for 1 day	4 ± 2.10[b]	6[a]	30	44 ± 4.64	0
10' on, 80' off for 1 day	0[c]	4[b]	49	16 ± 10.74	0
5' on, 85' off for 7 days	13 ± 6.86[a]	6[a]	9	66 ± 4.89	1 ± 0.83
10' on, 80' off for 7 days	0[d]	4[b]	26	15 ± 4.38	0

Note:
1= numbers followed by the same letter in the same column indicate results that are not significantly different, with the Mann-Whitney test (α =5%); 2 = refers to Table 1.
1= Angka-angka yang diikuti huruf yang sama pada kolom yang sama mengindikasi hasil yang tidak berbeda nyata menurut uji Mann-Whitney (α =5%); 2 = merujuk pada Tabel 1.

the cells to produce secondary metabolites in the epidermal tissue in the form of phenolic compounds (Müller-Xing et al., 2014). Explant mortality can be carried on by browning from cell damage that results in the production of phenolic chemicals as well as by disruptions in the genome brought on by the induction of mutations.

A decrease in the capacity to produce chlorophyll may be the cause of albino PLBs. This result is similar to the research from Afza & Iriawati (2016), UV_{254} radiation has an inhibitory effect on carnation plants, resulting in reduced chlorophyll levels, shorter plant segments, and suppression of axillary shoots.

Many absorbing hairs were grown on the surface of PLBs that had been given the UV_{254} treatment (5' on, 85' off for 7 days) (Figure 1). PLBs contain absorbing hairs that have a fine hair-like appearance at the base and do not cover the bipolar poles (Mose et al., 2017). Absorbing hair can optimize the absorption of nutrients and water (Ningrum et al., 2017; Novak et al., 2014). This complimentary tool also plays a role in the ability of PLBs to recover as well as correlated with the speed of growth and multiplication of PLBs.

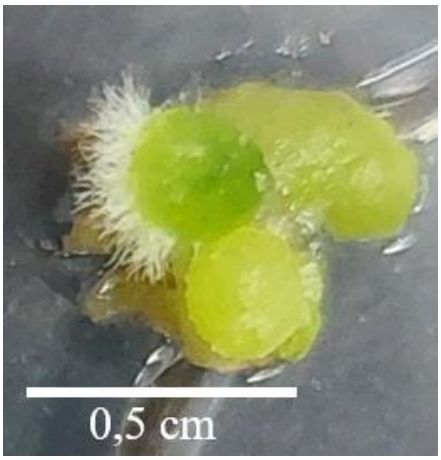


Figure. 1. Absorbing hair on PLBs surface induced by UV_{254} (5' on, 85' off (7 days))

Gambar 1. Absorbing hair pada permukaan PLBs yang diinduksi dengan UV_{254} (5' menyala, 85' mati (7 hari))

PLBs that generate new leaves and/or roots are referred to as regenerating PLBs. As can be observed, the amount of PLBs that regenerate depends on how long they are exposed to UV_{254}, with a one-day

treatment resulting in a higher proportion of PLBs than a seven-day treatment (Table 2). This may be related to the reasons that PLBs take longer to recover from long exposures, which can cause more severe damage, and that cells are more concerned with maintaining their viability than with regenerating.

Immersion of EMS

In this research, the EMS single and combination treatments were compared to the wild type using a mutagen solvent control (distilled water + DMSO 2%) in both cases. DMSO has an antibacterial effect, can enhance cell division and cell biomass, and is employed as a solvent for water-insoluble chemicals and mutagens like EMS (Xia Chen & Thibeault, 2013).

Table 3. Comparison of wild type with mutagen solvent (distilled water + DMSO 2%) to the measured parameters in 7 weeks after planting (WAP) explants

Tabel 3. Perbandingan dari wild type dengan pelarut mutagen (akuades + DMSO 2%) pada parameter terukur pada eksplan berusia 7 minggu setelah tanam (MST)

Treatment *Perlakuan*	Average Increase in Number of PLBs[1] *Rerata Peningkatan Jumlah PLB*[1]	Color[1,2] *Warna*[1,2]	Regeneration (%) *Regenerasi (%)*	Survival Rate (%) *Tingkat Survival (%)*	Albino (%) *Albino (%)*
Wild type	14 ± 6.10[a]	6[a]	3	70 ± 14.22	2 ± 1.5
0% EMS	16 ± 8.91[a]	6[a]	4	96 ± 1.25	1 ± 0.80
0% EMS for the combination treatment	10 ± 5.38[b]	6[a]	37	82 ± 9.71	7 ± 4.18

Note:
1= numbers followed by the same letter in the same column indicate results that are not significantly different, with the Mann-Whitney test (α =5%); 2 = refers to Table 1.
1= Angka-angka yang diikuti huruf yang sama pada kolom yang sama mengindikasi hasil yang tidak berbeda nyata menurut uji Mann-Whitney (α =5%); 2 = merujuk pada Tabel 1.

Table 4. Effect of EMS concentrations on measured parameters in 7 weeks after planting (WAP) explants

Tabel 4. Pengaruh konsentrasi EMS pada parameter terukur pada eksplan berusia 7 minggu setelah tanam (MST)

Treatment *Perlakuan*	Average Increase in Number of PLBs[1] *Rerata Peningkatan Jumlah PLB*[1]	Color[1,2] *Warna*[1,2]	Regeneration (%) *Regenerasi (%)*	Survival Rate (%) *Tingkat Survival (%)*	Albino (%) *Albino (%)*
Wild type	14 ± 6.10[a]	6[a]	3	70 ± 14.22	2 ± 1.5
0% (solvent control)	16 ± 8.91[a]	6[a]	4	96 ± 1.25	1 ± 0.80
0.05%	4 ± 2.48[bcd]	4[c]	0	69 ± 4.32	53 ± 20.58
0.06%	4 ± 2.14[cd]	4[c]	13	42 ± 15.32	37 ± 19.92
0.07%	5 ± 2.44[bc]	5[bc]	6	54 ± 12.80	63 ± 22.54
0.08%	3 ± 1.56[d]	4[c]	8	45 ± 18.80	96 ± 3.70

Note:
1= numbers followed by the same letter in the same column indicate results that are not significantly different, with the Mann-Whitney test (α =5%); 2 = refers to Table 1.
1= Angka-angka yang diikuti huruf yang sama pada kolom yang sama mengindikasi hasil yang tidak berbeda nyata menurut uji Mann-Whitney (α =5%); 2 = merujuk pada Tabel 1.

The function of solvent control is to guarantee that the solvent has no mutation effect so that the mutagen effect can be accurately assessed. The results indicate that none of the two solvent controls has a mutation effect, allowing the solvent to be used (Table 3).

Giving EMS at a specific concentration affects the observed parameters since the concentration used exceeds the LC_{50} EMS value and results in increased PLB mortality (Table 4). High levels of EMS exposure can interfere with the function of enzymes involved in the germination process and inhibit auxin production from taking (Arisha et al., 2014). The optimal EMS concentration is 0.05% EMS, with a higher survival rate than other concentrations. The percentage of albinos at various EMS concentrations is high, indicating that this response is a response to cell damage. PLBs that survive will exhibit growth. Albinism can be caused by chlorophyll mutations, which result in discoloration due to a lack of chlorophyll. PLBs are unable to form chlorophyll or lose the ability to do so in all parts of the cell exposed to the mutagen (Romeida et al., 2012). 0.05% EMS can cause changes in the color of the leaves of *Aerides crispa* Lindl. from green to white due to a lack of chlorophyll (Srivastava et al., 2018).

Secondary PLB grew and regenerated from the leaf tips of PLBs exposed to 0.05% EMS as a result of the leaves coming into contact with *in vitro* medium (Figure 2). PLBs can be induced to proliferate by using leaves, and these PLBs grow from the epidermal cells of the leaves, which can then be regenerated to form plantlets (Chookoh et al., 2019; Huang et al., 2014).

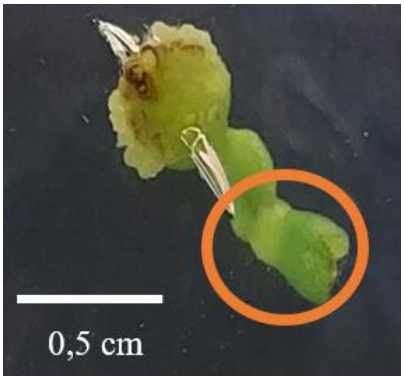


Figure. 2. Mutant secondary PLB formed from leaf tips induced by 0.05% EMS. Remarks: marked with an orange circle.

Gambar 2. PLB sekunder mutan yang terbentuk pada ujung daun yang diinduksi dengan 0,05% EMS. Keterangan: ditandai dengan lingkaran oranye.

Combination Treatment of UV_{254} Light Irradiation and EMS

The mutagens used in this research can cause random mutagenesis. Although the dose and duration can be planned, the results are difficult to predict (Mullins et al., 2021). The survival rate increased as the concentration increased, but the average albino percentage increased as immersion concentration increased (Table 5). These albino PLBs did not exhibit any signs of growth or death. The percentage of albinos in the combination treatment was generally lower than in the single EMS treatment, which could be due to PLBs already receiving $UV2_{54}$ irradiation treatment, which may affect their increasing tolerance to EMS toxicity, allowing for less depigmentation.

This combination treatment was carried out continuously by exposing PLBs to UV_{254} light, then observing them for 7 weeks before immersing them in EMS, causing PLBs to require time to recover and adapt from cell damage caused by mutagens. Furthermore, it may cause more PLBs to develop chlorosis as a result of ongoing cell damage.

Table 5. Effect of combination treatment of UV_{254} exposure and various EMS concentrations on measured parameters in 7 weeks after planting (WAP) explants

Tabel 5. Pengaruh perlakuan kombinasi paparan UV_{254} dan variasi konsentrasi EMS pada parameter terukur pada eksplan berusia 7 minggu setelah tanam (MST)

Treatment *Perlakuan*	Average Increase in Number of PLBs[1] *Rerata Peningkatan Jumlah PLB*[1]	Color[1,2] *Warna*[1,2]	Regeneration (%) *Regenerasi (%)*	Survival Rate (%) *Tingkat Survival (%)*	Albino (%) *Albino (%)*
Wild type	14 ± 6.10[a]	6[a]	3	70 ± 14.22	2 ± 1.5
UV_{254} (5' on, 85' off for 7 days) + 0% EMS (solvent control)	10 ± 5.38[b]	6[ab]	37	82 ± 9.71	7 ± 4.18
UV_{254} (5' on, 85' off for 7 days) + 0.05% EMS	6 ± 2.99[b]	4[b]	10	50 ± 10.57	17 ± 10.36
UV_{254} (5' on, 85' off for 7 days) + 0.06% EMS	4 ± 1.98[bc]	4[b]	10	56 ± 10.33	57 ± 17.69
UV_{254} (5' on, 85' off for 7 days) + 0.07% EMS	5 ± 2.69[bc]	4[b]	20	62 ± 9.07	43 ± 13.63
UV_{254} (5' on, 85' off for 7 days) + 0.08% EMS	5 ± 1.95[bc]	4[b]	0	69 ± 6.32	71 ± 10.94

Note:
1= numbers followed by the same letter in the same column indicate results that are not significantly different, with the Mann-Whitney test (α =5%); 2 = refers to Table 1.
1= Angka-angka yang diikuti huruf yang sama pada kolom yang sama mengindikasi hasil yang tidak berbeda nyata menurut uji Mann-Whitney (α =5%); 2 = merujuk pada Tabel 1.

The results of this research revealed that EMS 0.06% performed better as compared to EMS 0.07% (in both single treatment and treatment). This can be caused by EMS, which is unstable at 0.07% concentrations, reducing its mutagenic effect. EMS is a water-soluble reagent, but it will be decomposed by water during storage, rendering it unstable (Amini, 2014).

Most Likely Mutant Phenotype Character

Most likely mutant explants at 7 WAP were classified based on their proliferative ability, and after forming plantlets, they were classified based on the visual difference in plantlet phenotype to the wild type (Table 6; Table 7). In this research, plantlets with characters that are visually different from the wild type are referred to as mutants because they have the potential to become mutants, but the further characterization is needed. At 7 WAP, the UV_{254} treatment (5' on, 85' off for 1 day) produced the highest percentage of mutants with various multiplication abilities. In general, the UV_{254} radiation treatment produced mutants with different leaf characters from the wild type, some EMS treatments produced mutants with visual differences from the wild type, and some combination treatments and EMS produced albino mutants that did not grow (Table 7).

Table 6. Percentage of mutant PLBs with various characteristics of multiplication capability resulting from various mutation induction treatments in 7 weeks after planting (WAP) explants

Tabel 6. Persentase mutan PLBs dengan karakteristik kemampuan multiplikasi dari berbagai perlakuan induksi mutasi pada eksplan berusia 7 minggu setelah tanam (MST)

Treatment *Perlakuan*	Mutant Multiplication Characteristics *Karakteristik Multiplikasi Mutan*			Number of Mutant *Jumlah Mutan*	Mutant Percentage (%) (Ratio)
	Slow *Lambat*	Stagnant *Stagnan*	Fast *Cepat*		
UV_{254} (5' on, 85' off for 1 day)	5	10	2	17	45 ($\frac{17}{38}$)
UV_{254} (10' on, 80' off for 1 day)	2	4	1	7	23 ($\frac{7}{30}$)
UV_{254} (5' on, 85' off for 7 days)	14	2	4	20	27 ($\frac{20}{75}$)
UV_{254} (10' on, 80' off for 7 days)	4	0	2	6	33 ($\frac{6}{18}$)
0.05% EMS	0	2	5	7	32 ($\frac{7}{22}$)
0.06% EMS	0	2	2	4	17 ($\frac{4}{23}$)
0.07% EMS	0	0	3	3	14 ($\frac{3}{22}$)
0.08% EMS	2	1	1	4	19 ($\frac{4}{21}$)
UV_{254} (5' on, 85' off for 7 days) + 0.05% EMS	2	1	2	5	22 ($\frac{5}{23}$)
UV_{254} (5' on, 85' off for 7 days) + 0.06% EMS	0	3	2	5	18 ($\frac{5}{28}$)
UV_{254} (5' on, 85' off for 7 days) + 0.07% EMS	0	3	1	4	16 ($\frac{4}{25}$)
UV_{254} (5' on, 85' off for 7 days) + 0.08% EMS	6	5	1	12	35 ($\frac{12}{34}$)

Table 7. Percentage of mutant plantlets from various mutation induction treatments

Tabel 7. Persentase mutan planlet dari berbagai perlakuan induksi mutasi

Treatment *Perlakuan*	Mutant Age (WAP[1]) *Umur Mutan (MST[1])*	Mutant Percentage (%) (Ratio[2])	Mutant Characteristic *Karakteristik Mutan*
UV_{254} (5' on, 85' off for 1 day)	44	15 ($\frac{2}{13}$)	Wavy leaf edges (Figure 3B)
UV_{254} (10' on, 80' off for 1 day)		0 ($\frac{0}{2}$)	-
UV_{254} (5' on, 85' off for 7 days)		3 ($\frac{1}{37}$)	Leaves bases with purplish color and have rounded roots (Figure 3C)
		3 ($\frac{1}{37}$)	Elongated and twisted leaves (Figure 3D)
		3 ($\frac{1}{37}$)	Inward-curved leaf shape (Figure 3E)

Treatment *Perlakuan*	Mutant Age (WAP[1]) *Umur Mutan (MST[1])*	Mutant Percentage (%) (Ratio[2])	Mutant Characteristic *Karakteristik Mutan*
UV_{254} (10' on, 80' off for 7 days)		3 ($\frac{1}{37}$)	The leaves are shaped like a heart. (Figure 3F)
		3 ($\frac{1}{37}$)	Explant growth is slower and has a flat leaf tip (Figure 3G)
		3 ($\frac{1}{37}$)	Rounded leaf (Figure 3H)
		3 ($\frac{1}{37}$)	White spots on the leaf (Figure 3I)
		25 ($\frac{1}{4}$)	The yellow color on half of the leaf side and root (Figure 3J)
		25 ($\frac{1}{4}$)	Roots with 3 different colors (Figure 3K)
0.05% EMS	14	10 ($\frac{2}{21}$)	PLBs that are larger than the wild type (Figure 4B)
0.06% EMS		0 ($\frac{0}{2}$)	-
0.07% EMS		0 ($\frac{0}{19}$)	-
0.08% EMS		37 ($\frac{11}{30}$)	Non-growing albino PLBs (Figure 4C)
UV_{254} (5' on, 85' off for 7 days) + 0.05% EMS	17	27 ($\frac{3}{11}$)	Non-growing albino PLBs
UV_{254} (5' on, 85' off for 7 days) + 0.06% EMS		0 ($\frac{0}{3}$)	-
UV_{254} (5' on, 85' off for 7 days) + 0.07% EMS		0 ($\frac{0}{11}$)	-
UV_{254} (5' on, 85' off for 7 days) + 0.08% EMS		8 ($\frac{3}{37}$)	Non-growing albino PLBs

Note:

1 = Week After Planting; 2 = presented in the percentage of mutants with different phenotypic characters from the wild type from the total number of surviving mutants.

1 = Minggu Setelah Tanam; 2 = disajikan dalam persentase mutan dengan karakter fenotip berbeda dari tipe liar dari jumlah total mutan yang bertahan

The mutants produced by UV_{254} irradia-tion showed differences in leaf phenotypes when compared to the wild type, with the resulting various forms (Figure 3). The UV_{254} treatment (10' on, 80' off for 1 day) prevented mutants from forming and surviving. Treatment with various EMS concentrations revealed the presence of PLBs that were larger than the wild type as well as the presence of non-growing albino PLBs (Figure 4). The combination treatment also produced non-growing albino PLBs. Aside from muta-gens, factors influencing PLB growth res-ponse include differences in PLB cha-racteristics because one orchid contains hundreds to thousands of seeds that are sown and grown together *in vitro* and are not uniform in nature. Seed propagation results in heterozygous offspring (Utami et al., 2022).

The mutants obtained must be selected and further characterized in terms of genetic stability and mutant tolerance to extreme conditions that are not appropriate for their agro climate. The mutants that have been obtained must first be raised for the flowers that form to be identified, and it is hoped that the mutants that form will also produce unique flowers. The mutations induced in PLBs in this study resulted in

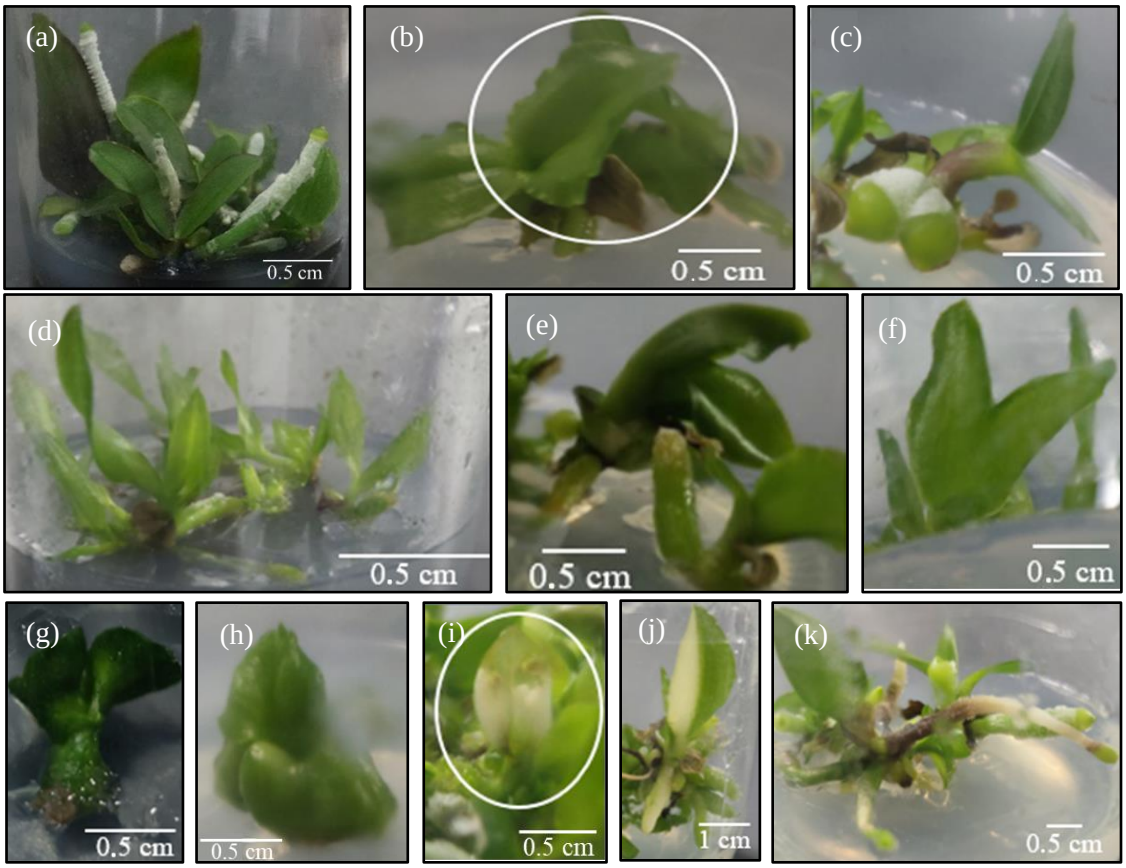


Figure 3. Mutant plantlets obtained from UV_{254} irradiation treatment: wild type (a), mutants from 5' on, 85' off (1 day) treatment (b), mutants from 5' on, 85' off (7 days) treatment (c to i), mutants from 10' on, 80' off (7 days) treatment (j and k).

Gambar 3. Planlet mutan yang didapatkan dari perlakuan iradiasi UV_{254}: wild type (a), mutan dari perlakuan 5' menyala, 85' mati (1 hari) (b), mutan dari perlakuan 5' menyala, 85' mati (7 hari) (c sampai i), mutan dari perlakuan 10' menyala, 80' mati (j dan k).

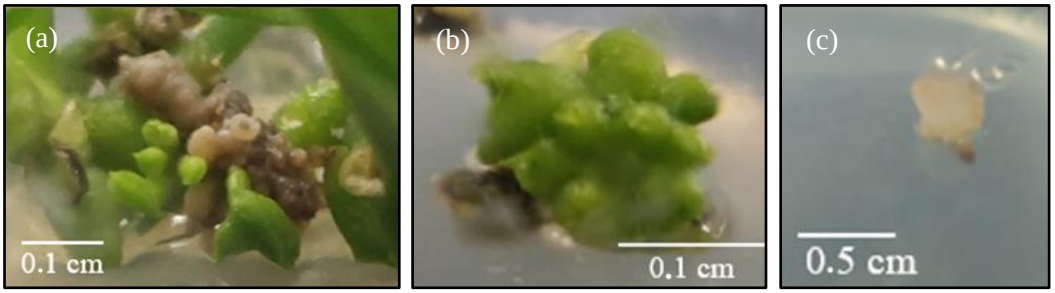


Figure 4. Mutant PLBs obtained from EMS treatment: wild type (a), mutants from 0.05% EMS (b), mutants from 0.08% EMS (c).

Gambar 4. PLBs mutan dari perlakuan EMS: wild type (a), mutan dari 0,05% EMS (b), mutan dari 0,08% EMS (c).

undirected mutations; the results obtained should be subjected to molecular analysis to ensure that mutations occur.

The mutants obtained must be selected and further characterized in terms of genetic stability and mutant tolerance to extreme conditions that are not appropriate for their agro climate. The mutants that have been obtained must first be raised for the flowers that form to be identified, and it is hoped that the mutants that form will also produce unique flowers. The mutations induced in PLBs in this study resulted in undirected mutations; the results obtained should be subjected to molecular analysis to ensure that mutations occur.

CONCLUSION

Mutation induction through UV_{254} irradiation (5' on, 85' off (1 day and 7 days) and 10' on, 80' off (7 days)) produced mutants with phenotype leaf shape and color which were visually different from the wild type (wavy leaf edges, leaves bases with purplish color, elongated and twisted leaves, inward-curved leaf, flat leaf tip, rounded leaf, leaf with white spots, and yellow color on half of the leaf side); 0.05% EMS resulted in PLBs mutant with a visually larger size than the wild type; 0.08% EMS and combination treatments (0.05% and 0.08% EMS for each treat-ment) resulted in non-growing albino PLBs.

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