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The Effect of in Vitro Modified Basic Media Types on the Morphological Growth of Dendrobium anosmum Lindl. Mutant Orchids Resulting from Gamma Ray Irradiation

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ABSTRACT

The demand for orchid seedlings continues to increase every year, resulting in significant price fluctuations. To meet market demand, a method for rapid plant propagation is needed. The solution is to multiply plants through *in vitro* culture. The choice of growth media in *in vitro* culture is a crucial factor; Therefore, the media used must have sufficient nutrients for plant growth. This study aims to determine the effect of in vitro modified basic media types on the morphological growth of *Dendrobium anosmum* Lindl. mutant orchids resulting from gamma ray irradiation. This study was conducted at the Tissue Culture Laboratory, Faculty of Agriculture, Udayana University, from January to April 2025, using a Completely Randomized Split Plot Factorial Design and 8 replications. The first factor was the type of gamma-ray irradiation mutant dose and the second factor was the type of *in vitro* modified base medium. The results showed a significant interaction between the gamma-ray irradiation dose and the type of in vitro modified base medium on the observed variable of plantlet height, but no interaction was observed for the other research variables. The type of modified FFM base medium produced the highest value for each type of *D. anosmum* mutant.

Keywords: Basic Media, D. anosmum Lindl., Gamma Ray Irradiation, In Vitro Culture

INTRODUCTION

Orchid is member from family *Orchidaceae* , estimated there are around 6000 species orchid which is spread across all over world (Fandani *et et al.,* 2018). Dendrobium is commonly found in area tropical, orchid This is Wrong One type orchid Which relatively easy to cultivate. This type of orchid is the largest genus among orchid other, with more from 1300 species Which different (Maitra, *et al*., 2020). Dendrobium is an orchid species that has a variety of flower colors. And Lots made into as flower decoration so that own mark the economy Enough tall.

Request will seeds orchid Keep going increase every Annually, prices also fluctuate significantly. According to the Center for Agricultural Data and Information Systems (2015), Indonesia imported orchid seedlings from several major countries until 2014, including Taiwan (51.52%), Thailand (30.90%), and Japan (17.58%). Therefore, a balance is needed between demand from

orchid enthusiasts and the demand from orchid enthusiasts. orchid and availability seeds orchid In breeding efforts, plant diversity can be achieved through tissue culture techniques and enhanced with the use of mutagens. Qualitative traits such as flower shape and color in some plant species can be improved through mutation induction. The mutation induction process can increase diversity genetics something species (Yamagucha, 1988). Induction Mutations can be carried out through physical or chemical methods. In plants that develop breed in a way vegetative like orchid, treatment induction mutation physically with use irradiation more liked than method chemical.

One of the methods common mutation induction used in plant breeding is gamma ray irradiation. Factors that influence the formation of mutants covering dose irradiation. Giving dose tall can cause death or sterilization of the mutated plant material, while at low doses, the plant Still can recover with damage physiological which limited and no fatal (Soedjono, 2003).

The success of orchid propagation and cultivation using tissue culture methods depends greatly on the type of media used. Media which used for growth shoots orchid in tissue culture has a significant impact on the growth and development of explants and the resulting seedlings (Tuhuteru *et al*., 2012). Media plays a crucial role in the success of tissue culture, particularly in the use of plant growth regulators (PGRs) and organic extracts. These two elements function to stimulate cell division and trigger shoot formation (Suryowinoto, 1996).

Potato extract contains polysaccharides and elements essential for root growth in orchid explants. Explant growth also depends on the availability of elements such as phosphorus, calcium, manganese, iron, and boron. The use of Ambon banana extract in orchid plantlet subcultures *Dendrobium* has proven can stimulate its growth. Banana fruit Also contain hormone experience auxin and gibberellin Which can encourage plant growth (Kasutjianingati and Irawan, 2013).

MATERIALS AND METHODS

Time and Place of Research

Study This implemented in Laboratory Culture Network Garden Experiment, Faculty of Agriculture, Udayana University, Jl. Pulau Moyo No.16X, Pedungan, South Denpasar District, Denpasar City, Bali. This research was conducted in January – April 2025.

Tools and materials

Tool which used in study this is bottle culture orchid, stove, glass baker, cup petri dish, spatula, tweezers, bottle glass, pipette drops, *hand sprayer,* knife, ruler, *Erlenmeyer flask,* digital scale, autoclave, *magnetic stirrer,* pH meter, *oven,* Bunsen lamp, culture rack, refrigerator, *Laminar Air Flow Cabinet* (LAFC), rubber band, label paper, *plastic wrap,* brown paper, tissue, *aluminum foil,* stationery and camera.

Material which used is media *New Phaleopnopsis* (NP), Media *Murashige skoog* (MS), FFM (*Foliar Fertilizer of Medium*), sugar, agar, 70% alcohol, spirits, potatoes and Ambon bananas. Planting materials used in the study This is explanation plant mutant orchid *Dendrobium anosmum* Lindl. which has been exposed to gamma rays and is sown in the Tissue Culture Laboratory of the Faculty of Agriculture, Udayana University.

Research Design

This study used the Completely Randomized Design (CRD) Split Plot Factorial method. Factor Which First consists of from 3 level treatment and factor Which The second consisted of 3 levels of treatment. The first factor was the mutant with a dose irradiation ray gamma with 3 levels, namely: M_0=0 gy (control without irradiation); M_5=5 gy; M_{10}=10 gy. The factors Which both types

of basic media with the addition of 100 gr/L potato extract and 100 gr/L banana extract with 3 levels of treatment, namely: D_n = NP Media; D_m = MS Media; D_f = FFM Media.

Environmental Sterilization

In conducting research, sterilization of the work environment is one of the important aspects that must be considered, because it directly affects the success of the research. Tissue culture laboratories or work areas are cleaned by sweeping and mopping the floor using carbolic acid solution to maintain sterility. In addition, room sterilization can also be done by spraying 70% alcohol evenly. Not only the workspace, the cleanliness and sterility *of the Laminar Air Flow Cabinet* (LAFC) must also be maintained. Before use, the Laminar Air Flow must be disinfected or sterilized by spraying 70% alcohol and turning on the ultraviolet light. After that, the Laminar is closed and left for at least 30 minutes to ensure contamination on the work surface is removed. Workers are required to wear masks, lab coats, and spray alcohol on their hands before putting on rubber gloves (*handscoons*).

Sterilization of Tools and Media

Autoclaving is performed at a pressure of 15 psi and a temperature of 121°C. For liquid volumes up to 100 ml, the required sterilization time is 15–20 minutes, while for larger volumes (between 2 and 4 liters), it takes 30–40 minutes once the temperature and pressure have reached the specified conditions.

***New Phaleonopsis* (NP) Media**

The type of planting media used is *New Phaleonopsis* (NP) media as the basic media which is then added with organic materials such as potato pulp. 100 gr and banana porridge 100 gr. First prepare a *beaker glass* with a size of 1000 ml, NP Which used is NP instant as much as 22, 03 gr, further added sugar as much as 30 gr, added also agar-agar as much as 7 grams and all the ingredients that used weighed use analytical balance. Put all the weighed ingredients into a *beaker*, then add 750 ml of distilled water and then homogenize using *magnetic stirrer*, after Enough homogeneous add water until it reaches a size of 1000 ml. The homogenized media is heated using stove until boiling. Measuring pH media use Litmus paper with a range of 5.6 – 5.8, if the pH is appropriate, the media is ready to be poured into a sterilized 4.5 cm diameter culture glass, then cover the bottle with plastic and tie it with a rubber band. After that, put all the bottles in the autoclave to be sterilized for 25-30 minutes at a temperature of 121˚C. If sterilization has finished so media saved on stock room and wait for 7 days to see whether or not the media is contaminated.

***Murashige and Skoog* (MS) Media**

The type of planting media used is *Murashige and Skoog* (MS) media as the base media which is then supplemented with organic materials. like potato porridge 100 gr and porridge banana 100 gr. First prepare a 1000 ml *beaker glass*, the MS used is instant MS as much as 4.43 grams, then add 30 grams of sugar, also add 7 grams of agar-agar and all the ingredients used are weighed using an analytical balance. Put all the ingredients that have been weighed into the *beaker glass* then add 750 ml of distilled water then homogenize using *magnetic stirrer*, after Enough homogeneous add water until it reaches a size of 1000 ml. The homogenized media is heated using stove until boiling. Measuring pH media use Litmus paper with a range of 5.6 – 5.8, if the pH is appropriate, the media is ready to be poured into a sterilized 4.5 cm diameter culture glass, then cover the bottle with plastic and tie it with a rubber band. After that, put all the bottles in the autoclave to be sterilized for 25-30 minutes at a temperature of 121˚C. If sterilization has finished so media saved on stock room and wait for 7 days to see whether or not the media is contaminated.

Making *Foliar Fertilizer of Medium* (FFM)

The type of planting media used is *Foliar Fertilizer of Medium* (FFM) as media base Which Then plus with Organic ingredients such as 100g potato pulp and 100g banana pulp. Prepare a 1000ml *beaker first, then add 30g granulated sugar, 7g agar-agar,* 2ml *fish emultion , 1ml atonik, and growmore.* 2 gr, A B mix 2 ml each, charcoal active 2 gr and vitamin B1 as much as 1 ml. All material Which already available to be weighed using an analytical balance. Enter all material Which Already weighed to in glass *beaker* then add distilled water as much as 750 ml Then homogenized using *magnetic stirrer*, after Enough homogeneous add water until it reaches a size of 1000 ml. The homogenized media is heated using stove until boiling. Measuring pH media use Litmus paper with a pH range of 5.6–5.8. If the pH is correct, the media is ready to be poured into sterilized 4.5 cm culture bottles. Then, cover the bottles with plastic and tie them with rubber bands. After that, place all the bottles in an autoclave to be sterilized for 25–30 minutes at a temperature of 100°C. 121˚C. If sterilization has finished so media saved on stock room and wait for 7 days to see whether or not the media is contaminated.

Planlet Subculture

On stages subculture done replacement media planting with media the new one so that nutritional needs for growth can fulfilled. Principle The basis of subculture is cutting, dividing and replanting explants that have grown so that the number of plants will increase. In this study, explant planting was carried out in *a Laminar Air Flow Cabinet,* the explants used were *Dendrobium anosmum orchid plantlets* that had been given treatment ray gamma with concentration 5 And 10 gy and natural *Dendrobium anosmum orchids (without gamma ray treatment).* Step the beginning done in process This is insert media, explanation and tools into *the Laminar Air Flow Cabinet* (LAFC), then UV is applied for 30 minutes. After That UV turned off, blower and light turned on, as well as light Bunsen burner is lit. Explanation Which Already sterile direct planted on media Treatment, each bottle contains 4-5 plantlets and the bottle cap is covered with plastic *wrap* and tied with rubber to keep it secure. sterile. Storage done on room growth under 20 watt neon lamp with a temperature of approximately 25ºC for 4 weeks and checked every 7 Days After Planting (DAP).

Observation Variables

The growth of the plantlets observed in this study was 1) the height of the plantlets (cm), the measurement was carried out by measuring the plantlets from the base of the stem to the longest leaf. done by using ruler. Measurement done on at the end research. 2) Number of plantlet leaves (strands), observations were made by visually observing the number of leaves per plantlet. The number of leaves counted were leaves that were fully developed and clearly visible. The number of leaves was counted at the end of the research. 3) Number of plantlet roots (fruit), the roots counted were all the roots formed. The number of roots was counted at the end of the research during the acclimatization of the plantlets. 4) Length of plantlet roots (cm), observations were made by measuring from the base of the root to the tip of the longest root. The tool used was a ruler. Root length was measured during the research. 5) Weight fresh total plantlets (g), the results of the wet weight were calculated by removing the plantlets from the culture bottle and weighing all parts of the plantlets using a digital scale. The total fresh weight was measured at the end of the study. 6) Percentage *Browning* Plants (%), *browning percentage observations* can be made at the end of the study. Explants that experience *browning* will show changes color from white or green pale become chocolate or black. This can occur in the entire explant or only in certain parts.

Data analysis

Observation data were analyzed using ANOVA (*Analysis of Variance).* If the ANOVA or analysis of variance shows that F.Hit > F.Table with a significance of 5%, further testing is carried out using the 5% Duncan test to more clearly determine the differences between existing treatments. If the ANOVA shows an interaction between treatments, further testing is carried out using the 5% BNT test.

RESULTS AND DISCUSSION

in vitro modified basic media analysis test on the morphological growth of *the Dendrobium anosmum mutant orchid* showed that there was a significant interaction effect on the plant height variable. As a single factor, the gamma ray mutant dose treatment showed a very significant effect on each variable. As a single factor, the modified basic media type treatment showed a very significant effect on the plant height and number of roots, and on the root length and total fresh weight variables showed a significant effect, and on the number of leaves variable showed no significant effect on the morphological growth of the *Dendrobium anosmum mutant orchid* based on the results of the ANOVA analysis of variance test (Table 1).

Plantlet Height (cm)

The results of the study showed that there was an interaction between the dose of gamma ray irradiation mutants and the type of modified base media with the highest value in the $M_0 D_{f\ treatment}$ with a value of 2.34 cm (Table 2).

Table 1. Significance of the influence of the type of *in vitro* modified base media on the morphological growth of the mutant orchid *Dendrobium anosmum* Lindl

No	Variables	Treatment		
		Gamma Ray Mutants	Basic Media Types	Interaction
1	Plantlet Height (cm)	**	**	*
2	Number of leaves (blades)	**	ns	ns
3	Number of Roots (fruit)	**	**	ns
4	Root Length (cm)	**	*	ns
5	Total Fresh Weight (g)	**	*	ns
6	Percentage of Planlet *browning* (%)	td	td	td

Information:
td = not statistically analyzed
ns = no significant effect (P≥0.05)
* = significantly affected (P<0.05)
** = very significant effect (P≤0.01)

Table 1Interaction between the dose of gamma ray irradiation mutants and the type of *in vitro modified base media* on the height variable of *D. anosmum* orchid plantlets

Gamma Ray Mutant (M)	Basic Media Type (D)		
	D_f	D_m	D_n
M_0	**2.34 a**	0.91 ab	**1.28 ab**
M_5	0.83 ab	0.31 b	0.68 ab
M_{10}	0.94 ab	0.3 b	**0.2 b**

Description: Numbers followed by the same letter in one observation variable indicate no significant difference based on the Duncan test at 5% level.

Orchid plants treated with gamma ray irradiation at doses of 5 and 10 Gy experienced shorter growth compared to orchids that did not receive the treatment. This is due to the effects of gamma rays which can damage cells, tissues, and disrupt the physiological processes of plants. This irradiation inhibits cell division, triggers oxidative stress, and disrupts the balance of growth hormones, so that the speed of plant growth, including the height of the stem, decreases. FFM media is specially formulated with a balance of macro and micro nutrients that suit plant needs, such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), as well as micro elements such as iron, manganese, boron, zinc, copper, and molybdenum. According to Wardhani *et al.,* (2007) gamma rays affect the growth of *Brachypeza indusiata orchids* and the most optimal dose of 0 Gray, this is indicated by the higher the dose given the higher the number of orchid plantlets that do not grow. Plant cell death due to irradiation can occur directly through DNA damage, or indirectly due to the toxic effects of free radicals such as H_2O_2 and OH_2 ions formed through radiolysis (Soeranto 2003). The addition of organic matter in the form of potatoes and bananas also contributes to providing a source of carbohydrates, vitamins, and microelements that support optimal plant growth.

Number of leaves (blades)

The treatment of gamma ray irradiation mutant dose showed significant results. very real to amount leaf plantlets *D. anosmum*. Amount leaf Which most produced on dose ray gamma M_0 (control) with mark 0.66 strands. The treatment of the modified basic media type showed influential results not real to amount leaf plantlets *D. anosmum*. The largest number of leaves was produced on the $D_{f\ media\ type.}$ with mark 0.54 strands (Table 3).

According to Srivastava *et al.* (2018), induced treatment using gamma rays as a mutagen can produce random or unpredictable traits. Gamma ray irradiation can also cause chromosomal aberrations, which are damage or breaks in chromosome threads in plants. Another study by Oktavina (2011) also showed that exposure to gamma rays can cause a significant reduction in the number of leaves in orchid hybrid D. *schulerii* x May Neal wrap.



A B C

Figure 1. Comparison of plantlet height between treatments M $_0$ (A), M $_5$ (B) and M $_{10}$ (C) with a bottle diameter of 4.5 cm
Source: Personal Documentation

Table 2Effect of gamma ray irradiation mutant doses with in vitro modified basic media types on *the* number of leaves, number of roots, root length and total fresh weight of plantlets

Treatment	Observation Variables			
	Number of leaves (blades)	Root Length (cm)	Number of Roots (fruit)	Total Fresh Weight (g)
Gamma Ray Mutant (M)				
Ms0	**33.33** a	**4.08** a	**32.67** a	**0.66** a
Ms5	14.00 b	0.65 c	14.33 b	0.17 b
Ms10	12.67 c	2.52 b	10.00 c	0.11 c
BNT 5%	0.97	0.28	1.96	0.05
Basic Media Type (D)				
Df	**27.33** a	**12.91** a	**27.67** a	**0.54** a
Dm	14.67 c	4.86 b	7.67 c	0.14 c
Dn	18.00 b	3.99 b	21.67 b	0.26 b
BNT 5%	2.62	1.53	1.69	0.05

Information: The numbers Which followed with letter Which The same showed no significant difference in the 5% BNT test.

Number of Roots (fruit)

The treatment of gamma ray irradiation mutant dose showed significant results. very real to amount root plantlets *D. anosmum*. The highest number of roots was produced at a gamma ray dose of M_0 (control) with mark 32.67 pieces. The treatment of modified basic media types showed influential results. very real to amount root plantlets *D. anosmum*. Amount root which most produced on type media D_f with value of 27.67 pieces (Table 3).



A B C

Figure 2. Comparison of the number of leaves, number of roots and root length between treatments M_0 (A), M_5 (B), M_{10} (C).
Source: Personal Documentation

According to Wattimena (2007) the root formation process begins with the activity of meristem cells that continuously divide and form a group of small cells called root primordia, which then develop further. If meristem cells are exposed to irradiation at too high a dose, serious damage can occur to the tissue. As a result, plantlet growth will be disrupted, so that the plantlets are unable to develop into roots or leaves. This is in line with research by Rahmawati (2003) which showed that inhibition of *Phalaenopsis amabilis root growth* occurred in all irradiation treatments.

Root Length (cm)

The treatment of gamma ray irradiation mutant dose showed significant results. very real to long root plantlets *D. anosmum*. The highest root length was produced at a gamma ray dose of M_0 (control) with a value of 4.08 cm. Treatment type media base modification shows results influential significant effect on the root length of *D. anosmum plantlets*. The highest root length was produced on the D_f media type. with a value of 12.91 cm (Table 3).

Treatment with gamma radiation doses also affects root growth. Differences in root size at each dose level indicate that the gamma radiation administered can inhibit plant growth. The higher the radiation dose used, the greater the level of damage to the plant, thus inhibiting overall growth, including root growth (Sulistianingsih, 2009).

***Browning* Percentage (%)**

This observation variable was not analyzed for variance but rather the research data was tabulated and described descriptively because there was a lot of missing data, so the number of sample data did not meet the requirements for analysis of variance. In table 4 it can be seen that the average *browning percentage* is shown by the $M_5 D_m$ and $M_5 D_n$ treatments with a value of 62.5% plantlet *browning* (Table 4).

Table 4. Effect of in vitro modified base media type and gamma ray irradiation dose on *browning percentage* (%) of D. anosmum orchid mutant

Observation Variables	
Treatment	Percentage *(%)*
M_0D_f	0
M_5D_f	37.5
$M_{10}D_f$	0
M_0D_m	0
M_5D_m	25
$M_{10}D_m$	**62.5**
M_0D_n	0
M_5D_n	25
$M_{10}D_n$	**62.5**

Information: Column Which filled number zero (0) show that the plantlets did not experience *browning.*

A change in color to brown (chocolate) in the plantlets is an indication of cell damage caused by by stress oxidative consequence Gamma radiation exposure. In general, increasing doses of gamma ray irradiation cause increased browning rates in *Dendrobium orchid plantlets* in *vitro*. This *browning* is a physiological response to irreversible cell damage caused by radiation exposure. In other words, the higher the gamma ray dose administered, the higher the level of *browning* that occurs, which ultimately negatively impacts the growth and survival of the plantlets.

Total Fresh Weight (g)

Treatment mutant dose irradiation ray show results Which gamma has a very real effect on the total fresh weight of *D. anosmum plantlets*. Heavy fresh the total highest produced on dose ray gamma M_0 (control) with a value of 0.66 g. Treatment media types base modification show influential results real to heavy fresh total plantlets *D. Anosmum*. Fresh weight total Which highest produced on type media D_f with value of 0.54 g (Table 3).

Wijiono's (2016) research found that irradiation of this species often causes a decrease in several growth parameters, such as dry weight, fresh weight, and plant height. However, this decrease did not show a significant effect. Plant dry weight reflects growth results, which are the accumulation of assimilation processes and serve as an indicator of successful plant growth.

CONCLUSION

The basic media type FFM (*Foliar Fertilizer of Medium)* provides the best response to the morphological growth of orchids. mutant *D. anosmum* Which added light irradiation gamma rays (0.5,10) gy compared to commercial media such as MS (*Murashige and Skoog)* and NP media (*New Phalaenopsis).* Dose irradiation ray gamma influential very real to plant height variables, number of leaves, number of roots and length of orchid roots mutant *D. anosmum.* Type doses of 5 and 10 gy can cause orchid plantlets to become shorter compared to orchid plantlets that are not exposed to gamma ray irradiation. There is a significant interaction between the dose of gamma ray irradiation (0, 5, 10) gy with the type of *in vitro modified base media* (MS, NP, FFM) on the observation variable of plant height. Showing the highest value in the $M_0D_{f\ treatment}$ with a value of 2.34 cm. Based on the research

results, for planting the mutant orchid *D. anosmum,* you can use the basic media type FFM (*Foliar Fertilizer of Medium)* because this media contains more nutrients than other media and media. This Also more own price Which more cheap compared to media others such as MS, NP etc.

REFERENCES

Asza, AA, Moeljani, IR, Koentjoro, Y., 2022. GAMMA60CO Ray Radiation Induction Dose 3 Gy To Genetic Diversity Population Mutant (M4) of Bauji Variety of Red Onion Plant. AGROHITA, 7(2), pp.332- 336.

Data, P. 2015. Agricultural Information System. 2015. Food Consumption Bulletin, 5(1), 1-59.

Fandani, HS, & NengahKorja, SNMI 2018. Diversity of orchid species in several breeding centers in Ampera Village and Karunia Village, Palolo District, Sigi Regency. Warta Rimba Journal E-ISSN, 2579 (6287), 2014.

Kasutjianingati and R. Irawan. 2013. Alternative Media for In Vitro Propagation of Moon Orchids (*Phalaenopsis amabilis*). Agroteknos Journal. Vol 3 (3): 184 - 189.

Maitra, S., Sairam, S., Shankar, T. and Gaikwad, DJ 2020. Cultivation of Dendrobium Orchids in Greenhouse. In: Protected Cultivation and Smart Agriculture. New Delhi Publishers, New Delhi, India.

Oktavina, Z. 2011. The effect of gamma ray irradiation on the growth of hybrid orchids: *dendrobium sclerii* x *may neal wrap* in *vitro*.

Qosim, WA, PURWANTO, R., & WATTIMENA, GA 2007. The effect of gamma ray irradiation on the regeneration capacity of nodular callus of mangosteen plants. Journal of Biological Biosciences, 14 (4), 140-144.

Rachmawati, A. (2019). Hybrid Moon Orchid Callus Induction (*Phalaenopsis Black Jack* X *Yupin Sweety*) on Alternative Culture Media with Compound Leaf Fertilizer (Doctoral Dissertation, Uin Sunan Kalijaga).

Soedjono, S. 2003. Application of induced mutation and somaclonal variation in plant breeding. Journal of Agricultural Research and Development, 22(2), 70-78.

Soeranto, H. 2003. The Role of Nuclear Science and Technology in Plant Breeding to Support the Agricultural Industry. Center for Research and Development of Isotope and Radiation Technology. National Nuclear Energy Agency. Jakarta. 20 pp.

Srivastava, D., Gayatri, MC, and Sarangi, SK 2018. In vitro mutagenesis and characterization of mutants through morphological and genetic analysis in the orchid Aerides crispa Lindl. Indian Journal of Experimental Biology, 56(6), 385–394.

Sulistianingsih.R, Mangoendidjojo.W, Purwantoro.A and Semiarti.E. 2006. The Effect of Gamma Ray Irradiation on the Growth of Moon Orchid Planlets *Phalaenopsis amabilis* (L.) BI. Agricultural Journal. Faculty of Agriculture, UGM Yogyakarta.

Suryowinoto, M.1996. In vitro Plant Breeding. Kanisius Publisher. Yogyakarta.

Tuhuteru, S., Hehanussa, ML, and Raharjo, SH 2012. Growth and development of *Dendrobium anosmum orchids in in vitro* culture media with several concentrations of coconut water. Agrologia, 1 (1), 288770.

Wardhani MUD, DM Puspitaningtyas, and D Dinarti. 2007. The Effect of Gamma Ray Irradiation on Growth Variation of *Brachypeza indusiata* (Reichb.f) Garay Orchids *In Vitro*. Indonesian Botanical Garden Bulletin. Vol 30 (2): 53-59.

Wijiono, Rudin, 2016. The Effect of Soaking Time and Mutagen Concentration of EMS (*Ethyl Methane Sulfonate*) on the growth of plants (*Glycine max* L.) Dering 1 Variety. UIN Maulana Malik Ibrahim, Malang.