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Diversity Induction of *Dendrobium sylvanum* Orchid through *In Vitro* Irradiation of Gamma Ray

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Abstract

Dendrobium sylvanum orchid is categorized as lowland habitus orchid and able to survive wihtout any shade. Orchid cultivation takes a very long time so it needs faster cultivation and producing large quantities. Therefore, it is necessary to do orchid culture by using tissue culture processes and then given gamma ray irradiation to bring up new characters in the orchid .The purpose of this research was to discover the impact of gamma ray irradiation in vitro to the diversity of D. sylvanum. The doses of gamma ray used in this research were 0 Gy, 15 Gy, 30 Gy, 45 Gy, and 60 Gy. The results obtained indicated that mutation mostly occurred on the treatment with 15 Gy (4.4 cm of plant height, 2.67 of leaves, 2.36 cm of leaf length, 0.49 cm of leaf width, and 5.33 of root strands) compared to the other doses and the control plants. The new finding in this research is the gamma ray dose that can optimally stimulate the mutation in *D. sylvanum*. This information is useful to generate the new variety in orchid cultivation in Indonesia. This research provides innovation in orchid cultivation and new variety that is possible to arise after the mutation.

How to Cite

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INTRODUCTION

Dendrobium is one of the genera of orchid plants which becomes the favorite in the Indonesian community and the second most favorite in the world. *Dendrobium* is the most favorite commodity because the nature of this flower is relatively more sustained compared to the other types of orchid and it has variation of colors, which make its market potential bigger. *Dendrobium* genus has been adequately distributed in the Asian regions, such as Indonesia and Philipines. In the regions of Borneo, there are 143 species of *Dendrobium* orchid and most of them are discovered in the forests (Sabran *et al*., 2003). *Dendrobium* orchid could also be found in Eastern part of Indonesia, such as Papua and Maluku (Widiastoety *et al*, 2010). One of the species of *Dendrobium* which becomes the favorite is *Dendrobium sylvanum*. *D. sylvanum* orchid could only be found in the areas of Papua New Guinea, New Ireland, Bougainville, and Solomon Island. Therefore this species required more cultivation. Flower of *D. sylvanum* has intermediate shape in greenish brown color with pink tinge and purple line. The flower is relatively last longer and in one blooming period, it could produce about 30-40 of flower buds.

The flower produced by *D. sylvanum* orchid has high economic value, therefore, the demand for this orchid continues to increase. In order to fulfill the market demand, quantity cannot be put as the only determining factor, but the quality of the orchid has to be considered as well. In Indonesia, the production of orchid is expected to increase. The obtained data shows 16,166,628 pots produced in 2005 had increased to 19,284,219 pots in 2010. This condition is in accordance with the quality standard of domestic and international markets (Dirjen Hortikultura, 2005).

The effort conducted to improve the gene and to multiply the quantity of plant can be done through the in vitro tissue culture technique. This technic is able to accelerate the multiplication of plant in vegetative or generative manner as well as facilitate the mutant selection. Then, the process continues with gamma ray irradiation (mutation) which is aimed to improve the characteristics of the plant, either qualitatively or quantitatively, therefore, the desired characteristics of plants' biochemistry, physiology, and morphology can be produced. In the breeding of plant, irradiation mutation has important role to improve the desired characteristics without changing the other characters, moreover, the plant provided with the exposure of gamma ray irradiation is having heterozygote characteristic that is able to produce a high variety after the irradiation process. The factor which is able to influence the gamma-ray irradiation is the difference of irradiation dose that will be given to the plant. A high dose could kill the materials of the plant which will be mutated, causing sterilization and death of the plant as well, while, plants exposed to the low dose could still be recovered from the received damage and will not cause death on plant (Soedjono, 2003).

The similar research conducted by Suwarno *et al*. (2012) showed that the application of higher dose will accelerate the initiation of leaf and root, however, the growth and height of *Phlaenopsis amabilis* become blocked. In addition, in the research of Dehgahi *et al* (2017), it was mentioned that the life sustainability of plant and the weight of PLBs (*Protocorm Like Bodies*) are on the contrary with the dose of gamma ray irradiation applied, namely controlled treatment of vitality and weight of plant, which are multiplied by tenfold through 200 Gy of irradiation dose which only has fivefold of vitality.

The purpose of this research was to discover the impact of gamma ray irradiation in vitro to the diversity of *D. sylvanum*. This research was expected to provide contributions in biology or agriculture field, especially in cultivation of *D. sylvanum* orchid through gamma ray irradiation.

METHODS

Media Production and Sterilization

The tools used in this study were glassware, scalpel, scissor, forceps, petri-dish, Beaker glass, and so on. The tools used in the planting process were sterilized to prevent contamination. Sterilization was performed using autoclave on 180^0C for ± 3 hours. After that, 40 gr/l of granulated sugar, 250 ml of coconut juice, 100 gram/l of banana juice, thiamine 20 ppm, 14 gram/l of gelatin powder, and 2 gram/l of active charcoal were made. Distilled water was added until the volume became 2000 ml, then the solution was stirred until it became homogenous by using magnetic stirrer. The level of media pH was set to reach 5.4 by adding HCl if the pH is higher than 5.5 and adding the NaOH if the pH was less than 5.5.

Sterilization of Orchid Seeds Explants

Orchid seeds used as explants needed to be sterilized in prior to prevent contamination in the planting of plant tissue culture. The orchid seeds were sterilized by immersing them into the spirits and then they were burned on a Bunsen burner.

The Planting of Orchid Seeds

After the sterilization of explants materials, the orchid seeds were divided into four parts with scalpel and distributed equally on VW media that has been made in prior. The tissue culture media which had been planted were tightly sealed using the plastic wrap.

Irradiation of Gamma Ray on Plantlet

The growing orchid plantlet that was then irradiated using 4000 A of Gamma Chamber with 5 different doses of 0 Gy, 15 Gy, 30 Gy, 45 Gy, and 60 Gy. After that, the plantlet was sub-cultured to ensure the sufficient nutrition of plantlet.

Acclimatization

Plantlets were then moved into the new growing media as acclimatization places. The media used were charcoal and fern. Acclimatization was conducted for 3 months.

RESULTS AND DISCUSSION

The acclimatization stage was a transformation stage from the controlled and sterilized condition in which the plant was cultured in a culture media bottle to maintain its temperature and humidity (Widiarsih & Ita, 2013). The acclimatization stage caused stress on the plant treated by gamma ray irradiation. The stress on the plant was due to the change of environment from anaerobe condition. This condition occurs because the plantation medium located in culture room was in a stable condition. The plant was transferred to aerobe condition by using non-sterile medium that causes the death on treatment without gamma ray irradiation (control) after the first acclimatization week. Some acclimatized orchid plants with low doses of radiation were able to perform recovery on the following week. However, the plants with higher doses were having a longer recovering process and as a result they were unable to survive.

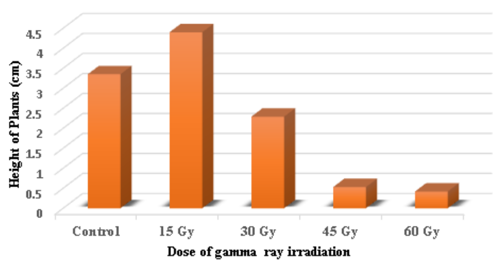

Figure 1. Height of *D. sylvanum*

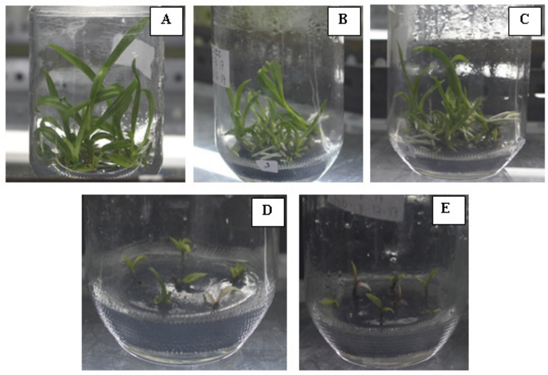

Figure 2. The results of the culture process after the plants are given gamma ray irradiation (a) Control *D. sylvanum* (b) *D. sylvanum* with 15 Gy (c) *D. sylvanum* with 30 Gy (d) *D. sylvanum* with 45 Gy (e) *D. sylvanum* with 60 Gy.

According to the results obtained (Figure 1), it is known that the highest height of plants was obtained from 15 Gy dose of gamma ray irradiation (4.4 cm). The average height of plants with 15 Gy is better compared to the control plant with 3.35 cm . While the highest dose (60 Gy) resulted in the lowest growth of plants with only 0.43 cm in height. Before acclimatization was performed on plant, explants with 45 Gy and 60 Gy of doses were mostly unable to survive until the end of research. The death of explants occurred due to the contamination of mosh on explants after sub-cultured process. The treatment of gamma ray irradiation through the application of 0-30 Gy doses was not generating any negative impact towards the growth of explants.

The result of this study shows that the height of the plant with 15 Gy dose is better compared to the control plant. Similar results were also obtained in the research of Wardhani *et al.* (2007) in which the plant with 20 Gy of dose provides better vitality compared to the plant without irradiation treatment (control). Besides, similar research was also produced by Yadav (2016) in which the effect of gamma ray provision on *Canscora decurrens* at low doses, i,e, 10, 15, and 25 Gy had generated better results compared to controlled treatment. These doses were called as stimulation (positive development) because they were able to produce heights of plant, quantity of *nodus*, the number of leaves and significant length of root. On the treatment with higher doses, i.e. 30, 35, 40, 45, and 50 Gy, the significant decrease of growth in *Canscora decurrenns* occurred, therefore, these doses were determined as inhibitor (negative development) (Yadav, 2016).

The provision of gamma ray generates effect that might happen spontaneously, thus, it causes a mutagen. In this research, the effect of gamma ray irradiation has been tested on different growth parameters (heights of plant, number of leaves, width and length of leaves, and the number of roots). The exposure of gamma ray produces positive impact at low doses but generates negative impact on high doses that cause inhibition as well as decreasing level of growth and maturity of plant. Besides the irradiation applied on the plant, the effect produced by the exposure of gamma ray irradiation on microalgae also occurs as performed by Ermavitalini *et al*. (2017) in which gamma ray with 10 Gy dose reveals deceleration phase in microalgae growth. The gamma ray radiation caused the cells on microalgae to experience oxidative stress which then triggered the cell to perform defensive process to maintain the growth and reduce the generated stress effect. The gamma ray irradiation provides different levels of sensitivity in one type of plant with another. High dose of gamma ray irradiation will cause the damage and affect the working system of plant's meristem cells as well as causing the death of plant.

The provision of gamma ray irradiation generates significant impact towards the post-irradiation growth of plant. Gamma ray irradiation could inhibit the growth of plant since the irradiation is the radiation applied with radioactive, therefore, it could generate mutation. This condition occurs because higher dose of irradiation applied will make the growth rate of plant lower. The obtained heights of plant on 45 Gy dose of irradiation treatment, are 0.53 cm, and the lowest heights of plant acquired on 60 Gy of dose with 0.42 cm of result. This result is in line with the research conducted by Anshori *et al*. (2014) in which the growth of the heights of *Curcuma domestica* Val. plant acquires its lowest value on 40 Gy of dose caused by high number of the death plant. This condition is determined by the existence of high radio-sensitivity level of turmeric rhizome towards irradiation dose. Gamma ray irradiation which causes random mutation on plant will construct physiological damage in the metabolism of cell development, therefore, the potential of the growth will become faster or slower (Anshori *et al*., 2014).

Higher irradiation dose application will make the growth of the vegetative state continue to decrease. In a research of Rashid *et al*. (2013), the gamma ray irradiation on ginger plant had resulted in the decrease of the growth of sprout along with higher and longer exposure of gamma ray.

The Improvement on the Number of Leaves

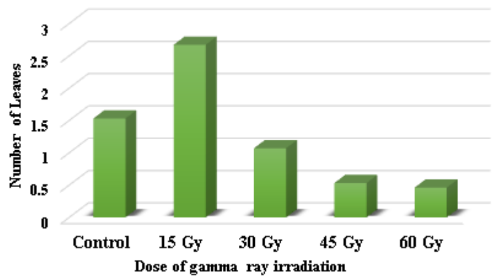

Figure 3. The improvement of the number of *D. sylvanum* leaves

The obtained results indicate that the treatment of gamma ray irradiation provides significant impact on the number of leaves. This result means that through the gamma ray irradiation, the increasing number of leaves can be stimulated. According to Figure 3, it is shown that the highest average of leaf quantity improvement is obtained in 15 Gy with 2.67 and the lowest number of leaves is in 60 Gy with 0.47. The 15 Gy irradiation generates more leaves compared to the control plants. This result is similar with the research of Wardhani *et al*. (2007) that the highest improvement of number of leaves is obtained in 10 Gy of dose with 1.16, 10 G in which 10 Gy of dose is higher compared to control plants. This result is also in line with the research of Suwarno *et al*. (2013) that showed that the optimum number of leaves was obtained from 20 Gy of dose with 7 and control with 5.14.

In 45 Gy and 60 Gy, the leaves experiences inhibition on its growth because a higher dose will generate more inhibition on the growth of plant. Similar condition occurred in the research of Suwarno *et al*. (2013) in which 45 Gy of dose indicated significant difference towards the speed of initiation. Physical damages generated through the gamma ray irradiation are the death of cell, the inhibition of cell division, and the occurrence of mutation, therefore, the leaves will be smaller compared to the control plants (without gamma ray irradiation). Gamma ray irradiation with high dose will disrupt the processes of protein synthesis, the balance of plant hormone, and the gas exchange on leaf (Borzouei *et al*., 2010). The small number of leaves obtained from the treatments with 45 Gy and 60 Gy is caused by high dose of irradiation which is able to cause damage or change of chromosome that will affect the growth rate of leaves. The smaller number of leaves as a result of gamma ray irradiation compared to the control plants is a common phenomenon that occurs in the plant with mutation treatment. The research conducted by Devy & Sastra (2006) showed that gamma ray irradiation on cultured ginger resulted in plants that was not able to form leaf even when they were already had sprout. In addition, similar research conducted by Widiarsih and Ita (2013) mentioned that 60 Gy dose on *Phalaenopsis amabilis* orchid resulted in smallest average number of leaves (0.34).

Length and Width of Leaf

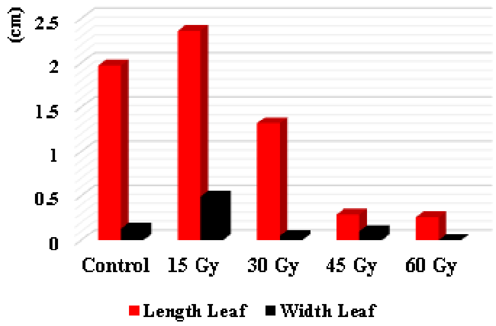

Figure 4. Length and width of *D. sylvanum* leaves

According to Figure 4, the results show that the highest average length of leaf of *D. sylvanum* orchid is obtained in the treatment of 15 Gy with 2.36 cm. This provides a real difference towards average length of plant's leaves on control plants. The lowest average of length of leaves is acquired in 60 Gy with 0.26 cm. The research of Tongpong *et al.* (2009) stated that 0-30 Gy provision on *Anubias congenis* plant produced better development of width and length of leaves compared to the provision of higher doses. In the time the *Anubias congensis* plantlet was exposed by severe gamma ray radiation, the width and length of leaves experienced decreases on the growth and the development. Plantlets with 105.99 Gy and 120.30 Gy showed statistically significant decrease on the number of new sprouts, as well as the width and length of leaves compared to the control plants.

Results of observation show that the highest average of *D. sylvanum* orchid leaves width found in 15 Gy of dose with 0.49 cm and the lowest average is in 60 Gy of dose with 0.05 cm. The results of treatment with 15 Gy shows the significant difference towards the width of leaves on control plants. The treatment of gamma ray irradiation with high dose will cause a disruption of plant's development. This condition is seen from the narrowing form of leaf on gamma ray irradiation with high dose, namely 45 Gy of dose with 0.1 cm of width, and 60 Gy of dose with 0.05 cm of width.

The Number of Roots

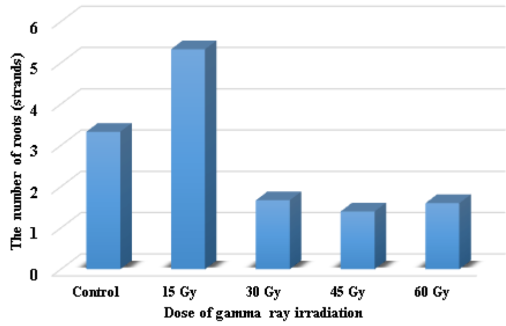

Figure 5. The number of roots of *D. sylvanum*.

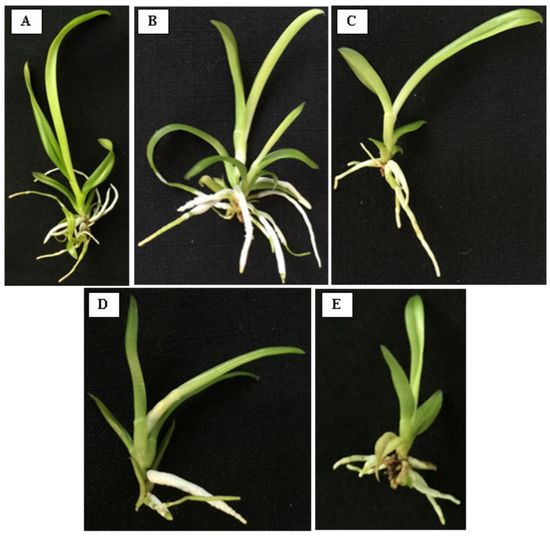

Figure 6. Results of acclimatization of *D. sylvanum* orchids after gamma ray irradiation (a) Control *D. sylvanum* (b) *D. sylvanum* with 15 Gy (c) *D. sylvanum* with 30 Gy (d) *D. sylvanum* with 45 Gy (e) *D. sylvanum* with 60 Gy.

Gamma ray irradiation with 15 Gy produces the average number of roots which is adequately high compared to the both other treatments and the control group. It can be seen that 15 Gy of dose generates 5.33 root strands and the lowest number of roots is obtained from 45 Gy. This condition is assumed due to the light exposure resulted from gamma ray irradiation with 15 Gy dose, therefore, the plant in this dose is able to recover itself faster compared to the other doses, and able to grow normally. In a research conducted by Gonzales (2007), 10 Gy dose on ground orchid had resulted in decreasing number of root and inhibition on the growth of plant. Gamma ray irradiation caused the decline of the cell that affects the development of plant root. Otherwise, research by Suwarno *et al*. (2013) stated that 10 Gy dose administered to *Phalaenopsis amabilis* did not provide a significant result towards the number of roots compared to the plant without irradiation treatment.

In addition, Cahyo (2015) also stated that the treatment of 20 Gy on *Dendobrium lasianthera* (JJ. Smith) produced the highest average number of roots compared to others doses. Meanwhile, in the treatment of gamma ray irradiation with 45 Gy and 60 Gy of doses, the average number of roots waere lower compared to the treatment with 15 Gy and control group. On 30 Gy and 60 Gy doses, 1.67 leaves was obtained. The research conducted by Suwarno *et al*. (2013) stated that 30 Gy dose of gamma ray irradiation on *Phalaenopsis amabilis* orchid produced more significant acceleration of the root initiation compared to the plant without the irradiation. It means that the provision of gamma ray irradiation does not inhibit the speed of root initiation.

Meanwhile, 45 Gy dose of gamma ray irradiation resulted in lower average number of roots compared to the 15 Gy dose and the control group. This condition also occurred in the research by Tongpong *et al*. (2009) in which 40 Gy dose on *Anubias congensis* showed no effect on the development on the plant's root. This result was assumed to occur due to the excessive dose of gamma ray irradiation that causes the damage to cell of *plb* (*Protocorm Like Bodies*), therefore, numerous deaths were found on *plb*, while the *plb* which is able to survive experiences sluggish growth. Faster root initiation has correlation with the leaf initiation. After leaf is formed, the part of radicle will differentiate to form root by absorbing the content of micro and macro elements available in the media (Suwarno, 2013).

The meristem cells which continue to be exposed by gamma ray irradiation with high dose will experience severe damage and then the plantlet growth will be inhibited and unable to form roots and leaves. The research of Devy & Sastra (2006) stated that the gamma ray irradiation was able to inhibit the root development on ginger plant as a result of disruption on the activities of endogen auxin which occurred after the exposure of the irradiation, therefore, the concentration of endogen auxin will decrease and the root will not be formed.

The benefits of this study can be used for further research such as doses to be used in irradiation, and for orchid farmers can help in generating new characters from mutations produced by orchids after gamma ray irradiation treatment.

CONCLUSION

According to the results of this research, it can be concluded that through gamma ray irradiation, the variety of *Dendrobium sylvanum* orchid is able to be influenced on its growth. The occurrence of mutation on the treatment of 15 Gy of dose indicates the most optimal result from the entire measurements, including the height of plant, the number of leaf, the length of leaf, the width of leaf, and the number of roof compared to the controlled treatment as well as the treatments with the other irradiation doses.

REFERENCES

Anshori, Rosyidah, S., Aisyah, S.I. & Darusman, L.K. (2014). Induksi Mutasi Fisik dengan Iradiasi Sinar Gamma pada Kunyit (*Curcuma domestica* Val.). *J. Hort. Indonesia,* 5(3), 84-94.

Borzouei, A., Kafi, M., Khazaei, H., Naseriyan, B. & Majdabadi, A. (2010). Effect of gamma radiation on germination and physiological aspect of whea (Triticum aestivum L.) seedling. *J. Bot.,* 42(4), 2281-2290.

Cahyo, Fitro Adi., dan Diny Dinarti. (2015). Pengaruh Iradiasi Sinar Gamma Terhadap Pertumbuhan *Protocorm Like Bodies* Anggrek *Dendrobium lasianthera* (JJ. Smith) Secara In Vitro. *J. Hort. Indonesia,* 6(3) : 177-186.

Dehgahi R., dan A. Joniyasa. (2017). Gamma Irradiation-Induced Variation in Dendrobium Sonia-28 Orchid Protocorm-Like Bodies (PLBs). *Fungal Genom Biol,* 7, 151.

Devy, L., dan Sastra DR. (2006). Pengaruh iradiasi sinar gamma terhadap kultut in vitro tanaman Jahe. *J Sain dan Teknologi,* 8(1) : 7-14.

Dirjen (Direktorat Jenderal) Hortikultura. (2005). *Kinerja Pembangunan Sistem dan Usaha Agribisnis Hortikultura.* Jakarta: Departemen Pertanian Press.

Ermavitalini, Dini., Niki Yuliansari, Endry Nugroho Prasetya, Triono Bagus Saputro. (2017). Effect of gamma 60 Co irradiation on the growth, Lipid content and fatty acid composition of *Botryococcus* sp. microalgae. *Biosaintifika,* 9(1), 58-65.

Gonzales, MA. (2007). Radiosensitivity of three species of ground orchid (*Spatoglotis plicata, S. kimballiana var. angustifolia* and *S. tomentosa*) to acute gamma radiation. *Thesis.* Philippines (PH): Central Luzon State University.

Rashid, K., A.B.M. Daran, A. Nezhadahmadi, K. Hazmi, S. Azhar, S. Efzueni. (2013). The effect of using gamma rays on morphological characteristics of ginger (*Zingiber officinale*) plants. *Life Sci J,* 10(1), 1538-1544.

Sabran, M., Krismawati, Galingging, dan Firmansyah. (2013). Eksplorasi dan Karakterisasi Tanaman Anggrek di Kalimantan Tengah. *Buletin Plasma*

Nutfah, Vol.9. No.1, 1-6.

Soedjono, S. (2003). Aplikasi Mutasi Induksi dan Variasi Somaklonal dalam Pemuliaan Tanaman. *Jurnal Litbang Pertanian,* 22 : (2).

Suwarno, A., N. A. Habibah dan Herlina. (2013). Respon Pertumbuhan Planlet Anggrek *Phalaenopsis amabilis* L. var. Jawa Candiochid Akibat Radiasi Sinar Gamma. *Unnes Journal of life science,* 2 (2), 78-84.

Tongpong, Pakorn., Thanya Taychasinpitak, Choosak Jompuk and Peeranuch Jompuk. (2009). Effect of Acute and Chonic Gamma Irradiations on In Vitro Culture of *Anubias congensis* N.E. Brown. *Kasetsart J. (Nat. Sci.),* 43, 449-457.

Wardhani, Maria Utami Dewi., Dwi Murti, dan Diny Dinarti. (2007). Pengaruh iradiasi sinar gamma terhadap variasi pertumbuhan anggrek *Brachypeza indusiata* (Reichb.f). *Buletim Kebun Raya,* Vol 10. No 2, 53-59.

Widiarsih, Sasanti., dan Ita Dwiwahyuni. (2013). Gamma Irradiation Application for Mutation Breeding in Earli Flowering Moth Orchid (*Phalaenopsis amabilis* B1). A scientific *Journal for the Application of Isotopes and Radiation,* 9(1), 59-66.

Widiastoety, D., N. Solvia, dan S. Kartikaningrum. (2009). Pengaruh Tiamin terhadap Pertumbuhan Planlet Anggrek *Oncidium* Secara *In Vitro*. *J. Hort,* 19 (1), 35-39.

Yadav, V. (2016). Effect of gamma radiation on various growth parameters and biomass of *Canscora decurrens* Dalz. *Internasional Journal of Herbal Medicine,* 4(5), 109-115.