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Effect of Gamma Irradiation on Callus of Handeuleum (Graptophillum pictum L. Griff) *Kalimantan and Papua Accession*

Efek Iradiasi Sinar Gamma pada Kalus Handeuleum *(Graptophillum pictum* L. Griff) Aksesi Kalimantan dan Papua

Arrin Rosmala[1*], Nurul Khumaida[2], Dewi Sukma[2]

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ABSTRACT

Handeuleum (Graptophyllum pictum L.Griff) is a medicinal plant that is widely used as a traditional medicine for its healing properties, hence it should be developed as one of the leading Indonesian medicinal plants. The combination of tissue culture and gamma irradiation are two common methods to improve vegetatively-propagated cultivars. This experiment aims to determine the effect of gamma irradiation dose-rate on the callus culture of the two handeuleum accessions. Callus generated from proliferation were irradiated with gamma rays (0 Gy, 15 Gy, 25 Gy, and 35 Gy) and then grown on regeneration medium containing BAP (4.44 µM and 8.88 µM) and TDZ (4.44 µM and 8.88 µM). The result of the experiment shows that RD50 was at 19.25 Gy. The interaction among the Papua accession, gamma irradiation dose-rate of 15 Gy, and regeneration media 4.44 µM BAP produced the highest weight of callus subculture 1 and 2. Irradiation dose-rate of 25 Gy on Kalimantan accession resulted in the highest relative fresh weight growth of irradiated callus. The treatment with a 25 Gy dose-rate produced brownish-white callus and produced the highest value of variability in the relative growth rate of irradiated callus.

Keywords: accession, irradiation dose-rate, purple leaves, variability

ABSTRAK

Handeuleum (*Graptophyllum pictum* L.Griff) adalah tanaman obat yang telah digunakan secara luas sebagai obat tradisional karena memiliki kandungan yang berkhasiat obat, dan dapat dikembangkan sebagai salah satu tanaman obat asli Indonesia. Kombinasi antara kultur jaringan dan iradiasi sinar gamma adalah metode yang efektif untuk memperbaiki kultuvar yang diperbanyak secara vegetatif. Penelitian ini bertujuan melihat efek dosis radiasi sinar gamma pada kalus aksesi Kalimantan dan Papua. Kalus diiradiasi dengan sinar gamma (0 Gy, 15 Gy, 25 Gy, dan 35 Gy) lalu diregenerasikan pada media regenerasi yang mengandung BAP (4.44 µM dan 8.88 µM) dan TDZ (4.44 µM dan 8.88 µM). Hasil penelitian menunjukkan bahwa RD_{50} ada di 19.25 Gy. Interaksi antara aksesi Papua, iradiasi sinar gamma dosis 15 Gy, dan media regenerasi 4.44 µM BAP menghasilkan bobot kalus subkultur 1 dan 2 paling berat. Dosis iradiasi 25 Gy pada aksesi Kalimantan menghasilkan laju pertumbuhan relatif kalus hasil iradiasi paling tinggi. Dosis iradiasi 25 Gy menghasilkan kalus warna putih kecoklatan sedangkan perlakuan lain tidak. Iradiasi sinar gamma dosis 25 Gy menghasilkan nilai ragam paling besar pada laju pertumbuhan relatif kalus hasil iradiasi.

Keywords: aksesi, daun ungu, dosis iradiasi, keragaman

[1]Program Studi Agroteknologi Pertanian, Universitas Perjuangan Tasikmalaya.
Jl. Peta No.177, Kahuripan, Kec. Tawang, Kec. Tasikmalaya, 46115, Indonesia.
[2]Departemen Agronomi dan Hortikultura, Fakultas Pertanian, Institut Pertanian Bogor.
Jl. Meranti, Kampus IPB Dramaga, Bogor, 16680, Indonesia.
Email: arrinrosmala@unper.ac.id (*Penulis korespondensi)

INTRODUCTION

Handeuleum (*Graptophyllum pictum* L.Griff) is generally used by Indonesian community to cure various diseases since ancient times. The results of phytochemical analysis and active component compound of thirteen accession *Graptophyllum pictum* L. Griff) conducted by Lestari (2015) have proven that accession number 1 (West Java) and accession number 9 (Papua) to be high in phytochemical content (anthocyanin and carotenoids) that is useful for treatment. According to Juniarti *et al*. (2021) ethanolic purple leaves extract of handeuleum has antibacterial activity against *Lactobacillus acidophilus*. Jiangseubchatveera *et al.* (2015) observed that the essential oils in handeuleum have the ability to poison (cytotoxity) KB, NCI-H187 and Vero. Handeuleum essential oils have antioxidants ability, also have antibacterial against *S.areus* and *E.coli*.

Gamma irradiation may affect plant growth and development by altering physiological, biochemical, and genetical processes as well as leading to morphological changes in cells and tissues (Hanafy and Akladious 2018). Gamma irradiation is performed on callus culture because their cells are meristematic, making it more responsive to radioactive compared to adult cells (Hartati *et al.* 2020). Khalil *et al*. (2015) found that various gamma irradiation dosage (5 Gy, 10 Gy, 15 Gy, and 20 Gy) on 30-day-old leaf derived callus cultures of stevia (*Stevia rebaudiana* Bert.). Gamma rays dosage 15 Gy slightly enhanced stevioside content (0.251 mg g^{-1}) over the control (0.232 mg g^{-1}) along with total phenolics and flavonoids contents. The combination of in-vitro culture technique and gamma ray-induced mutation improve vegetatively-propagated cultivars, such as on gerbera (Ghani *et al*. 2013) and grapes (Munir *et al*. 2015).

There has been no information and results of research on the effect of invitro culture and gamma ray irradiation on the variability of handeuleum. Therefore, in vitro and gamma irradiation technique are expected to generate new mutants as a source of variability in handeuleum. This study is aimed to determine the effect of gamma irradiation dose-rate to the variability of callus culture in handeuleum, Kalimantan and Papua accession.

MATERIALS AND METHODS

Procedures

The experiment was conducted in 2010 at the Laboratory of Plant Biotechnology and Laboratory of the Department of Agronomy and Horticulture, IPB. The Applications of irradiation was performed at Pusat Aplikasi Isotop dan Radiasi (PAIR) / Center for Isotope and Radiation Applications BATAN, Jakarta.

The experiment utilized factorial completely randomized design (CRD) with 3 (three) factors. The first factor is the handeuleum accession consisting of two (2) levels; Kalimantan and Papua accession. The second factor is the regeneration medium comprising 4 (four) levels: R1 (4.44 μM BAP), R2 (8.88 μM BAP), R3 (4.44 TDZ), R4 (8.88 μM TDZ). The third factor is the dose-rate of gamma irradiation consisting of 4 (four) levels, ie without irradiation 0 Gy (control), 15 Gy, 25 Gy, and 35 Gy. There were 32 combined treatments, where each experimental unit was repeated 10 times by 2 (two) explants per culture bottle.

The experiment used callus obtained from callus proliferation age 3 weeks (eksplant leaves were used to make the callus, and the callus used weighed 0.5 g.), which previously were cultured to the medium WPM 0 for 1 (one) week to eliminate the influence of the previous medium. Callus was then irradiated with gamma rays at some predetermined different dose-rates of treatment. The explants were then grown on the treatment medium for regeneration. The basic medium used consisted of macro- and micronutrients WPM medium, 3% sucrose, 0.2% gelrite, active charcoal, casein hydrolyzate, and growth regulators (BAP and TDZ) in accordance with the treatment.

Observations after callus have been irradiated include:

1. RD50, defined using Curve Expert Software1.4.
2. Interaction among accession, irradiation dose-rate and regeneration media on the weight of callus subculture 1 and callus subculture 2
3. Callus relative fresh weight growth (CRFWG) = (weight of callus subculture 2 - weight of callus subculture 1)/ weight of callus subculture 1. (Huang *et al*., 2015).
4. Colour of callus

Data Analysis

The experimental results were analyzed by using test F. If the result of the test real followed by Duncan's Multiple Range Test (DMRT). The distribution of the normality of data was analyzed by the Shapiro-Wilk system. Data was transformed with a scale of normalcy $\sqrt{X + 0.5}$ to approximate normal distribution. Contrast test was carried out on the callus proliferation experiments to determine the comparisons of treatment.

RESULTAND DISCUSSION

Reduce Dosage 50 (RD50)

According to Werdhiwati *et al.* (2020) the use of mutation techniques to induce diversity requires information on the LD50, which is the dose that causes 50% of individuals in the population to die. Based on Sinusoidal Fit Graph (Figure 1) Reduce Dosage 50 of callus culture is 19.25 Gy. The mathematical equation for the graph is y = a + bcos (cx + d). Where a = 9.26113122860E-001, b = 4.61372914461E-002, c = 1.18166181993E-001 and d = -102659525896E+000.

Figures 1 show that the increase in irradiation dose in line with the increase of percentage of dead callus. Irradiation dose of 19.25 Gy causes death in callus above 50%. Increased radiation dose of less than 30 Gy callus death more than 70%. Determination of lethal dose is important factors that must be known to obtain the desired mutant. By theoretical and strengthened by some results research, the LD20-LD50 range can be produce the highest diversity.

Interaction among Accession, Irradiation Dose-rate and Regeneration Media on the Weight of Callus Subculture 1 and Callus Subculture 2

Gamma irradiation very tangibly affected the weight of callus subculture 1 (Figure 2) and callus subculture 2 (Figure 3), as well as the rate of the relative fresh weight growth of callus (Figure 4). Accession, media, and dose-rate interacted to affect the variables.

Figure 2 shows that the highest weight of callus subculture 1 is produced by the Papua accession irradiated by 15 Gy of gamma rays and planted on the medium of 4.44 μM BAP (0.570 g). The highest weight of callus subculture 2 (Figure 3) is produced by Papua accession irradiated by 15 Gy of gamma rays, and grown on medium containing 4:44 μM BAP (0.595 g).

Genotypes that is used determine the success of induced mutations, parts of the plants irradiated and the dose-rate of applied mutagens also play critical role. Nonetheless, the ability to generate high weight of callus was not related to the ability of the respective accession to regenerate after irradiation. The Papua accession handeuleum irradiated with 15 Gy of gamma rays produced the highest weight of callus compared to other treatments, and its weight decreased along with increasing dose-rate of irradiation. Purnamaningsih *et al.* (2014) states that the irradiated soybean callus is influenced by plant genotype.

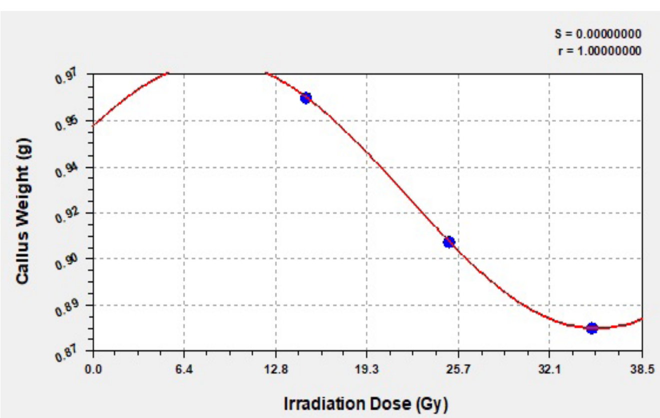

Figure 1. Callus weigt responses curve in different level of gamma ray irradiation

Callus Relative Fresh Weight Growth (CRFWG) of Handeuleum Callus Caused by Gamma Ray Irradiation

Gamma ray irradiation has a very significant effect on the relative fresh weight growth of Handeuleum callus on both accessions (Figure 4). The results presented in Figure 4 suggest that The CRFWG is influenced by the interaction between the dose-rate of gamma rays and handeuleum accession.

The highest CRFWG rate of irradiated callus was found in the Kalimantan accession irradiated with dose-rate of 25 Gy (0.448), while the lowest relative growth rate occurred to the Papua accession, irradiated with dose-rate of 35 Gy (0.083).

Gamma ray irradiation affects the relative fresh weight growth randomly; however, there is a general overview of changes in relative fresh weight growth of callus of both accessions. Figure 4 illustrates that the Kalimantan accessions generally have a higher relative fresh weight growth in all irradiation treatment compared to the Papua accession (except treatment of 15 Gy), thus a conclusion can be drawn that the Kalimantan accession is more resistant to gamma ray irradiation treatment compared to the Papua accession. It is in line with the research of Abdelnour-Esquivel *et al.* (2020) that each ricegenotype generates differing sensitivities for response of several parameters such as survival, regeneration, tissue growth, fresh weight, or dry weight after callus being exposed to gamma rays. Yunita *et al.* (2020) describe that Batugegi dan Situpatenggang rice variety also show different result in sensitivities in every observation of parameters.

Variability of Relative Fresh Weight Growth of Handeuleum Callus Caused by Gamma Ray Irradiation

This experiment shows that gamma ray irradiation can cause variability in the weight of post-irradiation callus (subculture 1 and 2) and also can be seen from the relative fresh weight growth. Based on Table 1, of the irradiated callus

Table 1. The variability of the relative growth rate of irradiatedcallus of handeuleum, Kalimantan and Papua accession.

Irradiation Dosage (Gy)	Variable			
	×	s	σ^2	CV (%)
0	0.07*	0.05	0.0025	74.09
15	0.05*	0.04	0.0019	87.22
25	0.07*	0.07	0.0047	92.11
35	0.04*	0.04	0.0013	102.77

Note: The figures of mean value (×) marked with (*), significantly different according to Duncan test 5%, s = standard deviation, σ2 = variability, CV = coefficient of variation.

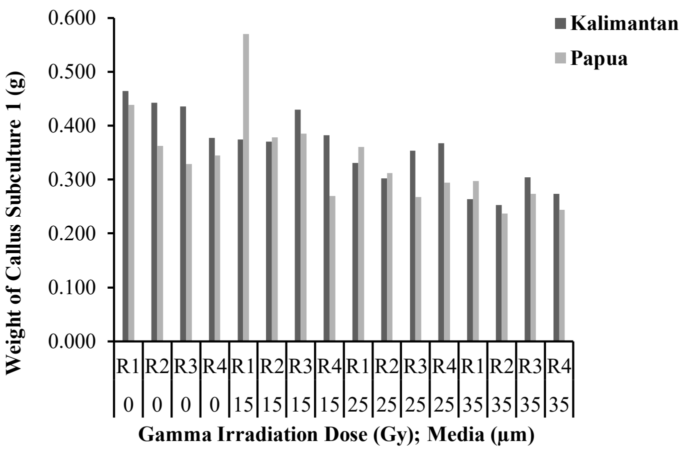

Figure 2. Callus weight from different level of gamma ray irradiation at subculture 1 after treatment

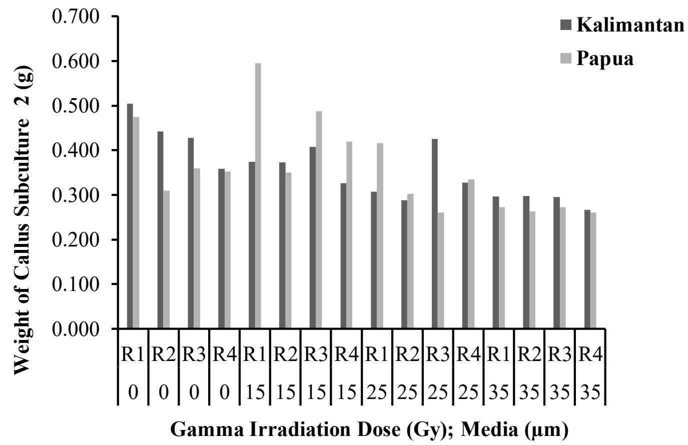

Figure 3. Callus weight from different level of gamma ray irradiation at subculture 2 after treatment

populations show variability in relative fresh weight growth. Based on the findings, it seemed that the relative fresh weight growth of callus on the control callus population (0 Gy), and the gamma irradiated ones at the dose-rates of 15 Gy, 25 Gy, and 35 Gy resulted in significantly different average values from each other. It shows that the gamma-ray irradiated callus populations generated the highest mean, standard deviation, and variance values, followed by non-irradiated callus population (control).

Table 1 shows that the CV value increases as the dose of gamma ray irradiation increases. The greatest CV value is produced by a 35 Gy gamma ray dose, which means that at this dose the variability is large. According to Dhanavel *et al.* (2012) morphological diversity in cowpea is caused by the effect of mutations.

Gamma ray irradiations have different effects on the weight of callus, as could be seen from the varying results of the variability analysis. Increasing dose-rate of gamma ray irradiation normally causes a decrease to the regeneration of callus (Nikam *et al.* 2015) as indicated by the weight loss and discoloration.

A research by Purnamaningsih *et al.* (2014) states that the regeneration of irradiated callus becomes more restricted than non-irradiated one (control). This is in line with research conducted by Yunita *et al.* (2020), which describes that irradiation dose of 30 Gy causes death in callus above 50% of Batutegi and Situpatenggang variety rice giving. Increased radiation dose of less than 30 Gy callus death more than 70%.

Hong *et al.* (2018) indicate that treatment with high doses of gamma irradiation has harmful effects on plants growth and development through the increase of free radicals. Oxidative stress caused by highly Reactive Oxygen Species (ROS) that generates by ionizing radiation. ROS can damage nucleic acids, peroxidation and carbohydrates, resulting cellular damage (Suzuki *et al.* 2012). Gamma ray irradiation may affect the primary metabolism, which in turn also affects the level of secondary metabolism in a plant (Jan *et al.* 2013).

Effect of Gamma Irradiation on the Colors of Two Handeuleum Accessions

The results showed that gamma ray irradiation has a very tangible effect on the color of callus (Figure 5). The colors of callus indicate the state of callus after irradiation treatment. There are four kinds of callus colors; brownish-white, whitish-brown, brown, brownish-black, and black. Black callus indicates dead callus.

Figure 5 and 6 showed that brownish-white callus could be found only in the treatment of 0 Gy (control), amounting to 5%, whereas the 25 Gy irradiation treatment produced brownish-white callus by 2%. The whitish-brown callus existed at all dose-rate of treatment with gamma rays, the dose-rate of 0 Gy (control) made up 24% of whitish-brown callus, dose-rate of 15 Gy and 25 Gy produced whitish-brown callus by 21% and 46% respectively.

Lastly, 35 Gy dose-rate of gamma rays produced whitish-brown callus by 5%. 0 Gy of irradiation produced brown callus of 22%, while the dose-rates of 15, 25 and 35 Gy resulted in 31%, 23%, and 40% respectively. Brownish-black callus is produced by gamma ray irradiation treatment successively as follows, 0 Gy (46%), 15 Gy (50%), 25 Gy (51%), and 35 Gy (49%). Black callus (dead callus) was generated only by the treatment of 25 Gy of gamma ray irradiation, amounting to 13%. There were no black callus in the other dose-rates.

The irradiated callus tends to be brown; it even can turn into black on a high-dose irradiation (indicating death of callus). Yunita *et al*. (2020) report that callus will experience browning after irradiated by gamma rays, because of messed

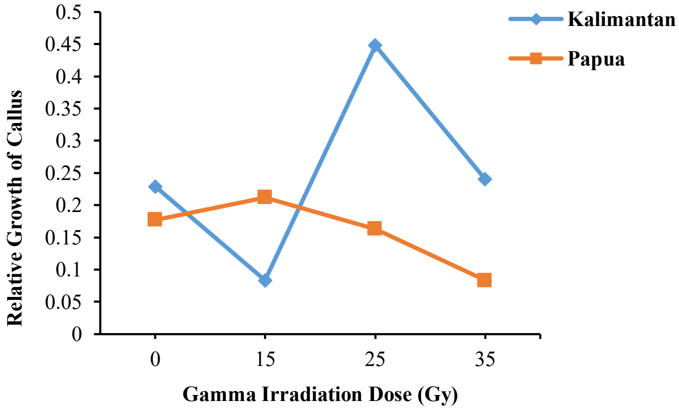

Figure 4. Relative growth of callus

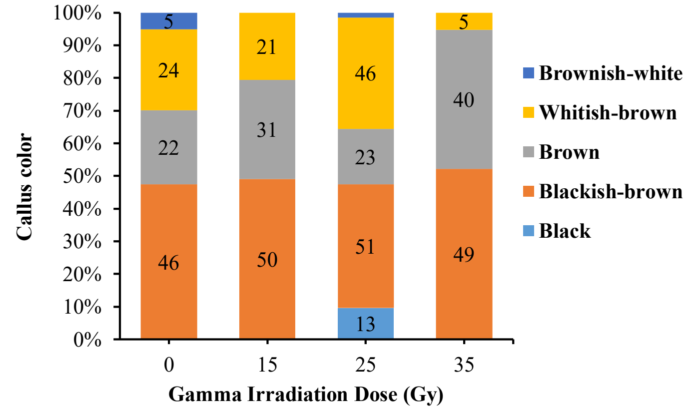

Figure 5. Callus color

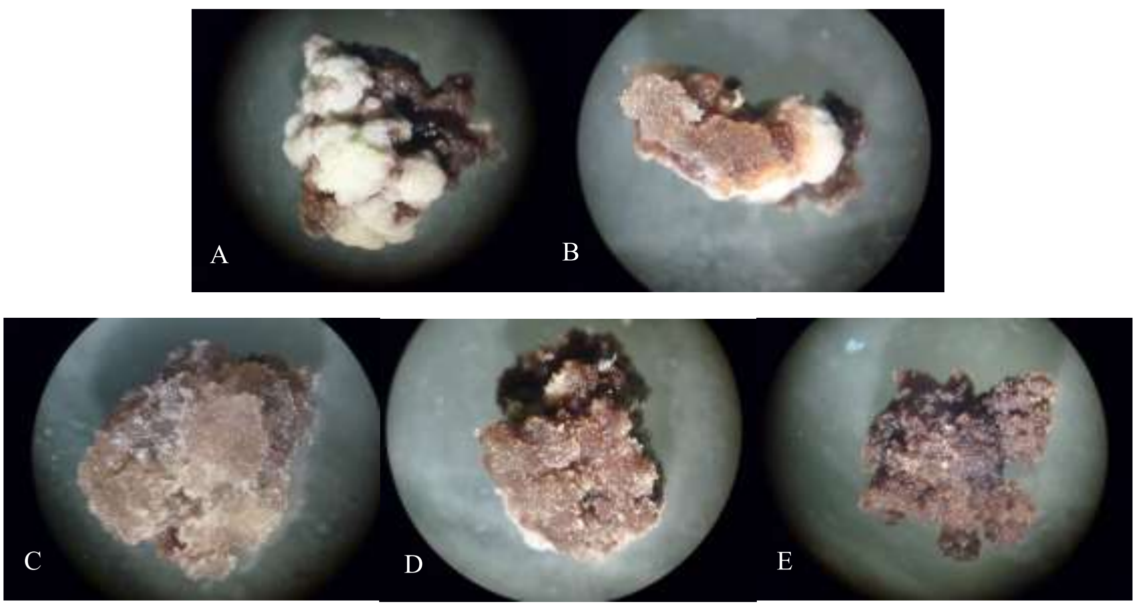

Figure 6. The colors of two irradiated handeuleum accessions: brownish-white (A), whitish-brown (B), brown (C), blackish-brown (D), and black (E).

up the biosynthesis of IAA that needed to regenerate somatic cell populations, because of the degradation of the enzyme indolacetol dehydrogenase which plays a role in the biosynthesis.

CONCLUSION

The interaction among the Papua accession, gamma irradiation at the dose-rate of 15 Gy, and regeneration media 4.44 μM BAP produced the highest weight of irradiated callus subculture 1 and 2, 0.570 g and 0.595 g respectively. Irradiation dose-rate of 25 Gy on Kalimantan accession resulted in the highest relative fresh weight growth of irradiated callus (0.448). Dose-rate 25 Gy produced a brownish-white callus of 13%, while other treatments did not. It also produced the highest value of variability in the relative fresh weight growth of irradiated callus (0.0047).

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