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IRRADIATION OF GAMMA RAYS IN *TYPHONIUM FLAGELLIFORME* CLUSTERS OF TISSUE CULTURE

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ABSTRACT

Typhonium flagelliforme is a group of grass plants from the Araceae family that has activity as an antioxidant and has the potential to be developed. Irradiation using gamma rays has been done to increase the antioxidant activity of the *Typhonium flagelliforme* clusters of tissue culture results. The purpose of this study was to investigate the effect of gamma ray irradiation on *Typhonium flagelliforme* clusters on antioxidant activity, growth, ploidy type, and DNA content. The antioxidant activity test was performed by DPPH method on cobs and *Typhonium flagelliforme* clusters which had been irradiated with gamma rays. Ploidy and DNA content analysis was performed using Flow Cytometer. The results of observation on the growth showed that the higher the dose of irradiation, the growth of *Typhonium flagelliforme*clusters more inhibited. Irradiation with gamma rays can increase antioxidant activity where the highest activity is obtained by cluster of *Typhonium flagelliforme* dose 10 Gy with IC50 value equal to 857,69 µg / ml. The result of ploidi and DNA content analysis showed that cluster of 10 Gy rat rats had decreased amount of DNA content.

INTRODUCTION

Air pollution is one source of free radicals in the form of reactive oxygen that can cause the oxidation process that can trigger the occurrence of various diseases[1]. WHO estimates 6.5 million people worldwide died from exposure to indoor and outdoor air pollution[2]. Free radical activity can be prevented by antioxidant compounds that can reduce the negative effects of oxidants in the body. Antioxidants work by donating one of its electrons to an oxidant compound, so that the activity of the oxidant compound can be inhibited[1]. Antioxidant compounds can be obtained from plants and synthetics. The use of synthetic antioxidants in the long term can have an effect on the body, so the use of antioxidants is naturally healthier and safer[3]. The use of plants as a natural antioxidant compounds have a very high prospect. One of them is *Typhonium flagelliforme* which has the potential to be used as a raw material of traditional medicine because it has various kinds of properties such as antioxidants and anticancer. However, *Typhonium flagelliforme* grown in the trenches or in rice fields have low quality[4]. Induction of mutations using gamma rays is a very potential technique used to improve the quality or antioxidant activity of *Typhonium flagelliforme*. Gamma rays have high penetrating power into tissue, which can lead to changes in DNA and chromosomes in plants[5,6]. When the DNA or chromosome changes, it automatically

changes the activity and quality of the mutated plant. This technique has been used by Fang et al.[7] of the plant *Artemisia annua* L. to increase the content of the active compound is artemisinin. Application of mutant gamma mutation induction technique is expected to improve the quality or antioxidant activity of *Typhonium flagelliforme*. In this study is expected to be a new innovation that nuclear energy or radiation is not always harmful, but can also be used as a plant breeding techniques.

MATERIALS AND METHODS

Plant Preparation of *Typhonium flagelliforme*: The *Typhonium flagelliforme* plant is obtained from Research Institute for Spices and Medicinal Plants (BALITRO), Bogor, West Java, Indonesia.

Preparation of *Typhonium flagelliforme* Clusters with Tissue Culture Technique

The material used in this research is *Typhonium flagelliforme* cluster. Clusters are obtained from the laboratory of Rhin Biotechnology, Bandung, West Java, Indonesia.

Irradiation Using Gamma Ray: The Typhonium flagelliforme cluster is irradiated using gamma rays sourced from Co60 located at BATAN, South Jakarta, Indonesia. Irradiation was performed on clusters with four dose variations ie 10 Gy, 20 Gy, 40 Gy, and 0 Gy as controls. The number of irradiated clusters were 7 bottles in which one bottle contained 3 clusters for each dose, so it needed cluster of 84 clusters for 28 bottles.

Cluster Multiplication

Cluster multiplication is done in Laminar Air Flow (LAF). Prepare sterile paper as a base and sterile tissue to dry clusters. Then the dried cluster is cut approximately 1 x 1 cm and then grown in growing medium.

Materials Processing

Typhonium flagelliforme clusters of multiplication and *Typhonium flagelliforme* plant collected, then cleaned by washing with running water to remove any dirt still clinging to clusters. Then done chopping on the *Typhonium flagelliforme* tuber to facilitate the drying process. Then the *Typhonium flagelliforme*tubers and *Typhonium flagelliforme* clusters were dried by oven at 40 °C. The drying process aims to get the simplicia that is not easily damaged, so it can be stored for a longer time. After drying is done dry sorting to separate foreign objects and other impurities that are still present and left behind on dry simplicia. Then the dried simplicia is weighed and pollinated and then placed in a clean, airtight container so as not to affect the content of the substance contained in the simplicia.

Extraction: The *Typhonium flagelliforme* cluster and the dried *Typhonium flagelliforme*tubers are smoothed with mortar. Then the *Typhonium flagelliforme* and *Typhonium flagelliforme*tubers powder were extracted using a maceration method for 5x24 hours with 96% ethanol solvent. After the extraction process is completed the filtering until obtained liquid extract. Furthermore, liquid extract concentrated using rotary evaporator with temperature 40-50^{o}C

Antioxidant Activity Test: *Typhonium flagelliforme* cluster extract and *Typhonium flagelliforme*tubers extract were reconstituted with solvent. Each test solution was taken as much as 2 ml and added with 2 ml DPPH solution, then homogenized and incubated for 30 min. The solution of the solution was measured its absorbance by UV-Visible spectrophotometry at 517 nm wavelength. A mixture of 2 ml of methanol and 2 ml of DPPH solution was used as control. Antioxidant activity is expressed as a percentage of inhibition of free radicals.

$$\% \text{ inhibition} = \frac{abs_{control} - abs_{sample}}{abs_{control}} \times 100\%$$

The IC50 value of the sample concentration was calculated using the linear regression equation formula.

Testing Antioxidant Activity From Ethanol Extract *Typhonium flagelliforme* Cluster: DPPH solution was made 60 µg / mL to determine the wavelength and absorbance, then made a solution of 2000 µg / mL then made series concentrations of 700, 900, 1100, 1300, 1500, 1700 µg / mL, then added with DPPH 60 µg / mL (1: 1). Then it was incubated for 30 minutes and measured at 516 nm wavelength. From the measurement results obtained value of inhibition of each series of concentrations to then made calibration curve and linear regression equation and calculated the value of IC50.

Analysis of Ploidi and DNA Content: The cluster with the best antioxidant activity was then analyzed by ploidi and DNA content using flow cytometer which was in Balai Besar R & D Biotechnology and Agricultural Genetic Resources (BB Biogen), Bogor, West Java, Indonesia.

RESULT AND DISCUSSION

The Effect of Gamma Ray Irradiation on the Growth of*Typhonium flagelliforme* Clusters

The observations of the *Typhonium flagelliforme* cluster that were given irradiation treatment using gamma rays with a dose of 0 Gy (as a control), 10 Gy, 20 Gy, and 40 Gy were carried out for 1 month. The results show that the higher the radiation dose, the *Typhonium flagelliforme* cluster growth is increasingly inhibited. The effect of the dosage shows that the plant response differs according to the dose level given. The higher the dose given, the greater the difference with the control[5].

1. Growth of Typhonium flagelliforme Cluster: The effect of irradiation dose differences showed a difference in % growth of Typhonium flagelliforme cluster. Observations carried out from the beginning to 4 weeks after induction showed differences in the percentage of growth of each dose. Based on table 1, there is a difference in the average growth rate of the Typhonium flagelliforme cluster due to the dose treatment of gamma ray irradiation. Observations from the beginning to 4 weeks of observation, the dose of 10 Gy had a high average growth compared to the control. Based on the statistical results of One-way Anova, it was shown that growth from week 1 to week 4 showed a significant difference (sig = 0,000), meaning that gamma ray irradiation affected the growth of *Typhonium flagelliforme* cluster.Based on the results of Melki and Marouani[8] irradiation doses had a very significant effect on shoot height at 3-4 MSI. The height of the shoots of both chrysanthemum varieties is increasingly inhibited as the increase in irradiation dose is given. The effect of gamma ray irradiation can cause inhibition in the division and increase in the number of cells. This occurs because mutations cause a decrease in the ability of a group of cells in the meristem area which can also cause increased activity of other cell groups so that explant growth becomes disrupted[8]. The inhibition of growth is thought to be due to the inhibition of plant growth hormone activity, where irradiation treatment can affect plant growth hormone activity, namely the hormone auxin[9]. Based on Kravets's research and literature[10] showed that the administration of irradiation in ginger buds can inhibit elongation and cell division to be budded so that the formed buds do not increase in height. The inhibition of *Typhonium flagelliforme* cluster growth can be seen in Figure 2.

Figure 1. *Typhonium flagelliforme* cluster 1 month after dose gamma irradiation: (a). 0 Gy; (b). 10 Gy; (c). 20 Gy; (d). 40 Gy.

Table 1. The percentage of Typhonium flagelliforme cluster growth every week after irradiated with gamma rays dose 0, 10, 20, and 40 Gy.

Dose	$\bar{x}$ % Cluster Growth							
	week 1		week 2		week 3		week 4	
0	40.73	± 45.27	66.84	± 67.75	87.14	± 86.74	110.90	± 102.42
10	93.14	± 121.09	116.96	± 138.82	122.27	± 142.07	129.41	± 145.06
20	48.22	± 53.29	60.02	± 67.35	64.75	± 71.59	71.19	± 74.51
40	35.18	± 52.02	45.55	± 67.41	51.49	± 73.15	55.81	± 72.70

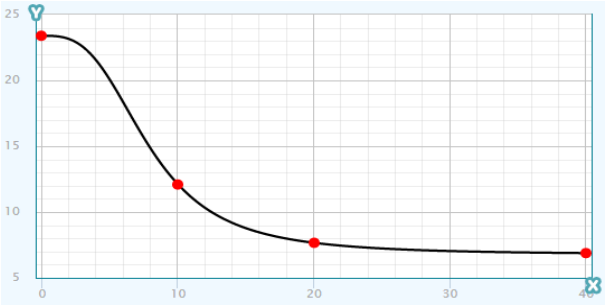

Figure 2. Graph of *Typhonium flagelliforme* cluster growth inhibition after irradiated with gamma rays dose 0, 10, 20, and 40 Gy.

Table 2. Percentage of color color of *Typhonium flagelliforme* 1 month after irradiated with gamma rays dose 0, 10, 20, and 40 Gy.

Dose	Week 0	Cluster Color After 1 Month Radiation (%)			
	Green	Green	Pale	White	Brown
0	100	68	28	-	4
10	100	45,33	52	-	2,67
20	100	33,33	32,00	13,33	21,33
40	100	20	50,67	8	21,33

2. ***Typhonium flagelliforme*** **Cluster Color:** Based on table 2 shows that gamma ray irradiation can cause changes in the color of the *Typhonium flagelliforme* cluster. These color changes vary between green, pale, white and brown. After 1 month of observation, the color of the radiated *Typhonium flagelliforme* cluster showed a difference with the control.

Color changes that occur in the *Typhonium flagelliforme* cluster are likely to occur due to a disturbance in chlorophyll synthesis. Based on the results of the study, there was a deficiency in the chlorophyll of mutant barley plants associated with the inhibition of the activity of the enzyme catalase in the synthesis process of chloroplasts[11]. Changes in color that occur due to mutations cause a deviation or physiological disturbance in the synthesis of chlorophyll in cells of the palisade tissue and mesophyll sponges so that symptoms appear that resemble chlorophyll deficiency in leaves[12].

3. Number of Leaves

Observation of leaves is very important as a reference whether plant growth and development is good.Irradiation can cause cell division to be inhibited which in turn can inhibit the process of organ formation[13]. This can occur because of damage to the meristem cells of plants. Irradiation can also inhibit thedivision and differentiation of ginger stem cells so that the leaves formed are very small and the development is very slow so it is difficult to be called a leaf.

Table 3. Number of leaves of *Typhonium flagelliforme* cluster every week.

Dose	Number of Clusters	Number of Leaves			
		Week 1	Week 2	Week 3	Week 4
0	75	8	20	37	49
10	75	21	24	24	24
20	75	2	5	5	5
40	75	12	22	22	22

Sample processing

1. Processing of *Typhonium flagelliforme* Clusters

Typhonium flagelliforme clusters obtained are then washed with running water to remove the impurities that stick. *Typhonium flagelliforme* cluster weighing 36.04 g dose 0 Gy, 41.39 g dose 10 Gy, 35.23 g dose 20 Gy, and 37.16 g dose 40 Gy dried using an oven at 40^{o}C. The dried simplicia is weighed and the simplicia yield is shown in table 4.

2. Processing *Typhonium flagelliforme*Tuber

*Typhonium flagelliforme*tuber obtained are then washed to clean from other impurities. The washing process is carried out in running water so that all the dirt attached to the hump can be lost. *Typhonium flagelliforme* tuber as much as 119.58 grams is then chopped and dried using an oven with a temperature of approximately 40^{o}C. Dry simplicia was weighed and 10.68 grams of dry simplicia were obtained with the obtained simplicia ratios of 8.93%. The results from processing *Typhonium flagelliforme*tuber material are shown in Figure 3.

Table4.Results of processing *Typhonium flagelliforme* clusters

Radiation Dosage	Gross weight (gram)	Dry weight (gram)	Rendemen of simplicia (%)
0 Gy	36,04	2,83	7,85
10 Gy	41,39	3,93	9,49
20 Gy	35,23	3,02	8,57
40 Gy	37,16	3,27	8,79

(a) (b) (c)

Figure 3. Results of Processing of *Typhonium flagelliforme* Plants; (a). *Typhonium flagelliforme* tuber; (b). Simplicia *Typhonium flagelliforme* tuber; (c). tuber powder

Table 5. Result of Typhonium flagelliforme cluster extraction

Dose	Powder Weight (Gram)	Extract Weight (Gram)	Rendemen (%)
0 Gy	2,79	0,8044	28,83
10 Gy	2,79	0,9601	34,41
20 Gy	2,79	1,011	36,24
40 Gy	2,79	1,1168	40,03

Extraction

1. ***Typhonium flagelliforme* cluster extraction:** *Typhonium flagelliforme* cluster simplicia doses of 0, 10, 20, 40 Gy each of 2.79 grams were extracted with 96% ethanol and 1,120 liters of total solvent used. So that we get the thick extract of *Typhonium flagelliforme* cluster with extract rendemen shown in table 5.

2. ***Typhonium flagelliforme* tuber extraction:** *Typhonium flagelliforme* as much as 10 grams of simplicia powder is extracted with 96% ethanol and 1 liter of the total solvent used. So that we obtained *Typhonium flagelliforme* extract as much as 2.8578 grams with extract yield of 28.578%.

Antioxidant Activity Test with DPPH Method in *Typhonium flagelliforme* Cluster and *Typhonium flagelliforme* Plant: Results From the determination of the calibration curve of the DPPH standard solution obtained a linear regression equation (y = bx + a), a = 0.2177, b = 0.0102 with the square of the correlation coefficient of r2 = 0.9992 with a maximum wavelength of 516 nm. The results of determining the DPPH standard solution calibration curve are shown in Figure 4.

Figure 4. Graph of the calibration of DPPH standard solution

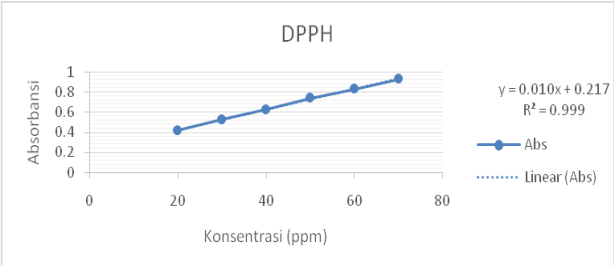

Table 6 Antioxidant activity test with DPPH method on Vitamin C, tuber ethanol extract and *Typhonium flagelliforme* cluster with irradiation dose 0, 10, 20, 40 Gy

Sample		IC_{50} (µg/ml)	
Vitamin C		8,70	± 0,01
Tuber		1389,73	± 3,01
Radiation dose	**0 Gy**	1048,85	± 0,71
	10Gy	865,66	± 1,29
	20Gy	1558,21	± 8,05
	40Gy	1305,57	± 8,16

The results of testing antioxidant activity by using the free radical reduction (DPPH) method on *Typhonium flagelliforme* which was treated by tissue culture techniques showed that there was an increase in antioxidant activity where *Typhonium flagelliforme* cluster activity with a dose of 0 Gy (IC50: 1048.85 ppm) was better than control plants namely *Typhonium flagelliforme tuber* (IC50: 1389.73 ppm). The ability of different antioxidant activities may be related to different levels of secondary metabolites. Technique of tissue culture of secondary metabolites can be found in callus and the metabolites produced from callus also have higher levels than the normal way (directly from plants)[14]. Increased antioxidant activity occurred in the *Typhonium flagelliforme* cluster at a dose of 10 Gy (IC50: 865.66 ppm) where the antioxidant activity was better than the dose of 0 Gy (IC50: 1048.85ppm). This increase can be caused by giving doses of gamma irradiation can increase the activity of the enzyme phenylalanine ammonialyase and the enzyme peroxidase which play a role in the biosynthesis of phenolic compounds[15]. The oxidative destruction process caused by gamma rays can break down chemical bonds in polyphenol compounds so that it will release low molecular weight phenol compounds that will donate hydrogen atoms to free radical compounds. However, in the *Typhonium flagelliforme* cluster with doses of 20 Gy (IC50: 1558.21 ppm) and 40 Gy (IC50: 1305.57 ppm) there was a decrease in antioxidant activity. A decrease in antioxidant activity by gamma rays was caused by degradation of phenolic acids which play a role in the biosynthesis of flavonoids such as cinnamic acid, p-coumarate, and hydroxybenzoic acid[15].

Ploidi Analysis and DNA Content Using Flow Cytometry: The ploidy analysis using flow cytometry produced a histogram that can be seen in Figure 5 showing a difference between the *Typhonium flagelliforme* cluster without irradiation and the *Typhonium flagelliforme* cluster after irradiation at a dose of 10 Gy. The histogram results from the *Typhonium flagelliforme* cluster without irradiation show 1 peak representing the G1 phase with the amount of DNA content 2C, and the radiated *Typhonium flagelliforme* cluster histogram shows 2 peaks, where the first peak shows the G1 phase with the amount of DNA content 2C and the second peak showing the phase G2 with the amount of DNA content 4C[16,17]. The number of *Typhonium flagelliforme* cluster content DNA without irradiation and *Typhonium flagelliforme* cluster after irradiation can be calculated by comparison with standards that have known DNA content. Corn is the standard plant used in this study because corn has a known genome size.

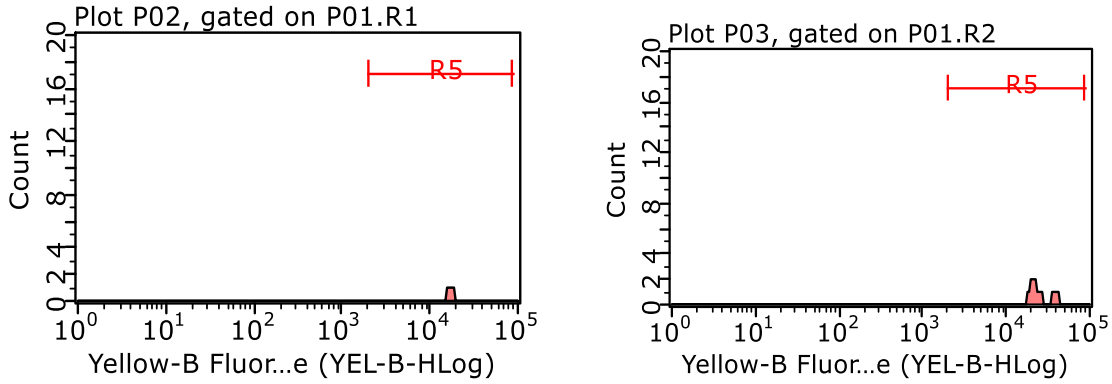

Figure 5. Histogram nuclear DNA content; (a). *Typhonium flagelliforme* Cluster Histogram 0 Gy, (b). Historgram *Typhonium flagelliforme* Cluster 10 Gy

Table 7. The nuclear DNA content estimation results using flow cytometry in the *Typhonium flagelliforme* Cluster with different gamma doses.

	2C DNA Content (pg)	± SD	1C Genome size (Mbp)	Differences (%) from kontrol
Typhonium flagelliforme 0 Gy	8.20771146	1.01197479	4021.77862	-
Typhonium flagelliforme 10 Gy	6.15097380	0.75991942	3013.97716	1007.80 (25.06)

Irradiation using gamma rays on the *Typhonium flagelliforme* cluster has caused variations in the amount of DNA content. Based on table 7 shows that there has been a decrease in the amount of DNA content in the *Typhonium flagelliforme* cluster which has been irradiated compared to the *Typhonium flagelliforme* cluster without irradiation. The difference in genome size between controls and irradiated clusters is 25.06%. a decrease in the amount of DNA content may be caused by gamma rays can cause deletion of DNA on a large scale[18].

CONCLUSION

Irradiation using gamma rays can inhibit *Typhonium flagelliforme* cluster growth. Gamma ray irradiation can increase antioxidant activity where the highest activity is found in the *Typhonium flagelliforme* cluster with a dose of 10 Gy with an IC50 value of 865.66 µg/ml.*Typhonium flagelliforme* cluster dose 10 Gy undergoes polyploidization and decreases the amount of DNA content.

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