Micropropagation and gamma irradiation mutagenesis in *Philodendron billietiae*

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ABSTRACT

Background and Objective: *Philodendron billietiae* is an ornamental plant. To date, mutated ornamental species with variegated traits are in high demand and command higher prices. Thus, the study was carried out in order to optimize the protocol for micropropagation and to induce mutation of *P. billietiae* using gamma rays.

Methodology: The shoot tips were cultured on Murashige and Skoog (MS) medium supplemented with growth regulators. The shoots were proliferated on MS medium supplemented with 1–4 mg/L BA, and the roots were performed on half-strength MS medium supplemented with 1–3 mg/L NAA for 60 days.

Main Results: The best shoots of *P. billietiae* were obtained on MS medium supplemented with 3 mg/L BA, resulting in a significantly greater number of axillary shoots (2.97 ± 0.20 per explant) compared to the control after 60 days of culture. On the other hand, using 2 mg/L NAA in half-strength MS medium resulted in the highest number of roots (6.11 ± 2.26 per explant), but it was not significantly different from the control. For *in vitro* mutation induction, the cutting nodes of *P. billietiae* plantlets were exposed to 20–80 Gy of gamma rays. At 60 days, the lethal dose of gamma ray that causes a 50% reduction in survival (LD_{50}) was 60.03 Gy.

Conclusions: The M_1V_3 plantlets in the 20 Gy treatment can grow and have pale leaves. In high gamma dose-irradiated cultures, there is no regeneration.

Keywords: *Philodendron billietiae*, micropropagation, gamma rays, mutation, tissue culture

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INTRODUCTION

Philodendron billietiae (Figure 1) is a tropical plant species in the family Araceae. The plant is often grown as a houseplant for its attractive foliage because of its distinctive orange-yellow petioles and wavy, ridged leaf edges (Bown, 2000). *P. billietiae*, particularly the variegated variety, is highly valued. Most *P. billietiae* plants are spread by cutting off new shoots, but this method is not preferred because the plants grow slowly. So, tissue culture methods could be used to meet the needs of the plant. Micropropagation is a common method used to propagate philodendron, which involves the use of plant growth regulators (PGRs). Different types of PGRs have different effects on plants. Cytokinins are a group of PGRs that are widely used in tissue culture. They promote cell division, shoot formation, and growth (Gaspar *et al*., 1996; Hönig *et al*., 2018). Auxins are one of the most commonly used types of PGRs responsible for promoting cell elongation, root initiation, and branching (Ivanchenko *et al*., 2010; Laskowski, 2013). Gibberellins (GA) are a group of PGRs that are involved in stem elongation, seed germination, and flower induction (Nickell, 1958; Digby *et al*.,1964).

However, the optimized concentrations of PGRs on plant species need to be investigated to avoid abnormal growth and physiological disorders. Several studies have investigated the effects of PGRs on the micropropagation of philodendron species, such as *Philodendron erubescens* "Imperial red" (Hesheng, 1998), *Philodendron xanadu* (Jirakiattikul and Limpradithtanont, 2006), *Philodendron cannifolium* (Han and Park, 2008) and *Philodendron bipinnatifidum* Schott ex Endl. (Alawaadh *et al*., 2020). However, no information for *P. billietiae*.

Figure 1 *Philodendron billietiae* (green type)

Gamma rays are one of the most powerful methods for inducing genetic changes in cells and tissues of plants. Several improvements in ornamental crops could be achieved in new traits through gamma mutation, including characteristics of flowers, leaves, growth habits, and physiological traits. Using gamma rays to develop new traits for conventional breeding is inefficient. Variegated plant varieties are always in demand as a commercial ornamental. Gamma irradiation has the potential to produce mutant leaf varieties such as *Philodendron erubescens* "Gold" (Karunananda *et al*., 2018) and *Monstera deliciosa* (Huang *et al*., 2017). The goal of this study was to develop an efficient micropropagation protocol and to investigate the effect of acute gamma irradiation on *P. billietiae* tissue culture. In tissue culture, gamma ray-induced mutations can be promising mutants to develop as a new Philodendron cultivar.

MATERIALS AND METHODS

Plant Material

The clean culture of *P. billietiae* shoots was maintained on an MS medium containing 30 g/L sucrose, 2.5 g/L agar, and pH 5.8. The cultures were then incubated for 8 weeks at 25 ± 2°C under a 16-hour photoperiod (light intensity of 2,000 Lux) before the experiments were conducted.

Axillary Shoot and Root Multiplication in Gelled Culture

Shoot tips (approximately 2–4 cm long, 4 tips per culture vessel) were cultured in MS medium containing 30 g/L sucrose, 2.5 g/L agar, and pH 5.8. The shoot induction was tested on MS with different concentrations of 6-Benzylaminopurine (BA) at 1, 2, 3, and 4 mg/L. For root multiplication, cultures were grown in a half-strength MS medium with varying concentrations of 1-Naphthalene acetic acid (NAA) at 1, 2, and 3 mg/L. The shoots were counted at three different time intervals during the cultivation period of 15, 30, and 60 days, while the roots were recorded at four different time intervals of 15, 30, 45, and 60 days of culture. Each treatment had four replicates.

Acute Gamma Irradiation

Pathogen-free plantlets of *P. billietiae* (green type) shoots were cultured on MS medium supplement with 3 mg/L BA. Single-node cuttings were produced after approximately 60 days. They were exposed to acute gamma irradiation using a Gamma chamber – GC 5000 with ^{60}Co source at Thailand Institute of Nuclear Technology (Public

Organization), Nakhon Nayok, Thailand. The irradiation doses were 0, 20, 40, 60, and 80 Gy, with a dose rate of 26.95 Gy/min. After exposure, 20 plantlets for each dose were transferred to a half-strength MS medium supplemented with 2 mg/L NAA to develop plantlets and rooting. The LD_{50}, or 50% survivability of plantlets, was calculated 60 days post-irradiation. Desirable variations were recorded and selected in the M_1V_1-M_1V_3 generation.

Experimental Design and Statistical Analysis

The experiments were designed using a completely randomized design. All data were subjected to analysis of variance and LSD (Least significant difference) tests using R software.

RESULTS AND DISCUSSION

Effect of BA on Shoot Multiplication *P. billietiae*

BA is one of the cytokinins that are commonly used in plant tissue culture to promote shoot formation (Schmülling, 2013). In this experiment, BA was added to media at 1, 2, 3, and 4 mg/L. The result found that the multiplication of *P. billietiae* shoots was significantly influenced by the concentration of BA added to the culture medium (Table 1).

Table 1 Effect of BA on shoot multiplication of *P. billietiae* after 15, 30, and 60 days in culture

BA concentration (mg/L)	No. of shoots		
	15 days	30 days	60 days
0 (control)	1.24 ± 0.14^{b}	1.35 ± 0.13^{b}	1.68 ± 0.50^{b}
1	2.06 ± 0.19^{a}	2.02 ± 0.36^{ab}	2.11 ± 0.10^{ab}
2	1.93 ± 0.07^{a}	1.97 ± 0.06^{ab}	2.57 ± 0.28^{a}
3	1.98 ± 0.24^{a}	2.41 ± 0.30^{a}	2.97 ± 0.20^{a}
4	1.76 ± 0.32^{a}	2.18 ± 0.74^{a}	2.34 ± 0.87^{ab}
F-test	*	*	*
LSD	0.38	0.72	0.87

Note: ^{a,b} Means within the same column with different superscripts differ (P < 0.01) level, according to Fisher's least significant difference (LSD) test. * Significant at P < 0.01. Control = MS without growth regulator, BA = N_6-Benzyladenine.

The number of axillary shoots per explant continuously increased at 15, 30, and 60 days of culture time, depending on the concentration of BA. Adding BA at 3 mg/L significantly increased shoot number (2.97 ± 0.20 per explant) at 60 days compared to the control (Figure 2). These results agree with a previous report that a concentration of BA at 4 mg/L produced a maximum number of 5.8 shoots of *Philodendron xanadu* (Jirakiattikul and Limpradithtanont, 2006).

Similarly, in lacy tree philodendron explants, the medium with BA at 1 mg/L yielded the greatest mean shoot number, and no proliferation was observed in the absence of cytokinin (Alawaadh *et al*., 2020). The different hormone activities could be due to differences in uptake rate, rate of metabolism, and genetic makeup in plants (Centeno *et al*., 1998; Kwon *et al*., 2019). The level of regulator hormone in the media plays a role in differently inducing the multiplication of shoots in each plant species. It is important to develop effective strategies for plant propagation and crop improvement.

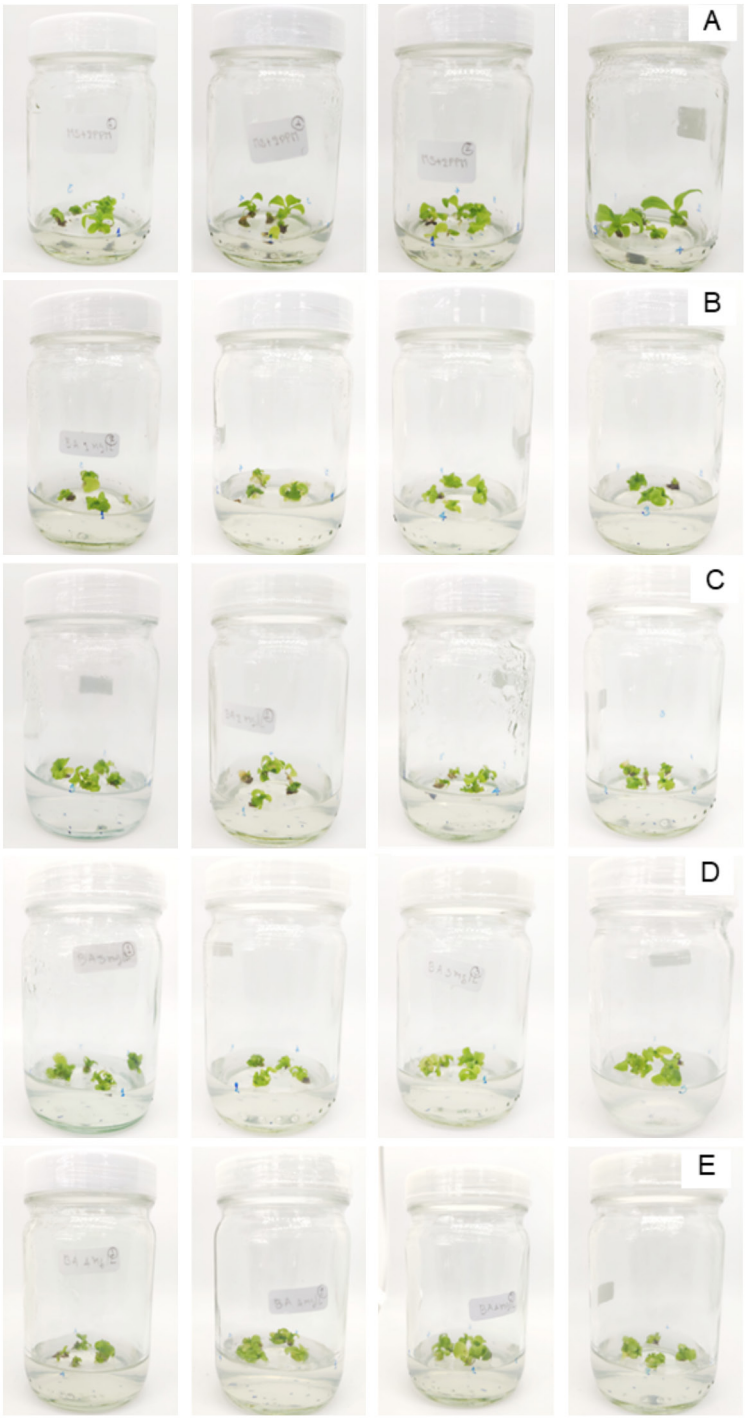

Figure 2 Axillary shoot multiplication of *P. ballistae* in MS medium supplemented without and with BA after 60 days. A = control, B = 1 mg/L BA, C = 2 mg/L BA, D = 3 mg/L BA, and E = 4 mg/L BA.

Effect of NAA on Root Multiplication *of P. billietiae*

NAA is an auxin of PGRs that is commonly used to promote root development and is often used in combination with other growth regulators to achieve specific growth effects (Yan *et al*., 2014). In this study, the NAA at 1, 2, and 3 mg/L were added to the half-strength MS. The result showed that adding NAA to the rooting medium did not significantly improve the rooting responses of *P. billietiae* compared to the control treatment at 15, 45, and 60 days. However, the number of roots increased at 15, 30, 45, and 60 days of tissue culture in each treatment. The half-strength MS medium was supplemented with NAA (2 mg/L), which yielded the greatest mean number of roots (6.11 ± 2.26 per explant) at 60 days (Table 2 and Figure 3). The optimum dose of NAA could affect rooting, as was observed in the rooting of *P. xanadu*, which was induced by a half-strength MS medium supplemented with 0.5 and 1 mg/L NAA (Jirakiattikul and Limpradithtanont, 2006).

Similarly, the best rooting number of lacy tree philodendron was obtained from MS supplemented with NAA (1–2 mg/L), and resulting plantlets were successfully acclimatized, with a survival rate of 100%, and were morphologically similar to the mother plant (Alawaadh *et al*., 2020). On the other hand, previous studies have reported that microshoots from six philodendron cultivars rooted easily in MS medium without PGR supplementation (Sreekumar *et al*., 2001). These results clearly demonstrate that exogenous auxin treatment is unnecessary to improve *P. billietiae* rooting. However, in some experiments, the combination of cytokinins (BA) and auxins (NAA) exerted a synergistic and positive influence on shoot proliferation of Araceae plant species, for example, *Philodendron van* "Serratum" (Patel and Shah, 2004). Therefore, further experiments are needed to investigate the effect of a combination of PGRs on shoot proliferation.

Table 2 Effect of NAA on rooting of *P. billietiae* after 15, 30, 45, and 60 days in culture

NAA concentration (mg/L)	No. of roots			
	15 days	**30 days**	**45 days**	**60 days**
0 (control)	1.50 ± 1.32	2.83 ± 0.29^{b}	4.50 ± 1.32	5.33 ± 1.61
1	2.50 ± 0.50	3.83 ± 0.76^{ab}	4.17 ± 1.04	5.00 ± 1.80
2	1.67 ± 0.29	4.78 ± 1.11^{a}	5.89 ± 2.11	6.11 ± 2.26
3	1.08 ± 0.52	3.58 ± 1.18^{ab}	4.00 ± 0.87	4.25 ± 0.43
F-test	NS	*	NS	NS
LSD	1.44	1.71	2.67	3.14

Note: ^{a,b} Means within the same column with different superscripts differ (P < 0.01) level, according to Fisher's least significant difference (LSD) test. NS = nonsignificant, * significant at P < 0.01, respectively. Control = half-strength MS without growth regulator, NAA = napthalene acetic acid.

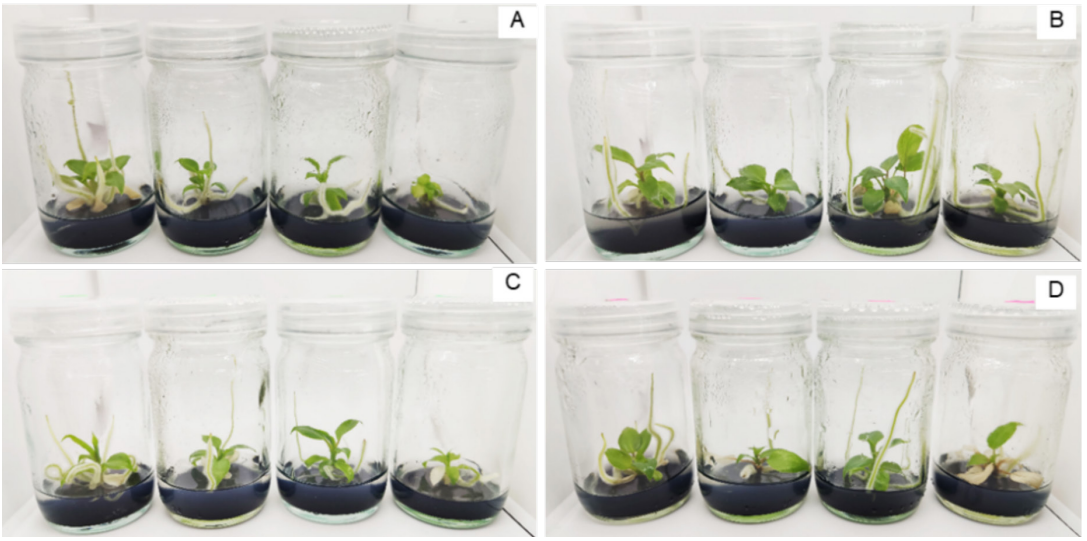

Figure 3 *In vitro* rooting of *P. billietiae* on half-strength MS medium that contained without and with NAA after 60 days in culture. A = control, B = 1 mg/L NAA, C = 2 mg/L NAA, and D = 3 mg/L NAA

Effect of Acute Gamma Irradiation on *P. billietiae* Plantlets

The results revealed a significant effect of gamma ray treatments on the survival percentage of *P. billietiae* plantlets. The survival percentage decreased with an increasing gamma radiation dose. Raising the gamma dose to 20, 40, 60, and 80 Gy led to 100%, 72.6%, 48.9%, and 27.8% survivability, respectively (Table 3). The plantlet treatments at 40, 60, and 80 Gy significantly reduced the survival percentage as compared to the control. Meanwhile, the 20 Gy treatment was not different from the control. The lethal dose of gamma rays that causes a 50% survival reduction (LD_{50}) at 60 days was 60.03 Gy (Figure 4). However, at 60 days, both the dose lower and higher than this LD_{50} value showed abnormal morphology of the survived plantlets, as indicated by dwarf plantlets with pale leaves (Figure 5). The growth decreased with an increasing gamma radiation dose. There were no differentiable plantlets in the 40–80 Gy treatment groups. Only in the 20 Gy treatment group were the plantlets able to grow. Thus, morphological variation was observed only in the M_1V_2 - M_1V_3 generation of the 20 Gy treatment group. The plantlets in the 20 Gy treatment group could grow and exhibited pale leaves, whereas the leaves of the control group were green (Figure 6). Theoretically, exposure to gamma rays affects plant growth, development, and morphology. The lower dose (100 Gy) of gamma rays is frequently used to obtain useful mutants in diverse plant species. In contrast, there is no report of gamma irradiation being used to develop new varieties of *Philodendron* sp. in tissue cultures.

These results are similar to those reported for gamma ray-induced mutation in stem cuttings of *P. erubescens* "Gold", where doses of 70 and 100 Gy resulted in three different color patches of leaves as dark bluish green, strong yellow-green, and brilliant greenish yellow (Karunananda *et al*., 2018). Stem cuttings of *Philodendron scandens* treated with 50 Gy of gamma rays also showed desirable genetic effects on some morphological traits (El-Khateeb *et al*., 2016). The appropriate dose of gamma rays for inducing mutation differs depending on the part of the plant. This study is the first report to assess the potential of a low dose (20 Gy) of gamma rays for *P. billietiae* mutation breeding in tissue cultures.

Table 3 Survival percentage of *P. billietiae* plantlets at 60 days after irradiation

Irradiation dose (Gy)	Survival (%)
0	100.0[a]
20	100.0[a]
40	72.6[b]
60	48.9[bc]
80	27.8[c]
F-test	*
LSD	24.65

Note: [a,b] Means with in the same column with different superscripts differ ($P < 0.05$) level, according to Fisher's least significant difference (LSD) test. * Significant at $P < 0.05$.

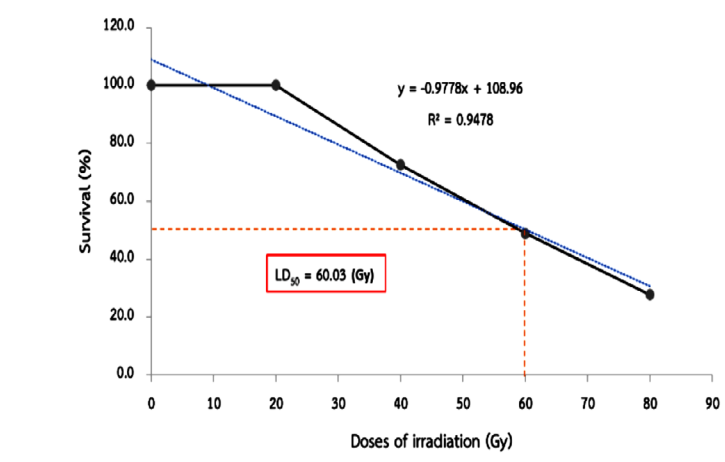

Figure 4 Effect of various doses of gamma on survival percentage of *P. billietiae* plantlets at 60 days after irradiation

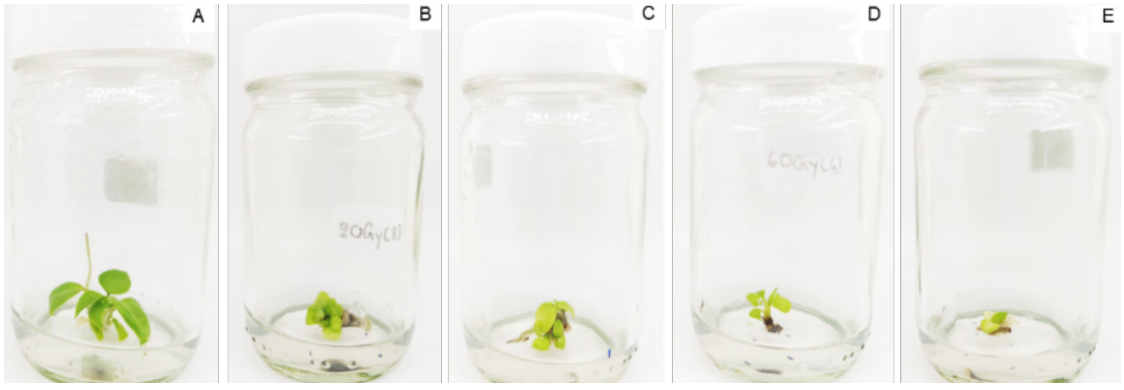

Figure 5 Effect of various doses of gamma irradiation on the morphology of *P. billietiae* plantlets at 60 days after irradiation. A = control, B = 20 Gy, C = 40 Gy, D = 60 Gy, and E = 80 Gy.

Figure 6 Variation observed in M_1V_2 - M_1V_3 generation of *P. billietiae* plantlets. A = control, B = 20 Gy of M_1V_2, and C = 20 Gy of M_1V_3.

CONCLUSIONS

Our results show that small pieces of *P. billietiae* plantlets can be used to produce hundreds of plants in a short time using an optimal PGRs concentration in the culture medium. The best shoots of *P. billietiae* were obtained on MS medium supplemented with 3 mg/L BA, resulting in a significantly greater number of axillary shoots (2.97 ± 0.20 per explant) than the control after 60 days of culture. On the other hand, using 2 mg/L NAA in a half-strength MS medium resulted in the highest number of roots (6.11 ± 2.26 per explant), but it was not significantly different from the control. To develop new varieties of *P. billietiae*, tissue cultures were exposed to 20–80 Gy of gamma rays. The lethal dose of gamma rays that caused a 50% reduction in survival (LD_{50}) at 60 days was 60.03 Gy. M_1V_3 plantlets in the 20 Gy treatment could grow and exhibited pale leaves, while no regeneration occurred in cultures irradiated with high doses of gamma rays.

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