Comparative effect of high energy electron beam and $^{137}$Cs gamma ray on survival, growth and chlorophyll content in curcuma hybrid ‘Laddawan’ and determine proper dose for mutations breeding

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ABSTRACT

Curcuma alismatifolia is an ornamental plant with a beautiful flower in the curcuma genus, which is popular in Thailand and other countries. C. alismatifolia has been improved as the market needs. This research aimed to induce mutations and to study the effects of electron beam and gamma ray on tissue culture of the Curcuma hybrid ‘Laddawan.’ Eight-weeks-old plantlets were irradiated by gamma ray with a Mark I irradiator at the dose rate of 3.74 Gy/min, at the Nuclear Technology Research Center, Kasetsart University. For the electron beam irradiation, samples were exposed to a high-energy electron beam. The lowest power of this machine is 10 MeV and the lowest radiation dose per cycle is 50 Gy (240 Gy/min). The comparison between the effects of gamma ray and electron beam irradiation at doses of 0, 50, 100 and 200 Gy showed that survival percentage, growth percentage, and chlorophyll content were significantly decreased (P<0.05) when radiation dose increased. The survival percentage, growth percentage and chlorophyll content of gamma-irradiated samples were higher than the electron beam-irradiated samples in the same dose. The results indicated the optimum dose range for gamma irradiation of Curcuma hybrid ‘Laddawan’ in tissue culture is 30 – 60 Gy and for electron beams the dose should be less than 50 Gy. New characteristics (variegated leaves and light green leaves) were found in $M_{V_2}$.

Keywords: Curcuma alismatifolia; Electron beam; Gamma ray; Mutation; Tissue culture

INTRODUCTION

Curcuma alismatifolia is a tropical flower that can be found in northern and north-eastern Thailand. C. alismatifolia is a species of plant in the family Zingiberaceae, genus Curcuma (Department of Agricultural Extension, 2005). A common name is Siam Tulip because they have beautiful leaves and flowers similar to the leaves and flowers of the European tulip. C. alismatifolia can be used as cut flowers, potted plants or ornamental plants in beds (National Research Council of Thailand, 2012), which has made them popular in Thailand and other countries. Thailand exports C. alismatifolia in the form of undeveloped rhizomes, rhizomes that are growing already, and fresh flowers. Annual export value is 3-6 hundred thousand dollars (Department of Agriculture, 2018). Improvement of C. alismatifolia is being worked on continuously to respond to the market demand, by selecting from the genes in nature or through the development of varieties by interspecific hybridization, such as the Curcuma hybrid ‘Laddawan’ of Dynamic Seeds Co., Ltd. It is a hybrid between C. alismatifolia and C. petiolata. There is a distinctive inflorescence with pink and purple flowers that can be used as cut flowers and potted plants (Fig. 1(A)) (National Research Council of Thailand, 2012). The offspring display traits and characteristics of both parents, but are often sterile, preventing gene flow between the species. Then they cannot interbreed with the parental species. Mutation breeding is a way to induce genetic mutations in plants by using x-rays, gamma-rays, ion beams, laser beams, neutrons and electron beams, which may result in gene mutation and

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chromosome aberration, often enabling breeders to gain new varieties. However, mutation is regarded as random and the success of obtaining a desired mutant trait depends on three factors (Hase et al., 2012). Tissue culture allows for large populations for mutagenic treatment, selection, and cloning of selected variants (Ahloowalia, 1995).

In vitro mutagenesis is a good technique for increasing the genetic variation in C. alismatifolia. New mutants previously induced by gamma irradiation include ‘Chiang Mai Red’ and ‘Chiang Mai Light Pink’ varieties, which were irradiated from ‘Chiang Mai Pink’ variety (National Research Council of Thailand, 2012). Abdullah et al. (2009) irradiated rhizomes of C. alismatifolia var. Pink with 10 different doses, and the (LD₅₀) was approximately 25 Gy. Taheri et al. (2014) irradiated eight different doses of acute gamma irradiation on three varieties of C. alismatifolia: Chiang Mai Red, Sweet Pink, Kimono Pink, and one Curcuma hybrid (Doi Tung 554) individual plants were investigated. Until now, no reports are available regarding the use of electron beam for induced mutagenesis in curcuma. The objectives of this study were to determine the optimum dose for induced mutation on tissue culture of the curcuma hybrid ‘Laddawan’ for mutation breeding using gamma ray and electron beam.

**MATERIALS AND METHODS**

**Plant materials (Curcuma hybrid ‘Laddawan’)**

Pathogen-free plantlets of the C. hybrid ‘Laddawan’ (supported by Chiang Rai Horticultural Crop Research Center, Thailand) were cultured on MS medium (Murashige and Skoog, 1962) with BAP (6-benzylaminopurine) 1 mg/l until enough plantlets (more than 1000) were produced for the experiments.

**Gamma and electron beam irradiation**

After being sub-cultured to new MS medium (without BAP), eight-week-old plantlets of C. hybrid ‘Laddawan’ were exposed to radiation treatments. The radiation doses were 0, 50, 100, 150 and 200 Gy, with 3 replications for each treatment, 20 plantlets per replication. For gamma irradiation, the plantlets were exposed to gamma radiation using a Mark I Gamma Irradiator with α²¹⁰Cs source at the Nuclear Technology Research Center, Kasetsart University, Bangkok, Thailand, at a dose rate of 3.74 Gy/min. For electron beam irradiation, the samples were exposed to electron beams with a high-energy electron accelerator at the National Institute of Nuclear Technology (Public Organization), Ministry of Science and Technology. The lowest power of this machine is 10 MeV and the lowest radiation dose per cycle is 50 Gy (at a dose rate of 240 Gy/min). After irradiation, the plantlets were sub-cultured to new MS medium for the M₁ generation. At 60 and 90 d after irradiation the number of surviving plantlets were recorded to calculate the median lethal dose (LD₅₀, (50% lethal dose at 60 and 90 d after irradiation)), the number of new shoots were recorded to calculate the median decreasing growth rate (GR₅₀) and the characteristics of abnormalities after radiation in the M₁ generation were observed. At 90 d after irradiation, new shoots (M₂) were subcultured to new media. Desirable variations were recorded and selected.

**Chlorophyll content measurement of C. hybrid ‘Laddawan’ leaves in the M₁ generation**

Chlorophyll content was determined in leaves of M₁ plantlets at 90 d after gamma and electron beam irradiation based on an adapted method described by Hipkins and Baker (1986). The chlorophyll content was measured in triplicate for each treatment.

**Experimental design and statistical analysis**

The experimental design was a factorial in a completely randomized design (Factorial in CRD) with two factors: radiation type (gamma ray and electron beam) and radiation dose. The radiation doses were 0, 50, 100, 150 and 200 Gy, with 3 replications for each treatment, 20 plantlets per replication. The data were analyzed using analysis of variance, after which means were compared using the least significant difference (LSD). The analyses were facilitated by the R program (Jompuk, 2012).

**RESULTS AND DISCUSSION**

**Effects of gamma and electron beam irradiation on the survival of C. hybrid ‘Laddawan’**

At 60 d after irradiation, the results showed that gamma and electron beam exposure affected the survival of C. hybrid ‘Laddawan’ plantlets. The irradiated samples had a percentage of survival less than the control, but it was not possible to calculate the LD₅₀ at 60 d of the two types of radiation (Fig. 3). The samples irradiated with gamma rays had a higher survival percentage than those exposed to electron beam at the same dose. The
The interaction between the radiation dose and the type of radiation was a highly significant difference (Table 1). At 90 d after irradiation (Fig. 2), the results showed that the doses of gamma and electron beam affected the survival percentage with highly significant differences ($p < 0.01$). The LD$_{50}$ at 90 d of gamma and electron beam irradiation were 80 and 55 Gy, respectively (Fig. 3). The results of the current study differed from Lamseejan et al. (2001). Taheri et al. (2014) studied the effects of different doses of acute gamma irradiation on three varieties of *Curcuma alismatifolia*. For electron beam irradiation it was found that the survival percentage decreased when the radiation dose increased, in accordance with the results of experiments on electron beam irradiation in rice (Guo et al., 1982), barley (Xu et al., 1983), soybean (Li et al., 1988), sorghum (Lu et al., 1995) and azuki bean (Luo et al., 2012). The LD$_{50}$ at 90 d of electron beam was lower than the gamma-ray. This was in agreement with Veni et al. (2017), who studied the effects of electron beam and gamma ray in blackgram and **Table 1: Survival percentage of *C. hybrid* ‘Laddawan’ *in vitro* plantlets at 60 and 90 d after gamma and electron beam irradiation compared with the control**

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Survival percentage at 60 days after irradiation (as % of control)</th>
<th>Survival percentage at 90 days after irradiation (as % of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gamma</td>
<td>e- beam</td>
</tr>
<tr>
<td>0</td>
<td>100 a</td>
<td>100 a</td>
</tr>
<tr>
<td>50</td>
<td>86.11 b</td>
<td>66.67 e</td>
</tr>
<tr>
<td>100</td>
<td>81.48 bc</td>
<td>72.22 cde</td>
</tr>
<tr>
<td>150</td>
<td>76.19 cd</td>
<td>56.67 f</td>
</tr>
<tr>
<td>200</td>
<td>69.64 de</td>
<td>52.38 f</td>
</tr>
<tr>
<td>average</td>
<td>82.69 a</td>
<td>69.59 b</td>
</tr>
</tbody>
</table>

F-test dose **
F-test rad **
F-test dose x rad **
C.V. (%)

7.17
9.06

**Significant at 1% level.
ns Non-significant difference

1 Different letters in the same column mean that the data are statistically different from the least significant difference ($p < 0.05$).

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**Fig 2. Control and irradiated samples at 90 d after irradiation: (A) Control; gamma irradiation, (B) 50 Gy; (C) 100 Gy; (D) 150 Gy; (E) 200 Gy and electron beam irradiation (F) 50 Gy; (G) 100 Gy; (H) 150 Gy; (I) 200 Gy.**

**Fig 3. The relationship between the dose of gamma ray, electron beam and survival percentage of *C. hybrid* ‘Laddawan’ *in vitro* plantlets at 60 and 90 d after irradiation compared with the control.**
found that the LD$_{50}$ dose for electron beam was around 300 Gy whereas, the LD$_{50}$ dose for gamma rays was around 320 Gy. Linear energy transfer (LET) is defined as the energy deposited to the target material per unit distance when ionizing radiation passes through it. Different kinds of radiation have different energy transfer patterns. The mutation effects of radiation are known to be a function of their LET. Both gamma rays and electron beams have low LETs of around 0.2KeV/µm (Magori et al. 2010). However, electron beam has a higher dose rate compared to gamma rays and is administered as short pulses while gamma irradiation is continuous (Hoglund et al. 2000).

Effects of gamma and electron beam irradiation on the growth rate of C. hybrid 'Laddawan'

The effect of radiation on growth was assessed by counting the number of new plantlets at 60 and 90 d after irradiation and calculating the growth rate (Table 2, Fig 4). Previous findings supports our study (Jompuk et al., 2009; Limtiyayotin et al., 2018; Lamseejan et al., 2002). The relative growth percentage with respect to non-irradiated control in the M$_1$V$_1$ generation was found to be reduced in a dose dependent manner (Table 2). However, there was no difference in the relative growth percentage of plantlets irradiated with either electron beam or gamma irradiation with similar doses. Waje et al. (2009) showed that broccoli seeds irradiated with the same dose of either electron beam or gamma rays had similar germination percentages. They also reported a decrease in sprout length of germinated seedlings with increase in dose of either gamma ray or electron beam. Our findings were similar to these results.

The GR$_{50}$ at 90 d of gamma and electron beam irradiation were 59 and 36 Gy, respectively (Fig 4). The results showed that, for gamma irradiation, the growth rate increased when the time after irradiation increased, but for electron beam irradiation, the growth rate decreased when the time after irradiated increased, because of electron beam has a higher dose rate when compared to gamma rays. The higher the dose-rate, the greater is the relative biological effectiveness (RBE). The absorbed dose rate of electron beam on biomaterials may reach $10^{10}$ Gy.s$^{-1}$, which is much higher than gamma rays (usually under 60 Gy.s$^{-1}$) (Zhu et al., 2008). For irradiation effects on cell division, the cells will be temporarily stopped from dividing and after some time they will be able to divide as before. It depends on the type of radiation, the dose and dose rate of irradiation (Yamaguchi, et al., 2010). In this study, the plantlets irradiated with both radiation types at doses of 100-200 Gy died (Table 2). It may be that the high radiation doses inhibit plant growth due to the meristem being destroyed or inhibiting the cell division (Zhu et al., 2008, Joshi-Saha et al., 2015 and Yadav, 2016)

Effects of gamma and electron beam irradiation on chlorophyll content in leaves of C. hybrid ‘Laddawan’ M$_1$V$_1$ plantlets

The chlorophyll contents were measured for all samples exposed to any doses except the samples that were exposed to electron beam at doses of 150 and 200 Gy. Statistical
analysis showed highly significant decreases in chlorophyll a, chlorophyll b and total chlorophyll content in the leaves of plants derived from irradiated plantlets. The irradiated samples (both gamma and electron beam) had chlorophyll content that was less than control and decreased when radiation dose increased. Comparing between gamma rays and electron beam, there were no significant differences in chlorophyll a, chlorophyll b and total chlorophyll content. As for the interaction between the dose of radiation and the type of radiation, the chlorophyll a and the total chlorophyll content were significantly different (p < 0.05) but the chlorophyll b content was not significantly different.

Conclusions

The tested doses of gamma rays and electron beam affected the survival and growth rate of C. hybrid ‘Laddawan.’ The survival percentage and growth rate decreased when the dose of radiation increased. The gamma irradiated samples had a higher survival percentage and growth rate than the electron beam irradiated samples. The LD_{50} at 90 d of gamma and electron beam irradiation were 80 and 55 Gy, respectively. The GR_{50} of gamma and electron beam irradiation were 58 and 36 Gy, respectively.

From this research the appropriate dose of gamma radiation to be used for inducing mutation in tissue cultures

Table 3: Chlorophyll content of leaves of C. hybrid ‘Laddawan’ M_{1}V_{2} plantlets

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Chlorophyll a (mg/g of fresh leaf weight)</th>
<th>Chlorophyll b (mg/g of fresh leaf weight)</th>
<th>Total chlorophyll (mg/g of fresh leaf weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gamma</td>
<td>electron beam</td>
<td>average</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>0.737</td>
<td>0.447c</td>
<td>0.592b</td>
</tr>
<tr>
<td>100</td>
<td>0.307</td>
<td>0.118 e</td>
<td>0.213 c</td>
</tr>
<tr>
<td>150</td>
<td>0.202</td>
<td>NA</td>
<td>0.202 c</td>
</tr>
<tr>
<td>200</td>
<td>0.084</td>
<td>NA</td>
<td>0.084 d</td>
</tr>
<tr>
<td>average</td>
<td>0.498</td>
<td>0.576</td>
<td>0.524</td>
</tr>
</tbody>
</table>

*Significant at 5% level.
**Significant at 1% level.
ns Non-significant difference
1 Different letters in the same column mean that the data are statistically different from the least significant difference test.

Variations in the M_{1}V_{2} generation

Morphological variations in the M_{1}V_{2} generation were observed, but only the 50 Gy treatment samples of gamma and electron beam produced new plantlets (M_{1}V_{2}). There were some plants with variegated leaves and light green leaves for both radiation types (Fig. 5). The results of this study were consistent with those of Priya et al. (2014), who reported that in gamma irradiation on tissue culture of turmeric, the chlorophyll mutations were viridis and xantha. In Abdullah et al. (2009)’s study on gamma irradiation on rhizomes of C. alismatifolia, the results showed that the variations observed were two inflorescences in the same stem, two real flowers in the same bract, changing the color intensity of flowers and chlorophyll mutation on leaves. For electron beam, in a study by Luo et al., (2012) dry seeds of azuki bean (Vigna angularisi) were irradiated by electron beam at 100, 300, 600, 700 and 900 Gy, respectively.
of C. hybrid 'Laddawan' ranged from 30 – 60 Gy, and for electron beams should be less than 50 Gy.

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Author's contributions

This work is part of the M.Sc. thesis of Chutiphorn Tosri (CT) advised by Peeranuch Jompuk (PJ). CT and PJ have designed the study, conducted the experimental work, and analyzed data. PJ wrote and revised the manuscript. Katatari Chusreeam is the committee of the M. Sc. thesis. Mayuree Limtiyayotin and Natnichaph Sukin are the scientists who take care the instruments and tissue culture lab.

REFERENCES


