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Effects of Acute and Chronic Gamma Irradiation on Tissue Culture of *Cryptocoryne wendtii* "brown"

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ABSTRACT

The value of aquatic plant exports from Thailand is rising and there is a demand for interesting new varieties of aquatic plants. The objective of this research was to find the appropriate dose of gamma irradiation to induce mutations in *Cryptocoryne wendtii* "brown." *C. wendtii* "brown" was cultured aseptically on MS medium supplemented with BA (2mg/l) and NAA (0.25 mg/l). Samples of the tissue-cultured plantlets were exposed to acute gamma radiation using a Mark I Gamma Irradiator with a Cesium-137 source at doses of 0, 10, 20, 30, 40 and 50 Gy. Other samples were exposed to chronic gamma radiation in a gamma room using a Cobalt-60 source at the Gamma Irradiation Service and Nuclear Technology Research Center of the Faculty of Science, Kasetsart University. The samples were placed at 1.5 and 2.5 m from the source with a dose rate of 1.614 and 0.537 Gy/hr, respectively. The samples at 1.5 m received a total dose of 238.87 and 477.74 Gy (0 for the control) and those at 2.5 m received a total dose of 10.75, 21.50, 32.24 and 42.99 Gy (0 for the control). The number of dead and surviving plantlets and the number of new shoots were recorded at 45 days after irradiation and compared with the controls to calculate the $LD_{50(45)}$ and $GR_{50(45)}$. Under acute irradiation, the $LD_{50(45)}$ and $GR_{50(45)}$ for *C. wendtii* "brown" were found to be 23 and 20 Gy, respectively. Some variations were observed, such as clumping, dwarfism, darker brown leaves, furled leaves and narrow leaves. Under chronic irradiation it was not possible to calculate the $LD_{50(45)}$ or $GR_{50(45)}$ because at 1.5 m from the source all the samples died and at 2.5 m from the source more than 50% of the samples survived. Some variations were observed, such as shorter plants, green leaves, increased branching, shorter petioles and longer, narrower leaves of a greenish-brown color.

Key words: *Cryptocoryne wendtii* "brown", gamma rays, acute irradiation, chronic irradiation, tissue culture

INTRODUCTION

Cryptocoryne is a genus of aquatic plants whose species are very popular with water garden enthusiasts and they can also be used for aquarium decoration. There are more than 50 species in the genus and they come in a variety of colors with attractive shapes. *Cryptocoryne spp*. are quite slow-growing, which is an advantage because aquarium owners do not have to cut them back or replace them often (Rajaj and Horeman, 1977). *Cryptocoryne wendtii* "brown" is a medium-sized

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aquatic plant with brown leaves that can be propagated by seed and by dividing new offshoots (Muhlberg, 1982).

To increase the value of aquatic plants for export, irradiation together with tissue culture (Jompuk *et al*., 2001) is one way to increase the diversity of products to meet the demand of consumers who are looking for something new and different. Since *Cryptocoryne spp*. have a slow growth rate in the natural environment, induced mutation is a way to increase the genetic diversity while tissue culture is another way to propagate large numbers of plants in a short time. Moreover, micropropagation is also an efficient way to increase the number of mutated varieties.

This research aimed to observe the effects of both chronic and acute gamma irradiation on tissue-cultured *Cryptocoryne wendtii* "brown" as a method to improve on the breeding outcomes for *Cryptocoryne spp*.

MATERIALS AND METHODS

Sterilization and tissue culture of *C. wendtii* "brown"

C. wendtii "brown" plants that had been grown in a hydroponic system were washed with water and the tips, leaves and roots were cut off. The remaining parts were immersed in 70% alcohol for 30 seconds. The explants then were soaked in 15% sodium hypochlorite solution for 15 min, followed by immersion in a 2% $HgCl_2$ solution for another 10 min. They were rinsed in sterilized distilled water three times before being placed on MS culture medium (Murashige and Skoog, 1962) supplemented with BA (2mg/l), NAA (0.25 mg/l), sucrose (30 g/l) and agar (8 g/l). The sterilized explants of *C. wendtii* "brown" were then subcultured on the same medium until there were enough samples for the gamma radiation experiments.

Study of acute and chronic gamma irradiation

Acute irradiation

C. wendtii "brown" plantlets were exposed to acute gamma radiation using a Mark I Gamma Irradiation with a ^{137}Cs source at the Gamma Irradiation Service and Nuclear Technology Research Center of the Faculty of Science, Kasetsart University, Bangkok, Thailand at doses of 0, 10, 20, 30, 40 and 50 Gy. There were three replications, with 20 plantlets per replication. Following irradiation, the plantlets were cultured on the same medium described above. After 45 days, the number of surviving plantlets and the number of new shoots were recorded to calculate the $LD_{50(45)}$ and the $GR_{50(30)}$. Desirable mutants were recorded and selected in M_1V_2 and M_1V_3 generations.

Chronic irradiation

C. wendtii "brown" plantlets were exposed to chronic gamma radiation in a gamma room with a ^{60}Co source at the Gamma Irradiation Service and Nuclear Technology Research Center of the Faculty of Science, Kasetsart University, Bangkok, Thailand. The samples were placed 1.5 and 2.5 m from the radiation source and were irradiated at a rate of 1.614 and 0.537 Gy/hr, respectively. At 1.5 m from the source, the samples were divided into three treatments receiving 0, 238.87 and 477.74 Gy, respectively. At 2.5 m from the source, the samples were divided into five treatments receiving 0, 10.75, 21.50, 32.24 and 42.99 Gy, respectively. Three replications were carried out for each treatment with 10 plantlets per replication. Following irradiation, the plantlets were cultured on the same medium as mentioned above. After 45 days, the number of surviving plantlets was recorded. Mutants were recorded and selected from the M_1V_2 generation.

Statistical analysis

A completely randomized design (CRD) was employed with three replications for each treatment. Twenty samples were used for each replication for the acute irradiation experiment

while 10 samples were used for each replication in the chronic irradiation experiment. The data were recorded and analyzed using analysis of variance (ANOVA) and least significant difference (LSD) analysis of the means.

RESULTS AND DISCUSSION

Acute irradiation

Exposing *C. wendtii* "brown" plantlets raised in aseptic conditions to acute gamma radiation at doses of 0, 10, 20, 30, 40 and 50 Gy showed that 45 days after irradiation the survival rate of plantlets (M_1V_1) that had received 10 Gy of radiation was no different from the control (100% survival). With a dose of 20 Gy, the survival rate was 75.56% and at a dose of 30 Gy or more, the survival rate was 0% (Table 1). The effect of radiation on growth was assessed by counting the number of new shoots at 45 days after irradiation. The average number of new shoots was negatively related to the radiation dose. That is, on the plantlets that were exposed to 0, 10, 20, 30, 40 and 50 Gy of radiation, the mean number of new shoots recorded was 2.5, 1.3, 1.2, 0, 0 and 0, respectively. Plantlets that were exposed to 30 Gy or more died and did not produce any new plantlets (Table 1). This was in agreement with Pongchawee *et al*. (2007) who studied the effects of acute gamma radiation on *Anubias nana* and Prerdpraiwong (2004) who worked *in vitro* on *Hygrophilla difformis* and found that the growth rate decreased with increasing radiation doses. The report by Ruangnarong (1999) on Amazon plant (*Echinodorus argentinensis*) and the study by Lamseejan *et al*. (2002b) of *Curcuma* spp. likewise gave similar results. In the current study the $LD_{50(45)}$ was 23 Gy (Figure 1) and the $GR_{50(45)}$ was 20 Gy. A previous study of *C. wendtii* "green"

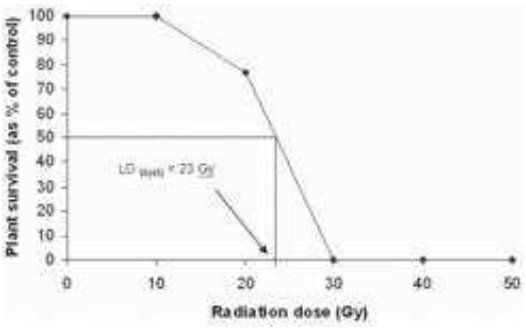

Figure 1 Relationship between radiation dose and survival of *Cryptocoryne wendtii* "brown" plants after acute irradiation with gamma-rays and culture on medium for 45 days.

Table 1 The number of irradiated plants, the number of plants surviving, the average number of new plantlets per plant and the growth rate of *C. wendtii* "brown" after acute irradiation of 60 *in vitro* plants with different doses of gamma rays.

Radiation dose (Gy)	No. of survived plants	Plant survival (as % of control)	Ave. no of plantlets per plants	Growth rate (as % of control)
0	60	100.00	2.5	100.00
10	60	100.00	1.3	52.01
20	46	76.67	1.2	48.30
30	0	0.00	0.0	0.00
40	0	0.00	0.0	0.00
50	0	0.00	0.0	0.00
F-test	**	**	**	**
C.V.(%)	5.08	5.31	10.95	8.79
$LSD_{(0.01)}$	1.17	6.11	0.23	7.31

** significant at 1 % level

by Tasananuttiya (2005) reported an $LD_{50(45)}$ of 15 Gy and a $GR_{50(45)}$ of 13 Gy, indicating that *C. wendtii* "brown" was more resistant to gamma rays than *C. wendtii* "green", since sensitivity to radiation may vary with plant species and variety (Fujii and Matsumura, 1958; Fujii, 1960; Tangsombatvitchit *et al*., 2008).

Variations in M_1V_2 and M_1V_3 generations

When plants were propagated from M_1V_1, some variations were observed in plants that had received 10 Gy of radiation, which showed paler, brown-colored leaves, green, smaller leaves and dwarfism (Figure 2, top row). The plants that were exposed to 20 Gy of radiation, produced variants that included: a clumped growth habit, dwarfism, darker-brown leaves, paler-brown leaves, furled leaves and narrower leaves (Figure 2, bottom row). At a rate of 30 Gy or more, none of the plants survived.

Plants from the M_1V_2 generation that showed variations were selected and propagated to form the M_1V_3 generation for testing variations and further selection. It was found that the M_1V_3 generation plants that were exposed to 10 Gy of radiation had characteristics such as green leaves, narrow pointed leaves, tapered leaves, small leaves, dwarfism and good bunching (Figure 3, top row). Those that were exposed to 20 Gy of radiation showed characteristics such as dwarfism, narrow pointed leaves, furled leaves, pinkish-brown leaves, green leaves and shorter petioles (Figure 3, bottom row). The plants with interesting variations were selected for further propagation and testing.

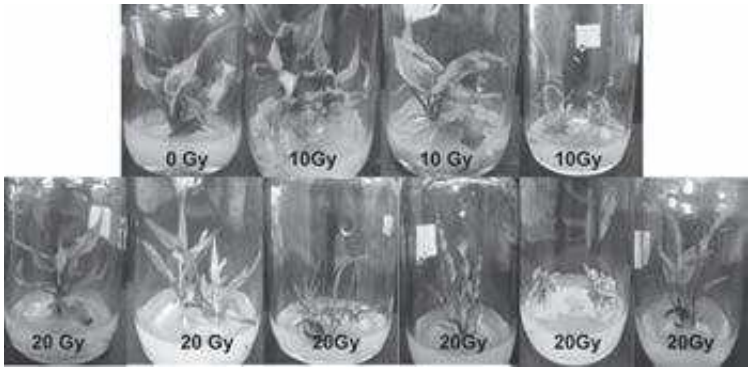

Figure 2 Variations in the M_1V_2 generation of *Cryptocoryne wendtii* "brown" exposed to 10 and 20 Gy of acute gamma radiation.

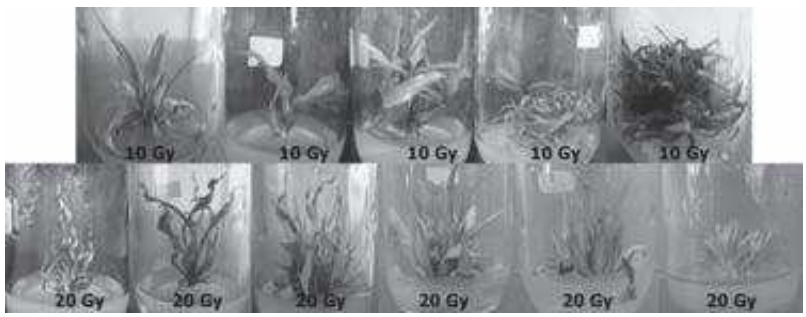

Figure 3 Variations in the M_1V_3 generation of *Cryptocoryne wendtii* "brown" exposed to 10 and 20 Gy of acute gamma radiation.

Chronic irradiation

In the experiment on chronic gamma irradiation, the plants were placed at 1.5 and 2.5 m from the source and received 1.614 and 0.537 Gy per hour, respectively. When *C. wendtii* "brown" plants grown in tissue culture received 0, 238.87 and 477.74 Gy at a distance of 1.5 m from the source, it was impossible to calculate the $LD_{50(45)}$ because at this high level of radiation all the samples died (Figure 4). The control plants grew an average of 2.58 new shoots per plant after 30 days. For the plants that were placed 2.5 m from radiation source, the survival rate and growth rate (number of new shoots) decreased with increasing radiation, but it was not possible to calculate the $GR_{50(45)}$ and $LD_{50(45)}$ (Table 2) because the radiation dose was not high enough.

Variations in the M_1V_2 generation

Some of the variations observed in the M_1V_2 generation samples of *C. wendtii* "brown" that were exposed to chronic gamma radiation at a rate of 0.537 Gy per hour included: small narrow leaves, green leaves, paler brown leaves, smaller leaves, dwarfism and increased branching (Figure 5).

Figure 4 *C. wendtii* "brown" plantlets from the M_1V_1 generation that were exposed to chronic gamma radiation at a rate of 1.614 Gy per hour for a total of 238.87 and 477.74 Gy.

Figure 5 *C. wendtii* "brown" plantlets from the M_1V_2 generation that were exposed to chronic gamma radiation at a rate of 0.537 Gy per hour for a total of 0, 10.75, 21.50, 32.24 and 42.99 Gy.

Table 2 The number of irradiated plants, the number of surviving plants, the average number of plantlets per plant and growth rate of *C. wendtii* "brown" after chronic irradiation of 30 *in vitro* plants with different doses of gamma-rays.

Radiation dose (Gy)	No. of survived plants	Plant survival (as% of control)	Average no. of plantlets per plants	Growth rate (as% of control)
0.00	30	100.00	2.53	100.00
10.75	27	90.00	1.87	73.68
21.50	23	76.67	1.70	67.06
32.24	20	66.67	1.97	77.86
42.99	22	73.33	2.00	78.94
F-test	*	*	**	**
C.V.(%)	14.54	14.54	9.42	8.22
$LSD_{(0.05)}$	2.15	21.53	0.35	11.90
$LSD_{(0.01)}$	-	-	0.49	16.91

* significant at 5 % level

** significant at 1 % level

A comparison of the results of the acute and chronic gamma irradiation showed that *C. wendtii* "brown" could withstand a higher dose of radiation when it was administered as chronic irradiation. When exposed to 30 Gy of radiation or more as acute irradiation, all the samples of *C. wendtii* "brown" died, while exposure to the same dose of chronic radiation resulted in most of the plants surviving and continuing to grow. This result agreed with Lamseejan *et al*. (2002a).

CONCLUSION

Acute irradiation

The most appropriate amount of acute gamma irradiation for inducing mutations in *C. wendtii* "brown" was 10-20 Gy, based on the $LD_{50(45)}$ and $GR_{50(45)}$ of 23 and 20 Gy, respectively. Plants that were exposed to 30 Gy of radiation or more ceased to grow and eventually died. Variations were observed in the M_1V_2 and M_1V_3 generations (10 and 20 Gy) in the form and shape of the leaves, the leaf color and the growth habit.

Chronic irradiation

In this experiment, tissue cultured *C. wendtii* "brown" plants were exposed to chronic gamma radiation at two distances from the source (1.5 and 2.5 m) with two different radiation dose rates (1.614 and 0.537 Gy per hour). The results showed that the total dose and dose rate of radiation had different effects on the plants. At a distance of 1.5 m from the source, the rate of radiation and the total dose of radiation were too high, causing all samples to die. At a distance of 2.5 m, many plants survived and displayed variations for selection. A greater dose of radiation could have been used because at the highest dose of 42.99 Gy the survival rate was still high.

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