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The Effect of Acute Gamma Irradiation on the Radiosensitivity and Morphology of *Gomphrena pulchella*

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ABSTRACT

Gomphrena pulchella, commonly known as the button flower, stands as an exotic ornamental species celebrated for its beauty in landscaping, fencing, and potted displays. Gamma irradiation-induced mutation has emerged as a promising strategy for induced-mutant breeding. Nonetheless, the application of gamma radiation on *G. pulchella* remain limited. This study aimed to determine the optimal effective dose by radio sensitivity test and evaluate the effects of radiations on *G. pulchella* cutting. The cuttings of *G. pulchella* were irradiated using acute gamma at 0, 10, 20, 40, 60, 80 and 100 Gy. Each treatment consisted of 35 cuttings. The irradiated cuttings were then planted into a perlite medium and after 60 days, the number of survived cuttings, the number of new leaves and shoots, the length of new shoots and days to flower bud emergence were measured. The survival rate of cuttings decreased as the dose of radiation increased. The highest mean survival rate was 97% obtained from the non-irradiated cuttings (0 Gy) followed by 89% from 10 Gy, 94% from 20 Gy, 69% from 40 Gy, 54% from 60 Gy and 31% from 80 Gy and only 9% survival at 100 Gy. Higher radiation rates also decreased the leaves and shoot numbers, shoot length and fluorescent emergence. Based on the survival rate graph, the lethal dose 50% (LD_{50}) value was 59 Gy. The results revealed that gamma irradiation had various effects on the growth of *G. pulchella* including vegetative and flower development. The value of LD_{50} will be used to determine the optimal effective dose for further radiation-induced mutation in *G. pulchella* research.

Keywords: *Gomphrena pulchella*, LD_{50}, radiosensitivity test.

INTRODUCTION

Gomphrena pulchella is a species from the large genus Gomphrena which belongs to the Amaranthacea family (Figure 1). Gomphrena which is also known as the button flower is an exotic ornamental plant famous for landscape decorations such as garden landscaping, fence plants or in pots. *Gomphrena* belongs to the family Amaranthaceae, native from America and comprises approximately 120 species found in America, Antartica and Indo-Malaysia (Ilyas et al., 2014). In 2020, MARDI has introduced *G. pulchella* as a new exotic ornamental plant suitable for decoration in landscape gardens (Ahmad et al., 2020). Perennial flower such as *G. pulchella* is often chosen as ornamental plant because of its fast growth rate and diligent flowering. However, the reliance on imported planting material and the limited availability of viable seeds hinders its propagation, necessitating innovative solutions to meet the demands of the floriculture industry. The development of new native and exotic varieties is expected to give a new injection to boost the floriculture industry in Malaysia.

Figure 1. *Gomphrena pulchella*

During the past decades, an induced mutation in plant improvement using gamma irradiation has become a proven way of creating a new variety. Mutation induction is the best way to develop new varieties with desirable traits in a short period of time compared to conventional breeding. Up to 90% of released mutant cultivars were derived from radiation-induced mutations (Pimonrat et al., 2012). Gamma irradiation is easy to use, safe for plant breeders and effective in many different plant species including flowering plants, fruits, vegetables and herbs. Many ornamental plant species such as chrysanthemum (*Chrysanthemum* spp.), rose (*Rosa* spp.) and carnation (*Dianthus caryophyllus*), are propagated vegetatively, making them relatively easy to be induced by mutation. Therefore, mutation breeding is well suited for breeding ornamental plants, as evidenced by the approximately 720 spontaneous and induced mutant cultivars that have already been produced (Yamaguchi, 2018).

In creating a new variant of *G. pulchella* by mutation induction, a database on the radiosensitivity of *G. pulchella* is essential to be established. The radiosensitivity test is a prerequisite step before the mutagenic treatment. This test is carried out to determine the most effective dosage of irradiation to be used and also to estimate the frequency and mutation spectrum using gamma irradiation (Abdullah et al., 2009). LD_{50} (lethal dose) is defined as the dose at which 50% of the irradiated objects die after irradiation and is considered as the dose which gives the highest frequency for mutation to occur. The half-reducing fatality by the lethal exposure to plants, the extended level of damage using gamma irradiation and the lethal exposure rate to plants can be used as an indicator for radiosensitivity tests (Abdullah et al., 2009). This study aimed to determine the optimal effective dose for the radiosensitivity test (LD_{50}) and to evaluate the effects of gamma rays on the growth and morphology of *G. pulchella*.

MATERIALS AND METHODS

Research work was carried out in several different areas. The preparation of plant material was carried out at the Flower Complex, Horticulture Research Center, MARDI Serdang. While acute gamma ray irradiation activities were carried out at the Malaysian Nuclear Agency, Bangi. Healthy and mature *G. pulchella* plants used in this study were obtained from the research plot of the Horticulture Research Center, MARDI Serdang. Cuttings from the second node with a diameter range of 1.5 – 2.5 mm and 7.0 – 14.0 cm long were used as explants (Figure 2). The number of cuttings taken depends on the number of nodes available on a single plant. All leaves on the cutting were cut in half and the cuttings were prepared 1 day before irradiation.

(a) (b)

Figure 2. Cuttings from the second node from a healthy and matured *G. pulchella* (a). Cuttings with diameter range of 1.5 – 2.5 mm and 7.0 – 14.0 cm long (b).

Cuttings were irradiated at 7 different doses of acute gamma rays, namely 0 (as control), 10, 20, 40, 60, 80 and 100 Gy using Biobeam GM8000. Each treatment consisted of 35 cuttings. A total of 245 *G. pulchella* cuttings were irradiated and then planted into perlite media for rooting (Figure 3). After two weeks, the rooted cuttings were then transferred to the media for further growth.

Figure 3. Cuttings in perlite.

The germinated cuttings were observed up to 60 days after planting. Data collected were the number of survived cuttings, number of leaves, number of new shoots, length of new shoots and days to flower bud emergence. The experimental design was a Completely Randomized Design (CRD) with 5 replications. Morphological data collected from the radiosensitivity study were analysed by Analysis of Variance (ANOVA) using SAS software version 9.3 and tested for significance using Least Significant Difference (LSD) at $p < 0.05$. The survival rate (%) reduction curve was constructed using a Simple Linear Regression for the determination of LD_{50}.

RESULTS AND DISCUSSION

The radiosensitivity of *G. pulchella* to radiation was evaluated by comparing the mortality rate (%) between treated and non-treated plants at 60 days after irradiation. The plant mortality rate increased with increasing irradiation dosage at 60 days after irradiation as shown in Table 1. Gamma rays have significantly reduced the mean survival rate of *G. pulchella* cuttings. At higher doses, the survival rates were decreased due to chromosomal damage, suppression of critical cell functions, or a combination of both, which finally led to bud death and prevented bud break in cutting (Ghasemi-Soloklui et al., 2023). The death of plants is attributed to the interaction of radiation with other molecules in the cell, particularly water, to produce free radicals. The free radicals could combine to form toxic substances, such as hydrogen peroxide (H_2O_2), which contribute to the destruction of cells. This indirect effect is especially significant in vegetative cells, the cytoplasm of which contains about 80% water (Taheri et al., 2014a). This decreasing trend was also observed in the number of leaves and shoots, length of shoots and days to flower bud emergence on germinated cuttings (Table 2).

Table 1. Mean survival rate percentage (%) of irradiated *G. pulchella* cuttings at 0, 10, 20, 40, 60, 80 and 100 Gy at 60 days after planting

Dose (Gy)	Survival Rate (%)
0	97.14[a]
10	88.57[a]
20	94.29[a]
40	68.57[b]
60	54.29[b]
80	31.43[c]
100	8.57[c]

Means with the same or common letter are not significantly different, by least square difference test (LSD), * $p < 0.05$

Table 2. Mean data of number of leaves, number of shoots, length of shoots and number of flower buds emerge at 60 days after planting

Dose (Gy)	Num. of leaf	Num. of shoots	Length of shoots (cm)	Num. of days flower bud emerge
0	39.71[a]	1.11[a]	3.56[a]	15[b]
10	33.00[a]	0.94[a]	1.79[b]	18[b]
20	35.71[a]	0.80[ab]	3.26[a]	21[b]
40	29.43[ab]	0.54[bc]	0.68[c]	36[a]
60	15.71[c]	0.33[cd]	0.81[bc]	36[a]
80	19.00[bc]	0.40[cd]	0.73[bc]	0
100	2.29[d]	0.10[d]	0.09[c]	0
Significant level	***	***	***	***

Means with the same or common letter are not significantly different, by least square difference test (LSD), * $p < 0.001$

Generally, non-treated cuttings produced more mean number of leaves and shoots compared to treated cuttings. The mean number of leaves and shoots, length of shoots and days to flower bud emergence decreased along with the increasing dosage of gamma rays. At 60, 80 and 100 Gy, mean number of leaves was just 15.71, 19.0 and 2.29, respectively, compared to non-treated (0 Gy), 10, 20 and 40 Gy which were 39.71, 33.0, 35.71 and 29.43, respectively. After 60 days, only non-treated cuttings produced shoots but none of the treated cuttings produced new shoots. This result showed that most cuttings were affected by the irradiation and their growth was slower than the control at dosages more than 50 Gy. This was consistent with the findings of Tangpong et al. (2019) who found that *Anubis congensis* N.E. Brown exposed to gamma irradiation at a dose of 80 – 100 Gy did not develop any shoots (Taheri et al., 2014b). Irradiation may lead to retardation or inhibition of cell division and cell death induction of mitotic activity which affect growth rate or growth habit and cause changes in plant morphology (Ahmadi et al., 2012). Days to flower bud emergence were also significantly different among all treatments. The delay in bud initiation eventually caused the late blooming. Delay in flowering might be due to disturbances in the biochemical pathway, which assists in the synthesis of flower-inducing chemical substances. The irradiation alters many biochemical pathways, which may be associated with the flower orientation (Anne and Lim, 2021). The result revealed that gamma irradiation had various effects on the growth of *G. pulchella* including vegetative and flower development (Figure 4).

Figure 4. Morphology of *G. pulchella* cuttings irradiated at 0, 10, 20, 40, 60, 80 and 100 Gy on the 60th day after irradiation

The radiosensitivity of the plant material to the gamma irradiation depends on the genetic constitution, dose of gamma rays employed, amount of DNA and type of plant material (Gupta et al., 2017). The plant may die if the dose of radiation is too high because the mutagen can have a direct or indirect adverse effect on the plant. However, there is no sufficient mutation induction effect if the dose of radiation given is too low. The radiosensitivity is determined by measuring the effects on plants when different doses of radiation are applied on them. Various factors such as biological, physical and chemical contribute to the diverse effects of radiation on plants.

From this study, it was indicated that the LD_{50} value of *G. pulchella* was 59 Gy (Figure 5). Different factors that influence the relative radiosensitivity of different plant species have been studied for a long time in different laboratories (Datta, 2019). These studies generate sufficient information about the radiation dose and its sensitivity to plants. Radiation-induced growth inhibition in plants is the result of genetic loss following chromosomal aberrations or physiological changes. The number of dividing cells, late mitosis, plant inhibition and pollen sterility are among the characteristics that can be used to measure the effect of radiosensitivity test. The decreasing or reducing rate of new shoot emergence and plant death can also be used as an indicator in radiosensitivity tests (Abdullah et al., 2009). This was shown by several radiosensitivity test studies involving major crops such as rice (Fifika et al., 2021; Solim and Rahayu, 2021) and wheat (Albokari et al., 2012; Hong et al., 2018). Mutagenic effects are different between various types of plant species and cultivars (Ahmad et al. 2012). According to the International Atomic Energy Agency (IAEA) Mutant Varieties Database (http//mvgs.iaea.org), many previous studies such as rice (seeds), orchids (tissue culture), chrysanthemum (seeds, cuttings, leaves), ginger and curcuma (rhizomes) and roselle (stem cuttings) produce similar radiosensitivity results but with different effects.

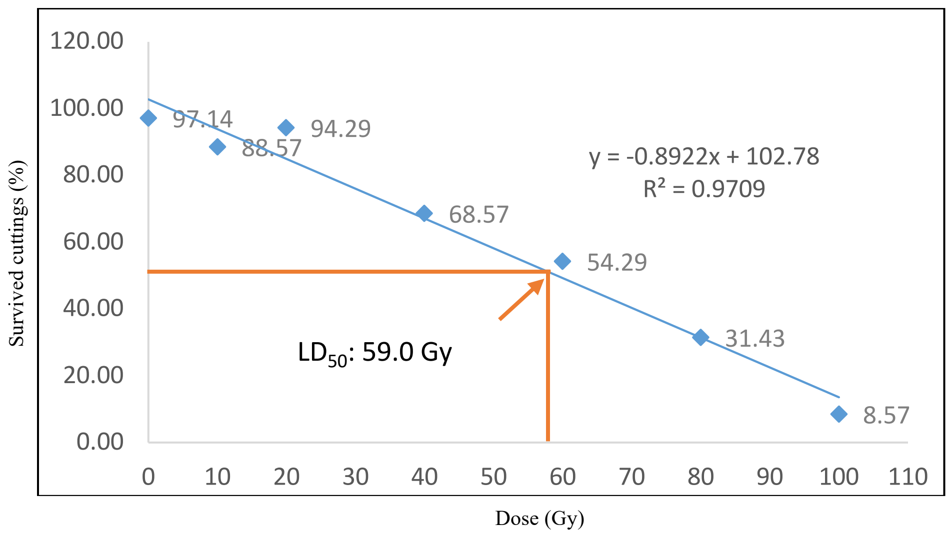

Figure 5. Data and regression equation for survival rate (%) of *G. pulchella* cuttings at 60 days after planting

CONCLUSION

In conclusion, the lethal dose 50% (LD_{50}) value for *G. pulchella* was 59 Gy. A lower dose of gamma rays gave no significant effect on the cuttings whereas the higher dose of gamma rays had caused a negative impact on the growth, in terms of higher mortality rate and reduction in growth. Data on this radiosensitivity test will be used to determine the optimal effective dose for radiation-induced mutation in *G. pulchella* research. This finding may be useful for induced mutations to accelerate the evolution of this ornamental species, generating novel varieties that align with market preferences and environmental resilience. Through this approach, the study aspires to enrich *G. pulchella* biodiversity and utility, ultimately enhancing its role in aesthetic landscapes and commercial viability.

ETHICAL APPROVAL

No ethical approval was required.

AUTHORS CONTRIBUTION

Conceived and designed the analysis: FZMN, CRCMZ, ZA, NHMS; collected the data: FZMN, CRCMZ, ZA: data or analysis tools: FZMN, ZA, CRCMZ, NHMS; performed the analysis: FZMN, ZA, NHMS; wrote the paper: FZMN.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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