# Effects of gamma radiation on morphology, survival and growth of *Allamanda cathartica* plants at different maturity

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**ABSTRACT**

*Allamanda cathartica* rooted cuttings were used to expose gamma radiation using Gamma chamber 1200 Cobalt-60 research irradiator and treatments were carried out at the Horticultural Crop Research and Developmental Institute, Gannoruwa. Two sets of experiments were conducted to determine the suitable dose of gamma radiation with plant maturity to recover the optimum amount of plants with favourable characters. The first experiment was conducted with six weeks old plants and they were exposed to a sequence of gamma radiation doses with 0 Gy, 17 Gy, 18 Gy, 19 Gy, 20 Gy and 21 Gy. The second experiment was conducted by exposing twelve weeks old plants with the same doses of gamma radiation as in the first experiment. Plants were pruned to a same height before exposing to radiation treatments. New flushes produced by radiated plants showed abnormal but attractive leaves. However, the changes gradually disappeared with time and visualized a normal growth. All radiated plants died at eight weeks after the gamma radiation treatment in the experiment one. Further, in experiment two radiated plants were survived longer. There was a gradual reduction in survival rate with increasing dose of gamma radiation. Also, a significant ($p>0.05$) reduction in plant height and internode length were observed in radiated plants compared to plants in the control. These results indicated that gamma radiation can retard the growth of plants. Therefore, it could be stated that changes occurred in leaf morphology due to gamma radiation may be mainly due to physical damage that was not persistent. Increased maturity of *Allamanda cathartica* plants exhibited resistance against lower doses of gamma radiation and which can be an effective way for the induction of mutations in *Allamanda cathartica*.

***Keywords:*** *Allamanda cathartica, gamma radiation, growth, morphology, survival*

## INTRODUCTION

Mutation induction is a powerful tool for creating new and innovative plant products (Penna *et al.,* 2012) and it is very much useful to improve natural genetic resources for vegetatively propagated crops (Mohan, 2006). Several advancements in ornamental crops could be attained by gamma radiation-induced mutation, including the plant characters such as flowers, leaves, growth habit and physiological features (Schum and Prell, 1998). Mutation induction through radio activities is one the most widely used method to develop and improve mutants compared to conventional breeding procedures; acclimatization, selection, hybridization, which are laborious, time-consuming, and also with limited genetic variations (Hanafiah *et al.,* 2010). Radiation mediated changes (morphological, structural and functional) in a plant are regulated by the intensity and duration of the gamma rays which generally induce changes as cytologically, biochemically, physiologically, morphologically and genetically in cells and tissues (Chandrashekar *et al*., 2013).

Gamma radiation is one of the vital physical agents used to enhance the characters and productivity of various plants. The technique

represented a significant role in plant breeding programs and genetic studies aimed to improve yield and produce favourable qualities in many crops under both normal and stress conditions (Borzouei *et al.,* 2013). Dose rate is one of the important factors of radiation treatment, and its effect has been examined by evaluating several traits, including lethality (Killion and Constantin, 1971, Rifnas *et al.,* 2020), growth (Killion and Constantin, 1971, Killion *et al*., 1971), and fertility (Killion and Constantin, 1971, Yamashita, 1964).

*Allamanda cathartica* Linn. (Apocynaceae) is one of the most studied species of the *Allamanda* genus. It is mainly multiplicated through vegetative means using stem cuttings. It is valued for its ornamental and medicinal characters but remains as a neglected plant (Madushani *et al.,* 2019). It is believed that the dose rate and plant maturity also influence radiation damage in *Allamanda cathartica*. Hence, the present study was conducted to evaluate the responses of different age-old *Allamanda cathartica* rooted cuttings to gamma radiation.

## MATERIALS AND METHODS

Experiments were conducted at the University of Colombo Institute for Agro-technology and Rural Sciences (UCIARS), Weligatta, Hambantota, Sri Lanka. Cuttings collected from UCIARS farm field were used to propagate *Allamanda cathartica* plants. Healthy and uniform plants with six and twelve weeks old were exposed to different doses of gamma radiation treatment. Before exposing for gamma treatments, the plants were pruned to same height using a sharp secateur and subjected to different doses (0, 17, 18, 19, 20 and 21 Gy) of gamma radiation using a $Co^{60}$ gamma chamber 1200 research irradiator source at the Horticultural Crop Research and Development Institute (HORDI), Gannoruwa, Sri Lanka. Plants of the control treatment (0 Gy) were not irradiated. Dose selection was based on our preliminary experiment on the *Allamanda cathartica* rooted cuttings, where a complete decrease in survival rate was observed above the dose of 21 Gy.

There were two sets of experiments; the first experiment was conducted using six weeks old plants and experiment two was conducted with twelve weeks old plants. Each treatment was replicated four times with ten plants in each replicate and the treated plants were allowed to grow in pots containing equal amounts of topsoil with compost. Those were arranged in a shade net house with Complete Randomized Design. The survival rate of the plants was recorded until it is getting stable. Thereafter morphological differences, plant height and internode length were noted. Collected data were analyzed using ANOVA by SAS 9.1.3 statistical software. Significant differences between the treatments were compared using DMRT at 5% significance level.

## RESULTS AND DISCUSSION

### Morphological changes

New flushes of radiated plants in both experiments showed abnormal leaves. Later on, the variation disappeared and leaves showed normal growth. The changes in the newly flushed leaves caused by gamma radiation may be due to the physical damages caused by radiation stress. Hence, the leaves recovered from the abnormalities showed during the early growth stages. As indicated by Minisi *et al.,* (2013), Exposing *Moluccella laevis* to irradiation caused leaf abnormalities, variegated leaves and calyx abnormalities. Further, an experiment on *Zinnia elegans* by Venkadachalam and Jayabalan (1997) has recorded that, gamma radiation caused significant morphological changes in leaf and flowers of *Zinnia elegans*.

### Experiment 1

***Survival rate***

There were significant ($p>0.05$) differences found in survival of radiated plants compared to control plants. Lethality effects of gamma radiation on plants were observed at 2$^{nd}$ week after exposing to radiation treatments. Increase in the dose of gamma radiation reduced the survival rate of the plants. Treated plants showed complete lethality at eight weeks after radiation treatment (Table 1). Gradual damage caused in meristematic cells and plant tissues may be caused these effects on treated plants (Kovacs and Keresztes, 2002). Experiments on

*Canscora decurrens* (Yadav, 2016), *Catharanthus roseus (*Mangaiyarkarasi *et al.,* 2014) have indicated that gradual reduction in the number of plants survived observed with increase in gamma radiation dose, further mutagenic effectiveness and efficiency increased with the decreased in the dose of gamma radiation.

**Experiment 2**

***Survival rate***

It was found that there were significant (p>0.05) differences between the different doses of gamma radiation treatments in the survival of *Allamanda cathartica* plants. Plants treated with gamma radiation showed a significant reduction in survival rate compared to control (Table 2). Increasing dose of gamma radiation caused a reduction in survival rate and the highest survival rate was obtained in the plants not exposed to gamma radiation treatment. The effects of radiation on survival of *Allamanda cathartica* was observed at two weeks after treatment. Survival rate got stable after 10$^{th}$ week. Comparatively highest survival rate was observed compared to experiment one. Maturity of *Allamanda cathartica* plant affected the survival of the plants. Twelve weeks old plants resisted highly compared to six weeks old plants. As indicated by Sawangmee *et al.,* (2011) The effect of gamma rays on plant survival was gradual depending on the exposure dose, irrespective of the irradiation method. Further Shakhs *et al.,* (2007) proved that a low dose of gamma-ray was most positively effective on the subsequent growth of the plant.

**Table 1:** Effects of dose rate of gamma radiation on percentage survival of *Allamanda cathartica*

| Treatments | Survival (%) | | | |
|---|---|---|---|---|
| | 2$^{nd}$ week | 4$^{th}$ week | 6$^{th}$ week | 8$^{th}$ week |
| T1 - 0 Gy | 100 | 100$^{a}$ | 100$^{a}$ | 100 |
| T2 - 17 Gy | 100 | 62.5$^{b}$ | 10.0$^{b}$ | 0 |
| T3 - 18 Gy | 100 | 55.0$^{bc}$ | 7.5$^{b}$ | 0 |
| T4 - 19 Gy | 100 | 40.0$^{bc}$ | 7.5$^{b}$ | 0 |
| T5 - 20 Gy | 100 | 37.5$^{bc}$ | 5.0$^{b}$ | 0 |
| T6 - 21 Gy | 100 | 25.0$^{c}$ | 5.0$^{b}$ | 0 |
| Sig. | - | * | * | - |

*Values represent the mean of four replications followed by the same superscripts in the same column are not significantly different at DMRT 5%. * represents significant at 5% and ns represents not significant.*

**Table 2:** Effects of dose rate of gamma radiation on percentage survival of *Allamanda cathartica*

| Treatments | Survival (%) | | | | | |
|---|---|---|---|---|---|---|
| | 2$^{nd}$ week | 4$^{th}$ week | 6$^{th}$ week | 8$^{th}$ week | 10$^{th}$ week | 12$^{th}$ week |
| T1 - 0 Gy | 100 | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ | 100$^{a}$ |
| T2 - 17 Gy | 100 | 75.0$^{b}$ | 72.5$^{b}$ | 52.5$^{b}$ | 20.0$^{b}$ | 20.0$^{b}$ |
| T3 - 18 Gy | 100 | 70.0$^{bc}$ | 65.0$^{b}$ | 42.5$^{b}$ | 17.5$^{b}$ | 17.5$^{b}$ |
| T4 - 19 Gy | 100 | 65.0$^{bc}$ | 60.0$^{b}$ | 40.0$^{b}$ | 15.0$^{b}$ | 15.0$^{b}$ |
| T5 - 20 Gy | 100 | 62.5$^{bc}$ | 50.0$^{b}$ | 40.0$^{b}$ | 12.5$^{b}$ | 12.5$^{b}$ |
| T6 - 21 Gy | 100 | 52.5$^{c}$ | 47.5$^{b}$ | 32.5$^{b}$ | 7.5$^{b}$ | 7.5$^{b}$ |
| Sig. | - | * | * | * | * | * |

*Values represent mean of four replications followed by the same superscripts in the same column are not different at DMRT 5%. * represents significant at 5% and ns represents not significant.*

**Internode length**

Exposing of *Allamanda cathartica* plants to gamma radiation significantly (p>0.05) reduced internode length when compared to control. Highest values in internode length were obtained in plants not exposed gamma radiation (Table 3).

An experiment on Chrysanthemum by Lee *et al.,* (2010) resulted that, when exposing chrysanthemum rooted cuttings to different doses of gamma radiation showed a remarkable decrease in internode length ranging from 2 to 4 times at 40-50 Gy dosage.

**Plant height**

It was found that there were significant (p>0.05) differences between the treatments on plant height. Highest plant height was recorded where plants not exposed to gamma radiation treatment (Table 3). Shorter internode length caused the reduction in plant height. An experiment in *Moluccella laevis* by Minisi *et al.,* (2013) proved that increase in gamma radiation dose decreased the height of *Moluccella laevis* plant. Further, they indicated that the higher dose irradiation caused growth retardation has been ascribed to the cell cycle arrest at G2/M phase during somatic cell division and or various damages in the entire genome.

**Table 3:** Effects of different doses of gamma radiation on plant height and internode length of *Allamanda cathartica*

| Treatments | Plant height (cm) | | | Internode length (cm) | | |
|---|---|---|---|---|---|---|
| | 4th week | 8th week | 12th week | 4th week | 8th week | 12th week |
| T1 - 0 Gy | 13.7$^{a}$ | 24.3$^{a}$ | 35.7$^{a}$ | 1.45$^{a}$ | 2.10$^{a}$ | 2.55$^{a}$ |
| T2 - 17 Gy | 8.3$^{b}$ | 15.4$^{b}$ | 23.4$^{b}$ | 1.25$^{a}$ | 1.75$^{ab}$ | 2.05$^{ab}$ |
| T3 - 18 Gy | 7.3$^{b}$ | 11.5$^{b}$ | 19.4$^{b}$ | 1.10$^{a}$ | 1.46$^{bc}$ | 1.83$^{b}$ |
| T4 - 19 Gy | 7.9$^{b}$ | 12.9$^{b}$ | 20.7$^{b}$ | 0.65$^{b}$ | 1.10$^{c}$ | 1.45$^{b}$ |
| T5 - 20 Gy | 6.8$^{b}$ | 13.0$^{b}$ | 19.9$^{b}$ | 0.43$^{b}$ | 1.06$^{c}$ | 1.46$^{b}$ |
| T6 - 21 Gy | 6.6$^{b}$ | 10.9$^{b}$ | 19.4$^{b}$ | 0.30$^{b}$ | 1.20$^{c}$ | 1.50$^{b}$ |
| Sig. | * | * | * | * | * | * |

*Values represent mean of four replications followed by the same superscripts in a same column are not significantly different at DMRT 5%. * represents significant at 5% and ns represents not significant.*

**CONCLUSIONS**

Increasing gamma radiation dose decreased the survival and retarded the growth of *Allamanda cathartica*. Increase in plant maturity increased the survival of the plants. Exposing more hardened plants of *Allamanda cathartica* to a lower dose of gamma radiation could recover plants with maximum survival and with favourable characters.

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**REFERENCES**

Borzouei, A., Kafi, M., Sayahi, R., Rabiei, E. and Amin, P.S., 2013. Biochemical response of two wheat cultivars (*Triticum aestivum* L.) to gamma radiation. *Pak J Bot*, *45*(2), pp.473-477.

Chandrashekar, K.R., Somashekarappa, H.M. and Souframanien, J., 2013. Effect of gamma irradiation on germination, growth, and biochemical parameters of *Terminalia arjuna* Roxb. *Radiation Protection and Environment*, *36*(1), p.38.

Hanafiah, D.S., trikoesoemoningtyas, T., yahya, S. and wirnas, D., 2010. Induced mutations by gamma ray irradiation to Argomulyo soybean (*Glycine max*) variety. *Nusantara Bioscience*, *2*(3).

Killion, D.D. and Constantin, M.J., 1971. Acute gamma irradiation of the wheat plant: effects of exposure,

exposure rate, and developmental stage on survival, height, and grain yield. *Radiation Botany*, *11*(5), pp.367-373.

Killion, D.D., Constantin, M.J. and Siemer, E.G., 1971. Acute gamma irradiation of the soybean plant: effects of exposure, exposure rate and developmental stage on growth and yield. *Radiation Botany*, *11*(3), pp.225-232.

Kovacs, E. and Keresztes, A. 2002. Effect of gamma and UV-B/C Radiation on plant cell. *Micron.*, 33: 19- 210.

Lee, J.H., Chung, Y.S., Joung, Y.H., Han, T.H., Kang, S.Y., Yoo, Y.K. and Lee, G.J., 2010. Induction of mutations for stem quality in chrysanthemum (*Dendranthema grandiflora*) by using gamma-ray irradiation. *Acta horticulturae*, *855*, pp.177-182.

Madushani, W.G.C., Silva, T.D., Geekiyanage, S., Weerasinghe, S., Vidanapathirana, N.P. and Subasinghe, S., 2019. Medicinal properties and morphological characters on *Allamanda cathartica* (Rukkathana). *Proceedings of the 5th International Symposium on Minor Fruits, Medicinal and Aromatic Plants*, pp. 56

Mangaiyarkarasi, R., Girija, M., Gnanamurthy, S. and Don, C. L. G. 2014. Mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in *Catharanthus roseus*. *Int. J. Curr. Microbiol. Appl. Sci*, *3*, pp.881-889.

Minisi, F.A., El-mahrouk, M.E., Rida, M.E.F. and Nasr, M.N., 2013. Effects of gamma radiation on germination, growth characteristics and morphological variations of *Moluccella laevis* L. *Am.-Eurasian J. Agric. Environ. Sci*, *13*, pp.696-704.

Mohan Jain, S., 2006, September. Mutation-assisted breeding for improving ornamental plants. In *XXII International Eucarpia Symposium, Section Ornamentals, Breeding for Beauty 714* (pp. 85-98).

Penna, S., Vitthal, S.B. and Yadav, P.V., 2012. In vitro mutagenesis and selection in plant tissue cultures and their prospects for crop improvement. *Bioremediation, Biodiversity, Bioavailability*, *6*, pp.6-14.

Rifnas, L.M., Vidanapathirana, N.P., Silva, T.D., Dahanayake, N., Weerasinghe, S.S. and Subasinghe, S., 2020. Effect of gamma radiation on survival rate of *Allamanda cathartica*–An indigenous medicinal plant. *International Journal of Minor Fruits, Medicinal and Aromatic Plants*, *6*(1), pp.50-53.

Sawangmee, W., Taychasinpitak, T., Jompuk, P. and Kikuchi, S. 2011. Effects of gamma-ray irradiation in plant morphology of interspecific hybrids between *Torenia fournieri* and *Torenia baillonii. Kasetsart J. Nat. Sci*, *45*, pp.803-810.

Schum, A., and W. Prell, 1998. Induced mutations in ornamental plants. Springer Netherlands, pp. 333-366.

Shakhs, M. H., El–Nagger, A. A. M. and El–Foully, A. A. 2007. Response of some ornamental palm seeds to gamma irradiation. *J Agric Sci Mansoura Univ.* 32(10):9629-9639.

Venkatachalam, P. and Jayabalan, N., 1997. Effect of gamma rays on some qualitative and quantitative characters in *Zinnia elegans* Jacq. *The Indian Journal of Genetics & Plant Breeding*, *57*(3), pp.255-261.

Yadav, V. 2016. Effect of gamma radiation on various growth parameters and biomass of *Canscora decurrens* Dalz. *International Journal of Herbal Medicine*, *4*(5), pp.109-115.

Yamashita, A., 1964. Some aspects of radiosensitivity of crop plants under chronic exposure. In *Gamma Field Symp.* 3, pp. 91-110.