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RESEARCH ARTICLE

Impact of Cobalt-60 gamma irradiation on growth, development, and morphology of *Acalypha hispida* (Cat's tail plant)

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Abstract: Gamma irradiation-induced mutagenesis has been utilized to improve many genotypes of crop species. Mutation induction has been identified as a significant method for the production of genetic variation in flowering plants. Hence, a series of experiments were conducted in a completely randomized design at the University of Colombo Institute for Agro-technology and Rural Sciences, Hambantota, Sri Lanka, to evaluate the influence of gamma rays on the mutagenicity of *Acalypha hispida*. The rooted cuttings were subjected to various dosages of Cobalt-60 gamma irradiation, including 0, 20, 25, 30, 35, and 40 Gy. Based on the findings, a second experiment was carried out in which the plants were exposed to 0, 45, 50, 55, 60, and 65 Gy gamma irradiation dosages. Treated plants were maintained inside a shade net house (30% shade) and morphological changes in plants, survival %, plant height, number of leaves, inflorescence length, and mutation % were recorded. Plants that showed improved characters were multiplied and observed the character expression in the second generation. Further, attempts were made to combine the improved characters into a single plant. There were significant differences in plant height and number of leaves but not in survival % and inflorescence length in experiment one. Further, a significant difference was observed in measured variables survival %, plant height, number of leaves, and inflorescence length in experiment two. A gradual reduction was observed in survival percentage, plant height and inflorescence length with the increase in gamma irradiation dose. Treated plants showed narrowed and malformed leaves but those were not persisted and discontinued. But, irradiated plants produced colour changed (pink and white), partial and no blooming inflorescences which was persisted in the second generation too. Hence, it could be stated that treating the *A. hispida* plants with gamma radiation has the potential to create mutation in plants.

***Keywords**: Induced mutation, Ornamental plant, Physical mutagen, Plant breeding, Variation*

Introduction

Gamma rays are a type of ionizing radiation particles that can interact with atoms or molecules in a cell, including water, to create free radicals. These free radicals can modify or harm essential components of plant cells, resulting in changes in plant morphology, anatomy, biochemistry, and physiology, which can vary based on the intensity of irradiation. Several researchers indicated the effects of Cobalt 60 gamma radiation on various plants (Bodele, 2013; Fadli et al., 2018; Astuti et al., 2020; Setia et al., 2020; Hajizadeh et al., 2022). Additionally, ionizing radiation can directly affect important targets in the cell. The impacts of gamma irradiation on plants depend on various factors, including plant traits (such as species, cultivar, stage of development, tissue architecture, and genome organization) and radiation aspects (such as quality, dose, and duration of exposure). Research has shown that changes in plant cellular structure and metabolism can occur after exposure to gamma

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irradiation (Kim et al., 2004; Wi et al., 2007; Jan et al., 2012).

In the early stages of assessing radiation's ability to generate mutations, various radiobiological measures are commonly employed. Mutagenic radiations have proven to be especially useful in breeding programs, particularly in flowering crops (Krasaechai et al., 2009) Gamma radiation can alter physiological characteristics (Kiong et al., 2008). As a result of the low genetic variability among current plant genotypes, mutation induction has become a well-established strategy in plant breeding, and a new era of crop development has begun, allowing for the improvement of cultivars in specific key qualities, while enhancing existing germplasm(Irfaq and Nawab, 2001).

Acalypha hispida, a member of the Euphorbiaceae family, also known as chenille plant, red hot cat's tail, monkey tail, and fox tail, is a genus of flowering shrubs primarily cultivated for ornamental purposes due to its attractive and brightly colored fuzzy blooms. Despite its aesthetic appeal, the plant has been overlooked due to its morphological characteristics such as vigorous growth. However, with some modifications to its traits, it has the potential to gain more popularity. Gamma irradiation can induce the formation of new variations of *A. hispida*, which can be reintroduced as a cut flower or potted plant, thereby advancing the floriculture industry.

This study may also be of interest to horticulturists who specialize in mutant breeding and the creation of new ornamental plant cultivars. Thus, this experiment aimed to evaluate the effects of various gamma radiation dosages on the performance of *A. hispida* plants.

Materials and Methods

Experimental Location

The University of Colombo Institute for Agro-technology and Rural Sciences in Weligatta, Hambantota, Sri Lanka, was selected as the study site. This region belongs to Low Country Dry Zone agro-ecological region (DL 5) in Sri Lanka, where the average annual temperature ranges from 29 to 33 °C.

Plant Materials

Acalypha hispida stem cutting type (Hardwood) with three nodes were separated and propagated using the technique described by Rifnas et al. (2020). The different doses of gamma irradiation were applied to rooted cuttings that were 6 weeks old.

Figure 1:a) Arrangement of irradiated A. hispida plants in a shade net house; b) Grafted root stock and scion

Gamma irradiation

To expose the plant materials, a Gamma chamber 1200 Cobalt-60 research irradiator installed at the Horticultural Crops Research and Development Institute (HORDI), Gannoruwa, Sri Lanka, was used as an irradiation source.

Experimental structure

A series of experiment was conducted as experiment 1 and 2. Experiment 1 was conducted with 6 treatments and 4 replications. The used doses were 0; 20; 25; 30; 35 and 40 Gy where the minimum dose can be given by the chamber is 17 Gy. Based on the observations of experiment 1, experiment 2 was conducted with 6 treatments and 6 replications. The plants were exposed to gamma irradiation doses 0; 45; 50; 55; 60 and 65 Gy. Treated plants were arranged in CRD manner inside a shade net house (30% shade).

Experiment 01

Rooted plants with 6 weeks old were used to expose to the gamma irradiation treatments. The plants were exposed to the irradiation doses 20 Gy, 25 Gy, 30 Gy, 35 Gy and 40 Gy with a control without exposing to the irradiation treatments. There were four replicates with each contained three plants. Treated plants were arranged in CRD manner under 30% shade condition (Figure 1a). Based on the results of experiment 01, 2nd experiment was conducted as follows.

Experiment 02

Rooted plants with 6 weeks old were exposed to the gamma irradiation doses 40 Gy, 45 Gy, 50 Gy, 55 Gy, 60 Gy and 65 Gy with a set of control. There were six replicates, and each contained twelve plants. Treated plants were arranged in CRD manner inside shade net house (30 shade).

Propagation of second generation of plants

Plants that showed variations in plant morphology were selected and attempts were made to produce the 2nd generation. The cuttings were separated from the mutant plants and propagated using the protocol developed during the early days of experiments. The propagated plants were observed for the expression of mother plant characters.

Combining the new characters to a single plant

Further, attempts were made to combine the improved characters into a single plant. Wedge grafting was practiced to combine the improved characters to a single plants with original characters (Figure 1b).

These plants were considered as the rootstocks and were established in black polyethylene bags to be grafted. The scions were obtained from the improved mother plants.

Data collection

Survival percentage of plants were measured up to 4 months after treating by expressing the ratio between total treated plants and survived plants as a percentage. With the onset of the first flower, flowering data such as inflorescence length was recorded. Plant height and no. of leaves were recorded up to 4 months.

Statistical Analysis

SAS 9.1.3 statistical software was used to perform statistical analysis on the recorded data. The Duncan Multiple Range Test (DMRT) was used to compare the means of the treatments at a 5% significance level.

Results and Discussion

Experiment 1

Morphological changes

Initial days of planting malformed leaves were observed, later the changes disappeared, and newly emerging leaves showed normal growth (Figure 2, and Fiure 3). Hence the changes occurred in the plant leaves due to gamma irradiation stress is temporary and not continued in later stages. The changes that occurred in leaves may be due to the physiological damage caused by the gamma radiation. Furthermore, treated plants showed colour-changed inflorescences while increasing the gamma radiation above 35Gy. Especially pink and white coloured inflorescences. Plants expressing both colors (white and red) in a single plant were also observed, and it may be due to the Chimera nature of the plants. The new character continued its changes in later development too.

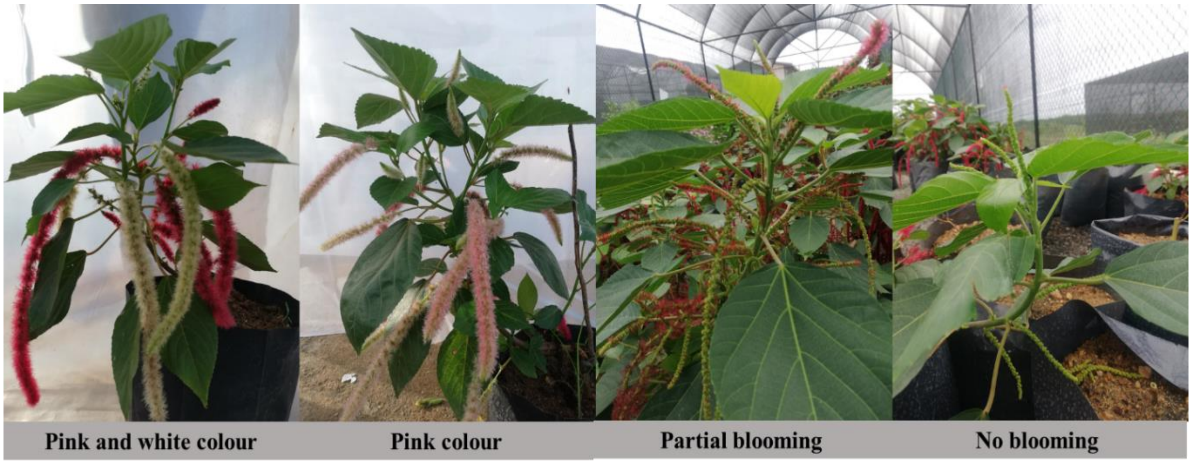

Figure 2: Variations found in inflorescences due to gamma irradiation treatments.

Figure 3: Misshapen and narrower leaves observed in A. hispida after gamma irradiation treatments.

As mentioned by Rifnas et al. (2020), when the *Allamanda cathartica* plants exposed to different doses of gamma radiation, morphological changes were observed in early growth stages but it was not persisted in later stages and leaves showed general morphology.

Furthermore, According to Minisi et al. (2013), *Moluccella laevis* developed variegated leaves, aberrant calyxes, and abnormal leaves after being exposed to irradiation. Venkatachalam and Jayabalan (1997) revealed that gamma radiation significantly alters the morphology of the leaves and flowers of *Zinnia elegans.*

Survival percentage

It was found that there were no significant differences between the treatments on survival % during 1st and 4th month after gamma radiation treatments (Table 1). The gamma irradiation dose up to 40 Gy did not influence the survival % of the plants. Hence the plants have the potential to be exposed to higher doses of gamma irradiation to create more variation.

El-Khateeb et al. (2016) showed a significant negative impact of gamma irradiation treatments on the survival rate of Philodendron plants. Further, they mentioned that, in comparison to the control, the survival percentage was considerably decreased by all treatment dosages such as 0.5, 2, 4, or 8 Krad. A reducing trend in survival rate observed while increasing the radiation dose.

This decline may be caused by plant tissue injury and the degeneration of meristematic cells (Tien et al., 2000; Kovacs and Keresztes, 2002)

Plant Height

It was revealed that, there were significant (P<0.05) differences among the treated gamma irradiation doses on plant height during the early growth stage and comparatively highest plant height was observed in plants that were not treated with gamma irradiation (Table 1). Increase in irradiation dose decreased the plant height and the lowest was recorded in the plants treated with the highest dose. But no significant differences were found among the treatments on plant height at later stages (4th month).

El-Khateeb et al. (2016) proved that the different doses ranging from 0.8 – 8 Krad significantly reduced plant height when compared to control (0 Krad). Cuttings treated with 0.5 krad significantly reduced plant height (27.21% reduction), while the highest dose resulted in the greatest reduction (69.1%) in plant height. Datta (1995) obtained comparable results for *Lantana depressa* and similar observation was reported by Gonzales (2007) in orchids. Suraninpong and Wuthisuthimethavee et al. (2012) showed that Anthurium plant growth decreased with increasing gamma doses, as did Patil (2014) on Gladiolus.

Table 01 : Effects of gamma radiation (20-40Gy range) on percentage survival, plant height, number of leaves and inflorescence length of *Acalypha hispida*

Treatments	Survival %		Plant height (cm)		No. of leaves		Inflorescence length (cm)	
	1st month	4th month	1st month	4th month	1st month	4th month	2nd month	4th month
T1 - 0 Gy	100^{a}	100^{a}	13.1^{a}	25.1^{a}	7.3^{a}	14.3^{a}	22.4^{a}	29.8^{a}
T2 - 20 Gy	88.9^{a}	88.9^{a}	8.0^{bc}	23.9^{a}	4.6^{b}	12.9^{ab}	21.9^{a}	27.2^{a}
T3 - 25 Gy	100^{a}	100^{a}	5.5^{cd}	24.7^{a}	4.0^{bc}	12.7^{ab}	20.0^{a}	27.03^{a}
T4 - 30 Gy	100^{a}	100^{a}	8.7^{b}	24.9^{a}	4.0^{bc}	12.3^{ab}	21.4^{a}	27.46^{a}
T5 - 35 Gy	88.9^{a}	77.7^{a}	7.6^{bcd}	24.0^{a}	2.6^{cd}	12.2^{ab}	21.0^{a}	23.0^{a}
T6 - 40 Gy	100^{a}	88.9^{a}	5.1^{d}	20.1^{a}	2.0^{d}	11.0^{b}	18.0^{a}	22.7^{a}
Sig.	ns	ns	*	ns	*	ns	ns	ns

Number of leaves

Early growth stages showed significant (P<0.05) values in number of leaves (Table 1). The highest value in number of leaves was observed in the plants that were not exposed to gamma irradiation and the lower values were recorded in plants exposed to maximum dose used during this experiment. Later days of plant growth showed no significant (P>0.05) differences among the treatments.

El-Khateeb et al. (2016) mentioned that irradiated plants produced considerably less leaves than the control group. The plants that weren't treated produced the most. The number of leaves dramatically reduced when the gamma dose was increased from 0.5 Krad to 2, 4, or 8 Krad. These findings concur with those made by Datta (1995) on *Lantana*, Gonzales (2007) on *Gladiolus*, and El-Khateeb et al. (2007) on *Melissa*, all of whom identified a significant decrease in the number of leaves with increasing gamma doses.

Inflorescence length

There were no significant (P<0.05) differences observed in length of inflorescences during the growth period among the applied gamma radiation doses

(Table 1). According to Taheri et al. (2016), a study conducted on *Curcuma alismatifolia* showed that

exposure to gamma radiation resulted in inflorescence shape mutations, including small inflorescences with fewer pink or purple bracts, inflorescences lacking true flowers, or true flowers with two flag petals, across all studied cultivars.

Experiment 2

Morphological changes

It was observed that, *A. hispida* emerged new leaves which appeared as narrow and misshapen as previous experiment due to increased gamma irradiation dose. But, the variation in the leaves were not found in later days. The varied leaf character discontinued in later growth stages.

Further, Gamma irradiation above 40 Gy produced more changes in inflorescences and changes persisted in later growth stages and second generations too. Irradiated plants showed inflorescences with pink, white, partial blooming (partial sterile) and no blooming (sterile) (Figure 2, 3). Raising the gamma dose up to 65 Gy induced the sterility of the plants (Table 2).

The leaves of *Curcuma alismatifolia* irradiated plants typically exhibit induced changes in their color, form, and texture, as stated by Taheri et al. (2016). Narrow leaves, split leaves, and certain chlorophyll mutations like *viridis*, *alboviridis*, and *striata* were all noted abnormalities after chronic gamma irradiation in irradiated plants.

According to earlier research on etiolated wheat and barley leaves, ionizing radiation may limit the production of chlorophyll and cause fragment deletions or insertions that ultimately affect the amino acids and alter the colour of the leaves and stems (Kovacs and Keresztes, 2002). According to Taheri et al. (2016), irradiation caused certain modifications in flower shape and color, including white, rather pale purple and purple white (marble pattern) in the coma bracts of *Curcuma alismatifolia*. The color of flowers can vary if a structural or regulatory gene's biosynthetic pathway is altered. White flowers will result from a blockage in the early stages of anthocyanin synthesis, but a blockage in the later stages results in varied flower hues because of the accumulation of different anthocyanins. Compared to other cell organelles, chloroplasts were particularly vulnerable to gamma radiation (Wi et al., 2007).

Table 02 : Effects of gamma irradiation on morphological variations in *Acalypha hispida*

Survival percentage

Treatments	Colour changed inflorescences	Sterile plants (no blooming inflorescences)	Partial sterile plants (Half blooming inflorescences)
T1 - 0 Gy	0	0	0
T2 - 45 Gy	8.3%	0	0
T3 - 50 Gy	8.3%	0	8.3%
T4 - 55 Gy	16.7%	0	0
T5 - 60 Gy	25%	8.3%	0
T6 - 65 Gy	0	0	0

It was found that, there were significant differences between the applied gamma irradiation doses on survival percentages during early and late growth stages (Table 3). Higher survival percentages (100%) were observed in the doses 0, 45 and 50 Gy during early growth stages. It was followed by the applied higher doses.

But later on, 4th month, the highest survival percentage (100%) was observed in the plants that were not treated with gamma irradiation. Increase in radiation dose decreased the survival percentage gradually. Further, by increasing the gamma radiation dose from 55 to 60 Gy, plant reached its LD_{50} value for survival at later growth stages.

According to research done by Lu et al. (2007) on *Narcissus tazetta*, the survival rate drastically declined as the radiation dose increased. An experiment on *Allamanda cathartica* by Rifnas et al. (2020) indicated that, lethality effects of gamma radiation on plants were observed in the second week after exposure to radiation treatments. An increase in the dose of gamma radiation reduced the survival rate of the plants and treated plants showed complete lethality at eight weeks after radiation treatment.

No matter the technique of irradiation, the effect of gamma rays on plant survival was gradual (Sawangmee et al., 2011), and a low dose of gamma rays had the greatest beneficial effects on the plant's subsequent growth (El-Shakhs et al., 2007).

irradiation dose decreased the plant height significantly (Table 3).

A study of *Allamanda cathartica* by Rifnas et al. (2020) indicated that plants that were not exposed to gamma radiation gave the highest value of plant height. A shorter internode length caused the reduction in plant height. Further, an experiment performed on the plant *Moluccella laevi*s by Minisi et al. (2013) demonstrated that an increase in gamma radiation dose reduced the plant's height.

The higher dose irradiation that resulted in growth retardation has been linked to cell cycle arrest at G2/M phase during somatic cell division and/or various genome-wide damages.

Number of leaves

There was a significant reduction in number of leaves recorded with an increase in the gamma irradiation

Table 03: Effects of gamma radiation (45-65 Gy) on percentage survival, plant height, number of leaves and inflorescence length of *Acalypha hispida*

Treatments	Survival %		Plant height (cm)		No. of leaves		Inflorescence length (cm)	
	1st month	4th month	1st month	4th month	1st month	4th month	1st month	4th month
T1 - 0 Gy	100[a]	100[a]	13.2[a]	29.2[a]	8.0[a]	16.3[a]	22.4[a]	29.7[a]
T2 - 45 Gy	100[a]	87.5[b]	6.0[b]	20.4[b]	4.0[b]	13.0[b]	15.8[b]	22.4[b]
T3 - 50 Gy	100[a]	76.4[b]	5.5[b]	18.7[c]	4.0[b]	13.0[b]	16.7[b]	21.7[b]
T4 - 55 Gy	91.7[b]	62.5[c]	5.5[b]	18.1[c]	2.7[bc]	12.8[b]	17.4[b]	20.6[b]
T5 - 60 Gy	91.7[b]	34.7[d]	5.3[b]	15.5[d]	2.5[c]	5.7[c]	10.7[c]	16.4[c]
T6 - 65 Gy	69.5[c]	27.8[d]	5.1[b]	14.5[d]	2.2[c]	5.0[c]	8.9[c]	15.3[c]
Sig.	*	*	*	*	*	*	*	*

Significant differences among means in the same column are indicated by different superscripts, as determined by DMRT at a 5% level of significance. The symbol "" denotes significance at the 5% level, while "ns" indicates no significance*

Plant height

Gamma irradiation had significant effect on plant height of *A. hispida* during the early and later growth stages of *A. hispida*. The highest values of plant height were found in the control plants. Increase in gamma

dose up to 65Gy. At the same time control plants produced the highest number of leaves (Table 3). It was noted that, leaf producing ability of the *A. hispida* was low during later growth stages compared to early growth stages. Gamma radiation can have various effects on plants, depending on the dose and duration of exposure. In general, exposure to high doses of gamma radiation can cause damage to the DNA and other cellular structures of the plant, leading to negative effects on growth and development (Sharma and Dubey, 2005). There have been several studies on the effects of gamma radiation on plants, including those of the family Euphorbiaceae. However, there is no specific study that directly investigates the effect of gamma radiation on the number of leaves of Euphorbiaceae plants. Nevertheless, there are a few studies that have examined the effect of gamma radiation on the growth and development of Euphorbiaceae plants, which indirectly relate to the number of leaves. One such study was conducted by Dhakshanamoorthy et al. (2010), who investigated the effect of gamma radiation on *Jatropha curcas* L., a member of the Euphorbiaceae family.

Inflorescence length

It was revealed that, there were significant differences among the treatments on inflorescence length. Plants exposed to the higher doses of gamma irradiation produced the significantly lower values of inflorescence length (Table 3). A study conducted to identify acute effect of gamma radiation on stable characteristics of *Spathoglottis Plicata* Blume reported short inflorescences with long spots on sepals and petals and undulate petal margin (Wuthisuthimethavee et al., 2012).

The evidence suggests that irradiation could decrease the sensitivity or quantity of endogenous growth regulators, particularly cytokines, either due to signal transduction or synthesis breakdown, or as a secondary effect of radiation. Furthermore, irradiation may harm essential plant cell components, causing diverse effects on plant morphology, anatomy, biochemistry, and physiology, as stated by (Ashraf et al., 2004).

Propagation of second generation of mutant plants

The attempts to produce the second generation of plants through asexual propagation also get succeeded. The plants with improved inflorescence characters showed the same variations as mother plants in inflorescences. The mutant plants were propagated using the technique invented by Rifnas et al. (2020) Gamma radiation can induce various types of mutations in plants, including point mutations, deletions, insertions, and translocations, among others.

These mutations can affect different parts of the genome and can result in a wide range of phenotypic changes, including alterations in growth, development, reproduction, and stress responses (Shu et al., 2012).

The expression of the same characters in the second generation of mutant ornamental plants induced by gamma radiation will depend on the nature and location of the mutations in the genome (Ibrahim et al., 2018). In mutagenesis experiments, whether with chemicals or physical mutagens, it is necessary to advance the treated material through few seed generations or vegetative propagations. In seed propagated plants, the recessive mutants are usually selected in the second (M2) or third (M3) generation after the treatment. In vegetatively propagated plants, following mutagenesis, several cycles of propagation are needed to obtain homo-histonts or to ‘dissolve’ chimeras and to obtain ‘solid’ mutants. It has been suggested that many of the mutants thus generated are sectorial chimeras (Ahloowalia and Maluszynski, 2001). In addition, the expression of the same characters in the second generation of mutant ornamental plants induced by gamma radiation may also be affected by the genetic makeup of the plants and how the mutations interact with other genes and traits. For example, some mutations may be lethal or deleterious, while others may have a neutral or positive effect on the plant's phenotype.

It's worth noting that the expression of the mutated traits in the second generation may require further breeding and selection to isolate and amplify the desired trait. This is because the mutations induced by gamma radiation are often random, and the likelihood of obtaining the desired traits may be low. Therefore, careful breeding and selection are necessary to improve the expression of the mutated traits and develop new ornamental plant varieties.

Combining the characters of improved plants

Attempts to combine the characters showed highest success rates due to the high compatibility of root stock of original plant and scion of improved plant. The Figure 4 indicates the pot plant developed with combined different characters developed through this research study.

The viability of a graft relies on the compatibility of the rootstock and scion. According to a research, grafts of various species within the same genus can form a successful graft union, whereas grafts of different genera within the same family are usually incompatible (Goldschmidt, 2014). Homografts, except for monocots, are generally compatible. Re-establishing the vascular system is essential for maintaining proper water and nutrient transport as the wound created during grafting disrupts the plant's vascular system (Asahina and Satoh, 2015). The lack of vascular cambia in the majority of monocots could be the cause of transplantation failure (Melnyk et al., 2015). This further indicates that successful grafting requires vascular differentiation during wound healing.

Figure 4: A potted plant with combined improved characters

Conclusion

In conclusion, the experiments conducted in this study demonstrate that treating *A. hispida* with gamma irradiation can lead to significant changes in the plants. However, it was noted that a gamma irradiation dose above 55Gy is lethal to the plants. The changes observed in the leaf morphology were temporary, while the color-changed inflorescences persisted in the plants even in the second generation and increasing the irradiation dose induced the sterility of inflorescences. Nevertheless, attempts to combine the varied characters to a single plant using grafting technique were successful in producing attractive pot plants with combined character. These findings highlight the potential of gamma irradiation-induced mutagenesis in creating genetic diversity in *A. hispida* that can be utilized for plant breeding purposes. Overall, this study provides valuable insights into the effects of gamma irradiation on *A. hispida* and can be used as a basis for future research in the field of plant genetics and breeding.

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