RESEARCH ARTICLE | MAY 25 2021

Morphological characterization and gamma irradiation effect on plant growth of *Curcuma heyneana* Val & Zijp FREE

Lia Hapsari ✉; Trimanto Trimanto; Yupi Isnaini; ... et. al

AIP Conference Proceedings 2353, 030012 (2021)
https://doi.org/10.1063/5.0052680

Morphological Characterization and Gamma Irradiation Effect on Plant Growth of *Curcuma heyneana* Val & Zijp

Lia Hapsari[1,a)], Trimanto Trimanto[1,b)], Yupi Isnaini[2], and Sasanti Widiarsih[3]

[1]*Purwodadi Botanic Garden – Research Center for Plant Conservation and Botanic Gardens, Indonesian Institute of Sciences, Jl. Surabaya – Malang Km 65, Pasuruan 67163, East Java, Indonesia*
[2]*Bogor Botanic Garden – Research Center for Plant Concervation and Botanic Gardens, Indonesian Institute of Sciences, Jl. Ir. H. Juanda 13, Bogor 16122, West Java, Indonesia*
[3]*Center for Isotopes Radiation and Application – National Nuclear Energy Agency, Jl. Lebak Bulus Raya No. 49, Pasar Jum'at, South Jakarta, 12440, DKI Jakarta, Indonesia*

[a)]Corresponding author: liah001@lipi.go.id
[b)]trim006@lipi.go.id

Abstract. *Curcuma heyneana* or temu giring is mainly popular for medicinal and beauty purposes; however due to its attractive inflorescence, it is potential for ornamental plant. This study aims to describe morphological characteristics and examine gamma irradiation effect on plant growth of *C. heyneana*. The method applied was acute gamma irradiation, with 6 doses of 0 Gy (control), 10 Gy, 20 Gy, 30 Gy, 40 Gy, and 50 Gy; 3 replications, 15 rhizomes per replication. *C. heyneana* is characterized as a perennial herb; rhizomes light yellow; leaves lanceolate to oblong; inflorescence on separate shoot; bracts white to green in lower part, pale to dark pink with dark tip in upper part; flowers yellow with yellow labellum. It is potential for landscape, pot and cut flowers. Irradiation treatment has been shown to affect the plant growth. The radiosensitivity test has indicated that LD_{20} and LD_{50} were approximately 40 Gy and 67.15 Gy, respectively. The lower doses of 10 - 20 Gy stimulated plant height and leaf number. The higher doses of 40 - 50 Gy stimulated shoots multiplication and stunted growth. High frequency of leaf mutations was noted; with spectrum including leaf deformation, apex splitted, narrowed, stiffed, wrinkled, twisted, and variegation.

INTRODUCTION

The genus *Curcuma* L. is one of the large genus in Zingiberaceae family, consists of many species estimates 50 to 120 spreads throughout South and South-east Asia with a few species extending to China, Australia and South Pacific [1,2]. The habit is a perennial rhizomatous herbaceous, comprising of underground parts (rhizome), leafy shoot and inflorescence. The inflorescence can be cylindric, conic or ovoid in shape; occurs either terminally on the leaf shoot or on the separate shoot. The flower is bisexual, zygomorphic, thin bracteoles, tubular petals, elongated crowns with pink to purple [3]. Some species have high economic values for medicinal purposes, cooking spice, cosmetics, colouring agent, religious substances, etc. [4,5]. In addition, due to its beautiful colors and compound spikes with prominent bracts, also relatively long vase-life inflorescences, some species have been used and developed as decorative plants or ornamentals, such as *Curcuma alismatifolia*, *Curcuma parviflora*, *Curcuma gracillima*, *Curcuma angustifolia*, etc. It has become increasingly popular as a cut flower, a potted plant and a garden plant [6]. In Thailand, ornamental *Curcuma* species is ranked second to orchids [7].

Specifically of *Curcuma heyneana* Valeton & Zijp, popularly known as temu giring is an important commodity mainly for medicinal and beauty treatments in Indonesia [8,9]. The rhizome contains of some antioxidant, antiviral, anticancer, antiaging, antimicrobial and anti-inflammatory activities such as heyneanone, phenolic, saponine, flavonoids, and essential oils [10,11]. This species can be found wild, cultivated commercially, or planted in home garden [12,13]. The morphological characteristics particularly the inflorescence of *C. heyneana* are potential for ornamental as garden plant, pot plant and also cut flower plant. The ornamental ginger species in Indonesia that have

International Conference on Life Sciences and Technology (ICoLiST 2020)
AIP Conf. Proc. 2353, 030012-1–030012-10; https://doi.org/10.1063/5.0052680
Published by AIP Publishing. 978-0-7354-4096-8/$30.00

been developed was still limited to *Alpinia purpurata* and *Zingiber spectabile* [14]. Therefore, a study of morphological characterization to fully describe this species is necessary. Furthermore, improvement efforts subjected to *C. heyneana* also required to respond the ornamental market demand of more variation and attractive morphology, larger or smaller flower size, brighter color, longer vase-life, etc.

Mutation induction on root and tuber crops, also vegetatively propagated ornamental plant species may become valuable improvement strategy to increase variability to develop new variety [15]. Gamma ray is widely used as a physical mutagen to induce mutation. The ionizing radiation gamma ray would interact with atoms or molecules within the cells to produce free radicals. These radicals may induce higher frequency of genetic mutation in plants since it could produce serious damage alteration to DNA or chromosomes within the cells [16,17]. Morphological alterations in various plant parts include stem, leaf, flower, fruit, etc. have been reported in many species. It also may create new characters in the irradiated plants such as higher yield and biomass, pest and disease resistance, higher bioactive compound, etc. through screening and selection process for putative mutant candidates [18-20].

Gamma irradiation has been successfully used to induce morphological variation in many species of Zingiberaceae such as in *Curcuma alismatifolia* [21,22], *Curcuma xanthorrizha* [23], *Curcuma longa* [23-25], *Curcuma aeruginosa* [23], *Etlingera elatior* [26-28], *Kaempferia rotunda* [29], *Zingiber officinale* [20], etc. Each species has specific radiosensitivity to gamma irradiation [15-17]. Determination of the optimum dose of gamma rays to induce mutation for each species is very important in order to get higher mutant frequency. Hence, the aims of this study were to conduct morphological description of *C. heyneana* and discuss its potential for ornamental; to determine the radiosensitivity; and to investigate the effect of various doses of gamma irradiation on plant growth and morphological variation. Hitherto, there is no previous report of induced mutation on this species. The result of this study is expected to provide basic information on morphological characteristics of *C. heyneana* as ornamental plant. Moreover, the gamma irradiation treatment is expected to increase genetic variation and obtained unique putative mutants of *C. heyneana* with aesthetic value for further research and development.

EXPERIMENTAL DETAILS

Plant Materials

The plant material used in this study is the living specimens of *C. heyneana* collection of Purwodadi Botanic Garden (Pasuruan, East Java), located at Vak V.E.I.19-19a, collected from Purwodadi (Pasuruan, East Java), with registration number P1978120096. For gamma irradiation treatment, fresh rhizomes were harvested, cleaned, and then selected. The rhizomes were selected with several criteria including healthy (not injured, not rotten, not infected by fungi), having 2 - 3 shoot buds, with similar size approximately 2 cm long.

Morphological Description

A complete morphological description was conducted directly to the living specimens of *C. heyneana* during 2019, both at vegetative and generative stages. Morphological characteristics observed includes habitus, rhizome, leafy shoot, leaf blade, inflorescence and flower. Detailed measurements in both vegetative and generative parts were made using a ruler, a digital calliper, and a long arm microscope.

Gamma Irradiation Treatment

Gamma irradiation treatment was carried out in November 2019 at Center for Isotope and Radiation Applications – National Nuclear Energy Agency, Pasar Jumat, South Jakarta, using Gamma Cell-220 facility. The method applied was acute irradiation, with 6 dose levels comprised 0 Gy (control), 10 Gy, 20 Gy, 30 Gy, 40 Gy, and 50 Gy; with three replications per dose, and each replication consisted of 15 rhizomes.

Post-irradiation Handling and Morphological Observation

After gamma irradiation treatment, materials were transported to Purwodadi Botanic Garden via cargo (two days). Immediately after the materials arrived, the irradiated rhizomes were planted at germination bed with planting distance 3 cm × 3 cm in the nursery greenhouse for further observation. The planting media is porous sand with sprinkler mist irrigation to provide humidity. Post irradiation experiment design used is completely randomized design (CRD) with single factor (gamma irradiation dose). Observation of plant growth variables were conducted starting from 2 to 8 weeks after planting (WAP). Plant growth variables observed including survival rate, plant height, number of shoots, and number of leaves. Mutation effects on the morphology of plant were visually observed over the respective controls.

Data Analysis

The observed data were compiled and analyzed with Microsoft Excel 2016 to show the data characteristics and distribution. The gamma irradiation radiosensitivity including lethal dose of 20% (LD_{20}) and 50% (LD_{50}) was forecasted using best curve fit analysis in Microsoft Excel 2016. One-way ANOVA test was conducted to determine statistical significances of the gamma irradiation effect to plant growth variables, using SPSS 16.0. Subsequently, post hoc test of Duncan's Multiple Range Test (DMRT) was performed to measure specific differences between pairs of means with 5% confidence level.

RESULTS AND DISCUSSION

Morphological Description of *C. heyneana*

The plant habitus of *C. heyneana* is perennial herbaceous; with leafy shoot height reaching 1.5 - 2 m; pseudostem 4 - 7 cm in diameter, light green to dark green. Leaves 6 - 8; the color of upper surface light green to green, lower surface pale green. Leaf blade size 72 - 105 cm × 15 - 20 cm; shape lanceolate to oblong, apex acuminate, base acute, surface plicate with entire margin; petiole length 21-38 cm, light green to dark green. Rhizome length 6 - 7 cm, diameter 2 - 4.5 cm; shape cylindrical and branched; skin/outer color light brown; flesh/inner color light yellow; medium aroma. Main rhizome length 4 - 6 cm, diameter 3.7 cm; first lateral rhizome length ca. 10 cm, diameter 2.2 cm. Stolon rhizome present; length between segments at main rhizomes 0.4 - 0.7 cm; length between segments at lateral rhizomes 1 - 2 cm. Inflorescence on separate shoot, length ca. 32.5 - 45 cm; peduncle length ca. 18.5 - 30 cm; fertile bract size 6.2 - 9.5 cm × 3 - 4.5 cm, light green at base and dark green at apex; sterile/coma bract size 5 cm × 2.6 cm, white at base and continue pale pink at center to dark pink at apex. Flower ca. 4 cm long, exerted from the bracts, yellow, glabrous; corolla ca. 4 cm long, whitish; labellum 25 mm × 13 mm, white to yellowish with dark yellow band at center; lateral staminodes longitudinally folded, yellowish; filament broad and flat, white; anther ca. 9 mm long, white; crest 1 mm × 1 mm; spur short, ca. 3 mm long, white to yellowish; style white, glabrous; stigma ca. 1 mm, white, glabrous; with epigynous glands. The documentation morphological characteristics of *C. heyneana* is present in Figure 1.

Potentials of *C. heyneana* as Ornamental Plant

This species is mainly popular for medicinal and beauty purposes, however due to its attractive morphological characteristics, it has high potential for ornamental plant. It grows in a clump, the leaves light green to green color, not very showy, but good as foliage plants for garden landscaping and pot plant (Figure 1.a). This species is easily propagated using rhizomes, leafy shoot appear within 2 weeks after planting. It requires well-drained, loamy or alluvial, fertile, and friable soil. It may grow well in open, sunny places, but also as undergrowth at shaded places [12,13]. Mutants with leaf variegation is expected to occur from gamma irradiation in order to enhanced the aesthetic value of the leafy shoots of *C. heyneana*. Gamma irradiation was reported effective to induce chlorophyll mutation in Zingiberaceae [20,22].

Furthermore, their inflorescence is the best part for ornamental purposes. The phenology habit of *C. heyneana* is unique, the leaves dry off during dormancy started at early dry season and then the flowers usually appear above the ground before the leaves during early rainy season. Thus, it makes them excellent as bedding plant and a good addition to mixed border plants (Figure 1.b & 1.c). The flowers are yellow with prominent and attractive pale pink to dark pink coma bracts. It has quite long petiole (21 - 38 cm) which make it suitable for cut flower (Figure 1.l). The inflorescence lasts in full bloom on the plants for about three weeks or more. It has a vase life of about 10 days or more, especially when the peduncle was freshly cut right before fully blooming.

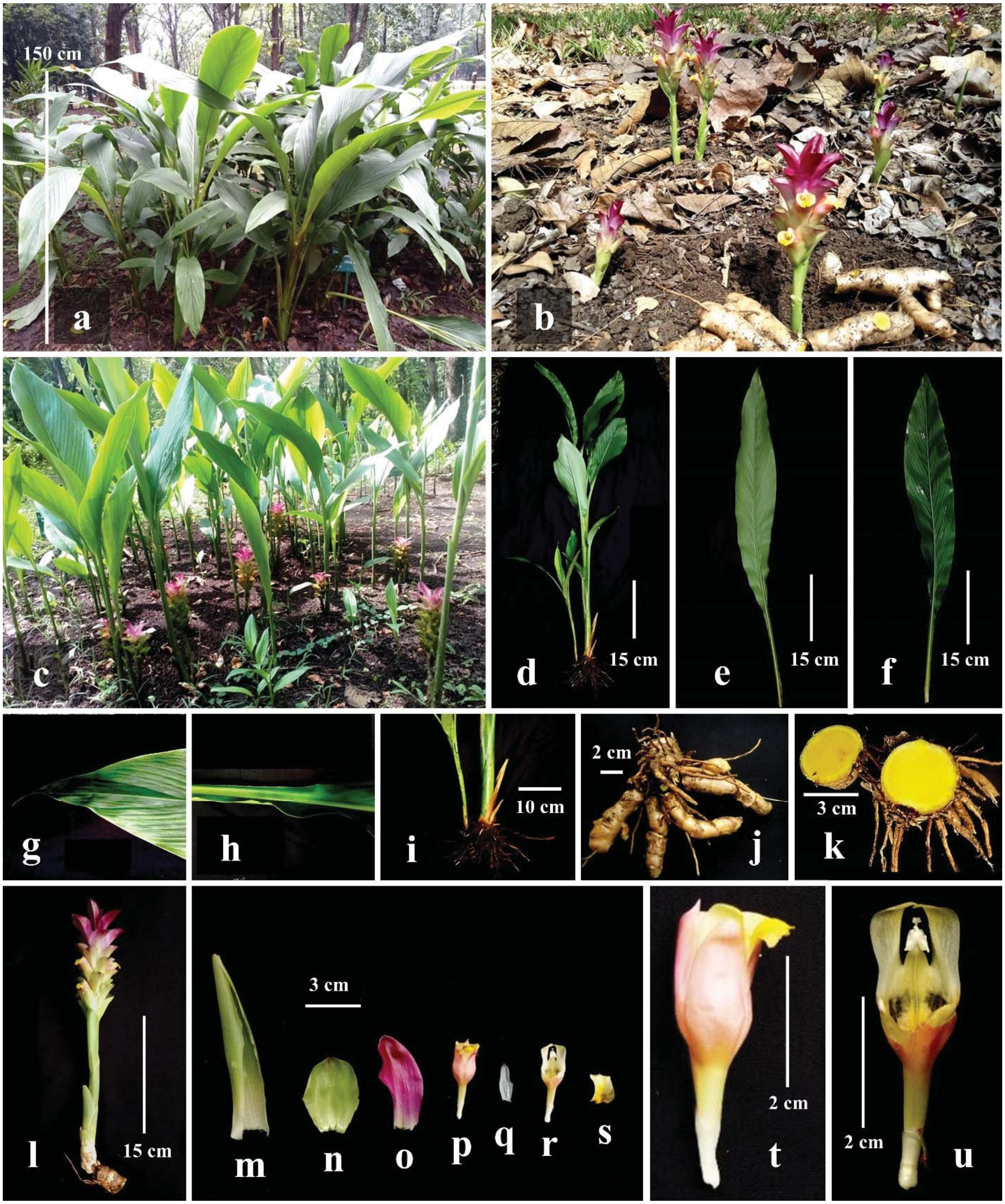

FIGURE 1. Morphological characteristics of *C. heyneana*: (a) Plant habitus, (b) Inflorescence emerged from the base of rhizome before leaves, (c) Leaves emerged after inflorescence, (d) Leafy shoots, (e) Leaf lower surface, (f) Leaf upper surface, (g) Leaf apex, (h) Leaf base, (i) Roots, (j) Main and lateral rhizomes, (k) Transversal section of main rhizome, (l) Inflorescence, (m-n) Fertile bracts, (o) Sterile bract, (p) Flower, (q) Calyx, (r) Incised of flower, (s) Labellum, (t) Flower with pinkish corolla lobe, and (u) Incised of flower, stamen at center with staminodes.

Survival Rate, Radiosensitivity LD_{20} and LD_{50}

Gamma irradiation treatments were affected to the germination of *C. heyneana* rhizomes and plant survivals, although no statistical differences among doses of irradiation. The survival rate at gamma irradiation dose 30 Gy is slightly higher compared to control *i.e.* 93% and 87% respectively. Whilst, at doses of 10 Gy and 20 Gy showed same survivals with control (87%). Later, at higher doses of 40 Gy and 50 Gy, the survivals were sharply declined to 73% (Figure 2.a). Low doses gamma irradiation may stimulate germination of rhizomes or seeds, possibly due to the activation of synthesis of RNA, or protein or enzymes involved in auxins formation for germination [24,30]. Gamma irradiation could also reduced bacterial population, mould fungi, bacterial and fungal spores thus induce germination [31,32]. Whilst, lethality of plant at higher doses of gamma rays possibly occurred by the serious damage of meristematic cells and important plant cell components both at physic and physiology levels [15-17].

The optimum radiosensitivity gamma irradiation dose which may produce the highest variability and mutation rate usually occurs around LD_{20} to LD_{50}. The LD_{50} is found quite arbitrary and might lead to high deleterious mutation. Meanwhile, LD_{20} with survivals of 80% appears to be more suitable for mutation breeding [18]. The best curve fit analysis showed that the survivals of gamma irradiated *C. heyneana* were following polynomial regression trend line, with strong correlation value ($R^2 > 0.5$) (Figure 2.a). It is indicated by drastic decline of the survivals with the increasing of gamma irradiation dose (after optimum dose of survivals). The LD_{20} of gamma irradiation on *C. heyneana* based on this study was predicted to occur around 40 Gy, and the LD_{50} around 67.15 Gy. Further research on those range of gamma irradiation doses is suggested for higher mutant frequency on *C. heyneana*. The radiosensitivity (LD_{50}) of each species varied, such as in *C. alismatifolia* was approximately at 25 Gy [21], *C. xanthorrizha* at 69.08 Gy [23], and *C. longa* at 47.26 Gy [25].

Effect of Gamma Irradiation on Pant Height

Analysis of variance on plant height showed a significant difference among doses of gamma irradiation ($p = 0.018$). Growth chart for eight weeks showed that at doses of 10 Gy and 20 Gy resulted in plants higher than control. The plant height was increased by 6 - 14 cm in 2 weeks at 10 Gy, and 7 - 15 cm at 20 Gy. Whilst at higher dose of 30 Gy causes a slowdown in growth, with height growth rate 4 - 7 cm in 2 weeks. Later, the height growth rate at dose of 40 Gy was slower to 1 - 3 cm per 2 weeks and even slower to only 0.5 - 2 cm in 2 weeks at 50 Gy. These results indicated that at lower doses or gamma irradiation 10 - 20 Gy were stimulates the plant height of *C. heyneana* compared to control, with optimum dose at 20 Gy. Then, the plant height growth was decreased sharply at higher doses of > 30 Gy, and significantly stunted at 40 Gy and 50 Gy (Figure 2.b).

Many previous studies on gingers reported similar results, rhizomes exposed to relatively low doses of gamma rays (1 - 25 Gy) normally developed, while the growth of plants irradiated with a higher dose gamma ray (≥ 40 Gy) were significantly inhibited [22,24,25]. Gamma irradiation causes random mutations resulted in physiological damage in the metabolism of cell development. Therefore, its growth potential can be faster or slower. The stimulating effect at lower doses of gamma irradiations on growth of plants possibly due to the stimulation of cell division, positive alteration of metabolic processes which affect synthesis of nucleic acids. Meanwhile, the decrease in plant growth (stunted) probably due to physiological disorders or chromosomal damage caused by mutagen at high doses [15-17].

Effect of Gamma Irradiation on Number of Shoots and Leaves

Effects of gamma irradiation doses on *C. heyneana* are not significantly different to the number of shoots ($p = 0.133$). However, the trend line showed that there were increases of shoot number with the increase of gamma irradiation doses. The highest number of shoots was shown at 40 Gy, statistically significant on average 1.67 shoots, or about 3 to 4 shoots per survived rhizome. Nevertheless, those multiple shoots were not being able to develop well or stunted in growth like normal shoots (Figure. 2.c & 3). This result was in the contrary of most previous studies on gingers and bananas which reported that gamma irradiation decreased the shoot number [20,22,27]. It possibly due to the activation and/or suppression of growth hormone synthesis that varies depending on each species radiosensitivity. Plant material conditions such as rhizome physiological age, size, and provenance source also influence irradiation. Young rhizome has meristematic cells, which is multiplied rapidly, thus being faster in recovery than older rhizome. Further, the damage also intervenes in cell division and differentiation processes, which made apical and/or axillary's buds failed to develop [33].

The number of leaves also not significantly different between doses of gamma irradiation ($p = 0.111$). The trend line showed that leaf number were increased at 10 Gy, 20 Gy, and optimum at 30 Gy with average 3.58 leaves. Then, the leaf number was drastically decreased at 40 Gy and 50 Gy *i.e.* 1.8 and 1.4 leaves (average), respectively. The leaf number at 50 Gy resulted in slightly higher than control although not significant, with some abnormality features (Figure 2.c & 3). Many studies have shown that gamma irradiation at higher doses were inhibitory, whereas at lower exposures were sometimes stimulatory. High doses of gamma rays were reduced the leaf number in *Brunfelsia pauciflora*, at 10 Gy resulted in mutant plantlets with the highest plant height and leaf number. Then, when the dose was increased to 30 Gy the leaf number was declined [34]. Another case, gamma irradiation at 10 Gy induced a significant stimulation effect on root length, leaf length and leaf width compared to non-treated of banana (*Musa* x *parasidisiaca*). Whilst, at higher dose of 30 Gy was induced dwarf plant and least number of leaves [35].

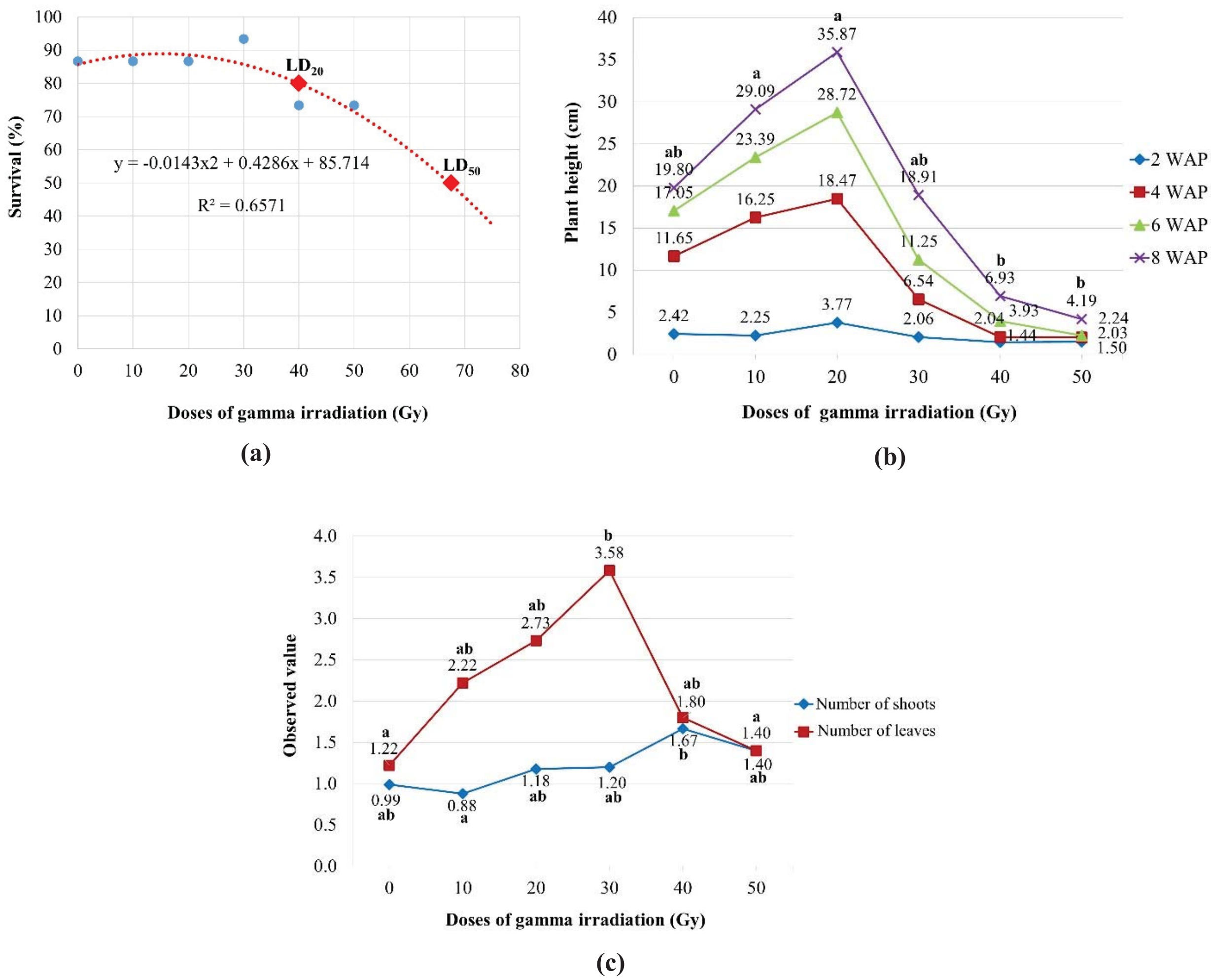

FIGURE 2. Effects of gamma irradiation on *C. heyneana*: (a) Survival rate and radiosensitivity at 8 WAP, (b) Plant height at 2 - 8 WAP, and (c) Number of shoots and leaves at 8 WAP. Note: Values within the same color line followed by the same alphabet were not significantly different based on DMRT 5% ($p = 0.05$)

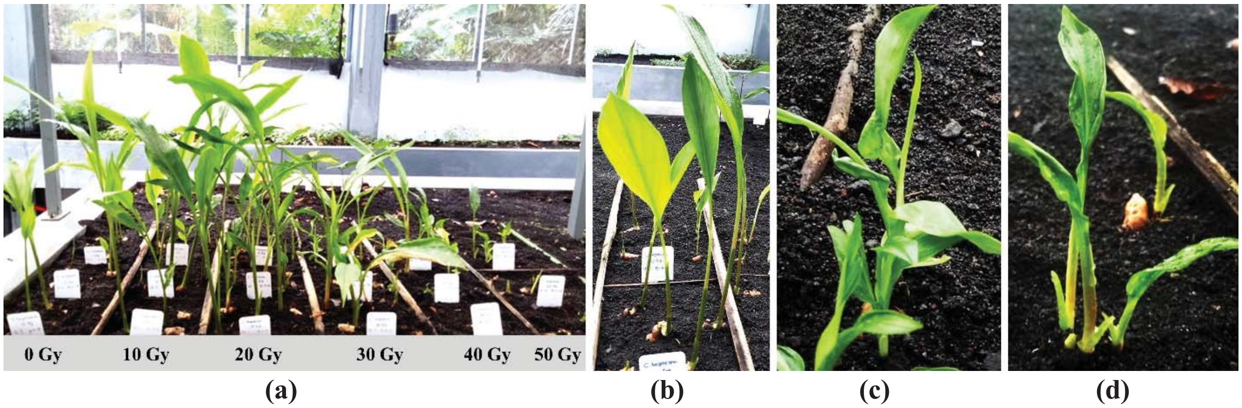

FIGURE 3. Effects of gamma irradiation on leafy shoots of *C. heyneana*: (a) All doses, (b) Normal shoots at 10 Gy, (c) Multiple shoots and abnormal growth at 40 Gy, and (d) Multiple shoots and abnormal growth at 50 Gy

Morphological Variation on Leaves

Interestingly, gamma irradiation induced high frequencies of leaf mutation in almost all of the irradiated plants, which indicated that rhizomes of *C. heyneana* were highly mutable. The spectrum of mutant characters observed on leaves were varied such as multiple shoots, leaf deformation, and variegation (Table 1, Figure 3 & 4). The changes in leaf shape may be due to abnormal mutant cells development into different tissues or organs compared to those from the parent cells, meanwhile leaf colour changes are caused by disruption of chlorophyll synthesis [28]. Further, ionizing gamma irradiation may disrupt chlorophyll synthesis and induces fragment insertion or deletions which lead to changes in amino acids and a modification of leaf (deformation) also pigmentation. The chlorophyll mutation is the most dependable index of gamma irradiation treatment that have been reported in various gingers. Chloroplasts were extremely sensitive to gamma irradiation compared to other cell organelles, particularly thylakoids being heavily swollen based on TEM observations [36]. However, the leaf variegation provides additional beauty attraction for ornamental purpose [22,25,37].

TABLE 1. Spectrum and frequency of leaf mutations induced by gamma irradiation on *C. heyneana*

Doses of gamma irradiation (Gy)	Spectrum of mutation on leaves	Frequency of mutation (%)
0	Normal, no mutation	0
10	Uneven color, variegation	13.33
20	Stiffed, wrinkled, curly surface, twisted, apex splitted, variegation	46.67
30	Multiple shoots, stunted, stiffed, wrinkled, curly surface, variegation	66.67
40	Multiple shoots, stunted, stiffed, narrowed, wrinkled, twisted, variegation	100
50	Multiple shoots, stunted, stiffed, narrowed, wrinkled, twisted, variegation	100

Genetic alteration in first generation after irradiation treatment (MV1) can easily be seen through morphological changes and may proposed as putative mutants. At lower doses of 10 Gy and 20 Gy, gamma irradiation given low to mild effects on leaves, such as stiffed, wrinkled, curly surface, twisted, apex splitted, chlorophyll mutation which appears as variegation/chimera. The leaf variegation patterns are varied comprises a mixture colour of yellow (viridis), white (albino) and green (normal). Further, the chimera in this study mostly considered as non-patterned sectorial [38]. At a dose of 30 Gy given mild to severe effects but it considered in good performance for growing. Meanwhile, at higher doses of 40 Gy and 50 Gy given more severe effects, in addition to leaf deformation and chlorophyll mutation, it also exhibited multiple shoots and stunted which make them poor in growing performance. (Table 1, Figure 3 & 6). It can be suggested that the appropriate dose to generate morphological variability in *C. heyneana* is around LD_{20} or lower (< 40 Gy), since on higher doses it displays poor plant performance which unlikely to survive in the future.

Previous studies of gamma irradiation treatments on vary ornamental plant habitus were also resulted in varying morphological changes. It induced abnormal leaves shape on herb *Orthosiphon stamineus* [39]. Likewise, on tree shrub *Jasminum* spp. some of the irradiated plants produced leaf anomalies includinge altered shape and texture, abnormal curvature, thickening, puckering and mosaic-like colour change [40]. Whilst, on herbaceous shrub *Coleus blumei*, gamma irradiation induced branching, and changes of leaf colour and pattern [41]. Leave variegation both viridis and albino also observed on irradiated plantlets of herbaceous epiphytic orchid *Phalaenopsis amabilis* [37]. Meanwhile on terrestrial pitcher plant *Nepenthes ampullaria* was obtained dwarf rosette plants, with small, wavy and curly leaves [42].

(a) **(b)** **(c)** **(d)** **(e)** **(f)** **(g)**

FIGURE 4. Morphological variation on leaves induced by gamma irradiation on *C. heyneana*: (a) Normal; (b) Stiffed, wrinkled, narrowed, variegation; (c) Wrinkled, variegation; (d) Variegation with yellow and white spots; (e) Variegation, yellow lines; (f) Abnormal shape, leaf apex splitted, variegation; and (g) Abnormal shape, apex splitted

Gamma irradiation can disrupt the genetic constitution within the plant cells, thus changes the morphological expression in plants. Gamma ray is a type of ionizing radiation with high penetration power due to its short wavelength. It is regularly used in breeding programs to induce genetic variability that almost does not exist in nature to complement existing germplasm. In most cases, random mutations cause unwanted changes in phenotypes of mutants. However, some changes may become beneficial to the breeder, such as taller or shorter plant, more or less branching, leaf variegation, and new color shades, early flowering, shape and flower color, higher yield, etc. [15-17,33].

Nevertheless, morphological variation observed on irradiated plants were not solely due to genetic mutation, instead it could be due to somatic mutation. Somatic mutations due to physiological damage on cells/tissues after irradiation usually appear in MV1 generation then possibly disappear in the next generations due to recovery [33]. Further, the occurrence of diplontic selection (intrasomatic competition) may also contribute to a decline in the mutated cells' regeneration. In chimeric tissue such as a variegated leaf, mutated cells are present along with the normal cells. During subsequent cell division, mutated cells compete with normal cells around them to survive, where if eventually the mutated cells fail to compete until a certain time limit then the plant tissue will grow back normally. However, if mutated cells survive in competition, they can potentially be expressed [27,42]. All of *C. heyneana* putative mutants in this study are well preserved (transplanted to polybag) for further observation and selection in the next generation, including mutants by characteristics on flowering and rhizome yield also phytochemical content.

SUMMARY

In summary, *C. heyneana* has high potential to use as an ornamental plant. Morphological characteristics showed it has good light green to green foliage for garden landscaping and as pot plants. The inflorescence is attractive yellow with prominent pale pink to dark pink coma bracts, with long petiole and relatively long vase life suitable as a cut flower. Further, gamma irradiation affected the plant height, number of shoots, and number of leaves also modified the leaf shape and color in MV1. It is suggested that the appropriate dose to generate morphological variability in *C. heyneana* is around LD_{20} or lower (< 40 Gy). Further observation followed by selection on the putative mutants are still in progress, hopefully to develop promising new variety of *C. heyneana* with potential characteristics for ornamental purposes.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Purwodadi Botanic Garden, Indonesian Institute of Sciences, for providing the living plant materials studied and Center for Isotopes Radiation and Application – National Nuclear Energy Agency for the gamma irradiation facility. This research is a synergy of collaboration between the leading centers of science and technology of Research Center for Plant Conservation and Botanic Gardens, Indonesian Institute of Sciences and Center for Isotopes Radiation and Application – National Nuclear Energy Agency.

AUTHORS' CONTRIBUTIONS

All authors declare that L.H. and T. are equal main authors of this article. Conceptualization, L.H., T.; Methodology, L.H., T., Y.I., S.W.; Performed gamma irradiation, Y.I., SW; Performed characterization and observation, L.H., T.; Data analysis, L.H., T.; Writing the original draft manuscript, L.H., T.; Supervised the research activities and results, Y.I., S.W.; Review and edit the final manuscript, L.H., T., Y.I., S.W.

REFERENCES

1. D.L. Wu, and K. Larsen. *Flora of China Vol. 24: Zingiberaceae* (Science Press & Missouri Botanical Garden Press, 2000)
2. J. Leong-Škorničková J, O. Šída O, M. Sabu, and K. Marhold. Taxon **57**, 949 (2008)
3. P. Sirigrusa, K. Larsen, and C. Maknoi. Gardens' Bull. Singapore **59**, 203 (2007)
4. N. Bhardwaj, and U. Dadsena. J. Innov. Appl. Pharm. Sci. **2**, 1 (2017)
5. D. Subositi, and S. Wahyono. Biodiversitas **2095**, 1356 (2019)
6. S. Ruamrungsri S. Eurasia J. Hort. Sci. **80**, 316 (2015)
7. S. Khumkratok, K. Boongtiang, P. Chutichudet, and P. Pramaul. 2012. Pakistan J. Biol. Sci. **15**, 929 (2012)
8. I. Kusumawati, K.O. Kurniawan, S. Rullyansyah, T.A. Prijo, R. Widyowati, J. Ekowati, E.O. Hestianah, S. Maat, and K. Matsunami. J. Ethnopharmacol. **225**, 64 (2018)
9. Lianah, Krisantini, and M. Wegener. 2020. IOP Conf. Series: EES **457**, 012025 (2020)
10. C. Nuraeni, and R. Yunilawati. J. Kimia & Kemasan **34**, 187 (2012)
11. E.D. Woelansari, A. Puspitasari, and Rachmaniyah. Folia Medica Indonesiana **49**, 62 (2013)
12. M. Jalil. J. Biol. Edu. **2**, 104 (2019)
13. L.S. de Padua, N. Banyaprophatsara, and R.H.M.J. Lemmens. *Plant Resources of South East Asia. Medicinal and Poisonous Plants 1. Curcuma L.* (PROSEA Foundation, 1999)
14. R. Soehendi, B. Marwoto, K. Budiarto, S. Kartikaningrum, K. Yuniarto, D.S. Badriah, L. Sanjaya, and D.H. Adriyani. *Katalog Varietas Unggul Florikultura* (IAARD Press, 2015)
15. C. Broertjes, and A.M. van Harten. *Applied Mutation Breeding for Vegetatively Propagated Crops* (Elsevier Science, 2013)
16. L.G. Iglesias-Andreu, P. Octavio-Aguilar, and J. Bello-Bello. *Gamma Radiation: Current Importance and Potential Use of Low Doses of Gamma Radiation in Forest Species* (In Tech Publishing, 2012)
17. J. Jankowicz-Cieslak, C. Mba, and B.J. Till. *Biotechnologies for Plant Mutation Breeding: Mutagenesis for Crop Breeding and Functional Genomics* (IAEA, 2017)
18. Y. Oladosu, M.Y. Rafii, N. Abdullah, G. Hussin, A. Ramli, H.A. Rahim, G. Miah, and M. Usman. Biotechnol. Biotechnologic. Equip. **30**, 1 (2016)
19. F. Ahmad, Z. Ahmad, A.A. Hassan, S. Ariffin, N. Noordin, S. Salleh, S. Hussein, M. Akil, M.Z. Sani, A.R. Harun, K.A. Rahim KA. J. Sains Nuklear Malay. **30**, 8 (2018)
20. S. Abdullah, N.Y. Kamaruddin, and A.R. Harun. Int. J. Adv. Sci. Eng. Info. Tech. **8**, 2085 (2018)
21. T.L. Abdullah, J. Endan, and B.M. Nazir. Am. J. Appl. Sci. **6**, 1436 (2009)
22. S. Taheri, T.L. Abdullah, Z. Ahmad, M. Sahebi, and P. Azizi. Eurasia J. Hort. Sci. **81**, 137 (2016)
23. R. Chosdu. Scientific J. Appl. Isotopes & Radiation 4, **109** (2008)
24. S. Ilyas, and S. Naz. J. Animal & Plant Sci. **24**, 1396 (2014)
25. S.R. Anshori, S.I. Aisyah, and L.K. Darusma. J. Hort. **5**, 84 (2014).
26. K. Dwiatmini, S. Kartikaningrum, and Y. Sulyo. J. Hort. **19**, 1 (2009)
27. M.F. Yunus, M.A. Aziz, M.A. Kadir, S.K. Daud, and A.A. Rashis. Turkish J. Biol. **37**, 716 (2013)
28. E.I. Azzahra, S.I. Aisyah, D. Dinarti, and Krisantini. J. Trop. Crop Sci. **5**, 111 (2018)

29. S. Soonthornkalump, P. Soontornchainaksaeng, and T. Jenjittikul. Thai J. Bot. **8**, 101 (2016)
30. R. Gaswanto, M. Syukur, B.S. Purwoko, and S.H. Hidayat. Agrivita **38**, 24 (2015)
31. N. Kumar, S. Rani, G. Kumar, S. Kumari, I.S. Singh, S. Gautam, and B.K. Choudhary. J. Biol. Physics **45**, 1 (2019)
32. D.P. Rahayu, F.C. Saputri, and D. Darwis. Atom Indonesia **42**, 53 (2016)
33. I. Dwimahayani, and S. Widiarsih. Atom Indonesia **36**, 45 (2010)
34. S.S. Sakr, A.M. Habib, M.A. El-Shamy, and H.B. Soliman. Middle East J. **6**, 916 (2017)
35. F.A. Kemal, F. Kayat, and S. Zakaria. Pak. J. Biotech. **15**, 265 (2018)
36. S.G. Wi, B.Y. Chung, J.S. Kim, J.H. Kim, M.H. Baek, J.W. Lee, and Y.S. Kim. Micron **38**, 553 (2007)
37. S. Widiarsih, and I. Dwimahayani I. Scientific J. Appl. Isotopes & Radiation **9**, 59 (2013)
38. M.H. Frank, and D.H. Chitwood. Developmental Biol. **419**, 41 (2016)
39. K.A. Rashid, M.I. Jamaludin, R. Farzinebrahimi, A. Nezhadahmadi, R.M. Taha, N.A. Abd Aziz, and M. Mamat. Life Sci. J. **15**, 45 (2018)
40. S.I. Aisyah, L. Hapsari, and D. Herlina. J. Agr. & Rural Dev. Trop. & Subtrop. **83**, 120 (2005)
41. E.R. Togatorop, S.I. Aisyah, and M.R.M. Damanik. J. Hort. Indonesia **7**, 187 (2016)
42. Y. Isnaini, and Y. Novitasari. Scientific J. Appl. Isotopes & Radiation **16**, 15 (2020)