

Phenotypic evaluation of gamma-induced dwarf mutants in *Dendrobium crumenatum* at M2 generation

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Abstract. Dwiyani R, Darmawati IAP, Kawuri R, Fitriani Y, Kamuhi RH, Hanifah WN, Santika IPB. 2026. Phenotypic evaluation of gamma-induced dwarf mutants in *Dendrobium crumenatum* at M2 generation. *Asian J Agric* 10 (1): g100150. <https://doi.org/10.13057/asianjagric/g100150>. Bali, Indonesia, has strong potential for the development of ornamental orchids, particularly compact cultivars with high commercial value. *Dendrobium crumenatum*, a native Indonesian orchid, is widely appreciated for its white, fragrant flowers; however, its tall growth habit limits its suitability as a potted ornamental plant. This study aimed to induce and evaluate dwarf phenotypes in *D. crumenatum* using gamma irradiation. Plantlets were exposed to 0, 5, 10, 15, and 20 Gy, arranged in a completely randomized design with five replicates and three plantlets per replicate (n=75). Morphological traits were assessed after in vitro culture and acclimatization. Data were analyzed using analysis of variance followed by the Least Significant Difference (LSD) test at a 5% significance level (p<0.05). Gamma irradiation significantly affected all measured traits. The 5 Gy treatment caused severe growth suppression, reducing plant height, leaf number, and shoot number by approximately 53.7%, 81.7%, and 84.4%, respectively, compared to the control. In contrast, the 10 Gy treatment achieved a similar reduction in plant height (~52.6%) while maintaining higher leaf number (~83.3% of control) and shoot number (~62.2% of control), indicating a more balanced morphological response. The 15 Gy treatment showed minimal deviation from the control, whereas 20 Gy produced semi-compact plants with moderate shoot retention. These results demonstrate a non-linear dose-response relationship, where intermediate doses (particularly 10 Gy) optimize the trade-off between dwarfism and vegetative performance. These findings indicate that medium gamma irradiation doses are effective for generating dwarf *D. crumenatum* mutants suitable for potted ornamental use and demonstrate the potential of mutation breeding to support orchid improvement and the floriculture industry.

Keywords: *Dendrobium crumenatum*, gamma irradiation, mutation breeding, ornamental orchid

INTRODUCTION

The Orchidaceae family represents one of the largest groups of flowering plants, with the genus *Dendrobium* recognized as one of its most diverse lineages. Indonesia is estimated to harbor approximately five thousand orchid species (Prayoga et al. 2022), highlighting its extraordinary biodiversity and its potential as a center for ornamental plant development. One widely distributed species is *Dendrobium crumenatum*, commonly known as the pigeon orchid, which naturally grows in tropical forests at elevations ranging from 5 to 2,500 m above sea level (Lestari et al. 2018). This species produces white, fragrant flowers and exhibits strong adaptability to various environmental conditions, making it a promising candidate for ornamental cultivation.

Despite these advantages, the utilization of *D. crumenatum* as a potted ornamental plant remains limited due to its tall and elongated growth habit. In the modern floriculture industry, there is increasing demand for compact and dwarf ornamental plants, particularly for indoor decoration, urban gardening, and commercial markets. Compact plant forms provide several benefits, including reduced transportation costs, efficient space

utilization, and improved aesthetic value. Therefore, the development of dwarf and compact plant architecture has become an important objective in ornamental plant breeding.

Various approaches have been applied to modify plant architecture, including the use of plant growth regulators, chemical mutagens, and physical mutagenesis. Growth regulators in orchids such as *Phalaenopsis* have been reported to influence plant morphology (Datta et al. 2018; Bidarnamani et al. 2021), but their application often requires repeated treatments to maintain the desired phenotype (Hsieh et al. 2020). Chemical mutagens such as colchicine can induce polyploidy and morphological variation (Revathi and Thomas 2022); however, their use is associated with toxicity risks and inconsistent outcomes, particularly in producing stable dwarf traits.

In contrast, gamma irradiation has emerged as an effective physical mutagenesis technique capable of inducing stable genetic variation without leaving chemical residues. Gamma rays interact with cellular components and DNA, causing mutations that may alter gene expression related to plant hormones, cell division, and growth regulation (Nilahayati et al. 2024). In ornamental plant breeding, gamma irradiation has been successfully

used to generate desirable traits such as reduced plant height and improved morphological characteristics (Li et al. 2022; Devi et al. 2023).

In orchids, several studies have demonstrated the effectiveness of gamma irradiation in inducing morphological variation. In *Dendrobium* species, low to moderate doses (generally 10-30 Gy) have been reported to induce compact growth and shorter internodes. Similarly, in *Phalaenopsis*, gamma irradiation has been shown to produce mutants with reduced plant size and improved ornamental value. The response to irradiation is dose-dependent, as low doses may induce beneficial variation, whereas high doses can inhibit growth or cause lethality. Therefore, identifying the optimal irradiation dose is essential in mutation breeding.

Evidence from other ornamental plants further supports the effectiveness of gamma irradiation in inducing dwarfism. Studies on chrysanthemum (*Chrysanthemum morifolium*) and rose have shown that low to medium doses can produce stable miniature phenotypes without negatively affecting flower quality (Susila et al. 2019). However, plant responses to irradiation are highly species-specific, requiring targeted evaluation for each species, including *D. crumenatum*.

Previous research by Dwiyani et al. (2024) demonstrated that gamma irradiation at doses of 10-15 Gy resulted in high levels of molecular variation in *D. crumenatum* based on SSR marker analysis. Although these findings confirm successful mutation induction, the study did not evaluate whether these molecular changes are expressed as desirable phenotypic traits, particularly dwarfism and compact growth. This gap is critical, as only mutations that result in stable and commercially valuable phenotypes can be utilized in breeding programs.

Therefore, this study aims to evaluate the effect of different doses of gamma irradiation on the phenotypic expression of dwarf characteristics in *D. crumenatum*. The research focuses on plant growth traits associated with compact architecture during the acclimatization stage. The study addresses the following research question: whether different doses of gamma irradiation can effectively induce stable and compact dwarf phenotypes suitable for ornamental pot plant development. It is hypothesized that intermediate doses of gamma irradiation (10-20 Gy) will

reduce plant height while maintaining sufficient vegetative growth, resulting in an optimal balance between dwarfism and plant vigor. The findings of this study are expected to contribute to the development of efficient mutation breeding strategies and support innovation in ornamental orchid production in Indonesia.

MATERIALS AND METHODS

Study site and experimental duration

This study was conducted from September 2023 to August 2024 at the Plant Tissue Culture Laboratory and acclimatization area, Faculty of Agriculture, Universitas Udayana, Denpasar, Bali, Indonesia. The experiment consisted of two main phases: an in vitro phase (September 2023-March 2024) and an ex vitro acclimatization phase (March-August 2024). Final observations were conducted when plants reached 12 months of age.

As an overview of the plant material used, Figure 1 shows the morphological appearance of *D. crumenatum* plantlets at 12 months of age after undergoing both in vitro culture and ex vitro acclimatization. The figure illustrates variation in plant height, leaf size, and shoot number across gamma irradiation treatments.

Plant material and gamma irradiation protocol

The plant material consisted of in vitro propagated *D. crumenatum* plantlets. Gamma irradiation was applied to three-month-old plantlets (M1V1 stage) using a Cobalt-60 (^{60}Co) gamma source at a certified irradiation facility.

Five irradiation treatments were used: 0 Gy (control), 5 Gy, 10 Gy, 15 Gy, and 20 Gy. The dose rate was approximately 60 Gy h⁻¹, and the exposure time was adjusted to achieve the desired doses. Dose calibration was performed using a standard dosimeter (e.g., Fricke dosimeter) following standard operating procedures.

Control plantlets (0 Gy) were handled identically to irradiated samples but without radiation exposure, ensuring experimental equivalence. After irradiation, plantlets were maintained in vitro for four months before acclimatization, representing the M1V2 stage.



Figure 1. Morphological appearance of *Dendrobium crumenatum* plantlets at 12 months of age following gamma irradiation at different doses (0, 5, 10, 15, and 20 Gy). Plants were cultured in vitro for seven months and acclimatized ex vitro for six months. Observed traits include plant height, leaf size, and shoot number. A scale bar of 5 cm is provided in each panel

Experimental design and layout

The experiment was arranged in a Completely Randomized Design (CRD) with five treatments. Each treatment consisted of five biological replicates, with three plantlets per replicate, resulting in 75 experimental units. During acclimatization, plantlets were grown under 70% shade, a temperature of 25-30°C, and a relative humidity of 70-85%. Plants were potted in black moss medium and arranged randomly on benches to minimize environmental variation.

Observed variables and measurement protocol

Five morphological parameters were evaluated: plant height (cm), number of leaves per plant, leaf length (cm), leaf width (cm), number of shoots. These traits were selected due to their importance in determining compact growth habit and ornamental quality for pot plant production. Measurements were conducted at the end of the acclimatization phase (12 months, M1V2 stage). Plant height and leaf dimensions were measured using a ruler (± 0.1 cm accuracy), while leaf and shoot numbers were counted manually.

Phenotypic stability assessment

The stability of dwarf traits was evaluated within a single growth cycle during the six-month acclimatization phase. Consistent expression of reduced plant height and compact morphology was considered indicative of phenotypic stability. However, heritability across subsequent generations was not assessed and requires further study.

Data collection and statistical analysis

Primary data were obtained through direct measurement, while supporting literature was used as secondary data. Data were analyzed using Analysis of Variance (ANOVA) at a 5% significance level ($p < 0.05$). Prior to analysis, normality and homogeneity of variance were tested using the Shapiro-Wilk and Levene's tests. When significant differences were detected, means were compared using the Least Significant Difference (LSD) test at the 5% level. Statistical analyses were performed using IBM SPSS Statistics version 26.

Morphological similarity analysis

Multivariate analysis was conducted to evaluate phenotypic relationships among treatments. Data were standardized using a z-score transformation, and a Euclidean distance matrix was generated. Cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in PAST software version 4.13. The resulting dendrogram was used to visualize morphological similarity and to support the interpretation of variation induced by gamma irradiation.

RESULTS AND DISCUSSION

Effect of gamma irradiation on the growth of *Dendrobium crumenatum* mutants based on the 5% Least Significant Difference (LSD) test

Analysis of Variance (Table 1) revealed that gamma irradiation significantly affected several morphological traits of *D. crumenatum*. The 5 Gy treatment caused the most pronounced growth inhibition, with plant height, leaf number, and shoot number reduced by approximately 53.7%, 81.7%, and 84.4%, respectively, compared to the control. This indicates that low-dose irradiation induced strong physiological stress, leading to substantial suppression of vegetative growth.

In contrast, the 10 Gy and 15 Gy treatments produced more compact plants while maintaining relatively stable leaf number and shoot production. Plant height in the 10 Gy treatment was reduced by approximately 52.6%, but shoot number remained within a comparable range to the control, suggesting a desirable dwarfing effect without severely compromising vegetative propagation potential. Similarly, the 15 Gy treatment showed minimal reduction in plant height (~3.9% increase relative to control) and maintained morphological stability, indicating tolerance to this irradiation level.

At 20 Gy, plant responses were intermediate. Although plant height decreased by approximately 37.4%, leaf number and shoot number were not significantly different from the control based on the 5% LSD test. This pattern may suggest a potential recovery response or could indicate the presence of more tolerant cell populations, although these mechanisms were not directly tested in this study.

Table 1 presents the mean values \pm standard deviation for all observed parameters. The relatively large standard deviation values observed in some treatments (e.g., leaf number and shoot number) indicate intra-mutant variability, suggesting heterogeneous responses among regenerants, likely due to chimeric mutation effects induced by gamma irradiation.

Percentage change analysis of morphological traits

To provide a standardized quantitative comparison of treatment effects across all measured traits, the percentage change ($\Delta\%$) relative to the control was calculated (Table 2). The percentage change analysis revealed clear dose-dependent patterns in morphological responses. The 5 Gy treatment resulted in the most pronounced reductions across all traits, particularly in shoot number (-84.4%) and leaf number (-81.7%), indicating severe growth inhibition. In contrast, the 10 Gy treatment maintained relatively smaller reductions in leaf number (-16.7%) while achieving substantial plant height reduction (-52.6%), suggesting a more balanced morphological response. The 15 Gy treatment exhibited minimal change in plant height (+3.9%) and an increase in leaf width (+28.3%), indicating potential compensatory growth. Meanwhile, the 20 Gy treatment showed moderate reductions across traits but relatively higher shoot retention (-15.6%), reflecting a less severe inhibitory effect compared to lower doses.

Morphological observation

Based on Table 1 and Figure 2, clear phenotypic differences were observed among treatments. The 5 Gy mutants exhibited severe growth suppression, characterized by shorter stature, fewer leaves, and reduced shoot formation, which negatively affects ornamental value.

Mutants treated with 10-20 Gy displayed a compact growth habit, with shorter stems but relatively stable shoot production. Notably, the 10 Gy treatment showed the most favorable phenotype, combining reduced plant height with adequate shoot number and healthy leaf development. This balance is particularly important for ornamental orchids, where compactness and fullness are desirable traits.

Although floral traits were not quantitatively measured in this study, the maintenance of vegetative vigor and shoot production in 10 Gy and 15 Gy treatments suggests a potential for maintaining floral quality despite reduced plant size, which is advantageous for commercial potted orchid production.

Genetic similarity analysis

Morphological similarity analysis using the UPGMA method was conducted based on five standardized traits: plant height, leaf number, leaf length, leaf width, and shoot number. It is important to note that this analysis was based solely on morphological data and did not include molecular markers such as SSR. The dendrogram (Figure 3) showed

that control plants (0 Gy) formed a distinct cluster with the lowest similarity index, indicating that gamma irradiation induced measurable morphological divergence. The 15 Gy treatment clustered closest to the control, suggesting minimal deviation from the natural phenotype.

Meanwhile, the 5 Gy, 10 Gy, and 20 Gy treatments formed a separate cluster, indicating greater similarity among irradiated mutants. Within this cluster, the 10 Gy and 20 Gy treatments were closely related, reflecting nearly identical morphological responses. Overall, the similarity ranking from most similar to most distinct was: 10 Gy \approx 20 Gy $>$ 5 Gy $>$ 15 Gy $>$ control. These results confirm that gamma irradiation induced consistent morphological variation and influenced clustering patterns based on dose.

Table 2. Percentage change ($\Delta\%$) of morphological traits in *Dendrobium crumenatum* under gamma irradiation (relative to control)

Dose (Gy)	Plant height (%)	Leaf number (%)	Leaf length (%)	Leaf width (%)	Shoot number (%)
0 (Control)	0	0	0	0	0
5 Gy	-53.7	-81.7	-60.2	-34.0	-84.4
10 Gy	-52.6	-16.7	-50.6	-18.9	-37.8
15 Gy	3.9	-28.3	-21.7	28.3	-33.3
20 Gy	-37.4	-31.7	-44.6	0	-15.6

Table 1. Growth performance of *Dendrobium crumenatum* mutants treated with different doses of gamma irradiation

Treatment	Variables observed				
	Plant height (cm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Number of shoots
Control	4.28a	15.00a	4.15a	0.53a	11.25a
5 Gy	1.98b	2.75b	1.65c	0.35b	1.75b
10 Gy	2.03b	12.50a	2.05c	0.43a	7.00a
15 Gy	4.45a	10.75a	3.25a	0.68a	7.50a
20 Gy	2.68a	10.25a	2.30b	0.53a	9.50a
LSD 5%	2.06	7.78	0.99	0.26	5.65

Note: Values represent mean \pm standard deviation. Superscript letters indicate statistical differences among treatments based on the LSD test at 5% significance level. Treatments sharing the same letter are not significantly different, while different letters indicate significant differences. Superscript letters are assigned based on pairwise comparisons using the 5% LSD values. All measurements should be verified to ensure accuracy and consistency across replicates. Observed parameters include plant height, leaf number, leaf length, leaf width, and number of shoots

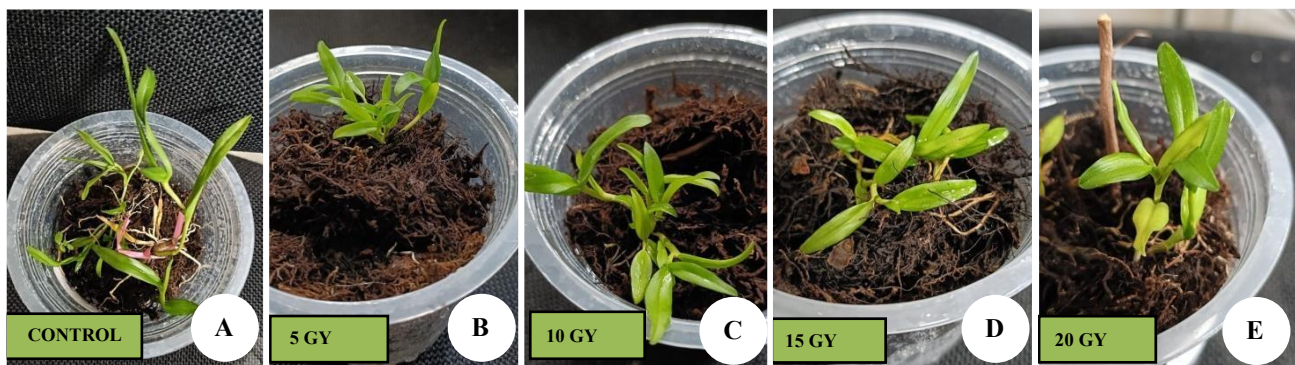


Figure 2. Phenotype of *Dendrobium crumenatum* mutants resulting from gamma irradiation at doses of 5, 10, 15, and 20 Gy. A culture vessel with a diameter of approximately six centimeters was used as a visual scale. A. Control at 0 Gy showed relatively normal growth with higher numbers of shoots and leaves, and some seedlings appeared elongated. B. At 5 Gy, plantlets were compact with bright green leaves and a moderate number of shoots. C. At 10 Gy, plantlets were compact with actively growing leaves and exhibited clear vigor. D. At 15 Gy, leaves appeared longer with a sufficient number of shoots, and growth was relatively stable. E. At 20 Gy, growth was more suppressed; one stem appeared desiccated, but several shoots continued to develop, indicating an intermediate response

Biological interpretation

The results demonstrate that gamma irradiation effectively induced morphological variation in *D. crumenatum*. The 5 Gy dose caused excessive growth suppression, while 10 Gy provided an optimal balance between dwarfism and vegetative performance. The 15 Gy treatment could maintain characteristics closest to the control, indicating lower mutagenic impact.

From a practical perspective, the 10 Gy treatment is the most promising for developing compact ornamental varieties, as it reduces plant size without significantly affecting shoot production or overall plant vigor. This highlights the importance of considering not only statistical significance but also biological relevance and commercial applicability in mutation breeding programs.

Multivariate analysis of morphological traits

To further examine the overall pattern of morphological variation across gamma irradiation treatments, a multivariate analysis was conducted using Principal Component Analysis (PCA) based on five traits: plant height, leaf number, leaf length, leaf width, and shoot number.

The PCA biplot (Figure 4) revealed a clear separation among treatments along the first two principal components. PC1 accounted for the largest proportion of total variation and was primarily associated with overall vegetative vigor, as indicated by strong contributions from plant height, leaf number, and shoot number. In contrast, PC2 represented variation related to plant compactness, mainly influenced by leaf dimensions.

The 5 Gy treatment was distinctly separated from other treatments, reflecting its strong inhibitory effects across most morphological traits. In contrast, the 10-15 Gy treatments were positioned closer to the center and aligned with balanced trait contributions, indicating more stable and proportionate growth. The 20 Gy treatment exhibited a

more dispersed distribution, suggesting increased variability in response to higher irradiation stress.

Discussion

The present study demonstrates that gamma irradiation induces significant morphological variation in *D. crumenatum*, with responses varying across irradiation doses and exhibiting a distinctly non-linear pattern. This non-linearity suggests that plant responses to ionizing radiation are not merely dose-dependent but are governed by a dynamic balance between cellular damage and adaptive repair mechanisms. Similar non-linear responses have been reported in mutation breeding studies of ornamental plants, where intermediate doses often produce more favorable phenotypes than either low or high doses (Kiani et al. 2022; Dwiyani et al. 2024).

At the physiological level, gamma irradiation primarily affects plant growth through DNA damage, Reactive Oxygen Species (ROS) accumulation, and hormonal imbalance. Ionizing radiation can induce single- and double-strand DNA breaks, which disrupt cell division in meristematic tissues. Additionally, excessive ROS production can damage cellular membranes and proteins, further inhibiting growth. One key mechanism underlying dwarfism is the disruption of the gibberellin (GA) biosynthesis and signaling pathway, which plays a central role in stem elongation. Reduced GA activity leads to decreased cell elongation and shorter internodes, resulting in compact plant architecture (Teixeira et al. 2017; Hinsley et al. 2018). At moderate doses, however, plants may activate DNA repair systems, antioxidant enzymes (e.g., SOD, CAT), and hormonal rebalancing involving auxins and cytokinins, allowing partial recovery and stabilization of growth, as indicated by the activation of DNA repair mechanisms, upregulation of antioxidant enzymes such as SOD and CAT, and the rebalancing of growth hormones that regulate cell division and elongation (Abbas et al. 2017; Magdalita et al. 2019).

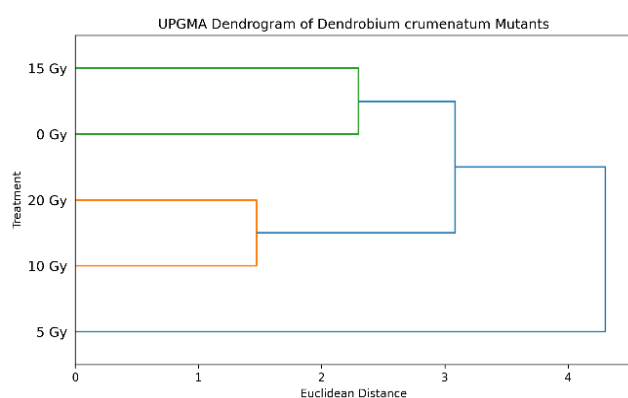


Figure 3. The similarity level of *Dendrobium crumenatum* mutants based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA)

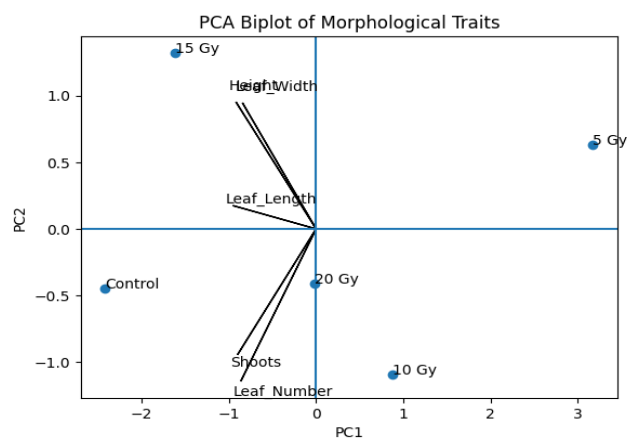


Figure 4. Principal Component Analysis (PCA) biplot showing the distribution of *Dendrobium crumenatum* under different gamma irradiation doses based on five morphological traits (plant height, leaf number, leaf length, leaf width, and shoot number). PC1 represents overall vegetative vigor, while PC2 reflects variation in plant compactness. Arrows indicate trait loadings contributing to each principal component

Plant height response and mechanistic interpretation

The strong suppression of plant height at 5 Gy suggests that even low-dose irradiation exceeded the tolerance threshold of *D. crumenatum* meristematic tissues. This response differs from several studies in orchids, such as *Dendrobium sonia* (Datta et al. 2018), where growth inhibition was observed mainly at higher doses (>10 Gy). The earlier onset of inhibition in this study indicates that *D. crumenatum* may have higher radiosensitivity, possibly due to differences in meristem organization, antioxidant capacity, or endogenous hormone balance.

At intermediate doses (10-15 Gy), plant height recovered and approached control values, reflecting activation of compensatory physiological mechanisms, including enhanced antioxidant defense and partial restoration of GA-mediated elongation. This pattern is consistent with the concept of radiation hormesis, where moderate stress stimulates adaptive responses (Dwiyanı et al. 2024). However, unlike some ornamental species where stimulation persists at higher doses (Handini and Aprilianti 2020), the decline at 20 Gy in this study indicates that cellular repair capacity was exceeded, leading to renewed growth limitation.

Importantly, these findings refine the concept of dwarfism in orchid breeding. Extreme dwarfism at 5 Gy resulted from severe tissue damage and reduced vigor, whereas moderate doses (10-15 Gy) produced proportionate dwarf phenotypes, which are more desirable for ornamental purposes.

Leaf development and radiosensitivity

Leaf number and leaf size (length and width) showed high sensitivity to gamma irradiation, particularly at low doses. The drastic reduction in leaf number at 5 Gy indicates severe impairment of leaf primordia initiation, likely due to disrupted auxin-cytokinin balance and meristem inactivity. Compared with previous studies (Hartati et al. 2021; Kim 2023), the magnitude of reduction observed here suggests that *D. crumenatum* has a lower tolerance threshold for maintaining vegetative organ formation under radiation stress.

At 10-20 Gy, leaf number and size showed an increase compared to higher doses, which may suggest a potential recovery response associated with the activation of ROS detoxification and DNA repair pathways. However, this response remained limited, indicating that the adaptive capacity of this species may operate within a relatively narrow dose range. Similar patterns have been reported in other ornamentals (Khor et al. 2020), although the optimal dose range in *D. crumenatum* appears more restricted.

Leaf width exhibited a particularly interesting response, with the highest value observed at 15 Gy. This suggests that moderate irradiation may enhance lateral cell expansion and mesophyll development, possibly through improved hormonal coordination. Unlike general hormesis that promotes overall growth, this response indicates trait-

specific stimulation, where leaf expansion is favored without excessive stem elongation—an important characteristic for compact ornamental plants.

Shoot formation and meristem stability

Shoot number is a critical indicator of meristematic activity and regenerative capacity. The severe reduction at 5 Gy confirms that low-dose irradiation can disrupt both apical and axillary meristems, leading to long-term developmental limitations. This response is more pronounced than in some other *Dendrobium* species (Sherpa et al. 2022), again highlighting species-specific radiosensitivity.

At 10-15 Gy, shoot number recovered substantially, suggesting that moderate irradiation supports functional stabilization of meristems rather than mere survival. This stabilization likely involves coordinated regulation of hormonal signaling and cellular repair processes (Nguyen et al. 2020). Importantly, shoot recovery occurred without excessive elongation, resulting in a compact and balanced canopy architecture, which is desirable for ornamental use.

At 20 Gy, an improvement in growth parameters was observed, which may suggest a potential recovery response, although it was accompanied by visible stress symptoms. This pattern may indicate that high-dose irradiation imposes a metabolic burden that could divert resources toward stress mitigation rather than growth optimization (Mondal and De 2024).

Morphological similarity and phenotypic stability

The UPGMA dendrogram revealed two major clusters, with the control and 15 Gy grouped, while 5, 10, and 20 Gy formed a separate cluster. This pattern confirms that 15 Gy induced minimal phenotypic deviation, while other doses resulted in more pronounced morphological changes.

Importantly, morphological similarity does not necessarily indicate the absence of mutation but may reflect phenotypic buffering, where genetic changes are not fully expressed due to regulatory mechanisms. This is consistent with findings from molecular studies (e.g., Dwiyanı et al. 2024), which show that genetic variation does not always translate directly into phenotypic divergence.

In addition to cluster-based analysis, multivariate ordination using PCA provides further insight into the integrated morphological responses to gamma irradiation. The clear separation of the 5 Gy treatment confirms its strong inhibitory effect across multiple traits, while the clustering of the 10-15 Gy treatments near balanced trait loadings reinforces their potential for producing compact yet stable phenotypes. The more dispersed distribution of the 20 Gy treatment suggests increased variability under higher stress conditions. These findings support the observed non-linear response pattern and highlight the importance of intermediate irradiation doses in optimizing morphological outcomes.

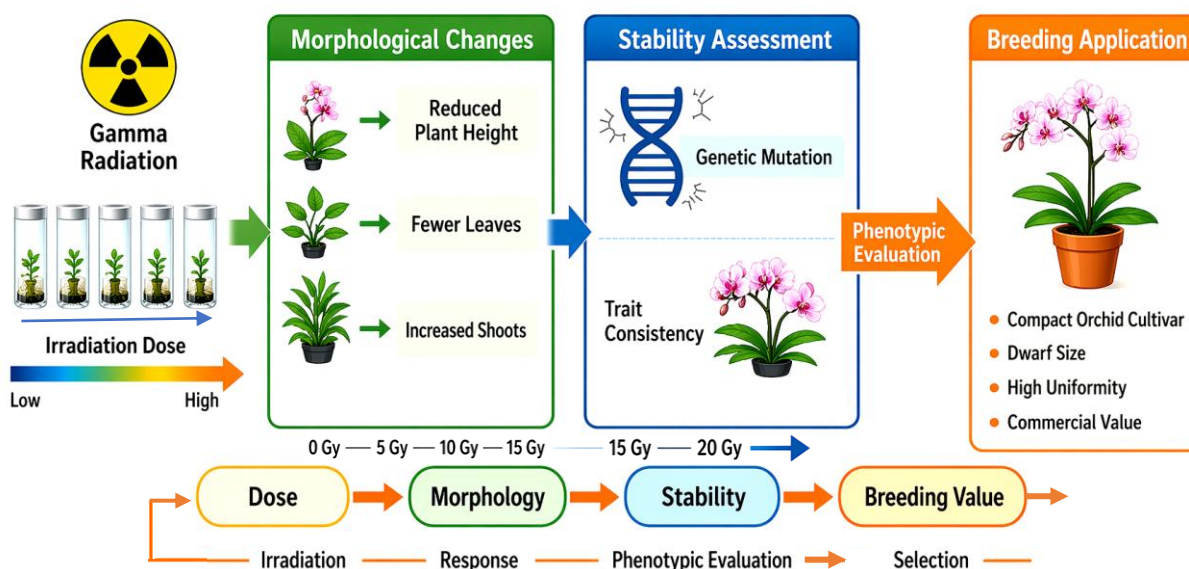


Figure 5. Conceptual model of dose-dependent responses to gamma irradiation in *Dendrobium crumenatum*

Implications for mutation breeding (refined interpretation)

The results suggest that gamma irradiation can be used to induce compact growth forms in *D. crumenatum*, particularly within the 10-15 Gy range. However, it is important to emphasize that the current findings are based solely on phenotypic observations at the early growth stage.

Therefore, The identified mutants cannot yet be considered stable breeding material. Further evaluation is required, including: genetic stability testing across generations, assessment of flowering traits and reproductive performance, molecular characterization (e.g., SSR, SNP markers). Thus, the present study provides preliminary evidence of phenotypic variation rather than definitive selection of elite mutant lines.

Study limitations and future directions

This study is limited to morphological evaluation without molecular validation. Additionally, intra-mutant variability observed in several parameters suggests the presence of chimeric mutations, which require further stabilization through subculture or clonal selection. Future research should focus on molecular analysis to confirm genetic variation, evaluation of floral traits and commercial quality, multi-generation trials to assess trait stability.

Conceptual synthesis of dose-dependent responses

To provide an integrative interpretation of these findings, the dose-dependent relationships among gamma irradiation, physiological responses, and morphological outcomes are summarized in a conceptual model (Figure 5). The model illustrates that low doses (5 Gy) are associated with strong growth suppression, intermediate doses (10-15 Gy) promote balanced dwarfism with maintained vigor and phenotypic stability, while higher doses (20 Gy) show more variable responses accompanied by stress symptoms. Overall, this synthesis suggests that optimal breeding value may be achieved at intermediate

doses, where compact growth is aligned with stability and performance.

In conclusion, the application of gamma irradiation in this study successfully induced morphological variation in *D. crumenatum*, particularly in plant height and vegetative architecture, with responses varying across irradiation doses and exhibiting a non-linear pattern. The 5 Gy dose caused severe growth suppression, reducing plant height, leaf number, and shoot number by approximately 53.7%, 81.7%, and 84.4%, respectively, indicating that this dose exceeded the physiological tolerance threshold and is unsuitable for ornamental application. In contrast, the 10 Gy treatment achieved a comparable reduction in plant height (~52.6%) while maintaining relatively high leaf number (~83.3% of control) and shoot number (~62.2% of control), producing a compact and balanced phenotype. The 15 Gy treatment showed minimal deviation in plant height (~+3.9%) with moderate reductions in vegetative traits, whereas the 20 Gy treatment resulted in semi-compact plants with moderate height reduction (~37.4%) and relatively high shoot retention (~84.4% of control), indicating partial tolerance at higher doses.

These findings demonstrate that intermediate irradiation doses, particularly 10 Gy, provide the most favorable balance between dwarfism and vegetative performance, supporting their potential use in mutation breeding for compact ornamental orchids. These results demonstrate that gamma irradiation can induce phenotypic variation in *D. crumenatum*, particularly in traits related to plant compactness. However, it is important to emphasize that these findings are based on early-stage morphological observations, and the observed traits have not yet been evaluated for genetic stability or heritability; the presence of intra-treatment variability also suggests possible chimeric mutations that require further stabilization. Future research should focus on multi-generation evaluation (M2-M3) to confirm trait stability, molecular characterization (e.g., SSR or SNP markers) to validate genetic changes,

and assessment of floral traits and commercial quality to ensure breeding relevance. This study provides a quantitative foundation for optimizing gamma irradiation as a targeted strategy to develop compact, high-value *D. crumenatum* cultivars for floriculture applications.

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