

Exploring phenotypic variability in gamma-irradiated melon (*Cucumis melo*) for early-generation putative mutant selection

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Abstract. Paramitha AI, Sugiharto AN, Waluyo B, Adiredjo AL, Arifin AG. 2026. Exploring phenotypic variability in gamma-irradiated melon (*Cucumis melo*) for early-generation putative mutant selection. *Biodiversitas* 27 (3): d270324. <https://doi.org/10.13057/biodiv/d270324>. The melon (*Cucumis melo*), an agriculturally pivotal crop globally esteemed for its economic value, faces intrinsic breeding limitations due to restricted genetic diversity. Confronting these inherent challenges, gamma irradiation has emerged as a robust mutagenic agent, purposefully employed to elicit phenotypic variability and strategically advance the enhancement of elite melon cultivars. This investigation systematically explored phenotypic modifications induced by six discrete gamma irradiation doses (0-150 Gy) in two melon varieties, CMA and DKN. Comprehensive assessments of fruit weight, flesh thickness, sugar content, and rind color were conducted in the M₂ generation, analyzed via descriptive and non-parametric statistics. Results revealed distinct dose-specific responses. Notably, G5 treatment yielded the highest mean sugar content in CMA (10.75°Brix), with DKN exhibiting substantial variation up to 15.00°Brix at G5. Fruit weight demonstrated considerable variability, exemplified by DKN at G1 (0.28-1.92 kg range). Both DKN and CMA consistently achieved their highest mean flesh thickness at G5. Statistical analyses confirmed significant differences across most irradiation doses for sugar content and flesh thickness in both varieties, while fruit weight showed significant effects exclusively at G4 for both CMA and DKN. Crucially, the study identified four novel putative mutant plants: one from CMA (G1) and three from DKN (G5). These putative mutants represent invaluable genetic resources, integrating dark rind color (absent in controls) with quantitative traits was not more than 5% lower than the control mean, thus proving highly advantageous for early generation selection. These findings unequivocally underscore gamma irradiation's profound potential in inducing beneficial phenotypic variability, offering critical foundations for breeding programs targeting enhanced melon fruit quality and marketability.

Keywords: *Cucumis melo*, gamma irradiation, Indonesia, M₂ generation, mutant selection, rind color

INTRODUCTION

Melon is a horticultural crop with economic value in fruit markets. Indonesia is a melon producer in Southeast Asia, yet the domestic sector faces structural constraints that can limit productivity and slow varietal development (Badami et al. 2020; Rohmatin et al. 2022). Production efficiency reported to be below global averages supports efforts to develop improved varieties adapted to local agro-ecological conditions (Badami et al. 2020). In some production systems, dependence on imported seed can increase cultivation costs, underscoring the importance of locally adapted cultivars that fit Indonesian environments and market preferences. Consumer preferences for fruit quality and typology shape breeding priorities; rind coloration, sweetness, and texture are key quality cues associated with market value (Nuñez-Palenius et al. 2008; Kyriacou et al. 2018). Fruit typologies and rind colors can also enable product differentiation for specific market segments (Perpiñá et al. 2025).

Despite notable achievements in melon breeding over the last century, conventional approaches retain limitations that can slow crop improvement. These constraints include the gradual pace of germplasm enhancement and restricted gene pools in wide commercial varieties, which can constrain the introduction of novel traits (Mahajan and Kapoor 2023). Breeders continue to pursue cultivars with improved disease resistance, enhanced flavor profiles, increased sweetness, and greater yield stability across environments (Sharma et al. 2025). Such targets often require combining multiple loci while maintaining performance in breeding backgrounds. The identification and use of useful genetic and metabolic variability, particularly from landraces and wild relatives, remain important for broadening the cultivated melon genetic base (Kesh and Kaushik 2020). Expanding genetic diversity can increase the range of selectable traits and support improvements in productivity and fruit quality while responding to changing market requirements (Sharma et al. 2025).

Induced mutagenesis offers an additional strategy to create genetic variability when natural diversity is

insufficient for specific breeding targets. Gamma irradiation is a well-established physical mutagen used in crop improvement because it can introduce DNA changes that generate novel genetic variants (Ntsefong et al. 2023). Such variants may express phenotypes relevant to breeding, including changes in yield components, disease response, and quality attributes (Riviello-Flores et al. 2022). A commonly noted advantage of gamma irradiation is its high penetrance and its capacity to induce many mutations from a single treatment (Puripunyanich et al. 2022). Radiation dose affects the balance between variability and viability, so dose selection is often a key protocol decision. Beneficial mutations, serving as indispensable genetic resources for accelerated breeding programs focused on specific agronomic or quality targets, require rigorous phenotypic screening for effective deployment (Prasanna and Jain 2017; Hassan et al. 2022).

The identification of desirable putative mutants from mutagenized populations depends on systematic phenotypic characterization and early-stage screening. Plant phenotyping is central to breeding programs because accurate trait measurement underpins the selection of promising genotypes (Abebe et al. 2023). Phenotypic variability reflects the interaction between genetic makeup and environmental influences, producing observable differences among individuals (Saibari et al. 2023). In induced mutagenesis, assessing variability in early generations is particularly important, because favorable allele combinations may be lost in later generations if selection is delayed (Alaguero-Cordovilla et al. 2020; Saibari et al. 2023). Early screening can therefore remove undesirable variants and prioritize individuals with advantageous morphological, physiological, or biochemical characteristics for subsequent evaluation (Saibari et al. 2023). Standardized descriptors and repeated measurements can further help distinguish stable phenotypes from transient environmental effects during early advancement, especially in field trials across repeated environments.

Despite the established utility of gamma irradiation, a significant knowledge gap persists regarding the specific magnitude and patterns of phenotypic variability induced in commercially Indonesian melon varieties. Current evidence is limited on dose-specific effects on key quantitative traits and the efficacy of early-generation phenotyping as a guiding principle for selecting improved fruit quality and novel rind characteristics. Clarifying these intricate dose-trait relationships is pivotal for optimizing mutagenesis protocols and establishing precise selection criteria that demonstrably increase the probability of identifying superior putative mutants for local breeding programs. Accordingly, this study aimed to: (i) quantify the effects of different gamma irradiation doses on fruit weight, flesh thickness, and sugar content in two Indonesian melon varieties, (ii) elucidate changes in phenotypic variability, and (iii) define explicit selection criteria to identify M_2 putative mutants suitable for advancement.

MATERIALS AND METHODS

Research location

The experiment was conducted from January to December 2024 in a greenhouse located at Permanu Village, Kepanjen Sub-district, Malang District, East Java, Indonesia. This controlled environment was deliberately selected to ensure consistent growing conditions for *Cucumis melo* throughout the study, reducing external environmental fluctuations and facilitating accurate monitoring of phenotypic traits under standardized conditions. Throughout the experimental period, the average air temperature was maintained at 24-26°C, while relative humidity ranged between 60% and 75%.

Genetic materials

The genetic materials for this study comprised seeds from two distinct commercial varieties of melon, namely CMA and DKN. These varieties were chosen for their established commercial value and genetic background. Prior to planting, dry seeds of both varieties underwent gamma-ray irradiation at the Isotope and Radiation Application Center of the National Nuclear Energy Agency, Indonesia. A spectrum of six different irradiation doses was applied to induce mutations: 0 Gy (G_0) serving as the non-irradiated control, 25 Gy (G_1), 50 Gy (G_2), 75 Gy (G_3), 100 Gy (G_4), and 150 Gy (G_5). This assay confirmed that the LD50 for the CMA variety was successfully reached within the selected range (100 Gy), whereas the DKN variety demonstrated a higher degree of radio-tolerance. The maximum dose was capped at 150 Gy to ensure the survival of a viable population for the CMA variety while still providing a robust mutagenic pressure for the DKN variety. Prior to planting, dry seeds of both varieties were equilibrated to a moisture content of 12% by storage in desiccators for 72 hours to ensure uniform responsiveness to irradiation treatment. Seeds were then subjected to gamma-ray irradiation at the Isotope and Radiation Application Center of the National Nuclear Energy Agency, Indonesia, using a ^{60}Co gamma source with a dose rate of 10 Gy min^{-1} . The exposure time was calculated according to the formula: exposure time = dose ÷ dose rate. After irradiation, seeds were stored at 4°C for 24 hours before sowing to allow repair of initial radiation damage and to ensure uniform germination timing across all treatments.

Procedures

The mutation generation advancement scheme was conducted as follows: seeds irradiated in 2024 represented the M_0 generation. M_0 plants were grown to maturity under greenhouse conditions, and individual plants were self-pollinated through bagging to prevent outcrossing and maintain the purity of the putative mutant. Seeds harvested from M_0 plants constituted the M_1 generation, which was not subjected to phenotypic evaluation due to the predominance of recessive mutations that may not be expressed phenotypically in the heterozygous condition. M_1 plants were again self-pollinated, and the resulting seeds from individual M_1 plants were harvested separately to produce M_2 families. The present study focused on

phenotypic evaluation of the M_2 generation, which represents the segregating generation where homozygous recessive mutations become visible for selection. For the M_2 generation, plants were also self-pollinated by bagging their flowers to prevent outcrossing and ensure the genetic purity of the observed putative mutant.

The experimental design was a completely randomized design with 48 M_2 plants per variety-dose combination ($n = 48 \text{ plants} \times 2 \text{ varieties} \times 6 \text{ doses} = 576 \text{ plants total}$). All plants were individually grown in 15-liter plastic pots filled with a commercial horticultural potting mix consisting of 60% peat moss, 30% perlite, and 10% vermiculite, arranged with 30 cm spacing between pots to minimize inter-plant competition. A single-plant-per-pot method was employed across all experimental units to facilitate detailed individual plant observation and to avoid the confounding effects of plant density on phenotypic traits. All cultivar-dose combinations were randomized across benches to minimize positional effects within the greenhouse. The individual plant served as the unit of analysis. Specifically, one representative fruit per plant was measured for phenotypic characterization. Notably, all M_2 plants successfully set fruit; thus, no data were excluded from the analysis due to reproductive failure.

Throughout the experimental period, all plants were maintained under standardized agronomic practices to ensure uniform growing conditions. Irrigation was provided through a drip system operating at 70% field capacity, with soil moisture monitored weekly using tensiometers to maintain optimal water availability without waterlogging. Plants received a complete N-P-K (14-14-14) fertilizer applied at the rate of 150 kg ha^{-1} split into three applications at vegetative, flowering, and fruit development stages. Pests and diseases were monitored through weekly visual inspections, and preventive applications of registered pesticides were conducted only when threshold levels were reached, following integrated pest management principles to minimize chemical intervention.

Data collection

Data collection took place at the fruit maturity stage, which was determined using a combination of objective and semi-objective criteria. Maturity was assessed by counting days after anthesis (35–45 days post-pollination), accompanied by visual evaluation of standard ripening characteristics specific to each melon variety including full slip formation at the peduncle, rind color change from green to variety-specific mature color, and development of characteristic aroma. The following quantitative traits were measured: fruit weight was measured in kilograms using a high-precision digital scale to the nearest 0.01 kg. Flesh thickness was determined by measuring the average thickness at the equatorial region of each fruit using a digital caliper in centimeters to the nearest 0.01 cm. The sugar content of each fruit was measured as °Brix using a handheld refractometer calibrated at 20°C, with juice extracted from the equatorial region of the fruit. Each trait was measured in triplicate for each individual plant, and the mean value was used for subsequent statistical analysis. Rind skin color was assessed as a qualitative variable using a combination of visual

scoring and photographic documentation. Color was evaluated through direct visual comparison to the Pantone color system to provide standardized color classification. Additionally, digital images of each fruit were captured under controlled lighting conditions using a calibrated DSLR camera positioned consistently at a fixed distance and angle.

Data analysis

All collected phenotypic data from the M_2 generation were subjected to a comprehensive statistical analysis. Data were first tested for normality using the Shapiro-Wilk test and homogeneity of variance using the Levene test. Since assumptions for parametric analysis were not consistently met across all datasets, the non-parametric Kruskal-Wallis H test was performed as an overall test to detect significant differences among all treatment groups within each variety, providing a comprehensive assessment of dose effects before conducting pairwise comparisons. When the Kruskal-Wallis test indicated significant overall differences, Mann-Whitney U tests with Bonferroni correction for multiple comparisons were conducted for pairwise comparisons between each dose and the control. The Mann-Whitney U test was employed to facilitate comparisons of fruit weight, flesh thickness, and sweetness content between each gamma-irradiated population and the non-irradiated control for each melon variety. In total, 30 individual Mann-Whitney U tests were performed (5 dose comparisons per trait $\times$ 3 traits $\times$ 2 varieties), ensuring no pooling of data across varieties.

To visually represent the distribution of phenotypic traits across all treatments and to effectively identify statistical outliers, boxplot analysis was performed. The identification of such outliers is a standard step in mutation breeding for detecting phenotypes deviating from the parental mean, which may represent potential genetic variants warranting further investigation. Outliers were operationally defined as data points exceeding 1.5 times the interquartile range beyond the first and third quartiles. Plants exhibiting outlier status for desirable traits (such as increased sugar content or unique rind color) were marked as candidate mutants for further evaluation. All statistical analyses were performed using R statistical software version 4.2.1, and statistical significance was established at a threshold of $p < 0.05$. However, to maintain a family-wise error rate of $p < 0.05$, a Bonferroni correction was applied, resulting in a more stringent significance threshold of $p < 0.01$ for individual comparisons. This adjusted threshold was strictly applied to all results presented in Table 3.

RESULTS AND DISCUSSION

Variability in quantitative phenotypic performance

The intentional induction of mutations through gamma irradiation serves as a powerful strategy to augment phenotypic variability, which is consistent with underlying genetic changes subsequently expressed as phenotypic diversity in quantitative traits. This artificially generated variation is crucial for crop improvement programs, facilitating the selection of novel and desirable phenotypes that might not be available within existing germplasm.

Table 1 presents the descriptive statistics for critical fruit quality parameters in the CMA melon population. It illustrates the impact of gamma irradiation on phenotypic shifts and broadening the range of expression for each trait (Widyapangesthi et al. 2022; Bammanakatti et al. 2023). For fruit weight, mean values varied, with G3 (75 Gy) yielding the highest mean of 1.03 kg, exceeding the G0 control (0.95 kg), while G4 (100 Gy) resulted in the lowest mean (0.62 kg). The widest range in FW was observed at G3 (0.51-1.83 kg), indicating the greatest induced variability in fruit size at this dose, alongside the highest standard deviation (0.28 kg). Conversely, G4 showed the narrowest range (0.36-0.95 kg) and lowest standard deviation (0.14 kg). This demonstrates the diverse and often bidirectional effects of radiation on this critical trait, consistent with findings in other irradiated fruit crops where gamma rays can both decrease and increase fruit size and weight (Ernest et al. 2020; Obok et al. 2023).

Flesh thickness also showed notable induced variability. The G5 (150 Gy) treatment produced the highest mean FT (3.18 cm), surpassing the G0 control (2.87 cm), while G4 (100 Gy) led to the lowest mean (2.23 cm). The greatest spread in flesh thickness, indicated by the highest standard deviation, was observed in the G0 control (0.55 cm), followed closely by G2 (0.50 cm). G4 exhibited the lowest standard deviation (0.34 cm). The maximum flesh thickness (4.20 cm) occurred at G5, representing a notable enhancement from the control, despite G5 not showing the broadest overall variability. These observed variations underscore the considerable potential for selecting mutants with enhanced physical attributes that meet consumer and market demands (Perpiñá et al. 2025).

Sugar content demonstrated significant variability across the irradiated populations. The G5 (150 Gy) treatment consistently exhibited the highest mean sugar content

(10.75°Brix), significantly exceeding the G0 control (8.56°Brix), highlighting strong potential for enhancing sweetness through induced mutagenesis (Goldenberg et al. 2014; Kantoğlu et al. 2023). In contrast, G3 showed the lowest mean (7.18°Brix). The highest standard deviation for BR was observed at G1 (2.12°Brix), indicating the greatest variability in sweetness at this lower dose, alongside the widest range (5.00-14.00°Brix). Conversely, G5, despite having the highest mean, showed a reduced standard deviation (1.32°Brix) and a narrower range (7.00-13.50°Brix) compared to G1, suggesting a more consistent increase in sugar content at this higher dose. Such variability in sugar content following irradiation has been observed in various fruits, indicating both positive and negative impacts depending on the specific treatment and crop (Sau et al. 2018; Cancino-Vázquez et al. 2020).

Table 2 presents the descriptive statistics for the DKN melon population. This data further illustrates the extensive phenotypic modifications observed. Such modifications are known to be achievable through gamma irradiation (Ernest et al. 2020). For fruit weight, mean values ranged from 0.77 kg at G4 (100 Gy) to 1.15 kg at G5 (150 Gy). This is compared to the G0 control, which showed 1.06 kg. The widest range (0.28-1.92 kg) and highest standard deviation (0.36 kg) for FW were observed at G1 (25 Gy). This indicates the greatest induction of phenotypic variability in fruit size at this lower dose. Conversely, G4 showed a narrower range (0.27-1.32 kg) and lower standard deviation (0.22 kg). This broadening of the phenotypic range, particularly at G1, expands the available phenotypic diversity for this economically important trait. Such induced variability is crucial for selecting diverse fruit sizes in breeding programs (Saibari et al. 2023; Bharath et al. 2024).

Table 1. Descriptive statistics of CMA gamma-irradiated melon population

T	FW (kg)				FT (cm)				BR (brix)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
G0	0.28	1.51	0.95	0.23	1.40	4.00	2.87	0.55	5.00	12.00	8.56	1.59
G1	0.38	1.56	0.87	0.27	1.80	4.00	2.79	0.48	5.00	14.00	9.75	2.12
G2	0.40	1.57	0.87	0.25	2.00	4.00	2.86	0.50	5.00	13.00	8.58	1.74
G3	0.51	1.83	1.03	0.28	1.90	3.80	2.70	0.41	5.00	11.00	7.18	1.76
G4	0.36	0.95	0.62	0.14	1.50	3.00	2.23	0.34	4.00	10.00	7.81	1.52
G5	0.51	1.53	0.90	0.21	2.30	4.20	3.18	0.45	7.00	13.50	10.75	1.32

Note: T: Treatment, FW: Fruit Weight, FT: Flesh Thickness, BR: Sugar content, Min: Minimum value, Max: Maximum value, SD: Standard deviation, G0: 0 Gy, G1: 25 Gy, G2: 50 Gy, G3: 75 Gy, G4: 100 Gy, G5: 150 Gy

Table 2. Descriptive statistics of DKN gamma-irradiated melon population

T	FW (kg)				FT (cm)				BR (brix)			
	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD
G0	0.57	1.50	1.06	0.24	2.20	4.00	3.30	0.38	4.00	10.00	6.75	1.65
G1	0.28	1.92	0.95	0.36	1.70	4.00	2.93	0.42	4.00	11.00	8.38	1.60
G2	0.57	1.93	1.10	0.32	2.00	4.30	2.98	0.53	5.00	11.00	7.59	1.55
G3	0.35	1.73	1.08	0.28	1.70	4.00	2.91	0.43	5.00	14.00	9.48	2.10
G4	0.27	1.32	0.77	0.22	1.40	3.30	2.56	0.35	4.00	15.00	8.65	1.95
G5	0.44	2.07	1.15	0.33	1.70	4.00	3.03	0.46	6.00	15.00	9.57	1.76

Note: T: Treatment, FW: Fruit Weight, FT: Flesh Thickness, BR: Sugar content, Min: Minimum value, Max: Maximum value, SD: Standard Deviation, G0: 0 Gy, G1: 25 Gy, G2: 50 Gy, G3: 75 Gy, G4: 100 Gy, G5: 150 Gy

Flesh thickness in the DKN population also exhibited a notable shift. The mean FT generally showed a tendency to decrease across irradiated populations compared to the G0 control (3.30 cm), with G4 (100 Gy) showing the most pronounced reduction to 2.56 cm. However, the highest standard deviation was observed at G2 (0.53 cm), suggesting the broadest variability in flesh thickness at this dose, despite not yielding the highest mean. The G0 control showed the highest mean FT (3.30 cm), with G5 showing an intermediate mean (3.03 cm) but with a substantial range. This enhanced variability within the population provides breeders with a broader pool from which to select individuals with potentially improved textural properties or other desirable fruit structural characteristics (Priyanka et al. 2021; Saadati et al. 2022).

Sugar content, a critical determinant of consumer acceptance, showed a largely positive response in the DKN population. Compared to the G0 control (6.75°Brix), most irradiated groups demonstrated an increase in mean sugar content, with G5 (150 Gy) showing the highest mean (9.57°Brix) and G3 (75 Gy) also high (9.48°Brix). The largest range for sugar content (11.00°Brix, from 4.00 to 15.00°Brix) was observed in both G4 (100 Gy) and G5 (150 Gy), representing the broadest spectrum of sweetness induced. The highest standard deviation was recorded at G3 (2.10°Brix), indicating considerable variability, while G0 had the lowest SD (1.65°Brix). This expanded variability is fundamental for genetic enhancement efforts, offering a richer genetic pool for breeders to select and develop melon varieties with superior sweetness characteristics (Goldenberg et al. 2014; Lamo et al. 2017).

The collective analysis of the descriptive statistics for all traits in the CMA and DKN M_2 generation unequivocally supports the notion that gamma irradiation is an effective tool for generating phenotypic variability in quantitative traits. The observed shifts in mean values, coupled with the consistent increase in standard deviations and expanded phenotypic ranges, underscore the potency of this method in creating a diverse genetic landscape. Specifically, in the CMA population, G3 (75 Gy) effectively increased fruit weight, while G5 (150 Gy) significantly enhanced both flesh thickness and sugar content. For the DKN population, G5 (150 Gy) effectively increased fruit weight and sugar content; however, no gamma irradiation treatment improved mean flesh thickness. This enriched variability is fundamental for facilitating the identification and selection of promising putative mutants, ultimately accelerating crop improvement by providing breeders with novel genetic resources for developing enhanced melon varieties with improved agronomic performance and quality attributes (Rad 2022).

Breeding for multiple desirable traits in *C. melo* often encounters significant challenges due to inherent biological trade-offs. A common example is the persistent negative correlation observed between fruit weight and soluble solids content in melon (Ercan et al. 2024). This implies that selecting for increased fruit size can lead to a dilution of sugars, impacting overall fruit quality and consumer acceptance. For instance, our data showed that in the CMA

population, G3 (75 Gy) increased fruit weight but reduced sugar content. Similarly, in the DKN population, G4 reduced fruit weight while elevating sugar content. Although some studies suggest a weak genetic correlation between total soluble solids variation and melon fruit size, indicating potential for breeding for sweetness independently (Tzuri et al. 2025), the observed phenotypic trade-offs necessitate a balanced approach. Therefore, effective subsequent generation selection strategies will prioritize individuals that exhibit an optimal balance of these interconnected traits, aligning with robust breeding principles for complex quantitative traits.

Figure 1 visually elucidates the intricate patterns of variability in fruit weight for both CMA (A) and DKN (B) melon populations following gamma irradiation, offering a detailed perspective on the induced phenotypic diversity. For the CMA melon population (Figure 1.A), the non-irradiated control (0 Gy) exhibits a relatively concentrated distribution of fruit weights, characterized by a narrower violin shape and a more compact interquartile range. Median FW increased from 0.95 at 0 Gy to 1.04 at 75 Gy, with the widest interquartile range at 50 Gy (0.35). It tends to broaden and display wider interquartile ranges, indicating an expansion of the overall phenotypic variation in the trait (Nobre et al. 2019). This visual broadening and increased spread suggest that gamma irradiation may induce a wider spectrum of genetic changes affecting fruit size, leading to a greater diversity in the resulting phenotypes in melon. The presence of outliers, represented by individual points beyond the whiskers, further highlights the generation of novel individuals traits significantly deviating from the central tendency of their respective populations, suggesting potential mutations influencing targeted traits (Nobre et al. 2019). Notably, G3 also produced a favorable outlier with a fruit weight of 1.83 kg.

In the DKN melon population (Figure 1.B), similar trends of increased variability are observed, yet with distinct response profiles to the various gamma doses. Median FW decreased from 1.06 at 0 Gy to 0.74 at 100 Gy, with the wider interquartile range at 150 Gy (0.49). With increasing irradiation, the distributions often become wider and less symmetrical, particularly noticeable at some higher doses, as indicated by the expanded violin shapes and altered median positions within the boxes. This broadening of the violin plots and the boxes signifies an enhanced phenotypic heterogeneity within the M_2 generation, providing a richer genetic pool for selection in muskmelon (Priyanka et al. 2021). G5 yielded an exceptionally desirable outlier with a fruit weight of 2.07 kg, indicating its potential for producing large-fruited varieties. The greater dispersion of data points, indicating the generation of both smaller and larger fruits compared to the control, expands the scope for breeders to select specific fruit sizes adapted to different market preferences or agronomic requirements. This reinforces the role of mutagenesis in creating novel variation, as induced mutations can contribute to fruit improvement without compromising the requirements of the consumers (Lamo et al. 2017).

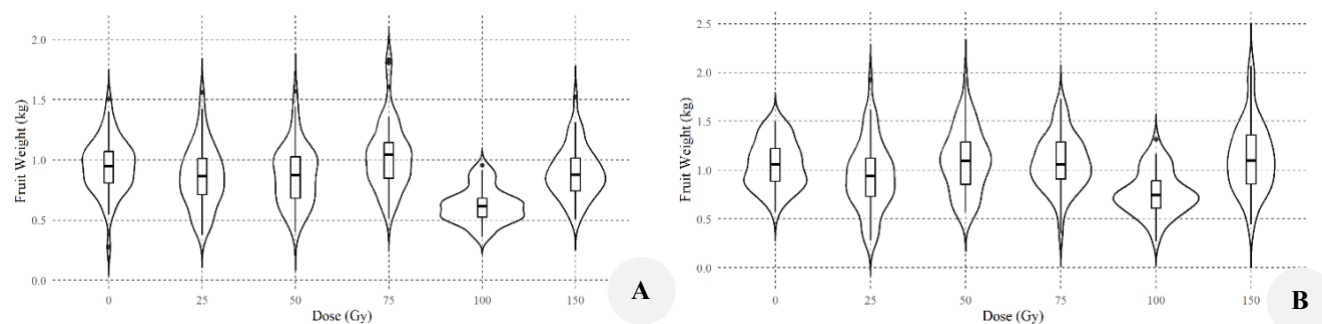


Figure 1. Boxplot and Violin plot analysis showing: A. Variability in fruit weight of CMA gamma-induced melon populations, B. Variability in fruit weight of DKN gamma-induced melon populations

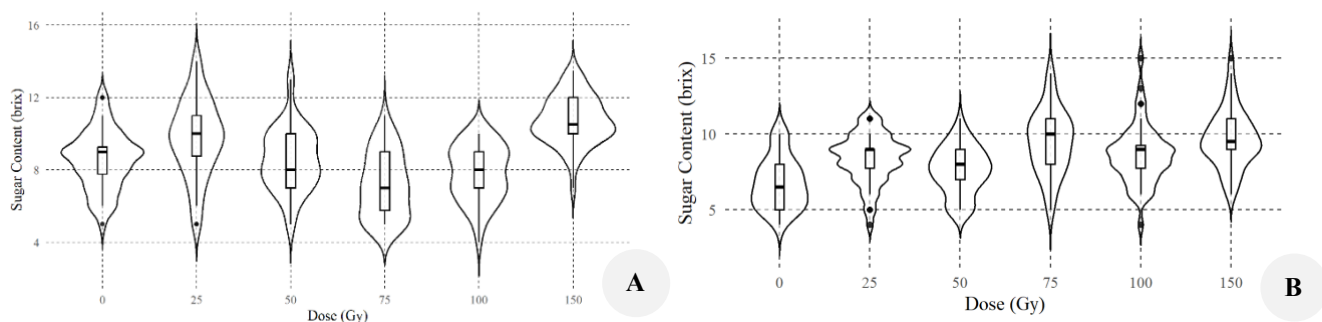


Figure 2. Boxplot and Violin plot analysis showing: A. Variability in sugar content of CMA gamma-induced melon populations, B. Variability in sugar content of DKN gamma-induced melon populations

Figure 2 visually represents the variability in sugar content (measured as °Brix) within the CMA (A) and DKN (B) melon populations following different doses of gamma irradiation. These boxplot and violin plot analyses offer a detailed perspective on how mutagenic treatment influences both the central tendency and the distribution spread of sugar accumulation. Sugar content is a critical quality trait influencing consumer acceptance (Argyris et al. 2017; Tzuri et al. 2025). Notably, the expansion of variability observed at higher irradiation doses highlights the potential of gamma-induced mutations to create a broader spectrum of sugar content, which could be pivotal for breeding programs aiming to enhance sweetness levels in melons. This variability not only provides more options for selecting desirable fruit characteristics but also increases the likelihood of discovering novel phenotypes with exceptional sweetness. Such phenotypic variability can lead to the development of melon varieties that meet the evolving demands of consumers, particularly those seeking fruit with specific taste profiles.

For the CMA melon population, as depicted in Figure 2.A, the non-irradiated control (0 Gy) establishes a benchmark with a particular median sugar content and a characteristic distribution range. As the gamma irradiation dose increases, the visual representation of the sugar content distributions tends to show discernible changes. For instance, the boxes and violin shapes for certain irradiated groups often appear more elongated or shifted, indicating an expansion in the interquartile range and a broader overall phenotypic spread

of sugar content compared to the control. Median sugar content increased from 9.00 at 0 Gy to 10.50 at 150 Gy, with an interquartile range of 1.50 at 0 Gy to 3.25 at 75 Gy. This heightened heterogeneity suggests that gamma irradiation effectively induces a wider array of genetic changes impacting sugar metabolism and accumulation pathways (Hayashi and Kawashima 1982; Eliwa and Ahmed 2025). This diversity is invaluable for targeted selection in breeding programs aimed at enhanced sweetness, allowing for the identification of individuals with exceptionally high sugar levels (Kawaguchi et al. 2021; Kantoğlu et al. 2023).

The DKN melon population (Figure 2.B) showed a distinct response to gamma irradiation regarding sugar content. Median sugar content increased from 6.50 at 0 Gy to 10.00 at 75 Gy. Consistent with findings, some specific gamma doses appear to promote an upward shift in the median values, indicating an overall positive influence on average sugar accumulation within the population (Sau et al. 2018). The widening of violin plots and box ranges across treatments indicates a significant increase in phenotypic variability within the M_2 generation (Kantoğlu et al. 2023). Both G4 (100 Gy) and G5 (150 Gy) exhibited the largest ranges for sugar content, reaching up to 15.00°Brix. These high values denote promising individuals valuable for targeted selection in breeding programs aimed at enhanced sweetness. Induced variability is fundamental for genetic enhancement, offering a richer genetic pool for breeders to develop superior sweetness characteristics (Lamo et al. 2017; Soneji and Nageswara-Rao 2018).

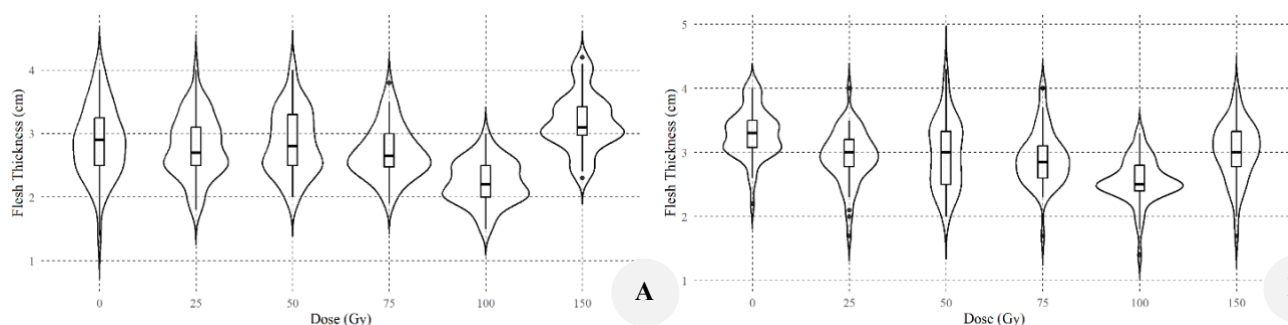


Figure 3. Boxplot and Violin plot analysis showing: A. Variability in flesh thickness of CMA gamma-induced melon populations, B. Variability in flesh thickness of DKN gamma-induced melon populations

Table 3. Comparison of median on the Mann-Whitney U test

T	CMA			DKN		
	FW	FT	BR	FW	FT	BR
G1	0.87 ^{ns}	2.70 ^{ns}	10.00*	0.94 ^{ns}	3.00*	9.00*
G2	0.87 ^{ns}	2.80 ^{ns}	8.00 ^{ns}	1.10 ^{ns}	3.00*	8.00 ^{ns}
G3	1.04 ^{ns}	2.65 ^{ns}	7.00*	1.06 ^{ns}	2.85*	10.00*
G4	0.62*	2.20*	8.00 ^{ns}	0.74*	2.50*	9.00*
G5	0.88 ^{ns}	3.10*	10.50*	1.13 ^{ns}	3.00*	9.50*
G0 (control)	0.95	2.90	9.00	1.06	3.30	6.50

Note: T: treatment, FW: fruit weight (kg), FT: flesh thickness (cm), BR: sugar content (brix), G0: 0 Gy, G1: 25 Gy, G2: 50 Gy, G3: 75 Gy, G4: 100 Gy, G5: 150 Gy, ns: P-value not significant based on the Mann-Whitney U test at the 5% significance level, *: P-value significant based on the Mann-Whitney U test after Bonferroni correction (P value<0.01)

Figure 3 visually presents the boxplot analyses of flesh thickness for both the CMA (A) and DKN (B) melon populations following different doses of gamma irradiation. In the CMA melon population (Figure 3.A), the boxplot for the non-irradiated control (0 Gy) establishes a reference for flesh thickness, showing its median and interquartile range. As the gamma irradiation dose increases, the boxplots reveal diverse responses. For instance, some doses might lead to a slight shift in the median, indicating a change in the average flesh thickness. Median flesh thickness increased from 2.90 at 0 Gy to 3.10 at 150 Gy, with an interquartile range of 0.43 at 0 Gy to 0.83 at 50 Gy. More importantly, alterations in the length of the boxes or the whiskers signify changes in the overall spread of the data. These results are consistent with other studies, which indicate an induced increase in phenotypic variability for flesh thickness within the population (Bammanakatti et al. 2023). However, G5 (150 Gy) produced the highest maximum flesh thickness (4.20 cm), representing a favorable outlier for increased flesh thickness, despite not having the broadest overall spread. It could be promising candidates for breeding programs aimed at specific fruit textural characteristics.

Similarly, the DKN melon population (Figure 3.B) exhibits its own unique patterns of flesh thickness variability under different gamma irradiation doses. Compared to the DKN control, certain irradiation treatments result in noticeable changes in the boxplot structures. For some doses, the median might shift downwards, suggesting a tendency towards thinner flesh, while the box itself could become longer, indicating a wider spread of flesh thickness values

among the central individuals. Median flesh thickness decreased from 3.30 at 0 Gy to 2.50 at 100 Gy, with the wider interquartile range at 50 Gy (0.80). Conversely, other doses might lead to a relatively stable median but with altered whisker lengths or a greater number of outliers. Gamma irradiation may demonstrate an expansion of the extreme range in the population (Saadati et al. 2022). These varied responses highlight the utility of mutagenesis in exploring the genetic potential for different flesh thickness profiles. Flesh thickness improvement is important to adapt melon varieties to different culinary uses or processing demands (Krurup et al. 2016; Pidigam et al. 2021).

Table 3 presents the results of the Mann-Whitney U test, offering a statistical evaluation of the differences in quantitative phenotypic performance between the gamma-irradiated melon populations and their respective non-irradiated controls for fruit weight, flesh thickness, and sugar. In the CMA melon population, the Mann-Whitney U test revealed variable responses to doses on quantitative traits. For fruit weight, significant differences from the control were observed at dose G4, indicating that gamma irradiation impacts fruit weight. Non-significant p-values for G1, G2, G3, and G5 suggest these doses did not significantly affect fruit size. Regarding flesh thickness, only doses G4 and G5 showed significant differences, indicating that higher doses had a greater impact. In contrast, G1, G2, and G3 had minimal or no detectable effect. Sugar content exhibited significant changes across most irradiated groups (G1, G3, G5). This high frequency of significant p-values is consistent with other studies that underscore the sensitivity of sugar accumulation pathways to gamma irradiation (Li et al. 2022; Saadati et al. 2022).

The DKN melon population exhibited more consistent and pronounced significant differences across all three quantitative traits, indicating a broader response to gamma irradiation than the CMA population. For fruit weight, only the G4 (100 Gy) dose showed a significant difference, suggesting that fruit weight in DKN is less affected by gamma irradiation compared to flesh thickness and sugar content. Flesh thickness showed significant differences across all doses (G1 to G5). This widespread significance indicates that gamma irradiation consistently induced statistically distinct changes in flesh thickness across a broad spectrum of radiation levels. Gamma irradiation may potent effect on this physical attribute (Bammanakatti et al. 2023) tent impact of gamma irradiation on sugar accumulation. This

makes the population a promising candidate for selecting putative mutants with improved sweetness profiles (Hooghvorst et al. 2020; Jariani et al. 2021).

Variability in qualitative phenotypic performance

The M₂ generation, following mutagenic treatment, is particularly significant as it allows for the segregation and visible expression of induced genetic changes, leading to observable phenotypic diversity (Amri-Tiliouine et al. 2018; Saibari et al. 2023). This includes not only subtle variations in measurable traits but also distinct alterations in qualitative characteristics, such as fruit rind color. The emergence of such novel traits underscores the capacity of induced mutagenesis to broaden the genetic base of a crop, which is essential for breeding efforts aimed at developing varieties with enhanced aesthetic appeal or unique market value (Lamo et al. 2017; Riviello-Flores et al. 2022).

Figure 4 clearly demonstrates the effect of gamma irradiation on a qualitative trait, namely the rind skin color of the CMA melon variety. The visual comparison highlights a notable difference between the non-irradiated control plant (0 Gy) and a melon treated with a 25 Gy dose of gamma radiation. The irradiated sample, observed in only 1 plant out of 48 plants exposed to the 25 Gy treatment, exhibits a markedly darker rind skin (Black Ink-19-0506 TPX) compared to the control. This contrast is visually striking and suggests a substantial alteration in the phenotypic expression of rind coloration. Although molecular or biochemical analyses were not conducted in this study, the emergence of this distinct phenotype strongly indicates the successful induction of a mutation affecting pigment-related pathways. Previous studies have reported that gamma irradiation can induce random genetic changes that influence qualitative traits, including fruit color, by modifying gene expression or function involved in pigmentation processes (Lamo et al. 2017).



Figure 4. Variability of rind skin color in CMA. A. 0 Gy (Control), and B. 25 Gy showing gamma-irradiated melon with dark skin

The pronounced alteration in rind skin color observed in the irradiated melon serves as strong evidence of a phenotypic mutation, strongly indicative of underlying genetic changes influencing pigment formation. This mutation likely affects the biosynthesis, accumulation, or regulation of key pigments such as carotenoids or anthocyanins within the fruit pericarp, which are known to play critical roles in determining melon rind coloration (Diao et al. 2023; Naeem et al. 2023). The appearance of the “dark skin” phenotype in the M₂ population underscores the effectiveness of gamma irradiation as a mutagenic technique for generating novel phenotypic variability beyond what is typically present in natural germplasm. Such induced variation is highly valuable for plant breeding programs, as unique rind colors can enhance fruit attractiveness, influence consumer perception, and increase market competitiveness. Moreover, gamma irradiation has been widely reported to induce cytological, biochemical, and physiological changes that contribute to morphological variation, including modifications in fruit color and appearance (Soneji and Nageswara-Rao 2018; Tarigan et al. 2023).

Figure 5 comprehensively presents a detailed visual assessment, meticulously showcasing the qualitative phenotypic variability specifically observed in the rind skin color within the DKN melon population. Panel A of this insightful figure clearly illustrates the characteristic and typical rind appearance of the non-irradiated DKN control group, which received a 0 Gy dose, thereby effectively establishing a fundamental baseline for all subsequent comparisons and analyses. In striking contrast, Panel B vividly and expansively displays the diverse and significantly broad range of rind skin colors that were observed among the DKN melons following their exposure to a substantially high dose of 150 Gy gamma radiation. This pronounced and unambiguous differentiation critically underscores the remarkable and inherent capacity of induced mutagenesis as a powerful tool to successfully generate a wide spectrum of distinct and novel color phenotypes, thus enriching the genetic diversity available for selection (Riviello-Flores et al. 2022; Tarigan et al. 2023).

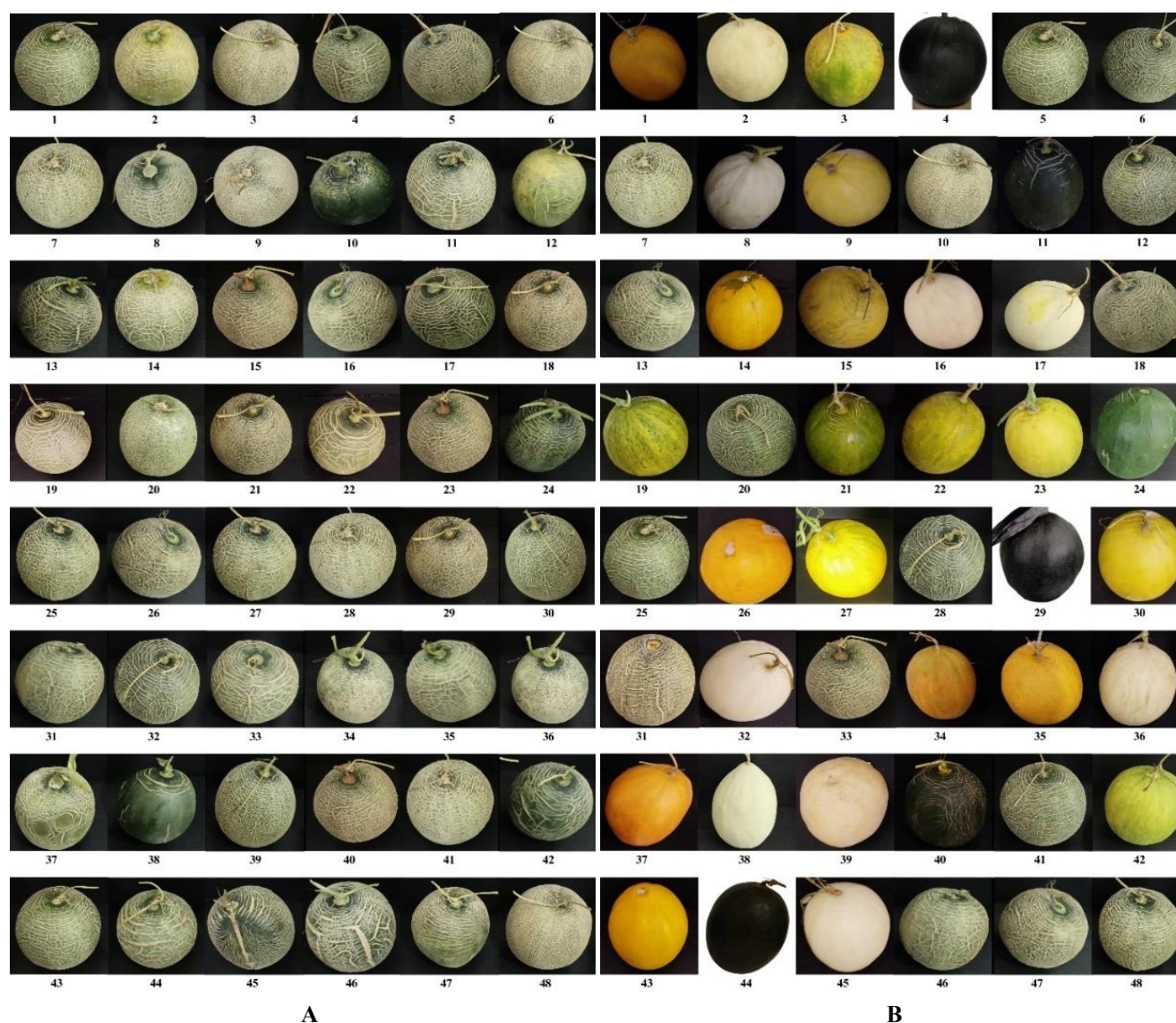


Figure 5. Variability of rind skin color in DKN. A. 0 Gy (Control), and B. 150 Gy showing gamma-irradiated melon with dark skin

The distinct shift towards a darker rind coloration observed in some DKN putative mutants implies that the 150 Gy gamma radiation treatment (3 out of 48 plants or 6.25% of the plants in the 150 Gy treatment) successfully induced phenotypic modifications. The 3 plants include number 4 (Black Ink-19-0506 TPX), 29 (Jet Set-19-5708 TPX), and 44 (Reven-19-0000 TPX). Although not tested here, previous studies suggest that mutation can affect the pathways responsible for pigment synthesis or accumulation in the melon rind (Li et al. 2025). Melon fruit color is determined by the composition and content of various pigments, including chlorophylls, carotenoids, anthocyanins, and flavonoids (Huang et al. 2021; Li et al. 2025). Gamma irradiation can cause cytological, biochemical, and physiological changes, which may alter gene regulation, protein synthesis, or enzyme activity involved in pigment biosynthesis, leading to observable changes in fruit color (Kumar et al. 2021; Raina and Khan 2023; Tarigan et al. 2023). For example, studies have shown that gamma irradiation can alter the levels of photosynthetic pigments like chlorophyll and carotenoids, and mutations can affect carotenoid-synthesizing genes, directly influencing fruit pigmentation (Arulbalachandran 2022; Raina and Khan 2023; Rodge et al. 2023).

Identification of promising gamma-induced melon putative mutants as genetic materials for breeding program

The identification of promising putative mutants from the M₂ generation represents a pivotal step in mutation breeding, offering novel genetic resources for future melon improvement programs. This meticulous selection process, focusing on the distinctive qualitative trait of dark rind skin alongside quantitative performance, underscores a strategic approach to enhancing crop attributes. Putative mutants were chosen based on their dark rind skin, a phenotype absent in the control group, and demonstrating non-inferiority compared to the non-irradiated control group for all three quantitative traits. This means a putative mutant's mean for a trait was considered non-inferior if it was not more than 5% lower than the control mean. This systematic identification of desirable phenotypes in mutagenized populations is crucial for accelerating crop improvement, providing breeders with a diverse pool of genetic resources (Lamo et al. 2017; Riviello-Flores et al. 2022; Penna and Jain 2023).

The presence of a novel and visually striking trait like dark rind skin is a crucial asset for melon breeding programs, profoundly impacting market dynamics and consumer preferences. Visual evidence, as demonstrated in Figures 4 and 5, clearly illustrates the significant shift in rind color induced by gamma irradiation in both CMA and DKN melons. This pronounced alteration suggests successful mutations affecting pigmentation pathways responsible for rind coloration. For example, similar studies indicate that such shifts imply changes in pigment synthesis or accumulation (Diao et al. 2023). Furthermore, gamma rays can induce genetic alterations affecting the "cumulative mass of pigments" and "chromatic properties" in plants, generally reinforcing the principle that irradiation can influence pigment-related characteristics (Rodge et al. 2023).

Further analysis of the quantitative traits associated with these dark-skinned putative mutants reveals their varying performance, which contributes to their value as genetic materials (Table 4). For fruit weight, putative mutants B-143-29 and B-143-44 from the DKN population demonstrated the most significant relative increases, both achieving 141.5% of their DKN control's fruit weight. Similarly, for sugar content (°Brix), putative mutant B-143-29 showed an exceptional improvement, reaching 222.2% of its DKN control's °Brix, highlighting strong potential for enhancing sweetness. These specific improvements illustrate the capacity of gamma irradiation to induce beneficial genetic diversity, consistent with observations in other fruit crops where radiation impacts fruit size and sweetness (Sau et al. 2018; Saadati et al. 2022; Kantoğlu et al. 2023).

Regarding flesh thickness, putative mutant B-143-44 also stood out by exhibiting 103.0% of its DKN control's flesh thickness, making it the only selected putative mutant to surpass its control for this specific trait. While some putative mutants showed a reduction in FT, the overall induced variability across all quantitative traits in both CMA and DKN populations (as detailed in Tables 1 and 2) signifies a broadening of the phenotypic base. This expansion of phenotypic ranges and consistent increase in standard deviations underscore the potency of gamma irradiation in creating a diverse phenotypic landscape. This enriched variability is fundamental for facilitating the identification and selection of promising putative mutants, ultimately accelerating crop improvement by providing breeders with novel genetic resources (Arulbalachandran 2022; Saibari et al. 2023).

While the unique dark rind skin trait offers significant market appeal, it is important to acknowledge the inherent trade-offs often encountered in plant breeding when simultaneously selecting for qualitative and quantitative characteristics. Improving one trait can sometimes adversely affect another, making it challenging to achieve optimal solutions across all desired attributes (Dwivedi et al. 2021). Therefore, these dark-skinned putative melon mutants are not immediately envisioned as direct commercial cultivars. Instead, their primary value lies in serving as promising candidates or putative mutants for introgression breeding programs. This strategy involves meticulously crossing these putative mutants with elite melon lines that already possess superior quantitative traits, such as high yield, optimal fruit weight, and excellent sweetness. Subsequent cycles of selection and backcrossing can then be employed to effectively transfer the gene(s) responsible for the desirable dark rind skin into these high-performing genetic backgrounds.

Table 4. Identified promising gamma-induced melon putative mutants

Pedigree	Code	Dose (Gy)	Uniqueness	FW	FT	BR
B-105-22	CMA	25	Darker rind skin	1.29	2.50	12.00
B-143-04	DKN	150	Darker rind skin	1.26	3.00	10.00
B-143-29	DKN	150	Darker rind skin	1.50	2.00	15.00
B-143-44	DKN	150	Darker rind skin	1.50	3.40	10.00

Note: FW: Fruit Weight (kg), FT: Flesh Thickness (cm), BR: Sugar content (brix)

Therefore, these dark-skinned putative melon mutants are invaluable as genetic resources for future breeding efforts, even if their current quantitative traits are not fully optimized for direct commercial application. They can be strategically incorporated into cross-breeding programs by using them as parents to transfer the dark rind skin gene(s) into elite lines that already possess superior quantitative traits, such as high yield or excellent sweetness. Subsequent selection and backcrossing can then combine the desirable dark rind skin with improved quantitative characteristics, leading to new cultivars that offer both unique aesthetics and robust agronomic performance. Strategies for combining genetic resources and elite lines in breeding programs to achieve desired traits can be used, which directly supports the proposed approach of using these putative mutants as parents for cross-breeding (Zhang et al. 2021; Sanchez et al. 2024). This approach leverages induced phenotypic variability to accelerate the creation of novel and commercially competitive melon varieties, ensuring the unique qualitative benefits are realized in future, high-performing melon cultivars.

Despite promising outcomes, this study is subject to several limitations. The current study involved extensive statistical analysis with multiple comparisons. This approach, even with Bonferroni correction, inherently risks Type I errors, potentially yielding spurious significant findings (García-Pérez 2023; Guillermou et al. 2023). The experiment was conducted in a greenhouse to ensure consistent growing conditions. However, evaluation solely under such controlled conditions may not fully reflect field performance. This challenge is attributed to significant genotype-environment interactions (Shimono et al. 2023; Barreto et al. 2024). Furthermore, phenotypic evaluation focused on the M₂ generation. Phenotypes assessed in this generation may still be segregating, thus requiring further generational advancement for stable trait confirmation and homozygosity. The M₂ generation represents the segregating generation where homozygous recessive mutations become visible (Dhasarathan et al. 2021; Pramanik et al. 2023). Finally, molecular or biochemical analyses were not conducted in this study. This absence impedes the full elucidation of underlying genetic mechanisms. Such mechanisms are crucial for precise breeding strategies (Acién et al. 2023; Singh et al. 2023). Future research will address these gaps through multi-environment trials, comprehensive segregation analysis in subsequent generations, and advanced molecular techniques to identify causal mutations and their biochemical effects.

In conclusion, this study indicates that gamma irradiation can be an effective method for inducing phenotypic variability in the two commercial melon varieties investigated under our single greenhouse conditions. Specifically, the 150 Gy dose proved most promising within the context of this study, yielding the highest mean sugar content and flesh thickness in CMA, and notable fruit weight and sugar content variation in DKN. This research successfully identified four novel dark-rind putative melon mutants: one from CMA at 25 Gy and three from DKN at 150 Gy, a phenotype entirely absent in the control groups. These specific putative mutants will be advanced for

further selection and rigorous evaluation of their inheritance patterns and agronomic stability in subsequent generations. Nevertheless, these initial findings suggest the potential for these dark-skinned lines to be utilized as donor parents in cross-breeding and backcrossing initiatives, potentially contributing appealing aesthetic novelty and improved agronomic performance, thereby aiding in accelerating the development of new and improved melon varieties within relevant breeding programs.

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