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Gamma-ray irradiation alters the morphology, anatomy and agronomic characters of the groundnut (*Arachis hypogaea*) bison cultivar in M1 generation

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Abstract. *Nilahayati, Handayani RS, Nazimah, Neni, Saputra D. 2024. Gamma-ray irradiation alters the morphology, anatomy and agronomic characters of the groundnut (*Arachis hypogaea *L.) bison cultivar in M1 generation. Biodiversitas 25: 4179-4189.* Groundnut is the world's most important agricultural commodity, serving as a major source of protein and vegetable oil. Enhancing the genetic diversity of groundnuts is essential for improving yield, early maturity and environmental resistance such as drought tolerance. This study aims to determine the effect of gamma irradiation on the morphological and agronomic diversity of bison groundnut cultivar in the M1 generation. Gamma irradiation was carried out at the Radiation Process Technology Research Center, National Research and Innovation Agency of Indonesia, Jakarta. This research was designed using a single-factor Randomized Complete Block Design. The dose of gamma irradiation was 0 Gray (Gy), 100 Gy, 200 Gy, and 300 Gy, with three replications. Consequently, there were a total of 12 experimental units. We generated 600 groundnut plants in total. The observed variables included morphological diversity such as variation in leaf, stem, seed color and seed shape. The agronomic variability was recorded to the seed growth percentage, plant height, number of branches, flowering age, harvesting age, number of pods, number of empty pods, dry pod weight per plant, dry seed weight per plant, 100 seed weight per plot, dry seed weight per plot, length, width, and number of leaf stomata. The results showed that gamma irradiation treatment induced morphological and agronomic changes in the bison groundnut cultivar. Morphological changes included alterations in leaf color, pod shape, and seed coat color. Higher doses of gamma irradiation can negatively impact certain agronomic traits such as plant height and yield, beginning at 200 Gy, but at 100 Gy all parameters slightly increased. Higher dose of gamma irradiation also delayed the flowering and harvesting time. Further studies are also needed to evaluate the heritability of these traits in the M2 generation to ensure the stability of desirable traits.

Keywords: M1 generation, mutant, mutation breeding, seed-coat, variation

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important food crop that serves as a significant source of protein and vegetable oil globally. According to Balasubramanian et al. (2023), the crop is rich in essential nutrients, such as vitamins (namely thiamine, riboflavin, niacin, pantothenic acid, pyridoxine and folic acid), minerals, antioxidants, which play a crucial role in promoting longevity, reducing chronic diseases, and strengthening the immune system. This shows the importance of groundnuts in addressing hunger in underdeveloped nations and ensuring food security. In addition, Mishra and Rasmi (2022) showed that peanuts made from groundnut contained 50% monounsaturated fatty acids (MUFAs), 33% polyunsaturated fatty acids (PFAs), and 14% saturated fatty acids, along with protein, fiber, and various vitamins. For example, 100 g of peanuts can provide 75% of the daily value for niacin, 60% folate, 53% thiamin, 10% riboflavin, 35% pantothenic acid, 27% pyridoxine, and 55.5% vitamin E.

The efforts to enhance groundnut production can be approached through a range of strategies, such as using superior varieties. Yadav et al. (2021) stated that genetic variability is essential to achieving the new features in the variety development program. Successful results will surely result from using different breeding strategies like hybridization, recombination, and mutation breeding, which will produce notable changes in plants. According to Akhtar et al. (2015) and Mamo et al. (2023), hybridization is a common conventional method for genetic variability, but it requires considerable effort. Emasculation and pollination are challenging and time-consuming, and the small number of progenies created by artificial crosses in groundnut makes this approach less feasible than other technologies. Thus, alternative method such as mutation breeding is often more effective for inducing genetic diversity for crops like groundnuts.

Mutation breeding techniques employ mutagens to induce genetic variation. These mutagens facilitate genetic changes in organisms, disrupt linkages, and lead to the emergence of beneficial traits for the enhancement of crop plants. Among the most widely utilized mutagens are chemical agents, such as ethyl methane sulfonate (EMS), and physical agents, such as gamma-ray irradiation. Celik and Atak (2017) stated that gamma irradiation is a highly effective physical mutagen often used in cultivated plants, which applies nuclear technology. Previous studies have successfully used gamma irradiation to enhance genetic diversity of various cultivated plants, such as *Celosia cristata* L. (Muhallilin et al. 2019), *Echinacea purpurea* (L.) Moench (Cahyaningsih et al. 2022), sunflower (Habib et al. 2022), cowpea (Vanmathi et al. 2021; Azzam et al. 2023), rice (Tiwari et al. 2018), wheat (Dwinanda et al. 2020) and soybean (Nilahayati et al. 2016; Nilahayati et al. 2019; Nilahayati et al. 2022a; Nilahayati et al. 2022b). These studies showed that nuclear technology used in plant breeding can lead to the development of high-yielding varieties with various benefits. The benefits include improved nutritional content, enhanced resistance to pests and diseases, shorter growth cycles, and better adaptation to climate change, such as drought tolerance.

Several studies have also investigated the effect of gamma irradiation on different genotypes of groundnut plants. Saibari et al. (2023) found that the treatment at a dose of 100 Gy increased flowering age and height in groundnut varieties Kp 29 and Fleur 11 in the M1 generation. Thenuja et al. (2024) showed that it influenced the growth and yield of indi cultivar groundnut, with the most diversity induced at a dose of 200 Gy. Spencer et al. (2018) stated that mutagen treatment frequently causes physiological abnormalities in the first generation of a mutated population (M1). As a result, phenotypic selection for mutations cannot be carried out in the M1 generation. It is noted that most induced mutations are recessive, meaning that the mutant phenotype remains hidden until the mutation becomes homozygous. Induced mutations are initially a one-cell event and are not present in every cell of the plant, leading to M1 plants being considered as chimeric plants. Furthermore, the most common effects observed include growth retardation, sterility, and plant death in the M1 generation.

The bison groundnut cultivar exhibited partial resistance to rust, leaf spot, and *Aspergillus flatus*, as well as showing tolerance to 25% shade intensity, yielding 3.6 tons/ha. However, it was characterized by a prolonged harvest age (90-95 days), low protein content, and susceptibility to drought. Therefore, strategies to enhance genetic diversity within this cultivar are necessary to unlock superior traits such as shorter maturity periods, improved drought tolerance, and higher yields. The genetic improvement of bison cultivar using gamma irradiation has not been previously explored by previous researchers. Therefore, this study aims to determine the effect of gamma irradiation on the morphological and agronomic diversity of bison groundnut cultivar in the M1 generation. The changes observed in these traits are expected to serve as a source of new genetic diversity for future generations. Following the selection of subsequent generations (M2-M5), we anticipate obtaining putative mutant lines with improved traits compared to their parent.

MATERIALS AND METHODS

Plant material

The groundnut bison variety seed was collected for this work sourced from The Indonesian Legume and Tuber Crops Research Institute, Malang, Indonesia. The seed samples with a moisture content of 12% were carefully packaged in envelopes and coated with clear plastic standing pouches to ensure their safety during delivery. Each envelope contained 150 groundnut seeds for each gamma irradiation dose treatment.

Gamma-rays irradiation treatment

Bison groundnut seeds were irradiated with gamma-ray nuclear technology at the Radiation Process Technology Research Center, a sub-division of the Organization for Nuclear Power Research, National Research and Innovation Agency of Indonesia (BRIN), Jakarta. The irradiation process was carried out using a ^{60}Co gamma irradiator Gamma Cell 220 with a 2.9 kGy/hour dose rate. The seed of bison groundnut was subjected to three doses of gamma-rays viz 100 Gy, 200 Gy, and 300 Gy (Tshilenge-Lukanda et al. 2012; Thenuja et al. 2024)

Experimental design and field research

The research was carried out from May to August 2024. The field experiment was conducted at Paloh Lada Village, Dewantara District, North Aceh Regency, Indonesia. A single-factor Randomized Completely Block Design (RCBD) method was used to investigate the effect of gamma irradiation on bison cultivar groundnut seeds, which were subjected to 4 different doses, namely 0 Gy (control), 100 Gy, 200 Gy, and 300 Gy. Each treatment was repeated in three replications. To raise M1 generation, a total of three gamma-ray irradiation dose treatments along with the control were sown in the field at the rate of 150 seeds for each treatment. Consequently, there was a total of 600 plants in the M1 generation.

The land was cleared of weeds manually. Twelve plots, each measuring 2×1.4 m, were made for planting. Seeds were planted with a spacing of 30×20 cm. Fertilization included Urea (50 kg/ha), SP-36 (100 kg/ha), KCl (100 kg/ha), and manure (10 tons/ha). Plant maintenance involves watering, weeding, and fertilizing. During harvesting, the plants were pulled out one by one, and the signs of harvesting in groundnut plants were observed.

Data collection

In the study, we carefully observed and documented the changes in the morphology characteristic of bison groundnuts, including variations in leaves, flowers, stems, pods, and seeds. Morphological changes were observed visually. Observations were made by counting the number of plants that experienced morphological changes, and each morphological variation was photographed.

Observations of the length, width, and number of leaf stomata were made on leaf samples of each dose of gamma irradiation with the stomatal printing methods. Observations of the length, width, and number of leaf stomata were made at the age of 35 days after planting. Stomatal samples were taken from the surface of the upper and lower leaves. The leaves were cleaned with tissue then given clear cuttings and left for 5-10 minutes. The latex that has dried is then given masking tape and then pulled, the leaves that have been pulled with tape are then placed into an object glass and labeled with an identity label. The preparations were observed using a binocular Olympus microscope and optic lab software with 40x10 magnification. The number of leaf stomata was counted in its intact or unbroken form. We closely examine the agronomic traits of the M1 generation such as seed germination (%), plant height (cm), number of branches, days of flowering (DAP), days of harvesting (DAP), number of pods (pods), dry seed weight per plant (g), 100 seed weight per plant (g) and seed weight per plot (g).

Statistical data analysis

One-way analysis of variance (ANOVA) for stomatal anatomy and the agronomic characters studied in the experiments was carried out at a 5% significance level to test whether the observed averages of the treatment levels were significantly different. We further conducted the test using the Duncan Multiple Range Test (DMRT). All statistical analyses were performed using SAS V.9.1

RESULTS AND DISCUSSION

Variation of leaf color

In this study, there was morphological diversity, namely changes in the color of the leaves of bison groundnut cultivar due to gamma irradiation treatment. Changes in leaf color in plant populations due to the treatment of a mutagen were often called chlorophyll mutants. The type and number of leaf color mutants due to gamma irradiation dose treatment on M1 generation bison groundnut cultivar can be seen in Table 1.

Table 1 shows that in this study, there were 3 different types of chlorophyll mutants in the M1 generation. Among these doses, the highest number of chlorophyll mutants was found in the 300 Gy gamma irradiation dose with a total of 147 plants. The most common type of chlorophyll mutant was striata with 141 plants (0.24%), albina with 5 plants (0.002%), and chlorine with 1 plant (0.001%). At a dose of 200 Gy, the most types of chlorophyll mutants were striata 138 plants (0.24%) and albina 1 plant (0.001%), while at a dose of 100 Gy, there were only 36 plants (0.06%) of striata type chlorophyll mutants.

In this M1 generation population, distinct chlorophyll mutants were observed, including the striata type, characterized by green leaves with longitudinal stripes that emerged 2-3 weeks after planting. The albina type showed green leaves with white spots, appearing at 2 weeks and persisting until harvest. Lastly, the chlorine type presented light yellowish-green leaves and emerged when the plants were 10 weeks after planting, lasting until harvest.

Chlorophyll mutants were categorized based on Gustafsson's classification and characteristics (Gustafsson 1940; Spencer et al. 2018). According to Pramanik et al. (2023), chlorophyll mutations were commonly used as reliable markers to assess the effectiveness of different mutagens in promoting genetic diversity to enhance crops. Chlorophyll mutations occurred in various crops after exposure to physical mutagens. Nilahayati et al. (2016) saw striata, albina dan clorina chlorophyll mutants in soybeans and Sasipriya et al. (2023) observed them in *Abelmoschus esculentus*. Similar findings were reported by Nura et al. (2021), who examined the frequency and spectrum of chlorophyll-deficient mutants in *Digitaria exilis* that were generated by varying dosages of gamma irradiation. The findings demonstrated that varying dosages of gamma radiation caused seven distinct chlorophyll viz. albina, chlorina, lustescent, striata, viridis, viriscent, and xantha. The most common chimeras are xantha, chlorina, and albina, and their incidence rises with an increase in irradiation doses. Figure 1 illustrates the emergence of different types of leaf discoloration (chlorophyll mutants) due to gamma irradiation doses in bison groundnut cultivar in the M1 generation.

Figure 1 shows that different gamma irradiation treatments led to mutant leaf colors, with the specific outcome depending on the dose. A total of types of chlorophyll mutants were observed in the field, striata, albina, and chlorine. This observation aligned with the results of Bara et al. (2017), who also discovered various chlorophyll changes in chickpea plants following treatment with different gamma irradiation doses. The spectrum of chlorophyll mutations included xantha, chlorina, albina, and viridis, which were observed when the plants were 8-20 hours post-treatment. Du et al. (2022) stated that due to the genetic chimera of M1 plants, very few investigations have been conducted with this generation. Individual embryonic cells were impacted by mutagens independently, dividing to create cell populations with distinct DNA damage or mutations. Additionally, M1 leaves were one of the best examples of a genetic chimera caused by gamma irradiation because of their white or light green color.

Pod-shape variation

In this study, we discovered a remarkable amount of morphological diversity, particularly in the form of changes in pod shape. We definitively identified several types of pod-shaped mutants. Figure 2 unequivocally shows the shifts in pod shape mutants attributable to varying doses of gamma irradiation.

Table 1. Type and number of chlorophyll mutants of bison groundnut cultivar due to gamma irradiation doses in the M1 generation

Gamma-ray irradiation doses		Chlorophyll mutant			
		Normal	**Striata**	**Albina**	**Klorina**
0 Gy		145	0	0	0
100 Gy		107	36	0	0
200 Gy		1	138	1	0
300 Gy		0	141	5	1
Total	575 plant	253	315	6	1

Figure 2 shows that the different doses of gamma irradiation had led to changes in the shape of pods in bison peanut varieties in the M1 generation. The pods exhibited morphological diversity in terms of shape, specifically deep or very deep. A deep-type mutant characterized by a deep-waisted pod shape was identified during the observation of pod-shape mutants. Additionally, a very-deep type mutant was characterized by a pod with a very deep waist, a feature that was present across all gamma irradiation dose treatments. Saibari et al. (2023) also reported the presence of pod shape mutants, such as the slightly-lipped, very deep-lipped, and deep-lipped, at gamma irradiation doses of 100 Gy, 150 Gy, and 200 Gy.

Seed-coat color variation

In this study, there was morphological diversity, namely changes in seed-coat color. The type and number of seed coat colors of bison peanut varieties due to gamma irradiation dose treatment can be seen in Table 2.

Table 2 shows that 3 different types of seed-coat color mutants were observed in the M1 generation. The highest number of seed coat color mutants was found at an irradiation dose of 300 Gy. At this dose, 2 plants showed the purple seed-coat color mutation, characterized by purple-colored seeds. Additionally, 10 plants exhibited the dark red mutation, which was characterized by dark red seeds. Only 1 plant showed the white stripe mutation, characterized by red seeds with white stripes. At doses of 200 Gy and 100 Gy, 2 and 10 plants respectively showed the dark red mutation. The type of seed coat color due to gamma irradiation dose of groundnut cultivar bison in the M1 generation could be seen in Figure 3.

Figure 3 shows that there were color mutants of groundnut seed coats resulting from gamma irradiation in the M1 generation, depending on the given dose. The observation showed 3 types of seed coat color mutants namely, purple, dark red, and white strips. Gaafar et al. (2016) defined that gamma irradiation (50 Gy) caused various morphological changes in cowpeas, including mutations in seed-coat color. Additionally, it was observed that the seed color of some M1 plants (M2 seeds) was changed from a normal white color to a dark black color. Gnanamurthy and Dhanavel (2014), who studied cowpeas planted with brown and white seed coat colors, were treated with EMS mutagen and found that this caused mutations in genes, leading to changes from dominant pigmentation factors to recessive forms.

Figure 2. Pod-shape variation. A. Normal; B. Deep; C. Very deep

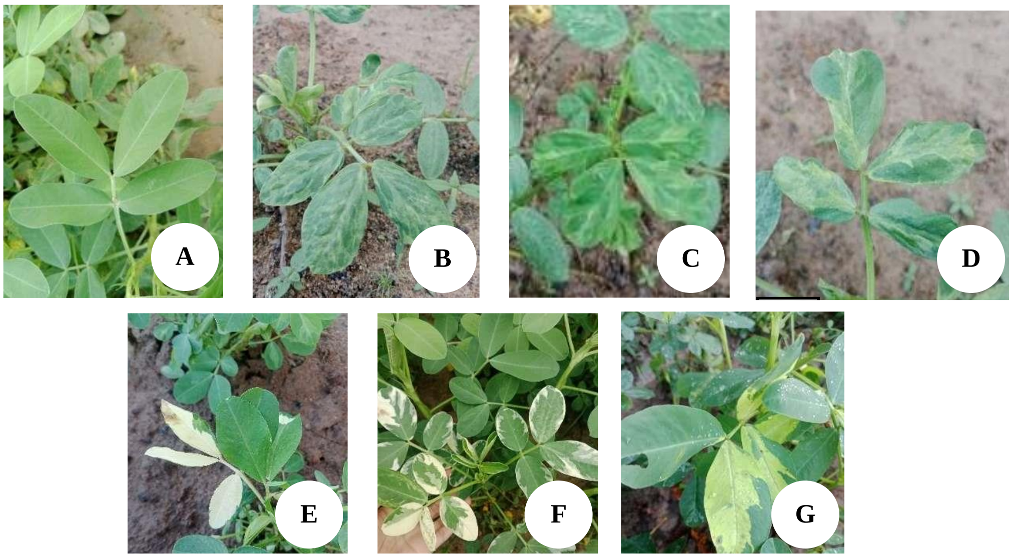

Figure 1. Leaves color variation. A. Normal (0 Gy); B. Striata (100 Gy); C. Striata (200 Gy); D. Striata (300 Gy); E. Albina (200 Gy); F. Albina (300 Gy); G. Klorina (300 Gy)

Table 2. Type and number of seed coat colors of bison groundnut due to gamma irradiation doses in the M1 generation

Gamma-ray irradiation doses	Seed coat color variation			
	Normal	Dark red	Purple	White strips
0 Gy	145	0	0	0
100 Gy	141	2	0	0
200 Gy	128	10	0	0
300 Gy	128	10	2	1
Total	542	22	2	1

The effect of gamma-ray irradiation on agronomic characters

Seed germination percentage

The results showed that the dose of gamma irradiation did not significantly affect the variable of the percentage of seed germination at the age of 2 weeks after planting (WAP) in the M1 generation of bison groundnut cultivar. The results of further tests on the average percentage of seed germination at 2 weeks after planting are presented in Figure 4.

Figure 4 shows that the dose of gamma irradiation did not significantly affect the percentages of growth. However, the highest growth percentage was at dose 0 Gy, which was 96.66%, and was not significantly different from doses 100 Gy and 300 Gy, while the lowest growth percentage was at dose 200 Gy, with a growth percentage of only 92.00%. Plants exposed to modest amounts of gamma radiation had better germination traits in their seeds, and this was because the radiation affected the genes that governed these traits, which in turn stimulated hormones, activated enzymes involved in germination processes, and sped up DNA repair. Low levels of gamma radiation could also hasten cell division in meristematic tissues, which could enhance plant germination. Following the study, Kiani et al. (2022) found that the wheat seedling survival rates were higher and lower when 19% seed moisture was combined with irradiation dose of 100 Gy, and when 19% seed moisture was combined with a dose of 400 Gy, respectively. Thenuja et al. (2024) supported these results, and according to their results, groundnut seed germination was unaltered at lower gamma radiation doses (0-100 Gy). The germination percentage for every treatment, ranging from 20 to 100 Gy, was higher than that of the control treatment (0 Gy).

Plant height

The results showed that the dose of gamma irradiation had a significant effect on the variable of plant height at the age of 6 and 8 weeks after planting, but had no significant effect on the variable of plant height at the age of 2 and 4 weeks after planting in M1 generation of bison groundnut cultivar. The results of further tests on the average of plant height variables are presented in Figure 5.

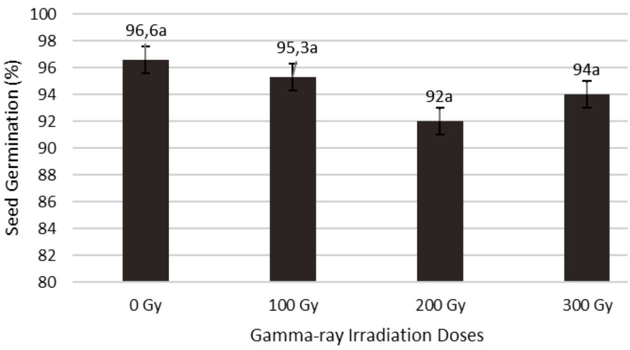

Figure 4. The average seed germination of groundnut bison cultivar due to gamma-ray irradiation doses in the M1 generation. Note: The numbers followed by the same letter in the bar are not significantly different according to the 5% DMRT test

Figure 3. Seed coat variation. A. Normal (0 Gy); B. Dark red (100 Gy); C. Dark red (200 Gy); D. Dark red (300 Gy); E. Purple (300 Gy); F. White strips (300 Gy)

Figure 5 shows that the dose of gamma irradiation affected plants' height at the age of 6 and 8 weeks after planting, but did not significantly affect the height of plants at the age of 2 and 4 weeks after planting. The tallest plants were found at dose 100 Gy with a height of 29.41 cm and 45.48 cm, which was not significantly different from dose 0 Gy and 200 Gy. The shortest plants were found in the 300 Gy dose treatment population with plant heights of 17.47 cm and 29.06 at the age of 6 and 8 weeks after planting.

The dose of gamma irradiation caused a decrease in plant height, starting at a dose of 200 Gy. The higher the dose of gamma irradiation given to the bison groundnut cultivar, the greater the reduction in plant height. This was in line with Saibari et al. (2023) which found that the height of kp29 and Fleur11 groundnut varieties decreased at all doses of gamma irradiation treatment compared to untreated plants. This also occurred in the study of Ganesan et al. (2022), which showed that the height of groundnut plants with increasing doses of gamma irradiation caused a maximum decrease at a dose of 600 Gy compared to the control plants.

According to Amir et al. (2017), the height of *Abelmoschus esculentus* plants increased with higher gamma irradiation doses, reaching the maximum height at 500 Gy and 300 Gy compared to the control. However, lower doses such as 100 Gy, 200 Gy, and 400 Gy led to a decrease in plant height compared to the control. This reduction in plant height may be attributed to damage to apical meristem, hindered respiratory enzyme synthesis, reduced amylase activity, and temporary suspension of cell division or delay in mitosis caused by ionizing radiation. Furthermore, the inactivation of growth regulators due to radiation could lead to retarded plant growth. The retardation in plant height could also be related to increased production of active radicals, which were responsible for lethality, or to an increase in radiation-induced gross structural chromosomal changes.

Length, width, and number of stomata

The results showed that the dose of gamma irradiation of groundnut bison variety M1 had a very significant effect on the variable number of stomata and the width of the upper leaf stomata. However, the variable length of upper leaf stomata, length of lower leaf stomata, and width of lower leaf stomata showed no significant effect. The results of further tests on the variables of length, width, and number of leaf stomata were presented in Tables 3 and 4.

Based on Table 3, the stomatal width of upper leaf stomata was the widest stomata at doses of 0 Gy with a width of 17.53 µm, and not significantly different from upper leaf stomatal width at 100 Gy and 200 Gy. The smallest stomata width upper leaf was found in 300 Gy with a width of 12.52 µm. The observation of the stomatal length and width of the stomata lower leaf showed no significant difference in size between the control plants and gamma-irradiated plants. Based on the results of the DMRT 5% test, only the width of the lower leaf stomata experienced a significant difference in irradiated plants. This was in line with the results of a study by Bajpay and Dwivendi (2019) that the size of stomata decreased with increasing doses of gamma irradiation, and stomata were not able to carry out gas exchange activities optimally and also caused a decrease in the ability to live.

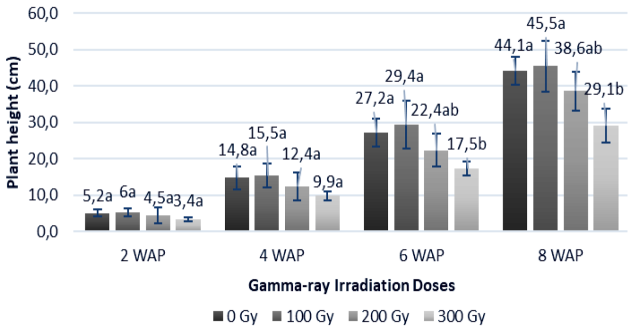

Figure 5. The average plant height of bison groundnut cultivar due to gamma-ray irradiation doses in the M1 generation. The numbers followed by the same letter in the bar are not significantly different according to the 5% DMRT test

Table 3. The average stomatal length and stomatal width of groundnut leaves of bison cultivar due to gamma irradiation doses in the M1 generation

Gamma-ray irradiation doses	Length and width stomata (µm)			
	Stomatal length upper leaf	Stomatal length lower leaf	Stomatal width upper leaf	Stomatal width lower leaf
0 Gy	24.80 a	26.84 a	17.53 a	17.03 a
100 Gy	23.42 a	25.67 a	14.99 ab	18.15 a
200 Gy	23.07 a	25.23 a	15.62 ab	17.75 a
300 Gy	18.91 a	22.22 a	12.52 b	15.08 a

Notes: Numbers followed by the same letter in the same column are not significantly different according to the 5% DMRT test

Table 4. The average number of stomata on groundnut leaves of bison cultivar due to gamma irradiation dose in M1 generation

Gamma irradiation doses	Number of stomata	
	Number of stomata (upper leaf)	Number of stomata (lower leaf)
0 Gy	9.66 b	12.00 ab
100 Gy	8.66 b	10.00 bc
200 Gy	9.33 b	7.66 c
300 Gy	15.33 a	13.33 a

Notes: Numbers followed by the same letter in the same column are not significantly different according to the 5% DMRT test

Table 4 shows that in the variable number of stomata of the upper leaves and the number of stomata of the lower leaves, the highest was found at dose 300 Gy with 15.33 and 13.33 stomata. The lowest number of upper leaf stomata was found in dose 100 Gy and not significantly different from 0 Gy and 200 Gy. The least number of lower leaf stomata was found at 200 Gy and 100 Gy, with 7.66 and 10 stomata, respectively. The observation of the number of stomata showed a difference between the control plants and gamma-irradiated plants. The appearance of the stomata of bison groundnut cultivar due to the dose of gamma irradiation can be seen in Figure 6.

Figure 6 shows that the length and width of the stomata of groundnut leaves due to gamma irradiation dose treatment were smaller than the control. The length of stomata in the 0 Gy treatment was 27.44 µm while 100 Gy was 26.90 µm. In the width of the stomata, it was also clear that gamma irradiation dose treatment was shorter than the control. The treatment of 0 Gy was 16.57 µm, while 100 Gy was 14.22 µm, although statistical analysis was not significantly different. The occurrence of properties that could reduce stomatal size due to gamma irradiation occurred randomly.

Similar findings were reported by Bajpay and Dwivedi (2019), gamma radiation significantly impacts the stomatal morphology of *Dendranthema grandiflora*. Higher doses, ranging from 300 to 450 Gy, unequivocally reduce stomatal size, severely impairing gas exchange and leading to a decline in plant survival. Handini et al. (2021) also obtained the results by measuring the length and width of the protocorm stomata of *Grammatophyllum scriptum* showing differences between control plants and irradiated plants. Although the size of stomata is not significantly different between control plants and irradiated plants, descriptively it can be seen that the greater the dose of irradiation, the smaller the stomata size.

Number of branches

The results showed that the dose of gamma irradiation significantly affected the variable number of branches in bison groundnut cultivar. The results of further tests on the average number of branches are presented in Figure 7.

Based on Figure 7, the dose of gamma irradiation had a significant effect on the number of branches. The highest number of branches was found at doses of 100 Gy, with 20.73 branches at 8 WAP, which was not significantly different from 0 Gy. The lowest number of branches was found at doses 300 Gy, with 16.76 branches. Additionally, it could be concluded that the dose of 100 Gy produced the most number of branches, while the dose of 300 Gy produced the least number of branches.

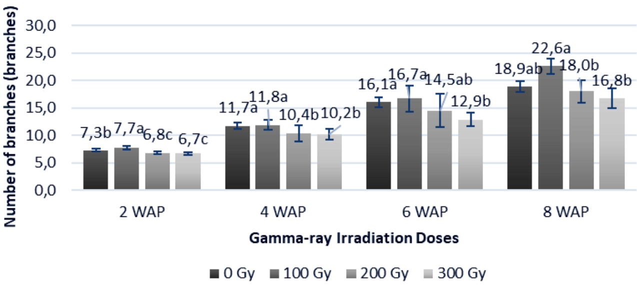

Figure 7. The average number of branches of bison groundnut cultivar due to gamma-ray irradiation doses in the M1 generation. The numbers followed by the same letter in the bar are not significantly different according to the 5% DMRT test

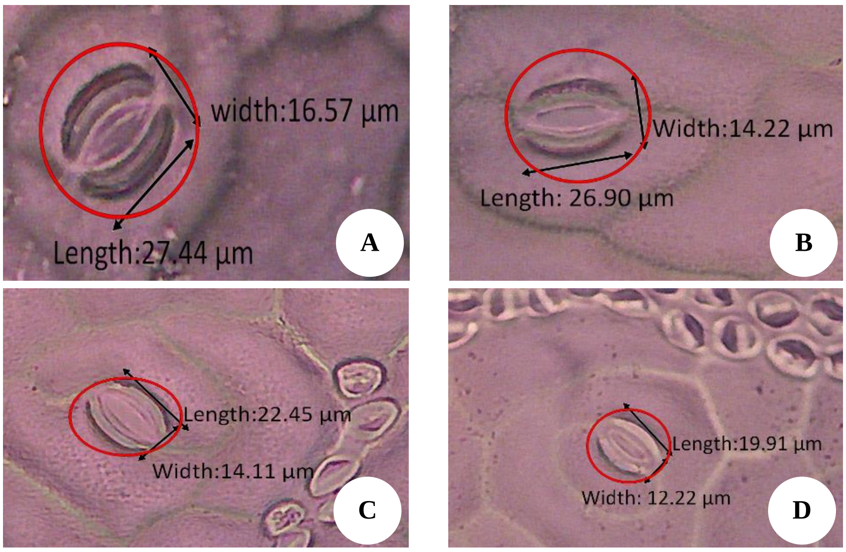

Figure 6. Stomata display of gamma irradiation treatment using binocular Olympus microscope with 400× magnification. A. 0 Gy; B. 100 Gy; C. 200 Gy; D. 300 Gy

Gamma irradiation dose could reduce the number of branches on bison groundnut cultivar. The decrease in the number of branches began to appear in the treatment population of 200 Gy gamma irradiation dose. Ganesan et al. (2022) found that gamma irradiation on groundnut showed a gradual decrease in the number of branches per plant. The maximum reduction in the number of branches was observed at 600 Gy gamma irradiation dose, while the minimum reduction in the number of branches at 100 Gy dose compared to the number of branches of control plants. Vanmathi et al. (2021) added that there was a decrease in quantitative characters with increasing doses of gamma irradiation in cowpea plants at M1 generation.

Flowering and harvesting age

The results showed that the dose of gamma irradiation had a very significant effect on the variables of flowering age and harvest age of bison groundnut cultivar in the M1 generation. The results of further tests on the average flowering age and harvest age are presented in Figure 8.

Figure 8 shows that the dose of gamma irradiation could slow down the flowering and harvesting time of bison groundnut cultivar. The earliest flowering age was found at dose 0 Gy, which was 27.30 DAP (Days After Planting). The longest flowering age was found at dose 300 Gy, which was 34.8 DAP, significantly different from 0 Gy, 100 Gy, and 200 Gy, which was 27.3, 29.3, and 32.4 DAP, respectively. The fastest harvesting age was at dose 0 Gy with a harvesting age of 91.6 DAP, which was significantly different from doses 100 Gy and 200 Gy. The longest harvesting age was found at dose 300 Gy, with a harvesting age of 103.2 DAP.

We found that gamma irradiation treatment in the bison groundnut variety can delay flowering and harvesting time at all doses of gamma irradiation in the M1 generation. These findings align with the results of the study by Nilahayati et al. (2015) who found that the flowering and harvesting age of gamma-irradiated kipas putih soybean were slower than the control plants. The higher the dose of gamma irradiation, the slower the flowering and harvesting age compared to the control. According to Choi et al. (2021), immediately after irradiating rice plants, both direct and indirect markers of radiation-induced damage, such as DNA degradation, free radical content, and malondialdehyde (MDA) content, increased in a dose-dependent way. These results implied that higher dosage levels caused more severe harm.

Number of pods/plant and number of empty pods/plant

The results showed that the dose of gamma irradiation of the bison groundnut cultivar did not significantly affect the variable number of pods per plant and the number of empty pods per plant. The results of further tests on the average number of pods and the number of empty pods are presented in Figure 9.

Figure 9 shows that the dose of gamma irradiation did not affect the number of pods and empty pods. On average, the highest number of pods was found at dose 100 Gy with 17.10 pods, which was not significantly different from dose 0 Gy, 200 Gy, and 300 Gy. On average, the highest number of empty pods was found at dose 200 Gy with 2.56 pods, which was not significantly different from doses 0 Gy,100 Gy, and 300 Gy. Similar results were obtained by Majeed et al. (2016), who found that increasing the dose of gamma irradiation did not change the number of pods in pea plants. In contrast to Saibari et al. (2023), higher irradiation doses could decrease the number of pods. Non-irradiated plants had the highest counts of pods, while the lowest counts were recorded at a dose of 200 Gy. Majeed et al. (2017) highlighted that the growth response of various plants to gamma irradiation treatment is largely influenced by factors such as the radiation dose, exposure duration, and the specific plant species involved.

Weight of dry pods per plant and dry seeds weight per plant

The results showed that the dose of gamma irradiation did not significantly affect the variable weight of dry pods and dry seed weight of bison peanut varieties in the M1 generation. The results of further tests on the weight of dry pods per plant and dry seed weight per plant are presented in Figure 10.

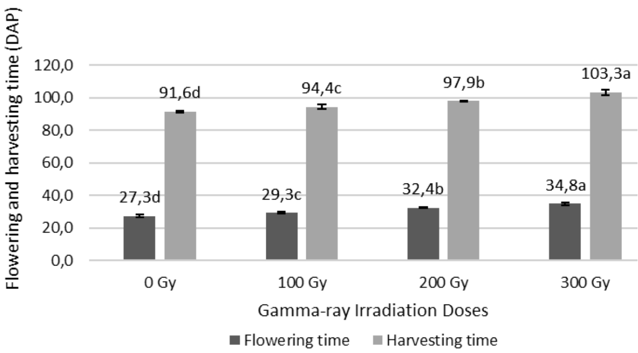

Figure 8. The average flowering and harvesting age of bison groundnut cultivar due to gamma-ray irradiation doses in the M1 generation. The numbers followed by the same letter in the bar are not significantly different according to the 5% DMRT test

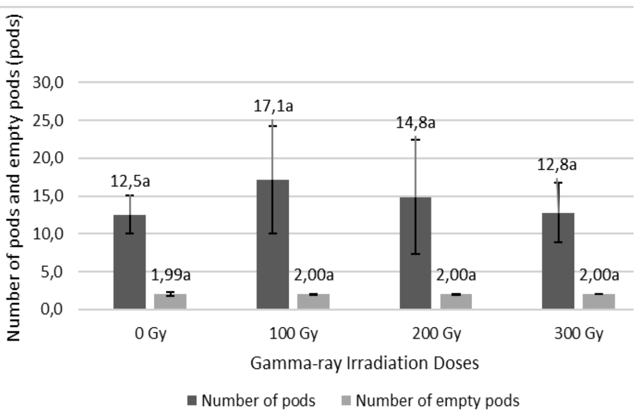

Figure 9. The average number of pods and number of empty pods of bison groundnut cultivar due to gamma-ray irradiation doses in the M1 generation. The numbers followed by the same letter in the bar are not significantly different according to the 5% DMRT test

Figure 10 shows the highest dry pod weight per plant and dry seed weight per plant was found at dose 100 Gy with a weight of 14.6 g and 9.8 g, which was not significantly different from doses 0 Gy, 200 Gy, and 300 Gy. On average, weight of dry pods and weight of dry seed slightly decreased at a gamma irradiation dose of 300 Gy. Ganesan et al. (2022) said that each dose of gamma irradiation could generally cause a decrease in pod yield per plant in groundnut compared to untreated. Manova and Gruszka (2015) stated that mutagenic agents can impact the stability of the plant genome. This can harm development and lead to lower yields in crops.

Weight of 100 seeds, and dry seeds weight per plot

The results showed that the dose of gamma irradiation of groundnut variety bison M1 had a very significant effect on the variable weight of dry seeds per plot, but had no significant effect on the variable weight of 100 seeds. The results of further tests on the variables of weight of 100 seeds and dry seed weight per plot are presented in Figure 11.

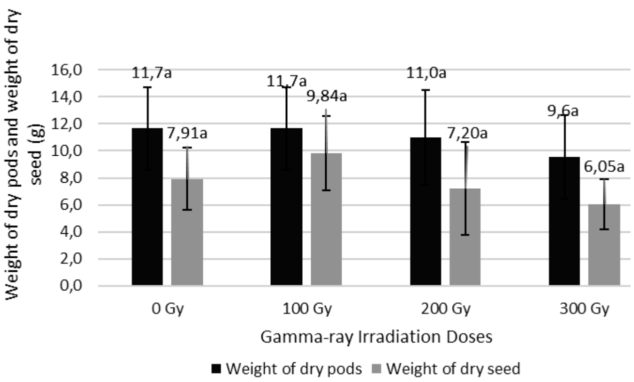

Figure 10. The average weight of dry pods and dry seed weight of bison groundnut cultivar due to gamma-ray irradiation doses in the M1 generation. The numbers followed by the same letter in the bar are not significantly different according to the 5% DMRT test

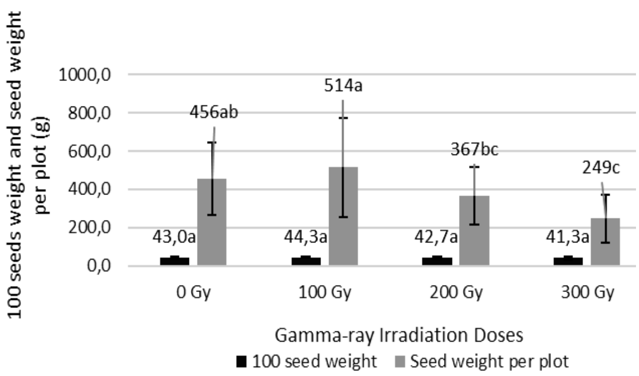

Figure 11. The average weight of 100 seed and dry seed weight per plot of bison groundnut cultivar due to gamma-ray irradiation doses in the M1 generation. The numbers followed by the same letter in the bar are not significantly different according to the 5% DMRT test

Figure 11 shows that the dose of gamma irradiation did not significantly affect the variables of dry seed weight per plant and the weight of 100 seeds per plot but had a significant effect on the variable weight of dry seeds per plot. The highest seed weight per plot was at dose 100 Gy, which was 514 g and which was not significantly different from dose 0 Gy, and the lowest seed weight per plot was at dose 300 Gy, which weighed 248.73 g. However, it was observed that gamma irradiation at 200 Gy and 300 Gy led to a decrease in yield.

These results were consistent with a study by Nura et al. (2021) that a lower dose of gamma rays (100 Gy) is more effective and efficient as it induces favorable mutation that could be utilized in the genetic improvement of *Digitaria exilis*. Ahmad et al. (2023) stated that higher doses of gamma radiation (120 Gy) on the sunflower variety produced negative effects on most of the parameters including seed weight per head. This was supported by Ganesan et al. (2022), who observed a decrease in the weight of 100 seeds in groundnut plants treated with gamma irradiation compared to untreated plants. Vanmathi et al. (2021) also reported cowpeas' morphological and quantitative characteristics decreased as gamma irradiation dosages increased, except for days to first flowering in the M1 generation. With increasing dosages of gamma irradiation compared to control, the number of fruit clusters per plant, number of pods per plant, number of seeds per pod, pod length, 100 seed weight, and seed yield per plant were steadily reduced.

Majeed et al. (2018) noted that gamma irradiation, particularly on seeds, can induce mutations in cells. This process involves high energy and penetrating power, leading to changes in DNA through direct exposure or the generation of reactive oxygen species, such as hydrogen peroxide and hydroxyl ions. These free radicals can interact with DNA and cellular components, causing ionization and altering protein and enzyme functions. The effects can be permanent and potentially lethal, or temporary, as DNA can repair itself. These DNA changes may introduce diversity in mutant offspring, which can show abnormalities in germination, morphology, and growth. While many traits can be negative, some may have beneficial effects, influenced by DNA repair and gene rearrangement. The irradiation dosage is crucial for producing either abnormal or desired plant mutants.

In conclusion, this study discovered that gamma irradiation dose caused morphological changes in the M1 generation of bison groundnut cultivar. Morphological changes observed included leaf color, pod shape, and seed-coat color alterations at all gamma irradiation doses. The doses of gamma irradiation applied to the M1 generation of bison groundnut cultivar reduced plant height, number of branches, and dry seed weight per plot beginning at 200 Gy, but at 100 Gy, all parameters slightly increased. However, it delayed the flowering and harvesting time at all irradiation doses. For future breeding programs, it is crucial to evaluate genetic variability dan heritability in the next M2 generation before selecting the desired traits in enhanced bison groundnut cultivar.

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