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Gamma Irradiation Affects the Growth and Yield Performance of Adlay (*Coix lacryma-jobi* L.)

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ABSTRACT

Watani Wado is a locally grown cultivar of adlay (*Coix Lacryma-Jobi* L.) that has the potential to develop in Indonesia. Its long maturity time can be improved by mutation breeding. The mutagenic effects of gamma irradiation on adlay have yet to be investigated. The purpose of this research is to assess the impact of gamma irradiation treatments on the growth and yield of adlay plants. Adlay seeds of Watani Wado variety were exposed to gamma irradiation in six different doses: 0 (control), 200, 250, 300, 350, and 400Gy. The study found that as the gamma irradiation dose increased, the percentage of viable seedlings declined, as did seedling height and root length. The lethal dose (LD_{20}-LD_{50}) ranged from 179.27 to 296.03Gy based on various seedling growth parameters. Gamma irradiation also delayed the time to flowering and maturity, except at 250Gy. The highest frequency of chlorophyll mutation on leaves was observed at a dose of 250Gy. Furthermore, higher doses tended to increase pollen sterility, whereas other traits such as plant height and number of tillers, internodes, and leaves similarly tended to rise until a certain dose, after which they began to decline at the higher dose. Meanwhile, the culm diameter remained unchanged. Gamma irradiation impacted on yield by increasing seed numbers, increasing the percentage of empty seed, and reducing seed the weight. The findings suggested that gamma irradiation might increase genetic diversity in plant populations. This provides valuable information for further plant breeding programs to generate improved adlay mutant varieties.

Keywords: Adlay, Agronomic traits, Gamma irradiation, Genetic diversity, Mutant lines, Yield.

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INTRODUCTION

Adlay (*Coix lacryma-jobi* L.) is an underutilized crop which has a great potency to be developed as an alternative and functional food (Suyadi et al., 2019). Adlay has a high nutritional content and therapeutic benefit, and it is utilized as a food and in traditional medicine (Feng et al., 2020). Since it is an underutilized crop, it is only grown and developed in a few regions. West Java is one of the areas in Indonesia that is growing and developing adlay. The adlay is cultivated by people sporadicaly across the districts of Bandung, Sumedang, Sukabumi, Ciamis, Indramayu and Garut (Nurmala, 2011). Exploration and identification of adlay have been carried out in 5 districts in West Java Province. The results of the exploration found 41 accessions of adlay originating from Bandung, Sumedang, Purwakarta, Cianjur and Indramayu Regencies (Qosim and Nurmala, 2011). Watani Wado is a local variety that has been developed as a result of this exploration. This variety has a potential yield of 6t.ha^{-1} with an average of 2 to 3t.ha^{-1} (Center for Plant Variety Protection, 2017). However, this variety has a fairly long time to harvest, reaching about 154 days normally.

Mutagenesis can be used as an effective tool to improve and modify genotypes of popular cultivars suitable for modern agricultural and commercial needs (Andrew-Peter-Leon et al., 2021). Gamma irradiation is one

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of the important physical mutagen used to improve the characters and productivity of many plants (Hanafy and Akladious, 2018). It has been used to produce various food crop cultivars including rice (*Oryza sativa* L.) (ICAR, 2023), wheat (*Triticum aestivum* L.) (Bayarsaikhan et al., 2022), sorghum (*Sorghum bicolor* L.) (Human et al., 2020) and adlay (Nakagawa and Kato, 2017). It has also been successfully used to induce mutation in breeding of various crops and ornamental plants (Songsri et al., 2019; Banyo et al., 2020; Li et al., 2022; Saadati et al., 2022). The effect of gamma irradiation as a mutagen on plant breeding has been widely studied in several crops such as wheat (Kiani et al., 2022), maize (*Zea mays* L.) (Yadav et al., 2019), rice (Jiya et al., 2018), soybeans (*Glycine max* (L.) Merr.) (Nilahayati et al., 2016) and barley (*Hordeum vulgare* L.) (Hussein, 2022). The effect of gamma irradiation on adlay has not been widely studied even though it is very important for an assessment. The purpose of this study is to assess the effect of gamma irradiation treatments on the growth and yield of adlay.

MATERIALS & METHODS

Adlay seeds of the Watani Wado variety with a water content of 11-12% were treated with gamma irradiation from a Cobalt-60 source at doses of 0 (control), 200, 250, 300, 350 and 400Gy. Adlay seed was irradiated in June 2022 using the gamma irradiator *Gammacell 220* at the Research Center for Radiation Processing Technology (PRTPR) – National Research and Innovation Agency (BRIN).

Field experiments were conducted from June 2022 to January 2023 at the Ciparanje experimental station, Padjadjaran University, Jatinangor, Sumedang, West Java. The radiosensitivity test was carried out by sowing 100 seeds from each irradiation treatment and control in a germination box containing soil mixed with manure (1:1) with three replications in the screenhouse. The percentage of viable seedlings, seedling height and root length were assessed 3 weeks after seed sowing. The optimal irradiation doses were determined based on Lethal Dose (LD), which range between LD_{20} and LD_{50} (Hapsari et al., 2021). The value of LD_{20} and LD_{50} was determined based on the regression equation using the mycurvefit.com program.

The M_1 generation was planted in the field from July 2022 to January 2023 at the end of the dry season (DS2 – RS1). Three-week-old seedlings were transferred to experimental plots with a spacing of 60x40cm in the field. Manure as basic fertilizer was given as much as five $tonsha^{-1}$. During fertilization, up to $200kg.ha^{-1}$ of NPK 16-16-16 is used (Qosim et al., 2014). To obtain optimal plant development, plant maintenance was done, including weed control, watering, and pest and disease management.

Observation of physiological damage resulting from the effects of mutations that occur in the leaves of M_1 plants in the form of chlorophyll mutations. Chlorophyll mutations were categorized using Muszyński (1968) classification. Mutated plants are counted in order to compare their numbers to those of normal plants. The frequency of chlorophyll mutations was calculated using Equation 1 (Gaul, 1964).

$$Mutation\ Frequency = \frac{Number\ of\ mutated\ plants}{Number\ of\ plants} \times 100\% \quad (1)$$

To assess the damage caused by induced mutation to generative phase, pollen sterility studies were conducted. For observation, a sample of 15 plants was collected from each irradiation treatment. Between 9:00 and 10:00 am, three anthesis flowers in anthesis from each plant were collected and placed in plastic. Preparation of observation glass was conducted using a modified version of the Virmani et al. (1997) method. On an object glass, three flowers were shaken to release their pollen, which then dropped to the glass. A single drop of Sigma Aldrich's 1% lugol (I_2KI) solution was dropped on the object glass. The object cover glass was placed on top of the object glass.

Adlay plant pollen was observed under a microscope with a 100× magnification in order to determine the pollen sterility. Three different fields of view were used for observation. Observation results are documented using a camera. Pollen counts were conducted using openCFU software (Geissmann, 2013) and manual verification. The classification of pollen was based on Virmani et al. (1997). Sterile pollen includes wrinkled and unstained (unstained withered, unstained spherical) or partially colored (partially stained round) pollen. Fertile pollen has a round shape and pigmented (stained round). Observations were made on the time to flowering and time to maturity, plant growth includes the number of leaves, plant height, culm diameter, number of tillers, number of internodes and number of side branches. Observations on yield components include the number of seeds per plant, weight of seeds per plant and weight of 100 seeds. Data were analyzed using the Z test by comparing treatment with the control (wt).

RESULTS

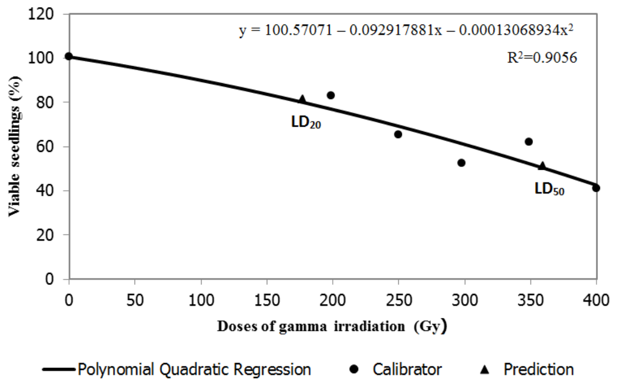
Lethal Doses

Gamma irradiation causes a decrease in the percentage of viable seedlings in adlay seeds as the irradiation dose increases as shown in Fig. 1a. Gamma irradiation treatment also affected the seedling height and length of the roots. This treatment significantly reduces both the height and root length of adlay seedlings as the gamma-ray irradiation dose increased as shown in Fig. 1b and Fig. 1c.

Plant sensitivity to gamma irradiation can be determined through the physiological response of irradiated plant material, including determining the dose that causes death in irradiated plants of 20-50% or a lethal dose of 20-50% (LD_{20}-LD_{50}) (Anggraini and Astuti, 2023). Based on the percentage of viable seedlings, LD_{20} - LD_{50} are in the range of 177.21Gy to 360.98Gy. Meanwhile, based on the height of the seedlings, LD_{20} - LD_{50} are in the range of 121.25Gy to 296.03Gy. While based on the length of the seedling roots, LD_{20} - LD_{50} are in the range of 179.27Gy to 366.20Gy.

Time to Flowering and Maturity

Gamma irradiation had a significant effect on the time to flowering of adlay of the Watani Wado variety except that at the dose of 250Gy. The time to flowering of adlay was distributed normally as shown in Fig. 2a. The flowering time was delayed with increasing irradiation doses except that at 250Gy. The longest average time to flowering was observed at 350Gy, with 116.36 days compared to 109.75 days in the control.



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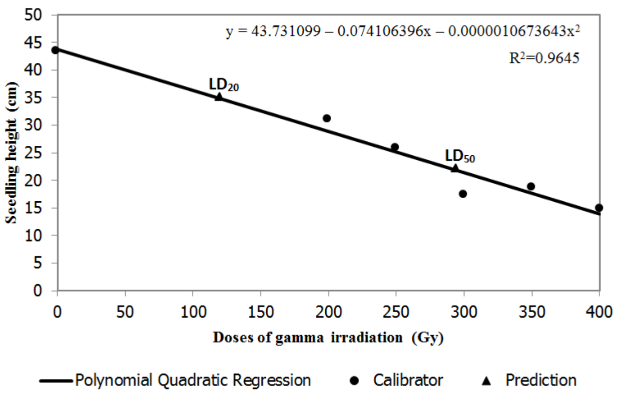

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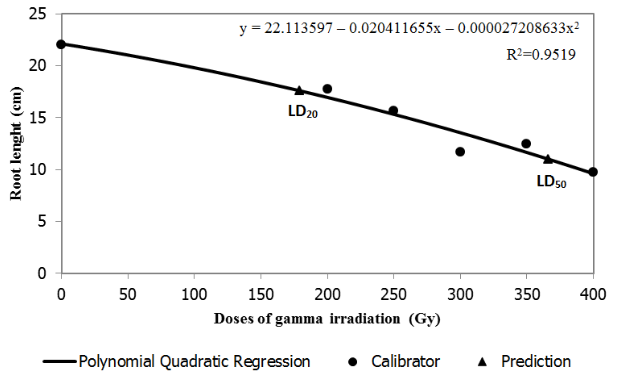


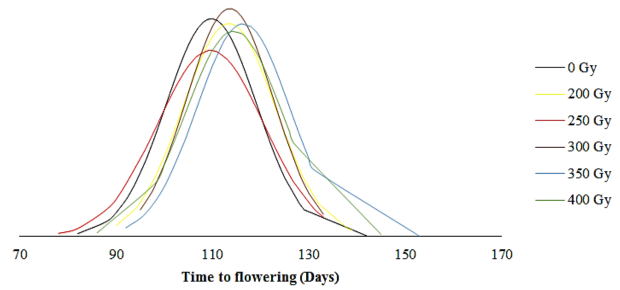
C

Fig. 1: A) Germination rate, B) seedling height, and C) seedling root length of adlay cv. Watani Wado in several doses of gamma-ray irradiation treatments.

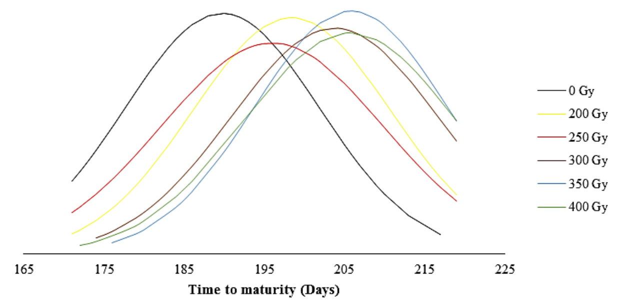
The time to maturity of adlay was distributed from 171 to 219 days. Gamma irradiation caused the time to maturity more lately compared to the control. The time to maturity of adlay was distributed normally as shown in Fig. 2b. Based on this Fig., the time to maturity progressively extends as the dosage treatments increase. Gamma irradiation had a significant effect on the time to maturity of the Watani Wado variety of adlay, where higher doses resulted in further delays. The longest average time to maturity was observed at 350Gy, reaching 205.81 days, compared to 189.77 days in the control.

Chlorophyll Mutations

Application of gamma irradiation produced various types of mutations (Muszyński, 1968) which showed an increase in physiological diversity in the M_1 adlay population (Fig. 3). Gamma irradiation treatment at a dose of 200 to 400 Gy produced different types of chlorophyll mutations with different frequencies in the adlay M_1 mutant population (Table 1). Irradiation with a dose of 250Gy resulted in the highest frequency of M_1 chlorophyll mutations (26.67%). The irradiation treatment with a dose of 300Gy resulted in a high degree of variation among the five types of chlorophyll mutations: white striata, yellow striata, yellow marginata, irregulare and albina-virescens.



(a)



(b)

Fig. 2: Distribution of the time to flowering (a) and time to maturity (b) of adlay in several doses of gamma irradiation treatments.

Table 1: Chlorophyll mutations in M_1 Adlay leaves of the Watani Wado variety after treated by several dose of gamma irradiation treatments

Doses (Gy)	Chlorophyll mutation						Number of mutant	Number of plant	Mutation. Frequency (%)
	Str-w	Str-y	Mar-w	Mar-y	Irr	Al-vir			
0	-	-	-	-	-	-	0	132	0
200	2	4	-	1	-	-	7	106	6.60
250	7	7	2	4	-	-	20	75	26.67
300	3	4	-	1	2	1	11	55	20.00
350	1	6	-	2	-	-	9	58	15.51
400	8	2	-	2	1	-	13	51	25.49

Note: Str-w: Striata white; Str-y: Striata-yellow; Mar-w: Marginata-white; Mar-y: Marginata-yellow; Irr: Irregulare; Al-vir: Albina-virescence.

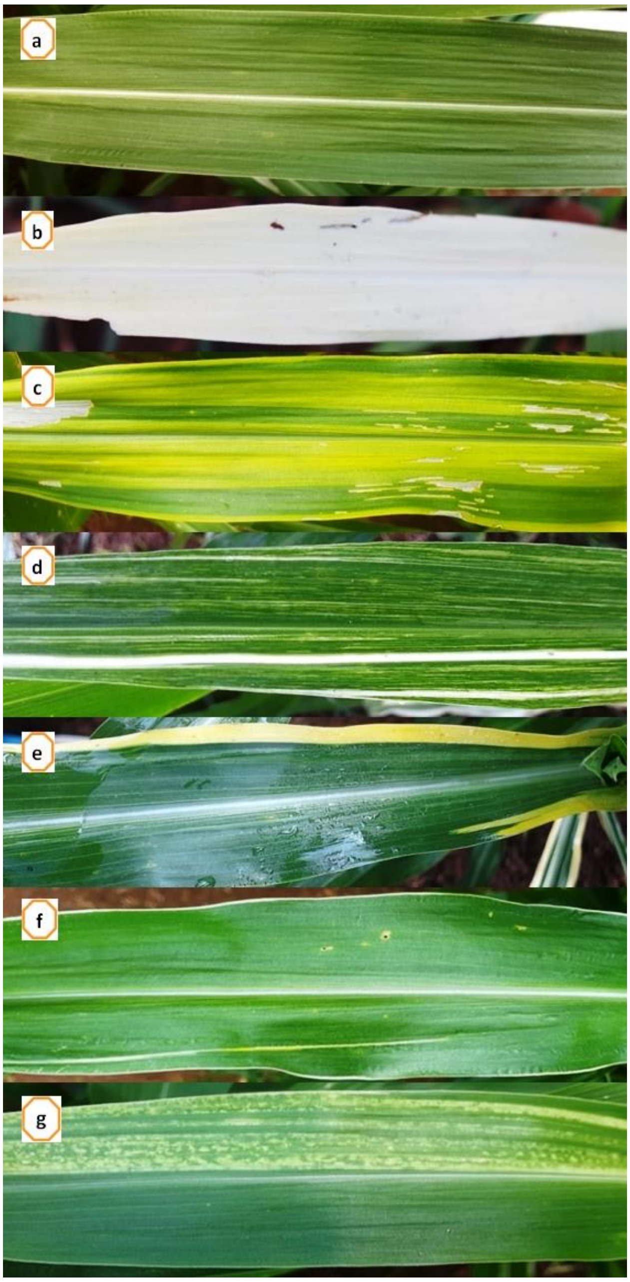


Fig. 3: Chlorophyll mutations in adlay leaves as a result of gamma irradiation treatment: a. normal leaves; b. Albina; c. Striata-yellow; d. Striata-white; e. Marginata-yellow; f. Marginata-white; g. Irregulare.

Pollen Sterility

Gamma irradiation treatment caused sterility of adlay pollen (Fig. 4). Pollen sterility increased with higher irradiation doses (Fig. 5). The average of sterile pollen on control plants was 4.27%, while the highest pollen sterility occurred in plants treated with 400 Gy of gamma irradiation, at 32.28%.

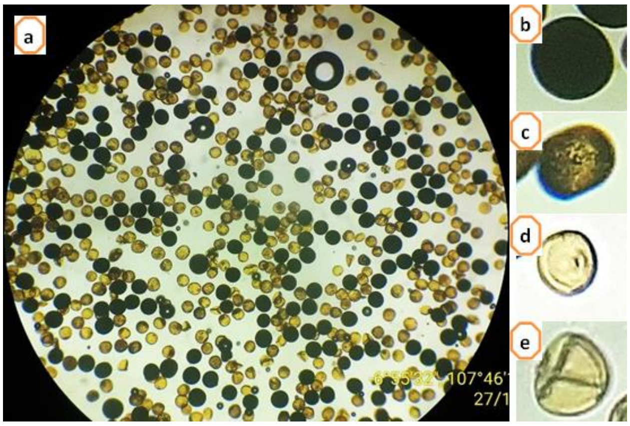


Fig. 4: Pollen sterility (a) on adlay after being treated with gamma irradiation of 400 Gy. Stained pollen (b) indicates fertile pollen; while light stained pollen (c); unstained pollen (d); and wrinkled unstained pollen (e) indicated sterile pollen. Microscope magnification was 100x.

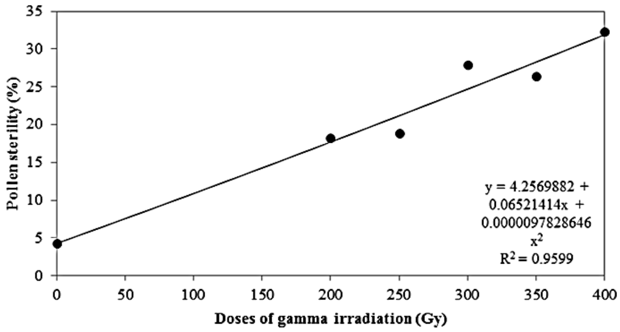


Fig. 5: Pollen sterility of adlay in several doses of gamma irradiation treatments.

Plant Development

Gamma irradiation significantly increased plant height, number of tillers, number of internodes and number of leaves of adlay but had no impact on culm diameter (Table 2). Plant height, number of side branches and number of leaves increased up to the dose of 350 Gy, then decreased. Meanwhile, the number of tillers and the number of internodes increased to the doses of 200Gy and 250Gy, and then decreased afterwards.

Crop Yield

Gamma irradiation had a significant effect on the number of seeds per plant, empty seeds, weight of 100 seeds and seed weight per plant of the adlay var. Watani Wado (Table 3). The number of seeds per plant increased to 350Gy, after which it decline at higher irradiation dose. With increasing irradiation dose, the number of seeds increased, but so did the number of empty seeds. This affected the weight of 100 seeds which declined with increasing irradiation dose. The seed weight per plant decreased at the dose of 400Gy.

Table 2: The effects of gamma irradiation treatments on some agronomic traits of adlay

Doses (Gy)	Plant height (cm)	Culm diameter (mm)	Number of tillers	Number of internodes	Number of side branches	Number of leaves
0 (control)	153.36	8.61	8.73	10.61	14.03	56.56
200	165.08*	8.50	10.44*	10.92*	14.79*	64.24*
250	164.04*	8.33	12.05*	10.29*	15.53*	66.69*
300	166.54*	8.89	10.96*	10.65	15.21*	71.13*
350	172.14*	8.77	11.43*	10.74	16.17*	76.67*
400	169.80*	8.24	9.39	10.37	14.65	64.06*

Note: The * sign indicates a significant difference between treatment and control at a significance of 5% of the 1-way critical Z value.

Table 3: Data of yield components of adlay after being treated by several doses of gamma irradiation

Doses (Gy)	Number of seeds per plant	Empty seeds (%)	Weight of 100 seeds (g)	Seeds weight per plant (g)
0	1,253.74	3.16	10.68	126.29
200	1,322.17	11.00*	8.73*	105.54
250	1,791.39*	20.67*	8.17*	126.20
300	1,848.49*	24.98*	7.87*	123.37
350	1,876.48*	23.69*	7.92*	125.45
400	1,683.94*	27.98*	7.16*	96.20*

Note: The * sign indicates a significant difference between treatment and control at a significance of 5% of the 1-way critical Z value.

This increase in the number of seeds was thought of correlated to the number of tillers and the number of branches. The number of seeds per plant increased with the number of tillers and branches formed. The percentage of empty seeds increased with increasing gamma irradiation dose. The highest average percentage of empty seeds (27.98%) was found at the dose of 400Gy, compared to the control of 3.16%. This increase in the empty seeds was believed to be caused by an increase in pollen sterility. The weight of 100 seeds decreased with an increasing irradiation dose. The average seed weight per plant at the dose of 400Gy reached 7.16grams compared to the control of 10.68. The decrease in the weight of 100 seeds of adlay plants was correlated to the sterility of the pollen on the plants and the percentage of empty seeds. Higher gamma irradiation treatment doses would result in more sterility. As a result, there were more empty seeds, which had an effect on seeds' weight.

Gamma irradiation at a dose of 400Gy had a significant effect on seed weight per plant of the adlay cv. Watani Wado but had no significant effect at other doses. The average seed weight per plant at the dose of 400Gy reached 96.20g compared to the control of 126.29g. Seed weight per plant decreased with increasing irradiation dose levels.

DISCUSSION

Lethal Doses

The decrease in germination is likely caused by the effect of mutagens on the meristematic tissue of the seeds as well as chromosomal aberrations and interference with DNA replication and growth regulators (Asare et al., 2017). Furthermore, at certain doses the seeds are able to germinate but cannot survive due to DNA damage and are unable to repair (Sood et al., 2016). High doses of gamma irradiation can damage the integrity and permeability of cell membranes, causing inhibition of nutrient and water absorption, thereby disrupting plant growth and physiological activity (Li et al., 2021). Previous researchers also reported a decrease in seed germination rates due to gamma irradiation treatment in okra (*Abelmoschus esculentus* (L.) Moench.) (Asare et al., 2017), coffee (*Coffea arabica* L.) (Spinoso-Castillo et al., 2021), wheat (Kiani et al., 2022), chili (*Capsicum annum* L.) (Shamsiah et al., 2022), tulip (*Tulipa gesneriana* L.) (Li et al., 2022) and corn (*Zea mays* L.) (Kikakedimau et al., 2022).

The decrease in seedling growth due to mutagenic treatment was thought of being caused by a decrease in mitotic activity in the meristematic tissue (Yadav et al., 2019). Mutagen treatment could cause damage to cell division and cell elongation processes (Iqbal, 1969). This was because irradiation causes DNA damage in plant cells which results in various types of damage to plant cell division, plant growth and development processes (Li et al., 2021). Decreased seedling growth after gamma irradiation treatment also occurred in chili (Sood et al., 2016), okra (Asare et al., 2017), maize (Yadav et al., 2019), and wheat (Ahumada-Flores et al., 2020), and groundnut (Arachis hypogaea L.) (Saibari et al., 2023).

The percentage of germinating seeds may not always accurately reflect the calculation of the optimal dose. Talebi et al. (2012) used the LD_{25} and LD_{50} ranges to determine the optimal dose based on plant height and root length parameters. Growth reduction calculations can be used to determine the optimal dose. This was also in accordance with the statement of Astuti et al. (2019) that the LD_{20} and LD_{50} values showed a wide range so that the optimum dose was determined not only based on the lethal dose but also on plant height parameters. Meanwhile, Ghasemi-Soloklui et al. (2023) uses Growth Reduction ($GR_{25,\ 50,\ 75}$) based root, leaves, and shoot morphometric traits. In this study, the LD_{20} and LD_{50} dose ranges varied based on the percentage of viable seedlings, height of the seedlings and length of the seedling roots. The LD_{20}-LD_{50} dosage range, which is 179.27-296.03Gy, was obtained by overlaying the three dose ranges to create a more concentrated range (Fig. 6). This dose range can be used to determine the genotype that will be planted in the M_2 generation. The genotypes that will be planted in the M_2 generation are prioritized to be derived from M_1 plants that have been treated with gamma irradiation at doses of 200, 250 and 300Gy.

Time to Flowering and Maturity

The different flowering times might be related to seed metabolism and initiation of DNA synthesis as a consequence of gamma irradiation treatment as those stated by (Shah et al., 2008). The late flowering time was thought to be influenced by the late germination and development of the plant. This was in accordance with (Asare et al., 2017) which stated that plants would flower more lately due to later germination and plant development as the irradiation dose increased. According to Sankar et al. (2021) the physiological process was impacted by the increased gamma-ray dosage, which resulted in late flowering. Shamsiah et al. (2022) stated that higher exposure to irradiation caused more chromosome damage. This damage causes disruption in integration with normal cells, causing a delay in flowering.

The effect of gamma-ray irradiation on the time to flowering was reported by several previous researchers. Jiya et al. (2018) reported that gamma irradiation treatment with increasing doses caused the flowering process delayed in rice plants (*Oryza sativa* L.). Similar result also reported by Suliartini et al. (2023) in rice lines G16 and by Saibari et al. (2023) in groundnut. Ahmed et al. (2017) reported different results on two wheat plant varieties. Increasing the dose of gamma irradiation causes the initial flowering period to be earlier in the Batoor variety, whereas in the Janbaz variety the initial flowering period was delayed. Shamsiah et al. (2022) reported there was no effect on the time to flowering of chili at the dose of 20Gy. Chili plants showed an earlier flowering at irradiation doses of 40Gy and 80Gy, but the plants showed later flowering above the dose of 80Gy.

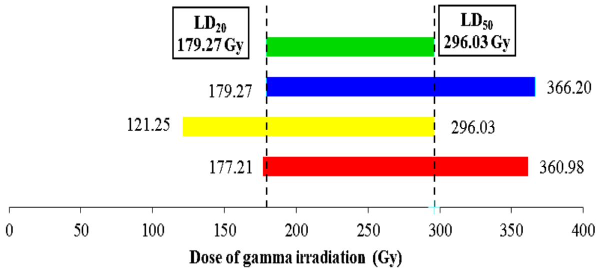


Fig. 6: Estimated LD_{20} and LD_{50} (green), which is an overlay of LD_{20}-LD_{50} on the percentage of viable seedlings (red), seedling height (yellow) and seedling root length (blue).

There might be several causes for the late maturity of adlay. Late maturity time could be related to late flowering time. The time to flowering was positively correlated with the time to maturity, which was also reported in rice by Tsenov (2009) and Safitri et al. (2011) and in eggplant (*Solanum melongena* L.) by Sari et al. (2021). Time to maturity might also be related to the type of plant growth. Shamsiah et al. (2022) stated that determinate plant types would flower 50 percent earlier than indeterminate plant types, which ultimately caused a delay in the seed ripening process. Several researchers reported the effect of gamma irradiation on the duration of crop maturity. Animasaun et al. (2014) reported that the time to maturity of fonio (*Digitaria exilis* Haller) delayed as the irradiation dose increased. The same condition was also reported in wheat by Ahmed et al. (2017) and in rice by Jiya et al. (2018). Different result were reported by Pujiyanti et al. (2021) that increasing the dose of gamma irradiation had no significant effect on the maturity time in rice.

Chlorophyll Mutations

The spot and streaks phenomenon that occurred on the M_1 leaves might be of a chlorophyll deficiency due to mutations happened in nuclear or cytoplasmic genes (Travis et al., 1975). Chlorophyll mutations could be as an indicator for evaluating the effect of mutagen treatment (Nilahayati et al., 2016). According to Kolar et al. (2011), chlorophyll mutations could be of useful for identifying mutagen dose limits that might increase genetic diversity in the M_2. According to Bhoi and Mishra (2021) there are up to 300 genes that are active in the synthesis of chlorophyll. The first genetic impacts of the mutagen utilized appear to be chlorophyll mutations in mutagenic treated populations, which act as a marker of induced genetic variants in M_1 and subsequent generations.

Several researchers have documented chlorophyll mutations resulting from gamma radiation. Sasipriya et al. (2023) reported spectrum of several chlorophyll mutation in okra (*Abelmoschus esculentus* L.). Kumar and Sharma (2024) also reported a wide spectrum of chlorophyll mutants (Chlorina, Xantha, Albina, Viridis and complex type) in mungbean (*Vigna radiata* L.) at various crop growth stages. According to Hasib (2022) the frequency of chlorophyll mutations in traditional rice cultivar was high in higher doses. Vaithiyalingan et al. (2023) also reported higher dose produced high frequency of albino mutant.

Pollen Sterility

Pollen viability could be an important trait that might be stable and genetically controlled (Monica and Seetharaman, 2015). Low pollen viability could cause low success of flowers to become fruits (Hayati et al., 2022). Low pollen fertility due to irradiation might be caused by aberrations in meiosis which impacted on abnormal pollen formation (Monica and Seetharaman, 2015). According to Bione et al. (2002) sterility due to induced mutations was mainly caused by degeneration of pollen generative cells in Meiosis II which impacted in low pollen viability. Pollen sterility due to induced mutations was also reported occur in hyacinth bean (*Lablab purpureus* (L.) Sweet) (Monica and Seetharaman, 2015), rice (Gowthami et al., 2016), jicama (*Pachyrhizus erosus* L.) (Hayati et al., 2022) and lentil (*Lens culinaris* Medik.) (Pramanik et al., 2023).

Plant Development

Plant height and number of tillers might be influenced by two factors: cell damage due to gamma irradiation and environmental influences. Cell damage due to irradiation caused delays in plant growth (Li et al., 2021). This impacted differences in plant growth phases. Higher doses tend to prolong the plant's vegetative phase. Environmental factors, especially water availability, could also influence plant height and number of tillers. In this study, the control plant population had entered the full generative phase when the time entered rainy season (in October) while those from irradiation treatments were still in the vegetative or early generative phase. The control plants, having entered full generative phase performed no longer culm elongation and form new shoots even though they received sufficient amounts of water. In contrast, plants still in the vegetative phase or in the early generative phase responded to increased water availability by continuing culm growth and forming new shoots.

The effect of gamma irradiation on plant height had been also reported by previous researchers. Animasaun et al. (2014) reported that fonio plant height increased up to irradiation dose of 80Gy, then decreased as the irradiation dose increased. Different responses were shown by rice plants as reported by Pujiyanti et al. (2021). Gamma irradiation with increasing doses tended to decrease rice plant height. Elizar et al. (2018) also reported that irradiation doses up to 100Gy had no effect on plant height of kenikir (*Cosmos caudatus* Kunth.), but it did on higher doses. Several previous researchers reported the effect of gamma irradiation treatment on the number of tillers of several crop commodities. For instance, Ahmed et al. (2017) reported that gamma irradiation increased the number of tillers on wheat up to the dose of 15kR, and then tended to decrease it at the higher doses. A similar pattern was observed in fonio, with increased tiller up to the dose of 40Gy (Animasaun et al., 2014). Conversely, Harding et al. (2012) reported that gamma irradiation had no effect on the number of rice tillers up to the dose of 300Gy, with a decline noted at the higher doses. Additionally, Jiya et al. (2018) found that gamma irradiation increased number of tillers in rice variety of FARO 44, while the FARO 60 variety showed a reduction. Furthermore, Hussein (2022) reported that the number of tillers in barley increased along with increasing doses of gamma irradiation, contrasting with findings from Khan et al. (2003). Culm diameter showed a correlation with plant height, with taller plants having larger culm diameter, contributing to their stability and firmness. The smaller culm diameter as the growing was usually caused by the environmental factors. Insufficient exposure to light resulted in plants undergoing etiolation, a phenomenon typically attribute to planting them to closely together. The effect of gamma irradiation on culm or stem diameter was also reported by Thiede et al. (1995) on sunflowers (*Helianthus annus* L.) and on Elizar et al. (2018) on kenikir. According to Elizar et al. (2018), the stem diameter of kenikir decreased as the irradiation dose increased. A similar result also observed in sunflower, particularly at irradiation doses above 5Gy (Thiede et al., 1995).

Animasaun et al. (2014) reported that in fonio, the number of internodes decreased as gamma irradiation doses increased. Similarly, Ahmed et al. (2017) found that in the wheat Batoor variety, gamma irradiation reduced the number of internodes with increasing doses. However, in the Janbaz variety, irradiation up to 15 kR tended to increase the number of internodes, followed by a decrease at higher dose levels. Several researchers previously reported the effects of irradiation on the number of plant branches. Hegazi and Hamideldin (2010) reported that gamma irradiation up to a dose of 400Gy increased the number of okra branches, then decreased starting at the dose of 500Gy. Similar result reported Shala (2019) in Basil (*Ocimum basilicum* L.) and Vinodh and Kannan (2020) in Crossandra (*Crossandra infundibuliformis* (L.) Nees.), where gamma irradiation increased number of branches up to a certain dose, after which it declined. However, Elizar et al. (2018) reported that gamma irradiation reduced the number of branches of kenikir as the irradiation dose increased. According to Elizar et al. (2018), branch formation was associated with internodes development; as more internodes form, the potential for branch formation increases. Irradiation affected the process of plant physiological damage which had an impact on the number of branches per plant. Gamma irradiation up to a certain dose could increase the number of branches. The higher the irradiation dose given; the fewer branches would be formed. The number of leaves was correlated with the number of branches. The more branches that could be formed, the greater the potential number of leaves could increase. Irradiation affected the process of plant physiological damage which had an impact on the number of leaves. Up to a certain dose, gamma irradiation can increase leaves numbers, but at higher doses, leaves formation decreases. Several researchers reported the effect of gamma irradiation on leaf number. Supanjani et al., 2024) reported that gamma irradiation with the dose up to 200Gy had no effect on the number of leaves of green bean. However, increasing the irradiation dose caused the number of leaves to decrease significantly. Asare et al. (2017) reported that increasing gamma irradiation caused a decrease in the number of leaves on okra. Atteh and Adeyeye (2022) reported different results in mung bean (*Vigna radiata* L.), pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.). Increasing the dose of gamma irradiation had no effect on the number of leaves in mung bean plants, but it did cause an increase in the number of leaves in pea and faba bean, although it was not significant.

Crop Yield

Several researchers reported the effect of gamma irradiation on seed number. Animasaun et al. (2014) reported that gamma irradiation with a dose of up to 80Gy increased the number of seeds per panicle in fonio, then decreased as the irradiation dose increased. Ahmed et al. (2017) reported different results on two wheat varieties. Gamma irradiation caused an increase in the number of seeds per panicle in the Batoor variety up to the dose of 15 kR, then decreased as the higher irradiation dose. Meanwhile, in the Janbaz variety, the number of seeds per panicle decreased as the irradiation dose increased. Khah and Verma (2015) reported that gamma irradiation treatment on wheat resulted in a decrease in the number of seeds per plant as the irradiation dose increased. A similar result was also reported by Khan et al. (2003) in barley. An increase in the number of empty seeds with rising radiation doses has been reported by Yunus et al. (2017) in rice plants. This occurrence of empty seeds is thought to be caused by pollen sterility (Ridha, 2019). Higher pollen sterility reduces fertilization success, leading to failure in seed formation. This caused the empty seeds to increase and impacting on the weight of the seeds to decrease.

Several studies have reported the effects of gamma irradiation on the weight of 100 or 1000 seeds. Khah and Verma (2015) observed a decrease in the weight of 100 seeds in wheat cv. KH-147 as irradiation doses increased. In contrast, Khan et al. (2003) found that the weight of 1000 seeds in barley increased with higher irradiation doses, a trend also observed by Ahmed et al. (2017) in wheat cv. Batoor. However, wheat cv. Janbaz showed an increase in 1000-seed weight up to 15kR, followed by a decrease at higher doses. Similar patterns were reported by Jiya et al. (2018) in rice cultivars FARO 44 and FARO 60, Pujiyanti et al. (2021) in red rice cv. Barak Cenana, Thenuja et al. (2024) in groundnut, and Rubasinghe et al. (2024) in black gram, where gamma irradiation increased 100 seed weight up to a certain dose, after which it declined.

Several researchers reported the effect of gamma irradiation on seed weight per plant. Rahimi and Bahrani

(2011) reported that gamma irradiation doses up to 50 Gy did not affect seed weight per wheat plant, then decreased as the increasing of irradiation doses. Different result was reported by Sankar et al. (2021), where seed weight per plant of cowpea plants decreased with the increasing irradiation dose. Hegazi and Hamideldin (2010) reported different results in okra. Gamma irradiation up to a dose of 400 Gy increased seed weight per plant, then decreased as the increase of irradiation dose. A similar result was observed in barley (Hussein, 2022), corn (Kikakedimau et al., 2022), and groundnut (Thenuja et al., 2024), where gamma irradiation increased plant yield up to a certain dose, after which it declined.

Conclusion

Gamma irradiation had a significant impact on the growth and development of adlay plants. These effects encompassed a reduction in the percentage of viable seedlings, reduction of seedlings height and root length, extended time to flowering, induction of chlorophyll mutations, increased pollen sterility and delayed maturity. Additionally, gamma irradiation had implications for adlay plant yield, leading to an increase in the number of seeds per plant, a higher proportion of empty seeds, reduced 100-seeds weight, and decrease in seed weight per plant. The optimal dose of gamma irradiation on Adlay was found to be in between 200 to 300Gy, making plants derived from these doses promising candidates for future adlay breeding process.

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