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RESEARCH ARTICLE
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Mutagenic Effectiveness and Efficiency of Gamma-rays Treatment on Adlay (*Coix lacryma-jobi* L.)

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ABSTRACT

Adlay has the potential to be developed as an alternative and functional food. However, this plant has a low yield and takes a long time to harvest. Mutation breeding is carried out to increase diversity and obtain adlay plants that exhibit early maturing and high-yield traits. The effectiveness and efficiency of mutagens are important factors in determining the success of mutation breeding. This study evaluated the effectiveness and efficiency of mutagens from various doses of gamma rays in inducing variability that can be utilized in the genetic improvement of adlay plants. Adlay seeds of the Watani Wado variety were treated with gamma-ray irradiation at doses of 0 (control), 200, 250, 300, 350, and 400Gy. A total of 100 seeds with three replicates of each treatment dose were grown to become M_1 plants. A total of 3960 seeds from M_1 plants were grown to become M_2 plants. The results showed that gamma-ray irradiation at 250Gy resulted in the highest chlorophyll mutation frequency, mutagenic effectiveness, and efficiency in adlay. Higher doses led to a decline in effectiveness and efficiency, accompanied by increased biological damage, such as reduced seedling height and higher pollen sterility. These findings suggest that lower doses of gamma irradiation are more effective in inducing beneficial mutation while minimizing adverse effects. Understanding the optimal mutagen dose can be used by breeders to identify populations effectively at early generations to reduce costs and increase selection scope.

Keywords: Adlay; Gamma-rays; Mutagenic effectiveness; Mutagenic efficiency; Mutation

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INTRODUCTION

Adlay (*Coix lacryma-jobi* L.) is one of the underutilized crops that is grown for human consumption. This plant originated from tropical and subtropical regions of Asia. It is potentially to be developed as an alternative and functional food (Suyadi et al. 2019). Adlay seeds are usually processed into porridge, but they are also presently processed into various products such as ice cream (Khongjeamsiri et al. 2011), butter cake (Kutschera and Krasaekoopt 2012), yogurt (Keeratibunharn and Krasaekoopt 2013), and saltine crackers (Andoy et al. 2019). Adlay flour can be used as a substitute for wheat flour as a raw ingredient for cakes. It is appropriate for making brownies and other pastries that do not require a lot of dough swelling power (Nurmala et al. 2013). Adlay seeds are a functional food with a low glycemic index that is advantageous for diabetics (Tensiska et al. 2019) and used as a dietary therapy for obese patients (Kim et al. 2004).

Watani Wado is one of the Indonesian local varieties that are grown in West Java. This local variety is widely cultivated, especially in the Sumedang area. Watani Wado is a soft-shelled adlay with a white seed shell. According to Center for Plant Variety Protection (2017), this local variety has a yield potential of up to 6.04 $tons.ha^{-1}$ with an average yield of 2 to 3 $tons.ha^{-1}$. The cultivation of this local variety could take up to 154 days, which is one of its weaknesses.

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The constraint in adlay breeding is the low level of genetic diversity of the plant (Ma et al. 2010; Fu et al. 2019; Xuan et al. 2020). One of the efforts to increase genetic diversity is through mutation induction (Wei et al. 2013). Mutation induction has been successful in obtaining new cultivars of numerous grain commodities, including wheat (Singh and Balyan, 2009), rice (Viana et al. 2019), and sorghum (Human et al. 2020). Shen (2017) performed mutation induction in adlay using gamma irradiation and EMS and found a high genetic variation.

The effectiveness and efficiency of mutagens are very important in mutation breeding. The frequency of mutations that occur as a result of induction from each dose of mutagen is considered the effectiveness of the mutagen, while the efficiency of the mutagen describes mutations based on biological damage which includes sterility, injury, and death. The effectiveness and efficiency of mutagens are necessary for mutation breeding programs to obtain the desired mutations in plants (Khursheed et al. 2018). The effectiveness and efficiency of a mutagen depend on the type of genotype used and the mutagen applied. Several researchers have reported the effectiveness and efficiency of several mutagens in various crops, including faba bean (Khursheed et al. 2018), black gram (Goyal et al. 2020; Makeen and Babu 2010), and cowpea (Raina et al. 2022).

This research was conducted to assess the effectiveness and efficiency of mutagen from various doses of gamma rays in inducing variability that can be utilized in the genetic improvement of adlay plants. This is carried out to determine the optimal dose of mutagens that can be used by breeder to identify populations effectively at early generation to reduce costs and increase selection scope.

MATERIALS & METHODS

Seeds of the Watani Wado variety were treated with gamma-ray irradiation from Cobalt-60 sources with doses of 200, 250, 300, 350, and 400Gy at the Research Center for Radiation Processing Technology (PRTPR) – National Research and Innovation Agency (BRIN). Field trials were carried out in June 2022 to January 2023 for the M_1 generation and February to August 2023 for the M_2 generation in the Ciparanje experimental station of Padjadjaran University, Jatinangor, Sumedang, West Java. One hundred seeds from each treatment and wild-type were planted in the germination boxes and repeated three times in the screen house to grow the M_1 generation. Germination boxes were arranged randomly without an experimental design. Three weeks of seedlings were transferred to the field in experimental plots with a spacing of 0.6m×0.4m. Experimental plots were arranged randomly without an experimental design. A half $kg.m^{-2}$ of manure was applied as a base fertilizer while $20g.m^{-2}$ of composite fertilizer (NPK 16-16-16) was used as the primary fertilizer. Maintenance such as irrigation, weed control, and pest and disease control was carried out to obtain optimal crop yields.

A total of 3960 seeds from M_1 and wild-type plants were planted in the M_2 generation. Seeds are sown directly in the field with a spacing of 0.6 m × 0.4 m. The trial plots were arranged using an Augmented Block Design. Maintenance was carried out to obtain optimal M_2 plant yields.

Mutation frequency was calculated based on chlorophyll mutations that occurred in three weeks plants for lethal chlorophyll mutation and eight weeks plants for viable chlorophyll mutation. Chlorophyll mutations were detected by changes in the leaves according to the classification of Muszyński (1968). The frequency of chlorophyll mutations was calculated using Equation 1 (Gaul 1964).

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

The effectiveness of a mutagen is a measurement of the frequency of mutation induction at each dose of a given mutagen. The effectiveness of the mutagen in the M_1 and M_2 generations was measured using Equation 2 (Konzak et al. 1965).

$$Mutagenic\ effectiveness\ (gamma-rays) = \frac{Mutation\ frequency\ at\ M1\ or\ M2\ generation}{Doses\ (Gy)} \quad (2)$$

Biological damage is measured by two parameters, namely seed injury (injury = I) and pollen sterility (sterility = S) (Goyal et al. 2020). Seedling height was measured three weeks after planting. The percentage of seedling injury was calculated from the percentage reduction in shoot height growth compared to wild-type plant height. Pollen for sterility observation was taken at the time of anthesis. A total of 15 plants at each treatment dose were taken randomly as a sample and 3 flowers were taken from each plant. Preparation of the object was carried out using the method according to Virmani et al. (1997) with modifications. The pollen is placed on the object glass then dripped with 1% Lugol's (I_2KI) solution (Sigma Aldrich) and covered with a coverslip. Observations were made under a microscope with a magnification of 100x. Pollen that is darkly colored and normally shaped is fertile while pollen that is colored but light, empty, and wrinkled is counted as sterile pollen (Gowthami et al. 2016). Pollen sterility is calculated from the percentage of sterile pollen compared to the total pollen count. Mutagenic efficiency in the M_1 and M_2 generations was measured using Equation 3 (Konzak et al. 1965).

$$Mutagenic\ efficiency = \frac{Mutation\ frequency\ at\ M1\ or\ M2\ generation}{Biological\ damage\ at\ M1\ generation} \quad (3)$$

where the biological damage in this study included two criteria, namely 1) Injury (seed injury): percentage reduction in seedling height; and 2) Sterility: percentage reduction in pollen fertility (Goyal et al. 2020).

RESULTS & DISCUSSION

Gamma-ray irradiation is a form of ionizing radiation that has enough energy to induce mutation by breaking chemical bonds in DNA, leading to various genetic alterations (Çelik and Atak, 2017). In this study, gamma-ray irradiation at doses of 200 to 400Gy produced chlorophyll mutations (Fig. 1) with various frequencies in the adlay (Table 1). The results show a non-linear relationship between the radiation dose and the mutation frequency. The frequency of mutation increases as the dose of gamma rays increases from 200Gy to a certain threshold

Table 1: The effectiveness of several dose of gamma-rays irradiation treatments on adlay var. Watani Wado

Irradiation doses (Gy)	Number of plants		Number of Mutated plants		Mutation frequency (%)		Mutagenic effectiveness	
	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
0	132	440	0	0	0	0	-	-
200	107	1188	7	9	6.54	0.76	0.03	0.0037
250	75	1034	20	12	26.67	1.16	0.11	0.0046
300	56	582	11	6	19.64	1.03	0.07	0.0034
350	58	144	9	2	15.51	1.39	0.04	0.0040
400	51	150	13	0	25.49	0	0.06	0

Fig. 1: Chlorophyll mutation in adlay leaves a. normal leaf; b. albina; c. striata; d. marginata.

(250Gy in M_1 and 350Gy in M_2). This trend is likely due to the increasing extent of DNA damage with higher radiation doses, which raises the probability of mutation occurring (Li et al., 2021). However, mutation frequency declines at doses above these thresholds (300Gy for M_1 and 400Gy for M_2).

This decline at higher doses might be attributed to several factors. High doses of gamma irradiation can cause extensive DNA damage, leading to cell death rather than mutation. Cells that suffer from irreparable DNA damage may not survive or reproduce, reducing the overall number of mutant cells in the population (Hall and Giaccia, 2012). At higher doses, cellular repair mechanisms might become overwhelmed, leading to lethal mutations or a complete failure in repairing the DNA, resulting in cell death rather than survivable mutations (Jeggo and Löbrich, 2006). High doses might impose a strong selection pressure, where only a few cells with specific mutations survive, leading to a reduced overall mutation frequency (Datta and Chakrabarty, 2005).

Mutagenic effectiveness refers to the ability of a specific dose of a mutagen to induce mutations within an organism (Konzak et al. 1965). This concept is critical for understanding the efficiency of different mutagenic treatment and their potential to generate genetic variation. The effectiveness of a mutagen is not uniform across doses and can vary significantly depending on the dose applied and the genotype of the organism being treated (Raina et al. 2022).

The genotypic response to increasing doses of mutagen is an essential aspect of mutation breeding. It reflects the mutagen's effectiveness, which is influenced by the genotype's inherent sensitivity to the mutagen (Khursheed et al. 2018). According to Raina et al. (2022), the sensitivity of the genotype to a particular mutagen is reflected in a mutagen's effectiveness. This means that there is an optimal mutagen dose that maximizes mutation frequency without causing excessive cellular damage or lethality.

The most effective mutagen dose in this study was 250Gy with mutagenic effectiveness values of 0.11 in the M_1 generation and 0.0046 in the M_2 generation. The identification of the most effective dose, 250Gy in this case, is consistent with the need to optimize treatment for different generation (Bhosle and Kothekar, 2010). These values indicate the efficiency of the dose in producing mutations that are expressed in the subsequent generations (Goyal et al., 2020). The mutagenic effectiveness increased with gamma irradiation up to 250Gy but decreased at higher doses (Table 1), suggesting a threshold beyond which the mutagen's ability to induce viable mutations diminishes (Raina et al., 2022).

A decrease in mutagenic effectiveness at higher doses has been observed across various plant species. For example, in finger millet (*Eleusine coracana*), Ambavane et al. (2015) reported a similar decline in mutagenic effectiveness at higher doses of gamma irradiation. Similarly, Julia et al. (2018) In Indian mustard (*Brassica juncea*), mutagenic effectiveness decreased with higher doses of mutagenic agent. A study in fonio (*Digitaria exilis*) by Nura et al. (2021) Also, it supported these findings by showing that after a certain dose, the mutagen's ability to induce beneficial mutation was reduced, likely due to increased cellular damage or lethality.

The decrease in the effectiveness of mutagens with increasing doses of gamma-ray irradiation may be associated with a more prominent mutational effect in cells that causes meiotic anomalies, bio-physiological changes, and reduced cell survival (Raina et al. 2022). These effects can impair the ability of the cells to survive and proliferate, thereby increasing the overall frequency of observable mutations. This concept is supported by the work of Bhosle and Kothekar (2010) on cluster beans (*Cyamopsis tetragonoloba*), where gamma irradiation at doses of 5 to 15kR led to reduced mutagenic effectiveness due to increased cellular damage. Similarly, Nair and Mehta (2014) reported that a decrease in the effectiveness of higher doses of mutagens can be attributed to the failure to increase the frequency of mutations proportionally with increasing doses or concentrations. This could be due to the saturation of cellular repair mechanisms or the induction of lethal mutation that prevents the survival and expression of viable mutants.

While gamma-ray irradiation is an effective tool for inducing mutations, its effectiveness is highly dependent on the dose (Shu et al., 2011). There is a critical balance between inducing mutations and causing excessive cellular damage, with an optimal dose maximizing the mutagenic effectiveness (Jain, 2010). The decrease in effectiveness at higher doses is a common phenomenon across species and highlights the importance of careful dose optimization in mutation breeding programs (Ahloowalia et al., 2004). Understanding the relationship between dose and mutagenic effectiveness is crucial for developing strategies that enhance genetic diversity while minimizing the adverse effects of irradiation (Shu et al., 2011).

Gamma-ray irradiation is widely recognized as a mutagenic agent that causes significant biological damage to plants, including adlay. The inhibition of plant germination at increased doses of gamma-ray irradiation (Fig. 2) suggests that radiation causes considerable damage at the cellular and molecular levels, leading to impaired metabolic functions necessary for germination and early seedling growth (Khursheed et al., 2018). This effect is confirmed by a dose-dependent increase in the reduction of seedling height, as shown in Table 2, indicating that higher doses of gamma-ray irradiation result in more severe growth inhibition (Ambavane et al., 2015).

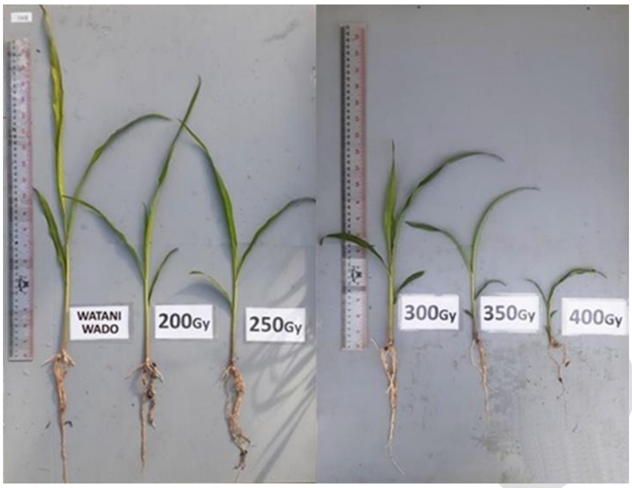

Fig. 2: Reduction in the height of adlay seedlings due to several doses of gamma ray irradiation treatment.

Moreover, gamma-ray irradiation has been shown to cause pollen sterility in adlay (Fig. 3), with sterility increasing alongside the irradiation dose (Table 2). The highest recorded sterility was at dose of 400Gy, where 32.28% of the pollen became sterile, significantly affecting the plant's reproductive success (Raina et al., 2022). This finding is consistent with earlier studies that have shown similar effect in other species. For instance, Hayati et al. (2022) reported that gamma-ray irradiation in yam bean (*Pachyrhizus erosus*) resulted in a marked increase in pollen sterility, which was also dose-dependent. This emphasizes the broad-spectrum impact of gamma-ray irradiation on plant fertility across different species.

The concept of mutagenic efficiency, defined as the ratio of mutations to undesired biological effects like sterility, injury, and death, is crucial for understanding the efficacy of mutagenic treatments (Khursheed et al. 2018; Konzak et al. 1965). Mutagenic efficiency can be calculated based on seed injury or pollen sterility approaches. The highest mutagen efficiency resulted from an irradiation dose of 250Gy, with value of 0.66 and 0.029 when assessed using injury approach, and 1.41 and 0.062 when evaluated using the sterility approach (Table 2). This dose appears to achieve an optimal balance between inducing beneficial mutation and minimizing the harmful effect of irradiation (Ambavane et al., 2015). Beyond this dose, mutagenic efficiency declines, likely due to excessive biological damage caused by a higher irradiation level (Raina et al., 2022). The same pattern occurs in other crops, such as black gram (Goyal et al. 2020) and cowpea (Raina et al. 2022).

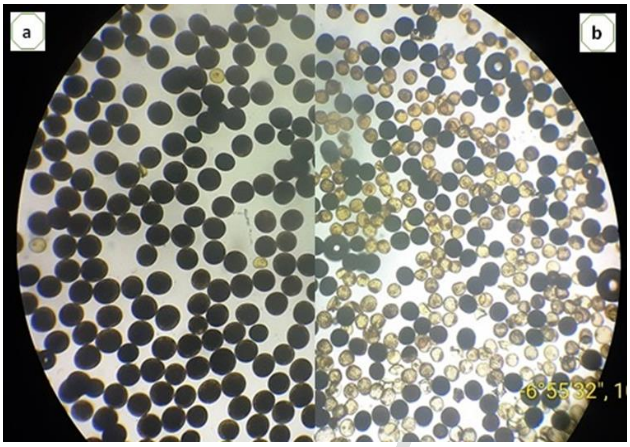

Fig. 3: Pollen sterility in adlay due to gamma ray irradiation treatment. a. pollen in normal plant; b. pollen in 400Gy treatment plant. Pollen that is darkly colored and normally shaped is fertile while pollen that is colored but light, empty, and wrinkled is counted as sterile pollen.

The efficiency of a mutagen depends on many factors such as the reactivity of the mutagen with the substance, its application to biological systems, and the biological damage that occurs (Shah et al. 2008). The higher efficiency reflects relatively less biological damage (seedling injury, pollen sterility, meiotic anomalies) to the induced mutations (Raina et al. 2022). Mutagenic efficiency in this study is evaluated based on criteria such as seedling injury (Mf/I), and pollen sterility (Mf/S). The efficiency determined by pollen sterility was higher than that determined by seedling injury. This might be due to sterile pollen occurs less frequently than seedling injury rates.

The calculation of mutagenic efficiency based on seedling injury is often preferred in practical breeding programs because it allows for early-stage evaluation of mutagenic effect (Khursheed et al. 2018). Seedling injury can assessed soon after germination, offering a rapid method for evaluating the impact of mutagenic treatment. this early-stage assessment is particularly useful in large scale mutation breeding programs where rapid screening of numerous lines is necessary. Further supporting this approach, Goyal et al. (2020) demonstrated that seedling injury was reliable indicator of mutagenic efficiency in black gram, where early-stage injury assessment correlated well with later-stage phenotypic mutation. Similarly, studies on cape goosberry (*Physalis peruviana*) by Gupta et al. (2018) found that seedling injury provided a consistent measure of mutagenic efficiency, helping breeder to identify potentially valuable mutant lines quickly.

However, it is important to note that while seedling injury is a convenient and early measure, it might not capture all the mutagenic events that occur later in plant development. For instance, Das and Prusti (2020) observed in Greengram (*Vigna radiata*) that some mutations affecting pollen sterility were not evident until later stages

Table 2: Mutagenic efficiency of several doses of gamma-rays irradiation treatments on adlay var. Watani Wado

Iradiation doses (Gy)	% Injury (I)	% Sterility (S)	Mutation frequency		Mutagenic efficiency			
			M_1 (Mf1)	M_2 (Mf2)	Mf1/I	Mf1/S	Mf2/I	Mf2/S
0	-	4.27	0	0	-	-	-	-
200	29.51	18.25	6.54	0.76	0.22	0.36	0.026	0.041
250	40.18	18.85	26.67	1.16	0.66	1.41	0.029	0.062
300	59.69	27.95	19.64	1.03	0.33	0.70	0.017	0.037
350	57.13	26.41	15.51	1.39	0.27	0.59	0.024	0.052
400	66.30	32.28	25.49	0	0.38	0.79	0	0

Note: I represents the percentage of injury based on reduction of seedling height; S represents the percentage of pollen sterility, Mf1 denotes the mutation frequency in M_1 generation, Mf2 denotes the mutation frequency in M_2 generation.

of growth. This suggests that a combination of early and late-stage evaluation might be necessary to comprehensively assess mutagenic efficiency.

The success of mutation breeding programs depends on the careful selection of mutagen dose. Lower doses of gamma irradiation on adlay are preferred to produce higher mutation frequencies and fewer biological damage. Understanding the optimal mutagen dose is crucial for the success of mutation breeding programs because it shows us how to balance beneficial and undesired mutations.

Conclusion

In conclusion, gamma-ray irradiation at dose of 250Gy was found to be the most effective in inducing chlorophyll mutations in adlay, while minimizing biological damage. This dose may be utilized as a reference point in the future mutation breeding programs.

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Conflict of Interest: The authors declare that there is no conflict of interest.

Authors' Contribution: RKA, WAQ, MR, and FD conceived and designed the experiment. RKA performed the study. WAQ supervised and coordinated the experiments. RKA performed statistical analyses of experimental data. RKA prepared the draft of the manuscript. SA and A provided advice on experimental activities. All authors critically revised the manuscript and approved the final version.

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