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## INDUCED MUTAGENESIS USING GAMMA RAYS, ETHYL METHANESULFONATE, AND COLCHICINE TO ENHANCE THE GENETIC VARIABILITY IN GARLIC (*ALLIUM SATIVUM* L.)

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### SUMMARY

The Indonesian garlic (*Allium sativum* L.), with small bulbs and cloves, requires improvement. Mutation breeding offers a valuable approach for inducing variability in vegetatively propagated crops. This study aimed to determine the LD<sub>50</sub> and assess the morphological responses of two garlic cultivars, Sangga Sembalun and Lumbu Putih, to gamma rays, ethyl methanesulfonate (EMS), and colchicine for identifying effective mutagens for bulbs and cloves' improvement. Gamma rays applied were 0, 6, 7, 8, 9, 10, and 11 Gy, while EMS and colchicine rates were at 0%, 0.2%, 0.4%, and 0.6%. Both cultivars differed in germination and growth under mutagenic treatments, with LD<sub>50</sub> values of 9.54 and 11.35 Gy (gamma), 1.19% and 0.67% (EMS), and 1.25% and 3.58% (colchicine) for Sangga Sembalun and Lumbu Putih, respectively. Foliage color was the most responsive trait. Gamma irradiation caused the greatest growth suppression, whereas EMS and colchicine treatments enhanced bulb and clove size by 8.35% and 36.4%, respectively. Putative mutants predominantly appeared at the LD<sub>50</sub> level and served as key genetic resources for developing superior Indonesian garlic cultivars.

**Keywords:** Indonesian garlic (*A. sativum* L.), genetic variability, mutation breeding, physical mutagens, chemical mutagens, LD<sub>50</sub>

**Key findings:** For garlic (*A. sativum* L.) mutagenesis, the LD<sub>50</sub> of gamma rays, EMS, and colchicine has succeeded in their determination as the optimal dose to generate the genetic variability. The breeding material comprising garlic mutants can be applicable to developing superior cultivars.

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## INTRODUCTION

Garlic (*Allium sativum* L.) is a widely cultivated vegetable crop used for seasoning and medicinal purposes. Likewise, in Indonesia, it is a typical ingredient in almost all their cuisines. Indonesia's garlic demand is nearly 600,000 tons per year; but ironically, the country's production is only 89,000 tons per year, with 85% of the garlic being imported (Data Center and Agriculture Information, 2020). The local garlic has smaller bulbs, making it less popular, with farmers uninterested in its planting because of a lesser market value. Therefore, it is crucial to improve the local garlic, especially the bulb and clove sizes.

The absence of true seed production in garlic results in vegetative propagation, thereby restricting hybridization, genetic exchange, and overall genetic variability (Yousef and Elsadek, 2020). Unfortunately, without a broad genetic diversity, it is difficult for the breeders to select promising garlic genotypes. Therefore, genetic variability requires enhancement through mutation in garlic.

Improving Indonesian garlic quality, particularly bulb size, can succeed through local variety selection and mutation breeding. However, the insufficiency of superior cultivars necessitates mutation induction as a critical strategy for generating novel clones with larger bulbs. Mutation refers to a heritable genetic alteration that may occur spontaneously or inductively by mutagens, resulting in changes to nucleotide sequences or chromosomal structure. Induced mutagenesis has had effective employment to enhance crop genetics, producing significant morphological, physiological, and biochemical variations (Mahajan *et al.*, 2015; Benke *et al.*, 2019). It is particularly valuable for improving vegetatively propagated or sterile crops, where conventional breeding is lacking (Pawar *et al.*, 2020). Physical and chemical mutagens' usage has succeeded in generating genetic variability in garlic by modifying biochemical processes (El-Fiki and Adly, 2020). Previous mutagenesis research on local Indonesian garlic cultivars has employed various mutagens on Doulu

(Gultom *et al.*, 2020), Tawangmangu Baru, Lumbu Kuning, and Ciwidey (Pangestuti *et al.*, 2020; Winarni *et al.*, 2022; Ulum *et al.*, 2023).

Ionizing radiation is a widely used technique to induce beneficial mutations in crops due to its efficiency, simplicity, and ability to generate high mutation frequencies and promote recessive gene expression (El-Azab *et al.*, 2018; Hong *et al.*, 2022). Gamma rays—highly penetrative electromagnetic waves emitted during radioactive decay—effectively induce DNA decay leading to mutation (Kiani *et al.*, 2022; Puripunyanich *et al.*, 2022). Among physical mutagens, they are the main preferences for their effectiveness and relative safety (Afram *et al.*, 2024). In garlic, radiosensitivity varies with cultivar, bulb size, and dormancy status (Mahmoud *et al.*, 2019). Determining the lethal dose 50% (LD<sub>50</sub>)—the dose causing 50% mortality—is essential to balance mutation frequency and plant survival (Fitriani *et al.*, 2021; Ghasemi-Soloklui *et al.*, 2023).

EMS has become the most effective chemical mutagen for inducing point mutations in plants, and the successful use of chemical mutagens relies on the dosage, exposure duration, and their ability to produce beneficial genetic variations with minimum undesirable alterations (Singh *et al.*, 2021). EMS is an alkylating mutagen that introduces gene mutation at a higher frequency while causing chromosomal abnormalities at a lower rate (Hong *et al.*, 2022).

Polyploidy, the heritable condition of possessing more than two complete chromosome sets per nucleus, is a key evolutionary mechanism exploited in plant breeding to enhance genetic and phenotypic diversity (Nagat *et al.*, 2020; Priyadarshini *et al.*, 2021). It serves as a powerful tool for developing superior germplasm and introducing useful traits through chromosome set alteration in crop plants (Yousef and Elsadek, 2020). Lumbu Putih and Sangga Sembalun are Indonesian garlic cultivars characterized by high adaptability and productivity; however, improvement in bulb size remains necessary. This study aimed to determine LD<sub>50</sub> values and evaluate

morphological variations induced by gamma rays, EMS, and colchicine in these two Indonesian garlic cultivars.

## MATERIALS AND METHODS

### Breeding material

Two Indonesian garlic cultivars, Sangga Sembalun (from Nusa Tenggara Barat) and Lumbu Putih (from Yogyakarta), were specimens selected for the study due to their adaptability to lowland conditions. Uniform cloves totaling 5,400, comprising 2,700 cloves per cultivar, underwent mutagenesis treatments. Each mutagenesis treatment utilized 60 cloves per cultivar per replication.

### Treatments used

Garlic cloves sustained irradiation with cobalt-60 gamma rays at seven doses (0, 6, 7, 8, 9, 10, and 11 Gy) using a Gamma Chamber 4000A at the Center for Application of Isotopes and Radiation (PAIR-BATAN), Indonesia. EMS and colchicine applications were at 0%, 0.2%, 0.4%, and 0.6%, each supplemented with 4% DMSO (dimethyl sulfoxide, five drops) to enhance tissue penetration. Cloves reached soaking in the mutagenic solutions for 12 h at room temperature before rinsing three times with distilled water to remove residues.

### Experimental site and procedure

The experiment commenced in Pacet District, Mojokerto Regency, Indonesia (600 masl), under temperatures of 21 °C–26 °C and relative humidity of 67%–89%. The study arrangement was in a randomized complete block design with three replications. Treated garlic cloves entailed planting at 15 cm × 15 cm spacing and harvesting 110 days after planting (DAP). Observations included plant survival at 90 DAP, LD<sub>50</sub>, and growth and yield traits. Quantitative measurements included leaf length, leaf number, bulb diameter, and bulb weight, while qualitative observations covered foliage color and leaf attitude following IPGRI (International Plant Genetic Resources

Institute, 2001) descriptors. LD<sub>50</sub> values estimation used Probit analysis in SPSS software.

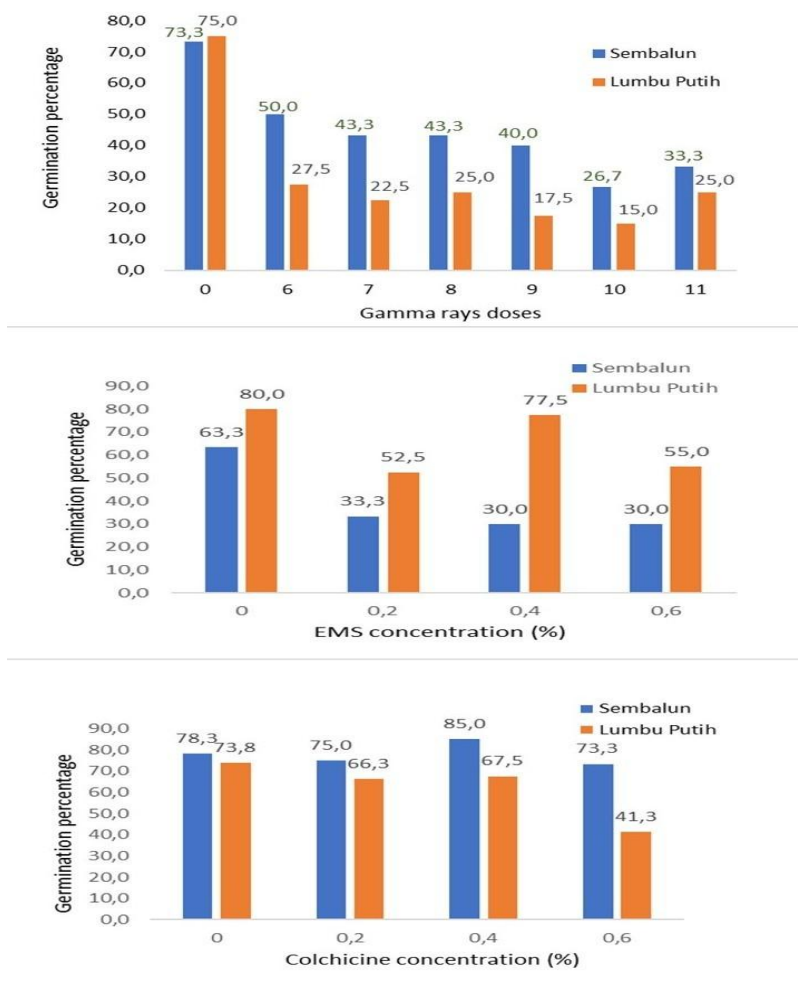
## RESULTS

### Mutagens effect on plant mortality and determination of LD<sub>50</sub>

All mutagenic treatments adversely affected clove germination, with increasing doses of gamma irradiation, EMS, and colchicine delaying emergence. In the control (non-mutagenized) plants, emergence began at 10 days after planting (DAP) and reached 100% by 16 DAP, whereas mutagen-treated plants first emerged at 12 DAP and, by 16 DAP, achieved only 33% emergence under gamma irradiation and EMS and 73% under colchicine treatment (data not shown). Gamma irradiation exerted the strongest inhibitory effect on growth and survival, and cultivar responses varied by mutagen, with Sangga Sembalun showing higher germination under gamma irradiation and colchicine, while Lumbu Putih performed better under EMS (Figure 1). The same observations also comprised reports in past studies while evaluating mutation-induced garlic plants (Mahmoud *et al.*, 2019; Benke *et al.*, 2019). Enhanced doses of gamma rays decreased the garlic clove germination in garlic cultivars (El-Fiki and Adly, 2020; Guerra *et al.*, 2022).

LD<sub>50</sub> determination depended on plant mortality at 90 DAP (Table 1), and the Probit model was statistically significant ( $P < 0.05$ ). Under gamma irradiation, 50% mortality occurred at approximately 9.5 Gy in cultivar Sangga Sembalun and 11.4 Gy in Lumbu Putih, with 95% confidence intervals of 8.8–10.3 Gy and 10.5–12.2 Gy, respectively. These values are comparable to those reported by Pangestuti *et al.* (2020) for Tawangmangu Baru (7.5 Gy) and Lumbu Kuning (10 Gy) and by Mahmoud *et al.* (2019) for Side-40 (6.4 Gy) and Balady (6.9 Gy), indicating a similar range of radiosensitivity among garlic cultivars.

However, EMS and colchicine treatments had varying effects on plant mortality (at 90 DAP) in both garlic cultivars.



**Figure 1.** Germination percentage of two garlic cultivars treated with gamma rays, EMS, and colchicine at 12 DAP.

**Table 1.** Effect of gamma rays, EMS, and colchicine on garlic plant survival and LD<sub>50</sub> at 90 DAP.

Cultivars	Gamma rays (gray)	Survived plants (%) over control	LD <sub>50</sub>	EMS (%)	Survived plants (%) over control	LD <sub>50</sub>	Colchicine (%)	Survived plants (%) over control	LD <sub>50</sub>
Sangga Sembalun	6	85.7	9.54	0.2	96.1	1.19	0.2	88.9	1.25
	7	67.8		0.4	88.5		0.4	96.3	
	8	71.5		0.6	73.0		0.6	70.3	
	9	53.6		-	-		-	-	
	10	53.6		-	-		-	-	
	11	17.9		-	-		-	-	
Lumbu Putih	6	96.9	11.35	0.2	97.3	0.67	0.2	96.9	3.33
	7	84.4		0.4	81.1		0.4	94.9	
	8	81.3		0.6	48.6		0.6	90.6	
	9	75.0		-	-		-	-	
	10	62.5		-	-		-	-	
	11	15.6		-	-		-	-	

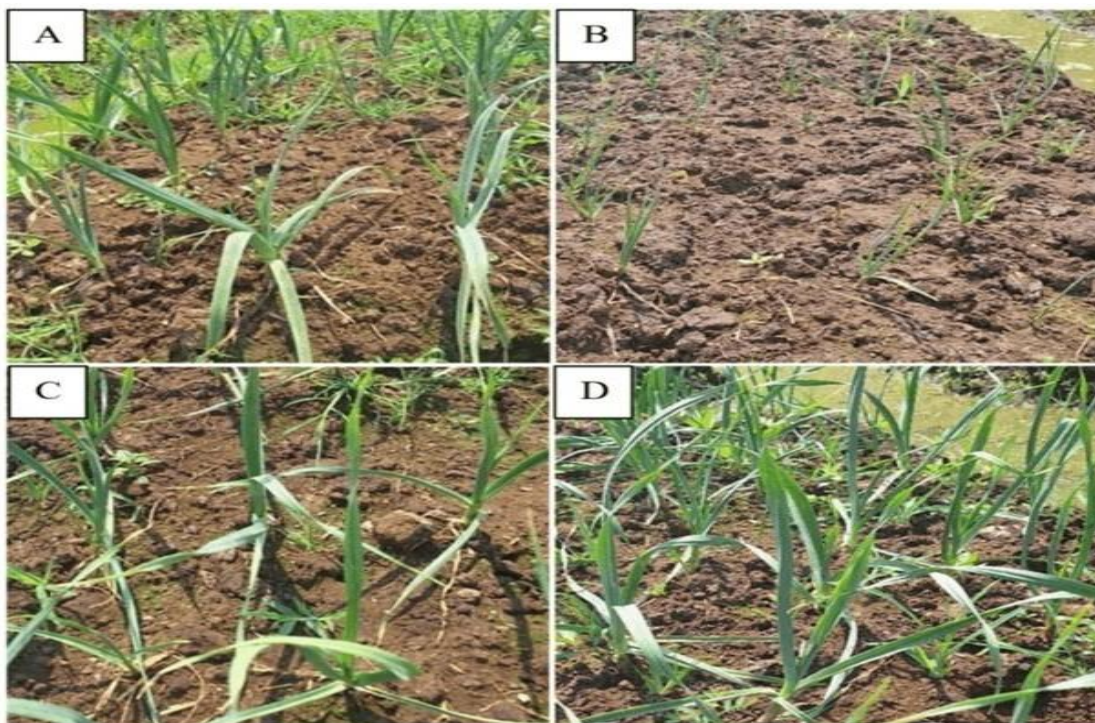
In the EMS treatment, the LD<sub>50</sub> concentration was twice as high in Sangga Sembalun as in Lumbu Putih, with 1.2% and 0.7%, respectively. This indicates the cultivar Lumbu Putih was more sensitive to this chemical mutagen than the cultivar Sangga Sembalun. Conversely, the cultivar Lumbu Putih had an LD<sub>50</sub> of 3.3%, which was about twice as high as that of Sangga Sembalun, at 1.3% under colchicine treatment. Similarly, Mahajan *et al.* (2015) reported 1.22% for EMS treatment and 0.097% for colchicine treatment. These results are consistent with the EMS LD<sub>50</sub> of 0.6% reported by Singh *et al.* (2021) and 1.6% by Banjare *et al.* (2017).

Among all mutagenic treatments, gamma irradiation exhibited the most pronounced effect on plant growth and survival. Although irradiated garlic cloves remained viable until harvest, their growth displayed a marked suppression compared with

those treated with EMS or colchicine (Figure 2). Conversely, EMS and colchicine treatments resulted in more vigorous plants that developed larger bulbs and cloves by 8.35% and 36.4%, respectively, compared with those exposed to gamma irradiation (Figure 3).

#### Mutagenesis effect on morphological traits

Mutagenesis induced morphological variations and enhanced genetic variability in garlic cultivars exposed to different mutagens. Most evaluated vegetative traits were foliage-related (IPGRI, 2001), with foliage color showing the highest responsiveness. Control plants typically exhibited dark to bluish-green foliage, while yellow-green variants were less frequent in both cultivars.



**Figure 2.** The growth of garlic cultivar Sangga Sembalun treated with mutagenic agents: without mutagen (A), gamma radiation at 8 gy (many plants developed in stress condition) (B), EMS at 6% (C), and Colchicine at 6% (D).



**Figure 3.** Garlic bulbs of cultivar Sangga Sembalun after mutagenic treatments, from left to right: gamma irradiation (0, 6, 7, 8, 9, 10, and 11 gy) (A); EMS (0%, 2%, 4%, and 6%) (B); and colchicine (0%, 2%, 4%, and 6%) (C).

Under gamma irradiation, the Sangga Sembalun mutant exhibited the greatest foliage color variation at 7 Gy, whereas Lumbu Putih showed the highest variation at 10 Gy (Table 2). Similarly, Benke *et al.* (2019) reported qualitative trait variations among gamma-irradiated garlic genotypes. EMS treatment induced noticeable foliage color changes at 0.2% and 0.6% in Sangga Sembalun and at 0.4% and 0.6% in Lumbu Putih (Table 3). Colchicine caused the strongest effect at 0.4% in Sangga Sembalun and across 0.2%–0.6% in Lumbu Putih. Despite these variations, dark green remained the predominant foliage color in both cultivars. Other foliage traits, including leaf width, density, attitude, and sheath characteristics, received less influence from mutagenic treatments. In both cultivars, increasing gamma doses reduced leaf length and leaf number, whereas EMS and colchicine caused only minor effects (Table 4).

Bulb size and weight expressed significant effects from mutagenic treatments, particularly gamma radiation. Increasing gamma-ray doses reduced bulb size and weight in both cultivars, whereas EMS and

colchicine treatments enhanced bulb and clove size (Figure 3). The cultivar Sangga Sembalun produced larger bulbs than the Lumbu Putih under EMS and colchicine treatments but showed no difference under gamma irradiation. Bulbs measuring 5–6 cm were most frequent under EMS (0.4%–0.6%) and all colchicine treatments, with colchicine yielding the highest proportion of large bulbs (>30 g). This aligns with reports that higher colchicine concentrations reduce survival rate (15%–51%) compared with the control but increase tetraploid frequency (16%) (Yousef and Elsadek, 2020). Taken together, the results reveal clear mutagen and genotype-specific responses that inform the selection of effective mutation strategies for garlic improvement.

## DISCUSSION

All mutagenic treatments inhibited garlic germination, with gamma irradiation exerting the strongest effect, likely due to physiological disruptions, such as oxidative stress and hormone imbalance. Gamma rays interact with cellular water to generate reactive oxygen

**Table 2.** Garlic traits following gamma ray-induced mutation in cultivars Sangga Sembalun and Lumbu Putih

Morphological traits	Gamma rays (Gray)													
	0		6		7		8		9		10		11	
	O	%	O	%	O	%	O	%	O	%	O	%	O	%
<b>Sangga Sembalun</b>														
Foliage color	D-G	61.5	B-G	95.2	G	8.3	Y-G	100	Y-G	100	Y-G	100	Y-G	100
	Y-G	38.5	Y-G		B-G	16.7								
					Gr-G	8.3								
				19.0	Y-G	66.7								
Leaf width (cm)	N	23.1	N	81.0	N	75.0	N	95.2	N	85.7	N	100	N	60
	M	30.8	M		M	25.0	M	4.8	M	14.3			M	40
	B	50.0		4.8										
Density of leaves	L	11.5	L	47.6	L	100	H	100	H	100	H	100	H	100
	M	50.0	M	47.6										
	H	46.2	H	28.6										
Foliage attitude	Sp	26.9	Sp	66.7	Sp	100	I	100	I	100	I	100	I	100
	I	61.5	I	4.8										
	E	15.4	E	100										
Leaf sheath	Sh	100	Sh	95.2	Sh	100	Sh	100	Sh	100	Sh	100	Sh	100
Leaf length (cm)	35.8		20.5		24.6		17.4		18.8		17.9		18.8	
Leaf number	9.6		5.5		7.7		6.3		6.2		6.7		7.6	
Bulb diameter (cm)	1.9	17.5	1.9	61.2	1.8	57.3	1.6	51.8	1.7	43.3	1.6	55.5	1.8	57.9
	2.8	28.3	2.9	25.6	2.8	32.2	2.7	36.5	2.8	45.3	2.6	37.1	2.6	42.1
	3.8	39.4	3.5	12.1	3.3	10.5	3.7	11.7	3.5	11.4	3.2	7.4		
	4.9	14.8	4.1	1.1										
Bulb weight (g)	3.7	15.6	3.2	55.1	3.4	60.1	3.5	55.2	3.7	53.1	3.9	63.3	3.2	57.4
	8.4	35.3	8.2	32.6	7.9	33.9	8.4	38.3	6.9	42.3	7.3	36.7	5.6	42.6
	13.1	31.8	11.8	9.2	11.3	6	12.2	6.5	10	4.6				
	19.6	17.3	15.7	3.1										
<b>Lumbu Putih</b>														
Foliage color	B-G	96.9	L-G	3.8	L-G	100	Y-G	100	Y-G	100	G	15.8	Gr-G	27.8
	Y-G	3.1	Y-G	96.2							GrG	15.8	D-G	16.7
											B-G	57.9	Y-G	55.6
											Y-G	10.5		
Leaf width (cm)	N	15.6	N	92.3	N	71	N	61	N	71	N	78.9	N	83.3
	M	6.3	M	3.8	M	29	M	29	M	29	M	21.1	M	16.7
	B	78.1	B	3.8										
Density of leaves	L	90.6	M	3.8	L	93.5	L	90.3	L	100	L	100	L	100
	H	9.4	H	96.2	H	6.5								
Foliage attitude	Sp	93.8	Sp	3.8	Sp	93.5	Sp	100	Sp	100	Sp	94.7	Sp	100
	I	6.3	I	96.2	I	6.5					E	5.3		
Leaf sheath	Sh	100	Sh	100	Sh	100	Sh	100	Sh	100	Sh	100	Sh	100
Leaf length (cm)	40.8		25.7		26.2		23.0		20.8		20.8		19.9	
Leaf number	7.7		7.1		6.3		6.4		6.2		6.2		4.2	
Bulb diameter (cm)	2.7	45.2	1.8	12.5	1.8	5.3	1.4	5	1.6	20	1.6	20	1.9	25
	3.5	54.8	2.7	68.8	2.6	79	2.5	80	2.7	40	2.2	60	2.4	75
			3.3	18.8	3.5	11	3.3	15	3.2	40	3.2	20		
				4.1	5.3									
Bulb weight (g)	3.3	3.2	3.3	37.5	3.8	21	3.5	50	2.9	40	3.6	60	3.0	75
	8.5	25.8	6.6	50	6.9	63	6.6	50	7.4	60	6.4	40	5.3	25
	12	48.4	10.8	12.5	13	11								
	19	22.6			16	5.3								

O: observation; D-G: dark-green; Y-G: yellow-green; Gr-G: gray-green; B-G: blue-green; N: narrow; M: middle; B: broad; L: low; H: high; Sp: spread; Sh: Short; I: intermediate; and E: erect.

**Table 3.** Garlic traits following EMS-induced mutation in cultivars Sangga Sembalun and Lumbu Putih.

Morphological traits	EMS concentrations (%)							
	0		0.2		0.4		0.6	
	O	%	O	%	O	%	O	%
<b>Sangga Sembalun</b>								
Foliage color	Gr-G	80.8	G	9.1	Gr-G	3.4	Gr-G	42.3
	D-G	19.1	Gr-G	27.3	D-G	96.6	D-G	53.8
			D-G	50.0			Y-G	3.8
			Y-G	13.6				
Leaf width (cm)	M	11.5	N	4.5	N	20.7	N	3.8
	B	88.5	M	50.0	M	79.3	M	11.5
		7.7	B	45.5	B		B	84.6
Density of leaves	L	84.6	L	22.7	L	24.1	L	84.6
	M	7.7	M	68.2	M	27.6	M	7.7
	H	69.2	H	9.1	H	48.3	H	7.7
Foliage attitude	Sp	23	Sp	36.4	Sp	27.6	Sp	76.9
	I	77	I	40.9	I	55.2	I	3.8
	E		E	22.7	E	17.2	E	15.4
Leaf sheath	Sh	100	Sh	100	Sh	100	Sh	100
Leaf length (cm)	43.9		44.3		44.7		41.4	
Leaf number	10.7		10.2		10.8		10.0	
Bulb diameter (cm)	1.8	11.2	1.9	16.4				
	2.9	23.9	2.8	27.9	2.8	18.4	2.7	12.9
	3.5	30.2	3.8	21.7	3.7	36.5	3.8	31.3
	4.5	19.9	4.7	20.9	4.3	25.3	4.3	39.4
	5.7	14.8	5.7	13.1	5.1	19.8	6.2	16.4
Bulb weight (g)	4.8	9.2	4.4	13.8				
	9.4	12.1	8.5	19.4	8.3	12.8	8.1	19.7
	13.3	25.3	14.6	22.6	12.8	28.3	13.8	26.9
	16.2	34.8	17.3	16.7	16.5	25.7	18.2	28.5
	23.7	10.3	24.8	10.3	22.1	17.1	23.3	14.9
	26.2	8.3	27.2	17.2	29.5	16.1	29.2	12
<b>Lumbu Putih</b>								
Foliage color	Gr-G	14.3	D-G	100	Gr-G	2.6	Gr-G	2.3
	D-G	85.7			D-G	60.5	D-G	29.5
					Y-G	36.8	Y-G	68.2
Leaf width (cm)	N	9.5	N	5.1	N	2.6	N	31.8
	M	26.2	M	20.5	M	42.1	M	59.1
	B	64.3	B	74.4	B	55.3	B	9.1
Density of leaves	L	100	L	100	L	100	L	100
Foliage attitude	Sp	16.7	E	100	E	100	E	100
	E	83.3						
Leaf sheath	Sh	100	Sh	100	Sh	100	Sh	100
Leaf length (cm)	49.0		50.1		45.6		36.2	
Leaf number	10.5		10.6		9.8		8.8	
Bulb diameter (cm)	1.8	4.4	1.6	1.7	2.4	35.4	2.7	35.7
	2.7	44.4	2.6	22.4	3.5	52.3	3.4	57.1
	3.4	37.8	3.5	58.6	4.2	12.3	4.2	7.1
	4.1	13.3	4.4	17.2				
Bulb weight (g)	3.5	11.1	3.4	3.4	3.2	3.08	3.1	7.1
	8.3	22.2	8.5	15.5	7.9	30.8	6.7	42.9
	11.7	40.0	13.3	36.2	12.4	35.4	12.2	35.7
	16.5	15.6	16.9	25.9	17.1	20	16.6	7.1
	22.1	6.7	22.8	12.1	23.4	10.8		
	26.8	4.4	27.9	5.2				

O: observation; D-G: dark-green; Y-G: yellow-green; Gr-G: gray-green; B-G: blue-green; N: narrow; M: middle; B: broad; L: low; H: high; Sp: spread; Sh: Short; I: intermediate; and E: erect.

**Table 4.** Garlic traits following colchicine-induced mutation in cultivars Sangga Sembalun and Lumbu Putih.

Morphological traits	Colchicine concentrations (%)							
	0		0.2		0.4		0.6	
	O	%	O	%	O	%	O	%
<b>Sangga Sembalun</b>								
Foliage color	D-G	88.9	D-G	100	Li-G	3.8	D-G	100
	Y-G	11.1			D-G	84.6		
Leaf width (cm)	N	11.1	N	8.3	N	26.9	N	20
	M	7.4	M	16.7	M	19.2	M	40
	B	81.5	B	75.0	B	53.8	B	40
Density of leaves	L	100	L	100	L	100	L	100
Foliage attitude	E	100	E	100	E	100	E	100
Leaf sheath	Sh	100	Sh	100	Sh	100	Sh	100
Leaf length (cm)	44.3		43.3		38.5		38.6	
Leaf number	9.8		9.5		8.6		9.9	
Bulb diameter (cm)	2.9	28.8	2.5	19.2	2.2	13.5	2.8	8.9
	3.3	39.9	3.8	38.7	3.2	19.9	3.5	42.4
	4.9	31.3	4.1	15	4.3	38.9	4.7	38.1
Bulb weight (g)			5.2	27.1	6.1	27.7	5.6	19.5
	7.5	16.8	8.2	12.9	6.6	5.1	7.4	10.1
	14.7	20.6	14.2	20.1	14.2	13.5	13.5	19.3
	18.5	29.3	19.2	27.2	17.8	20.2	18.3	37.8
	20.2	23.1	24.1	19.2	23.6	27.1	25.1	20.1
	26.8	10.2	29.4	12.2	28.4	20.9	29.3	12.7
			36.2	8.4	34.3	13.2		
<b>Lumbu Putih</b>								
Foliage color	D-G	92.2	Li-G	5.3	Gr-G	19.4	Gr-G	7.5
	Y-G	7.9	D-G	92.1	D-G	50	D-G	87.5
Leaf width (cm)			Y-G	2.6	Y-G	30.6	Y-G	5.0
	N	3.9	N	5.3	N	2.8	N	2.5
	M	15.7	M	5.3	M	5.6	M	47.5
Density of leaves	B	80.4	B	89.5	B	91.7	B	50
	L	54.9	L	71.1	L	77.8	L	97.5
Foliage attitude	M	45.1	M	28.9	M	22.2	M	2.5
	S	0	E	100	S	2.8	E	100
Leaf sheath	I	3.9			I	2.8		
	E	96.1			E	94.4		
	Sh	100	Sh	94.7	Sh	100	Sh	100
Leaf length (cm)	49.5		50.4		52.1		47.6	
Leaf number	8.9		8.4		8.3		9.9	
Bulb diameter (cm)	2.8	14.3	2.5	5.8	2.8	19.5	2.61	5.3
	3.5	57.1	3.6	77.1	3.7	48.8	3.62	65.8
	4.4	28.6	4.3	17.1	4.3	29.3	4.35	28.9
Bulb weight (g)					5.1	2.4		
	4.1	2.9	5.0	5.8				
	8.0	14.3	9.9	2.9	7.1	7.3	9.37	7.9
	13.7	20.0	13.1	17.1	12.4	19.5	12.9	13.2
	16.9	37.1	17.9	17.1	18.3	14.6	17.8	31.6
	22.2	14.3	21.8	40.0	23.2	41.5	22.6	31.6
	28.4	11.4	27.5	11.4	27.7	9.8	27.5	15.8
					39.1	7.3		

O: observation; D-G: dark-green; Y-G: yellow-green; Gr-G: gray-green; B-G: blue-green; N: narrow; M: middle; B: broad; L: low; H: high; Sp: spread; Sh: Short; I: intermediate; and E: erect.

species (ROS), which damage cellular structures and interfere with key metabolic processes, including photosynthesis, thylakoid organization, phenolic metabolism, and antioxidant activity (Kebeish *et al.*, 2015). Moreover, gamma irradiation alters endogenous growth regulators; the detection of indole-3-acetic acid in irradiated garlic cloves and perturbations in auxin homeostasis usually suppress shoot growth and early development (Crocchi *et al.*, 1990; Kurepa and Smalle, 2022).

Determining LD<sub>50</sub> is essential in mutation breeding to balance mutation frequency and plant survival. LD<sub>50</sub> values for Sangga Sembalun and Lumbu Putih emerged under gamma irradiation and EMS treatments. However, colchicine resulted in high survival rates, preventing LD<sub>50</sub> determination. This finding indicates the colchicine concentrations used were suboptimal and had a limited inhibitory effect on garlic growth. The optimal dose varies with genotype, developmental stage, tissue moisture, and treatment conditions (Singh *et al.*, 2021). The two garlic cultivars showed different mutagen sensitivities: the cultivar Sangga Sembalun was more sensitive to gamma irradiation and colchicine, while the Lumbu Putih incurred more effects from EMS. These differences reflect genotypic influences on radiosensitivity, consistent with previous reports (Mahmoud *et al.*, 2019; Kiani *et al.*, 2022). Variation in LD<sub>50</sub> values among studies and cultivars is largely due to differences in tissue age, anatomical structure, antioxidant capacity, and DNA repair efficiency, which together determine a plant's ability to mitigate gamma radiation-induced oxidative and genotoxic stress (Ghasemi-Soloklui *et al.*, 2025). Ionizing radiation induces structural and functional DNA alterations and generates ROS (Hong *et al.*, 2022).

High doses of gamma irradiation adversely affect plant viability, vigor, germination, growth, and fertility primarily through the generation of ROS and direct DNA damage, which impair cellular integrity and metabolic processes (Mahmoud *et al.*, 2019). The radiosensitivity varies among species and propagule types due to differences in genome

size, DNA repair capacity, antioxidant defense systems, and physiological status (Datta, 2019; Kiani *et al.*, 2022). In this study, garlic bulbs used as propagules were more sensitive to irradiation than seeds reported in chili crop (Makhziah *et al.*, 2023), reflecting tissue-specific susceptibility to oxidative stress and genomic damage. Despite reported cultivar-dependent variation in garlic radiosensitivity (El-Fiki and Fadly, 2020; Pangestuti *et al.*, 2020), the comparable LD<sub>50</sub> values observed for Lumbu Putih and Sangga Sembalun suggest similar physiological and molecular responses to gamma-induced stress.

EMS is an effective chemical mutagen that induces point mutations. The 12-h EMS treatment resulted in distinct LD<sub>50</sub> values, with Lumbu Putih showing greater sensitivity than Sangga Sembalun, indicating genotypic differences in repair or detoxification capacity. EMS predominantly induces genome-wide, non-lethal point mutations, mainly G/C to A/T transitions via guanine alkylation, with less frequent A/T to G/C transitions or G/C to T/A transversions arising from 3-ethyladenine mispairing and 7-ethylguanine hydrolysis (Shamshad *et al.*, 2023). Hong *et al.* (2023) identified 1.0% EMS for 8 h as optimal, whereas longer exposure markedly increased lethality, consistent with results in chili (Alcantara *et al.*, 1996) and soybean (Carroll *et al.*, 1986). These findings emphasize the importance of optimizing both EMS concentration and duration to balance mutation efficiency and plant viability.

The pronounced effect of colchicine on bulb enlargement suggests polyploidization can be effective in its usage to enhance garlic productivity. Lumbu Putih showed a higher LD<sub>50</sub> than Sangga Sembalun, reflecting genotypic variation in colchicine sensitivity, consistent with observations in rice anther culture (Ferrerres *et al.*, 2019). Increasing colchicine concentration suppressed germination and survival due to spindle disruption and abnormal chromosome segregation (Khah *et al.*, 2022). The significant rise in large-bulb frequency following 12-hour immersion demonstrates that optimal colchicine exposure can stimulate desirable morphological traits. Colchicine-induced

polyploidization enhances bulb growth through cell enlargement by disrupting microtubule formation and spindle assembly during mitosis (Sjahril *et al.*, 2023; Singh *et al.*, 2025). This process increases cell and tissue size, resulting in larger bulbs (Hasanah *et al.*, 2025). Overall, these results demonstrate that appropriate mutagen selection and dose optimization—particularly EMS and colchicine—provide an effective and practical strategy for improving bulb yield and quality in garlic breeding programs.

## CONCLUSIONS

Determining LD<sub>50</sub> is essential for optimizing mutation frequency in Indonesian garlic cultivars. Although both Sangga Sembalun and Lumbu Putih cultivars exhibited similar LD<sub>50</sub> values under gamma irradiation, their responses differed under EMS and colchicine treatments. Each mutagen distinctly affected mortality, viability, and growth, thereby influencing bulb development. Gamma irradiation caused severe growth inhibition, whereas EMS and colchicine demonstrated greater potential for inducing beneficial variation in bulb and clove traits. Therefore, the more suitable mutagens for garlic improvement programs targeting yield-related traits are EMS and colchicine.

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