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## INDUCED *LAVANDULA ANGUSTIFOLIA* MILL GENETIC VARIABILITY THROUGH IN VITRO GAMMA IRRADIATION

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### SUMMARY

Lavender (*Lavandula angustifolia* Mill.) contains chemical compounds useful for pharmaceutical, cosmetic, and culinary industries and controls the growth of several bacteria and fungi species. Combining in vitro propagation with induced mutation by gamma-ray irradiation can increase the speed of the breeding process to get new varieties with a diverse morphology and high metabolite contents. This study aimed to evaluate radiosensitivity by determining the lethal dose of 50% (LD50) of lavender on in vitro node explants by gamma-ray irradiation and assessing the genetic diversity of lavender putative mutants through morphological observations at MV2 and MV3 generations. Results of the experiment showed the LD50 of lavender was at a dose of 26.1 Gray (Gy). The higher the dose of gamma irradiation, the fewer irradiated shoots survive. Furthermore, a variability of quantitative and qualitative characters in the MV2 and MV3 generation putative mutants occurred. The greatest variability in quantitative characters resulted in putative mutants from 20 Gy. The variability of quantitative features revealed high heritability values for all variables ( $\geq 0.5$ ). Ten putative mutants, obtained through induction of gamma-ray mutation, exhibited a change in the leaf color (white or yellow variegation) at MV2 and MV3 generations.

**Keywords:** Lavender (*L. angustifolia*), heritability, lethal dose, putative mutants, qualitative characters, quantitative characters

**Key findings:** In lavender (*L. angustifolia*), 10 putative mutants characterized by morphological color changes in stem and leaf color emerged through gamma-ray mutation induction. This research shows these putative mutants exhibit improved quantitative characteristics compared to the control. Moreover, putative mutants from 20 Gy gamma irradiation have a high variability and biomass versus the control, and 40 Gy gamma irradiation is the potential material for further evaluation of their biochemical compound.

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## INTRODUCTION

Lavender (*Lavandula angustifolia* Mill.) is a herbaceous plant widespread across the Mediterranean Region, with extensive cultivation in various countries, especially in France, Bulgaria, China, Australia, Morocco, Spain, Ukraine, and England (Dong *et al.*, 2020). Lavender contains the chemical compounds linalyl acetate and linalool (Bejar, 2020; Ivanišová *et al.*, 2021) used by the pharmaceutical, cosmetics, and culinary industries and can control the growth of several species of bacteria and fungi (Koulivand *et al.*, 2013; Prusinowska and Smigielski, 2014; Smigielski *et al.*, 2018; Białoń *et al.*, 2019; Ogata *et al.*, 2020; Ivanišová *et al.*, 2021). Even though 39 species of lavender are common, only the species *L. angustifolia*, *L. x intermedia* and *L. latifolia* maintain commercial cultivation (Kara and Baydar, 2013).

*Lavender x intermedia* produces more biomass and becomes a preferred species for high-yield production. Meanwhile, *L. angustifolia* has shorter flower stalks than *L. x intermedia*, producing much lower biomass. However, *L. angustifolia* has superior oil quality and the highest economic value compared to other lavender species (Kara and Baydar, 2013). The phenotypic properties and biochemical composition of lavender sustain strong influences from seasons and environmental factors, such as soil type, temperature, and light (Lakušić *et al.*, 2013). Lavender's essential oil content and economic value for the fragrance industry hold significant potential for development. Developing new varieties with high secondary metabolite content and engineering in vitro production is crucial to achieving high biomass and metabolite production. In vitro culture techniques can increase the speed and efficiency of the breeding process and improve plant characteristics, which are unattainable through conventional breeding methods without depending on environmental conditions (Tazeb, 2017).

Mutation induction using gamma-ray irradiation is a widely used method in plant breeding that has been proven to produce

character improvements in plants. Reports on gamma irradiation have detailed to increase the diversity of patchouli (Tahir *et al.*, 2016), increase the genetic diversity of *Triticum aestivum* L. and tolerance to high temperatures in tropical areas (Setiawan, 2015), and reduce the bitter taste of *Coleus amboinicus* Lour (Sari, 2019). Further reports stated it changes petal color in *Tulipa gesneriana* L. (Li *et al.*, 2022) and increases the variation in flower size and essential oil content (Matricaria acid methyl ester) by 70%–80% in *Chamomilla recutita* L. Rauschert (Raj *et al.*, 2019).

Gamma rays are ionizing radiation with high penetrating power into plant tissue; thus, they can cause changes at the gene and chromosome level and can change a plant's phenotype (Shu *et al.*, 2012). Low irradiation doses can be useful for increasing the percentage and speed of seed germination and changing the plant at the physiological, biochemical, metabolic, and molecular levels, known as radiation hormesis (Villegas *et al.*, 2023). This study aimed to evaluate radiosensitivity by determining the LD50 of lavender and assess genetic diversity through morphological observations in the MV2 and MV3 generations following gamma-ray irradiation.

## MATERIALS AND METHODS

### The study area

The experiment to induce gamma irradiation mutations proceeded at the National Research and Innovation Agency, Lebak Bulus, South Jakarta. The in vitro selection took place at the Laboratory of Plant Molecular Biology, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Bogor, Indonesia, from May to September 2023.

### Plant material

The plant material used were lavender plantlets, which were in vitro propagated in Murashige and Skoog medium without plant growth regulators (MS0) at the Laboratory of

Plant Molecular Biology, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Bogor, Indonesia.

### Experimental design and statistical analysis

The experiment transpired using a randomized complete block design (RCBD) with five different irradiation doses, namely 0, 20, 40, 60, and 80 Gray (Gy) with four replications. Each experimental unit consisted of 25 single-node explants for irradiation treatment. Explant observations after irradiation treatment continued on quantitative and qualitative characters. Quantitative characteristics included the number of leaves, nodules, shoots, plant height, and LD50. The LD50 value calculation depended on the number of lavender explants that died during the first week to the sixth week after irradiation treatment by the curve fit analysis using *Curve Expert* 1.3. Observations on the number of leaves, nodules, shoots, and plant height ensued one week after subculture until the fourth week in generations MV1 to MV3.

Data obtained in the MV1 generation reached assessment using analysis of variances (ANOVA). When the F test showed a significant effect at a level of  $\alpha = 5\%$ , its further testing used the Duncan's multiple range test at the 5% level. The MV2 and MV3 generation data underwent quantitative boxplot analysis. Estimation of genetic parameter values in the MV3 generation included values of phenotype diversity coefficient (PDC), genotype diversity coefficient (GDC), and broad-sense heritability ( $h^2bs$ ). The classification of heritability values, divided into three criteria, comprised low, medium, and high. Heritability is high if the heritability value is greater than 50%, moderately high if it is 20% to 50%, and low if it is less than 20% (Tesfaye 2021). The qualitative characteristics observed were the leaf and stem color using the Royal Horticultural Society (RHS) color chart. The statistical software used was SAS® On Demand for Academics (<https://welcome.oda.sas.com/>), Microsoft Office Excel 2019, and Curve Expert 1.3.

## RESULTS

### The lethal dose (LD50) of gamma-ray irradiation

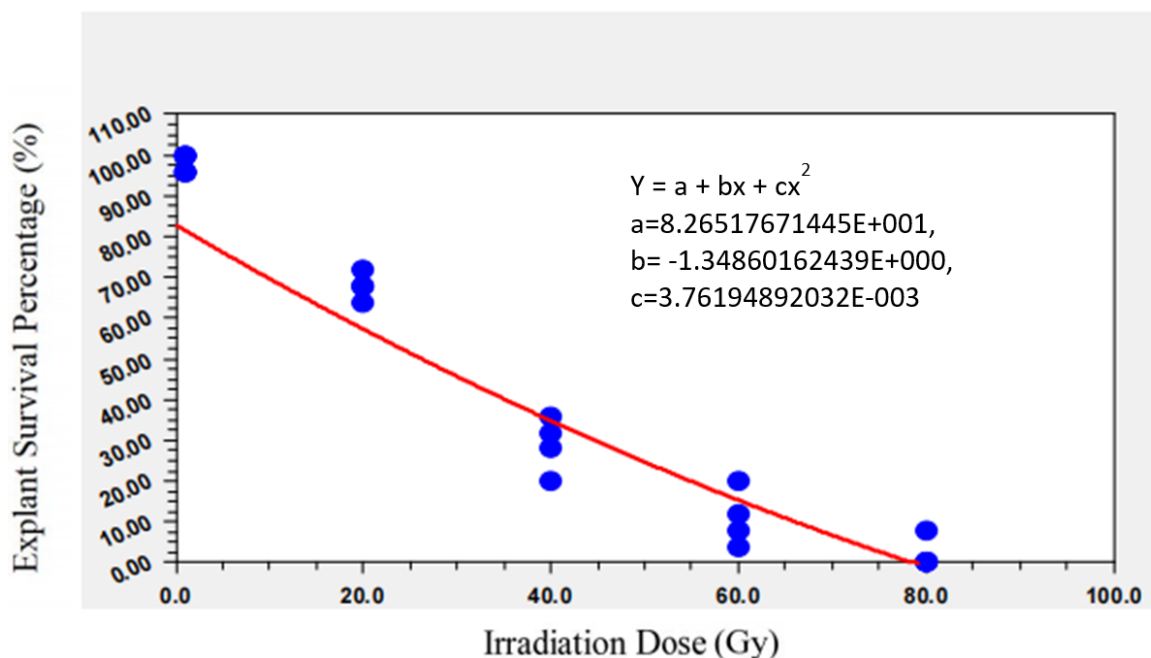
The survival percentage analysis of lavender explants after gamma irradiation used a quadratic fit model with an LD50 dose of 26.1 Gy. Graphic equation  $Y = a+bx+cx^2$  with  $a = 8.26517671445E+001$ ,  $b = -1.34860162439E+000$ , and  $c = 3.76194892032E-003$ , as shown in Figure 1.

### Quantitative characteristics of in vitro shoots

The number of putative mutants that survived after irradiation varied across doses and generations. The MV1 generation has a total of 500 lavender explants, consisting of 400 explants (100 explants in each gamma-ray dose) and 100 explants as controls. A significant decrease in explant survival appeared at the MV2 generation, with a total of 205 shoots (82 putative mutants and 123 controls) remaining to survive (Table 1). The control groups produced the largest number of sub-cultures in MV2 at 123 shoots, whereas doses of 20, 40, and 60 Gy only produced 65, 16, and one shoot, respectively. All generations of MV1 with an irradiation dose of 80 Gy experienced death after irradiation (Table 1). The number of shoots on the lavender putative mutants tended to increase in MV3 generation, and the vegetative growth of the mutants was better than with the control. The 20 Gy formulation treatment produced 271 shoots, 135 shoots in the control, 10 shoots from the 40 Gy, and only two shoots from the 60 Gy dosage treatment.

### MV1 in vitro shoot performance

MV1 shoot generation at each irradiation dose showed reduced growth compared to the control (0 Gy) in all observed variables (Table 2). Observation results at four weeks after irradiation at doses of 20, 40, 60, and 80 Gy gamma-ray irradiation treatments affected the growth of lavender explants and caused the



**Figure 1.** Curve fit analysis of survival percentage of lavender node explants on gamma irradiation treatment.

**Table 1.** Survival of lavender shoots per generation of MV1 to MV3.

Irradiation Dose (Gy)	Generation		
	MV1	MV2	MV3
0	100	123	135
20	100	65	271
40	100	16	10
60	100	1	2
80	100	0	0
Total Amount	500	205	418

The number in each row and column for each irradiation dose in generations MV1 to MV3 is the combined number of four experimental replications.

**Table 2.** The average number of leaves, nodes, shoots, and shoot height of MV1 generation lavender mutants at four weeks after irradiation.

Irradiation Dose (Gy)	Observation Variable			
	Number of Leaves	Number of Nodes	Number of Shoots	Plant Height (cm)
0	7.5 <sup>a</sup>	3.6 <sup>a</sup>	1.9 <sup>a</sup>	2.0 <sup>a</sup>
20	4.3 <sup>b</sup>	2.0 <sup>b</sup>	1.8 <sup>a</sup>	1.4 <sup>b</sup>
40	2.3 <sup>c</sup>	1.1 <sup>c</sup>	1.1 <sup>b</sup>	1.1 <sup>c</sup>
60	2.4 <sup>c</sup>	1.2 <sup>c</sup>	0.9 <sup>bc</sup>	1.1 <sup>c</sup>
80	2.2 <sup>c</sup>	1.1 <sup>c</sup>	0.8 <sup>c</sup>	1.1 <sup>c</sup>

Numbers followed by the same letter in the same column and character are not significantly different based on the Duncan test at  $\alpha = 0.05$  level.

explant's death. The data revealed the average number of leaves, nodes, shoots, and height of lavender decreased at higher doses of gamma-ray irradiation. Shoots in controls had the best growth in all observation variables versus irradiation doses of 20, 40, 60, and 80 Gy. The average number of leaves was 7.5 in the control, 4.3 at a dosage of 20 Gy, and between 2.2 and 2.4 in the treatment doses of 40, 60, and 80 Gy.

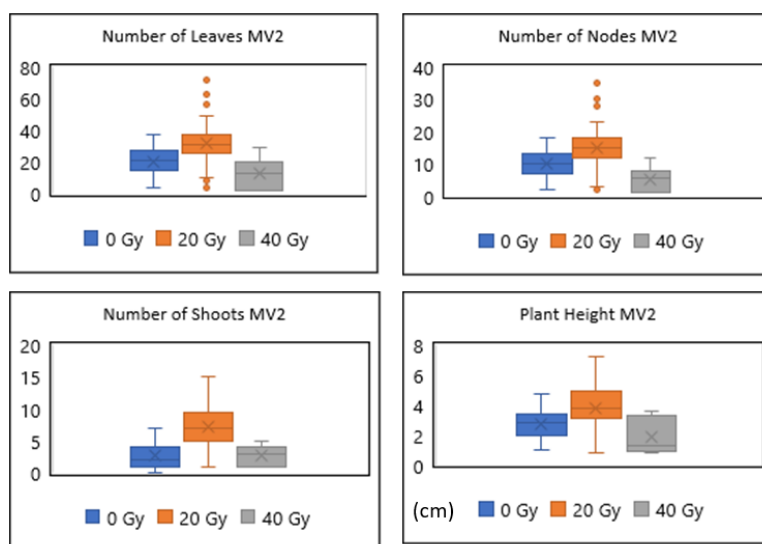
### MV2 in vitro shoot performance

The MV2 generation of lavender experienced a decline in shoot numbers because many explants had stunted growth; hence, they could not produce buds, and some died due to gamma-ray irradiation. Based on the results from the boxplot analysis (Figure 2), lavender shoots with an irradiation dosage of 20 Gy had the best growth compared to control shoots and those with a treatment strength of 40 Gy. The average number of leaves was 31.5, the average number of nodes was 15.1, the average number of shoots was 7.2, and plant height was 3.7 cm at a dose of 20 Gy. Meanwhile, in the control, the average number of leaves was 20.2, the average number of nodes was 9.9, the average number of shoots was 2.7, and the plant height was 2.7 cm.

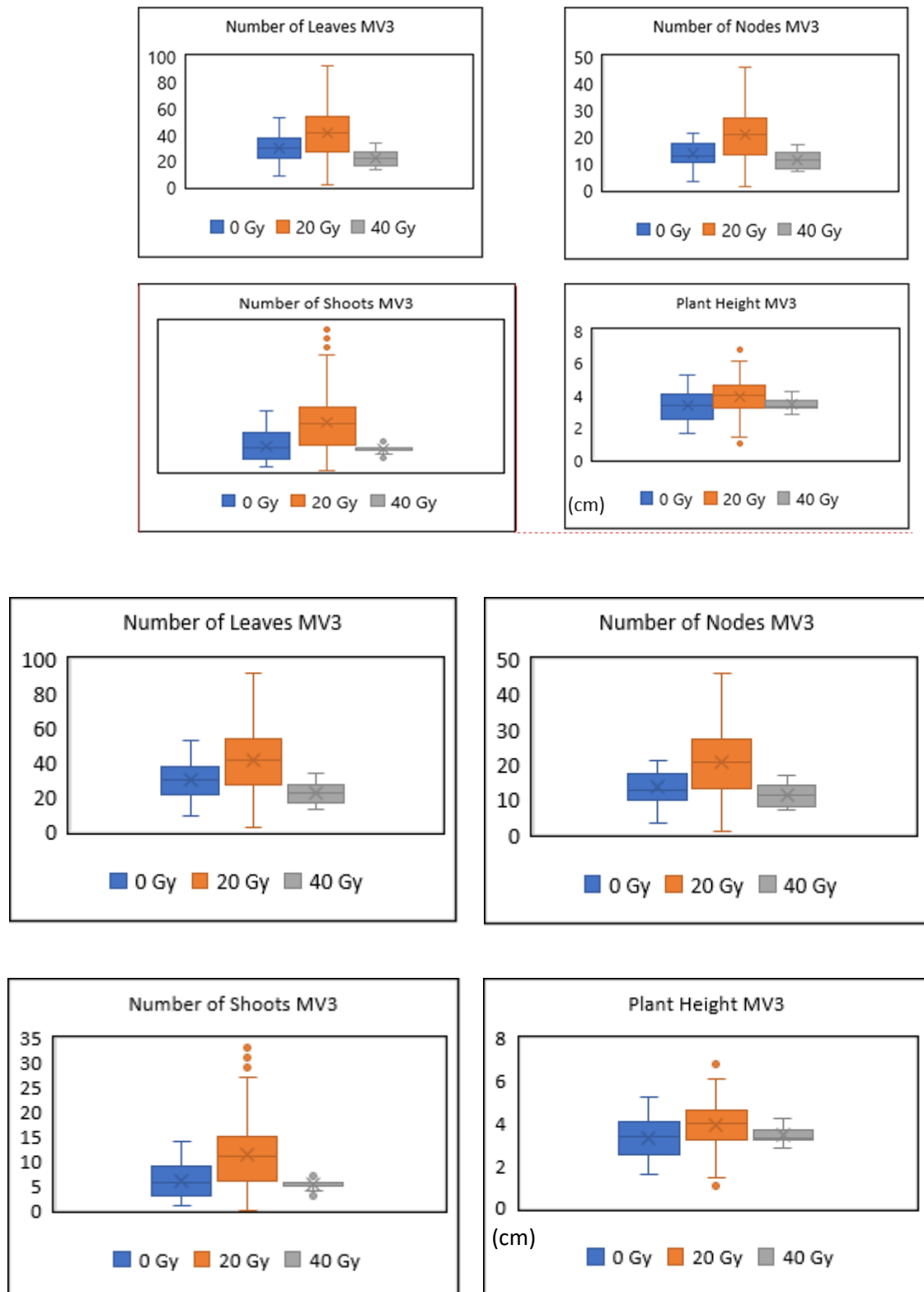
Extreme values occurred at a 20 Gy dosage, with 72 leaves and 35 nodes.

### MV3 in vitro shoot performance

The MV3 shoot generation exhibited an increase in the number of putative mutant shoots compared to the MV2 generation. There are 418 shoots, with the largest number produced by a strength of 20 Gy with 271 shoots, while the control only produced 135 shoots. The average number of leaves, nodes, shoots, and plant height in the 20 Gy treatment was higher than the control (Figure 3). The diversity and extreme values in the MV3 generation resulted in the variable number of shoots and plant height. This differs from the extreme values obtained from the MV2 for the number of leaves and nodes. The highest number of shoots produced by a 20 Gy dosage was 33 shoots, and the tallest lavender putative mutant shoot was 7 cm compared to the control. In the MV3 shoot generation, the data distribution for all irradiation doses is not symmetrical, as can be seen from the box and the length of the whiskers. These results show an irradiation level of 20 Gy gave extreme diversity in the number of leaves, nodes, shoots, and plant height in the MV2 and MV3 shoot generations.



**Figure 2.** Box plot analysis of the number of leaves, nodes, shoots, and plant height in the MV2 generation.



**Figure 3.** Boxplot analysis of the number of leaves, nodes, shoots, and plant height in the MV3 generation.

**Table 3.** Lavender mutants exhibiting changes in stem color and leaf color in the MV2 and MV3 generations.

Dose	Putative Mutants	% Color Change	MV2		MV3	
			Stem Color	Leaf Color	Stem Color	Leaf Color
0 Gy	L0-2	2,2	LG	DG	LG	WV
	L0-31		G	YV	G	LG
	L0-44		LG	YG	LG	YV
20 Gy	L20-8,3	2,9	WY	G	G	YV
	L20-8,6		LG	WV	G	WV
	L20-11		LG	WY	LG	YV
	L20-12		G	LG	G	WV
	L20-27		LG	DG	LG	YV
	L20-35		G	DG	LG	WV
	L20-42		LG	WV	LG	WV
	L20-45		G	YV	LG	G
40 Gy	L40-2	20	G	YV	G	WV
	L40-4		LG	YG	HM	YV

G=Green, DG=Dark Green, LG=Light Green, YG=Yellowish Green, WY=White Yellow, YV=Yellow Variegation, WV=White Variegation.

### Variation on MV2 and MV3 generations

Gamma irradiation produced variations in the leaf and stem color in putative mutants that differ from control plantlets. Changes in leaf and stem color in the MV2 and MV3 generations (Table 3) reached further classification according to color levels on the RHS color chart. Gamma irradiation doses of 0,

20, and 40 Gy produced changes in the leaf color to yellow and white variegation. The percentage of color changes occurring at a 0 Gy strength was 2.2%, at 20 Gy was 2.9%, and at 40 Gy level was 20%. However, the leaf color changes were still unstable because of continuing leaf color changes in MV2 and MV3 (Figure 4). Plantlet L20-45 MV2 had a yellow variegated leaf and changed to green on MV3.

**Figure 4.** The performances of several putative mutant lines with leaf variegation: L20-11 MV2 (white-yellow leaf), L20-27 MV3 (yellow variegation), and L40-2 MV3 (white variegation).

### Genetic parameters of MV3 generation

Analysis of the genetic parameters of the MV3 generation of lavender putative mutant shoots showed high phenotype diversity coefficient (PDC) values for four vegetative characters (Table 4). The diversity of mutant shoot phenotypes at 20 Gy had the highest value in the four traits observed compared to the control or dosage of 40 Gy. Based on the table, the PDC value is higher than the GDC value for all lavender mutant shoot features. Genetic diversity will influence the heritability value of the observed quantitative characters.

### DISCUSSION

LD50 revealed closely related to plant radiosensitivity values, namely the level of plant sensitivity, which results in morphological, physiological, and biochemical changes due to gamma-ray radiation. Chen *et al.* (2016) reported the gamma-ray irradiation applied to lavender seeds had an LD50 dose value of 48.1 Gy. In this experiment, the LD50 on in vitro nodes of lavender is lower (26.1 Gy), implying that in vitro explants are more sensitive to gamma irradiation than the seed. The LD50 found in this experiment is an important result for further implementation of

gamma irradiation on in vitro node explants for variability induction. Sari (2019) reported the gamma-ray irradiation on *Coleus* nodes had an LD50 dosage of 44.03 Gy, a higher value than the LD50 on lavender nodes, even though the type of explant used was relatively similar. Abdulhafiz *et al.* (2018) revealed the LD50 on banana shoots was 33 Gy. The differences in LD50 among species result from the irradiated explants' genetic factors or physiological characteristics. Lavender in vitro nodes could contain more water than the seed. The more oxygen and water molecules contained in the irradiated material, the more free radicals materialize, resulting in the material being more sensitive to gamma-ray radiation. The lower the LD50 of the explants, the higher their radiosensitivity level is.

In some cases, the performance of irradiated explants could be better or worse than controls called radio stimulation and radio inhibition, respectively (Hernández-Muñoz *et al.*, 2019). This phenomenon was also evident in this study. The irradiated explants from the 20 Gy irradiation treatment grew faster than the control. A similar result, as reported on the *P. amabilis* orchid, indicated 15 Gy gamma irradiation on protocorm produced more secondary protocorm, 83.3% higher than the control (Raihanun, 2017).

**Table 4.** Estimation of genetic parameter values for lavender mutant shoots based on quantitative observation variables in the MV3 generation.

Observation Variable	Irradiation Dose (Gy)	Average	$\sigma^2_f$	$\sigma^2_g$	PDC	GDC	$h^2_{bs}$	$h^2_{bs}$ Criteria
Number of Leaves	0	13.14	16.99	10.74	0.31	0.25	0.63	High
	20	25.64	156.64	140.40	0.48	0.46	0.89	High
	40	10.67	61.37	26.02	0.73	0.49	0.42	Medium
Number of Nodes	0	6.49	4.20	2.72	0.31	0.25	0.64	High
	20	12.49	38.32	34.31	0.50	0.46	0.89	High
	40	4.90	11.80	6.49	0.70	0.51	0.55	High
Number of Shoots	0	2.44	1.56	0.14	0.51	0.15	0.09	Low
	20	6.87	11.04	9.36	0.48	0.44	0.84	High
	40	2.84	1.29	1.17	0.40	0.38	0.90	High
Plant Height	0	2.60	0.11	0.10	0.13	0.12	0.87	High
	20	3.01	0.95	0.71	0.32	0.28	0.74	High
	40	2.05	0.82	0.31	0.44	0.27	0.38	Medium

$\sigma^2_f$  = phenotypic variance,  $\sigma^2_g$  = genotype variance, PDC = phenotypic diversity coefficient, GDC = genotypic diversity coefficient,  $h^2_{bs}$  = broad sense heritability.



The prominent phenomenon on irradiated shoots found in MV2 and MV3 generation was leaf variegation. Variegated color is an outcome of cells losing the ability to form chlorophyll in some or all parts of the cell exposed to gamma rays (Romeida *et al.*, 2013). Gamma irradiation also produced the putative mutants with variegated leaves in *Monstera deliciosa* (Huang *et al.*, 2017), Chrysanthemum (Bajpay and Dwivedi, 2019), and *Anubias minima* (Limtiyayotin *et al.*, 2024). The failure of the plant cell in chlorophyll biosynthesis could be due to a mutation in genes located in the chloroplast, mitochondria, or nucleus (Zhao *et al.*, 2020). Applying gamma rays can increase and accelerate the occurrence of diversity in plants through leaf variegation. The leaf variegation was also notable in controls, indicating the presence of other factors causing it, such as somaclonal variation along shoot multiplication. However, the percentage of leaf variegation increased by elevating the gamma dose, revealing the potential of the treatment to increase genetic variation in lavender (Table 3).

In plant selection activities, estimating genetic parameters, including genetic variability values, genotype variations, phenotypes and environmental variations, heritability values, genetic progress, as well as phenotype-genotype correlations, provides essential information for improving plant characters through further breeding activities (Priyanto *et al.*, 2023). The phenotypic appearance of a plant attribute is the influence of genetic and environmental factors and interactions between both. Therefore, in selection activities, it is vital to know and separate the magnitude of the influence of these three factors for a more effective and efficient carrying out of the selection. By estimating genetic parameters, the separation of values of genotype, phenotype, and environmental variations and their prediction from one another can succeed, making it easy to measure the value of genetic variability, heritability, and genetic progress. A high heritability value shows the genetic background is the main factor influencing the phenotype of a plant. The results reveal such

traits as the number of leaves, nodes, shoots, and plant height of putative mutants from 20 Gy gamma irradiation exhibit high heritability.

## CONCLUSIONS

The analysis results indicate the LD50 of *in vitro* lavender node explants is 26.1 Gy. The greatest diversity of quantitative characters in the number of leaves, nodes, shoots, and plant height emerged from a dosage of 20 Gy. The diversity of quantitative features produced high heritability values for all observed variables. Moreover, a change existed in the color of the white and yellow variegation leaves in the MV2 and MV3 mutant generations.

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