



## LETHAL DOSE 50 (LD<sub>50</sub>) OF MUNGBEAN (*Vigna radiata* L. Wilczek) CULTIVAR KAMPAR

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### SUMMARY

The first stage in plant breeding program by induced mutation technique using gamma rays is to determine the lethal dose, since the radio sensitivity of each species, varieties, and genotypes are different. This research was aimed to determine the lethal dose (LD<sub>50</sub>) of gamma ray of Kampar mungbean cultivar. One hundred seeds were exposed to different doses of gamma ray, such as 0, 100, 200, 300, 400, 500, 600, and 700 Gray, using <sup>60</sup>Co as a radiation source. The LD<sub>50</sub> was then determined and 500 seeds were exposed to the LD<sub>50</sub> of gamma ray. The LD<sub>50</sub> of Kampar mungbean cultivar based on number of survival plants was 619.875 Gray indicating that Kampar mungbean cultivar had low radio-sensitivity to gamma ray. The 619.875 Gray dose of gamma ray caused a delay in flowering and pods ripening periods. The same dose also resulted in the plants with higher plants, more branches, and more number of pods per plant as compared to control.

**Keywords:** gamma ray, irradiation, Kampar, lethal dose, Riau, *Vigna radiata*.

**Key findings:** LD<sub>50</sub> value of gamma ray irradiation is important for plant breeder to induce mutation in plant, because LD<sub>50</sub> value determines the effect of mutation in plant and it is different between species, varieties, and genotypes. The LD<sub>50</sub> in this study can be used by mungbean breeder to the next induced mutation using gamma ray in Kampar mungbean cultivar to obtain the expected mutant plants.

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### INTRODUCTION

Mungbean (*Vigna radiata* L. Wilczek), a member of Fabaceae family with  $2n = 2x = 22$  chromosomes, is one of the important food leguminous crops in Asia. Mungbean has special characters such as being rich in protein (240 g/kg), carbohydrate (630 g/kg), and micronutrients (Dahiya *et al.*, 2013). In addition, mungbean may become a major crop due to climate change, as mungbean may be harvested rapidly or it has short life (about 60 days) (Shanmugasundaram *et al.*, 2009a) as

compared to rice or corn. It may increase fertility of soils due to its ability to fix nitrogen together with soil bacteria, and it is also relatively tolerant to nutrient deficiency and drought (Lavanya *et al.*, 2008; Shanmugasundaram *et al.*, 2009b).

Kampar, one of the districts in Riau Province, is a central production of mungbean in Riau Province. Riau Province is predominately by acid soils, nutrient poor soils, and drought. Farmers in Kampar grow Kampar mungbean cultivar that is adaptable and suitable to those conditions. However, the Kampar mungbean

cultivar has some disadvantages, like pod dehiscence, asynchronous pod maturity, and trichomes. Although trichomes function as a defense of mungbean to the pest, their presence in the all parts of plant make the farmers difficult to harvest the pods. Therefore, farmers in Riau Province need the mungbean cultivar with less trichomes. Such mungbean cultivars with special characters can be obtained through induced mutation.

Mutation is a change of DNA sequence in a gene or a change of chromosome and is responsible for genetic variation. Mutation can occur spontaneously or resulting from exposure to radiation or chemicals. Some kinds of radiation are X-ray, gamma ray, and ultraviolet (UV). The gamma ray has been widely used for the improvement of various traits of crops (Khatiri *et al.*, 2005; Tah, 2006; Kanakamanay, 2008; Pandiyan *et al.*, 2008; Songsri *et al.*, 2011; Aney, 2013).

The success of mutation using the gamma ray depends on its dose. Low dose cannot cause mutation and therefore there are no changes in mutated seeds, but high dose can cause death of the mutated seeds, sterility, and other deleterious effect. Thus, we must first determine the LD<sub>50</sub> (lethal dose), a dose that causes 50% mortality to the seeds or a safe dose where 50% of the seeds can survive.

The LD<sub>50</sub> is different between species and varieties in a species. For example, the LD<sub>50</sub> of K-851 mungbean cultivar is 54.06 kR and for Sona is 53.20 (Tah, 2006). Furthermore, the LD<sub>50</sub> of *Jatropha crucas* L. is 600 Gray (Songsri *et al.*, 2011), *Manihot esculenta* L. is 27.5 Gray (Kangarashu *et al.*, 2014), *Pisum sativum* L. is 200 Gray (Aney, 2013). Therefore, this study was carried out to determine the LD<sub>50</sub> of Kampar mungbean cultivar.

## MATERIALS AND METHODS

### Plant material and mutagen treatment

The seeds of Kampar mungbean cultivar were collected from Kampar district, Riau Province, Indonesia and used in this study in March 2013. One hundred seeds were exposed to different doses of gamma ray, such as 0, 100, 200, 300,

400, 500, 600 and 700 Gray, using <sup>60</sup>Co as a radiation source at the Center for Application of Isotopes and Radiation (PATIR), National Atomic Energy Agency (BATAN) Jakarta, Indonesia. The LD<sub>50</sub> was then determined using CurveExpert software version 1.3 based on the number of survival plants at those different dose of gamma ray.

Then, another 500 seeds were exposed to the LD<sub>50</sub> to identify the effect of the LD<sub>50</sub> to the seeds germination time (days), plant height (cm), number of branches, flowering period (days), number of pods per plant, pod ripening (days), pod length (cm), number of seeds per pod, and 50-seeds weight (g), leaf color, leaf shape, leaf seed, flower color, pod color, and seed color.

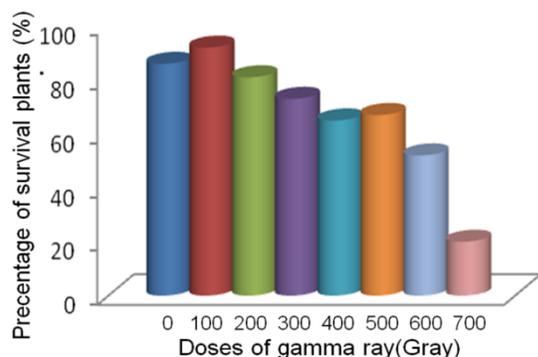
### Data analysis

After the seeds were treated with the LD<sub>50</sub>, the data was then analyzed using the F-test. If the variance between the treatment and the control was the same, the t-test would be performed using Two-sample assuming equal variances. In contrast, if it was different, the t-test would be performed using two samples assuming unequal variances. F-tests and the t-tests were calculated using Microsoft Office Excel 2007.

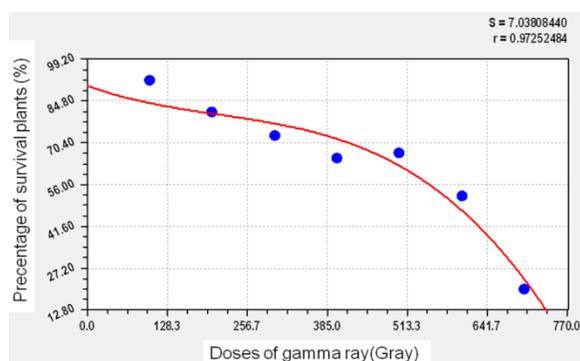
## RESULTS

### Determination of LD<sub>50</sub> of Kampar Mungbean Cultivar

The mortality of plants increased linearly with the increase of gamma ray doses. The percentage of the lowest survival plants was exhibited in 600 and 700 Gray doses (Figure 1). Percentage of survival plants (y) to gamma ray doses (x) was formulated by  $y = a + bx + cx^2 + dx^3$  where  $a = 90,0454$ ,  $b = - 0,0786$ ,  $c = 0,0002$ ,  $d = - 3.535e-0.07$ . Therefore, the LD<sub>50</sub> of Kampar mungbean cultivar observed in this study was 619.875 Gray. It means that 50% of plants population would be dead if exposed to 619.875 Gray of gamma ray (Figure 2).



**Figure 1.** Effect of gamma ray doses to percentage of survival plants at 21 days after germination.



**Figure 2.** Plot of log doses versus survival plants for calculation of LD<sub>50</sub> of Kampar mungbean cultivar.

### The LD<sub>50</sub> Affects the Vegetative and Reproductive Characters in Kampar Mungbean Cultivar

The LD<sub>50</sub> treatment, *i.e.* 619.875 Gray, decreased the percentage of seed germination as compared to the control. The F-test showed that the variances of vegetative characters - such as seeds germination time, plant height, and number of branches per plant - between the LD<sub>50</sub> treatment and the control were not similar (Table 1). Furthermore, the t-test showed that the LD<sub>50</sub> treatment affected significantly to those parameters at  $\alpha = 0.05$  and  $\alpha = 0.01$  and the mean value of those parameters in the LD<sub>50</sub> treatment was higher than the control (Table 2). In LD<sub>50</sub> treatment, the mean of the seed germination time, the plant height, and the number of branches per plant were 3.21 days, 41.91 cm, and 6.35, respectively. Whereas in the

control, for the same characters were 3 days, 38.35 cm, and 4.08, respectively. In the LD<sub>50</sub> treatment the number of branches per plant increased but the branches did not produce flowers and nor pods.

Generally, the leaf shape of mungbean is trifoliolate and oval with sharp tip, but in this research, the LD<sub>50</sub> treatment changed the leaf shape and it was permanent until harvesting period. The kinds of leaf shapes found in this study were trifoliolate, cuneate, four leaf, and wrinkle (Figure 3). Nevertheless, the LD<sub>50</sub> treatment in this study did not change the color of leaves and did not remove the trichomes.

The F-test between the LD<sub>50</sub> treatment and the control for flowering period was similar, but the number of pods per plant, the pod ripening period, the pod length, the number of seeds per pod, and the 50-seeds weight were not similar (Table 1). The t-test showed that the LD<sub>50</sub> treatment affected significantly the flowering period, the number of pods per plant, and the pod ripening period, yet not affected significantly the pod length, the number of seeds per pod, and the 50-seeds weight at  $\alpha = 0.05$  and  $\alpha = 0.01$  (Table 2). The flowering period in the LD<sub>50</sub> treatment was longer (32.9 days) than the control (29.2 days). The mean of the number of pods in radiated plants was 16.18 pods that were more than the control (8.94 pods). The mean of the pod ripening period in the LD<sub>50</sub> treatment was 46.48 days and in the control was 45.24 days. This study observed brown and black seed colors and wrinkle seed shape in LD<sub>50</sub> treatment (Figure 3). Green and brownish-green seed colors and rounded-box seed shape were observed in control. Flower color, trichomes, and synchronous pod maturity in control and LD<sub>50</sub> treatment were the same, *i.e.* greenish yellow, present, and asynchronous, respectively.

**Table 1.** The F-test of vegetative and reproductive characters in Kampar mungbean cultivar radiated with LD50 of gamma ray.

Characters	Mean ± Deviation Standard		Significance
	Control	LD <sub>50</sub> treatment	
<b>Vegetative characters:</b>			
Seeds germination time (days)	3 ± 0	3,12 ± 0,34	**
Plant height (cm)	38,35 ± 38,29	41,91 ± 117,14	**
Number of branches	4,08 ± 0,74	6,35 ± 4,04	**
<b>Reproductive characters:</b>			
Flowering period (days)	29,24 ± 17,80	32,91 ± 23,27	*
Number of pods per plant	8,94 ± 4,83	16,18 ± 58,89	**
Pod ripening (days)	45,24 ± 1,24	46,48 ± 3,89	**
Pod length (cm)	9,41 ± 2,00	9,15 ± 1,96	**
Number of seeds per pod	11,05 ± 1,99	10,38 ± 3,49	**
50-seeds weight (g)	4,26 ± 0,14	4,3 ± 0,23	**

\* : variances of control and LD<sub>50</sub> treatment were similar significantly.

\*\* : variances of control and LD<sub>50</sub> treatment were not similar significantly.

**Table 2.** The t-test of vegetative and reproductive characters in Kampar mungbean cultivar radiated with LD50 of gamma ray.

Characters	Mean ± Deviation Standard		t value	t table	
	Control	LD <sub>50</sub> treatment		0.05	0.01
<b>Vegetative characters:</b>					
Seeds germination time (days)	3±0	3.21±0.58	5.60**	1.65	1.97
Plant height (cm)	38.35±619	41.91±10.82	2.87**	1.66	1.99
Number of branches	4.08±0.86	6.35±2.01	6.75**	1.65	1.97
<b>Reproductive characters:</b>					
Flowering period (days)	29.24±4.22	32.909±4.82	4.364**	1.65	1.97
Number of pods per plant	8.94±2.19	16.180±7.67	11.684**	1.65	1.97
Pod ripening (days)	45.24±1.12	46.484±1.97	5.534**	1.66	1.99
Pod length (cm)	9.41±1.41	9.153±1.40	-1.053 <sup>ns</sup>	1.67	2.01
Number of seeds per pod	11.05±1.41	10.38 ±1.86	-2.559 <sup>ns</sup>	1.67	2.01
50-seeds weight (g)	4.26±0.37	4.30±0.48	0.559 <sup>ns</sup>	1.67	2.02

\*\* : differ significantly at α=0.05 and α=0.01.

<sup>ns</sup> : not differ significantly at α=0.05 and α=0.01.



**Figure 3.** Leaf shape, seed shape, and seed color of Kampar mungbean cultivar which was treated by LD50 of gamma ray irradiation. (a) Trifoliolate. (b) Four leaf. (c) Cuneate. (d) Wrinkle. (e) Rounded-box and green or brownish-green. (f) Wrinkle and black. (g) Green, brownish-green, brown, and black seeds.

## DISCUSSION

In this study, high dose of gamma ray affects seed germination and germinated seed at later stages could not grow well and died. Suggested that high dose of gamma irradiation had destructed cell components, like chromosomal damage (Kiong *et al.* 2008). Therefore, it is very important to determine the LD<sub>50</sub> value prior to inducing mutation to the plant.

The LD<sub>50</sub> is one of parameters to predict radio-sensitivity level in plants (Harding and Mohamad, 2009; Horn and Shimelis, 2013; Kumar *et al.* 2013). The LD<sub>50</sub> is a dose that causes 50% mortality to the seeds or a safe dose where 50% of seeds could survive. The higher the LD<sub>50</sub> is, the lower the plant radio-sensitivity to gamma ray is. The LD<sub>50</sub> in this study was determined based on survival plants derived from radiated seeds. The LD<sub>50</sub> of Kampar mungbean cultivar was 619.875 Gray and this showed that Kampar mungbean cultivar had low radio-sensitivity.

The radio-sensitivity varies from species and also genotype to genotype, depending on the content of water inside the seeds. The seeds which contain much water and oxygen are more sensitive to gamma ray because gamma ray can interact with atoms or molecules in the cell, particularly water, producing free radicals (Kovacs and Keresztes, 2002). Imbibed papaya (*Carica papaya*) seeds are more sensitive to gamma ray (LD<sub>50</sub> = 50-87 Gray) than the dry one (LD<sub>50</sub> = 300 Gray) (Chan, 2009). The LD<sub>50</sub> in physic nut (*Jatropha curcas* L.) is relatively lower, i.e. 425 Gray based on the survival percentage of 30 days after germination (Songsri *et al.*, 2011) than the long bean (*Vigna sesquipedalis*), i.e. 800 Gray (Kon *et al.*, 2007).

The LD<sub>50</sub> about 619.875 Gray in this study was able to increase the plant height and number of branches as compared to control, but such a dose had not been able to accelerate the seeds germination period. It showed that the mutation had been occurred and the gamma ray had resulted in damages to the cells and their components, so the ability of the seeds to germinate reduced. Soedjono (2003) stated that high dose of gamma ray could cause death and sterility of mutated material.

In this study, the plant height and the number of branches per plant increased as a result of gamma ray radiation using the LD<sub>50</sub>. Hanafiah *et al.* (2010) reported that the 50 Gray and 100 Gray of gamma ray could increase the plant height and the number of branches per plant in *Glycine max* (L.) Merr. The increasing of plant height and number of branches was also observed in K-851 and Sona mungbean cultivars (Tah, 2006).

Generally, the mungbean has a trifoliate and oval leaf shape with a sharp tip, but after radiation with the LD<sub>50</sub>, cuneate, four leaf, and wrinkle leaves shapes were observed in this study and those three leaves shapes were permanent and not seen in controls. Sangsiri (2005) reported eight variations of leaf shape of the second generation in mungbean that were radiated with gamma ray. Purnamaningsih *et al.* (2010) also reported the change of leaf form as a result of gamma ray in *Artemisia annua* L.

The LD<sub>50</sub> in this study did not change the leaf color. Hanafiah *et al.* (2010) also reported that gamma ray could not change the leaf color of *Glycine max* (L.) Merr. However, our results were not in agreement with what Pawar *et al.* (2010) observed in *Zingiber officinale* Roscoe, where the gamma ray could change the leaf color from green to become yellow and yellowish-green. Purnamaningsih (2010) also reported that gamma ray changed the leaf color of *Artemisia annua* L from green to become light green and dark green.

The delay of flowering time in this study was due to the LD<sub>50</sub> had altered DNA so that various metabolisms in the cells were disturbed, and finally the plant was late to flower and it followed by the delay of pod ripening. Suggested mutation in DNA which was caused by gamma ray irradiation could affect the photosynthesis process in plants. Consequently, the supply of nutrients for fruit was inhibited, and then the pod ripening was longer and the harvest was delayed.

In this study the number of pods per plant increased. This result was similar to the increasing of number of pods per plant in *Glycine max* (L.) Merr which was radiated by gamma ray (Hanafiah *et al.*, 2010).

The LD<sub>50</sub> of gamma ray did not affect the pod length, the number of seeds per pod, the

50-seeds weight, and the flower's color. The effects of gamma ray to those characters might be seen in the next generation as Soedjono (2003) reported that the effects of gamma irradiation were commonly detected in the next generation. However, the effects of gamma ray to many characters could be seen in the early generation (Hanafiah *et al.*, 2010; Lamseejan *et al.*, 2000, (Soedjono, 2003).

We found the change of seed color in the LD<sub>50</sub> treatment, namely from green or brownish-green to become brown and black. Besides the change of seed color, the LD<sub>50</sub> treatment also changed the seed shape, from rounded-box to become wrinkle. The change of the seed color was also found in *Arachis hypogaea* as reported by Satpute and Suradkar (2011). The seed color changed from pink to yellowish-pink and white at 15 kR dose which was detected in the third generation.

One of the methods in plant breeding to improve the properties or acquire high yielding varieties is doing induced-mutation using gamma rays. The first stage to perform that technique is determining the lethal dose (LD<sub>50</sub>) that vary between species and varieties or genotypes. In this study, the LD<sub>50</sub> has been determined and applied but have not managed to get a mutant plants as expected. Therefore, this study should be continued in the next generation, M2 or M3, or by doing induced-mutation again using the LD<sub>50</sub>. It is due to the effect of mutation usually emerged on the next generation, the changes in DNA caused by radiation of gamma rays occurs randomly, and sometimes induced mutation does not affect the genes involved in the expression of the characters expected.

## CONCLUSION

The LD<sub>50</sub> of Kampar mungbean cultivar based on the number of survival plants was 619.875 Gray which indicated that Kampar mungbean cultivar had low radio-sensitivity to gamma ray. The 619.875 Gray dose of gamma ray caused a delay in flowering time and pods ripening period. The same dose also resulted in the plants with higher plants, more branches, and more number of pods per plant as compared to control. This research will continue to develop

mutants with less trichomes, synchronous pod maturity, and pods were not dehiscent through next investigation on M2 and M3 generation.

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