Effects of gamma irradiation on the performance of Jatropha (Jatropha curcas L.) accessions

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Abstract. This study aimed to assess the effects of mutation by using gamma ray on the performance of jatropha plants. The study was conducted at PAIR BATAN. Jatropha seeds obtained from the collection farm of SBRC LPPM IPB and PT Indocement Tunggal Prakarsa Tbk in Gunung Putri, Bogor, were irradiated. The irradiated seeds were grown in Jonggol Trial Farm of IPB. Gamma irradiation was conducted by using a GCM 4000A device. Treatments consisted of irradiation doses, irradiation methods, and accessions. Irradiation doses given were 175, 200, 225 Gy, and no irradiation (control). Irradiation methods consisted of acute, intermittent, and split-dose. Accessions used in this study were Dompu, Medan, Bima, Lombok, ITP II, IP2P, and Thailand. Results of the study were analysed until 5 months after planting showed that gamma ray mutation gave stimulating and inhibiting effects on similar traits. Irradiation dose of 225 Gy was good to be given in acute, intermittent, and split-dose methods. Irradiation effects were found to be significant in jatropha accessions. Effects of irradiation on production will be published soon.

Key words: Jatropha, mutation, gamma ray, performance

1. Introduction

Jatropha produce fruits whose seeds contain oil that can be used as fuel for cooking, lighting, and biodiesel feedstock (1). Depending on their places of origins, jatropha seeds contain oil of up to 40.96% with an average figure of 36.43% (2). Jatropha fruits are found in inflorescencs formed from pollinated female flowers. Most fruits contain three seeds but some may have up to four seeds containing oil. The amount of oil that can be produced was found to be positively correlated with the number of inflorescences, number of harvested fruits per plant, and number of total fruits per plant, dry seed weight, and seed diameter (3).

Dry seed weight is the idiotype commonly used as an indicator of superior variety of jatropha plant as it is correlated with oil production. Dry seed production was significantly correlated with plant height, branch diameter, number of secondary branches per plant, number of productive branches per plant, number of inflorescences per plant, number of fruits per plant, number of seeds per plant, and dry weight of 100 seeds (4). Another research also shown that jatropha production was affected by number of inflorescences per plant and number of seeds per plant (5). Therefore, these characters can be used as important characters for the selection of jatropha plant.

However, not all characters which were correlated with high production of jatropha had high variability and heritability (3). Therefore, not all of those characters can be used as the selection
criteria for high producing jatropha plants. Other research showed that only number of inflorescents per plant and number of fruits per plant could be used selection criteria for high productivity of jatropha as they had wide genetic variability, high heritability, and high genetic advance (3,4,5,6). Narrow genetic variability from the observed characters indicated that the accessions of jatropha from various places in Indonesia were relatively uniformed although they came from geographically different origins. By using molecular markers to detect genetic variability of jatropha in India, it was found high level of uniformity among different genotypes. This meant that the accessions of jatropha currently available had narrow variability. In other words, current population was relatively uniformed, introduction from other countries, crossbreeding, or mutation was needed (7,8). Mutation is an effective method to improve genetic variability. It has been proven to improve the genetic variability of jatropha (9,10,11). Gamma irradiation is very important in mutation breeding. Mutagenesis can be used to improve genetic variation (12). Therefore, this study was aimed at analyzing the performance of jatropha produced by gamma irradiation.

2. Materials and Methods
The study was conducted at the Center for Isotopes and Radiation Application (PAIR), National Nuclear Energy Agency (BATAN). Jatropha seeds obtained from the collection farm of SBRC LPPM IPB and PT Indocement Tunggal Prakarsa Tbk in Gunung Putri, Bogor. The irradiated seeds were grown in Jonggol Trial Farm of IPB. As the jatropha seed availability was limited, the irradiation of it was done in two stages in March and April 2014. Gamma irradiation was conducted by using a GCM 4000A device. Treatments consisted of irradiation doses, irradiation methods, and accessions. Irradiation doses given were 175, 200, 225 Gy, and no irradiation (control). Irradiation methods consisted of acute, intermittent, and split-dose.

Accessions used in this study were Dompu, Medan, Bima, Lombok, ITP II, IP2P, and Thailand. In acute irradiation method, irradiation was done only once in the proposed doses. In intermittent method, irradiation was done twice in full doses. The irradiation was repeated within 1 week interval. In split-dose method, irradiation was done twice. The dose in the second irradiation was half of it in the first irradiation.

Irradiated seeds were grown on a nursery bed for 2 months before they were moved into the field. Planting was done in a planting distance of 2 m x 2 m. Planting holes were prepared in 3 weeks prior to planting time. Five kilograms of manure (organic fertilizer) was put into each planting hole. One seed was planted in each planting hole.

Morphological observation was done to assess the effects of irradiation on jatropha plants resulted from irradiated seeds (M1). The observation was done on seed germination, plant height, number of leaves, number of branches, number of inflorescents, and number of fruits per inflorescent. Means of plant growth rate in nursery bed were measured to assess the effects of irradiation and accessions used. Meanwhile, observation data from the field were obtained from individual plant. Data were subjected to an analysis of variance by using SAS 9 application.

3. Results and Discussions
3.1 Effects of irradiation on mutant seed germinability (M1)
The germination rate (%) of M1 irradiated seeds is shown in Table 1. Theoretically, irradiated seeds undergo genetic or physiological changes. Yet, when irradiated seeds can still germinate and grow into intact plants, then this is an indication that the irradiation done creates changes which are naturally acceptable (13). From the germination rate indicated that the irradiation dose and method applied were naturally acceptable for jatropha seeds.

Among all irradiated jatropha accessions, ITP II accession had the highest germinability (80.8%) although it was not different from those of Lombok (80.7%), Bima (60.6%), Medan (59.3%), and Dompu (79.25%) accessions. It was shown that some irradiated seeds had higher germination rates than control and lower doses groups. These included seeds of Lombok accession treated with intermittent dose of 175 Gy, Dompu accession treated with intermittent dose of 200 Gy, and Lombok, Bima, and Medan accessions treated with split dose of 225 Gy. Another research also found that gamma irradiation at the doses of up to 200 Gy did not cause a seed damage although it lowered seed germination rate especially in jatropha of IP-3A variety (14). The germination rates found in this
study also showed a stimulation effect of irradiation on seed germination. Higher doses of irradiation can effect on higher germination rate of jatropha seeds (14,15). Responses to gamma irradiation were also found to be different in each accession.

Table 1. Effects of gamma irradiation on jatropha seed germinability (%)

| Accession | Intermittent (%) | Split dose (%) | Acute (%) | Control |
|-----------|-----------------|----------------|-----------|---------|
|           | 175+175         | 200+200        | 225+225   | 175+87.5| 225+112.5| 175   | 200   | 225   |
| ITPII     | 90              | 90             | 80        | 70      | 90       | 80    | 80    | 67    | 90    |
| Lombok    | 92              | 67             | 69        | 90      | 100      | 92    | 67    | 69    | 90    |
| Bima      | 38              | 83             | 46        | 80      | 90       | 38    | 79    | 31    | 80    |
| Medan     | 58              | 67             | 21        | 90      | 100      | 50    | 67    | 21    | 90    |
| Dompu     | 83              | 92             | 67        | -       | -        | 83    | 92    | 58    | 90    |
| Mean      | 72              | 80             | 57        | 83      | 95       | 69    | 77    | 49    | 88    |

Effects of irradiation on mutant plant (M1) growth in nursery bed

In the nursery bed, mutant plants had various growth rates as indicated by their plant height, number of leaves, and number of branches. In stage 1 irradiation, in 2 months after planting (MAP), ITP II accession had the highest growth rate.

Jatropha plants of ITP II accession started to have branches in nursery bed. Similar development was shown by Lombok accession irradiated acutely with 225 Gy. Like germination rate, response of each accession on plant growth to gamma irradiation was varied. Gamma irradiation gave either stimulating or inhibiting effects on plant height. For Dompu, Bima and Lombok accessions, acute irradiation doses of 175 and 200 Gy inhibited plant height and number of leaves increment in nursery bed. In contrast, acute irradiation at 225 Gy was found to result in higher plant height and number of leaves. However, in nursery bed, two jatropha plants of Dompu accession in control group were found to have, on average, six branches. In stage 2 irradiation, increased growth rate and number of leaves were found in jatropha plants of Medan and ITP II accessions treated with 225 Gy acute irradiation. Growth rate of mutant plants in 2 MAP is shown in Table 2.

At second stage irradiation, in 1 month after planting (MAP), jatropha plants of Medan accession tended to have better growth as shown by their higher number of leaves (11.10) and plant height (24.91 cm). For plants of ITP II accession, repeated irradiation was found to inhibit their growth in nursery bed as indicated from their plant height and number of leaves. Inhibiting effect of gamma irradiation on plant height was also observed in Medan accession.

Gamma irradiations at 225 Gy (acute) and 175 Gy (split dose) increased plant height and number of leaves of Lombok accession in nursery bed. Similar effect on plant height and number of leaves was found in Bima accession treated with split dose irradiation at 225 Gy. On the contrary, inhibiting effects on plant height and number of leaves of Bima accession in 1 MAP were shown by acute irradiation at 225 Gy and split dose irradiation at 175 Gy. These findings indicated that both stimulating and inhibiting effects of irradiation were already experienced by plants since 1 MAP. Plant height and number of leaves in jatropha plants in second stage irradiation are given in Table 3.
| Jatropha accession | Irradiation doses (Gy) | Plant height (cm) | Number of leaves | Number of branches |
|-------------------|------------------------|-------------------|------------------|-------------------|
| Dompu             | 175                    | 9.2               | 6.1              | 0                 |
| Dompu             | 200                    | 12.1              | 7.2              | 0                 |
| Dompu             | 225                    | 25.1              | 20.4             | 0                 |
| Dompu Control     | 225                    | 22.2              | 13.8             | 2                 |
| Bima              | 175                    | 6.0               | 4.3              | 0                 |
| Bima              | 200                    | 10.8              | 5.6              | 0                 |
| Bima Control      | 225                    | 23.9              | 20.1             | 0                 |
| Lombok            | 175                    | 9.8               | 5.4              | 0                 |
| Lombok            | 200                    | 12.9              | 7.1              | 0                 |
| Lombok Control    | 225                    | 32.3              | 26.2             | 3.5               |
| Medan             | 175                    | 7.5               | 4.8              | 0                 |
| Medan             | 200                    | 11.6              | 6.4              | 0                 |
| Medan Control     | 225                    | 12.7              | 13.0             | 0                 |
| ITP II            | 200                    | 38.7              | 36.2             | 3.8               |
| ITP II            | 225                    | 43.0              | 32.0             | 2.75              |

| Treatment        | Mutation treatment (Gy) | Height (cm) | Number of leaves |
|------------------|-------------------------|-------------|------------------|
| ITP II           | control                 | 32.60       | 12.88            |
| ITP II 175+175   |                         | 26.43       | 9.28             |
| ITP II 200+200   |                         | 14.13       | 7.88             |
| ITP II 225+225   |                         | 19.60       | 9.56             |
| ITP II 225+112.5 |                         | 20.67       | 9.76             |
| ITP II 175+87.5  |                         | 22.57       | 12.21            |
| Lombok           | control                 | 15.00       | 5.33             |
| Lombok 175+87.5  |                         | 20.40       | 10.78            |
| Lombok 225       |                         | 17.33       | 5.14             |
| Lombok 225+112.5 |                         | 29.57       | 12.40            |
| Medan            | control                 | 36.00       | 21.50            |
| Medan 175+87.5   |                         | 14.33       | 4.11             |
| Medan 225        |                         | 30.14       | 16.00            |
| Medan 225+112.5  |                         | 30.25       | 13.20            |
| Bima             | control                 | 30.33       | 16.00            |
| Bima 175+87.5    |                         | 11.00       | 4.50             |
| Bima 225         |                         | 21.00       | 13.00            |
| Bima 225+112.5   |                         | 34.33       | 13.33            |
Effects of gamma irradiation in the field

Based on field observation within 5 months after planting, it was found that the treatments given to the seeds resulted in various plant growths. Gamma irradiation gave significant effects on the growth of jatropha plants of different accessions. The growth of treated jatropha plants is shown in Table 4. Like on seed germination, gamma irradiation gave stimulating and inhibiting effects on jatropha plant growth. These were indicated by plant height in 5 MAP, number of inflorescents and number of fruits per inflorescent in 4 MAP. The average plant height in 5 MAP was 84.5 cm with average number of branches of 5.3. In 4 MAP, the average number of inflorescents per plant was 2.7.

As shown in Table 4, irradiation doses and methods were found to be effective in improving plant growth. In plant breeding, induced mutation is an effective way to improve the variability of the existing germplasms and it can be used to improve plant varieties (16). The distribution frequency of plant height, number of branches, number of inflorescent per plant, and number of fruits per inflorescent of all M1 mutant plants is presented in Figure 1. It was known from this figure that M1 mutant plants had various number of branches, number of inflorescent per plant, and number of fruits per inflorescent.

It was revealed that plants resulted from irradiated seeds were either shorter or taller than those in control group. Irradiation dose of 225 Gy given in acute, intermittent, and split dose methods gave positive effects on plant height, number of branches, number of inflorescent per plant, and number of fruits per inflorescent in all plant accessions except ITP II accession. ITP II accession have negative impact of split dose irradiation at 225 Gy at plant growth and production. Meanwhile, irradiation doses of 175 and 200 Gy gave both positive and negative effects on plant height, number of branches, number of inflorescent per plant, and seed weight per harvested plant. Other report shown that mutation gave positive or negative effects on plant height (15,17). This phenomenon was also found in gladiolus plant, where gamma irradiation, at certain dose, was found to not only stimulate but also inhibit plant height growth (18). This might be caused by the fact that irradiation not only stimulates but also inhibits the division and elongation of meristematic cells including plant bud meristematic cells. Inhibition results in plant dwarfism.

For Bima accession, irradiation doses of 200 and 175 Gy which was repeated twice at a full dose resulted in plants which were shorter than those in control group. Meanwhile, irradiation of 225 Gy given in acute, intermittent, and split dose methods and irradiation of 175 Gy given in a split dose method resulted in plants which were taller than those in control group. The opposite results were found in Dompu accession for which irradiation doses of 200 and 175 Gy given twice in full doses resulted in plants which were taller than those in the control group. This result was similar to that given by 225 Gy irradiation.

For ITP II accession, acute and intermittent 225 Gy irradiation resulted taller plant than control group. Split dose 225 Gy irradiation, like acute 200 Gy irradiation, however, gave a negative effect on plant height. For ITP II accession, intermittent 200 Gy irradiation, acute and split dose of 175 Gy irradiation resulted in taller plant than control group plants. For Lombok accession, all irradiation treatments, except intermittent 175 Gy irradiation, resulted taller plant than control group plants. For Medan accession, acute, intermittent, and split dose 225 Gy irradiations resulted taller plant than control plants while 200 and 175 Gy irradiation in all methods produced plants shorter than control plants.
| Accession | Irradiation (Gy) | Plant height (cm) | Number of branches | number of inflorescents | Seed weight (g) |
|-----------|-----------------|-------------------|--------------------|-------------------------|-----------------|
| Bima      | control         | 73.46             | f     | 3.82                         | 2.36            | 17.5            |
| Bima      | 225             | 126.50            | a     | 6.75                         | bcd             | 7.50            | 45.02           |
| Bima      | 225+225         | 82.29             | c     | 5.00                         | bi              | 3.29            | 29.77           |
| Bima      | 200+200         | 67.00             | gi    | 5.18                         | bh              | 0.24            | -               |
| Bima      | 175+175         | 71.50             | f     | 4.00                         | d_i             | 0.33            | f               |
| Bima      | 225+112.5       | 99.67             | bcd   | 4.89                         | bi              | 5.89            | 71.48           |
| Bima      | 175+87.5        | 77.88             | ej    | 4.00                         | d_i             | 3.00            | f               |
| Mean      |                 | 85.47             | f     | 4.80                         | b_i             | 3.23            | 13.56           |
| Dompu     | control         | 73.67             | f     | 4.89                         | b_i             | 1.67            | f               |
| Dompu     | 225+225         | 91.2              | b-e   | 10.6                         | a-b             | 3.2             | f               |
| Dompu     | 200+200         | 84.14             | bi    | 6.43                         | bc              | 3.71            | f               |
| Dompu     | 175+175         | 74.82             | f     | 3.64                         | fi              | 0.09            | f               |
| Mean      |                 | 80.96             | -     | 6.39                         | f               | 2.17            | 15.31           |
| ITP       | control         | 84.57             | b-h   | 4.48                         | c     | 2.19            | f               |
| ITP       | 225             | 89.06             | b-h   | 5.31                         | b-h             | 1.50            | f               |
| ITP       | 200             | 84.19             | b-i   | 4.88                         | b_i             | 1.38            | f               |
| ITP       | 175             | 87.06             | b-h   | 4.22                         | d_i             | 1.44            | f               |
| ITP       | 225+225         | 109.25            | bc    | 6.50                         | be              | 2.33            | f               |
| ITP       | 200+200         | 103.56            | bc    | 6.11                         | b-f             | 7.56            | a               |
| ITP       | 225+112.5       | 83.71             | b-e   | 4.41                         | d_i             | 0.94            | f               |
| ITP       | 175+87.5        | 92.43             | b-e   | 5.50                         | b-b             | 2.79            | f               |
| Mean      |                 | 91.73             | -     | 5.18                         | f               | 2.52            | 27.42           |
| Lombok    | control         | 60.22             | j     | 2.67                         | ef              | 0.89            | f               |
| Lombok    | 225             | 67.50             | hij   | -                            | j                | 0.33            | f               |
| Lombok    | 225+225         | -                 | -     | 7.11                         | j              | 40.66           |
| Lombok    | 200+200         | 68.75             | gj    | 2.50                         | hj              | 0.12            | f               |
| Lombok    | 175+175         | 58.40             | f     | 2.00                         | gj              | 0.40            | f               |
| Lombok    | 225+112.5       | 92.00             | b-e   | 4.80                         | b_i             | 1.60            | f               |
| Lombok    | 175+87.5        | 79.67             | d     | 3.33                         | ghi             | 2.22            | f               |
| Mean      |                 | 71.09             | -     | 3.06                         | f               | 1.81            | 13.47           |
| Medan     | control         | 91.50             | b-e   | 5.67                         | bg              | 2.67            | f               |
| Medan     | 225             | 102.29            | bc    | 7.43                         | bc              | 7.36            | a               |
| Medan     | 225+225         | 98.00             | b-e   | 12.00                        | a                | 4.00            | b-c             |
| Medan     | 200+200         | 81.25             | cj    | 5.25                         | bh              | 1.50            | f               |
| Medan     | 175+175         | 69.00             | f     | 6.40                         | b-f             | 0.40            | f               |
| Medan     | 225+112.5       | 106.05            | b     | 7.55                         | b                | 8.25            | a               |
| Medan     | 175+87.5        | 72.65             | f     | 2.88                         | ghi             | 0.82            | f               |
| Mean      |                 | 88.68             | -     | 6.74                         | f               | 3.57            | 179.52          |

Plant height is one of the most important characters in the selection of superior jatropha plant. Farmers prefer shorter plants so it easier to reach during harvest time. However, plant height is not the only one to consider. Other production characters including number of inflorescents per plant,
number of fruits per inflorescent, and dry seed production are also important. Tall plants with higher number of inflorescents and fruits are preferred to shorter plants with fewer inflorescents and fruits.

In branch growth character, mutagen treatment resulted in plants with higher or fewer number of branches than control plants. However, the results were not significant in Bima, Dompu, ITP II, and Lombok accessions. For Medan accession, intermittent 225 Gy irradiation produced plants having 12 branches. Branch in jatropha plant is an important character as it relates to fruit production characters. Flower production in jatropha plant is always related to plant branch growth. Flowers or inflorescents can be formed in plant axial or terminal positions. If inflorescents are formed in stem terminal, branches could be formed after the plant forms flowers. Meanwhile, if flowers are formed in axial position, the plant would have indeterminate growth and few branches tended to be formed. Previous studies showed that branches in jatropha plants could be formed in early growth after the plants had 5-10 leaves (Nisya, 2010) or 60-70 leaves or within 4-5 MAP (19). In order to increase fruit production in jatropha plant, pruning is always done to increase number of branches. More inflorescents are expected with higher number of branches.

Number of inflorescents per plant was affected by irradiation. The effects of irradiation on number of inflorescents were both positive and negative. The highest number of inflorescents (8.25) was found in Medan accessions treated with split dose 225 Gy irradiation. However, it was not different with Medan accessions treated with acute 225 Gy irradiation (7.36), Lombok accessions treated with intermittent 225 Gy irradiation (7.11), ITP II accessions treated with intermittent 200 Gy irradiation (7.56), and of Bima accessions treated with acute irradiation (7.50).

Inflorescent in jatropha plants is a cluster of flowers consisting of female and male flowers. However, in some accessions in Indonesia inflorescents were found to consist of male and hermaphrodite flowers (3). In this study, within 1 MAP, some plants had already had flowers. For all accessions, intermittent irradiation treatment of 225 Gy was found to result in plants which produced flowers early within 1 MAP. It was indicated by the finding that more than 50% of all plant accessions treated with intermittent 225 Gy irradiation had flowers. Mutant plants having early flowering were also found in Bima and Medan accessions treated with 225 Gy split dose irradiation, ITP II accessions treated with 200 Gy intermittent irradiation, Lombok accessions treated with 175 Gy split dose irradiation, Medan accessions treated with 225 Gy acute irradiation, and Medan accessions treated with no irradiation.
This study shown that for different accession, the effects of irradiation were different. Radio sensitivity of the irradiation given varied among plant species (20). The difference in radio sensitivity among cultivars or breeding lines is directly related to their genetic. This might occur as each accession has different base sequence. The frequency of mutation occurrence is affected by different base sequences in various plant genetic (21).

Conclusions
It was concluded that gamma irradiation gave both stimulating and inhibiting effects on the morphological and agronomical characters observed. Acute, intermittent, and split dose 225 Gy irradiation tended to give stimulating effects in jatropha. Irradiation effects were different among accessions.

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