Growth responses of alfalfa (Medicago sativa (L.) as affected by gamma irradiation

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Abstract. Minimum availability of important feed resource for ruminant livestock was worldwide problem. Biotechnology like plant breeding is one of the problem solving to attain supplying forage. The purpose of this study was to assess the influence of gamma rays irradiation on growth responses of alfalfa seeds for increasing genetic variety of alfalfa. Sample of alfalfa seeds, categorized by different doses level (0 Gy, 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy), were irradiated by gamma rays. After irradiating, the seeds (n=350 replicates) were planted and grown on the polybags filled the soil mixed compost up. In 30, 45 and 60 days, plant height, total stem and total leaf, were measured manually. Related to the amount of total values growth responses, after irradiated and grew, total plant height, total stem and total leaf was highest with dose 400 Gy, 200 Gy and 100 Gy (R² = 0.99511, 0.99107, 0.99326). All of lowest responses were 500 Gy. It is concluded that, growth responses investigated, there are association between increasing rate of irradiation with alterations on growth responses. Increasing doses have impact on the decline in the responses of growth.

1. Introduction
Alfalfa (Medicago sativa L.) is a perennial legume which is important forage crop worldwide. Biotechnology, such as plant breeding, can be the problem solving to attain supplying forage.

Induced mutations by gamma irradiation is one of the way used to increase biodiversity and to provide breeding material for conventional plant breeding. Seeds tolerance to radiation exposure may vary widely among species due to differences in morphology, as well as differences in genetic.

Many research have been done about mutation induce but still lack of evidence that can be investigate more. Dose of gamma ray irradiation in soybean with 3.0 kGy [1], 25, 50 and 75 Gy [2], alfalfa (Medicago sativa (L.) dose 350 Gy [3] provide different responses. It is influenced by several factors such as morphology and plant physiology, age, type, size, gene composition, irradiation dose, and irradiation type. It is important to know an appropriate radiation dose for a species prior to initiating large-scale mutation research. Effect of radiation exposure to seeds capability being tolerance differ among species due to vary widely in morphology, as possible as differences in genetic or biochemical arranging.

There are not many research about gamma irradiation doses of alfalfa seeds. The present study was undertaken to assess the effect of gamma rays irradiation on growth responses of alfalfa seeds.
2. Materials and methods

2.1. Irradiation of the alfalfa seeds
Irradiated seedlings of alfalfa were performed in a Cobalt-60 in October 2017, at Radiation and Isotope Technology, Indonesia (PATIR BATAN). Alfalfa seeds were treated with different dose. Five dose level of gamma rays were used 100, 200, 300, 400, 500 Gy with dose rate was 5925.3 Gy/h. Un-irradiated seeds were used as a control (0 Gy). Each treatment was replicated 350 with 2 seeds in each replicate (figure 1).

2.2. Variables measured
In the present study, variables measured were plant height, total stem and number of leaves. Samples of alfalfa, using manual method, were assessed in 30, 45 and 60 days.

2.3. Statistical analysis
Data were subjected to analysis of variance (ANOVA) using Excel (2010) and the software SPSS (23.0). Means and standard deviations were calculated. The mean values were compared using Duncan’s Multiple Range Test (DMRT). Mean differences were separated by the least significant difference procedure using the significant level of p < 0.05.

3. Results and discussion

3.1. Effects of gamma ray irradiation on plant height
Results of irradiation effect on plant height are presented in table 1. The seeds irradiated with 100 Gy have shown highest plant height in 30 days but no significant difference with control. In 45 days, The highest was 100 Gy was compared with control and significant difference with all dose. Furthermore, control has shown maximum plant height (p<0.05) to the all dose. 500 Gy was noticed as the lowest plant height in all days.

Figure 1. Media growing seeds each dose of gamma irradiation.

Increasing the enzymatic that supporting activation and awakening of the young embryo may be caused by gamma rays irradiation with low doses, which stimulating the rate of cell division and affects not only germination, but also vegetative growth [4].
### Table 1. Effect of irradiation on alfalfa plant height.

| Irradiation Dose (Gy) | Days | 30       | 45       | 60       |
|-----------------------|------|----------|----------|----------|
|                       |      | 9.85±3.42| 14.51±5.77| 18.14±6.84|
| 0                     |      | 9.33±3.35| 15.65±6.76| 16.97±9.66|
| 100                   |      | 9.89±1.41| 12.20±3.99| 16.61±5.73|
| 300                   |      | 8.96±1.87| 13.03±3.76| 14.69±4.94|
| 400                   |      | 8.89±2.66| 12.22±4.59| 16.47±6.26|
| 500                   |      | 7.35±2.78| 8.39±3.59 | 10.80±5.18|

0 Gy was used as the control. Means with different letters in one column were significantly different at P < 0.05. Each value compared in the same days.

Plant height rate declined slightly as the radiation doses were increased (figure 2). The increasing dose of irradiation causes limited on plant height response.

![Figure 2. Dose respons curve of plant height.](image)

Gamma ray irradiation can influence plant growth and development in physiology, biochemistry, genetics and morphology of cells and tissues. Insignificant irradiation doses can stimulate the plant growth rate [5].

### 3.2. Effects of gamma ray irradiation on total stem

Effects of gamma ray irradiation on total stem are presented in table 2. The seed irradiated with 400 Gy have shown highest total stem in 30 days and significant difference (p<0.05) with control. In 45 days, The highest was 100 Gy but no significant difference compared with control whereas 100 Gy was significant with control in 60 days. According to [6] excessive radiation administration can induce chromosomal damage that causing cell death when condition of cell nucleus is an unbalanced atomic arrangement.

### Table 2. Effect of irradiation on total stem.

| Irradiation Dose (Gy) | Days | 30       | 45       | 60       |
|-----------------------|------|----------|----------|----------|
|                       |      | 4.50±1.5 | 9.80±3.97| 10.52±6.23|
| 100                   |      | 4.96±1.73| 10.05±3.57| 13.67±4.56|
| 200                   |      | 4.84±0.74| 7.46±2.46 | 9.34±3.60 |
| 300                   |      | 4.38±0.68| 8.37±2.14 | 9.50±3.05 |
| 400                   |      | 5.12±1.59| 8.74±2.91 | 10.84±4.00|
| 500                   |      | 3.99±1.46| 6.28±2.44 | 9.25±3.03 |

0 Gy was used as the control. Means with different letters in one column were significantly different at P < 0.05. Each value compared in the same days.
Total stem rate declined continuously as the radiation doses were increased (figure 3). Gamma irradiation dose increasing induce a decrease on total stem of alfalfa. figure 2 shows that a 500 Gy irradiated dose inhibits the increase in the number of alfalfa stem.

Symptoms like sterilization, death of plants, appearing a disruption on growth response physiological damage were happened due to the effects of gamma ray radiation [7]. Plant height related with stem elongation [8] that might be minimally affected by radiation.

3.3. Effects of gamma ray irradiation on number of leaves
Results of irradiation effect on number of leaves are presented in table 3. The irradiated seedlings with 100 and 200 Gy have shown significant increase (p<0.05) on the number of leaves in 30 days compared to control with the highest at 200 Gy and 500 Gy is the lowest. In 45 and 60 days, the highest number of leaves were found at 100 Gy but no significant difference (45 days) but there is significant difference in 60 days compared with control.

| Irradiation Dose (Gy) | Days  |          |          |          |
|-----------------------|-------|----------|----------|----------|
|                       | 30    | 45       | 60       |          |
| 0                     | 13.22±4.79 | 28.35±11.70 | 36.94±14.56 |          |
| 100                   | 14.10±5.01 | 29.16±49.75 | 40.46±16.69 |          |
| 200                   | 14.26±1.91 | 18.74±8.71 | 26.38±10.91 |          |
| 300                   | 12.88±2.18 | 23.89±6.21 | 26.40±9.22 |          |
| 400                   | 13.00±5.62 | 25.05±8.35 | 32.04±11.67 |          |
| 500                   | 11.41±4.16 | 17.81±7.20 | 27.47±9.13  |          |

0 Gy was used as the control. Means with different letters in one column were significantly different at P < 0.05. Each value compared in the same days.

Number of leaves responses rate declined continuously as the radiation doses were increased (figure 4). It was noticed that dose response curve to the number of leaves were relatively decline but it was not constantly among doses.
According to [9] ionizing irradiation exposure, especially gamma rays with high doses, may damage or modify cellular components of plants that impact on morphological, anatomical, biochemical and plant physiological changes such as thylakoid membrane damage, inhibition of photosynthesis rate, antioxidative system modulation and accumulation of phenolic compounds.

4. Conclusion
Induced gamma rays may impact on characteristic growth rate of alfalfa. Related to the amount of total values growth responses, after irradiated and grew, total plant height, total stem and total leaf was highest with dose 400 Gy, 200 Gy and 100 Gy ($R^2$= 0.99511, 0.99107, 0.99326).

Gamma ray irradiation, increasing dose, can be an obstacle to the plant height, number of leaves and stems to more developing.

This research can establish range of irradiation dose afterwards it will contribute to provide an information for investigating further biological and genetic effects in huge populations.

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