The effect of gamma rays irradiation on the growth and flavonoid content of kenikir (Cosmos caudatus Kunth.)

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Abstract. The aim of the research was to identify agronomical characteristics and flavonoid content of kenikir (Cosmos caudatus Kunth.) irradiated by gamma rays with several dosages. Seeds of kenikir were irradiated by gamma rays in radiator chamber 4000 A using ⁶⁰Co source at Isotope and Radiation Application Center, BATAN, Jakarta. Dosages of irradiation ranging from 50 Gy- 300 Gy with interval 50 Gy. Subsequently, irradiated seed along with unirradiated seeds (control) were planted in Greenhouse Agricultural Faculty, Universitas Sumatera Utara (± 32 meters above sea level). The variation of agronomical characters (plant height, leaf number, stem diameter, number of branches per plant and flowering age) were observed from 1st week up to 9th week after planting. Data were analyzed by t-test using minitab 18 program. The flavonoid content of leaves extract were analyzed using spektrofotometer UV-VIS in Research Laboratory, Faculty of Pharmacy, Universitas Sumatra Utara. The results showed that the mean values of M1 generation of all agronomical characters observed were lower than control plants whereas the highest flavonoid content was found in the population of gamma ray irradiated at 100 Gy.

1. Introduction

Kenikir (Cosmos caudatus Kunth.) is a vegetable that can be developed as an alternative crop to meet the increasing vegetable needs in Indonesia. The young leaves of kenikir are commonly used as fresh vegetables or appetizers because they have a distinctive flavor and aroma [1]. It is a vegetable and medicinal plant that has the potential to be developed. The vegetables has not been well known widely and usually these vegetables are only found in the traditional market [2].

Many bioactive constituents were isolated from C. caudatus leaves and roots, those are mainly flavonoids, phenolic acids, vitamins, minerals and phenylpropanoids are responsible for many pharmacological activities attributed to fresh C. caudatus leaves or extracts. Anti-diabetic, antihypertensive, antimicrobial, anti-osteoporotic and anti-inflammatory activities were proved in clinical, in vitro and in vivo studies [3]. High contents of phytochemicals, antioxidants, proteins, amino acids, vitamins, and minerals are associated with degenerative diseases risk reduction, such as cancer, diabetes, hypertension, cardiovascular diseases and osteoporosis, as it is thought that these diseases are connected with high free radical levels [4].

One of the efforts to obtain new improved varieties need to be supported by the high genetic diversity of plants. Mutation is one of the most effective ways to increase genetic diversity in plants of low genetic diversity and the conventional variety such as kenikir.
Genetically irradiated disorders will produce new mutants, then manipulated to obtain good crops and have the ability to produce high secondary metabolites. One of the irradiated rays that is often used as a physical mutagen is a gamma ray which is a short wavelength electromagnetic wave and has great penetrating power and velocity [5].

Gamma rays were reported to be the most efficient ionizing radiation of creating mutants in plants as they can induce high mutation numbers in plants. It could also modify physiological characteristics to create new mutants with improved properties that can produce higher amounts of commercially essential metabolites, developing varieties that are agriculturally and economically significant, and contain high productivity potential [6-7]. Mutations with gamma-ray irradiation were able to increase level of secondary metabolite compounds and plant productivity [8].

Flavonoids are the most important groups of secondary metabolism in plants that consider as good sources of natural antioxidant in human diets. In the last decade, gamma irradiation has been drawn the attention as a new and rapid method to improve the qualitative and quantitative characters of many crops. Irradiation with gamma rays is currently used as a tool in mutation breeding technology for enhancing the production of plant secondary metabolites like alkaloids or to increase biomass production in medicinally valuable plants [9].

Based on the above description, the authors are interested in conducting research entitled “The Effect of Gamma Rays Irradiation on The Growth and Flavonoid Content of Kenikir (Cosmos caudatus Kunth.)”

2. Materials and Methods

2.1 Preparing plant materials

Seeds of kenikir were irradiated by gamma rays in radiator chamber 4000 A using 60Co source at Isotope and Radiation Application Center, BATAN, Jakarta. Dosages of irradiation ranged from 50 Gy-300 Gy with interval 50 Gy. Subsequently, irradiated seed along with unirradiated seeds (control) were planted in Greenhouse Agricultural Faculty, Universitas Sumatera Utara (± 32 meters above sea level).

Analysis of total flavonoid content was conducted at the Research Laboratory of the Faculty of Pharmacy, Universitas Sumatera Utara. The material used is a leaf that is not too young and old that leaves to 4 to 9 from the growing point that has been opened perfectly when the plant aged 9 week after planting.

2.2 The maximum wavelet determination (λ) of quercetin.

A total of 10 mg of quercetin (comparison) was weighed and dissolved in 10 ml of methanol as a stock solution. Then dilutions of quercetin with concentration of 20, 30, 50, 60, 80 μg / ml as comparable quercetin solution. A total of 0.5 ml of comparative aqueous solution (quercetin) was diluted with 1.5 ml of methanol and then added 10% aluminum (III) chloride, 0.1 ml of 1M sodium acetate and 2.8 ml of aquadest. After incubation for 30 min, the absorbance of the reference solution was measured by UV-visible light spectroscopy at a wavelength of 429 nm. Each comparative solution was measured three times. Created calibration curve and obtained regression of linear equations.

2.3 Assay of flavonoid content

Ethanol extract 96% of kenikir was dissolved in methanol with a concentration of 2000 μg / ml, for 96% ethanol extract. A total of 0.5 ml of the test sample was added with 1.5 ml of methanol, then added 0.1 ml of aluminum (III) chloride 10%, 0.1 ml of 1 M sodium acetate, and 2.8 ml of aquadest. After incubation for 30 minutes, the absorbance of the reference solution was measured by a spectrophotometer. The light appears at a wavelength of 429 nm. Total flavonoid was calculated by using the linear regression equation of the quantified quercetine calibration curve.
3. Result and Discussion

3.1 Agronomical characteristic

The results of the irradiation several dosages of gamma ray on the observation of height of 1, 3, 5, 7, and 9 week after planting revealed that highly significant effect of gamma ray irradiation on plant height at age 1 week after planting was seen at dosages of 100 Gy to 300 Gy, this occurred every week until 3 week after planting. At 5 and 9 week after planting gamma ray irradiation had a very significant effect on the dosage of 150 Gy. At 7 week after planting gamma ray irradiation had a significant effect on 100 Gy dosage and had a very significant effect on the dosage of 150 Gy. Gamma ray irradiation was not significantly different from plant height at dosage of 50 Gy. The plant height at dosage of 50 Gy is still close to the average of control plants (0 Gy), so it does not give a real effect. The higher dosage of irradiation given will further suppress the growth of plant height, can even cause death in plants, this occurs at dosages of 250 Gy and 300 Gy, the crop was not able to survive at age 3 week after planting and at dosages of 200 Gy the plant is not able to survive at age 5 week after planting.

Data on the height of the plants in irradiation of several dosages of gamma ray can be seen in Table 1.

| No. | Dosages | Week 1 | Week 3 | Week 5 | Week 7 | Week 9 |
|-----|---------|--------|--------|--------|--------|--------|
| 1   | 0 Gy    | 6.30 ± 0.86 | 15.34 ± 2.33 | 28.36 ± 3.80 | 39.89 ± 6.38 | 57.49 ± 7.56 |
| 2   | 50 Gy   | 5.32 ± 1.83 | 15.16 ± 2.80 | 26.72 ± 3.71 | 39.63 ± 5.18 | 57.03 ± 7.05 |
| 3   | 100 Gy  | 3.62** ± 2.46 | 12.80** ± 2.21 | 26.55 ± 4.05 | 36.42* ± 5.92 | 55.12 ± 6.82 |
| 4   | 150 Gy  | 0.93*** ± 0.95 | 4.80*** ± 0.49 | 2.47** ± 3.59 | 1.63*** ± 4.15 | 2.41** ± 6.23 |
| 5   | 200 Gy  | 0.97*** ± 1.13 | 1.05*** ± 2.09 |        |        |        |
| 6   | 250 Gy  | 0.67*** ± 0.83 |        |        |        |        |
| 7   | 300 Gy  | 0.68*** ± 0.83 |        |        |        |        |

* and ** = Significant at 5% and 1 % level of probability at t test analysis.

Based on the results of the research that has been done showed that the effect of highly visible gamma ray irradiation on plant height at age 1 week after planting started to be seen at a dosage of 100 Gy to 300 Gy, this happens every week until age 3 week after planting. At the age of 5 and 9 week after planting gamma ray irradiation had a very significant effect on the dosage of 150 Gy. At age 7 week after planting gamma ray irradiation had a significant effect on 100 Gy dosage and had a very significant effect on the dosage of 150 Gy. Gamma ray irradiation was not significantly different from plant height at dosage of 50 Gy. The plant height at dosage of 50 Gy is still close to the average of control plants (0 Gy), so it does not give a real effect. The higher the dosage of irradiation given will further suppress the growth of plant height, can even cause death in plants, this occurs at dosages of 250 Gy and 300 Gy, the crop was not able to survive at age 3 week after planting and at dosages of 200 Gy the plant is not able to survive at age 5 week after planting. This corresponds to [10] stating that the higher the irradiation dosage significantly affects plant height, where the higher the dosage of irradiation given the higher the average of the plant will decrease. This is also supported by [11] assertion that gamma-ray irradiation causes damage to morphological characters that include inhibition of growth in a plant. The higher the dosage of irradiation given will cause more damage to the plant can even cause death. The performance at dosages of 50 Gy, 100 Gy, 150 Gy, 200 Gy, 250 Gy and 300 Gy when compared to control plants can be seen in Figures 1 and 2.
Figure 1. The performance of kenikir at 2 week after planting.

The leaves number of kenikir plant in irradiation of several dosages of gamma ray can be seen in Table 2.

Table 2. Mean leaf number of plant of 1, 3, 5, 7, and 9 week after planting.

| No. | Dosage of irradiation | Week 1     | Week 3     | Week 5     | Week 7     | Week 9     |
|-----|-----------------------|------------|------------|------------|------------|------------|
| 1   | 0 Gy                  | 1.50 ± 0.65| 6.29 ± 0.47| 12.07 ± 1.77| 18.71 ± 2.02| 19.57 ± 2.38|
| 2   | 50 Gy                 | 1.71 ± 0.47| 6.07 ± 0.73| 11.64 ± 1.55| 18.50 ± 2.21| 18.86 ± 2.41|
| 3   | 100 Gy                | 1.14 ± 0.77| 5.93 ± 0.73| 10.79* ± 1.19| 16.00* ± 2.04| 16.43* ± 3.01|
| 4   | 150 Gy                | 0.50** ± 0.52| 1.93** ± 0.27| 1.21** ± 2.39| 1.29** ± 3.29| 2.00** ± 5.14|
| 5   | 200 Gy                | 0.50** ± 0.52| 0.29** ± 0.61|            |            |            |
| 6   | 250 Gy                | 0.43** ± 0.51|            |            |            |            |
| 7   | 300 Gy                | 0.43** ± 0.51|            |            |            |            |

* and ** = Significant at 5% and 1% level of probability at t test analysis.

Number of leaves of 1 and 3 week after planting in irradiated gamma ray 0 Gy had a very real effect by giving gamma ray irradiation 150 Gy, 200 Gy, 250 Gy and 300 Gy but not significantly different with giving gamma ray irradiation 50 Gy and 100 Gy. On 3 week after planting of Kenikir plants with irradiated dosages of 250 Gy and 300 Gy can not survive the whole plant because the effect of the dosage
given was too high. On 5 week after planting with 0 Gy treatment was significantly different from 100 Gy gamma ray irradiation and had a very significant effect on the dosage of 150 Gy while the crop of 200 Gy dosages was not able to survive at age 5 week after planting. On 7-9 week after planting with 0 Gy treatment was significantly different from 100 Gy gamma ray irradiation and had a very significant effect on giving gamma ray irradiation 150 Gy but not significantly different with 50 Gy gamma ray irradiation. This was because the higher the dosage of irradiation given to the plant cause the inhibition of the number of leaves produced even at certain dosages cause the plant was not able to survive. Based on [12] study, it is said that the number of leaves for high dosages irradiation treatment that is 60 and 90 Gy on dendrobium orchid planlet has decreased and significantly different to control. This is also supported by the research of [13] which states that gamma-ray irradiation on the Samosir shallotplant causes a reduction in the number of irradiated leafs of plants irradiated with the number of unaturized plant leaves (control) in the first generation.

The development of the leaves number at aged 1-9 week after planting on administration of several doses gamma ray irradiation can be seen in Figure 2.

![Figure 2](image-url)

**Figure 2.** The performance of development the leaves number at aged 1,3,5,7,9 week after planting
The mean diameter of the plant stems in irradiation of several dosages of gamma ray can be seen in Table 3.

| Week | Dosage of irradiation (Gy) | Mean     |
|------|---------------------------|----------|
| 9    | 0 Gy                      | 6.64 ± 1.16 |
|      | 50 Gy                     | 5.63* ± 1.12 |
|      | 100 Gy                    | 4.50** ± 1.50 |
|      | 150 Gy                    | 0.06** ± 0.16 |

* and ** = Significant at 5% and 1% level of probability at t test analysis.

The stem diameter of the age crops of 9 week after planting in irradiated of gamma ray resulted from the analysis of variance showed that the dosage of 0 Gy resulted in larger stem diameter than other treatments. This is presumably because the seeds given the dosage of irradiation in the first offspring will affect the damage to the plants, including the decrease in diameter of the stem resulting from each treatment. According to research of [14] stated that the control plants have the highest average diameter of the stem while the lowest is owned by the plants with the highest irradiation dosage treatment. However, in general the diameter of the stem of the plant decreases at high irradiated dosages when compared with control.

Data on the branch number of the plants in irradiation of several dosages of gamma ray can be seen in Table 4.

| Week | Dosage of irradiation (Gy) | Mean     |
|------|---------------------------|----------|
| 9    | 0 Gy                      | 19.93 ± 6.57 |
|      | 50 Gy                     | 18.21 ± 8.22 |
|      | 100 Gy                    | 7.57** ± 4.78 |
|      | 150 Gy                    | 0.00** ± 0.00 |

** = Significant at 1% level of probability at t test analysis.

Giving several dosages of gamma ray irradiation also had a significant effect on the number of branches per plant at age 9 week after planting. The irradiation dosage of 0 Gy (control) yields the largest number of branches per plant compared to other treatments. This is allegedly due to the formation of branches associated with the stem. Higher stems of plants have greater potential to form branches and segments of plants. Irradiation is also influential in the process of plant physiological damage that will also affect the number of branches per plant. The higher the dosage of irradiation given the less number of branches per plant produced. This is in accordance with research [15] which states at dosages given the given gamma rays the higher the number of branches becomes greatly reduced compared with the control.

From the results of analysis of variance can be seen that giving various dosages of gamma ray irradiation gives a real effect on the age of flowering kenikir. Control plants (0 Gy) resulted in faster flowering age of plants compared to other treatments and even in plants with a treatment of 100 Gy there are some plants that are still not flowering in time, as well as on administration of gamma ray irradiation with a dosage of 150 Gy no single flowering plants. It is suspected that the higher dosages of gamma ray irradiation given to the kenikir plants can affect the length of flowering process in the plant due to the physiological damage caused by the irradiation effect. This is in accordance with the statement of [16] which states that the reduction of reproductive ability caused by mutagen has various phenomena,
including: growth barriers that block the flowering, the flowers are formed but not meet the required reproduction, the form of reproduction occurs, but the flour the juice is barren, and the seeds are formed but are unable to germinate.

The flowering age of kenikir plant in irradiation of several dosages of gamma ray can be seen in Table 5.

| Dosage of irradiation (Gy) | Mean     |
|---------------------------|----------|
| 0 Gy                      | 50.00 ± 2.20 |
| 50 Gy                     | 52.00* ± 1.34 |
| 100 Gy                    | 58.00** ± 28.20 |
| 150 Gy                    | 0.00** ± 0.00 |

* and ** = Significant at 5% and 1 % level of probability at t test analysis.

3.2 Flavonoid content

The total flavonoid content of leaves in giving gamma ray irradiation result of analysis of variance showed that dosage 0 Gy (plant control) have real effect to other treatment. This was presumably because the plant seeds given irradiated dosages give a real effect on the physiological changes and the content of the compounds contained in kenikir plant. This was in line with the results of a study conducted by [17], that the content of flavonoids of pea was treated with UV-B treatment with the status setting of the water larger than the single treatment of each. The total flavonoid content of kenikir plant in irradiation of several dosages of gamma ray can be seen in Table 6.

| Week | Dosage of irradiation (Gy) | Flavonoid content |
|------|----------------------------|-------------------|
| 9    | 0 Gy                       | 68.016 ± 7.18     |
|      | 50 Gy                      | 54.797 ± 0.08     |
|      | 100 Gy                     | 132.802** ± 0.20  |
|      | 150 Gy                     | 74.429 ± 0.08     |

** = Significant at 1 % level of probability at t test analysis.

4. Conclusion

The mean value of first generation (M₁) from gamma ray irradiation results in all observed agronomical characters is lower than the mean of control plants. The highest content of flavonoid obtained in the population of gamma ray irradiation with a dosage 100 Gy.

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