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Research Article
Effect of Gamma Rays Irradiation on M_1 Generation of Roselle (*Hibiscus sabdariffa* L.)

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Abstract

Background: Roselle have low genetic diversity, because its self-pollinating plant so it needs to be an effort to increase the genetic diversity of plants. The one of effort to increase genetic diversity is through mutation. Mutation induction of roselle plants aims to increase the genetic diversity of the plants that indicated by morphological changes and improve the character of the plant. **Materials and Methods:** Roselindo 2 variety seeds was used and evaluated the response on the growth and development of the first generation (M_1) through gamma ray irradiation treatment. These seeds subjected to: 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 Gy to obtain the value of Lethal Dose 50 (LD_{50}). Furthermore roselle seeds irradiated with doses of 0, 150, 300, 450 and 600 Gy to increase genetic diversity in roselle plants. **Results:** The results indicate that the value of Lethal Dose 50 (LD_{50}) at 477.803 Gy. The variation derived from the number of branches and canopy diameter on M_1 generation affects plant growth and development. Morphological changes occur in the form of branches, leaves and flower color than the control plants. **Conclusion:** Mutation induction with doses range of 0-600 Gy affects the growth and morphology diversity of roselle plants compared to plants without irradiation.

Key words: Radiosensitivity, irradiation effect, genetic diversity, morphological changes, gamma rays, M_1 generation, mutation, roselle, Roselindo 2 variety

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Utilization of roselle flowers as food is very diverse, among others, as an herbal tea, jam, vegetables, juice, flavor etc. Roselle is more widely known as the flower that can be used as raw material for the manufacture of herbal tea in Indonesia. Puspitowati *et al.*[1] stated that red roselle has benefits to overcome the various of disease and health problems as a medicinal plant. Among its many usefulness, roselle featured as a herbal anticancer, antihypertensive and antidiabetic. Ninety six percent ethanol extract of roselle flower petals contain flavonoid compounds, saponins and alkaloids. The content of phenols and flavonoids in the roselle flower petals thought to have immunostimulatory effects. Flavanoid compounds in roselle flower petals one of which is anthocyanin.

Roselle superior varieties obtained through plant breeding by improving yield and crop adaptation. The new varieties require a population base which has a high genetic diversity. Roselle have low genetic diversity, because its self-pollinating plant so it needs to be an effort to increase the genetic diversity of plants. The one of effort to increase genetic diversity is through mutation. Mutation breeding is useful to improve the character of the plant if desired character not found in a species of plant germplasm. Mutation induction is done by using a mutagen, one using gamma ray irradiation. Mutation induction of roselle plants aims to increase the genetic diversity of the plants that indicated by morphological changes, nutritional content and adaptation to the environment.

These results have been done by some researchers[2-5] whom studied gamma irradiation which it has been used to increase the genetic diversity, quantitative characters and improve the quality and nutrition of roselle plants. The results of this study are expected to reveal various information that useful in the development of roselle plants through gamma ray irradiation.

MATERIALS AND METHODS

This study was carried out in BATAN (National Atomic Energy Agency) for irradiation treatment. Field research was conducted at the University of Sumatra Utara, Medan. Roselle seeds of Roselindo 2 variety were treated with 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 Gy of gamma irradiation derived from irradiator gamma chamber. The effect of irradiation on germination was observed. Percentage germination data used to determine the value of Lethal Dose 50 (LD_{50}). The data were analyzed using curve expert program. Furthermore roselle seeds irradiated with doses of 0, 150, 300, 450 and 600 Gy for conducting the field experiment. A total of 20 seeds (M_1) in each treatment dose planted with a spacing of 100×100 cm^2 and one seed per hole. The variability in agronomic characters viz., plant height, number of branches and canopy diameter were evaluated. Genetic variation in M_1 generation was also observed.

RESULTS AND DISCUSSION

Based on the LD_{50} value obtained, it will be the determination of the doses of irradiation that used to induce diversity in plants and obtain the desired characters. Observations in 2 weeks after planting showed that gamma-ray irradiation at a dose of 500 Gy reduced the percentage ability to grow of roselle plant germination but increase in the dose from dose of 600-1000 Gy cause variations in the percentage of germination. This variation did not lead to a greater reduction (Table 1).

Increased gamma-ray dosage from 100-1000 Gy affected the growth of roselle seed. Increased doses of irradiation given is affecting seedling growth. Visually the sensitivity of plants in a given irradiation treatment can be observed in plant response, one of them on the ability of the plants grow.

The LD_{50} was different for each type of plant depends on the stage of growth and development of plants and parts of plants irradiated. The LD_{50} on germination percentage and ability to grow from the seeds of tomatoes 790 and 640 Gy, while the okra seeds 770 and 580 Gy. In general, a high dose of gamma rays, especially at 800 Gy had a negative effect on the morphology and the character from germination of tomatoes, okra and beans. Some studies have been done[6-8] and it found that range of LD_{50} was deviated among each plant. The LD_{50} value of soybean cultivars Pusa-16 and PK-1042 found in the value of 377 and 467.4 Gy while the LD_{50} in soybean varieties Argomulyo was 457.1 Gy. Germination percentage at high dose 50 Gy, only decreased by 28% germination of seed Jamaica (*Hibiscus sabdariffa* L.), under

Table 1: Germination percentage of roselle seeds at 2 weeks after planting in several doses of gamma ray irradiation

Doses (Gy)	Germination (%)
0 (Control)	100
100	100
200	90
300	90
400	70
500	60
600	90
700	80
800	100
900	70
1000	90

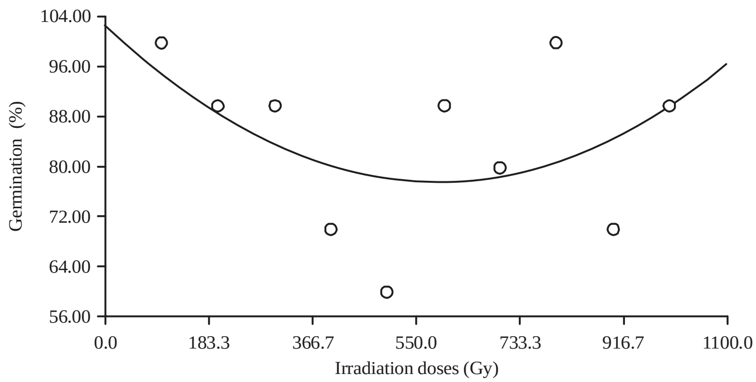

Fig. 1: Graph of gamma ray irradiation effect on the germination percentage of roselle seeds

Table 2: Percentage of roselle seeds germination in 3 weeks after planting

Treatment doses	Germination (%)
I0 (0 Gy)	100
I1 (150 Gy)	100
I2 (300 Gy)	95
I3 (450 Gy)	75
I4 (600 Gy)	80

^{60}Co gamma radiation. The radiosensitivity curve was adjusted to a decreasing linear model. Diaz-Lopez *et al.*[9] stated that variables germination percentage and radiation were found to be closely correlated negatively and by increasing the radiation dose germination tends to decrease.

This study showed that the LD_{50} of Roselindo 2 variety found in 477.803 Gy. The LD_{20} value found in 408.422 Gy. Best radiosensitivity as curves obtained for Roselindo 2 variety is illustrated by a quadratic curve based on the results of analysis by using curve expert 1.3 (Fig. 1). The regression equation obtained was:

$$Y(x) = 102.657-0.085x+0.00007x^2$$

where, Y (x) is the logarithm overall ability to grow of germination and x is the dose of radiation. Bhatia *et al.*[10] stated that LD_{50} is different for each type of plant depending on the stage of growth, the development of plants and parts of plants irradiated. In addition species of plants, ploidy level, stage of plant development and physiology (the biotic/abiotic) will lead to different responses to gamma-ray irradiation. On the other study, Pavadai[11] stated that the determination of optimal mutagenic dose is not easy. Because, low and high mutagenic doses are having some merits and demerits generally, higher mutagenic doses provide a higher number of possible mutants.

Irradiation effect of gamma rays on M_1 generation: Irradiation technology used by breeders to induce mutation and genetic diversity. Abdullah *et al.*[12] told that LD_{50} dose range is useful for estimating the dose or concentration that is suitable to induce mutations and determination level of sensitivity to the physical and chemical mutagens. This research using irradiation dose of 150, 300, 450 and 600 Gy for irradiation of Roselindo 2 variety seeds.

Based on data from Table 2 the germination percentage of 3 weeks after planting, showed that the percentage decrease in germination of seeds roselle influenced by the increased irradiation dose given. The decline in the percentage of germination began to occur from irradiationdose of 300-600 Gy. Table 1 shows that germination percentages decreased after gamma irradiation. Harding *et al.*[13] showed that the effect of gamma rays on seed germination in some rice varieties showed that increase in doses of gamma irradiation had no significant effect on seed germination from 0-7 days. The decrease in germination was not directly proportional to the increase in dosage nor was a definite pattern observed in all the 13 rice varieties.

Gamma ray irradiation treatment causing some roselle seeds do not grow and some seedlings began to die with symptoms of abnormal growth, the leaves dry and soon the plant dies. Death seedlings do not occur simultaneously. Generally some plants began to show abnormalities in 3 weeks after planting (Fig. 2). Based on studied by Islam[14], it was observed that gamma rays had significant effect on reduction in germination percent and percent plant survival at 30 DAS in Nerica-1 and Nerica-10 rice varieties, respectively at M_1 generation than their parents (control). There might have occurred physiological imbalance in the viability of the seeds due to the negative imbalance of gamma radiation which cause decreased seed germination and disturbed plant development.

The results showed that gamma ray irradiation from 150-600 Gy affects the performance of morphological characters from each population irradiation on M_1 generation. The difference can be seen from the average value of the

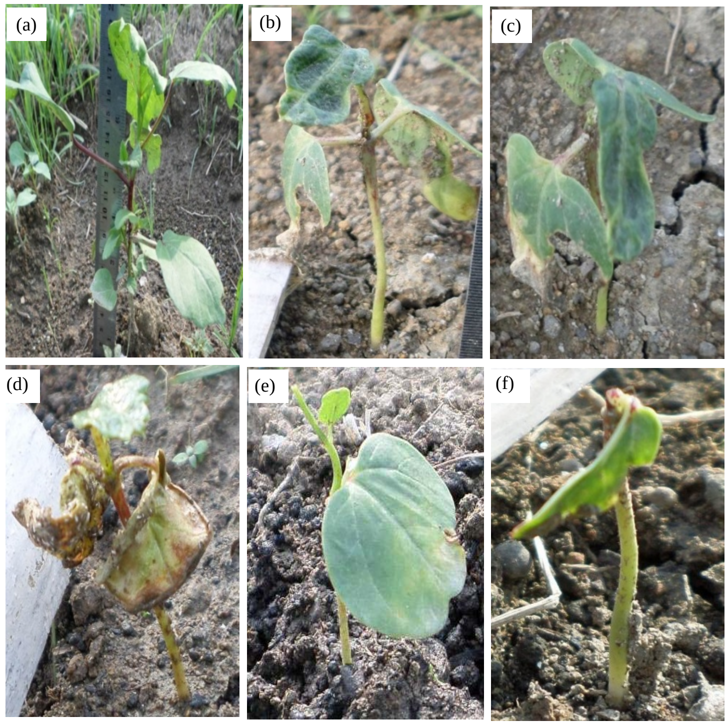

Fig. 2(a-f): Performance of roselle plant at the age of 3 weeks after planting showed symptoms of abnormalities compared with (a) Control plants, (b-d) An abnormality plant at 450 Gy and (e-f) An abnormality plant at 600 Gy (Scale 1:5)

Table 3: Effect of gamma irradiation on morphological characters Roselindo 2 variety

	Irradiation doses (Gy)				
Characters	0	150	300	450	600
Plant height (cm)	207.7	193.3	208.1	221.0	209.3
No. of branches	28.3	26.7	33.3**	25.8	30.7
Canopy diameter (cm)	165.1	135.9**	199.0*	203.3**	156.6

Significantly differences at **p<0.01, *p<0.05 compared to without irradiation

morphological characters compared to population without irradiation (Table 3).

Gamma ray irradiation dose of 300 Gy significantly increased the average value from number of branches character. Irradiation doses of 150, 300 and 450 Gy significantly increased the average value from canopy diameter character. Irradiation dose of 600 Gy was not significantly effect on the average value of plant height, number of branches, canopy diameter and not significantly different from population without irradiation (Table 3).

Piri *et al.*[15] and Van Harten[16] stated that M_1 generation is a population that changes physiological, biochemical, inhibition of growth and germination due to irradiation. Gamma rays produces free electrons that are radical, resulting in damage to the cells. Gamma ray irradiation dose is different for each species of plant in inducing changes in quantitative characters. Expected changes that occur towards a favorable development and not abnormalities.

The character changes that occur in several studies[17-19], such as sesame plant (*Sesamum indicum*), a dose of 300-400 Gy able to produce mutant plants with the desired character. The low dose gamma ray irradiation at 50 and 100 Gy in wheat (Durum wheat) is able to improve and enhance the quantitative character in plants. Research on Argomulyo variety (*Glycine max*), doses of 50-200 Gy able to improve crop quantitative character such as plant height, number of productive branches, number of productive pods per plant and seed weight per plant.

Gamma ray irradiation affects the variation of phenotypes in M_1 generation based on morphological traits of plants. This is indicated by the change in qualitative as in the form of branching, leaf shape and color of flowers. In general, roselle plant has erect upwards primary branch and secondary branches below surrounds primary branches. However, due to irradiation, there is a difference in the form of branching (Fig. 3).

Increased doses of gamma ray irradiation affects branching form changes of roselle plant. Dose of 600 Gy irradiation affects more branching form differently than the control plants (Table 4, Fig. 3).

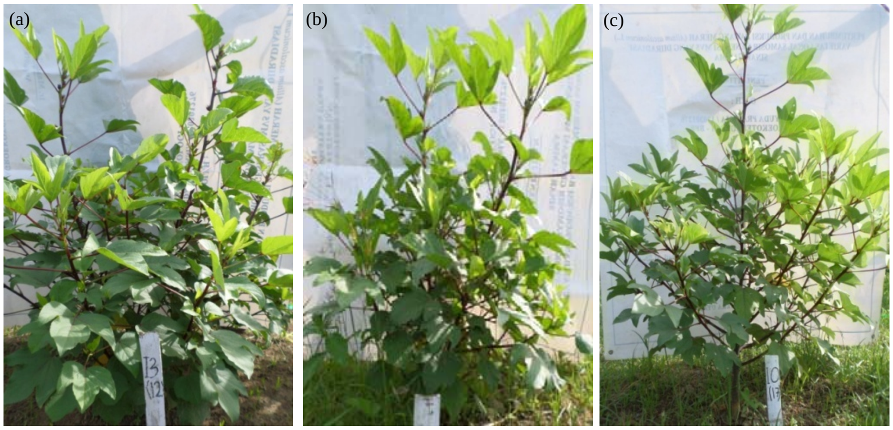

Fig. 3(a-c): Form branching of roselle plants (a) at 450 Gy, (b) at 600 Gy and (c) Control plant (0 Gy). (Scale 1:25)

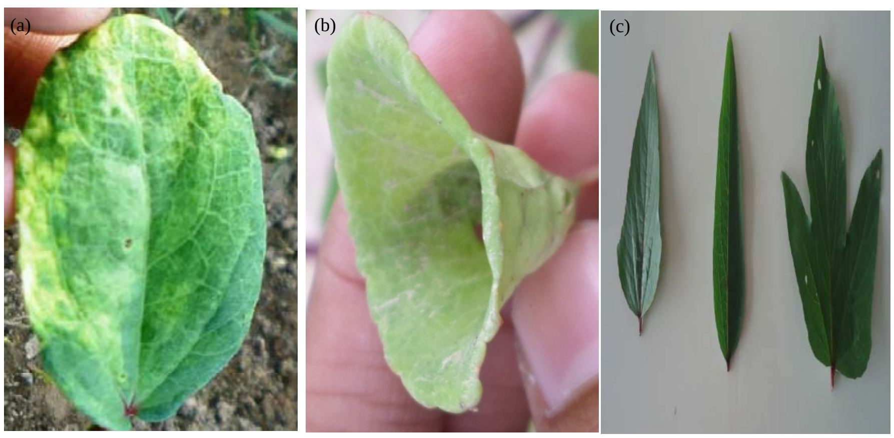

Fig. 4(a-c): Abnormal leaf of plants at 600 Gy (a) Scale 1:8, (b) Scale 1:3 and (c) Leaves of control plants (Scale 1:12)

Table 4: Effect of gamma ray irradiation to branching form of roselle plants

Irradiation population	No. of abnormality plants (%)
I0 (0 Gy)	0.00
I1 (150 Gy)	0.00
I2 (300 Gy)	5.26
I3 (450 Gy)	12.50
I4 (600 Gy)	18.75

The young plants of roselle have leaves that are unlobed but as the plant grows the later and developing leaves are shallowly to deeply palmate, 3 parted. Treatment of high dose irradiation (600 Gy) causes the appearance of leaf morphology different from control plant (Fig. 4).

Kumar *et al.*[20] reported that spectrum of macro-mutations is generally used to evaluate the genetic effects of various gamma treatments. Different types of morphological macro-mutations viz., plant stature, maturity, pod shape, seed color, seed shape observed in mutagenic treated populations of the Arkel variety (*Pisum sativum* L.). Among different types of morphological mutations, the most frequent types were early maturity followed by tall plants and long pod shape indicating high mutability of the gene for the character.

Generally, the flower color of Roselindo 2 variety was pink with dark red inside. Consisting of five corolla and the forms of corolla periphery was spherical. Gamma ray irradiation treatment causes changes in morphology and color of roselle flower in some individual plants from each population irradiation 150, 300, 450 and 600 Gy.

Changes occur in the flower color to red, white and yellowish-white. Changes on the periphery corolla become irregular or oval. Increased doses of irradiation increase the changes that occur in roselle corolla (Table 5, Fig. 5).

Moghaddam *et al.*[21] stated that the effect high-dose irradiation on plants causing disruption of hormone balance and activity of enzymes in the plant cell. Pavadai[11] also stated that the decrease in survival percentage has been attributed to the physiological disturbance or chromosomal damage

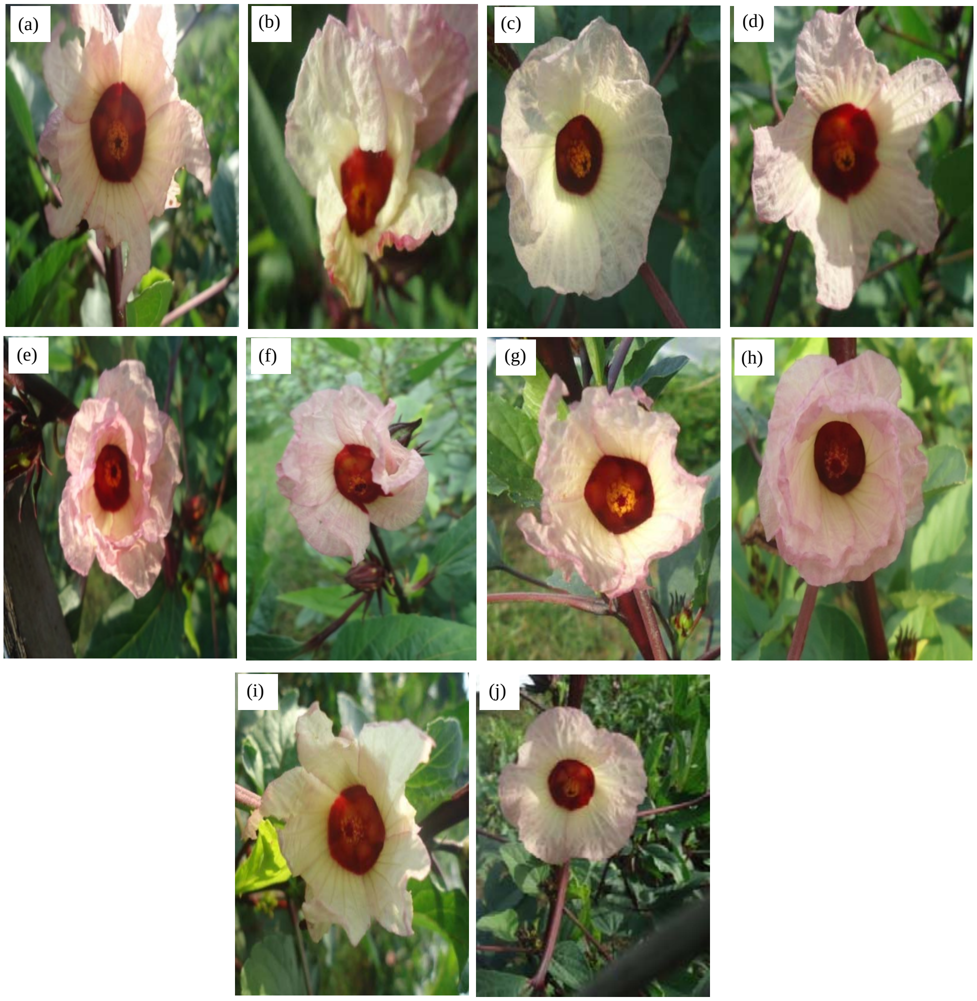

Fig. 5(a-j): Abnormal flower at (a-b) 150 Gy, (c) 300 Gy, (d-e) 450 Gy, (f-i) 600 Gy and (j) Flower of control plants (Scale 1:0.8)

Table 5: Effect of gamma ray irradiation to changes of roselle corolla

Population irradiation	Total interest abnormal (%)
I0 (0 Gy)	0.00
I1 (150 Gy)	10.00
I2 (300 Gy)	10.52
I3 (450 Gy)	12.50
I4 (600 Gy)	25.00

caused to the cells of the plant by the mutagen. Based on the results of the study, changes in the roselle plants due to the gamma ray irradiation are qualitative such as changes in the form of branching, leaf and flower color. Results of other studies[12,18,22,23] stated that forms mutation of long beans plants (Cowpea), green beans (Mung bean), turmeric plant (Curcuma) and soybeans are seen grouped in plant height, modification or abnormal leaf, variations branches, mutation on flowers, pods, seeds and flower was not develops.

Some studies[2-5] showed that gamma ray irradiation has been used to increase the genetic diversity, quantitative characters and improve the quality and nutrition of roselle plants. Kumar *et al.*[20] stated that in mutation breeding where large populations are handled, estimation of mutagenic effect and create genetic diversity help the breeders in identifying effective treated populations in early generation and to obtain desired characters and also enhancing scope of selection.

CONCLUSION

This study showed that LD_{50} value at 477.803 Gy and LD_{20} value at 408.422 Gy. The irradiation doses at 150-600 Gy affected the growth, development and morphological appearance of roselle plants.

SIGNIFICANCE STATEMENTS

The purpose of this study was to know radiosensitivity and LD_{50} value of Rosellindo 2 variety. This study also aims to increase the genetic diversity of roselle plant, as roselle plant is self-pollinated crops and have a low genetic diversity. Based on this research results obtained, known roselle plant responses to gamma-ray irradiation treatments give morphological changes that are different from each treatment irradiation dose given. This long-term study is expected to be selected to obtain the desired character roselle plant, to increase crop production and to improve nutrition quality of roselle plant.

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