VARIATION OF MORPHOLOGICAL CHARACTERS ON M_2 GENERATION OF ROSELLE (*Hibiscus sabdariffa* L.) INDUCED BY GAMMA IRRADIATION

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ABSTRACT

Roselle is a self-pollinating and has low genetic diversity crop species. Gamma ray irradiation is one of way to improve the plant genetic diversity. Seeds of Rosellindo 2 variety was treated with 150, 300, 450 and 600 Gy doses of gamma-rays to study morphological variations. The result showed that population of 150, 300, 450 and 600 Gy gave different appearance at plant height, canopy diameter, number of productive branches per plant, fruit calyx weight per plant, and age of harvesting. Overall, gamma irradiation can be effectively increased genetic diversity of roselle.

Keywords: genetic diversity, morphological characters, Rosellindo 2 variety

INTRODUCTION

Roselle is more widely known as the flower that can be used as raw material for the manufacture of herbal tea in Indonesia. Red roselle has benefits to overcome the various of disease and health problems as a medicinal plant. 96% ethanol extract of roselle flower petals contain flavonoid compounds, saponins and alkaloids. (Puspitowati *et al*., 2012).

Roselle have low genetic diversity, because its self-pollinating plant so it needs to be an effort to increase the genetic diversity of plants (Osman *et al*., 2011). 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.

Superior varieties obtained through plant breeding to the improvement of yield and crop adaptation. The new varieties require a population base which has a high genetic diversity. M_2 population is a segregation population after gamma-ray irradiation, in which each individual of the population have the possibility randomly mutated genes so that the genetic diversity in each character different from irradiation genotype population. The objective of this study is to increase the genetic diversity of the plants that indicated by morphological changes.

MATERIALS AND METHODS

Irradiation treatment was performed at BATAN (National Atomic Energy Agency) and field research was conducted at the University of Sumatra Utara, Medan. Roselle seed variety of Rosellindo 2 were treated with 150, 300, 450 and

600 Gy of gamma irradiation for conducting the field experiment. The effect of irradiation on the growth and development of roselle was observed in M_2 generation.

A total of 150 seeds (M_2) planted with a spacing 100 × 150 cm^2. The first one capsules of each M_1 were harvested (restricted bulk) and grown as an M_2 generation. The morpho-agronomic characters viz., plant height, canopy diameter, number of productive branches per plant, fruit calyx weight per plant, and age of harvesting were evaluated.

RESULTS AND DISCUSSION

Morpho-agronomic characters of Rosellindo 2 variety at different irradiation doses can be seen in Table 1. M_2 plants are expected to show segregation at the genetic locus that has mutation. Variation of morphological can be observed in M_2 generation.

The results showed that gamma ray irradiation from 150-600 Gy affects the performance of morphological characters from each population irradiation on M_2 generation. The difference can be seen from the mean value of the morphological characters compared to population without irradiation (Table 1).

Table 1. Effect of gamma irradiation on morphological characters Roselindo 2 variety

Character	Gamma dosage	Mean value
Plant height (cm)	0 Gy	182.93
	150 Gy	181.60
	300 Gy	164.10**
	450 Gy	173.70**
	600 Gy	182.60
Canopy diameter (cm)	0 Gy	160.10
	150 Gy	156.40
	300 Gy	146.30**
	450 Gy	159.20
	600 Gy	156.20
Number of productive branches per plant	0 Gy	27.23
	150 Gy	26.50
	300 Gy	25.76
	450 Gy	25.00
	600 Gy	30.00*
Fruit calyx weight per plant (g)	0 Gy	96.10
	150 Gy	98.00
	300 Gy	49.80**
	450 Gy	82.10
	600 Gy	49.40**
Age of harvesting (days)	0 Gy	116.33
	150 Gy	117.38
	300 Gy	126.79**
	450 Gy	119.07**
	600 Gy	127.62**

Significantly different at **p<0.01, *p<0.05 compared to control (0 Gy) for each variable

Gamma ray irradiation dose of 300 Gy significantly decreased the mean value from plant height, canopy diameter, and fruit calyx weight per plant character. Irradiation dose of 300-600 Gy increased age of harvesting. Meanwhile, irradiation dose of 600 Gy was significantly increased the mean value from number of productive branches compared to population without irradiation (Table 1). Generally, irradiation dose of 150 Gy did not give significantly different with the mean value from population without irradiation. Although, descriptively, it can be seen an increase in the mean values characters of the population 150 Gy than the control population.

Gamma ray irradiation affects the variation of phenotypes in M_2 generation based on morphological traits of plants. This is indicated by the change in the form of branching, and color of flowers. 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 1, Fig. 1).

(a) (b) (c)

Figure 1. Form branching of roselle plants (a) at 150 Gy, (b) at 300 Gy and (c) Control plant (0 Gy)

(a) (b) (c)

(d) (e)

Figure 2. Abnormal flower at (a-b) 150 Gy, (c) 300 Gy, (d) 450 Gy and (e) Flower of control plants

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 (Fig. 2).

(a) (b)

Figure 3. Unique colour calyx at 150 Gy (a); Normal colour calyx of control plants (b)

Generally, the color of the calyx on the roselle of Rosellindo 2 variety is purple in all parts of the calyx. However, there is a plant that has a unique calyx color that is pink but not in the entire calyx. There are irregularly green and white colors around the calyx (Fig.3).

DISCUSSION

Genetically, the diversity of agronomic characters in M_2 populations of roselle irradiated by gamma rays due to the segregation of genes within loci that have mutations in the M_2. The diversity that occurs as a result of segregation genes from affected population irradiation caused the diversity of quantitative characters on M_2 generation (Van Harten, 1998; Pavadai *et al*., 2010; Hanafiah *et al*., 2016).

Some studies showed that gamma ray irradiation has been used to increase the genetic diversity, quantitative characters and improve the quality and nutrition of roselle plants (Harding & Mohamad, 2009; El Sherif *et al*., 2011; Atmarazaqi, 2013; Sanni *et al*., 2015; Hanafiah *et al*., 2017). Based on the results of this study, changes in the roselle plants due to the gamma ray irradiation are qualitative and quantitative such as changes in the form of branching, flower color, plant height, number of productive branches and fruit calyx weight per plant.

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. Previous study showed that the LD50 of Roselindo 2 variety found in 477.803 Gy (Hanafiah *et al*., 2017). Moghaddam *et al*. (2011) stated that the effect high-dose irradiation on plants causing disruption of hormone balance and activity of enzymes in the plant cell. On the other study, Pavadai (2015) stated that low and high mutagenic doses are having some merits and demerits generally, higher mutagenic doses provide a higher number of possible mutants. Kumar *et al*. (2016) 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

Gamma ray irradiation doses from 150-600 Gy affects the variation of phenotypes in M_2 generation based on morphological traits of roselle plants. These changes occur on the qualitative and quantitative characters of the roselle plant.

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REFERENCES

Atmarazaqi, I.W. 2013. Analysis of phenotype and anthocyanin content of red roselle plant (*Hibiscus sabdariffa* L.) post gamma rays irradiation. www.uin.kalijaga.ac.id/pdf. [18 Desember 2015].

El Sherif, F., S. Khattab, E. Ghoname, N. Salem, K. Radwan. 2011. Effect of gamma irradiation on enhancement of some economic traits and molecular changes in *Hibiscus Sabdariffa* L. Life Science Journal. 8(3):220-229.

Hanafiah, D.S., R.R. Lahay, I. Safni, E.S. Bayu, I. Nuriadi. 2016. Assesment of gene action for agronomy characters in segregating generation of soybean (*Glycine max* (L.) Merr.). Journal of Agriculture and Environmental Science. 5(2):56-59.

Hanafiah, D.S., L.A.M. Siregar, D.P. Mutia. 2017. Effect of gamma rays irradiation on M_1 generation of roselle (*Hibiscus sabdariffa* L.). International Journal of Agricultural Research. 12:28-35.

Harding, S.S., O. Mohamad. 2009. Radiosensitivity test on two varieties of Terengganu and Arab used in mutation breeding of roselle (*Hibiscus sabdariffa* L.). African Journal of Plant Science. 3(8):181-183.

Kumar, A., A.K. Chaurasia, S. Markeri, P.K. Shukla, P.K. Rai, P.K. Verma, B.M. Bara. 2016. Effect of gamma radiation of macro mutations, effectiveness and efficiency under M_2 generation in Pea (*Pisum sativum* L.). Annals of West University of Timişoara, ser. Biology. 19(1): 71-76.

Moghaddam, S.S., H. Jaafar, R. Ibrahim, A. Rahmat, M.A. Aziz, E. Philip. 2011. Effect of acute gamma irradiation on physiological traits and flavonoid accumulation of *Centella asiatica*. Molecules Journal. 16:4994-5007.

Osman. M., F. Golam, S. Saberi, N.A. Majid, N.H. Nagoor, M. Zulqarnain. 2011. Morpho-agronomic analysis of three roselle (*Hibiscus sabdariffa* L.) mutants in tropical Malaysia. Australian Journal of Crop Science. 5(10):1150-1156.

Pavadai, P., M. Girija, Dhanavel. 2010. Effect of gamma rays on some yield parameters and protein content of soybean in M_2, M_3 and M_4 generation. Journal of Experimental Sciences. 1(6):8-11.

Pavadai, P. 2015. Studies on quantitative characters for gamma rays treatment in soybean (*Glycine max* (L.) Merr.) var. Co-. International Journal of Modern Cellular and Molecular Biology. 4(1):1-10.

Puspitowati, O.H., M. Ulfah, E. Sasmito. 2012. The immunostimulatory activity of water fraction test of roselle (*Hibiscus sabdariffa* L.) calyx ethanol extract on cell proliferation of lymphocytes of Swiss mice strain by in vitro as well as identification of chemical content. www.download.portalgaruda.org/pdf. [28 Februari 2016].

Sanni, T.A., J.O. Ogundele, E.M. Ogunbusola, O. Oladimeji. 2015. Effect of gamma irradiation on mineral, vitamins and cooking properties of sorrel (*Hibiscus Sabdariffa* L) seeds. 2nd International Conference on Chemical, Biological, and Environmental Sciences (ICCBES'15) Dubai, May 20-21.

Van Harten, A.M. 1998. Mutation Breeding, Theory and Practical Application. The United Kingdom: The Press Syndicate of the Univ. of Cambridge.