

The genotype selection of M₃ generation of Kipas Putih soybean with gamma-rays irradiation on agronomic characters, early maturity and high yielding mutants

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Abstract

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Selection is the important process in conventional plant breeding for character improvement. The objective of this study was to select individual plant in Kipas Putih M₃ generation to obtain early maturity and high yielding mutant lines. The M₃ generation seeds in each dose treatment were planted with a spacing of 40 x 20 cm. All technical cultivation practices such as fertilizing, watering, weeding and pest control were carried out during the period of plant growth. Plant selection was based on morphological appearance, early maturity and high yielding plant. The research findings show that the gamma-rays irradiation treatment resulted in a population with very different mean values with the control except for number of branches of 300 Gy doses, number of pod at 200 Gy and 300 Gy, number of empty pod at 300 Gy, seed weight per plant at 200 Gy and days to harvest at 200 Gy. In general, the means values of low agronomic characters were in the population of 100 Gy doses in all of observed characters except for days to flower and days to harvest. As a result of genotype selection on M₃ generation, 233 early maturity and high yielding mutants were obtained.

Keywords: soybean; mutation breeding; gamma-rays irradiation; early maturity

Introduction

Soybean is one of the most important crops in the world. In Indonesia, soybean is the third most important food crop after rice and maize. This plant is highly preferred because it has various benefits in its nutritional content. According to Chauhan et al. (1988) and Khan and Tyagi (2013), soybean contains 40% protein, high essential amino acids, 20% oils rich in unsaturated fatty acids, especially omega-6 and omega-3 fatty acids, 6-7% minerals, 5-6% crude fiber and 17-19% carbohydrates. In

addition, soybean also contains iron, vitamin B complex and isoflavones. The presence of calcium and iron makes it particularly useful for women who are suffering from osteoporosis and anemia. Pavadai (2015) called soybean as a wonder crop because it has a vast multiplicity of the uses of food and industrial product. He also notes that soybean is the richest, cheapest and easiest source of the best quality protein and fat.

The effort to increase soybean production must be done because of the increasing demand along with the growth of population. The superior variety is a farming

technology component that is easy for farmers to adopt. Assembling of new superior varieties can be done with plant breeding program either by using physical or chemical mutagens. Khan and Tyagi (2010) stated mutation is the ultimate source of variability in organisms. Mutations can be used in many different ways for plant breeding. Mutations can be used as a valuable supplementary approach to plant breeding when 1 or 2 characters are needed to increase easily on well adapted variety.

Mutation breeding of crop cultivation has been used for self-pollinated crop with limited genetic variability. Up to now, a lot of researchers have developed plants by mutation breeding techniques for sesame (Sharma, 1993), cowpea (Dhanavel et al., 2008; Horn et al., 2016), horsegram (Dhumal and Bolbhat, 2012), black gram (Thilagavathi and Mullainathan, 2009), mungbean (Tah, 2006) and soybean (Padmavathi et al., 1992; Pavadai et al., 2010; Arefrad et al., 2012). The use of gamma-ray irradiation has resulted in various mutants identified in soybean breeding programs (Rahman et al., 1994; Hanafiah et al., 2010; Mudibu et al., 2012).

In the previous study, the researchers use gamma-ray irradiation with 100, 200 and 300 Gy doses in the Aceh local soybean (Kipas Putih variety) to obtain early maturity and high-yielding soybean mutants in M₁ generation (Nilahayati et al., 2015) and M₂ generation (Nilahayati et al., 2016). In this study, the research continues in M₃ generation which aims to select individual plant based on the characters of early maturity and high yielding soybean.

Materials and Methods

This research was conducted at Reuleut Timu, North Aceh, Indonesia (± 8 m altitude). Kipas Putih soybean was treated with gamma-rays irradiation. In M₂ generation, each plant at each dose of gamma-ray irradiation treatment was harvested and all the seeds were grown as M₃ generation. M₃ seeds at each dose treatment were planted with spacing 40 x 20 cm. All technical cultivation practices such as fertilizing, watering, weeding and pest control were carried out during the period of plant growth. The plants were selected based on morphological appearance, early maturity and high yielding mutants.

The observation was done on plant height, number of branches, number of pods, number of seeds per plant, seed weight per plant, 100 seed weight, days to flower and days to harvest. The data analysis was done by calculating the means of each character observed, then the mean values of each population were tested by using t-test. Differential selection was obtained from the difference in the average

selected population of the selection results with the basic population average.

Results and Discussion

In M₃ generation, we found the morphological variations in gamma-ray irradiation population. The visible mutants in M₃ generation were not as large numbers as in M₂ generation. The types of visible morphological mutants included chlorophyll mutant (xantha, albino, variegated leaf, narrow leaf and sterile mutant (Fig. 1). Other authors have reported some common abnormalities at M₃ generation observed in cowpea genotypes derived through gamma irradiation. Mutants displayed visual phenotypic differences including chlorophyll mutant (yellow and striped leaves, albinos or yellow to pale leaf and stem pigmentation), spinach-like leaves, short pods, broad-dark leaves and single stem (Horn et al., 2016).

The results of t-test showed that the mean characters of gamma irradiation population showed highly significant difference with control except for number of branches 300 Gy, number of pods 200 Gy and 300 Gy, number of null pods 300 Gy, seed weight per plant 200 Gy and days to harvest at 200 Gy. In general, there were low agronomic characters in the population 100 Gy in all observed characters except for the days to flower and days to harvest (Table 1).

The plant height and the number of branches in irradiated population differed significantly with control at all irradiated doses except for number of branches 300 Gy. Plant height in irradiated populations was generally lower than control plants. The lowest plants were found at 200 Gy (66.48 cm) while the control was 72.70 cm. The number of branches in irradiated population 200 Gy (6.14 branch) was more than in the control (5.46 branch), while the number of branches in 100 Gy was slightly lower (5.06 branch) than in the control. Sadashiv and Kondram (2012) note that all of the mutagens are effective for inducing variability in plant height in M₃ generation of horsegram. Gamma radiation treatments have caused significant reduction in plant height. In M₃ generation the maximum plant height 49.85 cm was recorded in 300 Gy. The minimum plant height 44.57 cm was noted in 200 Gy. The average height of control plants was 50.99 cm. Reduction in plant height in M₃ generation is also noted by Khan et al. (2004), Gaikawad et al. (2005) and Sihombing et al. (2016).

The number of pods had very different mean values with control at 100 Gy population, while at 200 Gy and 300 Gy they were not significantly different. The lowest number of pods was found at 100 Gy (187 pod/plant),

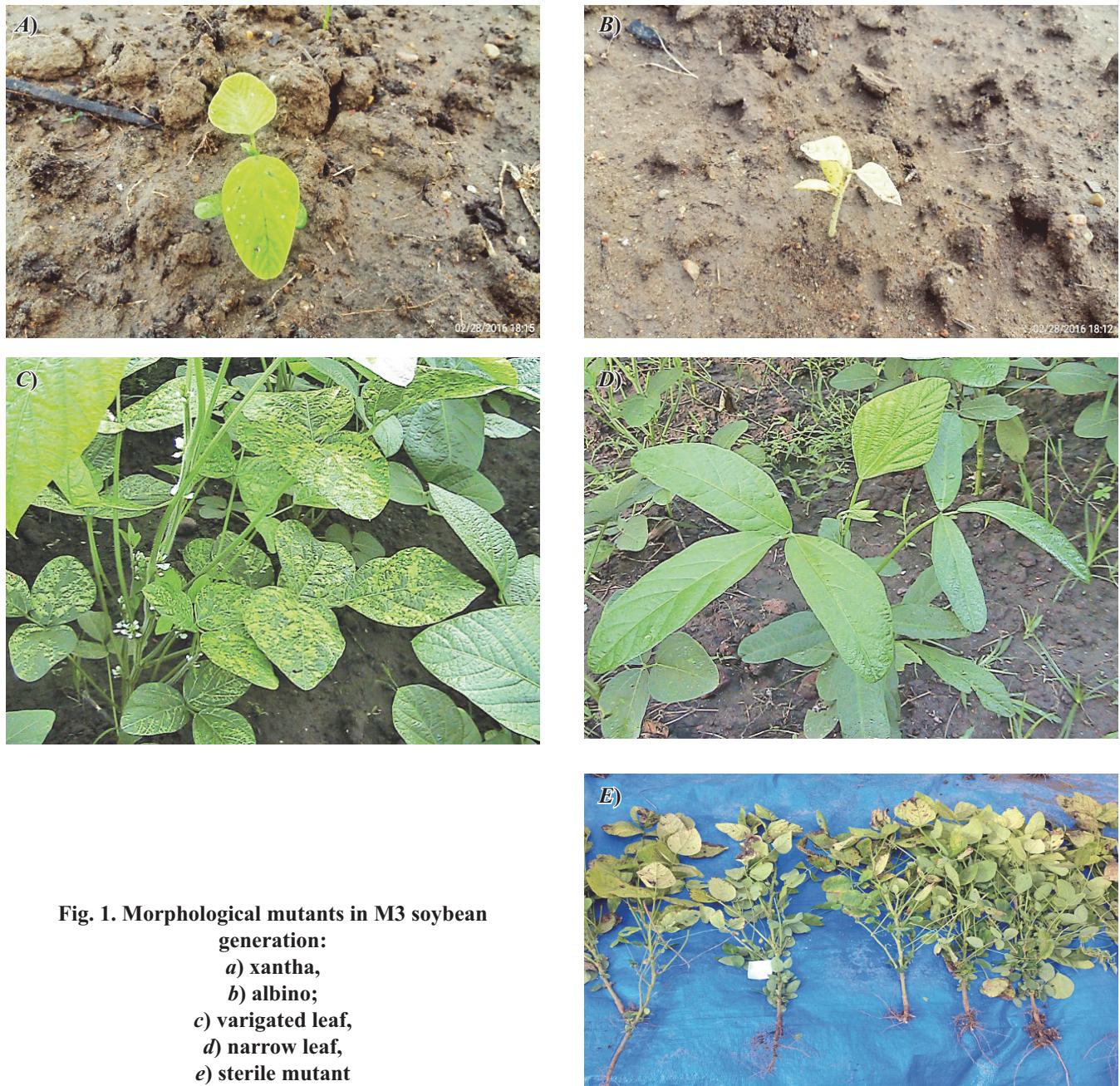


Fig. 1. Morphological mutants in M3 soybean generation:
a) xantha,
b) albino;
c) varigated leaf,
d) narrow leaf,
e) sterile mutant

while the highest number of pods was found at 200 Gy (253 pod/plant) although it was not significantly different from the number of control pods (241.5 pods). The character of seed weight per plant of irradiated population had very different mean values with control population except for 200 Gy population. The seed weight per plant at irradiated population was lower than the control except for 200 Gy. The highest seed weight per plant was found

at 200 Gy (52.7 g) which was not significantly different from the control (51.9 g), while the lowest was found at 100 Gy (41.3 g).

The character of days to flower and days to harvest of irradiated population had different mean values with control population (Table 2, 3). The earliest days to flower was found at 100 Gy (39.13), whereas the fastest days to harvest was at 300 Gy (88.98 days). In this study, early

Table 1. The effect of gamma irradiation on agronomic characters in M₃ generation

No.	Characters	Irradiation doses			
		P 0 Gy	P 100 Gy	P 200 Gy	P 300 Gy
1.	Plant height	72,70±9,79	68,2**±10,0	66,48**±9,66	67,45**±7,84
2.	Number of branches	5,46±1,62	5,06**±1,43	6,14**±1,68	5,46tn±1,22
3.	Number of pods	236,71±71,6	187,0**±67,7	253tn±108	218,0tn±65,7
4.	Seed weight per plant	51,3±16,2	41,3**±14,3	52,7tn±23,3	44,4**±14,5
5.	Days to first flower	40,20±0,65	39,13**±0,884	39,29**±1,18	39,67**±0,93
6.	Days to harvest	89,99±0,08	89,00**±2,30	90,01tn±3,63	88,98**±2,27

Note: ** = Significantly different with the control population (0 Gy) at level 1% by t-test

Table 2. The range values of agronomic characters of M₃ population resulted from gamma-ray irradiation

No.	Characters	Irradiation doses			
		P 0 Gy	P 100 Gy	P 200 Gy	P 300 Gy
1.	Plant height	36-105	40-102	34-87	43-88
2.	Number of branches	3-10	1-9	2-11	3-9
3.	Number of pods	30-343	29-460	30-600	51-598
4.	Seed weight per plant	6.24-83.03	5.79-79.5	10.11-135.29	6.6-83.25
5.	Days to first flower	39-43	37-41	37-42	37-42
6.	Days to harvest	88-90	77-90	82-99	84-99

Table 3. Differential selection in M₃ generation

No.	Characters	Irradiation doses			
		P 0 Gy	P 100 Gy	P 200 Gy	P 300 Gy
1.	<i>Days to flower</i>				
	Kipas Putih population	40,20			
	Baseline population		39,13	39,29	39,61
	Selected genotype		37,90	37,77	38,68
	Differential selection		1,23	1,52	0,93
2.	<i>Seed weight per plant (g)</i>				
	Populasi Kipas Putih	51,83			
	Baseline population		41,3	52,7	44,4
	Selected genotype		61,94	77,22	61,28
	Differential selection		20,64	24,52	16,88

maturity mutant showing early flowering within a short span of 37-41 days in 100 Gy population in comparison with the flowering duration of 49-43 days in control. The total duration of the crop was 77-90 days in 100 Gy against 88-90 days in control. Such type of mutants were recorded earlier in horsegram. These mutant are highly desirable to reduce the crop duration. Short duration variety of horsegram will play a key role in avoiding drought and water stress by rainfed agriculture (Dhumal and Bolbhat, 2012). Khan and Tyagi (2013) also reported early maturity soybean mutant with gamma-rays irradiation. Early maturing mutant showed normal growth and rapid productivity.

In M₃ generation, individual selection was performed to get early maturity and high yielding genotypes. The selection characters criteria were days to flower and seed weight per plant with 10% selection intensity. After the selection of the best crops in this generation, there was obtained an improvement in the mean of days to flower and seed weight per plant from gamma-ray irradiation populations. The selection of days to flower characters resulted the improvement of the early flowering genotype mean values in 200 Gy (37.77 days), the baseline population was 39.29 days and higher than control population with 40.20 days of mean values. The selection of the early

days to flower characters was also found in the 100 Gy population with 37.90 days of means values.

The selection of seed weight plant characters in the M₃ population also showed the highest selected genotype mean values in 200 Gy irradiation population with a mean value 77.22 g, the baseline population was 52.79 g and higher than the control population whose weight was only 51.88 g per plant. Hanafiah et al. (2015) conducts a selection of the total number of pods from the population of Anjasmoro soybean in M₃ generation resulting from gamma-ray irradiation under optimum conditions. The results of the study show the highest selected genotypes in the population of 150 Gy irradiation with 77.6 pods means values, the baseline population 45.2 pods and higher than Argomulyo population with 38.6 number of pods. The result of high pod number selection was also obtained from population 200 Gy with the selected genotype of 76.8.

Based on the selection criteria of days to flower and seed weight per plant, the genotypes were selected in M₃ generation. The selection results showed that there were 233 mutants putative which would be continued to the next generation.

Conclusions

Gamma rays' irradiation in M₃ generation affected plant height, days to flower, days to harvest, number of pods and seed weight per plant. In general, the low agronomic characters were in 100 Gy population for all observed characters except for days to flower and days to harvest. The result of genotype selection on M₃ generation was obtained 233 mutant putative soybeans with early maturity and high yielding mutant which would be continued in M₄ generation.

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References

- Arefrad, M., Nematzadeh, G., Babaian Jelodar, N., & Kazemitabar, S. K.** (2012). Improvement of qualitative and quantitative traits in soybean [*Glycine max* (L.) Merrill] through gamma irradiation. *Journal of Plant Molecular Breeding*, 1(1), 10-15.
- Dhanavel, D., Pavadai, P., Mullainathan, L., Mohana, D., Raju, G., Girija, M., & Thilagavathi, C.** (2008). Effectiveness and efficiency of chemical mutagens in cowpea (*Vigna unguiculata* (L.) Walp). *African Journal of Biotechnology*, 7(22): 4116-4117.
- Dhumal, K. N., & Bolbhat, S. N.** (2012). Induction of genetic variability with gamma radiation and its applications in improvement of horsegram. In *Gamma Radiation*. InTech, Chapter 10, pp. 207-228.
- Gaikawad, N. B., Wadikar, M. S., Kamble, S. S., & Kothekar, V. S.** (2005). Induced macromutations in *Lens culinaris Medic*. In: Proc. Nati. Conf. in Plant Sci., Pravaranganagar, M.S. India, 424-427.
- Hanafiah, D. S., Trikoesoemaningtyas, T., Yahya, S., & Wirnas, D.** (2010). Induced mutations by gamma ray irradiation to Argomulyo soybean (*Glycine max*) variety. *Nusantara Bioscience*, 2(3), 121-125.
- Hanafiah, D. S., Trikoesoemaningtyas, T., Yahya, S., & Wirnas, D.** (2010). Keragaan generasi ketiga (M₃) kedelai hasil iradiasi sinar gamma pada kondisi optimum dan pada kondisi kekeringan. *Jurnal Pertanian Tropik*, 2(1), 21-28 (Id).
- Horn, L. N., Ghebrehiwot, H. M., & Shimelis, H. A.** (2016). Selection of novel cowpea genotypes derived through gamma irradiation. *Frontiers in Plant Science*, 7, 262.
- Khan, S., Wani, M. R., Bhat, M., & Parveen, K.** (2004). Induction of morphological mutants in chickpea. *International Chickpea and Pigeonpea Newsletter*, 11(6-7), 8.
- Khan, M. H., & Tyagi, S. D.** (2010). Induced morphological mutants in soybean [*Glycine max* (L.) Merrill]. *Frontiers of Agriculture in China*, 4(2), 175-180.
- Khan, M. H., & Tyagi, S. D.** (2013). A review on induced mutagenesis in soybean. *Journal of Cereals and Oilseeds*, 4(2), 19-25.
- Mudibu, J., Nkongolo, K. K., Kalonji-Mbuyi, A., & Kizungu, R. V.** (2012). Effect of gamma irradiation on morpho-agronomic characteristics of soybeans (*Glycine max* L.). *American Journal of Plant Sciences*, 3(03), 331-337.
- Nilahayati, N., Rosmayati, R., Hanafiah, D. S., & Harahap, F.** (2015). Induction of genetic variability in Kipas Putih soybean with gamma ray irradiation (M₁ generation). Proceedings of the 1th Almuslim International Conference on Science, Technology and Society (AICSTS), November 7-8, 2015, Bireuen, Indonesia, 178-183.
- Nilahayati, N., Rosmayati, R., Hanafiah, D. S., & Harahap, F.** (2016). Gamma Irradiation Induced Chlorophyll and Morphological Mutation in Kipas Putih Soybean. *International Journal of Sciences Basic and Applied Research*, 30(3), 74-79.
- Padmavathi, T., Devi, P., & Kiranmai, V.** (1992). Induced variability for different biological parameters in soybean. *J. Cytol. Genet.*, 27, 175-177.
- Pavadai, P., Girija, M., & Dhanavel, D.** (2010). Effect of gamma rays, EMS, DES and COH on protein and oil content in soybean. *Journal of Ecobiotechnology*, 2(4), 47-50.
- Pavadai, P.** (2015). Studies on quantitative characters for gamma rays' treatment in soybean (*Glycine max* (L.) Merr.) var. Co-1. *International Journal of Modern Cellular and Molecular Biology*, 4, 1-10.
- Rahman, S. M., Takagi, Y., Kubota, K., Miyamoto, K., & Kawakita, T.** (1994). High oleic acid mutant in soybean in-

- duced by X-ray irradiation. *Bioscience, Biotechnology, and Biochemistry*, 58(6), 1070-1072.
- Sadashiv, B., & Kondiram, D.** (2012). Effect of mutagens on quantitative characters in M_2 and M_3 generation of horsegram (*Macrotyloma uniflorum* (Lam.) Verde). *International Journal of Scientific and Research Publications*, 2(10), 1-7.
- Sharma, S. M.** (1993). Utilization of national collections of sesame in India. In IBPGR-ICAR/NBGR Asian Regional Workshop on Sesame Evaluation and Improvement, 28-30th, Sep., Nagpur, India.
- Sihombing, Y. B. L., Hanafiah, D. S., & Husni, Y.** (2016). Seleksi Individu M_3 Berdasarkan Karakter Umur Genjah dan Produksi Tinggi pada Tanaman Kedelai (*Glycine max* L. Merrill)/ Individual Selection of M_3 based on the character of time early ripening and high production soybean plant (*Glycine max* L. Merrill). *Jurnal Agroekoteknologi Universitas Sumatera Utara*, 4(4), 2272-2283 (Id).
- Tah, P. R.** (2006). Studies on gamma ray induced mutations in mungbean [*Vigna radiata* (L.) Wilczek]. *Asian Journal of Plant Science*, 5(1), 61-70.
- Thilagavathi, C., & Mullainathan, L.** (2009). Isolation of macro mutants and mutagenic effectiveness, efficiency in black gram [*Vigna mungo* (L.) Hepper]. *Global Journal of Molecular Sciences*, 4(2), 76-79.

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