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Induction Of Genetic Variability In Kipas Putih Soybean With Gamma Ray Irradiation (M1 Generation)

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Abstract

This reasearch was done to study the effect of gamma ray on various characters of Kipas Putih soybean, including its height, number of pods per plant, number of seeds per plant, seed weight per plant, days to first flower and days to harvest. In this study, Kipas Putih Soybean (*Glycine max* L.) variety was treated with gamma ray with doses of 100 Gy, 200 Gy and 300 Gy. M1 generation showed that all the characters treated with gamma ray decreased, but days to first flower and days to harvest increased compared to non-irradiation Kipas Putih (as a control). This results indicated that different doses of gamma ray irradiation can be effectively used to create the genetic variability of plants.

Keyword: gamma irradiation, kipas putih soybean, M1 generation

Introduction

Soybean is an important crop in Indonesia. It is a protein-rich nutrient that plays an important role in improving people's nutrition. Soybean demand will continue to increase along with the growth of population and the needs of soybean as raw materials for the food, such as tofu, tempeh, soy sauce, soy milk, tauco and various snack products.

The increase in soybean demand is not in line with the increase in the production. According to BPS (2012), soybean production in 2012 was estimated at 779.74 thousand tons of dry beans or decreased by 71.55 thousand tons (8,40%) compared to 2011. Therefore, the increase in production of soybean is a must. Various attempts can be made, such as by using high yielding varieties. The development of varieties can be done through breeding programs.

Genetic variability of population is the basis of plant breeding programs. Extensive genetic variability can lead to the success of genetic improvement through selection of plant breeding. Mutation breeding is one of the most effective technologies in increasing the diversity of plant characters which in turn can produce

new varieties. Manjaya and Nandawar (1997) stated that mutation breeding is a way to increase the genetic variability of plant characters, both in quantitative and in qualitative manners. The important benefit of mutation breeding is that mutants with certain characters can be created without changing the essential character of the existing variety. Therefore, the mutants can be easily cultivated as a normal type.

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), black gram (Thilagavathi and Mullainathan, 2009) and soybean (Padmavathi et al., 1992 and Pavadai et al., 2010). A lot of soybean mutant lines have been identified by using mutation breeding programs based on morphological characteristics (Rahman et. al., 1994; Hanafiah et. al. 2010).

Doses of gamma ray is the most important thing in inducing genetic variability which in turn can generate positive mutants. Previous studies on soybean showed that at a dose of 200 Gy of gamma ray can effectively generate genetic diversity of plants (Mudibu et. al. 2012). Hanafiah et. al. (2010) also reported that this dose resulted higher genetic diversity than other irradiation doses and the control. Many studies showed that treatment with higher doses of gamma ray were inhibitory, whereas lower exposures were sometimes stimulatory. Gamma ray produces radicals that can damage and affect plant morphology, anatomy, biochemistry, and physiology depending on the irradiation level.

This research was conducted to study the effect of gamma ray on induction of genetic variability in M1 generation of Kipas Putih soybean. Kipas Putih is a local Aceh soybean variety becoming nationally leading soybean. Kipas Putih has a robust appearance, somewhat resistant to rust disease and well-adapted to the local climate. Unfortunately, for the farmers, this variety is not a favorite one due to its low production and long harvesting time span. According to Balitkabi (2009), Kipas Putih productivity is 1.69 tons/hectare and days of harvesting is 85-90 day. Induction of mutations by gamma ray irradiation on this variety is expected to result in genetic changes that could produce quality crops. The crops are more early maturing and high yielding.

Materials and Methods

Kipas Putih soybean seeds were irradiated by gamma ray irradiation undertaken by Indonesia's National Atomic Energy Agency (BATAN), located at Pasar Jumat, Jakarta. Meanwhile, field research was conducted at Reuleut Timu, North Aceh. This research took place from April to Agustus 2015. Kipas Putih soybean seeds were irradiated with gamma ray with doses of 0, 100, 200, and 300 Gray derived from cobalt-60 using 4000 A chamber. Each treatment were irradiated 200 seeds. Weeds that grew in the planting area was cleaned, then 4 plot with 9 m x 3 m was made. Tillage was done manually, two weeks before planting.

Irradiated soybean seeds were planted in the field immediately. A total of 200 seeds (M1) in each treatment dose were planted one seed per hole with plant spacing of 40 x 20 cm. Fertilization was done according to the recommendation of Indonesia's Soil Research Institute (Balittan) consisting of 50 kg

Urea / ha, 200 kg SP-36/ha and 100 kg KCl/ha. Fertilizer application SP-36 and KCl performed 2 weeks before planting, while urea is done at the time of planting.

Watering was done in the morning or afternoon in accordance with the conditions of the field. In case of rain, the plants were not watered. The weed control was done manually by pulling weeds in the plot in accordance with the conditions of the field. The observation was made as to plant height, number of pods, number of seeds, seed weight, days to first flower and days to harvest.

Results and Discussion

Soyben M1 generation resulted from the study had low percentage of germination and low percentage of survival plants compared to the control because of the increase of the irradiation dose. At a dose of 300 Gy, the germination percentage was 78%, but the percentage of survival plants only 15% (Table 1). At 200 and 300 Gy, most of plants were only able to germinate up to cotyledon leaves, but there was no growth occurs at a later stage. One month after planting, many plants in this population died (Figure 1). The same result had also been reported by some other research on rice (Ramesh et al., 2002), cowpea (Gnanamurthy et al., 2012) and soybean (Pavadai et al., 2010). The study of the effect of gamma irradiation in bhendi conducted by Jagajanantham et al. (2012) showed that there had been the decrease of the germination percentage and the number of survival plans compared to the control when treated with gamma irradiation.

Table 1. The percentage of germination and the percentage of survival plants due to gamma ray irradiation.

Doses (Gray)	% Germination	% Survival
0	85,5	87,5
100	60	70
200	67	50
300	78	15

In this study, there were many morphological variations especially leaf abnormalities, which were indicators of the effectiveness of mutagens treatment. In different treatment, there were morfological variations such as unifoliate, bifoliate, quadrifoliate, pentafoliate and fused leafs. There were founded chimera leaf, albino plants and undeveloped flower rasim plants. In M1 generation, the mutation of flower colour was not found.

In general, the appearance of the irradiated plants was very bad. The M1 generation was the one that suffered from direct damage as a result of gamma ray irradiation. This is consistent with what Van Harten (1998) stated, that is M1 generation is the population experiencing a direct physiological effect of gamma ray irradiation which produces free radical electrons that cause damage to cells.



Figure 1. The comparison of the growth of Kipas Putih soybean between 0 Gray (the control) and 300 Gray at one month after planting

Gamma ray irradiation treatment significantly reduced plant height (Table 2). Based on the results of the t test, there were distinct differences in plant height with the control plants (0 Gy) of 100 Gy, 0 Gy to 200 Gy and 0 Gy to 300 Gy. The tallest crops were in the control of 86.3 cm, followed by 100 Gy (79.1 cm) and 200 Gy (51.7 cm) respectively. The shortest plants was the irradiation treatment with a dose of 300 Gy and plant height of only 40.1 cm. Pepol and Pepo (1989) also showed that in general the treatment of mutagens causes plant height reduction compared to the control.

Table 2. The effect of gamma irradiation on agronomic characters in Kipas Putih soybean.

No.	Characters	Irradiation doses			
		0 Gy	100 Gy	200 Gy	300 Gy
1.	Plant height	86,3±14,3	79,1**±15,7	51,7**±13,4	40,1**±13,4
2.	Number of pods	213,7±77,0	174** ±69,2	63,8**±75,1	20**±42,5
3.	Number of seeds	364±29	266**±15	80,4**±9,5	19,9**±14
4.	Seed weight per plant	41,5±14,1	30,4**±12,7	10**±11,7	2,70**±5,95
5.	Days to first flower	41,10±1,37	43,23**±2,25	54,42**±6,64	58,33**±5,80
6.	Days to harvest	89,52±1,51	97,05**±4,04	120,96**±8,06	131,22**±6,67

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

The result of t test indicated that the average character of number of pods, number of seeds and seed weight per plant also showed a highly significant difference between the control and the treatment of various doses of irradiation. The number of Kipas Putih pods without irradiation (the control) were 213 per plant, which was highly significant compared to 174 pods (100 Gy), 63.8 pods (200 Gy) and 20 pods (300 Gy). The largest number of seeds per plant was in the control, that was 364 seeds per plant. This difference was highly significant compared to all doses of the irradiation treatment with the number of seeds per plant as many as 266, 80.4 and 19.9 respectively. Similarly, the character of seeds weight per plant of the control had the highest weight of seeds per plant that was 41.5 g/plant. Meanwhile, the plant population treated with irradiation doses had seeds weight of 30.4 g, 10 g and 2,7 g/plant respectively. This is consistent with research conducted by Girija and Danavel (2013) on cowpea plants which

concluded that all quantitative characters proportionally decreased with the increasing doses of gamma ray irradiation. A decrease in the number of pods, number of seeds per plant and seed weight on irradiation treatment because of the inhibition of enzyme activity, changes of enzyme activity and toxicity of mutagens. This causes disruption of the physiology and biochemistry of the development of the plant as reported by Laric (1975) in Girija and Danavel (2013).

In M1 generation, all quantitative characters decreased but days to first flower and days to harvest increased in plants treated with mutagen compared to the control. T-test of the character of days to first flower and days to harvest showed that there were distinct differences between the control plants and plants treated with the irradiation dose. The irradiation dose caused flowering and harvesting became longer. Table 2 shows that the control plants started flowering on 40.1 days after planting, while the irradiation dose flowered on 43.23 days (100 Gy), 54.42 days (200 Gy) and 58.33 days (300 Gy). The longest days to first flower was a dose of 300 Gy, which was late 18.23 days compared to the control. Days to harvest of the irradiation treatment was also very different from the control plants. Days to harvest became longer in line with the increase of irradiation doses. The control plants harvested on 89.52 days, while the irradiation treatment harvested on 97.05 days (100 Gy), 120.96 days (200 Gy) and 131.22 days (300 Gy). The delay of harvesting was in a dose of 300 Gy in which plants became late 41.7 days compared to the control. This is consistent with the results of Danavel Girija's research (2013), that was the gamma ray slowed cowpea where flowering was late 1-4 days compared to a dose of 40 kR. The shortest age of days to first flower was found in the control (34.78 days), while the longest were at the highest irradiation with a dose of 40 kR (49.32 days). Similar results were also recorded in soybean by Pavadai and Dhanavel (2005).

Jagajanantham et. al., (2012) noted that the biological effect of gamma ray was determined by the interaction among atoms or molecules in the cell. These radicals could damage or modify the important components of plant cells, and changed morphology, anatomy, biochemistry and physiology of plants depending on the radiation dose (Ashraf et al., 2003). The irradiation of seeds with high doses of gamma ray disturbs the synthesis of protein, hormone balance, leaf gas exchange, water exchange and enzyme activity (Toker et al., 2005). Girija and Danavel (2013) also stated the ability of mutagens to enter the cells of living organisms for interacting with the DNA produces toxic that associate with their mutagenic properties. Thus, mutagens can cause physiological damages mainly showed by growth retardation and death in M1 generation.

Conclusions

All quantitative traits observed in M1 generation decreased in line with the increasing doses of gamma ray irradiation, but the character of first days to flower and days to harvest increased compared to the control plants.

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