

Seed size selection from M₃ mutant soybean offspring of Kipas Merah Bireun variety in Aceh province

ZUYASNA^{1*}, M. MUCLISH ADIE², EFENDI¹, ZURAIDA¹

¹Faculty of Agriculture, Syiah Kuala University, Banda Aceh, Indonesia

²Indonesian Legumes and Tuber Crops Research Institutes, Malang, Indonesia

Abstract. Kipas Merah Bireun is one of the superior soybeans which was once widely cultivated in Bireun Regency, Aceh Province, used to be as a centre of origin of Kipas Merah Bireun variety. The weaknesses of the Kipas Merah Bireun variety are relatively small seed size (dry weight 12 g/100 seeds), long harvest period and drought susceptible. Therefore, it is necessary to improve the quality of Kipas Merah Bireun variety soybeans in order to get soybeans with superior properties; one way is to use mutation techniques. The objectives of the study were to evaluate the genetic variability for seed size of single plant selection for large seed in the mutants of gamma-irradiated population. The research successfully selected 41 lines soybean mutant with both of high weight seed per plant and large seed size. Several mutant lines of Kipas Merah Bireun were promising to be investigated and developed further in accordance with attempts to increase productivity in Aceh province. Therefore, further research is needed to see stability of selected mutant soybeans with large seeds in this study in multi environments.

Keywords: glycine max, high yield, large seed, mutation breeding

INTRODUCTION

In Indonesia, soybean is the third strategic commodity after rice and corn. Soybean seeds are used as a source of vegetable protein, fat, vitamins (phytic acid) and lecithin. Soybeans are commonly used as industrial raw materials and consumed in the form of processed products such as tempeh, tofu, soy sauce, tauco, soy milk and other types of snacks. In line with increasing population growth in Indonesia, the need for soybeans also increases. However, the need for soybeans cannot be fulfilled with domestic production, due to low soybean productivity as well as the area of planted area decreases from time to time. Professor Made Astawan from IPB, a Chairman of the Indonesian Tempe Forum, said that the average imports of soybean in Indonesia reached 2-2.5 million tons per year. Furthermore, he said that the average demand for soybeans in Indonesia reaches 2.8 million tons per year, meanwhile, currently soybean production has decreased drastically to just under 800,000 tons per year with the largest national need absorbed by the tofu and tempe industry [1].

Province Aceh has two local soybean varieties that have been released namely Kipas Merah Bireun and Kipas Putih. Kipas Merah Bireun is one of the superior soybeans which was once widely cultivated in Bireun Regency, Aceh Province, used to be as a centre of origin of Kipas Merah Bireun variety. Kipas Merah Bireun variety has such characteristics: deep and many root systems, pods are not easily broken, stems and branches are sturdy, resistant to fly flies and armyworms, somewhat resistant to leaf rust and fusarium and its ability to adapt well in the lowlands to hills (moor), rice fields, irrigated land and also peatlands [2]. The weaknesses of the Kipas Merah Bireun variety are relatively small seed size (dry weight 12 g / 100 seeds), long harvest period and drought susceptible. Therefore it is necessary to improve the quality of Kipas Merah Bireun variety soybeans in order to get soybeans with better properties, one way is to use mutation techniques.

Mutation is one of possible alternatives to conventional breeding for crop improvement program [3]. Mutation is a sudden heritable change in an organism and generally induces structural and composition changes in genome, chromosome, gene, or DNA [4] [5]. Exposing plant genetic material (seed, pollen, rhizome, callus, etc.) to mutagens enhances the chance for isolating unique genetic

*Corresponding Author:
zuyasna@unsyiah.ac.id

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material. Induced mutation can rapidly create the variability of inherited traits in crops, both quantitatively and qualitatively [6].

Gamma rays irradiation is an efficient tool to produce mutants in crop breeding and more than 1,800 cultivars either obtained as direct mutants or derived from their crosses have been released worldwide in 50 countries [7]. In Indonesia, gamma rays irradiation has been used in agriculture research and development since several decades ago, especially in fields of mutation breeding, pest control, plant nutrition, and animal health. In breeding program, this approach has not only contributed several crop varieties to national agriculture, but also generated hundreds of promising mutant lines that are ready for further multi-location trials.

In plant breeding program, genetic variability is the primary factor in developing a superior variety. Broad genetic variability allows superior variety can be developed faster. Plant breeders attempt to make their genetic material broader through many programs such as landraces exploration, artificial mutation, and germplasm introduction from other countries. Landraces exploration is very useful to find out genetic material for specific purposes, especially for adaptation in a specific agro ecology. Artificial mutation can be served as a technique to broaden genetic variability by damaging plant DNA. The damaged DNA will constitute new DNA sequence that can express the different performance of a character or whole characters in a plant [8].

Modern plant breeding has resulted in genetic erosion in many cultivated crop species [9] [9]. Identification of useful genetic diversity is expected to provide great opportunities to improve traits of interest in the future. At present, nearly 20,000 introduced accessions of the genus *Glycine* are available from USDA Soybean Germplasm Collection (GRIN, <http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?51>), but only a limited number of accessions have been extensively evaluated for seed composition traits. Publications available and have been used to investigate genomic diversity and population structure of the large collection of germplasm [10].

One of the criteria favoured by soybean consumers in Aceh, especially tempeh and tofu industry is soybeans with large seed sizes such as the size of Grobogan variety seeds. Grobogan weighs of 100 seeds is 18 g with 43,9% protein content and average yield per Ha is of 2.77 tons [2]. Soybean seed differences are vary between soybean production centers in the world. In

Indonesia, the size of the soybean seeds is divided into three groups, i.e. small seeds (<10g/100 seeds), medium (10 – 14 g/100 seeds), and large seeded (14 g/100 seeds) [11].

Despite the importance of both oil and protein contents, yield remains the ultimate driver of soybean cultivar selection to soybean breeders [12]. Nearly 95% of the soybean grown in the USA is commodity soybean for which farmers are paid by weight and not composition. Consequently, in cultivar development, breeders generally select for the best seed yield potential with little attention to seed protein or oil content. Since seed protein contents of US soybean cultivars have been declining slowly over many decades, while the seed yields have been increasing [13], developing high protein and high yield soybean cultivars would benefit farmers. [15] [16] [17].

Increasing the product and added value of soybeans from Aceh in the form of this new mutant is expected to be able to increase Aceh's economic growth towards a better direction and encourage the community to be more productive, and then be able to prosper the people of Aceh in general. The objectives of the study reported here were (1) to evaluate the performance of agronomic trait and yields, and (2) to select the superior M₃ line for large seeds size and high yield.

METHODOLOGY

Study Area

The research was conducted during the dry season (April to August 2016) at experimental station Faculty of Agriculture Syiah Kuala University (Darussalam-Banda Aceh, Indonesia) which located at the coordinate of 5°32'53.84"N and 95°19'25.52"E, 80 cm above sea level, climate C3 (Oldeman), range temperature 27,6-28,3°C, 80,63% relative humidity, and soil type of inceptisol.

Plant Materials

Plant materials used consisted of 370 lines of mutant population of Kipas Merah Bireun. The mutant population derived from M₃ segregating, which were obtained from M₂ mutants resulting from gamma rays of 200 and 300 Gy.

Field Study

The experiment was conducted by growing 370 mutant lines. The soil was tillage in two times and manure spreads after the tillage about 20 tons ha⁻¹. Each line was planted 2 seeds per hill in a plot size 1.5 m x 1.5 m with line spacing 30 cm x 20 cm. Plants were

fertilized with NPK *mutiara* (16-16-16) 150 kg ha⁻¹ and was applied one week after planting. Weeding, pest and disease were controlled when they needed.

Observations

The observed parameters were plant height, number of branch, number of productive branch, number of pods, number of filled pods, number of empty pods, number of seeds per plant, weight seeds per plant, and weight of 100 seeds.

Data Analysis

Data were subjected to descriptive analysis which consisted of mean, minimum value, maximum value, and standard deviation.

RESULTS AND DISCUSSION

Average agronomic and harvest character from 370 mutant line are very much differ in performance (Table 1). It was found the large diversity on plant height, number of branch, number of productive branch, number of pod per plant, number of fulfilled pod, number of empty pod, number of seed per plant, seed weight per plant and weight of 100 seed due to gamma irradiation on Kipas Merah Bireun variety. The condition of mutant lines grown in experimental station Faculty of Agriculture Syiah Kuala University can been seen in Figure 1.

The plant height of mutants line was distribute between 45 cm to 190 cm with average high 67.88 cm. Diversity of mutant line in number of branch, number productive branch, number of pod per plant, number of fulfilled pod, number of empty pod, number of seed per plant, seed weight per plant, and weight of 100 seed as shown in Figure 2, it is proven that gamma radiation can increase the diversity needed in plant breeding activities. This is in accordance with Muduli & Misra [6], induced mutation can rapidly create the variability of inherited traits in crops, both quantitatively and qualitatively. Post induced mutation has been effectively utilized in developing new and valuable

alternation in plant characteristics that have contributed to increase yield potential or disease resistance.

Table 1. Average agronomic and harvest characters mutant line

Parameter	Mean ± sd
Plant height (cm)	67.88 ± 22.25
Number of branch	7.46 ± 3.07
Number of productive branch	5.67 ± 2.81
Number of pod per plant	91.81 ± 40.96
Number of fulfilled pod	58.56 ± 33.19
Number of empty pod	32.23 ± 30.05
Number of seed per plant	89.10 ± 59.08
Seed Weight per plant	14.64 ± 7.85
Weight of 100 seed (g)	14.63 ± 4.14



Figure 1. Mutant line grew in experimental station Faculty of Agriculture Syiah Kuala University

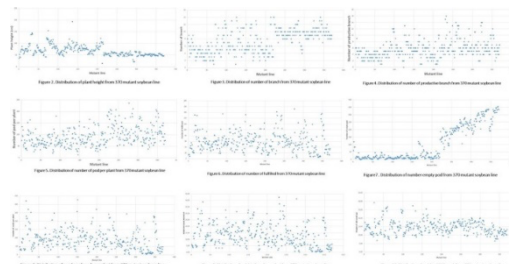


Figure 2. Diversity of mutant line

Based on weight of 100 seeds, our research divided mutant lines into small (39 lines), medium (135 lines), and large seed size (196 lines). The range of plant height with small seed size is between 42 cm to 90.06 cm, plant height with medium size is between 42.92 cm

Table 2. Average range of Agronomic Character based on the Seeds Size

Variable	Seed size		
	< 10 g	10 – 14 g	> 14 g
Number of lines	39	135	196
Plant height (cm)	66,03± 24,03	64,58 ± 21,66	70,52 ± 22,05
Number of branch	7,10± 3,10	7,41 ± 3,10	7,56 ± 3,05
Number of productive branch	4,90± 2,33	5,56 ± 2,86	5,89 ± 2,84
Number of pod per plant	77,38± 34,87	93,05 ± 41,46	93,82 ± 41,34
Number of fulfilled pod	42,97± 33,15	58,60 ± 36,46	61,63 ± 29,97
Number of empty pod	31,79± 38,10	34,68 ± 30,78	30,63 ± 27,72
Number of seed per plant	82,85± 75,52	90,74 ± 67,48	89,22 ± 49,41
Seed Weight per plant	10,84± 6,81	12,88 ± 7,31	16,45 ± 7,91

to 86.24 cm, while plant height with large size is between 48.47 cm to 92.57 cm. The range of seed weight per plant with small seed size is between 4.03 g to 17.65g, seed weight per plant with medium size is between 5.57 g to 20.19 g, while seed weight per plant with large size is between 8.54 g to 24.36 g (Table 2). Some examples of mutant seeds line can be seen in Figure 3 and also very much different from the parent Kipas Merah Bireun.

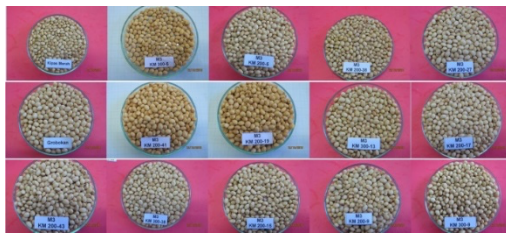


Figure 3. Sample of seeds mutant line

In this study, the mutant's lines derived from gamma irradiation 200-300 Gy were applied in 2013 to Kipas Merah Bireun variety in order to get large seeds of soybean. The used of Kipas Merah Bireun as source, because it originated from Bireun (Aceh) and has a great potential to adapt in Aceh province, however it has a character small seed in size (12 g/100 seeds). Our goal of soybean breeding is to obtain mutant line having large seed size as well as increasing the protein content. From this research we obtain several mutant lines with large seed size and high yield of seed per plant as shown in Table 2. There are forty-one line categorize large seed which high yield more than 22.49 g (above the average weight seed per plant) as shown in Table 3. These lines have the potential to be developed in soybean breeding in Aceh.

Large-sized soybean seeds is desirable trait in tempeh industry to fulfil the community's preference. Shifting preferences of soybean farmers and tempeh industries from seed medium into a large soybean seeded (14 g/100 seeds), become an important main reason to improve the genetic potential of soybean varieties in Indonesia. The both of high yielding and large seed size genotypes was an ideal combination.

Based on the data that has been presented, this research successfully selected 41 line soybean mutant with both of high weight seed per plant and large seed size (Figure 4). These selected genotypes prospective for source of tempeh material. Based on the requirements to obtain tempeh with good quality, therefore those genotypes need to examine its yield recovery, nutritional (protein) and sensory evaluation to produce tempeh suitable to consumer preference.

The quantitative characters are extremely affected by the environment, and the amount of such effect increases with the increase in the number of predominant genes. Thus, expression of a specific character which controlled by several loci were display greater genotype x environment (GxE) interaction. The elimination of GxE variance from the assessments of genetic variance forms an integral part of any endeavor to determine genetic variances without partiality [14]. Weight of 100 seeds and number of seeds per plant are among the quantitative characters on the crop including soybean. Therefore, further research is needed to see how the growth and production of selected mutant soybeans with large seeds in this study in various locations and planting seasons.

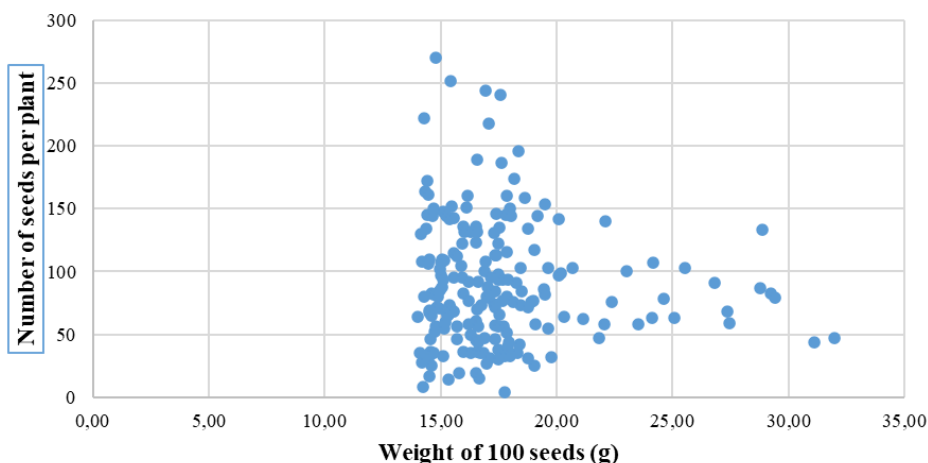


Figure 4. The both of high yielding and large seed size genotypes was an ideal combination

Table 3. Selection of Large Seed Size and Weight per Plant

Line No	Seed Weight/ plant (g)	100 seeds (g)	Line No	Seed Weight/plant (g)	100 seeds (g)
234	31,72	14,29	91	26,95	17,97
140	23,52	14,34	155	26,00	18,06
208	24,80	14,42	241	31,59	18,16
305	23,31	14,48	244	36,00	18,37
199	39,91	14,78	152	19,00	18,45
30	38,85	15,42	163	29,65	18,65
136	23,49	15,45	307	25,13	18,75
310	24,33	16,11	48	22,30	19,06
67	25,88	16,18	245	27,63	19,19
137	22,50	16,54	28	30,03	19,50
119	31,28	16,55	164	28,52	20,08
277	41,29	16,92	165	30,92	22,09
232	37,25	17,09	98	23,00	23,00
117	22,64	17,28	47	25,85	24,16
233	25,39	17,39	161	26,28	25,51
295	23,65	17,52	60	24,39	26,80
231	42,41	17,60	57	25,02	28,76
27	32,98	17,64	162	38,43	28,89
243	25,79	17,79	26	24,27	29,24
248	20,70	17,84	58	23,24	29,42
242	28,59	17,87			

CONCLUSION

There are 41 mutant lines selected from the populations with both of high weight seed per plant and large seed size. Mutant lines of Kipas Merah Bireun were promising to be investigated and developed further in accordance with attempts to increase productivity in Aceh province.

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