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Agronomic diversity of several soybean putative mutant lines resulting from gamma-ray irradiation in M_6 generation

NILAHAYATI[1,♥], NAZIMAH[1], RD. SELVY HANDAYANI[1], JAKA SYAHPUTRA[2], MUHAMMAD RIZKY[2]
[1]Department of Agroecotechnology, Faculty of Agriculture, Universitas Malikussaleh. Jl. Cot Tgk Ni Reuleut Timu, North Aceh 24355, Aceh, Indonesia. Tel./fax. +62-645-41373, ♥email: nilahayati@unimal.ac.id
[2]Student Department of Agroecotechnology, Faculty of Agriculture, Universitas Malikussaleh. Jl. Cot Tgk Ni Reuleut Timu, North Aceh 24355, Aceh, Indonesia

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Abstract. *Nilahayati, Nazimah, Handayani RDS, Syahputra J, Rizky M. 2022. Agronomic diversity of several soybean putative mutant lines resulting from gamma-ray irradiation in M_6 generation. Nusantara Bioscience 14: 34-39.* Soybean is one of the foremost commodities for Indonesian people. Therefore, increasing domestic production must continue to be pursued absolutely. One way to do this is to use the assembly technology of new superior varieties with better and adaptive properties. This study aims to determine the agronomic diversity and yield of soybean putative mutant lines resulting from gamma irradiation in M_6 generation. The research was conducted in Paloh Lada Village, Dewantara Subdistrict, Aceh Utara District, Indonesia, from July 2021 to October 2021. The tested genotypes consisted of 8: Kipas Putih variety (parent variety), Anjasmoro variety (comparison variety), M.1.1.3, M.5.2.1, M.5.2.3, M.1.1.8, M.1.1.9, and M.1.1.17 mutant lines. The results showed that the agronomic diversity of the tested mutants differed from the parents and comparison variety. In addition, we obtained early maturity and high yielding mutant in the M_7 generation. The mutant line with a high yield and equivalent to its parents was M.1.1.8, with a production of 4.37 tons.ha^{-1}. Mutant lines with early maturity were M.1.1.9, M.5.2.1, and M.1.1.3 lines with a harvesting age of 83 days after planting (DAP).

Keywords: Gamma-ray irradiation, genotype, M_6 generation, putative mutant lines

INTRODUCTION

Soybean is an important plant for Indonesian people (Lestari et al. 2017; Toyip et al. 2019). Increasing national soybean production is necessary to maintain food sovereignty (Harsono and Pratiwi 2017). Various efforts can be made to increase domestic soybean production by expanding the planting area, improving cultivation techniques, and using superior varieties. Assembling superior varieties can be done by plant breeding techniques to obtain genetic diversity. Various methods of plant breeding, both conventional and biotechnology, are currently available. One of the very popular breeding techniques is mutation breeding with gamma-ray irradiation.

Mutation induction with gamma rays is commonly used to obtain genetic diversity in cultivated plants, especially soybeans. Several researchers have previously used gamma-rays on various soybean genotypes, including Sohag, BARI Soybean-5, Bangladesh Soybean-4, and BAU-S/64, for genetic improvement of yield attributes (Malek et al. 2014), soybean line VX04-6828 for better agronomic performance and seeds composition (Nobre et al. 2019), Anjasari soybean for high seed yield and large seed size (Mawarni et al. 2019; Yoel and Rachmadi 2020), Korean cultivars Danbaek and Daepung for highest linolenic acid contents (Hong et al. 2019), Kipas Putih soybeans for early maturity and high yields (Nilahayati et al. 2016; Nilahayati et al. 2019), Denna 2 variety for shade-tolerant (Harsanti et al. 2020) and induction of genetic diversity of soybean genotypes PGRI-15, KY EXOTIC, NARC-, AJMERI-2, AJMER-I (Hussain et al. 2020).

It is possible to assemble local soybean varieties to obtain early maturity and high-yielding essential derivative variety using gamma-ray irradiation. A soybean mutant line from the Kipas Putih cultivar was obtained in the previous study. The selection in the M_5 generation obtained 33 putative mutant lines, including 6 early maturity mutant lines (4-14 days earlier than the parent), 3 mutant lines that harvested 8 days earlier than a parent, 19 mutant lines with high yields but not early maturity, and 7 putative mutant lines with high yields but not early maturity and have large seed weight (Nilahayati 2018). Putative mutant lines that are in early maturity are purified for several generations so that they can be continued for the release of new varieties.

The availability of early-maturity soybean varieties will overcome climate change because using short-lived varieties will reduce the risk of crop failure due to drought. Furthermore, early-maturity soybeans would be more profitable for farmers to alternate crops with rice and avoid water shortages for plants during their growth when planted after rice (Rehajeng and Adie 2013). In addition, early-maturity soybeans can provide various advantages, namely reducing pest infestations and increasing the cropping index in a year.

Therefore, the early maturity putative mutant lines obtained from the previous study need to be tested in the next generation to determine their genetic stability. The test was carried out by observing the agronomic diversity and yield of putative mutants selected by comparing their characters with the parents and the comparison varieties.

Therefore, a combination of plant breeding and agronomy is needed to improve plant characteristics and test line stability before releasing a variety.

This study used six soybean mutant lines of M_6 generation from the Kipas Putih soybean variety mutation with two checked varieties, Kipas Putih and Anjasmoro. All soybean seeds were obtained from the results of previous generations of research. These lines need to be tested for yield to determine agronomic performance with their parents.

MATERIALS AND METHODS

The research was conducted in Paloh Lada Village, Dewantara Subdistrict, Aceh Utara District, Indonesia, from July 2021 to October 2021. The plant materials used in this study were eight soybean genotypes: Kipas Putih variety (parent variety), Anjasmoro variety (comparison variety), M.1.1.3, M.5.2.1, M.5.2.3, M.1.1.8, M.1.1.9 and M.1.1.17 mutant lines. The agricultural production facilities are urea 50 kg ha^{-1}, SP-36 150 kg ha^{-1}, KCl 100 kg ha^{-1}, manure 3 tons ha^{-1}, Decis 25 EC, and Dithane-M45. A non-factorial Randomized Block Design (RBD) was utilized as the experimental design.

The planting area is cleaned of weeds that grow in the area. Then made an experimental plot with a size of 2 m x 1.5 m. A drainage ditch was made with a distance between plots and between replications of 50 cm. Seed planting is done by making planting holes in the plot with a depth of 2 cm and a spacing of 40 cm x 20 cm, then inserting 2 seeds per planting hole. Thinning was done at the age of 2 weeks after planting so that only 1 plant was left per planting hole.

Fertilization was carried out according to the recommended dosage of soybean fertilizer. The application of SP-36 and KCl fertilizers and manure was carried out 2 weeks before planting, while urea was applied at the planting time. Thinning was done at the age of 2 WAP. Weeding was done manually by removing weeds from the plot. Harvesting is done by pulling the stems of the plant by hand. The harvest criteria were that most of the leaves had turned yellow and fell, and the skin of the pods was brownish yellow in as much as 95% of the experimental plot units. In addition, observations were made on plant height, number of branches, flowering age, harvest age, number of pods per plant, seed weight per plant, seed weight per plot, and tons ha^{-1} production. Data analysis was performed by analysis of variance. If significantly different, it continues with the Least Significant Difference (LSD) Test.

RESULTS AND DISCUSSION

Plant height

The results significantly affected the character of plant height of several M_6 genotypes and their two comparison varieties. The results of further tests on the average plant height of several M_6 genotypes and their two comparison varieties can be seen in Figure 1.

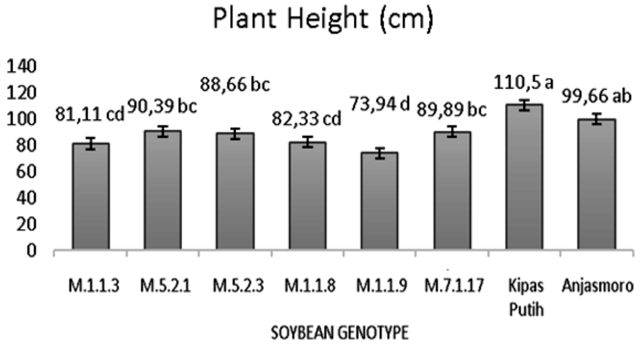

Figure 1. The effect of several soybean genotypes on plant height characters. Note: The numbers followed by the same letter in the same bar are not significantly different according to the 5% LSD test

The highest plants were found in the Kipas Putih variety, with a height at harvest reaching 110.50 cm, which was not significantly different from genotype Anjasmoro, with a height of 99.66 cm. Then followed by M.5.2.1, M.7.1.17, M. 5.2.3, M.1.1.8, and M.1.1.3 mutant lines. The shortest plant height was found in M.1.1.9 genotype with a height of only 73.94 cm, which was not significantly different from the height of M.1.1.8 and M.1.1.3 mutant lines (Figure 1). In the mutant lines of gamma-ray irradiation, the selection results in the previous generation that were tested still showed a decrease in plant height compared to Kipas Putih. The reduction of plant height due to gamma-ray irradiation treatment on plants has been found by many researchers before. Yadav et al. (2019) found that maize genotype HQGM-1 irradiated at several irradiation doses showed a reduction in plant height of 56% at an irradiation dose of 0.5 kGy. (MT et al. 2021) also reported the development of semi-dwarf, early maturing, and high-yielding mutant of rice cultivar Improved White Ponni with gamma-ray irradiation. In the M_6 generation, they found a significant reduction in plant height (up to 40% deduction). Similar results have been reported by (Mudibu et al. 2012) in soybean. In the 0.4 kGy gamma-ray treatment, plant height was shorter in the M_1 generation, with a decrease of 25%, 13%, and 38% for Kitoko, Vuangi, and TGX814-49D, respectively. In the M_2 generation, a 0.4 kGy gamma-ray irradiation dose caused a 14% decrease in plant height in the Kitoko genotype.

The plant height reduction in M_1 generation was observed for the 0.4 kGy treatment with 25%, 13%, and 38% decrease for Kitoko, Vuangi, and TGX814-49D, respectively. In M_2 generation, the 0.4 kGy irradiation significantly reduced plant height in Kitoko (14%).

Compared to planting in the previous generation (M5 generation), the plant height of the tested lines ranged from 39-59 cm, which means that it was shorter than the planting in this M_6 generation. That is due to different locations and different growing seasons. Plant height is included in the quantitative character, which is strongly influenced by the environment. Xue et al. (2019) stated that plant height is an important trait in soybean. The taller plants may give higher yields but will be more susceptible to lodging. There are many genes involved together in influencing plant height throughout development. Genetically, plant height is a quantitative trait controlled by multiple genes.

Days of flowering, harvesting age, and number of branches

The analysis of variance showed a very significant effect on the characteristics of flowering and harvesting ages of several M_6 genotypes and their two comparison varieties. However, there was no significant effect on the character of the number of branches of several M_6 genotypes and their two comparison varieties. Further tests on the average days of flowering, the number of branches and harvesting age, and their two comparison varieties can be seen in Figures 2, 3, and 4.

The longest days of flowering were found in M.5.2.3 mutant genotype with a flowering age of 42.77 days after planting (DAP), which was not significantly different from other mutant genotypes and the parent variety. The fastest flowering age was found in Anjasmoro, which started flowering at 35.55 DAP. The research results in this generation showed an unstable appearance on the character of flowering age compared to the planting of these lines in the previous generation. Nilahayati (2018) found that in the M_5 generation, the M.1.1.3, M.5.2.1, M.5.2.3, and M.1.1.9 lines were selected based on the criteria for flowering age, which were faster than other lines with flowering age at 36-37 DAP. In contrast, MT et al. (2021) reported reduced days to flower in the M_6 generation of Improved White Ponni (IWP) paddy mutants. Reduction days to 50% flowering of WP 5-4 mutant was reduced to 13 days (11.8% reduction from 110 days in the IWP control). In the other six mutants, there was a reduction of up to 11 days in their flowering time.

The longest harvesting age was found in genotype Kipas Putih with a harvest age of 89.66 DAP, which was significantly different from the harvest age of genotypes M.1.1.17, M.5.2.3 and M.1.1.8. The earliest harvesting age was found in genotype Anjasmoro with a harvest age 82.83 DAP, which was not significantly different from the harvest age of M.1.1.3, M.5.2.1 and M.1.1.9 with harvesting ages 83.89, 83.72 and 83.05 DAP, respectively. Compared with the previous generation, the genotypes of the M.1.1.3, M.5.2.1, and M.1.1.9 mutant lines showed an early harvesting age. These mutants can be called solid mutants for harvesting age because they provide stability in the character of early harvest in several generations of planting.

The mutant genotypes, namely M.1.1.3, M.5.2.1, and M.1.1.9, had an average harvest age of 83 DAP, so they were categorized as early-maturity soybeans (78-85 days). The Previous research to obtain early maturity soybeans using gamma-ray irradiation has also been carried out by (Puspitasari et al. 2021) on Argomulyo soybean with a radiation dose of 250 Gray. As a result, they get the harvest time of the mutant line was more early (70 days) than the control (>80 days). On the other hand, the GEE-5 mutant line showed the shortest harvest time of 66.79 days. The mutant line flowering days also showed a faster flowering time (31-32 days), while the control variety was around 35 days. The highest average seed yield was found in mutant lines GBB-2 and GEE-5 compared to control varieties and other mutant lines because this mutant had a higher number of filled pods than other genotypes. However, the seed size of the mutant line was smaller, only 10-11 g, compared to the 100 seed weight of the control varieties, which was 13-16 g.

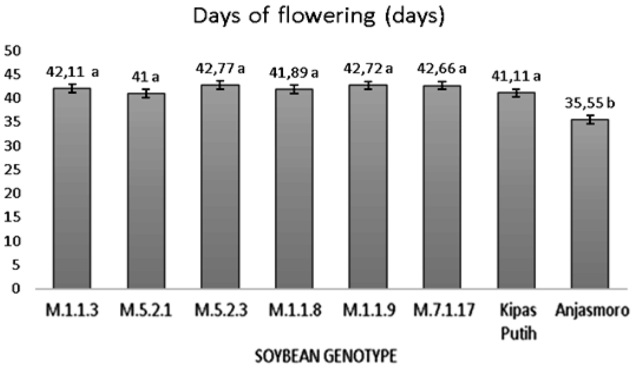

Figure 2. The effect of several soybean genotypes on the characters of days of flowering. Note: The numbers followed by the same letter in the same bar are not significantly different according to the 5% LSD test

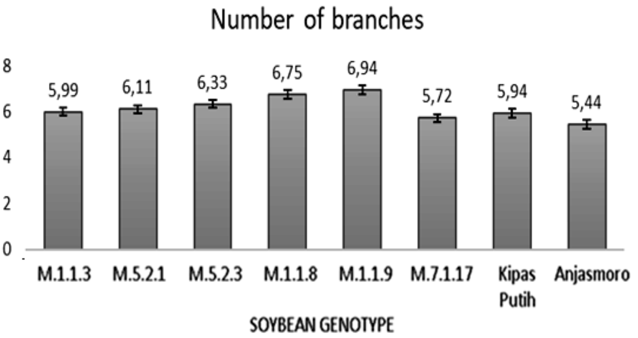

Figure 3. The effect of several soybean genotypes on the characters' number of branches. Note: The numbers followed by the same letter in the same bar are not significantly different according to the 5% LSD test

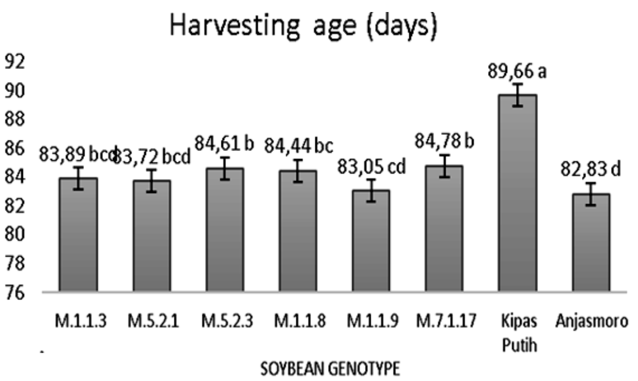

Figure 4. The effect of several soybean genotypes on the characters of harvesting age. Note: The numbers followed by the same letter in the same bar are not significantly different according to the 5% LSD test

The preference for early-maturity soybeans was higher than for late-maturity soybeans because it could increase the cropping index. It can also avoid crop failure due to drought stress that shortens pod filling mass. The length of time for pod filling is the most critical and vulnerable period in the event of a long dry season. Rahajeng and Adie (2013) said that early-maturity soybean varieties could be a solution for farmers to deal with climate change. Early maturing varieties are in great demand because they can provide various benefits, such as eliminating yield losses due to drought and pest infestations and increasing cropping in a year.

The number of the pod and 100-seed weight

The analysis of variance showed a very significant effect on the character of the number of pods and the weight of 100 seeds of several M_6 genotypes and their two comparison varieties. The results of further tests on the average number of pods and weight of 100 seeds on several M_6 genotypes and their two comparison varieties can be seen in Figures 5 dan 6.

Figure 5 shows that the highest number of pods was found in the Kipas Putih variety, which amounted to 239.89 pods, which was not significantly different from M.1.1.8 mutant line with 217.33 pods. On the other hand, the lowest number of pods was obtained in M.1.1.3 mutant line with only 97.05 pods, which was not significantly different from M.1.1.9, M.1.1.17 lines, and the comparison variety Anjasmoro.

Only one putative mutant genotype had the same number of pods as its parents, namely the M.1.1.8 genotype. There was no genotype whose number of pods exceeded the number of parent pods. Genotype M.1.1.8 in the previous generation test (M_5) had the highest number of pods compared to other genotypes, with an average of 500 pods (twice the number of parent pods). In the planting of the M_6 generation, there was a decrease in the average number of pithy pods, probably due to the different locations and planting seasons.

The largest weight of 100 seeds was found in the Anjasmoro variety at 17.34 g/100 seeds (Figure 6). The smallest 100-seed weight was found in the M.1.1.3 mutant line with 100 seed weight of 11.79 g, which was not significantly different from the Kipas Putih variety, the M.1.1.9 and M.1.1.8 mutant lines, which weighted 100 seeds, respectively 11.68 g, 11.82 g, and 12.55 g. The mutant line that increased seed size compared to its parents in the M_6 generation was the M.5.2.3 mutant line, which increased by 2 g/100 seeds. This mutant line on the previous M_5 generation planting showed a small seed size of only 10.21 g/100 seeds, which indicates that differences influence the increased seed size in the planting environment.

Soybean seed size was divided into three groups, namely small seed size (<10 g/100 seeds), medium seed size (10-14 g/100 seeds), and seed size >14 g/100 seed as large seed size (Krisnawati and Adie 2015). Based on these criteria, the putative mutant lines in this study had small seed sizes, and medium-sized seeds were not different from their parents, Kipas Putih. At the same time, the comparison variety is Anjasmoro, a large-seed soybean variety with a weight of 100 seeds 17 g, which follows the variety's description.

Seed weight/plant, seed weight/plot, and production tons.ha^{-1}

The results of the analysis of variance showed that there was a significant to a very significant effect on the character of seed weight/plant, seed weight/plot, and production of several M_6 genotypes and their two comparison varieties. Further tests on the average seed weight/plant, seed weight/plot, and production/ha on several M_6 genotypes and their two comparison varieties can be seen in Figures 7, 8, and 9.

Figure 7 dan 9 shows that the highest seed weight per plant and production per hectare was found in the Kipas Putih variety, with a seed weight of 42.25 g and production of 4.75 tons/h, which was not significantly different from the seed weight of M.1.1.8 mutant line, which was 38.84 g/plant and production of 4.37 tons.ha^{-1}. On the other hand, the lowest seed weight and production were found in M.1.1.3 mutant line with a seed weight of only 18.25 g and production of 2.05 tons.ha^{-1}, which were not significantly different from M.7.1.17 and M.1.1.9. In the M.1.1.9 mutant line, the low seed weight per plant was because many pods were contained, but the seeds produced were not good, such as the small and shriveled seeds' shape.

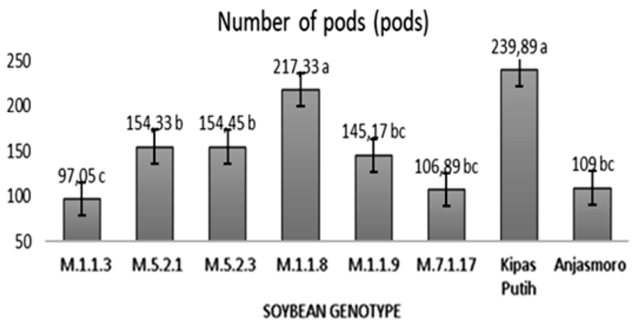

Figure 5. The effect of several soybean genotypes on the characters' number of pods and the weight of 100 seeds. Note: The numbers followed by the same letter in the same bar are not significantly different according to the 5% LSD test

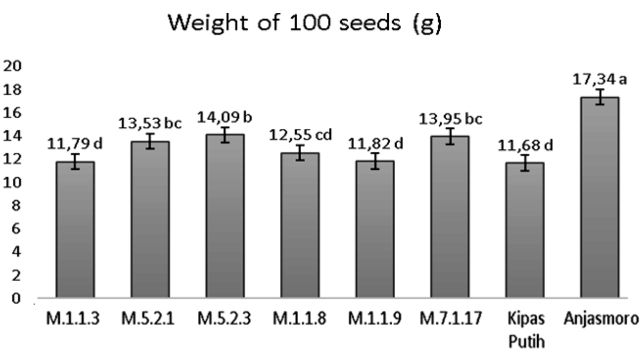

Figure 6. The effect of several soybean genotypes on the weight of 100 seeds characters. Note: The numbers followed by the same letter in the same bar are not significantly different according to the 5% LSD test

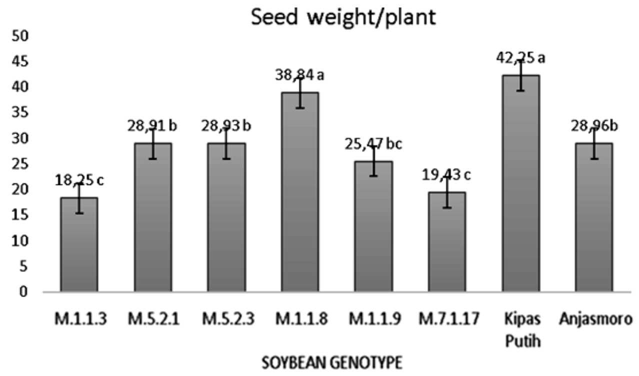

Figure 7. The effect of several soybean genotypes on seed weight/plant characters. Note: The numbers followed by the same letter in the same bar are not significantly different according to the 5% LSD test

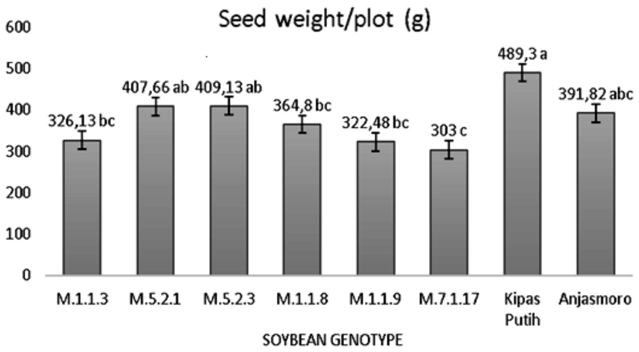

Figure 8. The effect of several soybean genotypes on seed weight/plot characters. Note: The numbers followed by the same letter in the same bar are not significantly different according to the 5% LSD test

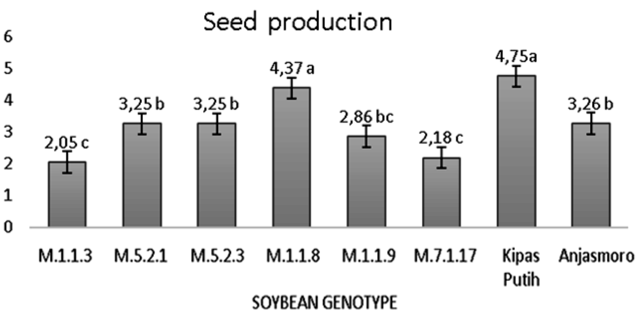

Figure 9. The effect of several soybean genotypes on production tons.ha^{-1}. Note: The numbers followed by the same letter in the same bar are not significantly different according to the 5% LSD test

Late-maturing soybeans (Kipas Putih variety) yield more seeds than early-maturing ones because their vegetative growth and reproductive periods are longer. Therefore, developing an early-maturing line without decreasing seed yield has not been easy. However, the present study showed that M.1.1.8 mutant had a significantly earlier maturing time than Kipas Putih (Figure 2), and M.1.1.8 had a seed yield similar to Kipas Putih (Figure 9). Therefore, we have successfully found an early-maturing mutant line without decreasing yield.

For measurement data of seed weight/plot (Figure 8), it can be seen that the highest seed weight/plot was found in the Kipas Putih variety, namely 489.30 g/plot, which was not significantly different from M.5.2.3, M.5.2.1 and M.1.1.8. On the other hand, the lowest seed weight per plot was found in the M.7.1.17 mutant line, which was 303 g/plot. There was a decrease in the seed weight/plots of the M.1.1.8 mutant line because the number of plants/plots that managed to grow in this line was only 50% of the total number of plants. The total 24 plants/plot managed to grow only 12 plants per plot, resulting in a decrease in seed weight/plot compared to seed/plant weight data.

These results are in line with (Asadi and Dewi 2020). They obtained soybean mutants resulting from gamma irradiation combined with crossbreeding. As a result, there were nine mutant soybean lines with better performance and 15-26% higher yield than the Panderman variety and 27-31% higher than the Anjasmoro variety. The nine selected lines will be tested for further adaptability in different locations and environments in the next generation.

Several mutant soybeans were also obtained through gamma-ray irradiation of the Panderman variety. The results on the M_5 generation of 10 tested mutants show two mutant lines, i.e., Kdl3 and Kdl8, that perform better than the other mutant lines under drought stress conditions. These mutant lines required 30.75 to 32 days to flower and 79.75 to 83.75 days to harvest. They also have relatively short plant heights of 28.25 and 23.35 cm, respectively (Yuliasti and Reflinur 2017).

In this study, sterile mutants were still found in M.1.1.3 mutant lines population. There was only one full sterility plant from the entire cultivated population, as shown in Figure 10. When entering the flowering phase, this plant shows the appearance of several flower candidates, but in 2 days they fall, so the flower candidates appear can not grow. In addition, this plant shows differences in growth when it reaches harvest age; the leaves turn yellow more quickly, and the leaves wither and fall off over time. Several other abnormal characteristics in full sterility plants were that the number of branches and leaves was less when compared to other plants.

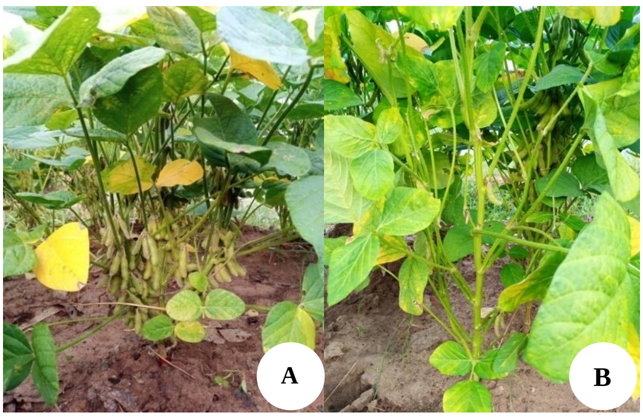

Figure 10. A. Fertile mutant lines, B. Sterile mutant lines

In a previous study, Nilahayati et al. (2016) also reported that full sterility plants showed normal vegetative growth but failed to produce flowers. Kumar and Rai (2006) investigated the study on soybean using gamma-ray irradiation and found some sterile mutants. The mutant cytology test showed the occurrence of desynapsis in the cells. In sterile plants, only slightly bivalent, while the univalent frequency is very high, which causes a very high percentage of pollen sterility. The later stages of meiosis are also severely disrupted. Plants were identified as male sterile plants because only a few pods were formed. They concluded that gamma rays may have acted on some of the genes responsible for forming synapses and chiasms at the stage of gamete cell division.

In conclusion, the genotype treatment affected all observed characters except for the number of branches and empty pods. The putative mutant line with a high yield and equivalent to its parents is the M.1.1.8 line, with a production of 4.37 tons/ha. The putative mutant lines with early maturity were M.1.1.9, M.5.2.1, and M.1.1.3 lines with a harvesting age of 83 DAP.

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