

The Effect of Gamma Co 60 Radiation on Soybean (Glycine max(L.) Merr) Shade Tolerants

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Abstract. Soybean is one of important national commodities for food and industry. Gamma rays are electromagnetic waves that have very strong penetrating power. One of the sources of gamma rays is from 60Co. With its strong penetration power, gamma rays can be used in plant breeding to create new genetic diversity in order to develop high-yielding varieties. Irradiation of gamma rays at 300 Gy dose on Denna 2 variety soybean was conducted, then it was planted as M1 plant and later continued to be planted as M2. In M2 generation, mutant lines were selected based on early maturity, better than of the parent variety. The criteria used was harvest age between 75-81 days, the plants should look robust and strong stems. Harvesting days of parent varieties (Denna 2) was 81 days, while the mutant strains obtained were between 75-81 days. Likewise, for the plant height, the parent variety was 42.30 cm, whereas mutant lines of 38.56 cm in average, making it more resistant to logging. The average pods for parent was 36.37 pods and mutant lines of 55.24 pods. In selected shade tolerant soybean of the M3 generation early aged mutant lines shade tolerant days, of the parent variety Denna 2 days.

1. Introduction

The use of gamma rays has developed in various fields for the welfare of humanity, including in the fields of health, industry, food preservation, agriculture and others. Gamma rays are electromagnetic waves that have a very strong penetrating power. One source of gamma rays is derived from 60Co (Cobalt-60), very strong translucency, gamma rays can be utilized in the field of plant breeding to create new genetic diversity in the assembly of superior varieties [1]. Plant mutation breeding with techniques for changes in plant in parent aims to obtain new properties from plants through genetic changes after receiving gamma ray radiation at certain doses in the parent plant [2].

Mutation is process in which genes changes of hereditary that can cause phenotypic and it is passed on from one generation to the others [1]. The advantage of mutation technique is can be improved without changing the other characteristic plant. This method is complementary to other techniques so that the technique can be used in conjunction with other techniques such as hybridization and biotechnology [3]. Gene linkages and the use of appropriate gamma ray irradiation techniques can increase the frequency and spectrum of mutations [4]. It reduces negative effects of physiological damage due to irradiation [5].

Soybean varieties breeding aims to obtain superior breed that are able to adapt to various environmental conditions. Plant breeding using crosses is still the main method in improving plant varieties in Indonesia. But with the limited genetic resources used and the crossing can be a constraint for this method. To expand genetic diversity in populations, one method that can be used is the mutation method [6]. Genetic resources through breeding programs to obtain early maturing soybean varieties and high yield productivity, carried out with various approaches, such as increasing plant genetic diversity through crossing, soma clonal variation, and mutation techniques [7].

Plant mutation breeding with gamma has developed quite rapidly in Indonesia, especially in the National Nuclear Energy Agency of Indonesia (BATAN) to get new good varieties rice [8], soybean, sorghum, peanut, wheat and cotton varieties have produced varieties that have been released by the
Ministry of Agriculture. Various superior varieties with the radiation mutation technique have contributed to increasing the production of agricultural commodities in Indonesia. One of the agricultural commodities that has been produced by this radiation mutation technique is soybean. BATAN has released 12 superior varieties of soybeans through plant breeding with mutation techniques. The decline in soybean production is one of them influenced by land area. Land available in Indonesia is more widely used by farmers to cultivate crops that have the potential to meet their economic needs, so very few farmers use their land for soybean. The development of soybean plants interrupted by the stands of estate crops, agroforestry environments or intercropping with other crops is one of the alternative cultivation to increase national production factor:

1. Effect intercropping of soybean to plant environmental factors shade affects the light intensity, temperature and humidity of the environment around the plant, physiological changes in plants, especially in photosynthetic activity.

2. Plant responses toward light intensity difference affects the availability of light energy which will be converted into heat energy and chemical energy, to overcome the problem needed genotype of soybeans needed tolerant of low light intensity. The rate of photosynthesis is lower than plants grown in an unshaded environment.

Soybean plants in plantation stands and agroforestry environments or intercropping with other crops is one of the alternatives for cultivation to increase national soybean production. Soybean planting as intercropping with different levels of shade affects the light intensity, temperature and humidity of the air in the plant environment, so the light intensity received by plants differs this difference affects the availability of light energy which will be converted into heat energy and chemical energy, to overcome the problem the soybean genotype is needed which is tolerant of low light intensity.

Soybean varieties that are shade tolerant stress is expected to be a more efficient way to prevent the decline in seed yield in a shaded environment. BPS 2015 Soybean production in Indonesia from 1993-2015 is 963,183 tons can be increased through the expansion of planting areas and increase production per unit area. For optimal areas and rice fields expansion of planting areas are constrained by competition with other commodities such as rice, so farmers are more interested in planting rice. While for suboptimal regions greater technological input and capital are needed, so it is also an obstacle in its application.

In line with these problems, planting shade tolerant soybeans as intercrops is considered as one of the efforts that can be taken to increase land productivity which is now declining shade resistant soybean research derived from Denna 2 varieties is an initial study using gamma ray irradiation at the Isotopic and Radiation Application Center. The use of good quality superior varieties, in this case high production is one of the criteria for making soybeans a superior commodity for shade resistant soybeans.

Generation M3 plant shade tolerant soybean, an agronomic trait was evaluated, among others: flowering age, harvesting age, plant height, number of filled pods. This agronomic trait is important to see the uniformity and homogeneity of the selected mutant lines. The selection is continued until the generations M4 and M5 until the plants are truly homogeneous and no longer segregating. The purpose of this study was to obtain a dose of irradiation which will produce mutant strains that can later be released as high yielding varieties that are of early maturity, high production and disease resistance.

2. Materials and Methods

2.1 Preparation Shading House
Bambo for house shade tolerant soybean with a height of 2 meters from the ground, the area of land in accordance with the distance of plots that have been provided with 50% sun exposure.

2.2 Seed Material
Varieties Denna 2 soybean seeds used is 500 grams with a moisture content below 12%, then irradiated with 300 gy dose of gamma rays from a 60Co source. The method used was the pedigree selection
method, which is a selection method based on origin. The irradiated soybean seeds are then planted in the field as M1 plants. M1 plants are fully nurtured and harvested so that M2 seeds are obtained.

2.3 Crop Establishment
Generasi plant M2, pedigree selection was carried out, which has characteristics such as: strong stem appearance, good and healthy plant growth, large number of filled pods, resistance to pests and diseases. The plants are marked in the field to determine harvest time. This selection on the M2 generation will determine the success in obtaining the desired mutant strain in accordance with the breeding purpose.

Generation M2 there will be wide genetic diversity such as, plants can be taller or shorter, more rapid or in their age, the shape of the phenotype will also change. The purpose of breeding is to get the early maturity and high production. The selection is directed to plants that are early maturing and have many branches and pods. The selected plants in the M2 generation are then planted as M3 plants with separate rows for each plant. Plants originating from one plant in M2 generation are planted in one particular row. Then the pedigree selection was carried out again on the M3 generation such as the M2 selection time, the appearance of the plant is sturdy and strong, the plant grows healthy and in unison and is resistant to disease pests [1].

Generation plant M3, character agronomic trait was evaluated, among others: flowering age, harvesting age, plant height, number of filled pods. This agronomic trait is important to see the splendor and homogeneity of the selected mutant lines. The selection is continued until the generations M4 and M5 until the plants are truly homogeneous and no longer segregating.

2.4. Data Analysis
Analyzes were performed with standard deviation (SD) using the microsoft excel-program.

3. Results and Discussion
Shade resistant soybean plants that are selected M3 include the shade house with 50 % irradiation: a. Early maturity, sturdy and strong appearance, plants grow healthy and resistant to disease pests, b. Evaluate agronomic traits to obtain uniformity or homogeneity data from selected plants. Evaluation of agronomic traits includes: flowering age, harvest age, plant height, and number of filled pods. Figure 1 shows growth of shade tolerant soybean in shade houses. In this M3 generation an evaluation of agronomic traits is carried out to get data and see the uniformity or homogeneity of selected plants. Evaluation of agronomic traits includes: flowering age, harvest age, plant height, and number of filled pods.

3.1 Flowering age and Harvest Age
Flowering age and Harvest Age are the main indicators for the selection of early maturing soybeans. Soybean plants flower faster and harvest under the age of 75-80 days, which should be noted after 80% of
flowering plants and how many days of flowering of each plant. While the age of harvest is observed after 90% of physiological ripe plants, which are characterized by yellowing leaves and some have started to wilt and fall out, and the pods are dark brown. Table 1 shows the flowering age and harvest age of the M3 mutant lines selected early maturity along with the parent variety Denna 2.

Table 1. Flowering Age and Harvest Age of Mutant lines tolerant Soybean Hold Shade M3. in Bogor (taken an average of 5 plants sample)

| No | Strain / Genotype | Flowering age (days) | Harvest Age (days) |
|----|-------------------|----------------------|--------------------|
| 1  | Denna 2.1.3       | 36                   | 76                 |
| 2  | Denna 2.5.3       | 35                   | 75                 |
| 3  | Denna 2.6.3       | 37                   | 80                 |
| 4  | Denna 2.9.3       | 35                   | 75                 |
| 5  | Denna 2.10.3      | 37                   | 77                 |
| 6  | Denna 2.11.3      | 35                   | 75                 |
| 7  | Denna 2.12.3      | 35                   | 77                 |
| 8  | Denna 2 (parent)  | 35                   | 81                 |
|    | Average           | 35.62                | 76.75              |
|    | SD                | 0.916                | 2.43               |

Table 1 showing the age of flowering plants at an average age of 35 days and flowering faster than the parent is a variety of Denna 2 which has a flowering age of 35 days. While the harvest age of mutant lines between 75-80 days, is sooner than its parent variety Denna 2 whose harvest age is 81 days.

With the influence of gamma rays at a dose of 30 gray will provide the opportunity and influence of the radiation mutation. Radiation mutations provide wider genetic diversity so that breeders have the choice to make more selections. Radiation mutations influence the age of flowering to be faster, so also the age of harvest to be faster than the parent varieties. Age of harvest is more early in the age range of 75-80 days, more early than the parent variety Denna 2 harvest age is 85 days. Plant shade tolerant soybean for Flowering Age on mutant lines faster flowering in vegetative and go to generative high production. With the effect of radiation mutations will give effect to the age of flowering and age of harvest so that it becomes more early [19]. Gamma rays produce energy, this can cause molecular damage through reactants where radiation energy is absorbed by DNA molecules. In an indirect reaction the energy is not absorbed (absorbed) by DNA, but by other molecules in the cell that produce free radicals resulting in changes in DNA molecules [3].

3.2 Plant height

Plant height has an effect on plant resistance to lodging. Plants that are too high will easily fall, which will reduce production. Observation of plant height is taken from a sample of 5 plants randomly from each selected line, then averaged. The plant height of each mutant line is shown in Figure 3. This may be caused by the appearance of the trait in M2 not in a homozygous form and the possibility of mutation in the direction of dominant or semi-dominant, or other possibilities for epistatic or non-allelic gene interaction is greater [14].

Table 2 show that the plant height is still segregated and has not been homogeneous which is characterized by the high standard deviation (SD) in one treatment number. This is common in plant breeding with mutation techniques, where in the M3 generation plants are still not homogeneous and need purification in further generations. This can be compared with the parent control of Denna 2 varieties whose plant height is homogeneous, which is characterized by the low standard deviation 3.51 and mutan line Denna 2.9.3 (3.41) of 5 observed plant samples. Interactions between genotype x environment significantly affected plant height [19].
Table 2. Average Plant Height of the Mutant Shade tolerant Soybean M3 mutant lines in Bogor, West Java (taken on average from 5 sample plants)

| No | Strain / Genotype | Flowering age (days) | Harvest Age (days) |
|----|-------------------|----------------------|-------------------|
| 1  | Denna 2.1.3       | 39.16                | 4.87              |
| 2  | Denna 2.5.3       | 39.66                | 4.30              |
| 3  | Denna 2.6.3       | 42.28                | 4.30              |
| 4  | Denna 2.9.3       | 48.23                | 3.41              |
| 5  | Denna 2.10.3      | 39.37                | 8.22              |
| 6  | Denna 2.11.3      | 38.56                | 4.54              |
| 7  | Denna 2.12.3      | 40.05                | 4.94              |
| 8  | Denna 2 (parent)  | 42.30                | 3.51              |
|    | Average           | 42.30                | 76.75             |
|    | SD                | 0.153                | 2.43              |

3.3 Filled pods
The number of filled pods was observed through a sample of 5 plants randomly selected from each line number. Each sample counts the number of filled pods, then averaged from the 5 observed samples. The number of filled pods from each soybean mutant strain is shown in Table 3.

Figure 4. Filled Pods Shade Tolerant Soybean

The number of filled pods reflects the level of soybean productivity, because the more filled pods in one plant, the higher the productivity. Selection is done by selecting lines that have more filled pods, which are then purified and planted in the next generation. Figure 4. showing like a shade resistant soybean plant with many filled pods, can increase shade tolerant soybean production.

Table 3. Average filled pod of the mutant lines of M3 Shade tolerant Soybean in Bogor, West Java (taken average of 5 sample plants)

| No | Strain / Genotype | Flowering age (days) | Harvest Age (days) |
|----|-------------------|----------------------|-------------------|
| 1  | Denna 2.1.3       | 55.24                | 11.51             |
| 2  | Denna 2.5.3       | 39.92                | 8.15              |
| 3  | Denna 2.6.3       | 43.36                | 8.29              |
| 4  | Denna 2.9.3       | 32.65                | 5.85              |
| 5  | Denna 2.10.3      | 36.45                | 4.98              |
| 6  | Denna 2.11.3      | 40.48                | 45.32             |
|    | Average           | 42.11                |                   |
|    | SD                | 6.78                 |                   |
The number of filled pods reflects the level of soybean productivity, because the more filled pods in one plant, the higher the productivity. Because of that selection is done by selecting lines that have more filled pods, to then it can be purified and planted in the next generation.

4. Conclusion
From the discussion presented above, the following items can be concluded:
1. The gamma ray treatment of 300 Gray in soybean variety Denna 2 has made way to the development of shade tolerant mutant lines in the M3 generation that are mature earlier than the parent.
2. The soybean shade tolerant mutant lines of the M3 generation with harvest age of 75-80 days were selected, these are earlier than the parent variety Denna 2 which is 81 days.
3. The plant height of the early mutant lines are ranged from 39.16 to 48.23 cm, while the parent cultivar has 42.30 cm of plant height.
4. The number of filled pods of the early mutant lines ranges from 32.65 to 55.24 pods / plants, and some mutant lines are still segregating.

Acknowledgments
The author thanks the Head of the Isotope and Radiation Application Center - National Nuclear Energy Agency for providing research funding sourced from DIPA - BATAN, the Head of the Citayam Experimental Garden Depok for being provided with facilities to use the experimental gardens and all those who have helped to carry out this research.

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