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International Journal of Sciences: Basic and Applied Research (IJSBAR) ISSN 2307-4531 (Print & Online) http://gssrr.org/index.php?journal=JournalOfBa sicAndApplied -- 74 Gamma Irradiation Induced Chlorophyll and Morphological Mutation in Kipas Putih Soybean Nilahayati a* , Rosmayati b , Diana Sofia Hanafiah c , Fauziyah Harahap d a Faculty of Agriculture, Malikussaleh University - Indonesia b,c Faculty of Agriculture, North Sumatera University - Indonesia d Faculty of Mathematics and Natural Science , UNIMED - Indonesia a Email: ichsan28@yahoo.com Abstract Kipas Putih soybean seed was treated by gamma ray irradiation with irradiation dose of 100 Gy, 200 Gy and 300 Gy to study number and types of mutant contained in M 2 generation.

The results showed that there are several types and numbers of soybean mutant in M 2 generation such as chlorophyll mutants (viridis, xantha, waxy and variegata), leaflet mutants (unifoliate, bifoliate, quadri foliate, pentafoliate, narrow -rugose leaflet, wrinkled leaf) and sterile mutants (undeveloped rasim flower, half sterility and full sterility). Some types of mutants in M 2 generation clearly show that gamma -ray irradiation can be effectively used to assemb le genetic diversity in plants.

Keywords: Kipas Putih Soybean; Chlorophyll mutant; Morphological mutant ; M2 generation. 1. Introduction Mutation breeding by irradiation of gamma rays in various plants can induce mutations. The breeding technique can be used to assemble a high-yielding variety. Mutation induced by gamma -ray irradiation is used to assemble genetic diversity in crops to improve its genetic.

-- * Corresponding author. International Journal of Sciences: Basic and Applied Research (IJSBAR) (2016) Volume 30, No 3, pp 74-79 75 The genetic diversity found becomes the main ingredient for breeders to acquire new more superior properties. Among the several ionizing radiations, gamma rays comprise the most widely used mutagen for mutation induction in crop plants.

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These are electromagnetic radiations of very short wavelengths similar to X -rays and thus very penetrating. Their energy may be absorbed by atoms in the tissue through which they pass, causing ejection of electrons resulting in ionization and consequent changes in chemical activity . Kinetic energy in ejected electrons produces further ionization [2]. The influence of mutagen materials especially radiation that more often occur in plant chromosome is chromosome breakage or chromosome aberration.

The breakage of chromosomes divided in to 4 groups: translocation, inversion, duplications, and deficiencies. In plants, the deficiencies occur as a result of mutagen treatment (radiation) is often indicated by the mutation of chlorophyll. The occurrence of chlorophyll mutation can usually be observed at seedling stage from the discoloration of plant leaves [11]. Many researchers have reported different abnormalities in mutation -induced plants with gamma ray irradiation.

Abnormalities that occur can be chlorophyll mutation and morphological muta tion. Abnormal growth in Argomulyo soybean caused by gamma rays irradiation were found by [6] . The abnormalities included bifoliate leaves and elongated, rasim flowers and buds (not develop) and white flowers. R eference [8] reported four types of chlorophyll mutant : viz, albina, xantha, chlorine and viridis in gamma rays and gamma rays + EMS treated population of soybean.

Gamma rays are found to be more effective to induce chlorophyll mutations. Xantha and clorine types of chlorophyll mutant in soybean were earlier reported by [5]. Since chlorophyll mutant are easily detectable as they have been extensively used to find out sensitivity of a crop plant to mutagen. This study used gamma -ray irradiation at a dose of 100 Gy, 200 Gy and 300 Gy on kipas putih soybean. It i s expected from the results of this study that there are various mutant types that can indicate mutations in the irradiated population.

In this paper, the discussion is limited to the type and number of mutant contained on the populations treated by dose of gamma rays irradiation treatment in the population of M 2 generation. 2. Matherials and Methode Kipas Putih soybean seeds were irradiated with doses of 100, 200, and 300 Gy using a 60Co gamma cell in National Atomic Energy Agency (Batan), Pasar Jumat, Jakarta. At every treatment, 200 seeds were irradiated to form four populations in M 1 generation .

In M 1 generation , 10 pods per plant were harvested from each plant in each treatment dose (restricted bulk) to obtain 4500 seeds to be planted as generations M 2. The seeds (M 2) in each treatment dose were planted with a spacing of 40 x 20 cm2 and one seed per hole. The tillage was done two weeks before planting. Fertilization was carried out in accordance with the recommended dosage of soybean fertilizer requirement , which is 50 kg Urea/ha, 200 kg SP -36/ha and 100 kg KCl/ha. Fertilization of SP -36 and KCl was done two weeks before planting, while urea was done at the time of planting.

Watering was done in accordance with the conditions in the field, in the morning or in the afternoon. In the event of rain, the plants were not watered. Weeding was carried out manually by removing the weeds in the plot International Journal of Sciences: Basic and Applied Research (IJSBAR) (2016) Volume 30, No 3, pp 74-79 76 in order to avoid competition for nutrients in the soil. It was done in accordance with the conditions of

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0% https://nucleus.iaea.org/sites/naipc/idi the field. So that the plants do not easily fall down and stand up straight and sturdy, bedding was done by making a mound of soil around the plant and it was done when the plants were 2 week after planting. Observation was conducted on chlorophyll mutations, leaves morphology diversity and sterile plants.

The data of chlorophyll mutants, leaflet mutants and sterile mutants were observed periodically from germination to harvest. 3. Resuls and Discussion A number of mutants were identified in the M 2 generation of Kipas Puti h soybean as a result of gamma rays irradiation. The percentage of mutant at doses of 100 Gy, 200 Gy and 300 Gy was 8.39, 8.91 and 3.5 respectively. The percentage of mutant contained in this research is more than has been reported by [1] in Black gram plant.

They got a number of mutant plants in M 2 generation and the percentage of mutations in gamma -ray irradiation dose of 10, 20, 30, 40, and 50 KR as many as 3.10, 5.09, 5.68, 4:29, and 3.96 respectively. The percentage data of chlorophyll, leaves and ster ility mutant contained in M 2 generation in Kipas Putih soybean with gamma ray irradiation is presented in Table 1. Table 1: Percentage of number of mutant contained in M 2 generation in each different treatment of gamma irradiation Treatment No.

of plant s tudied Mutan type Total Percentage of mutans Chlorophyll Leaf Sterile 100 Gy 1740 22 103 21 146 8,39 200 Gy 920 14 33 35 82 8,91 300 Gy 200 2 2 3 7 3,5 Total 3950 38 138 59 235 5,94 The most numerous mutant types in generation M 2 were leafl et, sterility and chlorophyll mutation. Leaflet mutant are like unifoliate, bifoliate, tetrafoliate, pentafoliate, narrow rugose leaflet, wrinkled leaf. Chlorophyll mutants found are light green, xanta, waxy and variegated. Sterile mutants found are undeveloped rasim flower, half sterility and full sterility.

Chlorophyll mutation is one indicator in evaluating the effect of mutagen treatment. It has been widely reported that chlorophyll mutation occurred in many plants irradiated with gamma rays such as in Black gram [1]. The frequency of chlorophyll mutations in the generation of M 1 and M 2 is the most dependable index for evaluating the genetic effects of mutagenic treatments. Chlorophyll mutations offer one of the most reliable indices for the assessment of genetic effects of mutagenic treatments [4].

Although the chlorophyll mutations do not have any economic value due to their lethal nature, such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability [9]. International Journal of Sciences: Basic and Applied Research (IJSBAR) (2016) Volume 30, No 3, pp 74-79 77 Table 2: Type and number of mutants found in M 2 plants of Kipas Putih soybean population No.

Mutant Character Treatment Total 100 Gy 200 Gy 300 Gy Number of plant Studied 1740 Plant 920 Plant 200 Plant 1 Chlorophyll mutation Wax y 1 - - 1 Xantha 13 11 - 25 Light green leaf/viridis 5 2 2 9 Variegated leaf 3 1 - 4 2 Leaflet mutation Unifoliate 2 - - 2 Bifoliate 2 1 1 4 Quadrifoliate 83 22 2 107 Pentafoliate 11 8 1 20 Narrow rugose leaflet 3 1 - 4 Wrinkled lea f 1 - - 1 3. Sterility Undeveloped flower rasim 7 14 1 22 Partial sterility 6 6 0 12 Full sterility 21 35 2 58 Total In this study, a wide spectrum of chlorophyll mutations found in the populations treated with mutagen in M 2 generation (Table 2).

Chlorophyll macro mutation such as waxy, xantha, viridis and variegated leaf was found in the populations treated with mutagen. No such mutation was observed in the controls. Of the various types of chlorophyll mutant, the most often found type o f chlorophyll mutant in this study was xantha. Similar results were also reported by [10] on greengram (Vigna radiata L) Sujata variety and TARM -1 treated with gamma radiation of variable doses (20, 30, 40, 50 and 60 kR). Chlorophyll macro -mutations viz., chlorina, xantha, Albina, viridis and sectorial were observed in the mutagen treated populations of both varieties.

Among different types of chlorophyll mutations, chlorina was the most frequent (2.15 percent in Sujata and 2.28 percent in TARM-1) in both cultivars suggesting high mutability of the gene controlling the phenotype. Furthermore [2] also got some type of chlorophyll mutant in Cyamopsis tetragonoloba (L.) Taub that was treated by gamma rays irradiation. They got three types of chlorophyll mutants such as xantha, chlorina and viridis. Xantha mutants displayed a bright yellow color.

In some mutants, the color was little lighter. The viability of this mutant was very less (2-3 days). Chlorina mutants showed yellowish green color. A few of them changed to the normal green type. The viridis mutants showed dull light green color. This color gradually changed to the normal color green color during subsequent growth phases of the plant. Only some of the International Journal of Sciences: Basic and Applied Research (IJSBAR) (2016) Volume 30, No 3, pp 74-79 78 chlorophyll mutants such as chlorina and viridis could be found growing well till maturity.

Various types of morphological mutation such as unifoliate, bifoliate, quadrifoliate, pentafoliate, narrow rugose leaflet, wrinkled leaf, undeveloped rasim flower, half sterility and full sterility many of which found in population treated by gamma ray irradiation (Table 2). Among the various types of morphological mutation, the most numerous mutants found were leaflet mutant of quadrifoliate. Similar results were also reported by [10] on Greengram. They got different types of morphological mutations viz.

unifoliate, bifoliate, quadrifoliate, pentafoliate, tall, dwarf, trailing type, early, late, profusely branched, more poded, erect branch type, serrated leaf, modified inflorescence and simple leaf type observed in mut agenic treated populations of both varieties. Among different types of morphological mutations, the most frequent types were quadrifoliate in Sujata variety and more poded type plants in TARM -1 variety. Morphological variations especially leaflets mutant s howed abnormalities on leaves is an indicator of effective mutagens given.

Bifoliate, tetrafoliate, pentafoliate and narrow rugose leaves showed normal growth and could produce pods and seeds, while unifoliate and wrinkled leaves showed only vegetative gro wth and eventually became sterile plants. The same leaflet mutant was also found by [1] on M 2 generation in Blackgram plant as a result of gamma ray irradiation. In this study, the most commonly found sterile mutant types were full sterility mutant.

This is in line with the results of [7], they state that among a number of mutants observed in soybean plants, it showed that sterile mutants were the most dominant types (complete sterile 7.02%, partial sterile 3.24%) followed by stunted growth mutant (1.89%) on soybean varieties of CO1 and C02 that were irradiated with gamma rays. Mutation that induces sterility in soybeans is caused by degeneration of pollen generative cells in Meosis II that produces a low pollen viability [3].

Full sterility plants showed no rmal vegetative growth but not managed to produce flowers let alone seeds and pods. Before reaching harvesting, these plants showed yellowing leaves and over time the leaves fell out. Full sterility plants were found many at a dose of 200 Gy in M 2 generation. Partial sterility plants had less number of leaves and branches. These plants could bloom and produced pods and seed.

Groose and Palmer (1987) i n [7] reported that partial sterility in soybean is marked by the reduction in the number of seeds per pod as a result of a very early embryo abortion. The mutant type of undeveloped rasim flower was the second largest type of sterile mutants in M 2 generation in this research. The dose of 200 Gy was the irradiation dose that generates most of the mutants with un developed rasim flowers. The same thing happened to M 1 generation of Kipas Putih soybean irradiated with gamma rays, in which undeveloped rasim flowers were most commonly found at this dose.

Undeveloped rasim flower plants could produce flowers but the flo wers could not bloom, had a big trunk with robust characteristic, the node and internode was ballooned and the leaves were always green even after entering the age of the harvest. 4. Conclusions Several types and numbers of chlorophyll and morphological mutant in M 2 generation of kipas putih soybean International Journal of Sciences: Basic and Applied Research (IJSBAR) (2016) Volume 30, No 3, pp 74-79 79 treated by gamma irradiation were found .

The chlorophyll mutants were viridis, xantha, waxy and variegat a. The leaflet mutants were unifoliate, bifoliate, quadrifoliate, pentafoliate, narrow -rugose leaflet, wrinkled leaf , and the sterile mutants were undeveloped rasim flower, half sterility and full sterility.