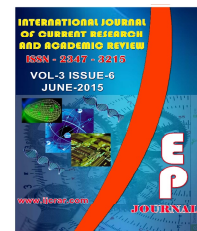




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Improving the genetic diversity of soybean seeds and tolerance to drought irradiated with gamma rays

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KEYWORDS

Soybean seeds,
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drought

A B S T R A C T

To increase the genetic diversity of soybean in order to adapt to agroecology in Indonesia conducted ways including introduction, cross, mutation and genetic transformation. The purpose of this research is to obtain early maturity soybean mutant lines, large seed tolerant to drought with high yield potential. This study consisted of two stages: the first is sensitivity of gamma rays carried out in the Laboratory BATAN. The genetic varieties used is Anjasmoro. The method seeds irradiated with gamma rays at a rate of activity with the old ci 1046.16976 irradiation 0-71 minutes. Irradiation doses of 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000gy. The results indicated all seeds irradiated with doses of 0 - 1000gy, just a dose of 200 and 300gy are able to show the percentage of germination, plant height, number of leaves, number of normal sprouts and green leaves of the best and can be continued for a second trial in order to assemble and to get mutants which is expected. The result of second stage of soybean M2 Population irradiated with diversity Gamma Irradiation performed that in the form of soybean planting, the seed planted is the first derivative of the M2 irradiated seeds. The result after the age of 30HST has already showing growth and development of plants that vary when compared to its parent, both in terms of plant height, number of leaves, leaf shape and leaf forage level. In the generative phase, a plant that has been irradiated 200 and 300 gy seen some plants flower form packs, but not formed pods, there is also a form packs of flowers, but few pods produce soybean morphological characters such as plant height, number of branches, pods, days to flowering, harvesting, seed weight and seed number. Keywords: Soybean, gamma ray Irradiation, gene mutations.

Introduction

Efforts are needed to increase soybean production in the country is the provision of

high yielding varieties of high yielding and adapted well in the paddy field and dry

land. One strategy to develop soybean on land that is often experienced water deficit conditions are assembling varieties tolerant to drought stress. The initial step for the assembly of these varieties are able to increase the genetic diversity of plants through hybridization and mutation induction, then selecting the character morfofisiologis early soybean genotypes to drought stress to determine tolerance to drought stress conditions. To increase the genetic diversity of soybean plants in order to adapt to agroecology in Indonesia conducted among others by way of introduction, crossover, mutation and genetic transformation. Soybean plants including self-pollinated crops where the plants will form a stable strains or not segregate. In certain characters such as character adaptation to drought on soybean varieties with early maturity, large seed size, not present in the local soybean germplasm varieties Indonesia. Mutation breeding is useful to improve the character of the plant if desired character is not found in a species of plant germplasm (Van Harten, 1998). Mutation breeding is useful to improve the character of the plant if desired character is not found in a species of plant germplasm (Van Harten, 1998). With a change in mutation breeding characters morfofisiologis very large and occur simultaneously, so that a change of this character can be used as a basis for selecting resistance to drought stress.

Mutations are changes in the genetic material of an organism that occurs in sudden, random, and are fundamental to the source of variation in living organisms that are heritable (heritable). Mutations can occur spontaneously in natural (spontaneous mutation), but the chance of occurrence is very small, which is about 10^{-6} and can also occur through induction (induced mutation) (BATAN, 2006). Based on the above description in order to increase the genetic

diversity of soybean to improve the quality and quantity of soybean plants, necessary to study the role of mutations in morfofisiologis about getting a mutant soybean genotypes with the expected character. Mutant soybean genotypes obtained with early selection to adapt to drought conditions with observations morfofisiologis approach and crop yield potential.

Research objectives and benefits research

This study aims to get early maturity soybean mutant lines, large seed tolerant to drought with high yield potential The specific objectives are (1) to study the response of soybean genotypes morfofisiologis character tolerant and sensitive to drought stress (2) determine the optimal dose of gamma ray irradiation may increase genetic diversity and (3) obtain a population of putative early duration, large seed and tolerant to drought stress The benefits of this research are to provide information about several soybean genotypes tolerant to drought stress. Another benefit data obtained will then be used as research material to be submitted to the local government for the development of soybean crops in dry land with the use of soybean varieties that are tolerant to drought

Research methods

Phase I studies the sensivity of gamma irradiation

The application of Gamma rays was carried out in the Laboratory of Batan, Friday Market, Jakarta. The genetic material used in this study is Anjasmoro variety. Seeds were irradiated with gamma rays at a rate of activity with the old ci 1046.16976 irradiation 0-71 minutes (Appendix Table). Irradiation doses used were 0, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 gy.

Soybean seeds were planted in plastic tubs, each planting hole consists of 2 seed / planting hole as much as 7 planting hole, so that the number of seeds planted every repetition is 14 seed. Field planting is done to verify the dose of gamma ray irradiation more heterogeneous environment. The experiment was conducted at the Research Institute and the Faculty of Agriculture Cereals Maros Indonesian Muslim University. The study was conducted from April to November, 2014.

Experiment Phase 2

The diversity of M2 Gamma Irradiation population. Soybean seed is soybean seed of M2 first line as the result of gamma ray irradiation Anjasmoro varieties, and the dose taken is a dose of 200 and 300 gy gy and control.

At this stage of the study parameters were observed in the form of diversity on plant height, number of branches, days to flowering, harvesting, productive pods, number of empty pods and seed weight per plant. In this M2 derivative pods from each plant in each dose irradiation harvested mass (bulk) where each plant was taken three pods (restricted bulk) to continue planting on the M3.

Preparation of planting. The first step is to dry the soil, and then sieved with a sieve diameter ± 6 mm while cleared of debris and dirt. Then the soil evenly mixed and put into a watertight plastic bag and tied tightly in order to keep the moisture content. To determine the characteristics of the soil physical and chemical soil analysis. Determination of soil water content to determine the weight of air-dry soil that will be included in a polybag done by the method of drying (oven). While the determination of water content at field capacity was conducted by Alrich.

Soil that has been provided inserted into polybag size of 10 kg. Watering is done through a plastic pipe insert into polybag. In the inside of a polybag plastic coated to maintain the levels of the treated field capacity. Polybag was arranged by the distance of experimental unit by 25 cm. The day before planting, the soil was given basic fertilizer N, P, and K in accordance with the results of the soil analysis.

Planting and maintenance. Seeds to be planted given Furadan in each planting hole. Each polybag planted four soybean seed depth 2.0 cm from the soil surface. At the age of 7 days only two the plants to be left per polybag. The second thinning is done at the age of two weeks, leaving only one plant per polybag which selected the best plant growth.

To protect plants from pests and diseases by using Decis spraying. Weeding regularly done by unplugging the weeds that grow in polybag. Maintenance is carried out continuously until the crop is harvested.

Results and Discussion

The sensivity of Gamma Irradiation

The results showed that radiosensivisitivity of gamma irradiation showed no significant effect on soybean seed germination percentage. The number of seeds germinated only decreased at doses of 500 and 600 gy, then the higher dose increased to 80%. Similarly, the number of normal seedlings showed that starting dose gamma irradiation 400 - 1000 gy normal seedling number has decreased to 50%. The higher dose of gamma irradiation caused an increasing number of abnormal sprouts and the number of seeds that do not grow. Visually this sensitivity level can be observed from a given plant response, both in morphology, sterility, and the lethal dose 50 (LD50).

Lethal doses (LD50) is the dose which causes the death of 50% of the population were irradiated. From many studies mutation induction, has been known that generally desired mutation lies in the range of LD50 or rather at a dose slightly below the LD50 (Sanada and Amano, 1998; Van Harten, 1998). This is different from that obtained in this study did not show a decrease in germination rate up to 50% to the highest dose. This study is in line with that report of Nur (2013) with wheat irradiation 100 gy - 1000 gy all doses experienced germination > 90%, after 15 days on leave until the dose from 400 gy already showed abnormal growth until the end of the dead. The percentage of germinated seeds (JB), the number of normal seedlings (JKN), and the number of abnormal sprouts (JKAB) in the treatment radiosensitivity gamma ray is presented in figure 2.

Subsequent observations by looking at the development of germination in which the dose of 300 gy normal germination is still around 90%, but after reaching a higher dose (300 gy upwards) then the germination rate has dropped, and instead the number of abnormal seedlings increased with the increasing dose after irradiation (300 gy upwards, Figure 1) Abnormal Sprouts characterized by abnormal growth (Appendix 1). This means that at the dose (irradiation doses higher than 300 gy) seed has undergone changes in morphology, anatomy and genetics. Furthermore, based on plant height, number of leaves and leaf greenness showed that at a dose of 400 gy has decreased the third character (Figure 2). At a dose of 400 gy plant height and number of leaves already showed abnormal development and some sprouts have been unable to form the first leaf trifoliate, while a dose of 500 gy start up initially normal germination, but once it is left up to 15 days can not be formed until the first leaf eventually die. Average plant height,

number of leaves and leaf greenness is presented in figure 2.

Based on these results the optimal dose can be continued into the next population establishment is a dose of 300 gy. This is in line with research Manjaya and Nandanwar (2007), at a dose of 250 Gy gamma irradiation successfully induce mutations and cause genetic diversity in soybean cv JS 80-21. The irradiation dose is different for each soybean cultivars and species that exist. The percentage of germination was also influenced by the state of conditions both in terms of seed vigor and the large size of the seed. The next-generation descendant of the seeds or plants treated with mutagens denoted by M1, M2, M3, and so on to distinguish the hybridization generation symbolized by F1, F2, F3, and so on. Plants usually linear to M1, M1 through plant growth, the chance of competition between mutant and non-mutant occurs (Nasir, 2002). Germination appearance soybean varieties radio sensitivitas Anjasmoro with gamma ray treatment is presented in Appendix 1.

The diversity of M2 Gamma Irradiation Results Based on the results of the first study, in which a dose of 300 gy is the optimal dose of gamma rays that can be continued on the formation of M2 generation, which in turn will be used as material for further research to obtain mutant strains of soybeans that are tolerant to drought and seeded larger than the original varieties of Anjasmoro. Plant growth and development until the age of 3 MST at doses of 200 and 300 gy still showing growth and development of plants together with its parent Anjasmoro. But after entering the age of 1 month (30 HST) have demonstrated the growth and development of plants that vary when compared with the parent (control) both plant height, leaf shape and leaf kehijaun level.

Differences in leaf shape and leaf greenness levels are presented in Figure 3. If we look at the color of green leaves, where a dose of 200 gy showed the older leaf color than the control (without irradiation) this means a change / mutation in the irradiation dose, which we know color older leaves have sunlight absorption capacity is higher than the color of the younger leaves, so it also affects the rate of photosynthesis (Yogi Sugito, 1999)

Generative phase is a phase which determines the soybean crop productivity levels. In this phase there is a difference that is very high between the parent plant (control) plants that had been irradiated with gamma rays at a dose of 200 and 300 gy. Plants that have been irradiated with gamma rays there are some plants that only a horde of interest, but not formed pods, there is also a form packs of flowers, but only a few that produce pods. This is the response of plants due to damage to the DNA level, which may occur as a result of plant DNA that has been irradiated with gamma ray sun dergo deletions, inversions or translocations and it will undergo a process of improvement and will appear plasticity

repair these characters in subsequent generations, especially the character that will be addressed disimprovedd rough tolerance and large seed. Appearance generative phase is presented in figure 4.

Looking at the general appearance of the plant in figure 5, indicates that plants that have been irradiated with gamma ray has mutated at the level of M1 plants, while to look at the stability of the mutations and changes that occur significantly character can be traced to the generation of M2, M3, and so on get in get mutated character and in accordance with the expected character.

Based on the results of the study showed that all of the characters were observed decreased with irradiation dose of 200 gray and 300 gray. The results of t test analysis showed higher plants. The number of pods, days to flowering, harvesting and seed number significantly different between the control (not irradiated) with irradiation dose of 200 gray and 300 gray, except for the character number of branches were not significantly different between the control (not irradiated) with two doses of irradiation.

Table.1 The average of soybean character at the treatment of gamma irradiation

Character	control	200 gray	300 gray
Plant height	124.53	72.40**	67.99**
Number of branches	4.13	3.03tn	2.83tn
Number of pods	19.14	14.23**	14.82**
Age of flowering	36.00	38.36**	43.63**
Age of harvest	80.33	85.08**	90.08**
Seed weight	8.26	8.07tn	5.31**
Number of seeds	61.99	48.13**	39.09**

Figure.1 Percentage of germination —, The number of normal seedling —, The number of abnormal sprouts — at the treatment of sensitivity gamma irradiation

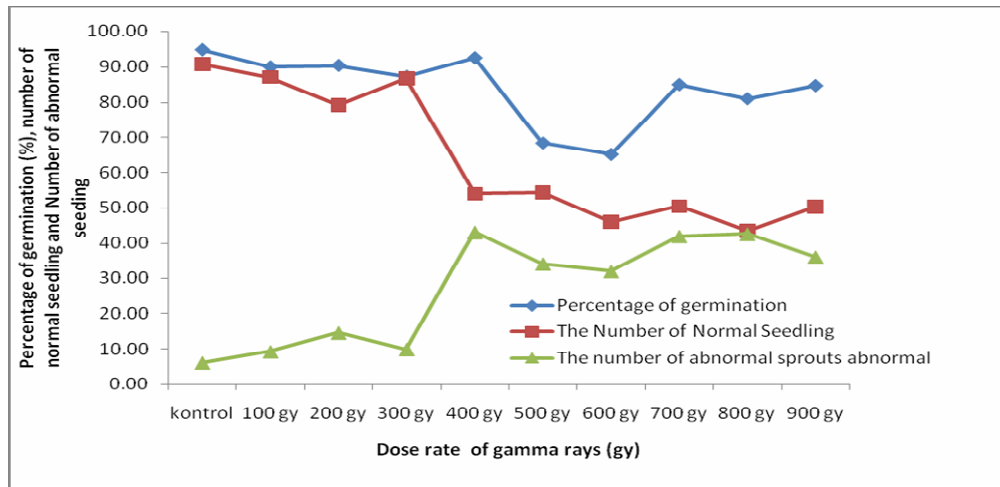


Figure.2 Plant height —, number of leaves — and level of greenness(chlorofil) — at the treatment of sensitivity gamma irradiation

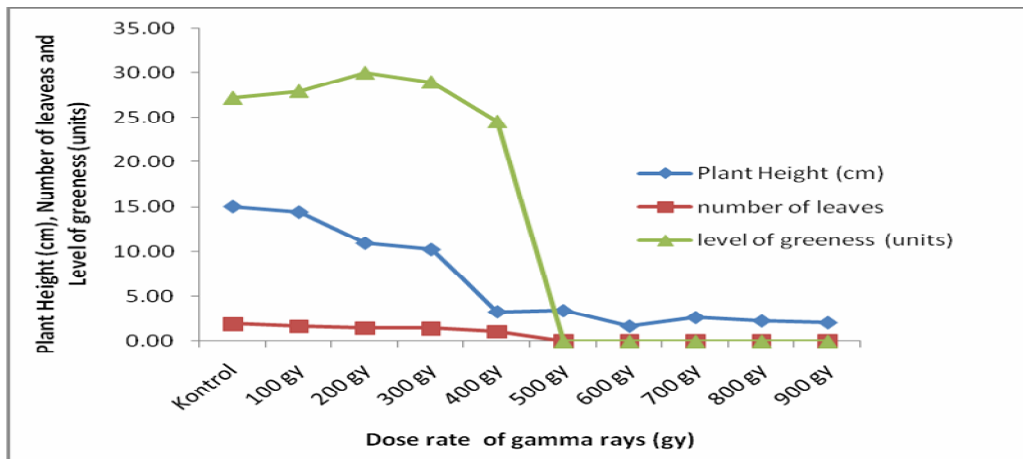


Figure.3 Appearance of leaves shape and level of greenness between parental of the plant. with the level of irradiation 200 gy and 300 gy



Control Home Anjosmoro

Irradiated Gamma 200Gy

Irradiated Gamma 300 Gy

Figure.4 Appearance of generative phase between parental of plants with the level of irradiation 200 gy and 300 gy



Control Home Anjosmoro Irradiated Gamma 200Gy Irradiated Gamma 300 Gy

Figure.5 Appearance of soybean between parental plant with the level of irradiation 200 gy and 300 gy

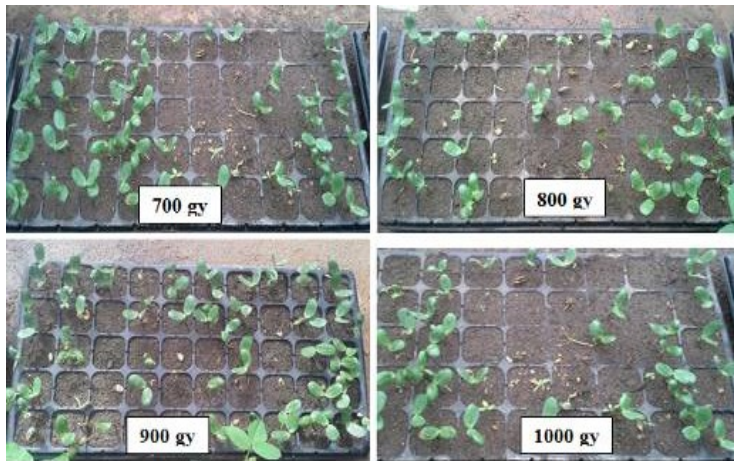


Control home Anjosmoro Irradiated Gamma 200Gy Irradiated Gamma 300 Gy

Appendix.1 Appearance germination of soybean varieties with Anjasmoro Gamma ray treatment radiosensitivitas At Age 10 Days After Planting



Kontrol (Induk Anjasmoro)



Differences that occur between the control and all the characters irradiation dose decreased caused by chromosome damage in plants. At this early generation (M1) is more characteristic changes caused by factors irradiation has not been caused by genetic factors. As a result of DNA damage induced by mutagens caused directly or indirectly by the service change passage that occurs in the process of transcription of DNA into chromosomes. So as to identify the specific damage and begin repair on DNA damage by maintaining the stability of DNA damage remains low by strengthening repair proteins to the damaged site (Van Harten, 1998).

Conclusion

The optimal dose gamma irradiation in the range of 200 and 300 gy. Appearance soybean plants were irradiated at doses of 200 and 300 gy different from the control (parent Anjasmoro), both in the number of pods plant height, days to flowering, harvesting, seed weight and seed number. Differences that occur between the control and all the characters irradiation dose decreased caused by chromosome damage in plants. At this early generation (M1) is more characteristic changes caused by factors

irradiation has not been caused by genetic factors.

Suggestions

It is advisable to conduct further research to continue penanaman M2, M3 and so on in the field either under optimum conditions (selection under optimum conditions) and in drought conditions (selection in drought conditions), because to look at the stability of the mutations and changes the character that occurred significantly can be traced to the generation of M2, M3, and so on until get the mutated character and in accordance with the expected character.

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