Mutagenic effects of gamma rays on soybean (Glycine max L.) germination and seedlings

by F Kusmiyati

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Abstract. Narrow genetic diversity is a main problem restricting the progress of soybean breeding. One way to improve genetic diversity of plant is through mutation. The purpose of this study was to investigate effect of different dose of gamma rays as induced mutagen on physiological, morphological, and anatomical markers during seed germination and seedling growth of soybean. Seeds of soybean cultivars Dering-1 were irradiated with 11 doses of gamma rays (0, 5, 10, 20, 40, 80, 160, 320, 640, 1280, and 2560 Gy [Gray]. The research design was arranged in a completely randomized block design in three replicates. Results showed that soybean seed exposed at high doses (640, 1280, and 2560 Gy) did not survive more than 20 days, the doses were then removed from anatomical evaluation. Higher doses of gamma rays siginificantly reduced germination percentage at the first count and final count, coefficient of germination velocity, germination rate index, germination index, seedling height and seedling root length, and significantly increased mean germination time, first day of germination, last day of germination, and time spread of germination. However, the effects of gamma rays were varies for density, width, and length of stomata. The LD_{50} obtained based on survival percentage was 314.78 Gy. It can be concluded that very low and low doses of gamma rays (5-320 Gy) might be used to study the improvement of soybean diversity.
Key words : seedling, stomata, morphological diversity

1. Introduction

Soybean is a major crop in many countries. Soybean can be used as food, feed, functional food and industrial products. Increasing interest in functional food and various soybean by- product is the reason for high demand of soybean [1]. However, soybean yield is decreased because of many stresses such as salinity stress. Salinity stress inhibited seed germination and seedling growth. The precise mechanisms of salt stress on seed germination and seedling growth of soybean are only partially understood [2]. Ionic and osmotic stresses are the effects of salinity stress. Ionic stress occurs when salt enters the plant reaching toxic levels, and may lead to a reduced ability to maintain water uptake, which is reffered to as the osmotic or water-deficit effect of salinity [3]. Water deficit or drought stress is one of the limiting factors which affect the numerous metabolic procces of crop [4]. Increasing salinity stress from 4 to 7 dS m^{-1} (1 dS m^{-1} = 700 mg L^{-1}) reduced soybean production by 20 – 60% [5]. Application of manure as organic fertilizer at saline soil increased growth and production of forage crops [6 – 7].

An effective way of maintaining sustainable production in salt-affected soil is through breeding of high salt tolerance soybean [8]. Narrow genetic diversity is a main problem restricting the progress of soybean breeding. One of the strategies perform crosses of parents differing in chloride accumulation tolerance, in which F_2 population segregated in a 3:1 ratio of non-necrotic to necrotic plant [9]. However, genetic improvement depends on the amount of genetic diversity present in the population, though the natural diversity can be improved through induced mutation [10]. There are various techniques to induce mutations and artificially increase variation viz. chemical mutagens such as sodium azide, methylnitrosourea, ethyl methanesulfonate and physical mutagens such as x-ray, neutron, gamma-ray etc.)[11]. Induced mutation combined with selective breeding, is highly efficient in order to screen for new traits [12].

Gamma-ray as mutagen has been used to induce changes in growth habits. Several research on the stimulative effects when using gamma rays as induced mutagens were reported. Recently, low doses of gamma-rays of seed treatment resulted significant effects on germination, shoot, and root systems of wheat [13], physic nut [14], rice[15], maize [16], pigeon pea [17], chick pea [18], lathyrus[19], castor bean[20].However, the impact of gamma rays on soybean cv. Dering 1 is still unknown. The major objective was to investigate effect of different dose of gamma rays as induced mutagen on physiological, morphological, and anatomical markers during seed germination and seedling growth of soybean. The result of such experiments could be used to study the improvement of plant diversity in soybean program for salinity tolerance.

2. Materials and Methods

2.1. Plant material

The soybean seed (*Glycine max* cv. Dering 1) were obtained from Seed Resources Management Unit, Indonesian Legumes and Tuber Crops Research Institute, Malang, Indonesia. The seeds were irradiated by gamma-rays.

2.2. Methods

2.2.1. Mutagenesis. Seeds mutagenesis were performed at the Center for Application of Isotope and Rays, National Nuclear Energy Agency of Indonesia, South Jakarta, Indonesia. The healthy seeds were irradiated by gamma-rays with doses of 0, 5, 10, 20, 40, 80, 160, 320, 640, 1280, and 2560 Gy, and arranged in a completely randomized block design in three replicates.After mutagenesis, the seeds were soaked for 24 hours in aquadest at a temperature of around 25 ^{0}C.

*2.2.2. Germination.*Three replicates of 50 seeds of each mutagen dose were germinated in plastic box containing sand, and kept under laboratory condition [21].It was supplied with water every day in order to maintain sand moisture.

2.2.3. *Physiological attributes of soybean as affected by mutagenesis*. The seeds from mutagenesis were considered germinating when the protrusion of radicle by≥ 2 mm [22].

2.2.3.1. Median lethal dose (LD_{50}) was calculated at 5th day after germination, based on the number of surviving seedlings in different mutagen doses [23].

2.2.3.2. Germination percentage at the 5th day (first count) and the 8th day the final count) were calculated following the formula of Marcu *et al.*[16], in which NT = proportion of the germinating seeds of each treatment; N = number of seeds used in bioassay.

$$GP\ (\%) = \frac{NT \times 100}{N}$$

2.2.3.3. Mean germination time (MGT) was determined using the formula of Σ f.x / Σ f; f = seeds germinated on the day "x". The lower MGT values indicate the faster a population of seeds has germinated [24].

2.2.3.4. First day of germination (FDG) was the day in which the first germination occurred. The faster initiation of germination has the lower value of FDG [25].

2.2.3.5. Last day of germination (LDG) was the day in which the last germination occurred. The lower the LDG values indicate a faster ending of germination [25].

2.2.3.6. Coefficient of velocity of germination (CVG) was determined using the formula of $N_1 + N_2 + \ldots + N_x/100 \times N_1T_1 + \ldots + N_xT_x$; N = number of seeds germinated each day, T = number of days from seeding corresponding to N. The CVG gives an indication of germination rapidity. The value of CVG increases when the number of germinated seeds increases and the time required for germination decreases. The highest CVG possible is 100, if all seeds germinated on the first day [26].

2.2.3.7. Germination rate index (GRI) was determined using the formula of G1/1 + G2/2 + ... +Gx/x; G1 = germination percentage x 100 at the first day after sowing, G2 = germination percentage x 100 at the second day after sowing. Germination rate index (GRI) calculates germination percentage on each day of germination period. Higher and faster germination has higher GRI value [27].

2.2.3.8. Germination index (GI) was determined using the formula of (10 x n1) + (9 x n2) + ... + (1 x n10); n1, n2, ... n10 = number of germinated seeds on the first, second and subsequent days until the 10^{th} day; 10, 9, ... 1 are weights given to the number of germinated seeds on the first, second and subsequent days until the 10^{th} day, respectively. In calculation of GI, value of ten as maximum weight is given to the seeds germinated on the first day and less to those germinated later on. The value of one as lowest weight will be for seeds germinated on the 10^{th} day. The GI emphasizes on both the germination percentage and its speed. Higher percentage and rate of germination has higher GI values [28].

2.2.3.9. Time spread of germination (TSG) was the time in days between the first and last germination occurred. Greater difference of germination speed between the fast and slow germinating sees has higher TSG values [25].

*2.2.4. Morphological attributes of soybean as affected by mutagenesis.*Seedling performance was evaluated through determination of seedling height and root length [29]. Seedling height was measured point of connection of the hypocotyl with the root, up to its point of connection of the apical bud to the stem. Seedling root length was measured from the tip of the root up to its point of connection with the hypocotyl.

*2.2.5. Anatomical attributes of soybean as affected by mutagenesis.*Stomatalsize (width and length) and density were determined using the rapid imprinting technique[30]. The abaxial leaf surface was taken firstly and washed using the tap water. Then it was dried, the transparent nail polish was applied uniformly with a brush on the surface. It was then dried at the room temperature for approximately 20 minutes. The nail polish imprints were placed on glass cover slips, and photographed under a model CX31 trinocularmicroscope (Olympus, Japan) with a mounted E330-ADU1.2X camera (Olympus, Japan).

*2.2.6. Statistical analysis.*Stomatal images were analysed to determine size using the Optilab Image Raster software. Data were analysed using CurveExpert 1.4 software for LD_{50}, and generalized linear model in the PROC-GLM procedure of SAS University Edition software for other parameters. The

means were compared through Dunnett option, at probability level of 5%, in order to determine the difference in means between non-mutated and mutated seeds.

3. Results and Discusions

Soybean seed exposure to gamma rays caused serious effects on seed germination. The survival percentage of irradiated soybean cv. Dering 1 decreased as the gamma dose increased (Figure 1). At this experiment, the LD_{50} obtained based on survival percentage was 314.78 Gy. The LD_{50} is the dose corresponding to 50% decrease of the survival percentage. The desired mutation can be found arround LD_{50} [31]. Similar experiments have been carried out in soybean [32] [33],The survival percentages as LD_{50} were 457.17 Gy for cv. Argomulyo, 583 Gy for cv. CO2, and 620 Gy for cv. CO1. It is explained the radiosensitivity to gamma rays were genotypes dependent. The irrays effect was also noticeable in the delay of seed germination according to the doses, and showed growth inhibition as a very common effect.

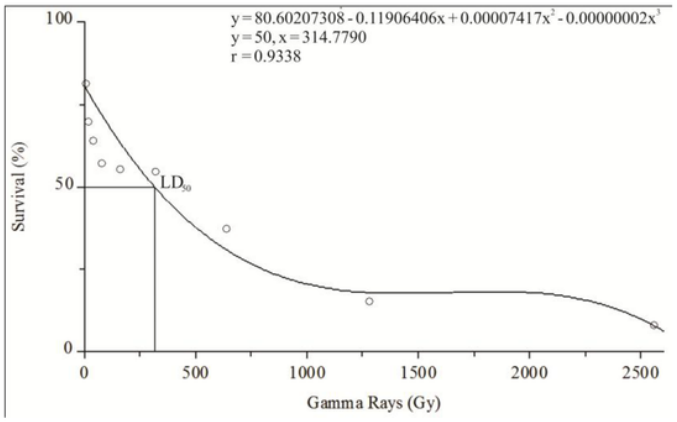

Figure 1. Dose – response curve with polynomial fit

Table 1. Effects of gamma rays on Soybean (*Glycine max* L. cv. Dering 1) germination

GR (Gy)	GP 1[1] (%)	GP 2[1] (%)	MGT[2] (day)	FDG[2] (day)	LDG[2] (day)	CVG	GRI[1] (%/day)	GI	TSG[2] (day)
0	100.00	100.00	2.08	2.00	3.00	48.08	48.67	346.00	1.00
5	92.00 **	100.00	2.47 **	2.00	6.00 **	40.47 **	45.25 *	326.33	4.00 **
10	81.33 **	91.33 **	3.36 **	2.00	6.00 **	29.73 **	29.01 **	257.33 **	4.00 **
20	70.00 **	90.67 **	3.90 **	3.00 **	6.00 **	25.72 **	25.28 **	231.33 **	3.00 **
40	64.00 **	86.67 **	4.24 **	3.00 **	6.00 **	23.61 **	22.30 **	206.33 **	3.00 **
80	57.33 **	86.00 **	4.55 **	3.00 **	6.00 **	21.99 **	20.37 **	191.33 **	3.00 **
160	55.33 **	86.00 **	4.85 **	3.00 **	6.33 **	20.64 **	18.80 **	178.67 **	3.33 **
320	54.67 **	82.67 **	5.09 **	4.00 **	6.33 **	19.64 **	16.65 **	161.67 **	2.33 **
640	37.33 **	78.67 **	5.37 **	4.00 **	6.67 **	18.64 **	14.99 **	142.67 **	2.67 **
1280	15.33 **	74.67 **	5.91 **	4.00 **	8.00 **	16.95 **	12.97 **	115.33 **	4.00 **
2560	8.00 **	62.00 **	6.01 **	4.67 **	7.67 **	16.64 **	10.42 **	92.33 **	3.00 **

GR = gamma rays; GP 1 = germination percentage at the 5th day (first count); GP 2 = germination percentage at the 8th day (final count); MGT = mean germination time; FDG = the first day of germination; LDG = last day of germination; CVG = coefficient of velocity of germination; GRI = germination rate index; GI = germination index; TSG = time spread of germination. Data were transformed by arcsine(1) and Log (X+1) (2)prior to analysis; nontransformed data are presented; * significant difference at $P \leq 0.05$; ** significant at $P \leq 0.01$

The result showed that the maximum germination percentage was observed at the control treatments (0 Gy) (Table 1). The germination percentage at the first count was significantly decreased with increasing gamma ray doses. The minimum and maximum decreased were reached at 8% and 92% for 5 and 2560 Gy, respectively. Furthermore, germination percentage at the final count was also significantly decreased with as gamma ray doses increased start from 10 Gy. The coefficient of germination velocity, germination rate index, germination index as seed germination potential showed the same decreasing pattern by increasing the rays doses. Besides, mean germination time, at the first day and the last day of germination, increased as gamma rays doses increased. However, time spread of germination increase not consistent with increasing gamma rays doses, it depends on the difference in germination speed between the fast and slow germinating . The higher gamma ray doses determined higher inhibiton of the germination process.

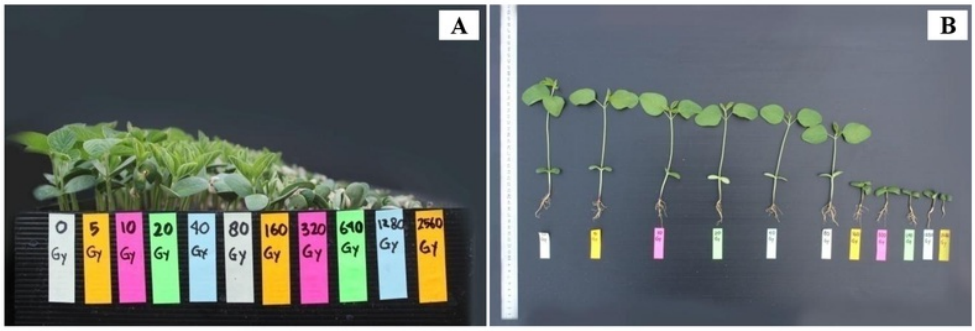

Figure 2. Effects of gamma rays on the seedling growth of Soybean (*Glycine max* L. cv. Dering 1).A = at 5th day after germination;B =at the 14th day after germination

Table 2. Effects of gamma rays on the seedling height and root length were taken at the 14th day of Soybean (*Glycine max* L. cv. Dering 1)

Gamma Rays (Gy)	Seedling Height (cm)	Seedling Root Length (cm)
0	15.07	6.97
5	15.70	5.67
10	14.20	7.57
20	14.40	5.87
40	13.47	4.30 *
80	13.20	5.77
160	8.53 **	3.20 **
320	3.77 **	3.93 *
640	2.67 **	4.20 *
1280	2.27 **	3.23 **
2560	1.33 **	2.50 **

* significant difference at $P \leq 0.05$; ** significant at $P \leq 0.01$

Gamma ray not only impact the germination potential,but also actual qualities of the germinated seedlings, such as seedling height and root length (Table 2). The biometric measurements show a significant decrease, the maximum seedling height and root length were recorded at the control (0 Gy), while the seedling height exposed to 160 – 2560 Gy decrease by 43.40 – 91.17 %, and the seedling root length exposed to 40 – 2560 Gy decreased by 38.31 – 64.13 %. Result showed that gamma ray with doses higher than 160 Gy significantly inhibited the seedling height and root length derived from irradiated seeds. These results were in compliance with the others researchers who reported that

increasing the gamma ray doses were decreased the seed germination and seedling growth. Very low and low doses of gamma rays were reported cause a significant delay of germination process, decrease of survival precentage, and seedling height and root length in wheat [13], maize [16], tumeric [34], and soybean [35]. Gamma ray induced inhibition of growth through restraining cell cycle during somatic cell and damaging entire genome [36].

Table 3. Effects of gamma rays on the density, width and length of stomata were taken at 21th day of Soybean (*Glycine max* L. cv. Dering 1)

Gamma Rays(Gy)	Stomata Width(µm)	Stomata Length(µm)	Stomata Density(mm^{-1})
0	1.43	2.42	187.84
5	1.30 *	2.40	217.19
10	1.26 **	2.29	158.49
20	1.21 **	2.08 **	300.84 **
40	1.29 *	2.58	149.69 *
80	1.06 **	2.03 **	218.66
160	1.06 **	2.31	158.49
320	1.00 **	2.21 *	227.46 *

* significant difference at $P \leq 0.05$; ** significant at $P \leq 0.01$

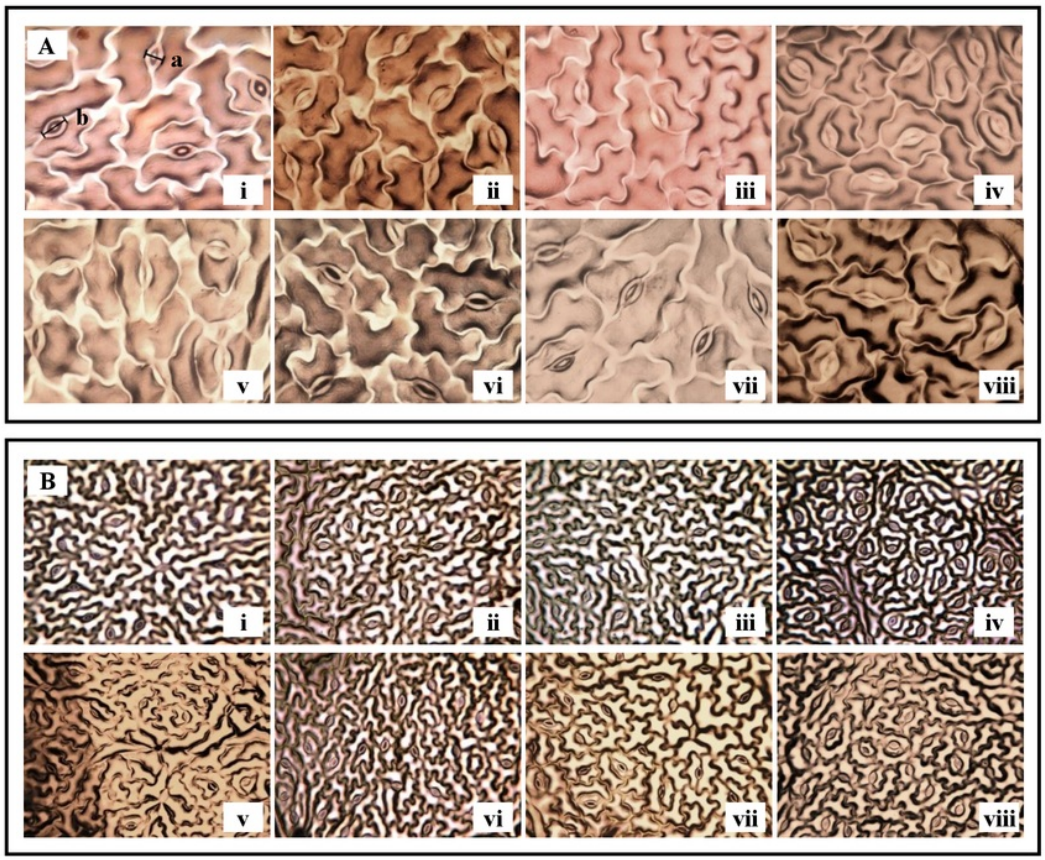

Figure 3. Panels show soybean stomatal measurements: (A) Stomata width (a) and length (b) were measured under 1.000x magnification; (B) Soybean stomata shown under 400x magnification; i = 0 Gy; ii = 5 Gy; iii = 10 Gy; iv = 20 Gy; v = 40 Gy; vi = 80 Gy; vii = 160 Gy; viii = 320 Gy

Auxin synthesis was inhibited by low doses of gamma rays, while auxin destruction was caused by higher doses of gamma rays. There were three ways of that reasons : 1) DNA is required for and is previously synthesized sequently to auxin formation, and gamma rays block occuring nucleic acid formation; 2) primary gamma rays block is in auxin synthesis, the auxin required for DNA formation; and 3) effect of gamma rays is on an undefined entity in reaction previous to and essential for both DNA and auxin synthesis [37]. The biological effect of gamma rays is also mainly due to the formation of free radicals by water hydrolisis, and results in modulation of antioxidative system, and accumulation of phenolic compounds and chlorophyll pigments [38]. However, these radicals can damage or modify important components of plant cell and affect differentially the biochemistry, physiology, anatomy, and morphology of plants [39]. The frequency of chromosomal damage cause the metabolic disorders and may be responsible for less germinability or to survive more than few days. In this study, seedling derived from seeds exposed to higher doses (640, 1280, and 2560 Gy) did not survive for more than 20 days, so it was not possible for stomata evaluations.

In figure 3, the stomatal apparatus of soybean irradiated by gamma rays were shown using nail polish imprint method. The stomatal size (width and length) and density were clearly inconsistent (decrease or increase) with increased gamma ray doses (Table 3). In this study, it was found that the stomatal width decreased 1.10 to 1.43 times, and the stomatal length decreased from 1.10 to 1.16 times. Stomatal density ranged from 149.69 to 300.84 mm^{-2} depends on gamma ray doses. It have significantly increased by 21.09 % and 60.16 % at 320 Gy and 20 Gy, respectively, and decreased 20.31 % at 40 Gy. However, Celik *et al.* [40] observed different results that soybean plant leaves developed from gamma irradiated seeds showed reductions in stomatal density compared to the control plant. The function of stomatal is correlated with process of transpiration and photosynthesis that occured in the leaves. Stomata biometric measurements have an important role in transpiration and photosynthesis process by adjusting gas exchange between the leaves and the atmosphere [41]. Stomatal size influences exchange of CO_2, hence the bigger stomatal size will increase the exchange of CO_2 [42]. It is explain the rate of photosynthesis is more efficient. Besides, the higher density of stomatal also allows the higher gas exchange, consequently, the rate of photosynthesis is higher than control [43]. The higher photosynthesis supports the plant growth. So, different stomatal size and density of each genotypes can be used as indirect selection criteria.

4. Conclusions

It was concluded that different doses of gamma rays influenced the germination and seedling growth of Soybean (*Glycine max* L.cv. Dering 1). Very low to low doses of gamma rays (5-320 Gy) might be used to study the improvement of soybean diversity.

5. Acknowledgements

Authors wish to convey gratitude to the Directorate General of Research and Community Service, the Ministry of Research, Technology and Higher Education the Republic of Indonesia for the research grant through scheme of "Superior Research of University", and Diponegoro University for the continuous support, particularly to the Institute of Research and Community Services.

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