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Induced mutations by gamma ray irradiation to Argomulyo soybean (*Glycine max*) variety

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Abstract. *Hanafiah DS, Trikoesoemaningtyas, Yahya S, Wirnas D. 2010. Induced mutations by gamma ray irradiation to Argomulyo soybean (*Glycine max*) variety. Nusantara Bioscience 2: 121-125.* Induced mutation by gamma ray irradiation is one way to increase genetic variability of plants. This research used gamma ray irradiation on low doses (micro mutation). The aim of this research was to know the respons of doses level by micro mutation on gamma ray irridation to the growing and development of Argomulyo variety of soybean [*Glycine max* (L) Merr]. The seeds were irradiated by gamma ray micro mutation doses, namely 0 gray, 50 gray, 100 gray, 150 gray, and 200 gray. Variations that were obtained of each characters at generation M_1 and M_2 influences plants growth and development either through qualitative and quantitative that finally will influence plant's production. The average highest genetic variation at M_2 generation of soybean was on 200 Gray doses. Results of the research indicated that gamma ray irradiation on 200 Gray doses effectively caused of plant variation genetic.

Key word: induced mutation, micro mutation, gamma ray irradiation, Argomulyo soybean variety.

Abstrak. *Hanafiah DS, Trikoesoemaningtyas, Yahya S, Wirnas D. 2010. Mutasi induksi irradiasi sinar gamma pada varietas kedelai Argomulyo (*Glycine max*). Nusantara Bioscience 2: 121-125.* Induksi tanaman dengan irradiasi sinar gamma merupakan salah satu cara untuk meningkatkan keragaman genetik tanaman. Penelitian ini menggunakan irradiasi sinar gamma pada tingkat atau dosis rendah (mutasi mikro). Tujuan penelitian ini untuk mengetahui respon pemberian tingkat irradiasi mikro sinar gamma pada benih kedelai. Benih kedelai [*Glycine max* (L) Merr] yang diuji adalah kedelai varietas Argomulyo yang diirradiasi sinar gamma dengan dosis 0 Gray, 50 Gray, 100 Gray, 150 Gray dan 200 Gray. Keragaman yang diperoleh dari setiap peubah amatan yang diperoleh pada generasi M_1 dan M_2 menunjukkan bahwa perlakuan irradiasi dapat mempengaruhi pertumbuhan dan perkembangan tanaman secara kualitatif dan kuantitatif, yang akhirnya akan mempengaruhi produksi tanaman. Variasi fenotipe pada tanaman kedelai generasi M_2 tertinggi rata-rata terjadi pada perlakuan 200 Gray. Hasil penelitian menunjukkan bahwa irradiasi sinar gamma pada dosis 200 Gray efektif menyebabkan terjadinya keragaman genetik tanaman.

Kata kunci : mutasi induksi, mutasi mikro, irradiasi sinar gamma, kedelai varietas Argomulyo.

INTRODUCTION

Indonesia is one of soybean importers [*Glycine max* (L.) Merr] in the world, especially from the United States. This need has not been adequated by domestic production; moreover there is a tendency of decrease of the production of local farmers recently. The Temporary Figures (ASEM/TF) of soybean production in 2009 is 972.95 thousand tons of dry beans. The Forecast Figures I (ARAM I/FF) of soybean production in 2010 is estimated at 962.54 thousand tons of dry beans. Compared to production in 2009 (ASEM), there is a decrease of 10.41 thousand tons (1.07%) (CSB/BPS 2010). Domestic soybean production can only meet 20-30% of the national soybean demand. The rest of 70-80% is filled by importing soybean (Sudaryanto and Swastika 2007; Purna et al. 2009). This is an opportunity as well as a challenge for Indonesian farmers to increase soybean production in the country. One of the attempts to increase national soybean production is done through improving desired plant traits, including productivity.

Soybean is a self-pollinated plant, which will form solid lines, or no segregation. The population is composed by lines, with a genetic diversity of a very thin intra lines or almost zero, and the diversity of a visible inter lines. New genetic diversity would appear in nature as a result of mutation or the occurrence of cross inter strains, even with a small degree, therefore the soybean genetic diversity is low (Jusuf 2004).

Soybean is not a local native plant of Indonesia; therefore, it has a poor genetic diversity. It is estimated that the central area of distribution of the *Glycine* genus is in Asia, where most endemic species are still alive. In China, several species of wild soybean can be found now, and become the source of genes from the cultivated soybean, where there are wild relatives of soybean species called *G. ussuriensis*. Exploration of the source regions of genes related to the provision of data and germ plasma resources

is needed for improving the varieties and breeding as well as adaptation to the cultivated soybean (Leppik 1971).

An assembling of the new varieties requires basic population that has a high genetic diversity which can be obtained through the introduction, cross over, mutation, and genetic transformation. Argomulyo soybean is one of the variety development introduced from Thailand, in addition to other introduced varieties such as Bromo, Krakatau and Tambora (Hidajat et al. 2000; Arsyad et al. 2007). Increasing genetic diversity of soybean crop will facilitate the selection efforts to obtain plants with desirable traits, such as plant characters for resistance to drought stress.

Mutation induction with radiation is the most frequently used method to develop direct mutan varieties, where improvement by acclimatization, selection, hybridization and also laborious have proven to be time consuming and also with limited genetic variation. Mutation breeding of plants is useful to improve the character if the character you want is not located in a plant germplasm of a species, and also for generating variability in the existing varieties (Van Harten 1998; Yaqoob and Rashid 2001; Khan and Goyal 2009).

Induction of plants with gamma ray irradiation is one way in improving plant genetic diversity. Gamma ray irradiation at low dose levels or (micro mutation) is less influencing changes in quantitative characters of plants and chromosomes compared with the macro mutation using gamma ray irradiation at high doses. Mutation induction can be done on the plants by mutagenic treatment of certain materials of plant reproductive organs such as seeds, stem cuttings, pollen, root rhizome, tissue culture and others. If a natural mutation process is very slow, the acceleration, frequency and spectrum of mutations can be induced by mutagen treatment of certain materials (BATAN 2006).

Micro mutation performed on Argomulyo soybean varieties aims at improving the quantitative plant characters that ultimately aims at increasing production and development of plant adaptation to marginal lands. The purpose of this research is to study the response of micro-level gamma irradiation on the growth and development of soybean plants first generation (M_1) and knowing the genetic diversity in the second generation (M_2).

MATERIALS AND METHODS

This research was conducted from February to June 2009. Irradiation treatment was performed at the Center for Biological Resources and Biotechnology, Bogor Agricultural University (IPB), Bogor and field research was conducted at the University Farm of Bogor Agricultural University, at Darmaga, Bogor, West Java.

Soybean seed varieties of Argomulyo were radiated with the gamma ray dose of 50 Gray, 100 Gray, 150 Gray and 200 Gray (micro dose) derived from ^{137}Cs using IBL 437C Irradiator type H (CIS Bio International, France) with a dose rate of 2.23 Gray/min . A total of 200 seeds (M_1) in each treatment dose planted with a spacing 40 x 20 cm^2 and the influence of gamma irradiation on morphological characters of plants including flowers, leaves, plant height, number of branches, number of productive pods, the number of empty pods and number of seeds per plant were then evaluated. For the M_1 generation, in each plant at each treatment dose, 10 pods per plant then were harvested (restricted bulk) and grown as an M_2 generation. Then 2000 seedlings of M_2 seeds were planted for each dose treatment with spacing of 40 x 20 cm^2, and the diversity in agronomic characters including plant height, number of branches, number of pods, number of empty pods and seed weight per plant were evaluated. Genetic variation in M_2 generation was calculated with the formula:

$$\sigma^2 = \frac{(\sum x^2) - [(\sum x)^2 / n]}{n-1}$$

$\sigma^2 M_2 = \sigma^2 p$;
$\sigma^2 p = \sigma^2 g + \sigma^2 e$;
$\sigma^2 g = \sigma^2 p - \sigma^2 e = \sigma^2 M_2 - \sigma^2 M_0$,

σ^2 = variance
n = number of members of the population
$\sigma^2 p$ = phenotypic variance
$\sigma^2 g$ = genotypic variance
$\sigma^2 e$ = environment variance
$\sigma^2 M_2$ = M_2 population variance
$\sigma^2 M_0$ = M_0 population variance
(Argomulyo population as a control)

Heritability values calculated using the following formula:

$h^2 = \sigma^2 g / \sigma^2 p$ (Singh and Chaudhari 1977)

Criterion of heritability:
$h^2 > 0.5$ = high heritability values
$h^2 = 0.2$ to 0.5 = value of heritability is
$h^2 < 0.2$ = low heritability values

Genetic variation is determined based on the coefficient of genetic variation (CGV) by using the method proposed by Singh and Chaudhari (1977) as follows:

$$CGV = (\frac{\sigma_g}{\bar{x}}) x 100\%$$

σ_g = genotypic standard deviation
$\bar{x}$ = character means

CGV the highest absolute value determined from the relative value of 100% CGV.

RESULTS AND DISCUSSION

Performance of M_1 plants micro-mutations result of gamma ray irradiation.

The results showed that the M_1 derivative of soybean varieties with micro mutation Argomulyo gamma ray irradiation of each variable observation showed the higher the dose of irradiation, the lower the parameters that were observed became (Table 1). Irradiation dose significantly

affected the plant height (Table 1). At a dose of 200 gray, the plant heights were shorter compared with other irradiation doses. The height of Argomulyo varieties that were irradiated with gamma ray at doses of 50 gray, 100 gray and 150 gray was higher than the control, but when the dose of the irradiation was raised the average height of the plant tended to decrease. Sakin (2002) stated that gamma ray irradiation treatment increased the average plant height compared with controls. This study is similar to the research conducted by Tah (2006) and Sing et al. (2001) who observed the effect of high dose treatment on plant derivatives M_1 mungbean [*Vigna radiata* (L.) Wilczek], where plant height decreased due to the treatment of gamma ray irradiation dose at 10 kR, 20 kR, 30 kR and 40 kR, with the highest decline occurred at a dose of 40 kR (1Gray=10 kR).

Research conducted by Shakoor et al. (1978) suggested that the treatment in the range of 10-30 kR doses was not significantly different but the dose of 40 kR caused plants to become stunted. Similar results were found in rice seed (Cheema and Atta 2007; Shah et al. 2008), where germination percentage decreased when irradiated with gamma rays, but its reduction is not proportional with the increase of the dose.

The effect of irradiation dose on the number of branches of M_1 plants Argomulyo soybean varieties was not significantly different (Table 1). The numbers of branches generally formed were two, which was not much different between the branches of control plants and that of irradiation produced ones. The maximum effect of dose occurred at 50 Gray with increase as much as 25.5% compared with the controls plants.

The number of branches at 150 Gray dose of irradiation is less than 200 Gray, where there were unproductive branches, no pods formed and flowers did not develop. This is consistent with the research conducted by Ganguli and Bhaduri (1980) who claimed there was a reduction in productive branches due to gamma ray irradiation and the numbers of main branches were more than the control plants at each treatment dose. Research conducted by Tah (2006) states that the influence of gamma ray irradiation dose on the number of branches at a dose of 30 kR increased by 30.55% compared with controls. With the increasing number of productive branches, number of pods per plant will increase.

Effect of gamma-ray irradiation makes the pod number greater than the control, with the varying increase (Table 1). The highest number of pods obtained at 150 Gray dose of irradiation, the increase is as much as 27.33% compared with controls. Number of pods showed an increase in M_1 plants, especially at 150 Gray dose of irradiation, which formed more pods on main stem, while the number of branches is only a few. Many pods formed on the main stem. In plants with radiation dose of 200 Gray irradiation, the number of pods formed is a few, many flowers did not grow and the flowers did not grow to form pods (experienced sterility and abnormality development). Increase in the number of pods at M_1 also occurred in the study conducted by Tah (2006), where an increasing number of pods as a result of gamma ray irradiation reached 15-23% and reached its maximum at the dose of radiation 30 kR. Number of empty pods were not significantly different at each dose of irradiation (Table 1). The highest numbers of empty pods of M_1 plants occurred at 200 Gray dose of irradiation, where the pods were empty, because the seeds failed to form due to the disruption on plant growth.

Irradiation dose significantly affected the number of seeds per plant (Table 1). At a dose of 200 Gray the number of seeds per plant was smaller compared with other irradiation doses. The number of seeds per plant of Argomulyo soybean varieties that were irradiated with gamma rays at doses of 50 gray, 100 gray, gray and 150 were higher than in control plants, but when the average dose of irradiation was increased the average plant height then decreased.

Figure 1 shows that the gamma-ray irradiation affects the diversity of phenotypes on the M_1 derivative by its characteristic morphology. This is indicated by qualitative changes, such as leaf shape changes from oval (normal) to elongate, there are unifoliat and bifoliat leaves on the first stem together with trifoliate leaf on a single plant, flowers color changes from purple to white, flower Rasim does not develop into pods, leaves still green even though the pods were ripe harvest.

These qualitative changes occured in some plants from dose irradiation treatment 150 and 200 Gray. The same things happened on mutation induction with gamma ray irradiation in soybean (Manjaya and Nandawar 2007) and green beans (Sangsiri et al. 2005) that indicated a change in shape and color of leaf and flower shapes and colors and also cause sterility in plants.

Table 1. The mean observation variables at different irradiation doses

Variables observations	Irradiation dose (Gray)				
	0	50	100	150	200
Plant height (cm)	34.20BC	38.00A	37.88A	36.42AB	30.47C
Number of branches (fruit)	1.75A	2.35A	2.30A	1.90A	2.20A
Number of pods (fruits)	27.65B	36.25A	35.75A	38.05A	29.20B
Number of empty pods (fruit)	0.35A	0.35A	0.35A	0.35A	0.45A
Number of seeds	70.60BC	88.35A	78.05AB	83.4AB	56.35C

Note: statistics analysis performed by t test

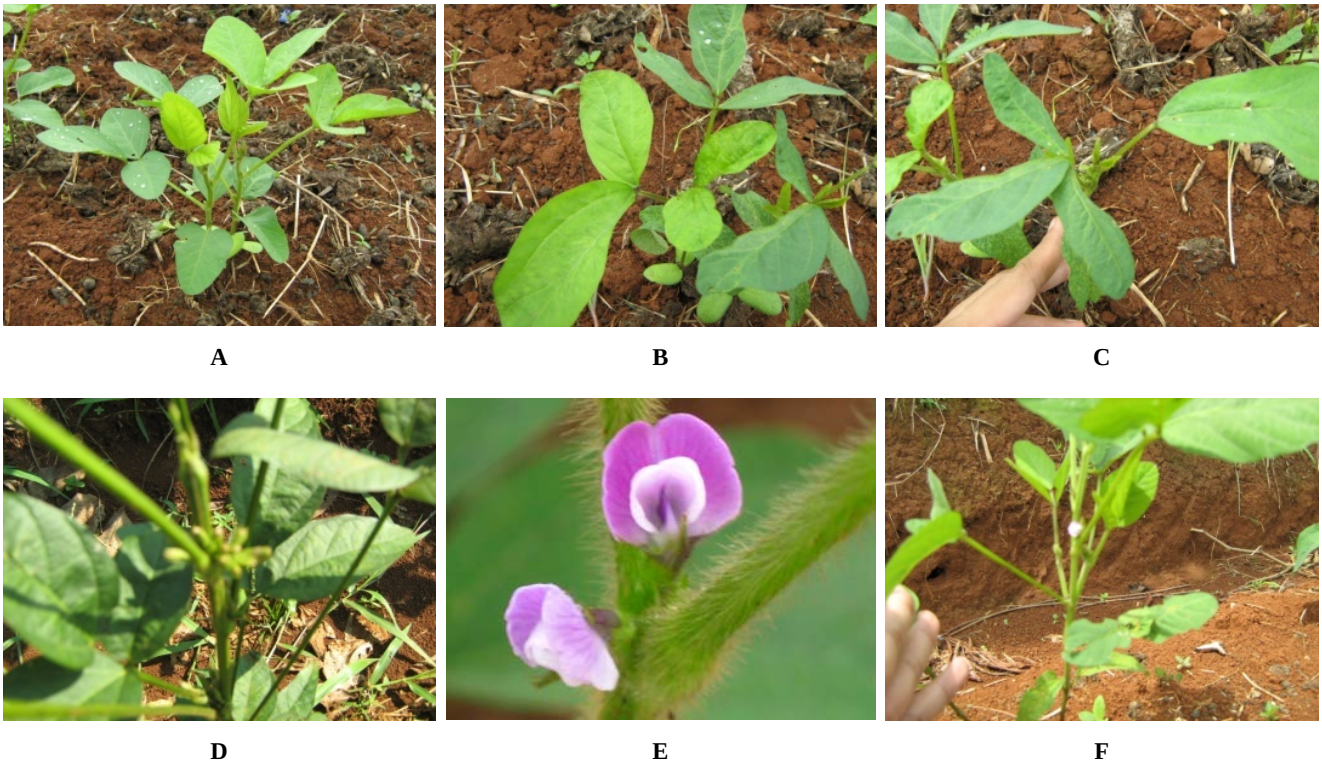

Figure 1. Growth abnormality of Argomulyo variety which is caused by the gamma ray irradiation: A. Trifoliate and oval leaves (normal), B. Bifoliat leaves and elongated, C. Unifoliat leaves and elongated, D. Rasim flowers and buds (not develop), E. Purple flowers (normal), F. White flowers.

Genetic variation and heritability of M_2 generation resulted from gamma-ray irradiation

Genetic variation and heritability of Argomulyo M_2 soybean variety plants at different irradiation doses can be seen in Table 2. Seeds which are harvested from M_1 plants are planted as seed M_2, and M_2 plants are expected to show segregation at the genetic locus that has mutation. Genetic variety can be observed in M_2 generation. In this study, observations focused on agronomic characters like plant height, yield components and yield.

The average plant height tends to increase with increasing dose of irradiation (Table 2). At a dose of 200 Gray, the average plant height is lower than the average plant height at the other doses. The average number of productive branches reaches the highest value at a dose of 200 Gray and lowest values found at a dose of 150 Gray. The highest average number of productive nodes is reached at dose 200 Gray and the lowest average value is at 50 Gray. The highest average number of productive pods is reached at dose 200 Gray and the lowest average value is at 150 Gray. The highest average value of empty pods is at 200 Gray and the lowest is at 50 Gray. The highest average value of the seeds weight is at 50 Gray dose.

The variance of morphology (phenotype) which increases with the increase of irradiation dose occurs at observation variables such as plant height, number of productive nodes and number of productive pods. For all these characters, the highest phenotypic variance comes in a dose of 200 Gray. Jamil and Khan (2002) reported promising fluctuation in germination (%), plant height, number of grains per plant, grain yield in the wheat through gamma irradiation. Gamma ray irradiation dose recommended by the IAEA (International Atomic Energy Agency) for the soybean crop is at 200 Gray irradiation, which is useful for improving quantitative characters of plants (Srisombun et al. 2009). For the other characters, the variance of phenotypes did not increase proportionally in line with the increase in the doses of irradiation, and they are the number of productive branches per plant and seed weight.

The results shows that low dose irradiation (micro dose) can produce the variance of characters desired. Sakin (2002) who observed mutation quantitative characters of wheat at low doses of gamma ray irradiation found the same thing. The advantage of using low-dose irradiation allows mutations to happen in the minor genes that can be observed in coming generation without harmful mutations. Sangsiri et al.(2005) reported many adverse mutations such as albino and leaf shape changes in green beans after irradiation treatment at a dose of 500 Gray.

The variance of agronomic characters in soybean after being irradiated by gamma rays is genetically controlled. High genetic diversity is very important in the selection process, because the genetic response to selection depends on the level of genetic diversity (Hallauer 1987). Previous researchers have reported hereditary changes in the desirable characters in crop plant by using gamma rays as a physical mutagen, which has been used to develop 64 % of the radiation-induced mutant varieties (Ahloowalia et al. 2004). High expectation value of wide heritability was

found in the observation variable such as plant height and number of productive pods. Selection for improvement of these two characters can be done to produce genotypes with the desired plant height and production. For other characters, the estimated expectation value of heritability range from low to moderate. Sakin (2002) who observed wheat found that heritability for some mutant population depends on the characters observed.

Table 2. Genetic variation and heritability of M_2 plants at different irradiation doses.

Character	Dose irradiation			
	50	100	150	200
Plant height				
$\bar{x}$	32.76	33.14	34.73	34.43
σ^2p	12.953	13.492	24.824	28.060
σ^2g	7.092	7.630	18.962	22.198
h^2	0.547	0.565	0.764	0.791
CGV (%)	8.129	8.334	12.536	13.683
Number of productive branches				
$\bar{x}$	2.73	2.81	2.48	3.01
σ^2p	2.572	1.055	1.237	0.951
σ^2g	1.924	0.406	0.588	0.302
h^2	0.748	0.385	0.475	0.317
CGV (%)	50.785	22.645	30.810	18.276
$\bar{x}$	17.24	18.00	17.28	19.02
σ^2p	15.587	23.611	25.468	24.507
σ^2g	1.105	8.611	12.468	14.507
h^2	0.071	0.365	0.490	0.592
CGV (%)	6.097	16.302	20.431	20.024
Number of productive pods				
$\bar{x}$	39.08	38.13	38.07	43.19
σ^2p	111.368	220.605	191.209	209.976
σ^2g	36.013	145.251	115.855	134.622
h^2	0.323	0.658	0.606	0.641
CGV (%)	15.355	31.601	28.269	26.862
Number of empty pods				
$\bar{x}$	1.40	2.53	2.60	3.05
σ^2p	2.783	5.792	4.866	7.705
σ^2g	0.131	3.140	2.214	5.053
h^2	0.047	0.542	0.455	0.655
CGV (%)	25.882	70.015	57.237	73.578
Weight of seeds per plant				
$\bar{x}$	10.93	9.85	9.47	10.17
σ^2p	10.775	16.458	16.574	12.116
σ^2g	0.033	5.716	5.832	1.374
h^2	0.003	0.347	0.352	0.113
CGV (%)	1.664	24.257	25.494	11.521

CONCLUSION

Phenotypic variations that occur on M_1 plants were caused by changes due to gamma ray irradiation, which affects plant growth and development. The highest average genetic variation in M_2 generation plants occurred in the treatment of 200 Gray. Gamma ray irradiation at a dose of 200 Gray effectively leads to genetic diversity in plants.

REFERENCES

Ahloowalia B, Maluszynski M, Nichterlein K. 2004. Global impact of mutation-derived varieties. Euphytica, 135: 187-204.

Arsyad DM, Adie MM, Kuswantoro A. 2007. The engineering of soybean variety specific agroecology. In: Sumarno, Suyamto, Widjono A, Hermanto, Kasim H (eds). Soybean: Production technique and development. Research centre and food plants development, Bogor. [Indonesia]

National Atomic Energy Agency [BATAN]. 2006. Mutation in plant breeding.

Central Statistic Bureau [CSB/BPS]. 2010. Rice, corn and soybean production (the temporary figures 2009 and the forecast figures I). CSB No.18/03/Th.XIII.

Cheema AA, Atta BM. 2003. Radiosensitivity studies in Basmati rice. Pakistan J Bot 35 (2): 197-207.

Ganguli P, Bhaduri P. 1980. Effect x-rays and thermal neutrons on dry seeds of Greengram (*P. aureus*). Gentica Agraria 34: 257-276.

Hallauer AR. 1987. Maize. In: Fehr WR (ed). Principles of cultivar development crops species, 2: 249-294. Machmillan, New York.

Hidajat JR, Harnoto, Mahmud M, Sumarno. 2000. The technology of soybean seed production. Research centre and food plants development, Bogor. [Indonesia].

Jamil M, Khan UQ. 2002. Study of genetic variation in yield components of wheat cultivar bukhtwar-92 as induced by gamma radiation. Asian J. Plant Sci., 1: 579-580.

Jusuf M. 2004. Exploration method, inventarisation, evaluation and conservation of germ plasma. Research center of Biotechnology, IPB. Bogor.

Leppik EE. 1971. Assumed gene centers of peanuts and soybeans. Econ Bot J 25 (2): 188-194.

Khan S, Goyal S. 2009. Mutation Genetic Studies in Mungbean IV. Selection of Early Maturing Mutants. Thai Journal of Agricultural Science, 42(2): 109-113.

Manjaya JG, Nandanwar RS. 2007. Genetic improvement of soybean vanety JS 80-21 through induced mutations. Plant Mut Rep 1 (3): 36-40.

Purna I, Hamidi, Prima. 2009. The efforts to increase soybean production. Secretariat state of Republic Indonesia, Jakarta.

Sakin MA. 2002. The use of induced micro mutation for quantitative characters after EMS and gamma ray treatments in durum wheat breeding. Pakistan Journal of Applied Sciences 2(12): 1102-1107.

Sangsiri C, SorajjapinunW, Srinives P. 2005. Gamma radiation induced mutations in mungbean. Sci Asia 31: 251-255.

Shah TM, Mirza JI, Haq MA, Atta BM. 2008. Radiosensitivity of various Chickpea genotpes in M_1 generation. Pakistan J Bot 40 (2): 649-665.

Singh, A., M. Diwakar, J. Singh and J. Singh, 2001. Mutagenic responses of mungbean *Vigna radiata* L. Wilczek. J. Applied Biol., 3: 75-79.

Shakoor A, Ahsan-ul-haq M, Sadiq M. 1978. Induced variation in mungbean. Env Exp Bot 18: 169-175.

Srisombun S, Benjamas K, Chitima Y, Jeeraporn K. 2009. Soybean variety improvement for high grain protein content using induced mutation. IAEA/RCA project RAS/5/045, Feb 16-20, 2009, Vietnam.

Sudaryanto T, Swastika DKS. 2007. Soybean economy in Indonesia. In: Sumarno, Suyamto, Widjono A, Hermanto, Kasim H (eds). Soybean: Production technique and development. Research centre and food plants development, Bogor. [Indonesia]

Tah, PR. 2006. Studies on gamma ray induced mutations in mungbean [*Vigna radiata* (L.) Wilczek]. Asian J of Plant Sci 5 (1): 61-70.

Van Harten AM. 1998. Mutation breeding, theory and practical application. University of Cambridge, Cambridge, UK.

Yaqoob M, Rashid A. 2001. Induced mutation studies in some mungbean (*Vigna radiata* L.) Wilczek cultivars. J. Biol. Sci., 1: 805-808.