

EVALUATION YIELD OF SOYBEAN MUTANT LINES ON MARGINAL LAND AND NUTRITION QUALITY

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ABSTRACT

The objective of this research was to evaluate soybean (*Glycine max* L. Merr.) Yield On marginal land and nutrition quality. Mutant soybean lines had been produced from soybean Muria variety by irradiation treatment using gamma radiation at 200 Gy. Yield test observation of four soybean mutant lines (G1M, G2M, G3M, G4M) and parent as control (Muria variety) had been conducted at Mataram, Central Indonesia, in marginal land. Seeds of these mutant lines from M5 generation were collected along with their parents and had been observed for nutrition content. Kjeldhal method was used to analyze protein content, AAS for nutrition content and HPLC for vitamin content. Based on the evaluation results, G4M soybean mutant line was identified as highest protein content (41.25%). The other mutant lines (G2M, G3M, and G1M) had protein percentage i.e. 40.57%, 39.63%, 38.95%, and 36.00% subsequently, while the parent check varieties Muria had average protein content 36%. For mineral content analysis showed that the P content G1M was the highest 583 mg/100g, but Muria 574.66 mg/100 g was lower than G1 M and G2 M 580.02, G3M and G4 M had P content , 568,72 and 557.42 mg/100g). G2M was the highest Ca content (224. 10 mg/100g). Subsequently Fe and vitamin B1 content of the mutants were achieved 8 mg/100g (G4M), 7.40 (G1M), 6.45 mg/100 g G3M, 6.09 mg/100g (G2M) and 5. 82 mg/100g (Muria). Vitamin B1 1.20 mg/100 g (G1M), 1.05 mg/100g Muria, 0.94 mg/100g (G4M), 0.82 mg/100 g (G3M) and 0.74 mg/100g (G2) respectively which were superior among the mutants and parent. The yield trial test had been performed on the mutant lines and control in marginal land locations in Mataram. The mutant line had the highest yield potential which was G2M (1.75 ton/ha) among mutants and parent (1.32 ton/ha).

Key words: *Glycine max*, gamma rays, Mutant lines, soybean, nutrition

ABSTRAK

Evaluasi hasil dan kualitas nutrisi galur mutan kedelai (*Glycine max* L. Merr.) di lahan marginal. Penelitian bertujuan untuk mengevaluasi produktivitas kedelai dan kualitas gizi. Galur mutan kedelai dihasilkan dari radiasi sinar gamma varietas Muria pada dosis 200 Gy. Penelitian terhadap empat galur mutan kedelai (G1M, G2M, G3M, dan G4M) dan kontrol tetua (Muria) telah dilakukan di lahan marginal Mataram. Benih galur mutan dari generasi M5 dengan tetuanya dianalisis kandungan nutrisinya. Analisis kandungan protein menggunakan metode Kjeldhal, kandungan nutrisi menggunakan AAS dan HPLC untuk vitamin. Berdasarkan hasil evaluasi, galur mutan G3 M mempunyai protein tertinggi (41,25%). Galur mutan yang lainnya (G2M, G4M, dan G1M) memiliki protein rata-rata 40,57%, 39,63%, 38,95% dan 36,00%, sedangkan varietas induk Muria memiliki protein 36%. Analisis mineral menunjukkan galur mutan G1M mempunyai kandungan P tertinggi 583 mg/100 g, tapi Muria mempunyai kandungan P 574,66 mg/100 g, lebih rendah dari G1M dan G2M 580,02. G3M dan G4M memiliki kandungan P masing masing, (568,72 dan 557,42 mg/100 g). G2M mempunyai

kandungan Ca tertinggi (224,10 mg/100g). Kandungan Fe dan vitamin B1 dari mutan G4M adalah (8 mg/100 g), G1M (7,40 mg/100 g), G3M (6,45 mg/100 g), G2M (6,09 mg/100 g) dan Muria (5,82 mg/100 g). Kandungan vitamin B1 G1M adalah (1,20 mg/100 g), lebih tinggi dari tetua Muria (1,05 mg/100 g Muria), G4M 0,94 mg/100 g, G3M 0,82 mg/100 g dan G2M (0,74 mg/100 g). Galur mutan G2M (1,75 ton/ha) memiliki hasil yang lebih tinggi dari dengan tetua (1,32 ton/ha).

Kata kunci: *Glycine max*, kedelai, radiasi gamma, galur mutan, nutrisi

INTRODUCTION

Soybean (*Glycine max* L. Merr.) breeders have made significant progress in improving the overall yield of soybean, which translates into more protein and oil on a per ha basis. Despite this, minimal advancements have been made in the selection of high-yielding genotypes, with major shifts in carbon flux for improvements in total oil or protein content (Mahmoud *et al.* 2006).

In Indonesia, consumption of soybean has been increased, but the production has been decreased. Therefore, to supply the domestic demand, it is important to increase domestic production. Expansion of growth areas and use of adapted varieties and high grain nutrient might overcome these problems among others. Besides increase in yield of a crop, quality and nutrition components are equally important in human diet. There is a necessity to enhance mineral elements (biofortification) and amino acids essential for human and animals, alteration of protein and fatty acid profiles for nutritional and health purposes, change of physicochemical properties of starch for different end uses, enhancement of phyto-nutrients in fruits and reduction of anti-nutrients in staple food. Induced mutations could play an important role in inducing mutations for enhancing nutritional quality in crop plants. According to IAEA Mutant Varieties Database (<http://www-mvd.iaea.org> 2010), 3000 mutant varieties have been officially released worldwide. Of these, 776 mutants have been induced for nutritional quality. Crop improvement by induced mutation using nuclear techniques (Jain 2000) intended to produce strains of cereals with higher concentrations of micronutrients and improvement of their bioavailability by reduction in the concentration of phytic acid. In this regard, strategies should be aimed at breeding plants that can contain high levels of minerals and vitamins in their edible parts to reduce substantially the recurrent costs associated with fortification and supplementation (Shetty 2009). Such a strategy will be successful depending on farmer's willingness to adopt such varieties, palatability of the edible parts of these varieties and consumer acceptability, and if the incorporated micronutrients can be absorbed by the human body (Bouis 2002).

The genetic variability is highly desirable for developing new cultivars, which is induced by mutagen treatments and natural spontaneous changes. The spontaneous mutation rate is pretty low and can't be exploited for breeding and that is why artificial mutations are induced with physical and chemical mutagen treatment. Quite many useful genetic changes have been induced by mutagen treatment including high yield, flower colour, disease resistance, and early maturation and so on in crop, vegetables, medicinal herbs, fruit, and ornamental plants. So far, over 3000 mutant varieties have been officially released over 60 countries including rice, wheat, barley, sorghum, legumes, cotton, edible oil, ornamental plants, and fruits (www-mvd.iaea.org 2010). The selection and development of mutants into recommended varieties for farmers have been successfully made in many countries (Wongpiyasatid *et al.* 1998). Sandhu and Saxena (2003) studied 34 mungbean

mutant lines and found high variation in yield per plant and nutritional quality, especially contents of protein, methionine, tryptophan, sulphur, phenol, and total sugars.

Mutation breeding has been employed successful for soybean (Lakshmi 2006; Ganapathy *et al.* 2008). Khan and Tyagi (2010) and Pavadai *et al.* (2010) obtained favorite traits using a number of chemical and physical mutagens in soybean. Likewise Sagel *et al.* (1995) registered a mutant variety of soybean with high oil (25.5%) and high protein content (39.2%). Today, soybeans are grown to some extent in most parts of the world and area primary source of vegetable protein and oil. Soybean protein can play an important role in the human diet (Erdman and Fordyce 1989). Mutant soybean lines have been generated from soybean cv. Muria by irradiation treatment using gamma radiation at 200 Gy. Selfing up to M5 generations have been conducted from the primary mutant lines in previous experiment. The purposes of generating those mutants were to developed new soybean mutant lines that were high yielding under dry land, highest protein and vitamin and nutrition content. In this experiments, characterizations of their yield under marginal land were evaluated. Evaluations were conducted on dry land, in order to inhibit growth and tested soybean genotypes. The purpose of the research was to obtain high yielding soybean varieties with large seeds, good quality, and high protein content.

MATERIALS AND METHODS

Mutant soybean lines have been generated from soybean Muria by irradiation treatment using gamma radiation at 200 Gy. Selfing up to M5 generations have been conducted from the four primary mutant lines (G1, G2, G3, and G4) in previous experiment.

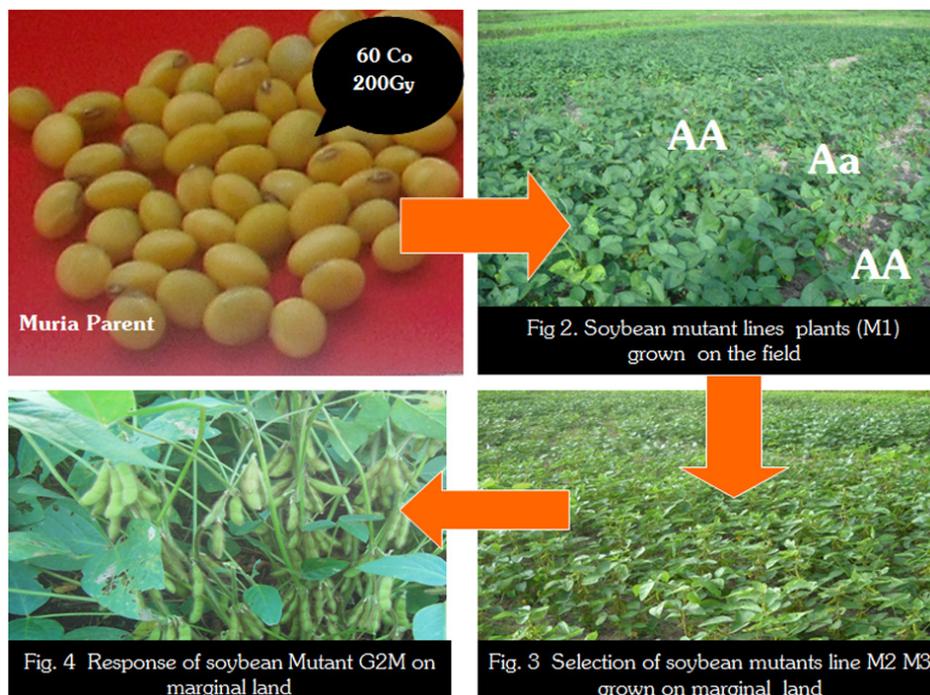
Yield test observation of four soybean mutant lines (G1M, G2M, G3M, and G4M) and parent as control (Muria variety) have been conducted at Mataram, Central Indonesia in marginal land during the 2010 (Juni to August).

A randomized block design with three replicates was used. Experimental plot size was 4 x 5 m, the distance between rows and between plants was 40 and 20 cm respectively, two plant per hill. Intensive techniques cultivation involves fertilizing with 75 kg of Urea, 200 kg of SP36, and 150 kg of KCl per ha, controlling of weeds, pests and diseases were carried out.

After harvesting, the average number of pods per plant and weight of dried seeds per plant were measured. After harvesting, the average number of pods and 100 seed weight yield (ton/ha) were measured.

For estimation protein content, nutrition and vitamin. were analyses at laboratry of post harvest, Ministry of Agriculture during October 2010.

The high protein mutant lines were tested for their protein yield in replicated trails. The Kjeldahl method (AOAC 1984) was used to determine seed protein percentages. Measurement of total nitrogen by Kjeldahl analysis is the historical reference method for determination of the protein content of dairy products. For quality assurance of the data, duplicated samples, laboratory-forti-fied matrix samples, and an internal reference, soybean seed sample were also analyzed and included in each batch of samples. The protein content was calculated as the total nitrogen (N) content multiplied by 6.25. The macro (P and Ca) and micro nutrient were analyzed by AAS. Vitamin B was determined by HPLC.



All data were subjected to an analysis of variance using the Proc Mixed procedure (Little *et al.* 1996 of SAS (SAS Inst 1995). Replicates and years were treated as random effects, and tillage system and crop rotation were considered fixed effects to calculate the expected mean squares and appropriate *F* tests in the analysis of variance.

RESULT AND DISCUSSION

The yield of G2M mutant line was the highest (1.75 ton/ha), significantly higher than parent Muria variety (1.32 ton/ha). Induction mutation for quantitative traits (yield) have been reported in soybean (Wakode *et al.* 2000). Mutant G2M had higher 100 seed weight than Muria. It was significant mutant was observed at 25 KR of gamma rays treatments.

Considering weight and pods number plant-1 is one of the major components of grain yield, the superiority of seed weight and pods number plant-1 is important in plant breeding to improve grain yield. As is shown in Table 1, mutant line G2M had higher grain yield (1.75 ton/ha) with higher 100 seed weight. Basavaraja *et al.* (2005), reported the significantly positive correlation for grain yield and 1000-grain weight. Iqbal *et al.* (2010) also reported that increasing the branch numbers in soybean, results in the increased grain yield. The mutant G2 M (1.75 ton/ha) had significantly ($p \leq 0.05$) higher grain yield than its parent Muria varieties (0.97 ton/ha).

G4M soybean mutant line was identified as highest protein content (41.25%). The other mutant lines (G3M, G2M, and G1M) have ranges of protein percentage were 40.57%, 39.63%, 38.95% subsequently, while the parent check varieties Muria had average protein content 36%. G1MP had the highest mineral P (583 mg/100 g). The mineral P Muria variety (parent) had P lower (574.66 mg/100 g) than the other mutant lines (Table

1). G4 M mutant line had the protein content 41,25% and Fe content (8,00 mg/100 g) higher than parent (36% for protein and 5,82 mg/100 g for Fe content). Soybean variety improvement for increased nutritional quality protein content using induced mutation was initiated. It is possible to select mutant lines with seed protein content higher than their respective parents by at least 1–2%.

Table 1. Yield performance of soybean mutant lines under dry land.

Mutant/variety	Yield (ton/ha)	Number of filled pot per plant	Number of Seed per plant	100 seed weight (g)	Plant Heigh
G1 M	1.41. ab	28.06. ab	70.3 ab	24.56. abc	28.4 a
G2 M	1.75. a	30.06. ab	60.1 b	25.56.ab	26.4
G3 M	1.27. ab	42.5.a	59.6 b	25.3.ab	23.63
G4 M	1.06. b	26. 26. b	63 b	21.96. abc	25.9
Muria	0.97.b	26.6. b	65.6 b	20 c	26.46

Mean with similar letters in at 0.05 probability level according to DMRT.

The highest protein content (41.25%) has been achieved in the G4M mutant, which is significantly higher than that of the parent Muria 36.00%. Induction mutation technique can used for crop improvement over the past few decades has shown that it is an effective plant breeding method to improved yield, quality, and resistance to biotic and a biotic stresses (Nichterlein *et al.* 2000, Pavaday 2009 Yuliasti *et al.* 2010). In breeding program, hybridization provides unlimited possibilities of generating new combination of characters, which can selected in the segregating population

Table 2. Nutrition performance of soybean mutant lines.

Mutant/variety	Protein Content	Makro nutrition (P) mg/100 g	Makro nutrition Ca mg/100 g	Mikro nutrition (Fe) mg/100 g	B1 Vitamin mg/100 g
G1 M	38. 50	583. 19	217. 55	7.4	1. 20
G2 M	39. 63	580. 02	224. 10	6.09	0. 74
G3 M	40. 57	568. 72	216. 20	6.45	0.82
G4 M	41. 25	557. 42	220. 48	8	0.94
Muria	36	574. 66	217.94	5. 82	1,05

Evaluation of oleic, linoleic, and linolenic acid contents of genotypes with modified fatty acid profiles is necessary to determine their utility in plant breeding programmes. The fatty acid profile of soybean oil from mutants and cultivars have been observed in several studies. The effects of year or location on the fatty acid content of soybean lines with different fatty acid profiles have been investigated by (Schnebly and Fehr 1993). The results of this study are in agreement with Chappell and Bilyeu 2006; Reinprecht *et al.* 2009 reported that mutations resulting in the low-lin oil phenotype in soybean.

Fatty acid analysis of seed from these mutants showed a range of variation for oleic acid content (1.513–1.710 g/100 g). Mutant G2 had oleic acid A higher than Muria. G4 soybean mutant lines had highest linolenat and linoleat (Table 3).

Table 3. Fatty acids performance of soybean mutant lines.

Mutant/ variety	The total fatty acids (g/100 g)						
	Laurat	Miristat	Palmitat	Stearat	Oleic acid	Linoleat	Linolenat
G1 M	0.005	0.008	0.615	0.276	1.513	2.86	0.31
G2 M	0.006	0.008	0.593	0.284	1.71	2.855	0.312
G3 M	0.004	0.008	0.664	0.298	1.605	2.913	0.326
G4 M	0.005	0.007	0.628	0.316	1.618	2.92	0.349
Muria	0.006	0.007	0.657	0.301	1.566	2.793	0.294

CONCLUSION

We conclude that the mutant line G4 M promising line with higher yield as well as protein and Fe content. We also conclude that the Gamma ray irradiation at 200 Gy is effective for generating genetic variation and selection for new high grain yield and nutrition content for soybean breeding programs.

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REFERENCES

- Basavaraja, G.T., G.K. Naidu, and P.M. Salimath. 2005. Evaluation of vegetable soybean genotypes for yield and component traits. *Karnataka J Agric Sci*, 18:27–31.
- Bilyeu, K.D., L. Palavalli, D.A. Slepser, and P.R. Beuselinck. (2003) Three microsomal omega-3 fattyacid desaturase genes contribute to soybean linolenic acid levels. *Crop Sci*. 43:1833–1838.
- Chappell, A.S., and K.D. Bilyeu. 2006. A GmFAD3A mutation in the low linolenic acid mutant C1640. *Plant Breed* 125:535–53.
- Graham, R.D., R.M. Welch, and H.E. Bouis. 2001. Addressing micronutrient malnutrition through enhancing the nutritional quality of staple foods: principles, perspectives and knowledge gaps. *Advances in Agronomy* 70,77*742.
- Graham, P.H., and C.P. Vance. 2003. Legumes: Importance and constraints to greater use. *Plant Physiol* 131:872–877.
- Iqbal, Z., M. Arshad, M. Ashraf, R. Naeem, M.F. Malik, and A. Waheed. 2010. Genetic divergence and correlation studies of soybean (*Glycine Max* (L.) Merrill.) Genotypes. *Pakistan J. Bot*, 42:971–976.
- Ngampongsai, S., A. Watanasit, S. Srisombun, P. Srinives and A. Masari. 1998. Current Status of Mungbean and the Use of Mutation Breeding in Thailand. *Kasetsart. J. Nat. Sci.* 32, 203–212
- Mahmoud, A.A., S.S. Natarajan, J.O. Bennett, T.P. Mawhinney, W.J. Wiebold, and H.B. Krishnan. 2006. Effect of six decades of selective breeding on soybean protein composition and quality: a biochemical and molecular analysis. *J. Agric Food Chem* 54:3916–3922.
- Nichterlein K., H. Bohlman, S. Niclen, and M. Maluszynski. 2000. Achievements and trends of

- using induced mutations in crop improvement. In: (DAE-BRNS Symposium on the Use of Nuclear and Molecular Techniques in Crop.
- Reinprecht Y., S.Y. Luk-Labey, J. Larsen, V.W. Poysa, K. Yu, I. Rajcan, G.R. Ablett., and K.P. Pauls. 2009. Molecular basis of the low linolenic acid trait on soybean EMS mutant line RG10. *Plant Breed LZB*:253–25.
- Sandhu, J.S., and A.K. Saxena. 2003. Variability and Association of Nutritional Quality Traits of Mungbean Mutants. *Indian J. Pulses Res.* 16(1):54–55.
- Schnebly, S.R., and W.R. Fehr, 1993. Effect of year and planting dates on fatty acid composition of soybean genotypes. *Crop Sci.* 33:716–719.
- The FAO/IAEA programme maintains a database of officially released mutant varieties worldwide (<http://www-mvd.iaea.org>). Currently ... *IAEA P.* 271–286.
- Wakode MM, R.S. Nandanwar and G.P. Patil. 2000. Radiation induced mutagenesis induced mutagenesis in soybean (*Glycine max L. Merr.*). In DAE- BRNS Symposium on use of Nuclear and Molecular techniques in crop Improvement. Dec. 6–8, 2000. Mumbai. Pp. 113–116.
- Wongpiyasatid, A., S. Chotchuen, P. Hormchan, and M. Srihattagum. 1999. Evaluation of Yield And Resistance to Powdery Mildew, *Cercospora* Leaf Spot and Cowpea Weevil in Mungbean Mutant Lines, Kasetsart. *J. Nat. Sci.* 33:204–215.
- Wongpiyasatid, A., S. Chotchuen, P. Hormchan, S. Ngampongsai, S. Lamsrijan, and S. Pichitporn. Mutant Mungbean Lines from Radiation and Chemical Induction, Kasetsart. *J. Nat. Sci.* 32, 203-212 (1998).