**BIODIVERSITAS**
Volume 23, Number 3, March 2022
Pages: 1231-1238

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230306

# Effect of gamma irradiation on the germination, pollen viability, and morpho-agronomic of *Pachyrhizus erosus* cv. Kota Padang

**P.K. DEWI HAYATI[1,♥], SISKA WITARI[1], NALWIDA ROZEN[1], SUTOYO[1], SASANTI WIDIARSIH[2]**
[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Andalas. Jl. Raya Unand, Limau Manih, Padang 25163, West Sumatra, Indonesia. Tel. +62-751-72701, fax. +62-751-72702, ♥email: pkdewihayati@agr.unand.ac.id
[2]Center of Isotopes and Radiation Application, National Nuclear Energy Agency of Indonesia. Jl. Raya Lebak Bulus Raya No. 49, Jakarta Selatan 12440, Jakarta, Indonesia

Manuscript received: 28 December 2021 Revision accepted: 13 February 2022.

**Abstract.** *Hayati PKD, Witari S, Rozen N, Sutoyo, Widiarsih S. 2022. Effect of gamma irradiation on the germination, pollen viability, and morpho-agronomic of* Pachyrhizus erosus *cv. Kota Padang. Biodiversitas 23: 1231-1238.* Yam bean or *Pachyrhizus erosus* (L.) Urb., an unexploited plant that produces the storage root tuber, is used in a variety scale of food processing and cosmetic industries in Indonesia. Novel approaches to induce variability in yam bean cv. Kota Padang will accelerate the breeding efforts and improvement of the cultivar. The purpose of the study was to elucidate the effects of gamma irradiation on seed germinability, pollen viability, and morpho-agronomic performance of yam bean cv. Kota Padang. Seeds were irradiated with gamma-rays on 0, 100, 150, 200, 300, 400, and 500 Gy, then $LD_{50}$ was determined based on the germination percentage. Observation of the irradiated yam bean growth in the field was carried out individually. Results showed that gamma irradiation decreased seed germinability, pollen viability, and agronomic traits. Still, it increased the variability of the traits among the mutant populations, except for the number of branches. Correlation analysis confirmed the results, indicating that selection in the next M2 generation must consider semi-dwarf and pod yield performances. The $LD_{50}$ was 150 and 176 Gy; hence, the irradiation doses of 150 and 200 Gy were appropriate in producing high genetic diversity with low physical damage in yam bean cv. Kota Padang.

**Keywords:** Bengkuang, correlation, $LD_{50}$, pollen fertility, variability

**Abbreviations**: Gy: Gray, $LD_{50}$: Lethal Dose 50%, M2: Mutant in 2$^{nd}$ generation

## INTRODUCTION

Yam bean, a legume crop that produces storage roots, originates from tropical America and has spread worldwide. There are three cultivated species, i.e., *Pachyrhizus erosus* (L.) Urb. or Mexican yam bean, *P. ahiapa* (Wedd.) Parodi or Andean yam bean, and *P. tuberosus* (Lam.) Spreng or Amazonian yam bean (Sørensen 1996). All three species are closely related and easily intercrossed, producing fertile interspecific hybrids (Grüneberg et al. 2003).

In Indonesia, the cultivated species of yam bean is Mexican yam bean or jicama, known as bengkuang. The root tuber is a good food source for health as it contains vitamins such as ascorbic acid, thiamine, riboflavin, pyridoxine, niacin, folic acid, and micronutrients, primarily Fe and Zn (Nugraheni et al. 2013; Ramos-de-la-Peña et al. 2013; Grüneberg 2016). The tuber contains starch and protein (Agaba et al. 2016; Grüneberg 2016). The seed contains a high amount of toxic rotenoids, but it also has the potential as a high palmitic acid oil source for the food industry (Grüneberg et al. 1999). Although the yam bean is an economically marginalized and underexploited plant (Sørensen 1996; Deletre et al. 2013), its edible tubers are used in many low- to medium-sized food processing industries in Indonesia. High water content and the sweetness from the inulin content of storage roots (Grüneberg et al. 2003; Sarkar et al. 2021) make yam tubers crunchy fresh fruit/vegetable. The majority of use of yam bean is as raw material in the cosmetics industry due to its ability to cleanse and brighten the skin as a beauty practice of traditional heritage.

The genetic variability of *P. erosus* accessions in Indonesia is narrow (Karuniawan and Wicaksana 2006) because all cultivated yam beans are mainly self-pollinating with outcrossing rates reported by Sørensen (1996) ranging from 2-4%. Mutation breeding is an effective tool in the hands of plant breeders, especially in crops having a narrow genetic base. Therefore, it is helpful to generate variability in the existing varieties. Induced mutation using physical mutagens is one method to create genetic variation and develop new cultivars with better characteristic traits. Gamma-ray is the primary physical mutagen to induce favorable mutations at high frequency, depending on plant species (Denissen and Den Nijs 1987), varieties (Addai and Safo-Kantanka 2006; Tshilenge-Lukanda et al. 2013; Muhammad et al. 2021), and dose of irradiation (Ji-Min et al. 2011; Tshilenge-Lukanda et al. 2013; Harsanti et al. 2020). Most mutant varieties released were developed using gamma irradiation (Ahloowalia et al. 2004; IAEA Mutant Variety Database 2021; Nilahayati et al. 2022).

Farmers prefer dwarf or semi-dwarf plants in producing seeds, while few inflorescences minimize pruning practices in producing tuber. Regular pruning flower buds is laborious (Grüneberg 2016), but essential to obtain a high tuber yield (Høgh-Jensen et al. 2008; Jean et al. 2017). There were still limited reports of induced mutation on yam beans. Gamma-rays and EMS were reported to cause a dwarf and a higher tuber yield mutant (Sørensen 1996); however, there are no further reports regarding the use of mutagens. In the present study, we examined the effects of gamma irradiation on seed germination, pollen viability, and growth performance of yam bean cv. Kota Padang. Yam bean cv. Kota Padang is a pure line variety from Padang city and is now widely planted in several regencies in West Sumatera Province. Furthermore, we tried to identify the optimum dose of gamma irradiation and provided M2 seed mutants that can be further selected for semi-dwarf, few inflorescences, and high tuber yield to be used in the yam bean breeding program through selection.

## MATERIALS AND METHODS

### Study area

Radiosensitivity and pollen viability tests were conducted at the Plant Breeding laboratory, while morpho-agronomic evaluations were conducted at the Research Station of the Faculty of Agriculture, Universitas Andalas in Padang, Indonesia. The order of soil is Inceptisol, with the soil pH being 5.20. The average rainfall at the site was 368.8 mm per month, ranging from 265-460 mm, while a temperature ranged from 25.6-26.5ºC and a relative humidity ranged from 82-85%.

### Procedures

Yam bean dry seeds cv. Kota Padang (14% moisture content) was exposed to $^{60}$Co gamma-cell irradiator model GC4000A at doses of 0, 100, 150, 200, 300, 400, and 500 Gy in the Center for Isotope and Radiation Application, National Nuclear Energy Agency (PAIR-BATAN), Jakarta, Indonesia. In each irradiation dose, 300 g seeds were procured with similar culture and post-harvest techniques before irradiation.

### Radiosensitivity of yam bean cv. Kota Padang

Totally 60 yam bean seeds cv. Kota Padang from each irradiation dose, *viz.* 0, 100, 200, 300, 400, and 500 Gy were germinated in seedbeds with the media mixture of soil and compost for two weeks. The seedbeds were laid out in a completely randomized design in three replications. The seeds were then scored for survival and measured for the seedling height. Seeds that germinated and produced both root systems and leaves were characterized as survival seedlings. The percentage of survival was determined by the formulae given below. The germination percentage was subjected to the regression analysis to determine the 50% Lethal Dose ($LD_{50}$).

$$\text{Survival (\%)} = \frac{\text{number of seedlings}}{\text{total number of seeds sown}} \ x\ 100\%$$

### Evaluation of morpho-agronomic of yam bean cv. Kota Padang

Seeds derived from irradiated doses 100, 150, 200, 300, 400, and 500 Gy and a control (non-mutant/wild type yam bean seed cv. Kota Padang) were germinated using organic polybags to minimize plot size in the field. The seedlings were subsequently transplanted into the field to observe the effect of irradiation mutation on growth, flowering, and pod yield. The plot size was 210 x 250 cm and 100 cm in space between, with the planting size being 30 x 25 cm. Fertilizing consisted of urea 100 kg ha$^{-1}$ applied at 2 and 7 weeks after planting and 75 kg ha$^{-1}$ both SP36 and KCl applied at two weeks after planting. Other agronomic practices followed as per standard recommendation to raise a healthy and good crop stand. Seeds from each mutant plant were harvested separately to obtain M2 seeds when plants attained maturity. The observation comprised variation in leaves and stem, plant height, the number of primary branches, days to first flowering (flowering date), pod maturity date, and the number of pods.

### Pollen viability assay of yam bean cv. Kota Padang

Pollens from fully opened flowers in four plant samples were selected randomly from each mutation irradiation dose. Flowers were handpicked at 09:00 am from the field, and separated to collect the anther. Anther was then extracted on glass slides, and 1% KI was dropped into pollen grains and incubated for 2 hours before observation was conducted using the microscope. A fully stained grain reflected fertile, while partial stained, shrunken and empty reflected sterile pollen grains (Monica and Seetharaman 2015). The viability of pollen by KI test was found out as formulae below.

$$\text{Pollen viability (\%)} = \frac{\text{number of viable pollen grains}}{\text{total number of pollen grains}} \ x\ 100\%$$

### Data analysis

The data collected was derived from an individual observation of each irradiation dose. The total irradiated and non-irradiated plants evaluated in the field were 247 plants. The quantitative data collected were analyzed using *t*-test, and descriptive statistics *viz.* mean and standard deviation. When the variance of the traits is more than two times of standard deviation, the phenotypic variability is classified as wide (Hayati 2018). The Simple Pearson phenotypic correlation analysis was conducted to investigate the association among traits affected by irradiation mutation. All analyses employed the Statistical Analysis System (SAS) computer software version 9.1.3.

## RESULTS AND DISCUSSION

### Radiosensitivity of yam bean cv. Kota Padang

The level of irradiation doses subjected to yam bean cv. Kota Padang *viz.* 100, 200, 300, 400, and 500 Gy referred to those applied to soybean (Addai and Safo-Kantanka 2006; Mudibu et al. 2012; Kusmiyati et al. 2018; Nobre et al. 2019; Ozdinc and Yalcin 2019), due to soybean (*Glycine max* L. Merr.) Fabaceae species is the closest relative for *Pachyrhizus* spp. (Deletre et al. 2013; Jean et al. 2017). The level of irradiation doses revealed a varied germination response. The survival percentage reflected the ability of seeds to germinate and produce all critical structures. The survival percentage of mutants derived from irradiation doses 200, 300, 400, and 500 Gy were significantly ($P<0.05$) lower than a control yam bean cv. Kota Padang (Table 1).

Despite the number of germinated seeds between irradiation doses 100 Gy was similar to control, the leaves size of the seedlings was smaller, and its height was shorter than those of control (Figure 1). Generally, seeds of irradiation dose 300 and 400 Gy revealed radicles but futile to produce plumule within germination, indicating the inability of seedlings to survive. There was no germinated seed in irradiation dose 500 Gy, indicating a high physiological damage effect on yam bean seed, leading to seed lethality. Similar to germination, there was a decrease in the seedling height and leaves size with increased irradiation doses.

Results obtained confirmed that seed germinability under controlled environment conditions indicates genotype response (Addai and Safo-Kantanka 2006; Kodym et al. 2011) to estimate the biological damage due to irradiation mutation. The seedlings of irradiation dose 100 Gy had a scantier reduction in germination. Germination rapidly decreased as irradiation dose increased to 200 and 300 Gy (Figure 2). The decrease of germinability with an increasing dose of irradiation directly affects the physiological damage of cells. The physiological damage increases rapidly with the use of higher doses of irradiation. Plant injury or physical damage was noticeable in growth inhibition. It will lead to null vitality of yam bean mutants in M1 generation when the doses of irradiation increase to 300 and 400 Gy. This condition is recognized as a deterministic effect, i.e., when the dose received exceeds the threshold accepted by the plant (Gaul 1977). Besides physiological damage, the damage of cellular components, including DNA and protein, exists and contributes to a reduction of seed germinability at M1 generation. The extent to which genetic change could be observed after the genetic inherited to the progeny in M2 generation (populations yet to be studied and will be published shortly). Inhibited seed germination due to mutagenic treatments may result from cell constituents damage at molecular (DNA) level or enzyme activity alteration (Khan and Goyal 2009).

**Table 1.** Means survival of yam bean cv. Kota Padang at various doses of gamma irradiation at two weeks after planting

| Irradiation dose (Gy) | Survival (%) |
|---|---|
| 100 | $88.30 \pm 5.00^{ns}$ |
| 200 | $33.89 \pm 5.09^{*}$ |
| 300 | $2.78 \pm 2.55^{*}$ |
| 400 | $0.56 \pm 0.96^{*}$ |
| 500 | $0.00 \pm 0.00$ |
| 0 (control) | $88.89 \pm 4.19$ |

Note: $^{ns}$ and $^{*}$: non-significant and significant, respectively at 5 % level based on *t*-test

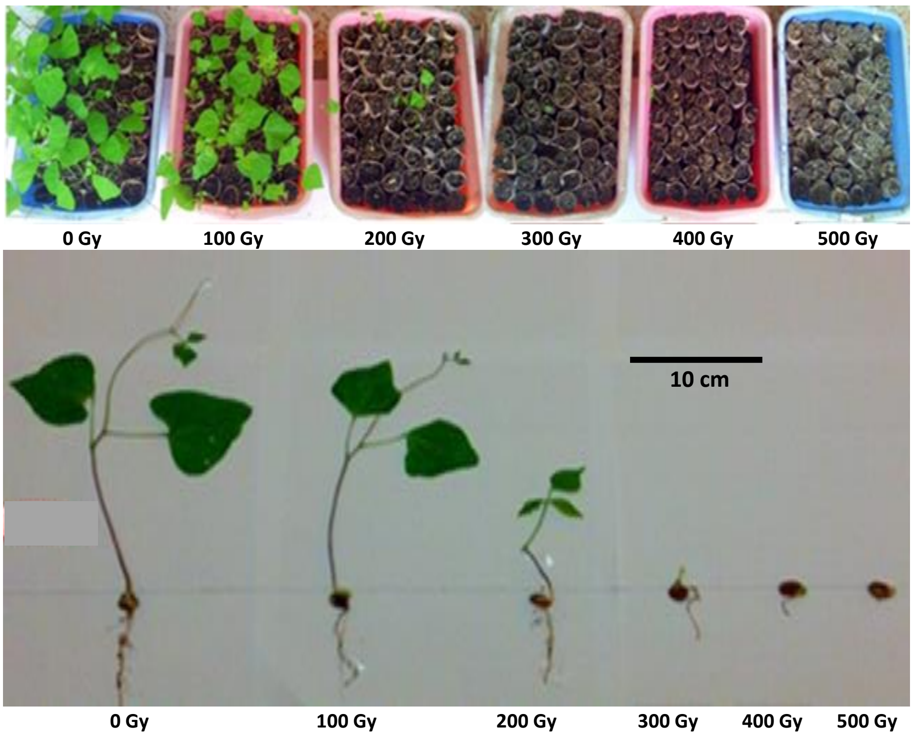


**Figure 1.** Seed germination and seedling growth of yam bean cv. Kota Padang in various doses of gamma irradiation at two weeks after planting

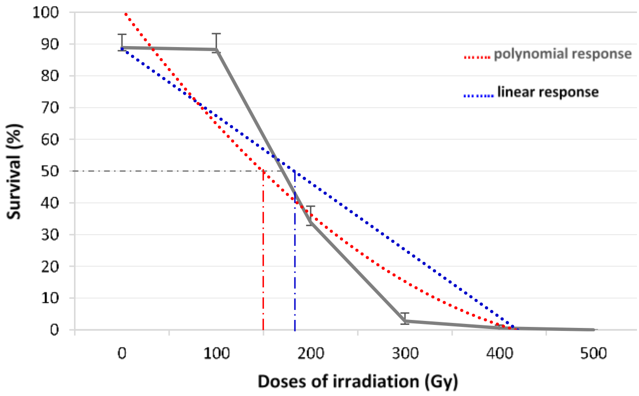


**Figure 2**. Response of survival percentage of yam bean cv. Kota Padang to various doses of gamma irradiation

Based on germination inhibition response to doses of irradiation in yam bean cv. Kota Padang (Figure 2), there were two possible response curves, *viz.* polynomial and linear responses with the coefficient of determination ($R^2$) values of 90% and 85%, respectively. The equation for polynomial response was $y = 3.7x^2 - 47.2x + 144.4$, while for linear response was $y = -21.1x + 109.6$. Therefore, the 50% lethal dose ($LD_{50}$) based on polynomial curve response was 150 Gy, and the linear curve response was 176 Gy. Polynomial response curve was previously reported on soybean by Kusmiyati et al. (2018), while linear response curve was reported by Ozdinc and Yalcin (2019). A half reduction in germination and seedling growth indicated that the $LD_{50}$ is between 150 and 200 Gy. Therefore 150 and 200 Gy were used as the appropriate dosage to induce mutation on yam bean cv. Kota Padang. The M2 mutant plants evaluated in the next generation were derived from seeds produced by mutants from both doses. Identifying the threshold dose is essential for obtaining high genetic variability and valuable mutants for practical purposes. Hanafiah et al. (2010a,b) reported that irradiation dose 200 Gy revealed the highest variability in plant height, yield component, and yield at soybean M2 generation. A similar report in using 200 Gy to induce high variability at M2 soybean generation was also reported by Mudibu et al. (2012). However, Kusmiyati et al. (2018) recommended very low and low irradiation doses (5-320 Gy) to improve the soybean diversity.

### Morpho-agronomic performance of yam bean cv. Kota Padang

There was no germinated seed from irradiation dose 500 Gy, while germinated seeds from irradiation dose 400 Gy did not complete the germination process. Only less than half germinated seeds from irradiation dose 300 Gy survived and attained maturity. Hence, morpho-agronomic traits were observed from irradiation doses 0, 100, 150, 200, and 300 Gy. Growth inhibition was a prevalent effect of irradiation mutation. The plant height of mutants from various irradiation doses tended to decrease when doses of irradiation increased (Figure 3). The amount of reduction was 30.9, 35.6, 41.7, and 34.5%, respectively, for 100, 150, 200, and 300 Gy. Based on the *t*-test, the means of plant height from the mutants were significantly shorter (P<0.01) than the control yam bean cv. Kota Padang. Many researchers also reported the decrease of plant height due to the increase of irradiation doses as in soybean (Hanafiah et al. 2010b; Mudibu et al. 2012), groundnut (Tshilenge-Lukanda et al. 2013). The decreased height in M1 generation directly affects physiological damage that occurs in mutant plants. Kodym et al. (2011) stated that most of the effect is direct injury, which results in a reduction in growth. In contrast, there was no difference (P>0.05) between the number of primary branches of mutants in each irradiation dose with that of the control plants. The variation found in the number of primary branches of each irradiation dose was similar to the response of Bambara groundnut to the acute irradiation dose (Muhammad et al. 2021).

Although the response of the mutants was different between plant height and the number of primary branches, there was a considerable variation in the performance of plant height and the number of branches at each irradiation dose. The irradiation dose of 200 Gy induced the highest variability in plant height and the number of primary branches, as indicated by the highest standard deviation value. Mutations reveal considerable variability in the population as mutagenesis can extend variability positively or negatively.

The means of flowering and harvesting time of mutants derived from irradiation dose 150, 200, and 300 Gy were significantly later (P<0.01) than those of a control yam bean cv. Kota Padang based on *t*-test. The delayed flowering was in line with maturity time, *viz.*16.3, 24.7, 31.2, and 36.8% for flowering and 8.3, 15.5, 24.0, and 22.4% for maturity, respectively, for irradiation doses 100, 150, 200, and 300 Gy. In contrast, the number of pods of mutants was significantly fewer (P<0.01) than that of a control. The reduction in the number of pods was 29, 30, 41, and 44%, respectively, for irradiation doses of 100, 150, 200, and 300 Gy. The gamma-rays stimulated late-flowering, which was in line with the increase of irradiation doses in the present study. Delayed flowering due to irradiation was reported on groundnut by Tshilenge-Lukanda et al. (2013), while early flowering on soybean due to irradiation was reported by Addai (2019).

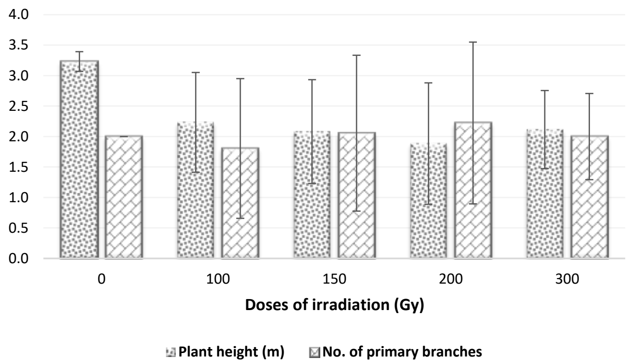


**Figure 3.** Response of yam bean cv. Kota Padang to various doses of gamma irradiation in plant height and the number of branches. Bars indicate the standard deviation values

In general, a high variation in flowering and maturity time and the number of pods was found within each irradiation dose. Irradiation dose of 200 Gy consistently revealed the highest variability for flowering and maturity time, while 150 Gy showed the highest variability in the number of pods based on standard deviation value (Figure 4). The late or early flowering time of mutants within a population is desired to select early or late flowering plants, remarkably variability of the traits in the M2 generation.

Gamma irradiation revealed variability in morphological traits *viz.* stem and leaves color (Figure 5). Gamma irradiation revealed four variations of stem color, *viz.* brown with streaked green, green with striped brown, green, and brown. Brown is a control stem color. At irradiation dose 100 Gy, brown dominated stem color (71.1%) followed by green with streaked brown (23.7%), and brown with striped green (5.2%). All variations in stem color were found in mutants from irradiation dose 150 Gy, *viz.* brown (44.4%), brown with streaked green (27.8%), green with streaked brown (15.3%), and green (12.5%). Variations were also found at the irradiation dose of 200 Gy, *viz.* brown (40.7%), brown with streaked green (29.6%), green with striped brown (24.1%), and green (5.6 %). There was no variation in the stem color at an irradiation dose of 300 Gy, i.e., brown. Four color types observed on the stem that occurred on irradiation doses of 150 and 200 Gy indicated high variability.

Dark green, the original color of leaves, was found at each irradiation dose. Variation in leaves was wrinkled, green with yellow leaves venation, bright green, green, and dark green. Wrinkled leaves with yellow midrib take a small portion of leaves' color and are found only at the irradiation dose of 150 Gy. Similar to stem color, this result indicated that high variability in leaves color was observed at an irradiation dose of 150 Gy. Irradiation doses of 150 and 200 Gy revealed high variability in morphological traits reported in soybean by Hanafiah et al. (2010a).

## Pollen viability

In the study, the effect of gamma-rays on pollen viability was also observed. Pollen viability is one of the essential stable and genetically controlled traits (Monica and Seetharaman 2015). Data presented in Table 2 represents the direct effects of irradiation mutation on pollen viability. Pollen viability varied and differed significantly from the control (P<0.05), except for irradiation dose of 100 Gy. Pollen viability of irradiated plants differed substantially from the control (P<0.05), except for irradiation dose of 100 Gy. Some pollen grains stained of control were high even though the percentage of pollen fertility of a control yam bean cv. Kota Padang in the study was not high (68.5%). Low viability of pollen might cause the low success of flowers into pods (fruit set). In contrast, the amount of unstained, shrunken, and empty grains was more significant with the increased irradiation doses (Figure 6), indicating that pollen viability decreased with an increasing dose of gamma irradiation. The reduction in pollen viability was 31, 51, 74, and 85%, respectively, for irradiation doses of 100, 150, 200, and 300 Gy.

Generally, the effect of primary injury on mutant growth and yield was a reduction in growth and fertility, which finally led to lethality (Kodym et al. 2011). The effect of irradiation mutation on low pollen fertility percentage was due to meiotic aberration resulting in abnormal pollen grains formation (Mathusamy and Jayabalan 2002; Monica and Seetharaman 2015). Reduction in pollen fertility due to irradiation and chemical mutation was reported earlier in cowpea (Bin and Dwivedi 2014), pigeon pea (Sangle et al. 2011), and garden bean (Monica and Seetharaman 2015).

## Phenotypic variability and association among traits observed.

Induced mutation of seeds using gamma-ray increases the phenotypic variability of the evaluated traits. Except for the number of primary branches, all traits showed a wide phenotypic variability among the mutant populations (Table 3). The variability observed in this M1 generation is a variation produced by the direct effect of irradiation on physiological damage to cells and the influence of genetic changes on both DNA and protein levels. The extent of genetic change can be observed in the M2 generation. The increased variability due to gamma irradiation has been reported by some researchers in soybean M2 generation (Addai and Safo-Kantanka 2006; Mudibu et al. 2012; Addai 2019; Harsanti et al. 2020).

**Table 2.** Pollen viability of yam bean cv. Kota Padang at various doses of gamma irradiation

| Irradiation dose (Gy) | Pollen viability (%) |
|---|---|
| 100 | $47.3 \pm 0.19^{ns}$ |
| 150 | $33.4 \pm 0.20^{*}$ |
| 200 | $18.0 \pm 0.14^{*}$ |
| 300 | $10.3 \pm 0.06^{*}$ |
| 0 (non-mutant) | $68.5 \pm 0.04$ |

Note: $^{ns}$ and $^{*}$: non-significant and significant at 5 % level based on *t*-test

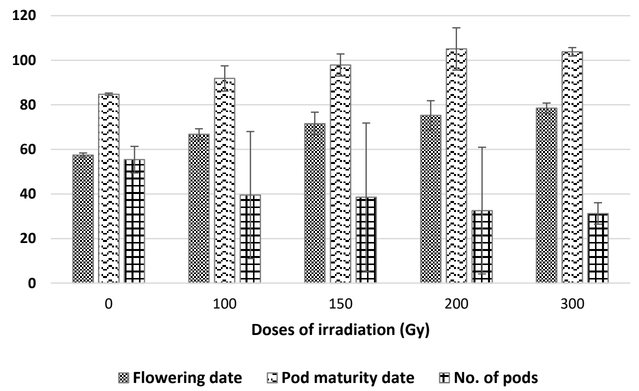


**Figure 4.** Response of yam bean cv. Kota Padang to various doses of gamma irradiation in flowering date, pod maturity date, and the number of pods. Bars indicate the standard deviation values

The correlation measures the extent to which the same gen or closely linked genes causes simultaneous variation in two different traits (Falconer 1989). Correlation studies have been reported earlier in chickpea (Khan and Wani 2005; Khursheed et al. 2016) to observe the pleiotropic effects of mutated genes. The correlations among traits observed were generally significant. Simple phenotypic correlation showed a significant and negative association between irradiation dose with plant height (r=-0.32), pollen viability (r=-0.84), and the number of pods (r=-0.18), while significant and positive associations between irradiation dose with the flowering date (r=0.74) and pod maturity date (r=0.67) (Table 4). The significant correlation coefficients indicated that the increasing irradiation doses decreased growth performance, pollen viability, and pod yield. The increasing irradiation doses also induced the lengthy flowering and pod maturity linearly.

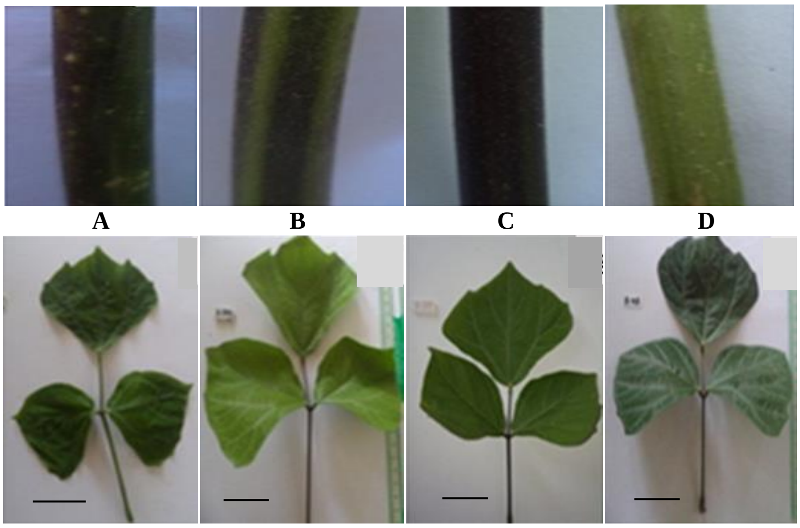


**Figure 5.** Variation in stem and leaves. Stem (*above*): A. Brown with streaked green; B. Green with striped brown; C. Brown; D. Green. Leaves (*bottom*): E. Wrinkled leaves with yellow midrib; F. Yellowish-green; G. Green; H. Dark green. The C and H were the stem and leaf colors of the control, respectively. Bar: 3 cm

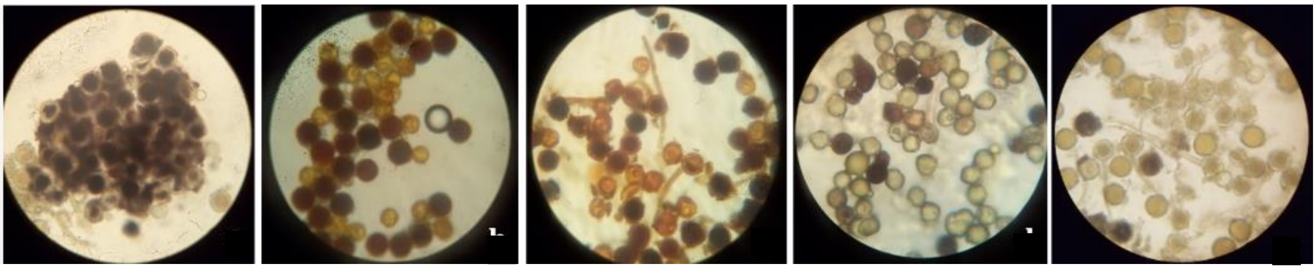


**Figure 6.** Pollen viability of yam bean cv. Kota Padang at various doses of gamma irradiation. A. Irradiation dose at 0 Gy; B. 100 Gy; C: 150 Gy; D. 200 Gy and E. 300 Gy

**Table 3.** Phenotypic variability of morpho-agronomic traits, and pollen viability of mutant and non-mutant on yam bean cv. Kota Padang

| Traits | Control | | Variability criteria | Mutans | | Variability criteria |
|---|---|---|---|---|---|---|
| | 2xSD | Variance | | 2xSD | Variance | |
| Plant height | 33.27 | 276.79 | Wide | 190.75 | 9096.71 | Wide |
| No. of branches | 0 | 0 | Narrow | 2.48 | 1.54 | Narrow |
| Flowering date | 1.05 | 1.09 | Narrow | 11.70 | 34.20 | Wide |
| Pod maturity date | 2.09 | 0.30 | Narrow | 16.87 | 71.13 | Wide |
| No. of pods | 12.20 | 37.20 | Wide | 60.42 | 912.58 | Wide |
| Pollen viability | 8.36 | 17.43 | Wide | 40.62 | 412.30 | Wide |

**Table 4.** Simple correlation among irradiation doses, morpho-agronomic traits, and pollen viability of yam bean cv. Kota Padang at various doses of gamma irradiation

| Traits | Plant height | No. of branches | Flowering date | Maturity date | Pollen viability | No. of pods |
|---|---|---|---|---|---|---|
| Irradiation dose | -0.32* | 0.09 | 0.74* | 0.67* | -0.84* | -0.18* |
| Plant height | | 0.04 | -0.23* | -0.21* | 0.60* | 0.27* |
| No. of branches | | | 0.08 | 0.11 | -0.21 | 0.24* |
| Flowering date | | | | 0.22* | -0.74* | -0.08 |
| Maturity date | | | | | -0.86* | -0.18* |
| Pollen viability | | | | | | 0.18 |

Note: *: significant at 5 % level based on *t*-test. The sample size of each variable was n: 147 except pollen viability, n: 20.

Interestingly, pollen viability had significant and negative correlations with the flowering date (r=-0.74) and pod maturity (r=-0.86), indicating an earlier flowering and pod maturity date. Further, the pollen viability had a significant and positive correlation with plant height (r=0.60), indicating that the decrease in height performance was in line with the low fertility of pollen. However, there was no association between pollen viability and the number of branches (r=-0.21), and the number of pods (r=0.18). The number of pods relies on the number of flowers and the success of the pollination and fertilization process. The correlation coefficient between both traits with pollen viability is the same and even higher than the correlation coefficient between the irradiation dose and the number of pods (r=-0.18) or the maturity date (r=-0.18). The difference in the association's significant response is due to the number of samples used. The larger samples provide a greater chance of significance at a similar correlation coefficient value.

Results of the present study revealed that exposing seeds of yam bean cv. Kota Padang to gamma-ray doses significantly decreased seed germinability, plant height, pollen fertility, the number of pods, and lengthen flowering and pod maturity. Correlation analyses confirmed the results. The correlation analysis results will be considered later in selecting M2 plants expected to perform semi-dwarf plants but not significantly reduce pod yields for seed production. Wide variability was found on morpho-agronomic traits and pollen viability among the mutant populations, indicating high variability induced by gamma irradiation. According to the curves responses of survival percentage, the $LD_{50}$ was 150 Gy and 176 Gy; hence, the irradiation doses of 150 and 200 Gy were an appropriate dosage in producing high genetic diversity and low physical damage in yam bean cv. Kota Padang. Genetic variation and selecting semi-dwarf, few inflorescences, and high yielding mutants in M2 generation are yet to be studied, and it will be published shortly.

## ACKNOWLEDGEMENTS

This research was conducted through a partnership between Universitas Andalas, Padang, Indonesia and the Center for Isotope and Radiation Application, National Nuclear Energy Agency (PAIR-BATAN), South Tangerang, Indonesia. This research was supported by a Research Grant from the Institute of Research and Community Services of Universitas Andalas with contract no. T/4/UN.16.17/ PT.01.03/Pangan-RD/2020. The authors thank Abdul Kudus, Ph.D., for his enlightenment in correlation analysis.

## REFERENCES

Addai IK, Safo-Kantanka O. 2006. Effect of $^{60}Co$ Gamma-irradiation on storability of soybean seed. Plant Sci 5 (2): 221-225. DOI: 10.3923/ajps.2006.221.225.

Addai IK 2019. Selection in the M2 generation of soybeans (*Glycine max* (L.) Merill) irradiated with Cobalt-60 gamma irradiation in the Guinea Savannah Agroecology of Ghana. Ghana J Sci Technol Dev 6 (1): 30-37. DOI: 10.47881/127.967x.

Agaba R, Tukamuhabwa P, Rubaihayo P, Tumwegamire S, Ssenyonjo A, Mwanga ROM, Ndirigwe J, Grüneberg WJ. 2016. Genetic variability for yield and nutritional quality in yam bean (*Pachyrhizus* sp.). Hortscience 51 (9): 1079-1086. DOI: 10.21273/HORTSCI10686-16.

Ahloowalia B, Maluszynski M, Nichterlein K. 2004. Global impact of mutation-derived varieties. Euphytica 135: 187-204. DOI: 10.1023/B:EUPH.0000014914.85465.4f.

Bin D, Dwivedi VK. 2014. Effect of mutagenesis on germination, plant survival and pollen sterility in M1 generation in cowpea (*Vigna unguiculata* (L.) Walp). Indian J Agric Res 48 (5): 398-401. DOI: 10.5958/0976-058X.2014.01322.5.

Deletre M, Soengas B, Utge J, Lambourdiere J, Sørensen M. 2013. Microsatellite markers for the yam bean *Pachyrhizus* (Fabaceae). Appl Plant Sci 1 (7): 1200551. DOI:10.3732/apps.1200551.

Denissen CJM, Den Nijs APM. 1987. Effects of gamma irradiation on in vitro pollen germination of different *Cucumis* species. Euphytica 36: 651-658. DOI: 10.1007/BF00041515.

Falconer DS. 1989. Introduction to Quantitative Genetics. 3rd Edition. Longman, New York.

Gaul H. 1977. Mutagen effects in the first generation after seed treatment. Manual on Mutation Breeding. 2nd Edition. IAEA, Vienna.

Grüneberg WJ. 2016. Enhancing the Nutrient-Rich Yam Bean (*Pachyrhizus* spp.) Storage Roots to Improve Food Quality and Availability and Sustainability of Farming Systems in Central and West Africa. Final Report. International Potato Center (CIP). Lima, Peru. DOI: 10.4160/9789290604747.

Grüneberg WJ, Goman FD, Velasco L. 1999. Characterization of yam bean (*Pachyrhizus* spp.) seeds as potential sources of high palmitic acid oil. J Amer Oil Chemists Soc 76 (11): 1309-1312. DOI: 10.1007/s11746-999-0144-x.

Grüneberg WJ, Freynhagen-Leopold P, Delgado-Vâquez O. 2003. A new yam bean (*Pachyrhizus* spp.) interspecific hybrid. Genet Res Crop Evol 50: 757-766. DOI: 10.1023/A:1025007918878.

Hanafiah DS, Trikoesoemaningtyas, Yahya S, Wirnas D. 2010a. Induced mutations by gamma-ray irradiation to Argomulyo soybean (*Glycine max*) variety. Nusantara Biosci 2 (3): 121-125. DOI: 10.13057/nusbiosci/n020303.

Hanafiah DS, Trikoesoemaningtyas, Yahya S, Wirnas D. 2010b. Agronomic improvement of Argomulyo soybean variety [*Glycine* *max* (L) Merr] through induced mutation by gamma irradiation in M1 and M2 generation. Biosfera 27 (3): 103-111. DOI: 10.20884/1.mib.2010.27.3.199.

Harsanti L, Anisyah, Parno and Tarmizi. 2020. The effect of gamma Co 60 radiation on soybean (*Glycine max* (L.) Merr.) shade tolerants. IOP Conf Ser Earth Environ Sci 518: 012055. DOI:10.1088/1755-1315/518/1/012055.

Hayati PKD. 2018. Analisis Rancangan dalam Pemuliaan Tanaman: Penerapan Statistika dalam Bidang Pemuliaan Tanaman. Universitas Andalas Press, Padang. [Indonesian]

Høgh-Jensen H, Mora AQ, Morera JAM, Sørensen M. 2008. Effect of planting density and prunings on yam bean tuber yields and quality. Res J Agron 2 (2): 56-63.

IAEA Mutant Variety Database. 2021. Available online:http://mvd.iaea.org [accessed on 31 July 2021]

Ji-Min S, Kim B, Seo S, Jeon SB, Kim J, Jun B, Kang S, Lee J, Chung M, Kim S. 2011. Mutation breeding of sweet potato by gamma-ray radiation. Afr J Agr Res 6: 1447-1454. DOI: 10.5897/AJAR10.936.

Jean N, Patrick R, Phenihas T, Rolland A, Placide R, Robert MOM, Silver T, Vestine K, Evrard K, Grüneberg WJ. 2017. Evaluation of performance of introduced yam bean (*Pachyrhizus* spp.) in three agro-ecological zones of Rwanda. Trop Plant Biol 10: 97-109. DOI: 10.1007/s12042-017-9188-5.

Karuniawan A, Wicaksana N. 2006. Genetic relationships of yam bean Pachyrhizus erosus based on morphological character of flowers and leaves. Bul Agron 34 (2): 98-105. DOI: 10.24831/jai.v34i2.1286.

Khan S, Wani MR. 2005. Genetic variability and correlations studies in chickpea mutants. J Cytol Genet 6: 155-160.

Khan S, Goyal S. 2009. Mutation genetic studies in mungbean IV: Selection of early maturing mutants. Thai J Agr Sci 42 (2): 109-113.

Khursheed S, Raina A, Khan S. 2016. Improvement of yield and mineral content in two cultivars of *Vicia faba* L. through physical and chemical mutagenesis and their character association analysis. Arc Curr Res Intl 4 (1): 1-7. DOI: 10.9734/ACRI/2016/24802.

Kodym A, Afza R, Forster BP, Ukai Y, Nakagawa H, Mba C. 2011. Methodology for physical and chemical mutagenic treatments. In: Shu QY, Forster BP, Nakagawa H (eds.). Plant Mutation Breeding and Biotechnology. FAO/IAEA Programme. DOI 10.1079/9781780640853.0169.

Kusmiyati F, Sutarno, Sas MGA, Herwibawa B. 2018. Mutagenic effects of gamma rays on soybean (*Glycine max* L.) germination and seedlings. IOP Conf Ser Earth Environ Sci 102: 012059 DOI: 10.1088/1755-1315/102/1/012059.

Mathusamy A, Jayabalan N. 2002. Effect of mutagens on pollen fertility of cotton (*Gossypium hirsutum* L.). Indian J Genet 62 (2): 187.

Monica S, Seetharaman N. 2015. Physical and chemical mutagenic effect on pollen fertility in M1 generation of garden bean (*Lablab purpureus* (L.)) sweet var. typicus cv. CO. Eur J Exp Biol 5 (9): 6-9.

Mudibu J, Nkongolo KKC, Kalonji-Mbuyi A, Kizungu RV. 2012. Effect of gamma irradiation on morpho-agronomic characteristics of soybeans (*Glycine max* L.). Am J Plant Sci 3: 331-337. DOI: 10.4236/ajps.2012.33039.

Muhammad I, Rafii MY, Nazli MH, Ramle SI, Harun AR, Oladosu Y. 2021. Determination of lethal (LD) and growth reduction (GR) doses on acute and chronic gamma-irradiated bambara groundnut (*Vigna subterranea* (L.) Verdc.) varieties. J Rad Res App Sci 14 (1): 133-145. DOI: 10.1080/16878507.2021.1883320.

Nilahayati, Nazimah, Handayani RDS, Syahputra J, Rizky M. 2022. Agronomic diversity of several soybean putative mutant lines resulting from gamma-rays irradiationin M6 generation. Nusantara Biosci 14: 34-39. DOI: 10.13057/nusbiosci/n140104.

Nobre DAC, Sediyama CS, Macedo WR, Piovesan ND, Arthur V. 2019. Gamma radiation to produce soybean mutants for better plant performance and chemical composition of seeds. Emirates J Food Agric 31 (7): 511-519. DOI: 10.9755/ejfa.2019.v31.i7.1983.

Nugrahaeni F, Wijayanti R, Notosandjojo YVP. 2013. Effectiveness of mahkota dewa (*Phaleria macrocarpa*) and bengkuang (*Pachyrhizus erosus*) seed extracts for controlling fruit pest in cocoa. Biofarmasi 11: 7-12. DOI: 10.13057/biofar/f120102.

Ozdinc N, Yalcin S. 2019. Effect of Gamma Radiation on different soybean varieties (*Glycine max* L. Merrill) in M1 generation. J Env Agric Sci 19: 01-09.

Ramos-de-la-Peña AM, Renard CM, Wicker L, Contreras-Esquivel JC. 2013. Advances and perspectives of *Pachyrhizus* spp. in food science and biotechnology. Trends Food Sci Technol 29:44-54. DOI: 10.1016/j.tifs.2012.09.003.

Sangle SM, Mahamune SE, Kharat SN, Kothekar VS. 2011. Effect of mutagenesis on germination and pollen sterility in pigeonpea. Biosci Dis 2 (1): 127-130. DOI:10.13140/2.1.4251.2003.

Sarkar R, Bhowmik A, Kundu A, Dutta A, Nain L, Chawla G, Saha S. 2021. Inulin from Pachyrhizus erosus root and its production intensification using evolutionary algorithm approach and response surface methodology. Carbohydr Polym 251: 117042. DOI: 10.1016/j.carbpol.2020.117042.

Sørensen M. 1996. Yam bean: Pachyrhizus DC. Promoting the Conservation and Use of Underutilized and Neglected Crops 2. International Plant Genetic Resources Institute, Rome, Italy.

Tshilenge-Lukanda L, Kalonji-Mbuyi A, Kongolo KKC, Kizunguet RV. 2013. Effect of gamma irradiation on morpho-agronomic characteristics of groundnut (*Arachis hypogaea* L.). Am J Plant Sci 4: 2186-2192. DOI: 10.4236/ajps.2013.411271.