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Determination of the Optimum Gamma Ray Lethal Dosage for Mutation Breeding of Indonesian Cassava Genotype Mentega 2

Penentuan Dosis Optimum Radiasi Gamma untuk Pemuliaan Ubi Kayu Indonesia Genotip Mentega 2

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ABSTRACT

This study aimed to determine the optimum lethal dosage (LD_{50}) of gamma rays in cassava and to study the effect of gamma irradiation on the morphological characters. Gamma rays is one of the physical mutagens that has been widely used for mutation breeding to improve the genetic traits of several crops including cassava. A total of 72 plantlets of Indonesian cassava genotype (Mentega 2) were cultured on Murashige & Skoog (MS) medium containing 2% of sucrose and were exposed to 0, 5, 15, 30, 50, and 75 Gray (Gy) using a Cobalt-60 source at dose rate of 600 Gy/h. Morphological characters such as plant height, leaf number, leaf width and length, and root number and length were measured. The percentage of plant survival decreased with the increase of gamma rays dose. The lowest survival rate (17%) was shown by 75 Gy-treated samples. The morphological characters' observation at 60 days of treatment showed the highest plant height was obtained from 15 Gy-treated samples (12.41±1.84 cm), while the lowest plant was from 75 Gy-treated samples (2.08±1.98 cm) compared to control (4.17±1.46 cm). The LD_{50} value was calculated to be 29.7 Gy which will be referred for further studies.

Key words: Cassavaplant breeding, gamma ray, optimum lethal dose (LD_{50})

ABSTRAK

Penelitian ini bertujuan untuk menentukan dosis letal (LD_{50}) optimal radiasi sinar gamma pada tanaman ubi kayu dengan menganalisis persentase kematian dan mempelajari pengaruh iradiasi sinar gamma pada karakter morfologi tanaman mutan. Sinar gamma adalah salah satu mutagen yang telah banyak digunakan dalam pemuliaan mutasi untuk meningkatkan keragaman genetik beberapa tanaman termasuk ubi kayu. Sebanyak 72 planlet genotipe ubi kayu asal Indonesia (Mentega 2) dikultur pada media Murashige & Skoog (MS) yang mengandung 2% sukrosa dan diberi paparan radiasi sinar gamma dengan dosis 0, 5, 15, 30, 50, dan 75 Gray (Gy) menggunakan Kobalt-60 (Co-60) pada laju radiasi 600 Gy/jam. Pengamatan dilakukan pada persentase hidup plantlet, tinggi tanaman, jumlah daun, lebar dan panjang daun, serta jumlah dan panjang akar. Hasil penelitian menunjukan bahwa persentase hidup tanaman menurun dengan meningkatnya dosis sinar gamma. Tingkat kelangsungan hidup terendah adalah 17% pada 75 Gy. Pengamatan morfologi pada 60 hari perlakuan menunjukkan bahwa tinggi tanaman tertinggi dicapai pada 15 Gy (12,41 ± 1,84 cm), sedangkan yang terendah adalah pada 75 Gy (2,08 ± 1,98 cm) dibandingkan dengan kontrol (4,17 + 1,46 cm). Nilai LD_{50} untuk genotipe Mentega 2 adalah 29.7 Gy yang akan diacu di penelitian selanjutnya.

Kata kunci: Ubi kayu, pemuliaan tanaman, radiasi sinar gamma, dosis letal optimum (LD_{50})

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the most important food crop in the tropics, which provides a staple food for approximately 800 million people worldwide [1]. In addition, cassava is also utilized for animal feed and nowadays, it is extensively used for many industrial purposes such as bioethanol, starch industries, modified cassava flour (mocaf) industries, bakery, and snacks industries. However, the utilization and the development of cassava as a crop and commodity face some problems such as low protein content, high cyanogenic glucoside content and poor shelf life after harvesting [2], [3] known as post-harvest physiological deterioration (PPD) that causes the roots unpalatable and unmarketable [4].

Mutation breeding has been widely used to improve various crop plants, including cassava, by creating variability of yield and yield component traits, which limit their productivity or enhance their quality and selection [5]. Although this technique has great contribution to increase food and agriculture production, it is still likely to have a great potential in genetic improvement of cassava and many other crops [6]. Mutation induction offers significant increase in crop

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production [7] and desirable traits that cannot be found in nature or have been lost during evolution. Conventional cassava breeding through hibridization has several limitations such as high heterozigosity, genetic overloading, poor flowering ability, low pollen fertility, self-incompatibility, and low fruit set rate [8]. Therefore, the mutation breeding is an alternative tool that can be used to compensate for those limitations. It also plays a key role in increasing the genetic variability for desired traits in various crop plants [9], [10], [11], [12], [13], [14], [15].

Mutations can be induced by physical and chemical mutagen treatment of both seed and vegetatively propagated crops. Gamma radiation also known as ionizing radiation, one of physical mutagens, has been widely used to induce mutations in various plants, for instance, *Antirrhinum majus* [16], *Chrysanthemum morifolium* [17], *Saintpaulia ionantha* [18, 19], rice [20], cassava [21] and sorghum [22]. Gamma irradiation (from a Cobalt-60 source) accounts for 61% of more than 200 direct-use mutant varieties released in Japan [23].

The mechanism of mutation induction is the mutagen treatment breaks the nuclear DNA and during the process of DNA repair mechanism, new mutations occur randomly and are heritable. It is a simple, efficient, rapid, and low cost option for obtaining desired genotypes of many crop varieties. Chemical mutagens and ionizing radiation have been used for long time as the plant mutagens in breeding research and genetic studies [24]. Production of mutants by chemical or irradiation mutagenesis is fairly economical [25].

The success of mutation breeding is much dependent on the rate of mutation, the number of samples, and the efficiency of mutation. The total dose of the mutagen affects the mutation rate, although it can be modified by either physical or biological factors. The use of higher doses of mutagen inevitably increases mortality and likely causes high pollen and seed sterility and deleterious mutations. In order to avoid the severe damage of the treated samples, the safe dose at which half of the planting material survive known as lethal dose 50 (LD_{50}) must be determined before massive irradiation of similar materials. Lethal dose, the percentage of treated samples die by a specific dosage (of chemicals or radiation), half will die at LD_{50} and is considered as a dose at which the highest frequency of mutation occurs [25], [26].

Research Centre for Biotechnology LIPI has been developing yellow cassava storage root, one of which genotype Mentega 2, that contain high beta carotene content and has good potential to be used as a healthy food source. However, some improvements are still required to produce even better traits related to nutrition content, starch content, and productivity. Genetic variation was conducted by mutation radiation. Prior to the mutation or radiation treatment, it is imperative to test an optimum lethal dose. Therefore, the aim of this study was to determine the optimum lethal dose (LD_{50}) of Gamma rays in selected Indonesian cassava genotype, Mentega 2.

METHODS

About one month-old in vitro cultures of cassava genotype, Mentega 2, were obtained from the Research Centre for Biotechnology-Indonesian Institute of Sciences (LIPI), Indonesia. The cultures were grown on MS (Murashige and Skoog) agar medium supplemented with 2% of sucrose under controlled condition (25-27°C, 50% relative humidity (RH)). Irradiation of plant materials was done at National Nuclear Energy Agency (BATAN), Indonesia using Gamma Chamber 4000 A with Cobalt-60 as a gamma rays source. The plant materials were irradiated with various doses of 0, 5, 15, 30, 50 and 75 Gy (1 Gy = 100 rad) at dose rate of 600 Gy/h.

Morphological characters including plant height, leaf number, leaf width and length, root number and root length were periodically observed at every seven days until 60 days after treatment. The optimum lethal dose (LD_{50}) was determined at 60 days after irradiation using graphical method [27]. The data set of plant height, leaf number, leaf width and length, root number and length were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's new multiple range test (DMRT) using SPSS software.

RESULT AND DISCUSSION

Determination of the optimum gamma ray lethal dose (LD_{50})

In order to reduce the detrimental impact of gamma irradiation on plant material, the optimum lethal dose, which is expressed as LD_{50} must be initially determined. Determining LD_{50} value is a key success in inducing mutation of any target plants or organisms. The LD_{50} value is considered as the dose of gamma ray that produces maximum

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of mutation frequency with minimum of damage to the samples. In the present study, the optimum lethal dose (LD_{50}), was determined at 60 days after irradiation. The LD_{50} for cassava genotype Mentega 2 was calculated to be 29.7 Gy using graphical method (Figure 1.) This finding was lower than that obtained in globular-stage for cassava somatic embryos which was 50 Gy [28]. This could be due to some factors affecting the response of samples to radiation exposures such as dose rate and use of different cultivars or species.

Effect of gamma irradiation on the survival rate of the treated samples

The current study showed that the survivability of *in vitro* cassava plantlets after treatment varied depending upon gamma rays dosage. The cassava plantlets survival rate decreased with the increase in gamma irradiation dose. It was found that over 80% of the plants were dead at 75 Gy after 60 days of irradiation compared to the lower doses (Figure 2). Gamma rays transfer energy to the molecules in the plant cells, particularly the DNA molecules resulting in point mutations [29]. However, if the DNA molecules undergo severe damage which can not be fixed by the cells, the cells will die [30]. The decrease in survival percentage after radiation treatment is due to the destruction of auxin [31], [32].

The plant growth hormone, auxin, or growth substance, is essential and within limits controls the growth of higher plants [31]. When gamma rays that belong to ionizing radiation interact with atoms or molecules, it induces reactive oxygen species (ROS) formation such as hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$), and hydroxyl radicals (.OH) in the cells. These ROS can damage or modify important components of plant cells [33].

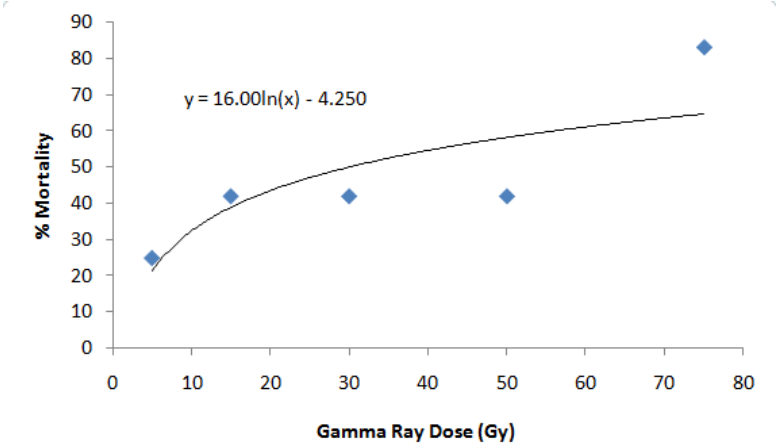

Figure 1. Effect of gamma rays dose on the mortality (%) of cassava plantlets at 60 days after irradiation

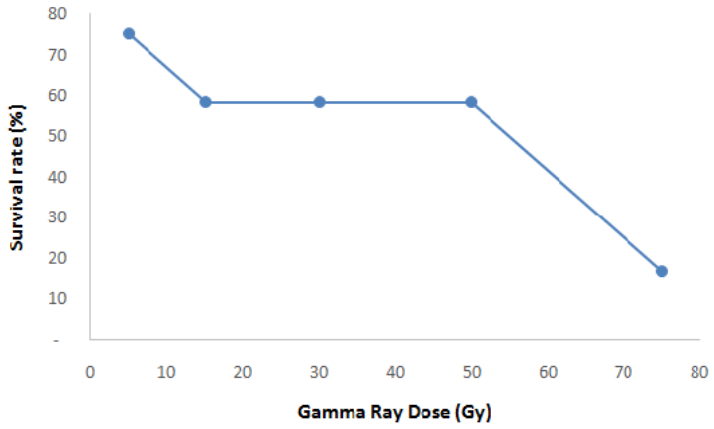

Figure 2. Effect of gamma irradiation on cassava survival rate after 60 days of treatment

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In terms of auxin, it therefore seemed to be destroyed by ROS produced by gamma irradiation [31]. To cope with the damage caused by ROS, cells possess a comprehensive and integrated endogenous enzymatic defence system such as peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) which are activated during cell injury [34].

Plant survival rate after irradiation indicates the capacity of treated population to withstand even the severe dosage of gamma rays. Sensitivity of the plant material to mutagens depends on the genetic constitution, dose employed, DNA amount, its replication time at initial stages, moisture content, stage of development, and genotype [35]. The dose rate also plays an important role in induction of mutations by irradiation [27]. When applying a high dose rate of irradiation, it was more possible to increase mutation frequency. In contrast, the possibility of mutations decreased when applying the lower gamma rays dose rate. This may have been due to the plant cells were less likely to be seriously damaged and they were able to repair themselves and return to the normal cell [36].

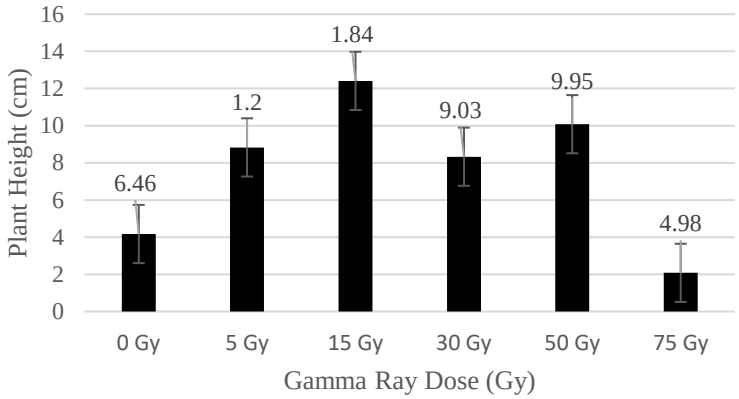

Figure 3. Effect of gamma irradiation on cassava plantlets growth at 60 days after treatment

Effect of gamma irradiation on plant growth

The growth of cassava plantlets decreased as the gamma doses increased. When the plantlets were exposed to over 30 Gy, the plantlets did not grow well and most of them were dead. The maximum growth of cassava plantlets was obtained from samples that were exposed to 15 Gy. The result after 60 days of treatment showed the highest plant height was 12.41±1.84 cm (15 Gy), while the lowest was 2.08±4.98 cm (75 Gy) compared to control (4.17±6.46 cm) (Figure 3).

The effects of ionizing irradiation on plant growth are largely deleterious and at high doses, the effect is lethal although different species of plants vary greatly in their sensitivity to the radiation. However, there was some evidence of a stimulating effect of growth when the seeds or seedlings were exposed to x-rays or other ionizing radiation. The symptoms frequently observed in the low- or high-dose-irradiated plants are enhancement or inhibition of germination, seedlings growth, and other biological responses [37], [38].

If the irradiation damages the genetic material of the cell then nucleic acid transcription process cannot proceed normally, and the organic molecules necessary for cell division may not be synthesized, leading to a stop in cell division and loss of viability. However, it was found that samples exposed to radiation at 50 Gy showed higher growth compared to irradiation dose of 30 Gy. This was possible that at this level of irradiation the enzymes involved in the synthesis of indole-3-acetic acid (IAA) from tryptophan were stimulated and causing a promotion of root growth [39].

Although there is no clear pattern on the plant growth, it showed that low-dose gamma irradiation has a stimulatory effect. The low-dose irradiation will induce the growth stimulation by changing the hormonal signalling network in plant cells or by increasing the anti-oxidative capacity of the cells to easily overcome daily stress factors [40]. On the other hand, the high dose-irradiation will inhibit the growth by arresting the cell cycle

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at G2/M phase during somatic cells division and/or various damages in the entire genome [41].

Effect of gamma irradiation on morphological characters

Gamma rays affect differentially the morphology, anatomy, biochemistry, and physiology of plants depending on the irradiation level. The morphological changes of cassava plantlets mutants were determined by measuring leaf number, leaf width, leaf length, root number, root length and shoot number. The results showed that the higher dose of gamma irradiation resulted in decreasing the root length to a statistically significant difference (Table 1). It would be possible that high dose of gamma irradiation could destroy the enzymes necessary for the synthesis of IAA from tryptophan, since IAA plays important role on root growth. Hence, the plantlets which were exposed to high dose of irradiation could not develop their roots [39]. A likely cause is that radiation affects organic molecules that are essential to the cell division process, causing cell division to stop.

Table 1. Effect of gamma radiation on morphological characteristics of cassava mutants.

Dose (Gy)	Root length (mm)	Root number	Leaf number	Leaf width (mm)	Leaf length (mm)	Shoot number
0	0.67±1.61b	0.50±1.16	0	0	0	0.75±1.35
5	10.0±15.81ab	1.83±3.12	0.08±0.28	0.08±0.28	0.25±0.85	1.08±1.78
15	12.5±19.24a	0.83±1.19	0.17±0.57	0.83±2.88	2.08±7.21	1.50±2.27
30	1.58±3.17b	0.33±0.65	0	0	0	1.41±1.44
50	5.25±8.65ab	0.92±1.08	0	0	0	1.58±1.67
75	0.83±2.88b	0.17±0.57	0	0	0	0.67±1.55
Sig.	*	ns	ns	ns	ns	ns

ns = not significant at the 0.05 level, * = significant at the 0.05 level based on DMRT
Numbers in the same column followed by the same letter is not significantly different on the DMRT at the 0.05 level

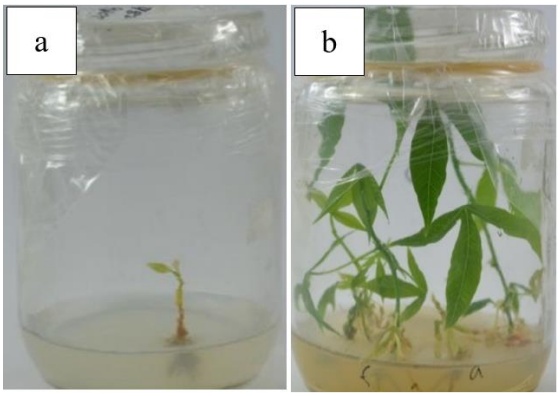

Figure 4. Plantlets of cassava genotype Mentega 2 after five months of irradiation. Plantlets were irradiated at 30 Gy (a), and 15 Gy (b).

CONCLUSIONS

The present study shows the optimum lethal dose (LD50) of gamma irradiation for cassava genotype Mentega 2 was 29,7 Gy at dose rate of 600 Gy/h. The LD_{50} will be further used as optimum dosage to induce mutation in larger number of samples. Plants materials exposed to gamma rays dosage ranged from 5 – 75 Gy showed variation of morphological characters in term of plant height, leaf number, leaf width and length, also root number and length. The decrease root length with significant difference was resulted by the higher dose of gamma irradiation.

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REFERENCES

[1] J. Liu *et al.*, "Cassava genetic transformation and its application in breeding", *Journal of Integrative Plant Biology*, vol. 53, pp. 552-569, 2011.

[2] Z. Keresztessy, *et al.*, "Identification of essential active-site residues in the cyanogenic β-glu cosidase (linamarase) from cassava (*Manihot esculenta* Crantz) by Site-Directed Mut agenesis", *Biochem. J*,

p ISSN 1907-0322
e ISSN 2527-6433

vol. 353, pp. 199-20 5, 2001.

[3] C.A. Iglesias, T. Sanchez, and H.H. Yeh, "Cyanogens and Linamarase Activities in Storage Roots of Cassava Plants from Breeding Program", *Journal of Food Composition and Analysis*, vol.15, pp. 379-387, 2002

[4] J.R. Beeching *et al.*, "Post-harvest physiological deterioration in cassava", *IVth Scientific Meeting of Cassava Biotechnology Network*, Salvador, Brazil, 1998.

[5] F. Novak and H. Brunner, "Plant breeding: induced mutation technology for crop improvement", *IAEA bulletin, vol.* 4, pp. 25-33, 1992.

[6] S.M. Jain, "Biotechnology and mutagenesis in genetic improvement of cassava (Manihot esculenta)", *Gene Conserve*, 6 (23): 329-343, 2007.

[7] M. Kharkwal and Q. Shu, "The role of induced mutations in world food security. induced plant mutations in the genomics era", *Food and Agriculture Organiation of the United Nations*, Rome, pp. 33-38, 2009.

[8] H. Ceballos *et al.*, "Casava: Root and tuber crops", *Springer*, pp. 53-96, 2010.

[9] P.R. Tah, "Induced macromutation in mungbean [*Vigna radiata* (L.) Wilczek]", *Int. J. Bot.*, vol.2, pp. 219-228, 2006.

[10] A.K.Adamu, and H. Aliyu, "Morphogical effects of sodium azide on tomato (*Lycopersicon esculentum* Mill)", *Science World Journal* vol. 2, no. 4, pp. 9-12, 2007.

[11] S. Khan and S. Goyal, "Improvement of mungbean varieties through induced mutation", *African Journal of Plant Science*, vol. 3, pp. 174-180, 2009.

[12] M.I. Kozgar, S. Goyal, and S. Khan, "EMS induced mutational variability in *Vigna radiata* and *Vigna mungo*", *Res J Bt*., vol. 6, pp. 31-37, 2011.

[13] G.G. Mostafa, "Effect of sodium azide on the growth and variability induction in *Helianthus annuus* L.", *Int. J. Plant Breed.& Genet*., vol. 5, 76-85, 2011.

[14] J. Chaudary, R. Deshmukh, and H. Somah. "Mutagenesis approaches and their role in crop improvement", *Plants*, vol. 8, no. 467, pp. 1-4, 2019

[15] S. Kumar, G. Katna, N. Sharma. "Mutation breeding in chickpea", *Adv Pl Agric Res 9*, No. 2 : 355-362, 2019.

[16] R.L. Cuany, A.H. Sparrow, and V. Pond, "Genetic response of *Antirrhinum majus* to a cute and chronic plant irradiation", *Zeitschri ft für Vererbungslehre,* vol. 89, pp. 7-13, 1958.

[17] P. Jompuk, S. Lamseejan, and S. Deeseepan, "The induction of mutations in chrysanthemum using gamma rays and in vitro culture techniques in radiation and life", *Proceedings of Eight Symposium on Science and Nuclear Technology* (NST8), 2001.

[18] K. Seneviratne and D. Wijesundara, "First African Violets (*Saintpaulia ionantha* H. Wendl.) with a Changing Colour Pattern Induced by Mutation", *American Journal of Plant Physiology*, vol. 2, pp. 233-23, 2007.

[19] A. Wongpiyasatid *et al.*,. "Effects of acute gamma irradiation on adventitious plantlet regeneration and mutation from leaf cuttings of african violet (Saintpaulia ionantha)", *Agriculture and Natural Resources,* vol. 41, No. 4, pp. 633-640, 2007.

[20] A. Esquivel *et al.*, "Use of gamma radiation to induce mutation in rice (*Oryza sativa* L.) and the selection of lines with tolerance to salinity and drought", *In Vitro Cellular & Development Biology-Plant*, vol. 56, pp. 88-97, 2020.

[21] E. Sudarmonowati, N. S. Hartati, Supatmi. "Enhancement of yield, starch, and amilose content of two Indonesian cassava

p ISSN 1907-0322
e ISSN 2527-6433

genotypes by producing gamma irradiated-induced mutants", *Annales Bogoriensis*, vol. 24, no. 2, 2020.

[22] M.A. Wanga *et al.*, "The effect of single and combined use of gamma radiation and ethylmethane sulfonate on early growth parameters in sorghum", *Plants 9*, no. 827, pp. 1-15, 2020.

[23] H. Nakagawa, "Induced mutations in plant breeding and biological researches in Japan", *Crops*, vol. 242, no. 48, pp. 48-54, 2009.

[24] J.L. Guenet, "Chemical mutagenesis of the mouse genome: an overview.", *Genetica*, vol. 122, pp. 9-24, 2004.

[25] S. Kangarasu, S. Ganeshram, and A.J. Joel, "Determination of lethal dose for gamma rays and ethyl methane sulphonate induc ed mutagenesis in cassava (*Manihot esculenta* Crantz.)", *Int.J.Sci.Res, vol.* 3, no. 1, pp. 3-6, 2014.

[26] M. Ramesh *et al.*, "Determination of lethal dose and effect of EMS and gamma ray on germination percentage and seedling parameters in barnyard millet variety Co (Kv) 2", *Electronic Journal of Plant Breeding*, vol. 10, no. 2, pp. 957-962, 2019.

[27] P. Tangpong *et al.*, "Effects of Acute and chronic gamma irradiations on in vitro culture of *Anubias congensis* NE Brown", *Kasetsart Journal*, vol. 43, pp. 449-457, 2009.

[28] R. Joseph, H.H. Yeoh, and C.S. Loh,"Induced mutations in cassava using somatic embryos and the identification of mutant plants with atered starch yield and composition", *Plant Cell Reports*, vol. 23, pp. 91-98, 2004.

[29] S. Lamseejan *et al.*, "Gamma-rays induced morphological changes in chrysanthemum (*Chrysanthemum morifolium*)", Kasetsart *J.(Nat. Sci.)*, vol. 3, no.4, pp. 417-422, 2000.

[30] M. Watanabe, K. Suzuki, and S. Kodama, "Molecular mechanism of cell death by radiation," *Nippon Igaku hoshasen Gakkai zasshi, Nippon acta radiological*, vol. 62, no.10, pp. 540-544, 2002.

[31] F. Skoog, "The effect of X-irradiation on auxin and plant growth, *Journal of Cellular and Comparative Physiology*, vol. 7, pp. 227-270, 1935.

[32] G. Smith and H. Kersten, "Auxin and calines in seedlings from X-rated seeds", *American Journal of Botany*, pp. 785-791, 1942.

[33] S.G. Wi *et al.*, "Effects of gamma irradiation on morphological changes and biological responses in plants", *Micron*, vol. 38, pp. 553-564, 2007.

[34] Y. Shindo *et al.*, "Enzymic and non-enzy mic antioxidants in epidermis and dermis of human skin", *Journal of Investigative Dermatology*, vol. 102, pp. 122-124, 1994.

[35] K. Deshpande, S. Mehetre, and S. Pingle, "Effect of different mutagens for induction of mutations in Mulberry", *Asian J. Exp. Biol. Sci. Spl.*, vol. 1, no. 2, pp. 104-108, 2010.

[36] R.M.Klein and D.T Klein, "Post-irradiation modulation of ionizing radiation damage to plants", *The Botanical Review*, vol. 37, pp. 397-436, 1971.

[37] J. Kim *et al.*, "Influence of low dose γ radiation on the physiology of germinative seed of vegetable crops", *Kor J Environ Agr.*, vol. 19, no. 1, pp. 58-61, 2000.

[38] S.G. Wi *et al.*, "Ultrastructural changes of cell organelles in arabidopsis stems after gamma irradation", *Journal of Plant Biology*, vol. 48, pp. 195-200, 2005.

[39] J.E. Gunckel, "The effects of ionizing radiation on plants: morphological effects", *Quarterly Review of Biology*, vol. 32, no. 1, pp. 46-56, 1957.

[40] J.H. Kim *et al.*, "Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (*Capsicum*

p ISSN 1907-0322
e ISSN 2527-6433

annuum L.) seedlings from gamma-irradiated seeds", *Journal of Plant Biology*, vol. 47, pp. 314-321, 2004.

[41] S. Preuss and A. Britt, "A DNA-damage-induced cell cycle checkpoint in arabidopsis", *Genetics,* vol. 164, pp. 323-334, 2003.