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The aplication of mutation induction by gamma irradiation on cultivars yam (*Dioscorea alata* L.) from banggai islands, Indonesia

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Key words: *Dioscorea alata* L., LD_{50}, *mutation-vegeative* (MV), *gamma-ray*.

Abstract

This study aims to obtain mutant plants through induced mutations gamma rays. Materials research 20 accessions of yam from Banggai Islands, Indonesia, which were irradiated with doses of 0 Gy, 10 Gy, 20 Gy, and 30 Gy using a Gamma Chamber 4000 A. The results showed the effect of growing LD50 at 0 Gy (100%), 10 Gy (88.33%), 20 Gy (86.67%), and 30 Gy (73.33%). Effect of irradiation dose of 20 Gy and 30 Gy causes accession BDa-05 and BDa-24 on the generation of MV1, MV2 and MV3, growing spread on the soil surface. Dose of 30 Gy irradiation led to the accession the round shape yam (BDa-30) into length the MV1, MV2 and MV3 generation. MV1 and MV2, shows the effect of irradiation dose of 30 Gy was significant different from 0 Gy and 10 Gy for duration of growth. Factors yam plant accessions and gamma radiation dose factors of significant influence on the length of growing crops, tuber number, stem diameter, length and weight of tubers. Exposure to gamma radiation doses higher tends to decrease the number of tubers, stem diameter, length and weight of tubers on MV1 generation. Exposure to irradiation 20 Gy and 30 Gy may cause growth patterns accession uwi some changes from pole climbing into climbing above the soil surface. Exposure to 30 Gy irradiation led to form the BDa-30 uwi accession changed (mutated) from the previous round shape becomes elongated.

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Introduction

Yam (*Dioscorea alata* L.) developed vegetatively by bulbs in the soil, generative propagatif very do difficult because it is rarely flowering and become a problem in the genetic expansion. One method that can be done to improved genetic yam is through mutation induction. Induction of mutation is one alternative to getting a new genetic variation in a relatively more quickly. In the field of plant breeding, mutation techniques can increase the genetic diversity of plants that allows breeders selecting plant genotypes according to the desired breeding objectives.

The success of the mutant plants formation, dependent on the dose of irradiation used. Induced of mutan plants is influenced by several factors among others, the type of plant, time of irradiation phase of plant growth, the size of the plant material, and the thickness of the material to be irradiated. Dose gamma irradiation induces plant diversity Dioscorea rotundata effective is 10-30 Gy (Nwachukwu *et al.*, 2009). This study aims to determine the lethal dose of gamma rays that may cause yam can grow 50 percent (LD_{50}) in MV1 generation, and also wanted to know the effect of gamma ray irradiation dose on variability of growth patterns, leaf shape, form of tubers, long time grows after planting, stem diameter, number of tuber, tuber weight, length of tuber, and evaluated of the MV2 and MV3 generations.

Material and methods

The material used is a tuber yam derived from Banggai Islands in Indonesia. Gamma ray irradiation was conducted in Badan Tenaga Nuklir Nasional (BATAN) Jakarta in September 2011 used Gamma Chamber 4000A. The experiment for MV1 consists of two factors, ie factor yam accessions with the symbol name BDa (*Dioscorea alata* from Banggai Islands) of 20 levels, and the second factor is dose gamma ray four levels(0, 10, 20, and 30 Gy. The treatment was repeated three times and each replication consisted of one sett of tuber. Irradiation setts were planted in polybags for 60 days, after the was transferred in the garden experiment. The design of the experiment using a completely randomized design (CRD) Robert and James (1991).

Observations began seedlings planted in polybags continued in the field until harvest time 5–9 months. Observation indicator is the percentage of plants that live in each treatment dose irradiation, the growth of duration, shape and color of leaves, growth pattern, shape of the bulbs, stem diameter, number of tubers, tuber weight, and tuber length.

The second experiment as the first trial evaluation to obtain MV2 only used 12 accessions of MV1 indicated mutations occur at each dose level gamma ray irradiation (0 Gy, 10 Gy, 20 Gy, and 30 Gy). Each treatment was repeated four times and each replication consisted of one seed sett. Experimental design using randomized block design (RBD) with two groups (Robert and James, 1991). Observations were made until the time of harvest for 5 to 9 months. Indicators observations for mutant plants are: the shape and color of leaves, growth model, shape of bulbs, growth duration, stem diameter, number of tubers, tuber weight, and tuber length. The procedure of data analysis using the MINITAB 14 program (Nur and Septin, 2006).

Results

LD_{50}

The results of the study after 60 days after planting showed all doses of irradiation treatment, greater growth response of 50% or below 50% LD_{50} (Table1). The first and second leaves, shape is not symmetrical (abnormal) in the treatment of irradiation dose of 10 Gy, 20 Gy and 30 Gy. Accession BDa-15 at a dose of 20 Gy leaves mottled purple color, and at a dose of 30 Gy leaves occurring combination of yellow and purple color as shown in (Fig. 1). The third leaf and so on emerge from all treatment doses of irradiation symmetrical shape. This might be due to an inhibitory effect on the early growth of cell division and the formation of chlorophyll.

The pattern of growth

The pattern of growth on MV1 generation with

irradiation doses of 0 Gy (control) and 10 Gy showed normal growth, namely climb the stakes, while the plant growth of accessions BDa-05 and BDa–24 with of gamma irradiation 20 Gy and 30 Gy dose to the generation MV1, MV2 and MV3 growth vines on the surface of the ground, despite supplied stakes (Fig. 2). Dose of 30 Gy irradiation led round shape to the accession of BDa-30 to be length (Fig. 3).

Table 1. Effect of gamma-ray dose on the growth and lethality percent of yam MV1 generation.

Dose gamma-ray	*grow*	*Growth (%)*	*Lethality (%)*
0 Gy	60	100	0
10 Gy	53	88.33	11.67
20 Gy	52	86.67	13.33
30 Gy	44	73.33	26.67

Note: Gy = gamma-ray.

Table 2. Analysis of variants the accession yam and gamma radiation dose factor of *MV1* and *MV2*.

		MV1		*MV2*	
Variable	Factors	F	P	F	P
The length of growing	Accession	3.75	0.000	11.34	0.000
	Dose Irradiation	17.17	0.000	4.96	0.003
	Accession * dose irradiation	1.27	0.125	3.25	0.000
Diameter rod	Accession	11.80	0.000	57.14	0.000
	Dose Irradiation	1.71	0.168	0.63	0.595
	Accession * dose irradiation	2.76	0.000	2.10	0.001
Number of tubers	Accession	8.38	0.000	9.08	0.000
	Dose Irradiation	3.67	0.014	0.24	0.871
	Accession * dose irradiation	2.07	0.000	1.81	0.009
Tuber weight	Accession	19.42	0.000	24.23	0.000
	Dose Irradiation	9.97	0.000	3.69	0.013
	Accession * dose irradiation	5.33	0.000	2.12	0.001
Long tuber	Accession	8.38	0.000	167.57	0.000
	Dose Irradiation	3.67	0.014	9.19	0.000
	Accession * dose irradiation	2.07	0.000	11.08	0.000

Description: all F values <p-value significant effect on the level of *0.05*.

Effect of gamma irradiation

Analysis of variance used the General Linear Model (GLM) of the study variables by two factors, namely accession (cultivars) yam and dose irradiation gamma-ray (Gy) and their interactions on showed in (Table 2). The value of the *F* factor all accessions and gamma irradiation doses and their interaction greater of *p-value* which means there is a significant difference between the levels in the accession yam factor, gamma irradiation dose, and the interaction there is a significant effect on the variable duration of growth, stem diameter, number of tubers, tuber length, and tuber weight. Accession to the value of the *F* factor variable duration grows less than the value of the *F* factor gamma irradiation doses compared with other variables that stem diameter, number of tubers, tuber weight and tuber length. This indicates that the long time to grown plants larger yam influenced by gamma irradiation doses compared with accession factor. In contrast to variable stem diameter, number of tubers, tuber length and tuber weight factor many influence by of the irradiation dose.

Further analysis showed significant differences between accessions characterized by different letters

(Table 3). The 0 Gy irradiation dose did not differ significantly with irradiation dose of 10 Gy and 20 Gy are marked with the same letter, but irradiation of 0 Gy and 10 Gy significantly different at a dose of 30 Gy irradiation to the variable length of growing plants marked with a different letter. Irradiation dose of 20 Gy did not differ significantly with of 30 Gy irradiation dose. The influence of gamma irradiation dose did not differ significantly to the variable number of tubers, stem diameter, length and weight of tuber are characterized by the same letter.

Table 3. Results evaluation of tuber production (kg) mutant accession BDA-30 in the generation of MV1, MV2, and MV3.

Dosis Irradiasi	*Generation*		
	MV1	MV2	MV3
0 Gy	1.1167 b	0.4367 a	1.0000 b
10 Gy	1.0167 b	0.4333 a	0.7833 c
20 Gy	1.2500 a	0.2367 b	0.6333 c
30 Gy	0.7333 c	0.2900 ab	1.2667 a

Description: numbers followed by the same letter in the same column do not differ significantly.

Table 4. Effect of gamma irradiation dose to the character of quantitative variables yam MV2 generation.

Dose (Gy)	*Growth of duration (days)*	*Number of tubers*	*Diameter rod (mm)*	*Length of tuber (cm)*	*Tuber weiht (kg)*
0	34.08 b	2.281 a	2.874 a	14.502 b	0.6249 a
10	32.04 c	2.458 a	2.811 b	15.760 a	0.5748 b
20	37.06 a	2.406 a	2.807 b	13.816 c	0.5315 b
30	34.25 b	2.250 a	2.785 b	13.167 d	0.4984 c

Description: numbers followed by the same letter in the same column means are not significantly different at the significance level α = 0.05%.

Average analysis to compare the value of each dose of irradiation to duration grown, number of tubers, stem diameter, bulb length, and tuber weight in the MV1 and MV2 can be seen in Table 3. Effect irradiation dose on the variable factor of tuber production be showed of MV1 and MV2. The yam accession BDa–15 in MV1 generation has the tuber production higher average 6.7 kg/plant, followed by BDa–11 and BDa-–35, respectively 3.7 and 3.6 kg/plant. The generally doses of gamma irradiation higher cause production tuber decline. Production of yam MV2 average is above 0.5 kg / plant, but well below average tuber production MV2 generation. It is thought likely caused by heavy rainfall in the growing season MV2 generation.

The results of the analysis of the average value of each accession for all variables showed significant differences between accessions to MV1 generation (Table 5). Accession longest BDa-15 an average 34.67 days, and the fastest grew BDa-34 15.58 average day. Highest number of tubers per plant on average 5.92 obtained accession number BDa-34 and an average of at least 1.25 acquired BDa-15 but did not differ with some other accessions such as the BDa-09, BDa-11, and BDa-31. Largest trunk diameter 4.21 mm average obtained BDa-15, and stem diameter on average 1.88 terkeci obtained accession BDa-07 but did not differ with accession BDa-17. Bulbs longest average gained 41.37 cm BDa-15 accession and accession no different from BDa-35. The highest tuber fresh weight average of 6.70 kg per plant accessions obtained BDA-15 and significantly different from the other accessions, whereas the lowest tuber fresh weight 0:11 kg obtained BDa-07.

The results of the evaluation (Table 6) shows the accession BDA-34 the average duration of most rapid growth (27.12 days), while the longest growth duration of each BDa-15 (42.72 days), BDa-08 and BDa-11 (42.16 days). The number of bulbs most BDa-05 (4,28 tubers / plant) and BDa-34 (4,06 tubers / planting). The most large diameter rod BDa-11 (3,73 mm), BDa-15 (3.63 mm) and BDa-35 (3.60 mm). Long bulb BDa-11 (33 65 cm) and BDA-15 (25,74 cm). The weight of the largest tubers BDA-15 (0.97 kg) and BDA-15 (0.92 kg).

Table 5. Average duration of effect of accession to grow, the number of tubers, stem diameter, bulb length, and tuber weight (α = 0.05).

Accession	Growth of duration (days)	Number of tubers	Diameter rod (mm)	Length of tuber (cm)	Tuber weiht (kg)
BDa 01	18.42 efg	2.25 defg	3.77 abc	29.57 b	1.77 cde
BDa 04	22.08 cdefg	1.75 defg	3.95 ab	19.62 de	2.03 c
BDa 05	20.83 cdefg	2.75 cde	3.51 bc	15.67 de	1.10 defg
BDa 07	18.33 efg	4.42 b	1.88 h	09.97 f	0.11 h
BDa 08	22.17 cdefg	2.42 cdefg	3.06 def	21.75 de	1.00 defg
BDa 09	24.92 bcdef	1.42 g	3.12 cdef	14.62 def	0.89 efgh
BDa 11	32.75 ab	1.33 g	3.75 ab	37.65 ab	3.73 b
BDa 14	20.50 defg	1.75 de	3.04 def	14.50 def	1.35 cef
BDa 15	30.08 abc	1.25 g	4.21 a	41.37 a	6.70 a
BDa 16	21.08 cdefg	3.58 bc	3.47 bc	34.70 abc	0.84 fgh
BDa 17	23.50 bcdefg	3.58 bc	1.91 h	18.43 de	0.44 gh
BDa 21	22.75 cdefg	2.08 defg	2.67 fg	25.69 cd	0.56 fgh
BDa 22	16.33 fg	2.67 cdef	3.64 abcd	21.63 d	1.08 defg
BDa 24	24.25 bcdefg	2.83 cd	2.87 eg	22.07 de	0.72 fgh
BDa 30	19.33 defg	2.17 defg	3.17 cdef	13.25 ef	0.75 fgh
BDa 31	28.33 abcd	1.33 g	2.28 gh	11.50 ef	0.32 gh
BDa 32	27.42 abcde	4.17 b	3.45 bcde	19.02 def	0.81 fgh
BDa 34	15.58 g	5.92 a	3.20 cdef	20.70 def	0.57 fgh
BDa 35	34.67 a	1.50 fg	3.29 cdef	41.00 ab	3.64 b
BDa 36	26.33 abcde	1.58 efg	3.47 bcde	17.88 def	1.87 cd
Mean	23,56	2,54	3,19	22,53	1,51

Description: Figures with the same letter in the column are not significantly different meaning.

Table 6. Evaluation of the average effect of accession to the character of quantitative variables yam MV2 generation.

Accession	Growth of duration (days)	Number of tubers	Diameter rod (mm)	Length of tuber (cm)	Tuber weiht (kg)
BDa 02	33.63 bc	1.66 cd	2.51 c	17.32 c	0.63 c
BDa 04	29.91 cd	3.34 ab	2.37 cd	6.99 g	0.38 de
BDa 05	33.34 bcd	4.28 a	2.28 d	6.36 g	0.30 e
BDa 08	42.16 a	1.97 bcd	2.18 d	11.57 de	0.34 e
BDa 11	42.16 a	1.53 d	3.73 a	33.65 a	0.84 b
BDa 14	30.47cd	2.06 bcd	2.30 d	8.53 fg	0.37 de
BDa 15	42.72 a	1.16 d	3.63 a	16.83 c	0.97 a
BDa 22	31.41 bcd	1.69 cd	3.17 b	13.40 d	0.42 de
BDa 30	29.75 cd	3.06 abc	2.09 d	7.34 g	0.31 e
Mean	34,36	2,35	2,79	14,31	0,56

Description: numbers followed by the same letter in the same column means are not significantly different at the significance level α = 0.05%.

Disccussion

The results of this study showed LD_{50} may be obtained by gamma irradiation dose, 10 Gy 11.67%, 20 Gy 13.33%, and 30 Gy 26.67%. The percentage of dead plants in this study is lower than the percent of dead plant *Dioscorea rotundata* ie 10 Gy (12%), 20 Gy (23.4%), and 30 Gy (49.1%) (Nwachukwu *et al*., 2009). However, both have the same trend that the higher doses of gamma irradiation increased the percentage of deaths. According to Khan and Goyal, (2009) gamma radiation has an inhibitory effect on the physical and physiological properties. Physiological effects may be its ability to withstand exposure to radiation causes damage to cell death. The results of this study demonstrate the physiological effects occur on the leaves of yam plants that grow abnormally due to radiation exposure of the cells on the surface of the skin where there are meristematic cells that will grow into shoots after a period of dormancy ends. Leaf color changes that occur in the BDa–15 akssesi spotty and there is also a combination of purple and yellow-green color, especially the formation of chlorophyll occurs inhibitory effect on the early growth. This occurs in *Hepper* or *black gram*, and M1 generation lost germination 40–50% due to the effects of radiation, some albino seedlings showed leaves and premature death. Mutant occurs primarily leaves of chlorophyll mutations such as albinism, copper leaf color, leaf lightgreen, variegated leaves shape, and waxy leaves (Bhosale and Hallale, 2011). But it this research does not become an obstacle to the growth of the next crop because supposedly still available nutrients in the bulbs so that the new plants that emerge can still survive even though the process of photosynthesis does not take place normally at the beginning of growth. Something similar happened to a study conducted Palamine *et al.* (2005) found exposure to 20 Gy of gamma radiation from the plant cuttings *Dracaena sanderiana* var. Displays an interesting yellow-green foliage than the dull appearance of a dark green color of the wild-type, and generally shortens leaf size 1.5–2.0 fold, and the average plant height was reduced 1.3–1.8 fold. Line mutant showed a decrease in the amount of chlorophyll *a* and *b* ratio of 1.6 fold lower compared to wild-type controls. Mark, (1992) said that in general beans (*Phaseolus vulgaris* L.), dry gets 20 Gy irradiation resulted in leaf chlorosis and lanceolate, leaves turn white dwarf, leaf bronzing and glossy.

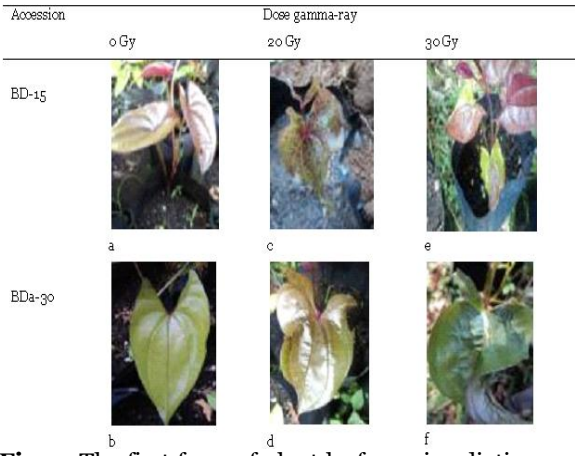

Fig. 1. The first form of plant leaf yam irradiation on generation *MV1*. (a, b). represent the 0 Gy normal leaves. (c, d). plant represents 20 Gy its leaf does not seem crimp symmetric. (e). represent 30 Gy leaves occurring combination of yellow and green color purple color. (f). represents 30 Gy leaves are not symmetrical, perming and abnormal.

In this research, is alleged to have occurred mutations in accession BDa–30 to 30 Gy irradiation dose treatment is estimated to have a mutation tubers shape. Mutations are changes in genetic material inheritance, individuals whose characteristics change will be inherited in the next generation and are known as mutants. In nature, the combination of errors that occur during replication of deoxyribonucleic acid (DNA) and damage to genetic material is passed on from generation to generation because individual exposure to sunlight and *ultra violet* (UV) radiation. Other types of mutations including duplications where the whole genome is duplicated and translocated part of chromosome is transferred to a non- homologous chromosome, this occurs as a result of an error during meiosis cell division. The effect depends on whether there is or not, and how, the genes that are affected in this mutation. The accumulation of such deviations has become the main basis of evolution and speciation. DNA chains form the basic unit of heredity, ie genes.

Small changes to the DNA sequence useful for crop improvement, while larger changes (at the level of chromosomal and cellular) always give some level of imperfections and even deadly. One way to classify mutations in the DNA sequence level mutations is to categorize on the basis of how the modification affects the gene's ability to synthesize proteins that are responsible for the character. In general, it is important mutations in crop improvement usually involves only one or a fundamental part and does not affect the synthesis of proteins.

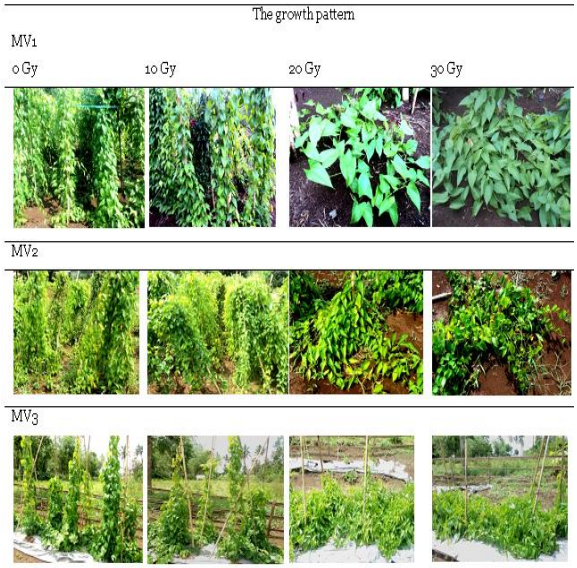

Fig. 2. Yam growth model according to the generation of gamma-ray irradiation dose MV1, MV2, and MV3.

The effect of gamma radiation doses of 0 Gy, 10 Gy and 20 Gy to the MV1 do not differ significantly on growth duration as well as radiation dose 20 Gy and 30 Gy did not differ significantly. But irradiation doses of 0 Gy and 10 Gy significantly different with 30 Gy radiation dose. Differences in the amount of plant grow control 0 Gy of 10 Gy and 20 Gy only approximately one day, and 30 Gy approximately ten days than the control plants. The most effective dose range for induction of mutations in vegetative crops such as yam which should between doses can be cause death no more than 50% and no growth reduction of more than 50%. The treatment dose should allow 40-50% survival and or growth slowdown was not more than 50% (Nwachukwu *et al.*, 2009). In this range, enough population of plants that can survive the lethal effects of mutagens and encourage genetic changes sufficient to allow the selection of mutants that is more efficient and desirable. Accession significantly influence the growth duration, it is thought to be caused the yam plants have a dormant period different and every cycle is fixed on the growth of each accession. According to Nwachukwu *et al.* (2009), that one of the objectives in addition to the genetic improvement of tuber yield increases, include the development of cultivars that can growth well with high yield production without stake. In this study the growth obtained yam plants propagate without stake. Result of gamma irradiation plants are divided into three types of effects, physiological damage, (primary injury), gene mutations and chromosomal mutations (Nwachukwu *et al.*, 2009). Radiation injury can reduce the ability of vegetative growth when used as a material organ radiation. This study shows that the ability to growth yam crops decreased with increasing doses of gamma irradiation. The same trend is occurring in the production of tubers decreased weight, stem diameter and length of tuber with increasing doses of gamma irradiation, contrary to the character of the number of tubers is likely to increase even though the amount is not significant and the size appear smaller tubers. (Abdul *et al.*, 2010) his study showed that gamma ray irradiation significantly affected the germination percentage, survival percentage, shoot and root length, number of branches and leaves per plant, fresh and dry weight of *Lepidium*, other growth parameters showed a trend declining with increasing doses of gamma irradiation .

Mutations of genes and chromosomes can be transferred from one generation to the next generation, but not physiological damage may derived. Leaf shape of yam not symmetrical on irradiation of 10 Gy, 20 Gy, and 30 Gy in this study is due to physiological damage that may not be passed down to the next generation. Therefore, for practical purposes, the selection of mutants should be started from the second generation after the physiological effects may have been lost.

Fig. 3. Tuber shape BDa-30 mutant and control the generation of (MV1, MV2 and MV3) according to the radiation dose.

Dwimahyani and Isaac, (2004) found that exposure 10–25 Gy irradiation of *Jatropha* cuttings sufficient to induce mutations. Rong *et al.* (2008) found that the LD_{50} dose for leaf segment, nodular stem segments and stem segments Pogostemoncablin in vitro were respectively 64, 66 and 72 Gy. The results of the study James *at al.* (2012) showed that all the non-irradiated yam grow at the end of month to 3 storage, while the 60 Gy grew 90%, 80 Gy irradiation grew 30%, and 100 Gy irradiation grew only 10% storage period. The gamma radiation dose in the range of 100–140 Gy can effectively inhibit the growth of yam for 7 months, and that the irradiation process can maintain tuber quality yam through inhibition of shoots, weight reduction, preservation of macronutrients, such as proteins, lipids, and carbohydrates (James *et al.*, 2012). Dhakshanamoorthy *et al.* (2010) found that seeds treated with 50 Gy of gamma rays showed a stimulatory effect except for germination, whereas a dose of 250 Gy showed inhibitory effects compared to other treatments. This can be caused by damage to the seed tissue and the severity of the damage depends on the dose of irradiation used (Datta, 2009). Gamma radiation has an inhibitory effect on the physiological and physical properties (Khan and Goyal, 2009). The inhibitory effect of gamma radiation is also reported in the green beans (Khan and Goyal, 2009), snap bean (Ellafa *et al.*, 2007), cowpea (Gnanamurthy *et al.,* 2012), and *Torenia fournieri* (Anchalee, 2011).

Conclution

All treatment doses of radiation (0, 10, 20, and 30 Gy) can cause a 50 per cent growth (LD50). Higher doses of gamma radiation slows time to growth yam seedlings, reducing the amount of plant life. Exposure to gamma radiation causes morphological diversity of leaf shapes and colors yam, the type of growth and tuber shape. Factors yam accessions and gamma radiation dose factors of significant influence on long time grow, tuber number, stem diameter, length and weight of tubers. Exposure to gamma radiation doses higher tends to decrease the number of tubers, stem diameter, weight and length of tuber yam on MV1 generation. Dose of 30 Gy irradiation led to the accession yam BDa–30 tuber shape round, becomes elongated.

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