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Gamma Irradiation on Growth and Development of *Amorphophallus muelleri* Blume.

Edi Santosa[1*], Sigit Pramono[1], Yoko Mine[2], and Nobuo Sugiyama[2]

[1]Department of Agronomy and Horticulture, Bogor Agricultural University
Jl. Meranti, Kampus IPB Dramaga, Bogor 16168, Indonesia
[2]Faculty of Agriculture, Tokyo University of Agriculture, Funako, Atsugi, Kanagawa 243-0034, Japan

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ABSTRACT

Iles-iles (Amorphophallus muelleri Blume) produces apomictic seeds lead to low genetic variation. In order to induce genetic variation, germinated seeds were exposed to Gamma irradiation (Co-60) at doses of 10 to 100 Gy. Seed irradiation was conducted at Center for the Application of Isotope and Irradiation Technology -National Nuclear Energy Agency (CAIRT), Indonesia. Morphology and yield of M1 generation were observed. Results showed that irradiation at a dose of 10 Gy close to LD_{50} with survival rate 56%. Gamma irradiation at a dose of 10 Gy delayed seeds germination. Germination rates gradually increased and reached maximum at 4 weeks after planting (WAP) for control plants, and 14 WAP of irradiated plants. At 16 WAP, germination rate of 10 Gy irradiated plants was 56% and 84% for those of control plants. Irradiation induced chimera as indicated by short petiole, variegated and abnornal shape of leaflets. Some irradiated plants entered dormancy at 8-10 weeks later than control ones. Prolong vegetative periode lead the plants to produce heavier corms. This study revealed the possibility to induce variation of A. muelleri by using gamma irradition.

Keywords: Amorphophallus muelleri, gamma irradiation (Co-60), morphological variation, mutation breeding

ABSTRAK

Iles-iles (Amorphophallus muelleri Blume) menghasilkan biji apomiksis yang menyebabkan keragaman genetik tanaman rendah. Dalam upaya meningkatkkan keragaman genetik, maka digunakan iradiasi sinar gamma (Co-60) dengan rentang dari 10-100 Gy. Iradiasi benih dilakukan pada Pusat Aplikasi Teknologi Isotop dan Radiasi-BATAN, Indonesia. Tanaman M1 hasil iradiasi diamati pertumbuhan hingga panen. Hasil penelitian menunjukkan bahwa dosis iradiasi 10 Gy hampir mendekati LD_{50} karena survival rate 56%. Iradiasi sinar gamma menyebabkan benih lambat berkecambah. Pertumbuhan tunas pada tanaman kontrol telah maksimal pada 4 MST, sedangkan pada tanaman iradiasi pada 14 MST. Pada minggu ke 16 setelah tanam, persentase tanaman tumbuh dari perlakukan 10 Gy adalah 56%, sedangkan pada kontrol mencapai 84%. Kimera muncul pada tanaman hasil iradiasi, berupa petiol yang memendek, varigata dan bentuk leaflet abnormal. Beberapa tanaman hasil iradiasi memasuki masa dorman 8-10 minggu, yang lebih lambat dibandingkan dengan kontrol. Lambatnya tanaman iradiasi memasuki dorman diduga menjadi penyebab meningkatnya bobot umbi pada tanaman tersebut. Penelitian ini menunjukkan adanya peluang penggunaan iradiasi sinar gamma untuk meningkatkan variasi pada tanaman iles-iles.

Kata kunci: Amorphophallus muelleri, iradiasi gamma (Co-60), keragaman morfologi, pemuliaan mutasi

INTRODUCTION

Amorphophallus muelleri Blume (*synonym Amorphophallus oncophyllus*), locally called *iles-iles* or *porang* is a new promising carbohydrate source in many Asian countries. Corm of *A. muelleri* contains large amount of glucomannan. Clinical study indicates that glucomannan is responsible for lowering lipids and glycemia (Sood *et al*., 2008). Glucomannan flour is used as food to relieve constipation and a raw material for many industries (Sugiyama and Santosa, 2008). In tropical climates, *A. muelleri* cultivation is preferable than *A. konjac* cultivation because *A. muelleri* is more tolerant to soil-borne diseases, viruses and shading, thus *A. muelleri* grow well in agroforests (Santosa *et al*., 2003; Sugiyama and Santosa, 2008).

Apomictic seeds and vegetative aerial bulbils are main propagation materials in *A. muelleri*, lead to low genetic variation (Sugiyama and Santosa, 2008). *A. muelleri* requires three years from seedlings to harvest of marketable corm size, ca. 1.5 kg or more (Sugiyama and Santosa, 2008). In spite of the high value of the crop in Indonesia, little research has been done to improve this species. Current productivity is considered low as compared with its potential yields ca. 3.5-4.0 kg plant^{-1}. Mine *et al*. (2010) stated that intraspecific competition on *A. muelleri* has contributed to low productivity. Low productivity and long cultivation period are main constrain for government

* Corresponding author. e-mail: edisang@gmail.com

to promote *A. muelleri* to farmers as commercial crop. Thus, breeding programs should be carried out in order to increase productivity.

Gamma irradiation is one of common practices to induce genetic variation in many plants species (de Micco *et al*., 2011) including tuberous crops. Gamma ray is an electromagnetic short wave with high energy. Mutation breeding using gamma irradiation has been applied in many food crops such as sorghum (Human and Sihono, 2010), upland rice (Ishak, 2012), lowland rice (Sobrizal, 2007), banana (Indrayanti *et al*., 2011), and in some Araceae such as Anthurium (Puchooa, 2005), and *Xanthosoma* sp (Blay *et al*., 2004), but till now no report about mutation breeding has been conducted on *Amorphophallus* species.

It is well known that sensitivity of plants to irradiation are dependent on many factors such as plant species or varieties, plant parts and irradiation dose (Esnault *et al*., 2010; de Micco *et al*., 2011). The optimum dose of gamma irradiation was 5 to 7.5 Gy for callus of *Anthurium* (Puchooa, 2005), and about 40 Gy for seed of *Anthurium andreanum* (Wegadara, 2008). The objective of this experiment was to study the effect of gamma irradiation on growth of *A. muelleri* seedlings.

MATERIAL AND METHODS

Gamma Irradiation

Application of gamma irradiation from Cobalt-60 (Gamma Chamber 4000A-USA) was conducted at Center for the Application of Isotope and Radiation Technology, National Nuclear Energy Agency (CAIRT), Indonesia on November 20, 2009. Field experiment was conducted at the Cikabayan Experimental Farm Bogor Agricultural University, Bogor (250 m above sea level) during November 2009-July 2010. The soil type was Latosol, with a pH 5.0, low total N (0.09%), medium phosphorus (18.0 ppm) and medium potassium (17.0 ppm) availability. Temperature during experiment was 25.7 ^{o}C (ranging from 20 to 34 ^{o}C) with relative humidity 84-86%.

Seed of *A. muelleri* from mother plants originated from Saradan Forest, East Java (planted in Bogor in 2002) was harvested on July 2009. Seeds were imbibed on moist paper at room temperature. Seeds with protruded leaf bud ca 2.0 mm were selected for treatment. Weight of selected seed was 20 g for 100 seeds. Gamma irradiation was applied for 10 levels with dose of 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 Gy, and non-irradiated seeds were used as control (0 Gy). One hundred seeds (±2.0 mm visual bud) were kept in a paper bag with three replicates per treatment. Seed is considered germinated when leaf bud had elongated ca 10.0 mm. Experimental procedure was presented in Figure 1.

Germination of Irradiated Seeds

Seedling bed was shaded with artificial shading net to reduce the light intensity by 50%. Growing media were composed of soil, goat manure and sand at ratio 4:2:1 (v/v). They were mixed well a week prior to planting. Seeds were planted on November 21, 2009 at distance 10 cm x 10 cm, 3-4 cm under soil surface. Carbofuran pesticide (Furadan 3G) was applied after planting at a rate of 2 kg ha^{-1} to prevent soil-borne diseases. Additional NPK (2:1:1) was applied at one month after planting at a rate of 200 kg ha^{-1}. Watering was carried out regularly by using overhead sprayers when rainfall below 10 mm per day. Weed and pest controls using fungicide Dithane M45 were carried out twice a month.

Variable data of germination, leaf size and morphology, the number of leaves, and yield were collected from M1 generation. Harvest was carried out individually after plant entered dormancy period, it was judged by leaf senescence. Outlier data was analyzed separately. Normal data was analyzed using F test; and significant variable was analysed Duncan's multiple range test at level 1% and 5%. Lethal dose 50% (LD_{50}) was calculated based on *curve-fit analysis.*

RESULTS AND DISCUSSION

Percentage of Germination

Gamma irradiation at dose of 10 Gy significantly delayed seed germination. At dose of 10 Gy irradiation, germination percentage of seedling increased steadily from 6 weeks after planting (WAP) until 14 WAP, and attained maximum at 56.3%. No seed germinated at a dose of 20 Gy and higher until 16 week after planting (WAP) (Table 1). In control plants without irradiation, germination rates attained its maximum within 4 (WAP). Most of non germinated seeds produced callus on its main bud, but failed to produce any leaf and roots. After 12 WAP, non developed callus and the seed died. According to Esnault *et al*. (2010), and Legue and Chanal (2010), exposure to irradiation causes the ionization of water, an integral component of living tissues, produces free radicals in addition to secondary reactive oxygen species (ROS), which triggers the activity of detoxifying enzymes to resist oxidative stress.

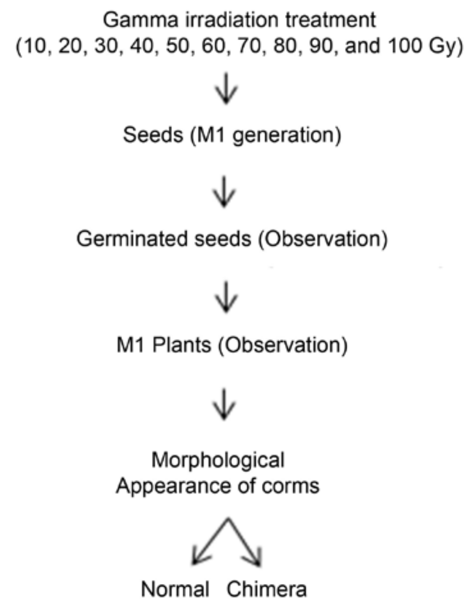

Figure 1. Experimental procedure

Irradiation at dose higher than 10 Gy and below 20 Gy is considered the lethal dosage (LD_{50}) for *A. muelleri* seedlings (Table 1; Figure 2). Similarly reported by Blay *et al*. (2004) mentioned that LD_{50} of plantlets of cocoyam is between 10-15 Gy, but it is lower than those doses for seeds of *Anthurium andreanum* (40 Gy) (Wegadara, 2008).

Morphological Variation

When seeds of *A. muelleri* were exposed to 10 Gy, 92% of seeds were germinated as indicated by elongation of their buds at 4 WAP. However, visual checking showed that some elongated buds were filled by a callus-like mass. Of these seeds, therefore, a total 43.7% of them died gradually up to 13 WAP, and 56.3% developed into seedlings at 14 WAP. Of the developed seedlings, 80.1% exhibited abnormalities in the first and the second leaves. The rest of seedlings had normal leaves. The most common abnormalities were stunted petiole, i.e., 94.2% of total abnormal plants. Petiole was classified as stunted when it was at least 50% shorter than those of control plants. The stunted petioles were also had malformed leaves such as twisted and cup shaped leaflets.

Variegate or chimera indicated by discoloration of leaflets was rarely found in irradiated plants, i.e., 4.3% of abnormal plants. The pattern of discoloration varies and appeared randomly from tiny white spot to half of leaflets or white stripe along the edge of leaflet or petioles. Loss of chlorophyll is common in plants after exposed to of high levels of irradiation (Kim *et al*., 2004; Abu *et al*., 2006; Ling *et al*., 2008; Al-Enezi and Al-Khayri, 2012). The effects of gamma rays on photosynthetic pigments vary among plant species and among cultivars (Kim *et al*., 2005), where carotenoid pigments are more sensitive than chlorophylls. Al-Enezi and Al-Khayri (2012) stated that in date palm (*Phoenix dactilyfera*) chlorophyll-a and carotenoids are more sensitive to irradiation than chlorophyll-b. Conversely, Abu *et al*. (2006) reported that an increase in chlorophyll-a and -b after irradiated gamma of cowpea (*Vigna unguiculata*), while in sweet orange (*Citrus sinensis*). Ling *et al*. (2008) obtained chlorophyll content was virtually insensitive to low doses of gamma irradiation. In *A. muelleri*, however, abnormalities including variegated in the first and second leaves resumed in the third and subsequent leaves. It is likely that variegated in *A. muelleri* is non permanent phenomenon. Puchooa (2005) stated that gamma irradiation leads to metabolism disturbance that causes the abnormal growth or necrosis. In *Dianthus*, Aisyah *et al*. (2009) reported that abnormal symptom of variegated flowers resumed in subsquence generation. It is speculated that the reversible symtom of variegated flowers is caused by diplontic selection where normal cells develop faster that those of abnormal ones.

Table 1. Percentage of germination of *A. muelleri* plants grown from seeds expossed to different level of gamma irradiation

Irradiation level (Gy)	Weeks after planting (WAP)						
	4	6	8	10	12	14	16
Control	83.7a	83.7a	83.7a	83.7a	83.7a	83.7a	83.7a
10	0b	1.0b	10.0b	29.8b	39.0b	56.3b	56.3b
20 to 100^{X}	0b	0c	0c	0c	0c	0c	0c

Means followed by the same alphabeth within a column were not significantly different by Duncan test at 1%. ^{X}Level irradiation on the basis of ten: 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy; Control-no treatment

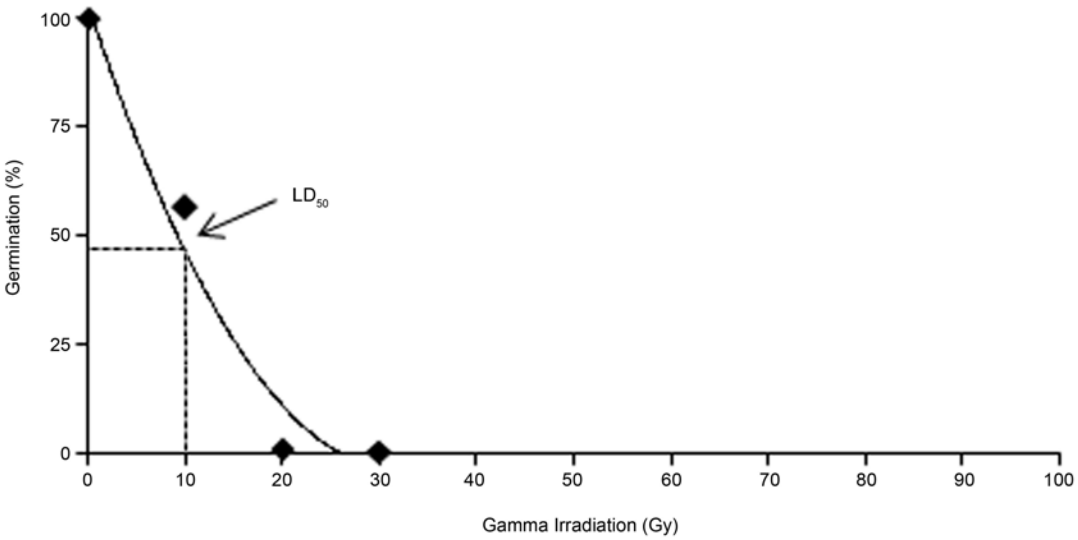

Figure 2. LD_{50}, 50% plants survive after gamma irradiation treatment

Leaf Characteristics

Number of leaf was affected by gamma irradiation (Table 2). Prior to dorman, control plants produced 2.6 leaves (2 to 4) while irradiated plants produced 2.8 leaves (1 to 5) on average. Leaves of irradiated plants were still green at 26 WAP (dorman at 27.3 WAP), while most control plants had entered dormancy at 18 WAP and leaves had withered. It is likely that leaves of irradiated plants stand longer than those of control plants. In *A. muelleri*, leaf life span was ca. 25 weeks and 18 weeks for irradiated and control plants, respectively. Longer leaf life span is desirable trait for *A. muelleri* because longer period of growing season may force plants to accumulate larger assimilates. This research implies that gamma irradiation is applicable to induce morphological variation in *A. muelleri*.

Petiole length and diameter, leaflets number and rachis width of the final leaf were larger on irradiated plants compared to control (Table 3). On average, first leaves of irradiated plants had smaller petiole (length and diameter), and rachis width. Santosa and Sugiyama (2007) reported that first leaf size is determined by corm size in *A. paeoniifolius*. It is probably that smaller size of first leaves of irradiated *A. muelleri* is caused by disturbance on nutrient mobilization from seed to growing point.

Merged and smooth (less serrate) leaflets were found, i.e., 11.5% and 10.0% of total abnormal leaves, respectively. Some abnormal leaflets also showed discoloration or excessive red color on the edges. Abnormal leaflets had reported in trifoliate white clover (*Trifolium repens* L) irradiated with gamma rays (Song *et al*., 2009). The abnormal leaves also included three leaflets with unusual angle, four to seven leaflets, and degenerated, merged and cup shaped leaflets that those were different from control plants.

Similar to discoloration, all abnormal leaflets recovered as of control plants in the third and subsquence leaves. It was likely that abnormal leaflets were temporary effect of gamma irradiation. It is probably that leaf of *A. muelleri* showed phenotypic plasticity. Song *et al*. (2009) stated that mutant phenotype of trifoliate white clover was not generated by genes that directly control leaf number because white clover shows morphological plasticity in response to various environmental stresses, in which those phenotypes were non heritable traits. Sugiyama and Santosa (2008) stated that normally *A. muelleri* seedling has 5 leaflets which distribute in all direction. Reduction of leaflets in seedling was possible because soil impedance during its emergence. Santosa and Sugiyama (2007) stated that diametral dissection main bud caused *A. paeoniifolius* produced abnormal initial leaf or flower. Nevertheless, physical soil impedance and bud damage were minimized in this experiment.

In irradiated plants, first leaf tended to have fewer leaflet than those of control plant (Table 3). However, many irradiated plants had larger leaf area because average leaflets width did not statistically different. Average ratio of leaflet (length to width) was 2:1 in control plants, but 3:1 in irradiated plants. Elongated leaflets of irradiated plants were thicker than those of control. Thus, larger size of the final leaves of irradiated plants was likely to have larger photosynthetic capacity, although number of leaf in early growth was significantly lower.

Table 2. Number of leaf of *A. muelleri* plants grown from seeds exposed to different level of Gamma irradiation

Irradiation level (Gy)	Week after planting (WAP)[X]							
	8	10	12	14	16	18	20	22
Control	1.0a	1.5a	1.9a	2.0a	2.4a	2.6a	-	-
10	1.0a	1.0b	1.1b	1.2b	1.6b	2.2b	2.5a	2.8a
20 to 100[Z]	- [Y]	-	-	-	-	-	-	-

Means followed by the same alphabeth within a column were not significantly different by Duncan test at 1%; [X]Measured from ten germinated sample plants; [Y]No germinated plant was found; [Z]Level irradiation on the basis of ten: 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy; Control-no treatment

Table 3. Leaf characteristics of *A. muelleri* plants grown from seeds expossed to different level of Gamma irradiation

Irradiation level (Gy)	Petiole length (cm)		Petiole diameter (cm)		Leaflets number		Rachis width (cm)	
	First	Last[Y]	First	Last	First	Last	First	Last
Control	3.5±0.5a	23.0±0.8b	0.3±0.01a	0.9±0.02a	5.7±0.5a	13.7±0.7b	4.8±0.2a	11.5±0.7b
10	2.6±0.5b	27.2±2.9a	0.2±0.03b	1.0±0.22a	5.5±0.5a	18.8±2.8a	3.5±0.6b	16.6±2.9a
20 to 100[X]	-[Z]	-	-	-	-	-	-	-

Means ±SD followed by the same alphabeth within a column were not significantly different by Duncan test at 1%; [X]Level irradiation on the basis of ten: 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy; Control-no treatment; [Y]First leaves measured at 6 weeks after emergence (WAE). Measured from third or fourth leaf for control plants at 16 WAE; from fourth or fifth leaf for 10 Gray irradiation at 21 WAE; [Z]no leaf emerged

Corm Yield

Control plants were harvested at 18 WAP, while some irradiated plants were harvested at 28 WAP, indicated that gamma irradiation prolonged vegetative period. Sugiyama and Santosa (2008) suggest that in order to enhance productivity of *A. muelleri*, agronomic treatments is aimed to extend vegetative period in a growing cycle or to prevent dormancy.

Since vegetative periode determined accumulation of assimililates, therefore, longer vegetative period in this experiment was desirable trait in *A. muelleri* growing. This confirmed by production of large corm from irradiated plant than control plant Table 4). Beside longer vegetative period, it was probably that heavier of corm from irradiated plants was due to longer leaf life span and larger leaf size (Table 3). According to Sugiyama and Santosa (2008), *A. muelleri* with larger number of leaf and longer leaf life span of each leaves produced larger corm.

Although, no corms abnormalities were found of irradiated plants, however corm diameter of gamma irradiated plants was smaller than that of control plants (Table 4). It was unclear whether the diameter was affected by gamma irradiation. Sugiyama and Santosa (2008) and Mine *et al*. (2010) stated that planting depth and limited rooting volume determine corm shape and diameter of *A. muelleri*.

This research implied that gamma irradiation enable to induce morphological variation in *A. muelleri*. However, it was unclear whether the morphological variation of irradiated plants would also present in M2 generation. In the field, one vegetative cycle generally resumed in 6 months followed by dorman period for another 6 months, and plants start to produce seeds after 3 to 4 years. Therefore, it would be interesting to investigate the production of second generation (M2) of promising lines in the field in the near future.

Table 4. Corm size of *A. muelleri* plants grown from seeds exposed to different level of Gamma irradiation

Irradiation level (Gy)	Fresh weight (g)	Diameter (cm)
Control	14.2b	3.1a
10	20.4a	2.6b
20 to 100[X]	-[Y]	-

Means followed by the same alphabeth within a column were not significantly different by Duncan test at 1%; [X]Level irradiation on the basis of ten: 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy; Control-no treatment; [Y]no leaf emerged

CONCLUSION

Gamma irradiation induced morphological variations in *A. muelleri* at M1 seedlings, e.g., leaf shape, number of leaves, time to harvest and daughter corm size. *A. muelleri* seeds had LD_{50} close to 10 Gy, and irradiation at doses of 20 Gy and higher caused lethal effect. Gamma irradiation delayed plant germination, and induced leaf abnormalities of first and second leaves, i.e., variegated, merged and elongate leaflets, and stunted. Abnormalities mostly recovered in third and fourth leaves. Some accessions from 10 Gy extended the vegetative period up to 8 weeks longer than control plants, leading to production of heavier doughter corms.

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