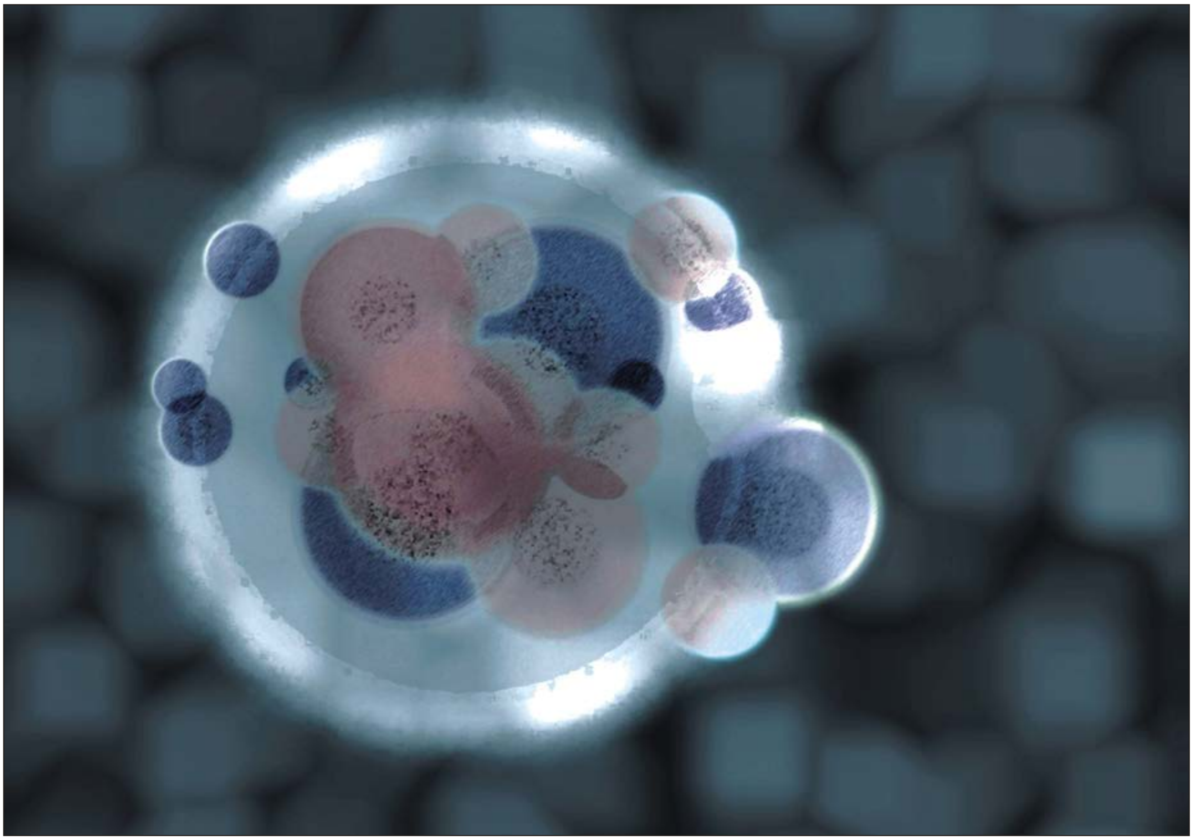

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Effects of Gamma Radiation on Germination and Growth Characteristics of Physic Nut (*Jatropha curcas* L.)

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Abstract: Narrow genetic base is a main problem hindering the progress of physic nut breeding. The objective of this study was to compare the effects of different levels of gamma radiation on germination percentage, survival percentage and growth of five physic nut genotypes. The seeds were treated with six levels of gamma radiation (0, 200, 400, 600, 800 and 1000 Gy) and the 5×6 treatment combinations were arranged in a randomized complete block design with three replications. Data were recorded for germination percentage, survival percentage at 15 Days After Germination (DAG) ($GR50_{(15)}$) and survival percentage at 30 DAG ($LD50_{(30)}$). As the seedlings did not survive at 600, 800 and 1000 Gy, these treatments were deleted from the successive evaluations of plant height, stem diameter and leaf number. Higher doses of gamma radiation resulted in the significant reductions in germination percentage, survival percentage and plant height. The germination percentages were from 4.0% for 1000 Gy to 82.5% for 0 Gy. The seeds treated with gamma radiation higher than 600 Gy resulted in complete mortality. Difference among genotypes was not significant for these characters. The dosages of gamma radiation to obtain LD50 based on survival percentage 30 DAG was 425 Gy and was not dependent on genotypes. The information is useful for gamma radiation induction of mutation breeding for physic nut.

Key words: GR50, induced mutagenesis, LD50, mutation breeding, co-gamma

INTRODUCTION

Physic nut (*Jatropha curcas* L.) is originated in Central America and the commercial plantations with economic importance were first established in the Cape Verde Islands for lighting oil and other products. Physic nut was introduced into Africa and Asia by Portugal traders and now it is important as the oil crop for biodiesel production in the tropics (Heller, 1996).

Ambrosi *et al.* (2010) found that seeds of physic nut commercialized worldwide include a few closely related genotypes which are not representative of the original Mexican gene pool, revealing high degrees of homozygosity for single varieties and very low genetic diversity between varieties. Narrow genetic base would be expected in the physic nut varieties used for cultivation in Asia and Africa. The lack of wide genetic base hinders the progress in physic nut breeding and the use of un-adapted varieties leads to low and unpredictable yield.

Tanya *et al.* (2011) found low ISSR variability within groups (37%) of physic nut germplasm collected in Thailand, Vietnam and China but the variability of related species was rather high (63%). Among 225 accessions of physic nut from Africa, Asia and Latin America, Montes *et al.* (2009) found low genetic variability in accessions from Africa and Asia but high in accessions from Guatemala and other Latin American countries. In Kenya, moderate to high genetic variation within populations and among populations of physic nut was reported and the high genetic variation in this case is possibly due to several introductions from many countries (Machua *et al.*, 2011).

Although genetic variability of physic nut is high in the center of origin, most physic nut breeding programs have been carried out outside the center of origin. To the best of our knowledge, the international organization for conservation and free transfer of germplasm has not been established for physic nut. Therefore, physic nut breeders outside the center of origin face difficulty in obtaining good germplasm sources for their breeding programs (Divakara *et al.*, 2010; Datta, 2009).

By using available germplasm, mutation breeding is an alternative way to increase genetic variations of economically important characters. This could be

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achieved through chemical and physical mutations. Gamma radiation is another way to induce mutation and create genetic variations in narrow-based germplasm (Datta, 2009).

In physic nut, attempts have been made to induce mutation through radiation treatment of the seeds. Treated seeds with gamma radiation at 50 to 250 grays showed no significant difference between treated seeds and un-treated seeds for germination percentage and survival percentage. Therefore, the medial lethal dose that causes 50% mortality of the seeds (LD50) is to be determined and seeds of different genotypes that may respond differently is also needed to be addressed. The objectives of this study were to find out the optimum doses for radiation treatment of physic nut seeds and to study the sensitivity of different physic nut genotypes to gamma radiation treatments. The information obtained will be useful as a guideline for mutation breeding using gamma ray.

MATERIALS AND METHODS

Five physic nut genotypes collected from different parts of Thailand were used in this study. These genotypes including 18/36, 19/18, 20/99, 18/42 and Check-I were kindly provided by the Khon Kaen Agricultural Research and Development Center at Thapra, Khon Kaen province. The Check-I is a promising variety selected from multiple yield trials. The seeds of these genotypes were treated with acute gamma radiation at the doses of 0, 200, 400, 600, 800 and 1000 grays at the Department of Applied Radiation and Isotope, Kasetsart University in Bangkok.

The M1 seeds were used in this study. Germination test was carried out in a greenhouse at the Horticulture Department, Khon Kaen University in 2010. The seeds were germinated in trays with holes for drainage containing sand. A 5×6 factorial experiment in a randomized complete block design with three replications was used. Five physic nut genotypes were assigned as factor A and six gamma radiation doses were assigned as factor B. There were 25 seeds for each sampling unit. The data were recorded for germination percentage at 5-10 days after sowing. The germinated seeds were later transferred to plastic bags at 15 days after germination when survival percentage at 15 days after germination was recorded. The plants were allowed to grow in plastic bags for 15 days and survival percentage at 30 Days After Germination (DAG) was recorded.

The survival percentage at 15 days after germination was then calculated as follows:

$$\text{Survival (\%)} = \left[\frac{\text{No. of survival plants at 15 DAG}}{\text{No. of seeds}}\right]\times 100$$

The survival percentage at 30 DAG was then calculated as follows:

$$\text{Survival (\%)} = \left[\frac{\text{No. of survival plants at 30 DAG}}{\text{No. of seeds}}\right]\times 100$$

The survival as percent of control at 15 DAG ($GR50_{(15)}$) was then calculated as follows:

$$\text{GR50 (15)} = \left[\frac{\text{No. of survival plants at 15 DAG}}{\text{No. of germinated plants}}\right]\times 100$$

The survival as percent of control at 30 DAG ($LD50_{(30)}$) was then calculated as follows:

$$LD50_{(30)} = \left[\frac{\text{No. of survival plants at 30 DAG}}{\text{No. of germinated plants}}\right]\times 100$$

The healthy seedlings with one-month old were later transplanted into pots containing rice husk, compost, chopped coconut husk and soil at the ratio of 2:2:2:1 by volume. As there was no survival seedling for gamma radiation treatments at 600, 800 and 1000 Gy, these treatments were deleted from the experiment. A 5×3 factorial experiment in a randomized complete block design with six replications was further carried out. Five physic nut genotypes were assigned as factor A and three gamma radiation doses were assigned as factor B. There were four pots for each sampling unit each of which contained one plant. The plants were allowed to grow until 72 days after transplanting and the data were evaluated for stem diameter, plant height and leaf number per plant at seven-day intervals from 30 days after transplanting until 72 days after transplanting. Other morphological characters such as leaf color and leaf shape were also observed.

Statistical analysis: The data were subjected to analysis of variance according to a factorial experiment in a randomized complete block design (Gomez and Gomez, 1984). Where the main effects were significant, mean separation was carried out using Duncan's Multiple Range Test (DMRT). All calculations were done in MSTAT-C and graphical presentation was done in Microsoft Excel.

RESULTS

Analysis of variance showed significant differences ($p\geq 0.01$) among gamma ray treatments for germination percentage, survival percentage at 15 Days After Germination (DAG), survival percentage at 30 DAG (Table 1), stem diameter, plant height and number of leaf per plant (Table 2). The genotypes were not statistically different for these characters and the interactions

Fig. 1: Seedlings at germination of physic nut subjected to different levels of gamma radiation

Table 1: Mean squares for germination, survival percentage at 15 days after germination (DAG) and survival percentage at 30 DAG of five physic nut genotypes subjected to six levels of gamma radiation

			Survival percentage	
Source of variation	df	Germination	15 DAG	15 DAG
Replication	2	308.1	60.8	65.7
Genotype (A)	4	171.6	20.5	48.2
Gamma dose (B)	5	17211.0**	21957.0**	22602.0**
A x B	20	73.6	35.4	39.0
Error	58	165.4	76.2	78.8

** Significant at 0.01 probability level, df: Degree of freedom

Table 2: Mean squares for stem diameter, plant height and number of leaves per plant of five physic nut genotypes subjected to six levels of gamma radiation evaluated from germination to 72 Days After Transplanting (DAT)

Source of variation	df	Stem diameter	Height	Number of leaves
Replication	5	4.19	19.9	45.10
Genotype (A)	4	1.24	6.1	0.85
Gamma dose (B)	2	156.00**	1367.0**	17.00**
A x B	8	1.74	10.9	3.82
Error	70	1.51	5.7	2.69

** Significant at 0.01 probability level df: Degree of freedom

Table 3: Germination percentage, survival percentage at 15 days after germination (DAG), survival percentage at 30 DAG, stem diameter (mm), plant height (cm) and number of leaves per plant of five physic nut genotypes subjected to six levels of gamma radiation evaluated from germination to 72 Days After Transplanting (DAT)

		Survival percentage				
Genotype	Germination	15 DAG	30 DAG	Stem diameter[1]	Height[1]	Number of leaves[1]
18/36	45.7^a	32.6^a	32.6^a	11.9^a	23.3^a	4.39^a
19/18	43.6^a	31.9^a	31.9^a	11.3^a	23.3^a	4.06^a
20/99	47.4^a	34.4^a	36.2^a	11.6^a	24.4^a	3.78^a
18/42	50.7^a	33.0^a	33.4^a	11.6^a	23.9^a	4.11^a
Check-I	50.6^a	34.2^a	34.2^a	11.9^a	24.6^a	4.06^a
Mean	47.6	33.2	33.6	11.6	23.9	4.10
CV(%)	27.0	26.2	26.3	10.5	9.9	40.20

[1] Stem diameter, height and leaf number were calculated from plants receiving 0, 200 and 400 grays and evaluated at 72 DAT. Means in the same column with the same letter(s) are not statistically different by Duncan's multiple range test (DMRT) at $p \leq 0.05$

Table 4: Effects of gamma radiation on germination percentage, survival percentage at 15 days after germination (DAG), survival percentage at 30 DAG, stem diameter (mm), plant height (cm) and number of leaves per plant of five physic nut genotypes subjected to six levels of gamma radiation evaluated from germination to 72 days after transplanting

		Survival percentage				
Gamma dose	Germination	15 DAG	30 DAG	Stem diameter[1]	Height[1]	Number of leaves[1]
0 Gy	82.5^a	79.6^a	79.6^a	12.8^a	29.2^a	3.20^b
200 Gy	77.3^a	74.6^a	77.1^a	13.1^a	26.2^b	4.50^a
400 Gy	67.7^b	45.3^b	45.3^b	9.0^b	16.3^c	4.53^a
600 Gy	42.9^c	nd	nd	nd	nd	nd
800 Gy	11.3^d	nd	nd	nd	nd	nd
1000 Gy	4.0^d	nd	nd	nd	nd	nd
Mean	47.6	66.5	67.3	11.6	23.9	4.08
CV(%)	27.0	26.2	26.3	10.53	9.92	40.22

nd = not determined (High germination was still observed at 600 Gy but the germinated seedlings did not survive.). [1] Stem diameter, height and leaf number were calculated from plants receiving 0, 200 and 400 grays and evaluated at 72 DAT. Means in the same column with the same letter(s) are not statistically different by Duncan's multiple range test (DMRT) at $p \geq 0.05$

between gamma ray treatment and genotype were also not significant ($p \geq 0.05$) (Table 1, 2). The overall means of the experiment across genotypes and radiation treatments were 47.6% for germination, 33.2% for survival at 15 DAG, 33.6% for survival at 30 DAG, 11.6 mm for stem diameter, 23.9 cm for plant height and 4.08 leaves for number of leaves per plant (Table 3).

The differences among gamma ray treatments were observed for germination percentage, survival percentage at 15 DAG and survival percentage at 30 DAG, stem diameter, plant height and number of leaves per plant (Table 4). On average, germination percentages were from 4.0% for 1000 Gy to 82.5% for 0 Gy. The seeds treated with 600-1000 Gy did not survive and hence the calculation of survival percentage was done for 0, 200 and 400 Gy (Fig. 1). Survival percentages at 15 DAG were in a range of 45.3% for 400 Gy and 79.6% for 0 Gy and germination percentages at 30 DAG were in a range of 45.3% for 400 Gy and 79.6% for 0 Gy.

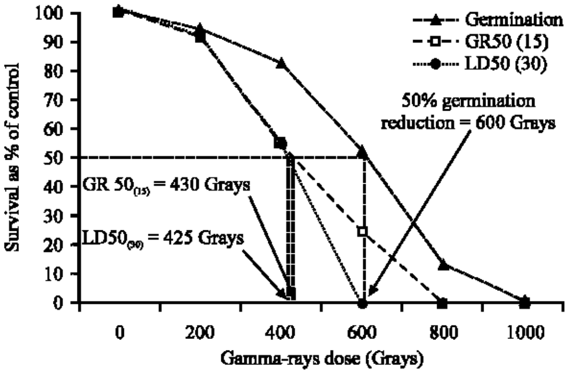

Fig. 2: Germination percentage, survival as percent of control at 15 days after germination and survival as percent of control at 30 days after germination of five physic nut genotypes subjected to six levels of gamma radiation

Stem diameters were lowest (9.0 mm) for the seeds receiving 400 Gy of gamma radiation and highest (13.1 mm) for the seeds receiving 200 Gy (Table 4). Plant heights were lowest (16.3 cm) for the seeds receiving 400 grays and highest (29.2 cm) for the seeds receiving 0 Gy. Leaf numbers, in contrast, were highest (4.53 leaves) for the seeds receiving 400 Gy and lowest (3.20 leaves) for the seeds receiving 0 Gy.

The seeds receiving 0 Gy and 200 Gy of gamma radiation were not statistically different for germination percentage, survival percentage at 15 and 30 DAG and stem diameter but they were statistically different for plant height and number of leaves per plant (Table 4). The reductions in germination, survival percentage at 15 DAG, survival percentage at 30 DAG and stem diameter were observed at 400 Gy but not at 200 Gy. Gamma radiation significantly reduced plant height observed at 200 Gy and 400 Gy. Numbers of leaves per plant were higher in the seeds receiving 200 and 400 Gy (4.53 and 4.50 leaves, respectively) than those receiving 0 Gy (3.20 leaves).

The germination of the control seeds was lower than 100% and the data were then converted to 100% germination and the germination of other gamma radiation treatments were calculated accordingly (Fig. 2). The data for germination percentage, survival as percent of control at 15 DAG ($GR50_{(15)}$) and 30 DAG ($LD50_{(30)}$) were plotted against gamma radiation dosages to determine Lethal Dose (LD50) of the radiation. LD50 for germination percentage could be as high as 600 Gy. However, at 600 Gy, the germinated seeds did not survive and germination percentage alone was not a good prediction for LD50. The $GR50_{(15)}$ and $LD50_{(30)}$ are good predictions for LD50 and the LD50 for these criteria was at 430 and 425 Gy, respectively (Fig. 2).

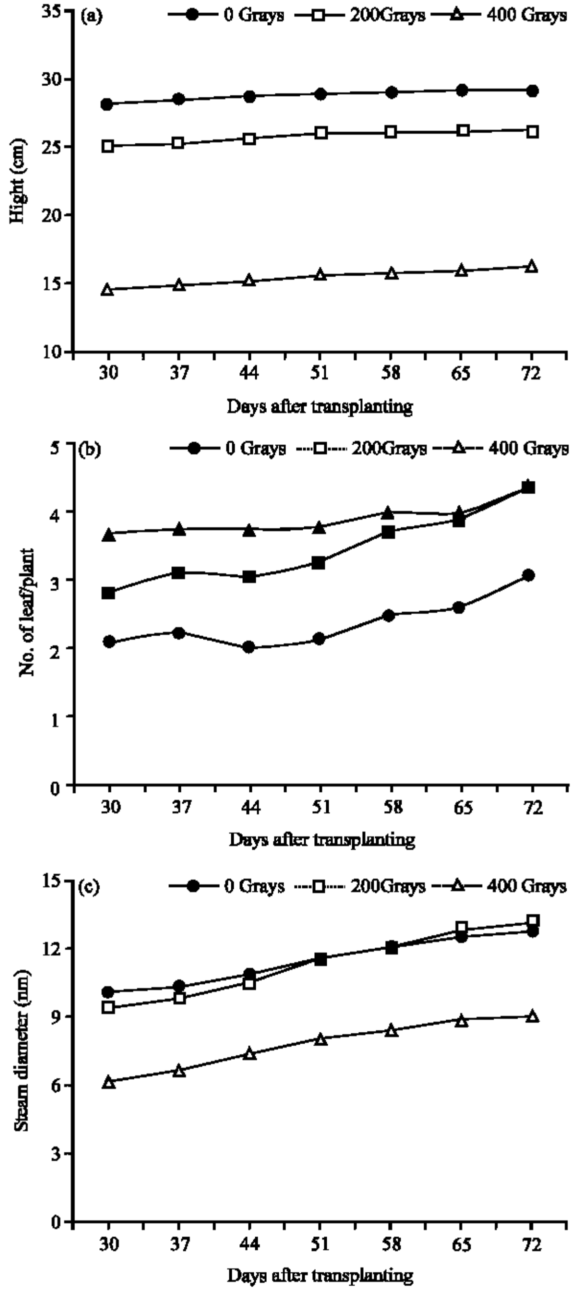

Fig. 3(a-c): Means for plant height (a), number of leaves per plant (b) and stem diameter (c) of five physic nut genotypes seedlings subjected to different levels of gamma radiation

Figure 4 showed growth patterns of the seeds treated with 0, 200 and 400 Gy for plant height, stem diameter and number of leaves per plant. Gamma radiation greatly reduced plant height and the effect was highest at 400 Gy (Fig. 3). Gamma radiation also reduced stem diameter but

Fig. 4: Seedlings at 30 days after germination of physic nut subjected to different levels of gamma radiation (0, 200 and 400 grays, respectively)

the effect was significant at 400 Gy. Gamma radiation significantly increased leaf number for both at 200 and 400 Gy.

DISCUSSION

Exposure to gamma radiation is known to produce morphological mutants, physiological mutants and biochemical mutants. As physic nut is a long-lived perennial shrub crops, these types of mutants have been sought to increase genetic variation (Nambisan, 2007). Gamma radiation has been reported to have beneficial effects on many crops. In African violets (*Saintpaulia ionantha* H. Wendl.), gamma radiation at 15 Gy improved plant architecture of the mutants (Seneviratne and Wijesundara, 2007). In wheat, gamma radiation improved germination, plant height, grain per plant and grain yield at 200 Gy (Jamil and Khan, 2002) and 300 to 400 Gy (Irfaq and Nawab, 2001). In this study, the estimation of medial lethal dose and the sensitivity of different physic nut genotypes to gamma radiation were reported.

In earlier studies, Wang *et al.* (2009) found that LD50 doses of gamma radiation were 178 to 198 Gy for seeds of physic nut from different sources in China and India. Dhakshanamoorthy *et al.* (2010) exposed physic nut seeds to gamma radiation at 50 to 250 Gy and found that these doses were still lower than LD50 dose. In this study, LD50 dose was estimated roughly as 425 Gy and there was no difference among seed sources. LD50 doses found in this study were rather higher than those reported previously.

In snap bean (*Phaseolus vulgaris*), however, the LD50 for germination could not be determined from the doses 300 Gy to 800 Gy because the radiation had small effect on germination and some treatments had higher germination than control (Ellafa *et al.*, 2007). Kon *et al.* (2007) reported in long bean (*Vignas esquipedalis*) that LD50 for germination ranged between 600-800 Gy and also in tomato and okra (Norfadzrin *et al.*, 2007). In contrast, gamma radiation doses of 200 to 800 Gy did not have significant effect on germination in pepper grass (*Lepidum sativum* L.) (Majeed *et al.*, 2009) and castor bean (*Ricinus communis*) was sensitive to gamma radiation higher than 2000 Gy for germination (Kobori *et al.*, 2010). LD50 dose of 425 Gy in this study was much lower than those in previous studies.

The differences in LD50 doses of different studies might be possibly due to seed moisture rather than seed source and LD50 doses were also different among species. Seeds with low moisture seem to be more tolerant to gamma radiation than the seeds with high moisture. Although seed moisture was not determined in this study, it could be assumed that the seeds had low moisture (8-9%) because the seeds were sun-dried and stored for a period in cool room.

In comparison to physic nut cuttings that had much higher moisture than seeds, Dwimahyani and Ishak (2004) found that exposure of physic nut cuttings to 10-25 Gy doses of gamma radiation was sufficient to induce mutation and Li-Rong *et al.* (2008) found that LD50 doses for leaf segments, nodular stem segments and stem segment of Pogostemoncablin *in vitro* were 72, 64 and 66 Gy, respectively.

Gamma radiation at higher doses resulted in the reduction in germination (200-400 Gy) to complete fatality (600-1000 Gy) of physic nut seeds. Dhakshanamoorthy *et al.* (2010) found that seeds treated with 50 Gy of gamma rays revealed stimulatory effect except for germination, whereas 250 Gy dose showed inhibitory effects on traits compared to other treatments. This could be due to the damage in seed tissues and the severity of the damage depending on the dosage used (Datta, 2009). Gamma radiation had inhibitory effects on physiological and physical traits (Khan and Goyal, 2009) and also caused mutation (Dhakshanamoorthy *et al.*, 2011). The inhibitory effects of gamma radiation were also reported in chickpea (Khan *et al.*, 2005) mungbean (Khan and Goyal, 2009) and snap bean (Ellafa *et al.*, 2007).

Dhakshanamoorthy *et al.* (2010) and Dhakshanamoorthy *et al.* (2011) used gamma radiation of 50-250 Gy and Datta (2009) used 60-240 Gy to induce mutation in physic nut. The previous studies used the rates that may be sufficient for induction of mutation inn physic nut seeds. In this study, we reported lethal dose of 600 Gy that induced complete fatality in physic nut seeds. The gamma radiation doses at the range of 200-400 Gy are suitable for the mutation induction in physic nut seeds. At these intensities, the M1 plants can survive and change in characters such as shorter plant, higher leaf number, shorter internodes and higher branch number that may contribute to higher yield.

In this study, the rates of gamma ray for mutation in physic nut were in the range of 425 to 430 Gy according to GR50 dose at 15 DAG and LD50 dose at 30 DAG, respectively. However, the observations were carried out in the seedlings of the M1 generation and further investigation in the more advanced generations are still required to ensure the stability of the traits such as shorter plant, early flowering, male and female flower ratio, fruiting and yield under field conditions. Molecular study for mutants and original plants is also necessary to identified genetic changes in the mutants.

CONCLUSION

Higher dosages of gamma radiation reduced germination of physic nut seeds, number of survival plants and plant height. The differences between physic nut varieties for these traits were not statistically significant. The dosages that are suitable for mutation induction in physic nut seeds are in the range of 425-430 Gy according to medial lethal dose at 15 and 30 days after germination. The information provides basic requirements for the use of gamma radiation for mutation induction in physic nut.

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