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Improvement of Coleus Performance through Mutation Induction using Gamma Ray Irradiation

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Abstract

The objective of this study is to study the effect of gamma ray irradiation (15, 30, 45, and 60 gray (Gy) to determine Coleus Lethal Dose 50 (LD_{50}) values, and to obtain new Coleus variances in a relatively short time. The study was conducted in a greenhouse at Cikabayan experimental field, Bogor Agricultural University, Darmaga, Bogor in May to July 2013. Gamma irradiation treatment significantly affected Coleus height, number of leaves, and number of nodes. Increasing the dose level of gamma irradiation tend to inhibit plant growth. LD_{50} for yellow/green, green/brown, variegated green/brown of *Coleus blumei*, and *Coleus amboinicus* Lour were 48.66, 65.2, 52.81, and 37.62 Gy, respectively. *C. amboinicus* irradiated at a dose level of 45 Gy had different leaf shapes compared to control.

Keywords: *Coleus*, gamma ray irradiation, LD_{50} values, ornamental plant, torbangun

Introduction

Coleus amboinicus, or torbangun, is one of the ornamental species that has medicinal properties. Torbangun consumption significantly increased iron, potassium, zinc, and magnesium in breast milk (Damanik, 2005). As a foliage ornamental plant, Coleus has been known to the general public as well as by ornamental plants lovers as potted plants, ground cover, or divider plants. The main attraction of Coleus is its bright leaf colors, and there are varieties of Coleus that have multiple color in a leaf. Coleus has serrated and ear-shaped leaves, with the height ranging from 60 to 90 cm (Croxton and Kessler 2007).

Aesthetic values of ornamental plants, including the diversity of leaf shape and leaf colors, will affect the economic values of the plant. New varieties with more new diverse phenotypes can be created through conventional breeding techniques or by induced mutations. Rose induced mutation through gamma irradiation could affect its flower color (Soedjono, 2003). Conventional breeding techniques such as hybridization or selection would require a long time, so it will be easier through a process of induced mutation.

Mutation is a sudden change in the genetic material of a cell that includes a change in the level of genes, molecular, or chromosome (Poehlman and Sleper, 1985). Spontaneous mutations that occur naturally take longer than artificial mutation. Induced mutations through gamma irradiation are an alternative to getting a new type of Coleus in a relatively shorter time.

In this research, induced mutation by gamma irradiation was conducted to find the lethal dose 50 (LD_{50}) levels of the Coleus plant. Crowder (2006) reported the use of gamma ray as an alternative to physical mutation breeding due to gamma ray emitted from a radioactive isotope has a shorter wavelength and has a penetrating power which is more powerful than X-ray. Poespodarsono (1988) added that artifical mutation could occur if the mutagen is used at appropriate dose and time of application.

The purpose of this study was to (1) study the effect of the application of multiple doses of gamma irradiation on the performance of four Coleus varieties, (2) obtain (LD_{50}) values of gamma irradiation to induce mutations in four varieties of Coleus, and to (3) obtain new variance of Coleus plants.

Materials and Method

The experiment was conducted at the Experimental farm greenhouse Cikabayan, Bogor Agricultural

University (IPB), Darmaga, Bogor. Gamma irradiation was conducted at the Center for Isotope and Radiation Technology Applications (PATIR), Nuclear Energy Agency (BATAN), Pasar Jumat, South Jakarta. This study was conducted from May to July 2013.

Coleus cuttings were obtained from plant stores at Padjadjaran Street, Bogor. Four types of *Coleus blumei* used are *yellow/green (V1)*, *green/brown (V2)*, *variegated green/brown of C. blumei (V3)*, *and C. amboinicus* (V4). Other materials are nursery medium charcoal, soil, cow manures, seedling trays, polybag 25 cm x 25 cm, and Rooton F. The radiation was conducted in IRPASENA Gamma Chamber. Leaf colors were evaluated using Royal Horticulture Color Chart (RHCC) as a reference.

The experiment was arranged in a randomized block design consisting of two factors: Coleus species and dose levels of gamma ray. Dose levels used are 0, 15, 30, 45, and 60 Gy. Each treatment was repeated three times and each replicate consisted of three plants, with a total of 180 plants

Scoring was conducted weekly starting one week after planting (WAP; starting at transplanting date) on (1) plants height (cm), measured from the soil surface to the highest growing point, (2) number of leaf, (3) number of stem per plant, (4) LD_{50}. Qualitative variables observed were (1) leaf color (using RHCC), and (2) phenotypic performance, particularly changes in leaf shapes and color after irradiation.

Ld_{50} values were obtained from the calculation of the percentage of plants alive in a certain period of time after irradiation at a particular dose level by using a curve fit analysis CurveExpert 1.3. The model with the highest correlation coefficient (r) was used as the best fit model.

Result and Discussion

Lethal Dose 50 (LD_{50})

The percentage of plants alive following irradiation at 45 and 60 Gy varies with varieties (Table 1). All *C. blumei* yellow/green, green/brown, and variegated green/brown survived following irradiation at 0 to 30 Gy, whereas many of the *C. amboinicus* died following 15 Gy gamma irradiation. In general, *C. blumei* green/brown had the highest percentage of survived plants than other varieties. The higher irradiation doses, the smaller the percentage of plants survived.

LD_{50} is the dose of irradiation resulting in the death of 50% of the plant population. This dose tended to produce the most mutants. According Boerjes and Van Harten (1988) the range of the applied irradiation dose level is very important in determining the optimum dose at which the plant will be irradiated. However, no previous studies reporting the optimum doses of gamma irradiation on Coleus cuttings. Therefore, the doses of 15-60 Gy were used.

Crop sensitivity to radiation levels depends on cultivar, plant parts that received radiation, types of radiation, and radiation techniques (Aisyah, 2006). Radio-sensitivity could be observed from the LD_{50} values, the presence of growth inhibition or lethality, somatic mutations, chromosomal fractures, as well as the number and size of chromosomes. Djojosoebagio (1988) added that radio-sensitivity was one of the important criteria used to determine the responses of cells to irradiation.

Table 1. Percentage of Coleus plants survived at eight weeks after Gamma ray irradiation

Dose (Gy)	Coleus species			
	Yellow/green *C. blumei*	Green/brown *C. blumei*	Variegated green/brown *C.blumei*	*C. amboinicus* Lour
	Plants alive (%)			
0	100.00	100.00	100.00	100.00
15	100.00	100.00	100.00	88.89
30	100.00	100.00	100.00	77.78
45	55.56	66.67	77.78	22.22
60	11.11	55.56	11.11	0.00

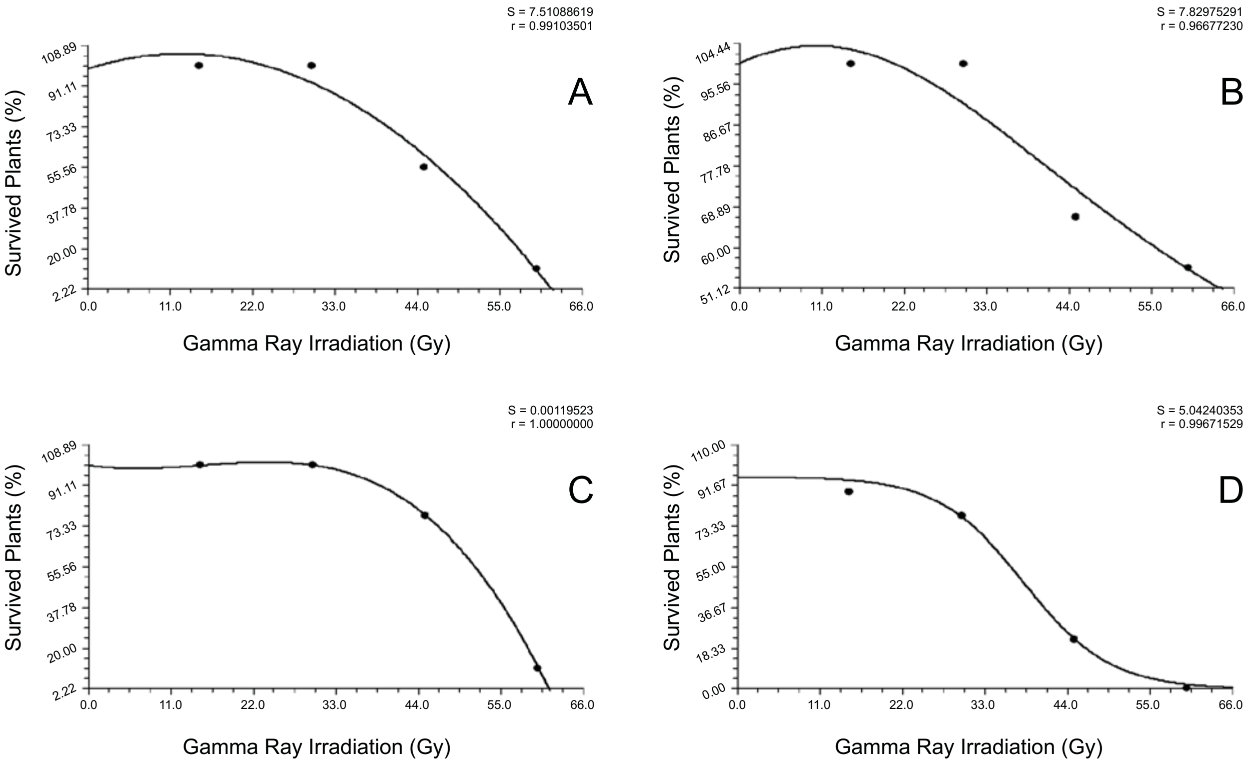

Figure 1. Percentage of Coleus plants alive following irradiation treatment; (A) Yellow/green (upper left) *C. blumei*, (B) *Green/yellow* (upper right) (C) *C. blumei*, variegated green/brown *C. blumei* (lower left), (D)*C. amboinicus* (lower right). Graphs were created using CurveExpert 1.3.

The percentage of plant survival of the four Coleus varieties was sequentially described by quadratic Fit, Reciprocal Quadratic, Polynomial Fit, Logistic Model functions (Figure 1). These functions are the best fit according to the fit curve analysis with the value of reliability models sequentially r = 0.991, r = 0.967, r = 1, r =0.997 and has equations sequentially $y = 98.729 + 1.058x - 0.042x^2$, $y = \frac{1}{0.009 - 6.959e^{-005}X + 3.42X6e^{-006}X^2}$, $y = 100 - 0.494x + 0.0494x^2 - 0.001x^3$, $y = \frac{a}{1 + 0.001e^{0.175X}}$. LD_{50} values obtained from the equation results in a sequence were 48.66 Gy, 65.21 Gy, 52.81 Gy, and 37.62 Gy (Figure 1).

Radio sensitivity varies with plant species (Ray 2000). Radio sensitivity depend on the content of the nucleus (the more DNA content, the more sensitive the plants), and ploidy level (the higher the ploidy level, the lower the sensitivity). Climatic factors and other environmental conditions before and after irradiation treatment also affected plants' radio sensitivity.

Coleus Height, Number of Leaves and Nodes

Varieties interacted with doses of gamma ray in affecting Coleus height, number of leaves and number of nodes (Table 2). Irradiation treatment inhibited Coleus growth in terms of height, number of leaves and number of nodes (Table 2). The higher the dose level applied, the more growth inhibition occurred (Table 2). In general the number of leaves of the irradiated Coleus were smaller than the Coleus without irradiation, except in green/brown *C. blumei* irradiated with 30 Gy. Green/brown *C. blumei* irradiated at 15 Gy had a fewer number of leaves compared to the same variety that was irradiated at 30 Gy. This is presumably due to the random effects of gamma-ray irradiation. The random mutation due to irradiation was resulting from the random energy emitted to the target plants (Isaac 2007).

Tabel 2. The effects of gamma-ray irradiation on Coleus height, number of leaves and nodes at 11 weeks after planting

Radiation Dose (Gy)	Coleus species			
	Yellow/green *C. blumei*	Green/brown *C. blumei*	Variegated green/brown *C.blumei*	*C. amboinicus*
Height growth (cm)				
0	25.94±4.05cde	30.83±0.59c	38.53±5.91b	44.14±6.17ab
15	29.63±2.68cd	26.87±1.27cde	30.51±3.80cd	46.54±2.68a
30	21.98±4.34e	22.76±2.26e	23.11±2.67e	24.16±6.75de
45	6.02±5.01fgh	2.86±0.51fgh	7.36±3.01fg	8.18±1.44f
60	2.06±1.65fgh	1.38±1.27gh	1.06±0.60gh	0.34±0.28h
Number of Leaf				
0	87.33±20.90[a]	31.90±3.29[de]	43.89±03.40[bcd]	48.67±4.67[bc]
15	59.78±06.08[b]	25.67±2.67[ef]	57.11±09.34[b]	43.33±3.28[bcd]
30	58.56±07.17[b]	33.00±3.79[cde]	45.56±22.39[bcd]	21.11±4.29[efgh]
45	25.00±18.66[efg]	8.79±2.14[ghij]	20.44±05.01[efghi]	10.67±0.88[fghij]
60	5.00±03.18[hij]	4.22±0.38[ij]	2.56±01.50[j]	2.67±0.67[j]
Number of nodes				
0	87.33±20.90[a]	8.67±0.67[cd]	9.78±0.38[bc]	10.11±1.39[abc]
15	59.78±06.08[b]	8.00±0.33[d]	10.22±0.19[ab]	11.33±0.00[a]
30	58.56±07.17[b]	7.22±0.51[de]	8.11±0.19[d]	7.67±1.33[d]
45	25.00±18.66[efg]	3.33±0.58[fg]	4.11±1.07[f]	5.89±0.84[e]
60	5.00±03.18[hij]	1.00±0.58[h]	1.44±0.84[f]	0.67±0.33[h]

Note: Values in each row and column within each parameter followed by the same letter are not significantly different according to DMRT at 5%.

Qualitative Characters

Table 3 shows the color classification of Coleus leaves in this study. Each type of Coleus has different color leaves. Gamma ray irradiation resulted in changes of the leaf color of the yellow/green and green/brown *C. blumei*.

Yellow/green *C. blumei* irradiated with 15, 30, or 45 Gy gamma ray had different patterns of leaf color compared to control. Irradiation gave rise to dark green leaf color and made it more dominant. In addition, there was a dark pink color that appeared on the leaves of yellow/green *C. blumei* on the 45 Gy dose level. This leaf color pattern was not observed in the whole plant, but only in one or two leaves of a single plant. The yellow/green colors and leaf shape of the control plants could still be found in the irradiated plants, but irradiation treatment resulted in a more flat leaf shape. Flat leaf shape was only recorded in the dark green leaves of the yellow/green *C. blumei*. None of the *yellow/green C.*

Table 3. Leaf colors of four Coleus varieties after gamma ray irradiation treatment

Dose (Gy)	Type of Coleus			
	Yellow/green *C. blumei*	Green/brown *C. blumei*	Variegated green/brown *C.blumei*	*C. amboinicus* Lour
0	RHS 15D/ 137CD[a]	RHS 187A/145A	RHS 187A/144A	RHS 137C
15	RHS 144A	RHS 187A/144A	RHS N77A/144A	RHS 137C
30	RHS 144A	RHS 187A/149A	RHS N77A/144A	RHS 137C
45	RHS 4D/144A/ N57D	RHS 187A/149A	RHS 187A/144A	RHS 137C
60	-	RHS 187A/149A	RHS 187A/144A	-

Note: RHS 15D and RHS 4D = *light yellow*, RHS 137C = *green*, RHS 144A = *dark green*, RHS N57D = *dark pink*, RHS 187A dan RHS N77A = *dark purple brown*, RHS 149A = *light yellow green* *blumei*. None of the *yellow/green C. blumei* plants treated with 60 Gy survived (Figure 2A).

Gamma ray irradiation treatment did not change the green/brown *C. blumei* leaf color, but inhibited leaf growth, resulting in smaller leaves. However, gamma-ray irradiation at 15 Gy produced larger leaves compared to control (Figure 2B).

The variegated green/brown *C. blumei* treated with 45 Gy produced a flat surface leaf shape. Coleus leaves of the control plants of this type was curving. The shape and color of the control leaves can still be found in the irradiated plants. Increasing the level of irradiation dose altered the original wavy leaf edge form to become smaller. Variegated colors that were the characteristic of variegated green/brown *C. blumei* became less obvious with the higher dose of irradiation. The leaves irradiated at 60 Gy were smaller compared to control (Figure 2C).

C. amboinicus irradiated with 15 Gy resulted in plants with more round leaves compared to control leaves, whereas those irradiated with 30 Gy had smaller leaves. Irradiation at 45 Gy generated a more wavy leaf edges with a tapered end like serrations. Control plants had leaves that formed a semi-triangular with a blunt tip. Edge of the control plant leaves ware also wavy with a blunt tip. Not all *C. amboinicus* treated with 45 Gy had leaf shapes as shown in Figure 2D. This leaf shape was only found in one plant irradiated with 45 Gy. Other plants tended to have the same leaf shape with a smaller size. Irradiation at higher doses inhibited plant growth; no *C. amboinicus* survived following treatment with 60 Gy gamma ray irradiation. *C. amboinicus* appears to be the most radio-sensitive compared to all three other varieties, so this plant mortality score of *C. amboinicus* was higher. In addition, *C. amboinicus* leaf color did not change (stayed green) following gamma ray irradiation at all doses (Figure 2D).

Special Phenotypic Performance

Gamma ray irradiation tended to suppress variegated color characteristics and resulted in pink leaf color. At 30 and 45 Gy, green leaf color became dominant, and pink color appeared on some leaves. This pattern was not observed on the yellow/green *C. blumei* treated with 15 Gy, but occurred on a few leaves of one plant treated with 45 Gy.

Variegated green/brown *C. blumei* after irradiation showed results that were not much different from yellow/green *C. blumei;* the leaf variegated colors were reduced and green color became more dominant. In addition at the 45 Gy the leaf surface became flat and smaller. The phenotypes were only found in a few of the plants treated with 45 Gy. Coleus leaves without irradiation could still be found on the variegated green/brown *C. blumei* treated with 15, 30, or 45 Gy. Gamma ray irradiation at 45 Gy

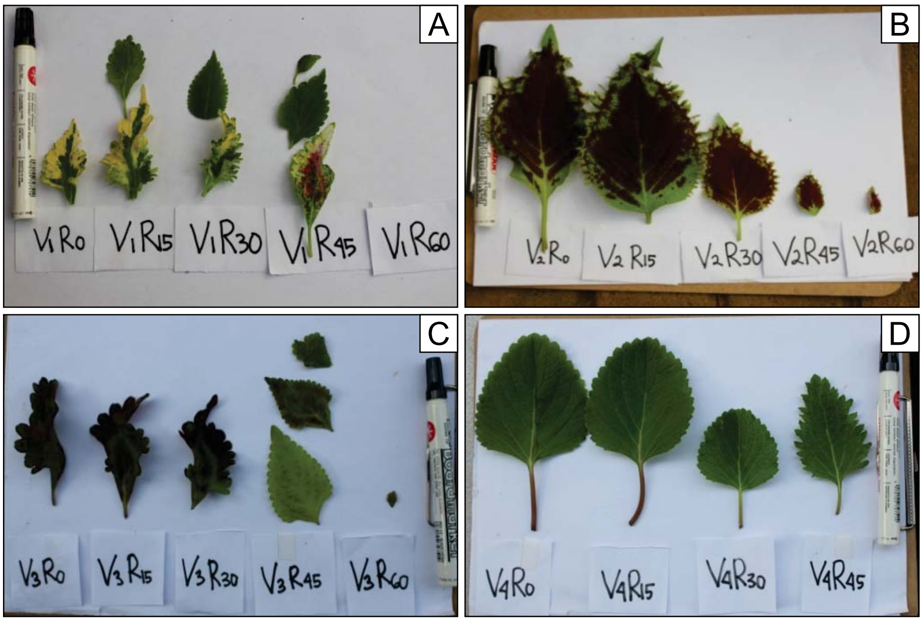

Figure 2. Shapes and colors of the Gamma ray-treated Coleus leaves: R0 = control; R15 = 15 Gy; R30= 30 Gy; R45 = 45 Gy; A = yellow/green *C. blumei* (V1), B = green/brown *C. blumei* (V2), C = variegated green/brown *C. blumei* (V3), D = *C. amboinicus*.

on *C. amboinicus* resulted in a plant with a different leaf shape and color pattern which is contrast to control plants. Untreated leaves were larger, rounded, and corrugated with a small blunt on the edge of the leaf, whereas the irradiated leaves were oval and wavy on the edge, or pointed.

Each plant species has a different level of radio sensitivity. According to Datta (2001) radio-sensitivity was the level of sensitivity or response shown by plant tissues after irradiated. One of the parameters to measure the radio-sensitivity level was LD_{50} value. Range of dose level irradiation applied became a very important factor in determining the optimum dose. At the low dose range, the ability of plants to live was higher than the high doses, but the frequency of occurrence of mutations would be lower. As observed, specific phenotypes of the yellow/green, green/brown, variegated green/brown *C. blumei*, and *C. amboinicus* tended to be more abundant in plants irradiated with 30 Gy and 45 Gy, and the number of plants survived were fewer compared to those treated with 15 Gy. At the dose level of 15 Gy every plant could survive until the end of the experiment.

Irradiation at 30 Gy, 45 Gy, and 60 Gy caused plants to be shorter, resulted in smaller leaf sizes compared to control and even caused plant death. According to Boertjes and Van Harten (1988) there were two kinds of influences that may occur after irradiation, i.e. the physiological and genetic damage (mutations). Physiological damage could occur in the form of cell death, inhibition of cell division and plant growth. Grosch and Hapwood (1979) added that the irradiation could cause growth inhibition (dwarfing), leaf thickening, changes in leaf shape and texture including shrinkage, abnormal folding, curling at the edges of the leaves, and leaf such as discoloration.

Conclusion

Gamma ray irradiation at a dose level of 15-60 Gy significantly reduced growth of Coleus. Gamma ray irradiation tended to suppress Coleus height, number of leaves, and number of nodes. The higher the dose applied, the smaller the percentage of plant survival. *C. amboinicus* had the lowest (LD_{50}) value among the three other types used in the study. LD_{50} values of yellow/green, green/brown, variegated green/brown *C. blumei*, and *C. amboinicus* were 48.66, 65.21, 52.81, and 37.62 Gy, respectively. Gamma ray irradiation treatment altered the Coleus phenotypes, especially the leaf color. At 30 and 45 Gy the variegated leaves color of yellow/green, variegated green/brown tended to turn into a dominant plain green. Yellow/green C. blumei irradiated with dose level of 45 Gy resulted in pink color leaves. The pink color was only found in two leaves on the plants irradiated at 45 Gy. *C.* amboinicus irradiated with 45 Gy had different leaf phenotypes with a more pungent smell. The physiological changes of C. amboinicus have potentials to be developed further if these mutations are stable in the next generation. Further studies are required to study repeated irradiation at doses around the LD_{50} to generate new phenotypes that add the commercial values of Coleus.

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