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# Effects of Co 60 gamma ray Ionizing radiation exposure on the variability of *Adenium obesum* growth

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**ABSTRACT**

This study aims to determine the effect of Co-60 gamma ray irradiation on the growth of *Adenium obesum* plants and obtain an effective dose of Co-60 gamma ray irradiation for the growth of *Adenium obesum* plants. A sample of 200 *Adenium obesum* seeds was divided into 5 groups. T0 is the control group without irradiation, T1 is the 2 Gy gamma ray irradiation treatment group, T2 is the 6 Gy gamma ray irradiation treatment group, T3 is the 12 Gy gamma irradiation treatment group and T4 is the 20 Gy gamma irradiation treatment group. Each group consists of 50 samples. Giving gamma rays to the seeds, done by exposing all parts of the seeds of the *Adenium obesum* plant which are arranged parallel. The treatment was observed every 3 weeks for 12 weeks. Observation of plant growth of *Adenium obesum* includes biomass, plant height and number of leaves. The data obtained were analyzed using the Variant Analysis Test and the Tukey Test for height and biomass variables, while for the number of leaves the variables were analyzed by the Kruskal-Wallis test, and then a regression test was performed. The results showed that the radiation dose treatment significantly affected the growth of biomass and plant height of *Adenium obesum* at the 3rd, 9th and 12th weeks of observation but did not significantly affect the number of leaves that grew. The optimum dose of irradiation to stimulate the growth of biomass and plant height of *Adenium obesum* at observations of the 3rd, 9th and 12th is 6 gray. So Co-60 gamma ray irradiation affects the growth of biomass and plant height of *Adenium obesum.*

***Key words :*** *Gamma ray irradiation, Co-60, Adenium obesum, Effective dose, Observation time*

## Introduction

*Adenium obesum* is an ornamental plant that is loved by many people. The beauty of Adenium lies in its semi-bonsai stem, its unique stump, and colorful flowers. Adenium which is a plant of the family Apocynaceae sp. whose flowers resemble frangipani flowers (*Plumeria* sp.), originating from South Africa, Somalia, and the Arabian Peninsula, such as Yemen and various places in the middle east.

*Adenium* is divided into several genera, including; *Oleifolium, Arabicum, Socotranum, Somalense, Bohemianum, Multiflorum, Obesum, Swazikum* (Oyen, 2008). Engineering techniques to make *Adenium* appear more beautiful and charming require ways that are not simple, one of which is by plant breeding (Rochayat *et al.,* 2017). Plant breeding includes conventional breeding and mutation breeding.

Plant breeding is conventionally done by hybridization (cross-breeding of various species of each plant), while breeding by mutation can be induced by physical mutagen or chemical mutagen. Physical mutagens are mutations in the form of physical material, with sources in the form of alpha, beta and gamma rays. Whereas chemical mutagens are mutations that have the ability to infiltrate nitrogen bases so that they can interfere with DNA replication. In general, physical mutagens can cause mutations at the chromosome stage, whereas chemical mutagens generally cause mutations at the gene or nitrogen base stage. Mutation induction is one of the effective methods to increase plant diversity (Soertini, 2003). Gene mutations occur as a result of changes in the induction of mutations is one of the effective methods to increase plant diversity. Physical mutations can also produce diversity faster than conventional breeding (large or large amounts). Breeding with physical mutations has several disadvantages, where the acquired traits are unpredictable and the instability of genetic traits that emerge towards the next generation.

Mutation breeding by applying radiation to ornamental plants can produce new varieties and can improve various plant characteristics, such as productivity, growth, age, resistance to pests and diseases, flower color, flower size, and nutrient content. The basis for carrying out plant breeding is the availability of genetic diversity that can be created in several ways, namely the introduction of plant genotypes from outside Indonesia, hybridization / crossing, in-vitro culture / tissue culture, and mutations both with radiation and with mutagenic chemicals (BATAN, 2006). Expressions and manifestations of mutations are seen in changes in plant properties (morphology, physiology, biochemistry) which are all summarized in the appearance of new varieties.

Radiation is the transmission of energy through a material or space in the form of heat, particles, or electomagnetic waves (photons) from an energy source (Broettjes, 1998). Radiation can induce mutations because the irradiated cells will get high energy, so they can change the chemical reaction of plant cells which can ultimately cause changes in the composition of plant chromosomes. Gamma rays are radiations emitted by a radionuclide such as Co-60 that decays towards a stable state (Astuti and Kholimatussa'diah, 2019). High energy radiation is a form of release of energy in large numbers which causes ionization in the material penetrated by the energy. Radiation exposure causes genetic effects in the form of gene mutations in reproductive cells. Physical mutagenic materials that are often used in plant breeding research include X-rays, Gamma rays, and ultraviolet light (Majeed *et al.,* 2018). Ionization process will occur in the tissue and can cause changes in the tissue itself, cells, genomes, chromosomes, and Nucleic Deoxyribo Acid (ADN) or genes. Such changes are known as mutations.

The frequency of mutations is linearly proportional to the radiation dose. This linear relationship can indicate that mutations can be induced by radiation. Increasing irradiation can cause an increase in mutation rate. Giving radiation causes changes in certain chemicals around genes that produce changes in the composition of nucleotides. This results in termination of chromosomes and results in deletions. The higher the dose, the greater the chance of termination, and can produce inversions and translocations. Changes that are genetic in plants are as a result of the end of the interaction process between radiation and genetic material contained in the seed embryo cells that are irradiated (Sutapa and Kasmawan, 2016).

Various studies show the effects of gamma ray radiation on the growth of wheat plants (Singh *et al.,* 2013), the effect of salinity on cowpea plants (Abdel Haleem *et al.,* 2013) and on Seeds Enhances the Tolerance of Sweet Osmanthus Seedlings to Salinity Stress (Geng *et al.,* 2020), germination characters of amaranth seeds (Aynehb and Afsharinafar, 2012) and effects on lettuce (*Lactuca sativa* var. Capitata) seedlings (Jun Cheng *et al.,* 2018). The results of research conducted by Acta Hoticulturae 607 on seeds (*Beta vulgaris* L.) irradiated with gamma rays Cobalt-60 with a dose variation of 2.5; 5.0; 7.5 and 10.0 Gy, indicating that the 5 Gy radiation dose is the optimum dose that can cause growth stimulation at the stem length and root length of the plant (Amilin *et al.,* 2015). At this dose there is a breakdown and integration of several DNA molecules that causes somatic or genetic abnormalities, so that certain irradiated cells will become much larger than normal cells. This automatically affects the growth in stem length and root length of plants.

This research is an attempt to get *Adenium obesum* variability in the form of faster or slower growth, in this case biomass, number of leaves and plant height since pre-germination through induction of gamma Co-60 ionization with varying dosages of 2 Gray, 6 Gray, 12 Gray, 20 Gray, and variations in the length of time the maintenance of plants to *Adenium obesum* ornamental plant seeds.

## Materials and Methods

The tools used in this study Gamma Chamber 4000-A made by Bhaba Atomic Research Center, India portable type initial activity of 10,000 Ci. The study was conducted at the BATAN Isotope and Radiation Technology Application Center (PATIR) Pasar Friday, South Jakarta. The radiation source is Cobalt-60 with 3474.6632 Ci activity.

### Treatment of Samples

The sample in this study was *Adenium obesum*. A sample of 200 *Adenium obesum* seeds was divided into 5 groups. T0 group is a control group without irradiation, T1 group is a treatment group with Cobalt-60 gamma ray irradiation by 2 Gy, T2 is a treatment group with Cobalt-60 gamma ray irradiation of 6 Gy, T3 is a treatment group with Cobalt-60 gamma ray irradiation 12 Gy and T4 are the treatment group with Cobalt-60 gamma ray irradiation of 20 Gy. Each group consists of 50 samples. Plant seeds are placed in a glass cup, inserted in an iron tube contained in the Gamma Chamber, then irradiated with a dose of 2, 6, 12, 20 Gray. The planting of *Adenium obesum* seeds in a plastic pot is done on the 7th day after irradiation. Observation of plant height, number of leaves and biomass is done every 3 weeks for 12 weeks. The measuring instrument used was the ruler and balance sheet of the Mettler Toledo AL 204.

### Statistical Analysis

Data obtained were analyzed by ANOVA test to determine the effect of irradiation on plant height and biomass. While testing the number of leaves using the Kruskal-Wallis test. To find out the optimal dose of gamma irradiation, Tukey's post hoc test was used.

## Results and Discussion

Statistical test results of observations of the 3rd week showed that there was no effect of gamma radiation doses on the plant height variable with a value of p = 0.127 (p> 0.05) and the biomass variable had an influence with a value of p = 0.00 (p <0.05). Tukey's test results showed that the biomass variable at a dose of 6 gray produced the highest average value of 0.511425. Kruskal-Wallis calculated statistical test results with statistical tables for df = 4 significance level of 5%, obtained 9,448, obtained a calculated value of 1,640 (<9,448), so that the radiation dose does not affect the growth variable of the number of leaves. The graph of the comparison of average biomass, plant height, and number of leaves for each treatment for the observation time is shown in Figures 1, 2 and 3. The results of the Co-60 gamma ray exposure treatment results on observation time are shown in Figure 4 and 5.

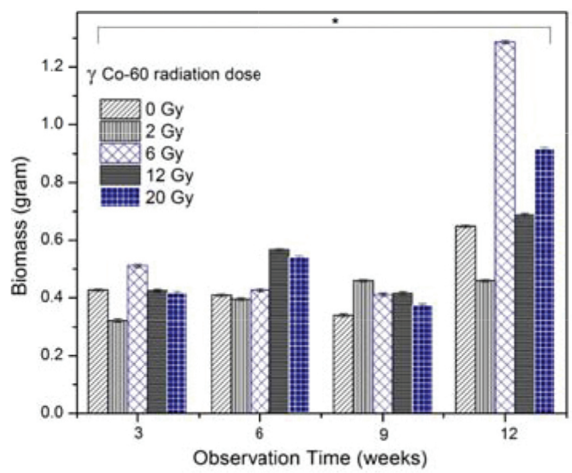


**Fig. 1.** Graph of average biomass of each treatment for the length of observation time

At the 6th week observations there was a real difference between control and treatment of all growth variables. Statistical test results showed that there was no effect of gamma ray irradiation treatment on plant height variables with a value of p = 0.350 (p> 0.05) and biomass value of p = 0.175 (> 0.05). Kruskal-Wallis calculated statistical test results are equal to 1,533 (<9,448) so that the radiation dose does not affect the growth of the number of leaves.

The results of the 9th week observation test statistic showed a difference between control and treatment of all growth variables (P = 0.00). Tukey test

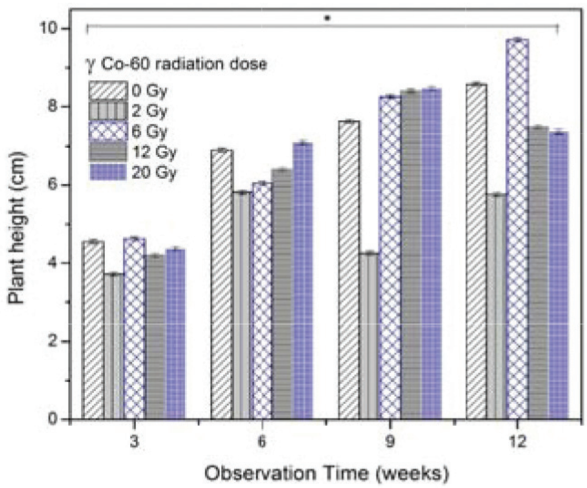


**Fig. 2.** Graph of average plant height of each treatment for the length of observation time

results showed the biomass variable with a dose of 12 gray produced the highest mean value of 0.416125 significantly different from the control group. In the variable height of 20 gray plants, the highest average value was 8,475, significantly different from the control group. The results of the Kruskal-Wallis statistical test were 7,851, so the radiation dose had no effect on the growth number of leaves.

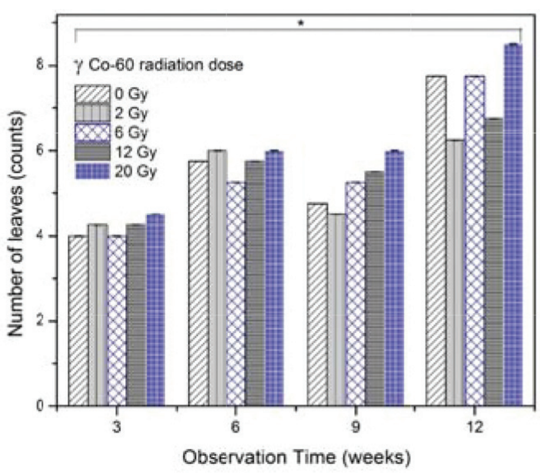


**Fig. 3.** Graph of number of leaves of each treatment for the length of observation time

The results of the 12th week observation test statistic showed a difference between control and treatment of all growth variables (P = 0.00). Tukey test results showed the biomass variable with a dose of 6 gray produced the highest mean value of 1.286650 significantly different from the control group. Plant height variable at dose 6 gray produced the highest mean value, which is 9,725, significantly different from the control group. The results of the Kruskal-Wallis statistical test were 8.466, so the radiation dose had no effect on the growth number of leaves.

Irradiation of Cobalt-60 gamma rays with an energy of 1.25 MeV carried out on the seeds of the *Adenium obesum* plant can cause a Compton effect due to the presence of perfect resilience collisions between a photon coming with the electron electrons which are initially at rest. The direct effect in the form of ionization is the release of electrons from atoms with certain kinetic energy accompanied by scattered photons (Astuti and Kholimatussa'diyah, 2019). While the indirect effect is hydrolysis of wa-

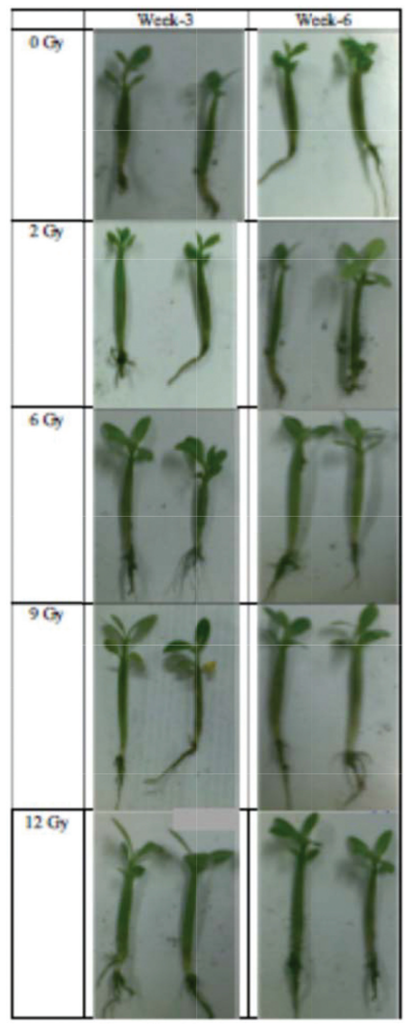


**Fig. 4.** The growth of the *Adenium obesum* plant at observation time week 3 and 6 at various doses of Co-60 gamma ray exposure

ter which produces reactive oxygen species (ROS), this generally occurs in absorbers with low atomic numbers such as carbon, air, water, and biological tissue (Astuti *et al.,* 2019).

High energy gamma ray irradiation in plant material, especially seeds, produces mutagenic changes in living cells due to the penetration of radiation energy into cells and tissues that are exposed so that DNA undergoes severe changes. Mutations are changes that occur in genetic material (DNA or RNA), both at the level of the sequence of genes (called point mutations) and at the level of chromosomes. Mutations in genes can lead to new variations in species. Mutations can be divided into small mutations (gene mutations) and large mutations (chromosome mutations). Small mutations are changes that occur in the arrangement of gene molecules (DNA). While large mutations are changes that occur in the structure and arrangement of chromosomes. Gene mutations are also called point mutations. This mutation occurs because of changes in the sequence of bases in DNA or can be said to be a change in nucleotides in DNA. Chromosome mutations are structures inside cells in the form of long series of molecules consisting of one DNA molecule that connects genes. Chromosomes have two arms, the length of which is sometimes the same and sometimes not the same, the arms are joined to the centromere (the location of the spindle thread attached during the division of mitosis and meiosis). The most common effect of mutagen material, especially radiation, on plant chromosomes is chromosome breakage or chromosome aberration.

These changes are caused by the direct effect of gamma irradiation in the form of ionization on DNA and the production of ROS such as hydrogen peroxide, hydroxyl ions and other active oxygen atoms. ROS products can then interact with DNA and other cellular components and biomolecules to produce ionization, functional changes in proteins and enzymes and overall metabolic activity (Majeed *et al.,* 2017). The reaction between free radicals and peroxide with a cell nucleus consisting of chromosomes and DNA molecules causes DNA damage and mutation. For cells that do not divide tend to be insensitive to radiation. This can be proven from the results of biomass research at the 3rd, 9th, and 12th weeks of the effect of gamma ray radiation between the control group and the treatment group, especially at doses of 6 and 12 Gray. Plant height variable was seen to have an influence at 9th and 12th weeks, especially at 6 Gray doses. As for the number of leaves, there is no effect of radiation dose on the number of leaves at any time.

Seed germination is an important initial phase. Seed germination that succeeds in overcoming internal and external pressure can produce stable morphological performance and plant growth. These obstacles in seed germination can be influenced in the positive and negative directions by radiation exposure as a result of induced mutations depending on cell abnormalities or stimulation modifications that are triggered by radiation doses. Aref *et al.,* (2016) revealed that 5 Gy has a stimulatory effect on germination. Verma *et al.,* (2017) have also reported that germination and survival of *Cuminum cyminum* seeds increases at lower doses (<100 Gy) but decreases at higher doses especially at 500 Gy. Germination and growth responses of various plants to gamma irradiation treatment generally depend on the amount of radiation dose, time of exposure, and plant species (Celik and Atak, 2017; Majeed *et al.,* 2017). Improved germination and plant growth properties exposed to low-dose gamma irradiation can be associated with positive mutational effects on genes that control these traits, the mechanism of rapid DNA repair and the stimulation of hormones and enzymes that are actively involved in the germination and growth process. Lower doses may accelerate cell division in meristematic tissue which can contribute to increased germination and plant growth (Verma *et al.,* 2017). In addition, lower doses can cause an increase in antioxidant potential and better communication among growth hormones in irradiated cells which can cause increased growth (Majeed *et al.,* 2018).

Plants are living organisms and will respond systematically to growth challenges. Plants with good genetic traits will be able to cope with environmental stress, through the mechanism of growth hormone mobilization, regulation of the cell cycle, activation of the relevant enzymes (Majeed *et al.,* 2018). Radiation dose affects plant growth. The 5 Gy radiation dose significantly increases the growth rate of roots and shoots of *Datura innoxia* L (Aref *et al.,* 2016). Hong *et al.,* (2017) showed that gamma radiation doses in the range between 10 and 15 Gy positively influenced the general growth traits of wheat (*Triticum aestivum* L.). Gamma rays can also suppress the growth of roots, stems and leaves (vegetative growth). The radiation dose given to get an individual exhibiting changes in properties (mutants) depends on the type of plant, growth phase, size, hardness, and material to be mutated. Utilization of gamma ray radiation at various doses is expected to get superior varieties that have better fruit character than before. The radiation dose used to induce diversity largely determines the success of mutant plants.

Some cells in the tissue have different sensitivity to gamma radiation. This sensitive area is generally related to the location of DNA in cells. Irradiation can affect one or both DNA strands in a double helix. If the DNA fracture consists of a single strand, then the linear integrity of the DNA molecule remains intact and the fault mechanism will work to form normal DNA again. If the fault involves both strands, then repair cannot occur quickly. This is the most important radiation effect that causes DNA damage that forms observable mutations (Hong *et al.*, 2017). Bergonie's and Tribondeau's Law states that cells with a high mitotic rate and cell division take place for most of their life time, so they can be easily damaged by ionizing radiation. Therefore, cells that relatively often divide are more likely to become easily damaged by exposure to gamma radiation. Besides the effects of gamma irradiation is also influenced by water content. The more oxygen and water molecules ($H_2O$) levels in the irradiated material, the free radicals are formed so that plants become more sensitive (Borzouei *et al.,* 2010).

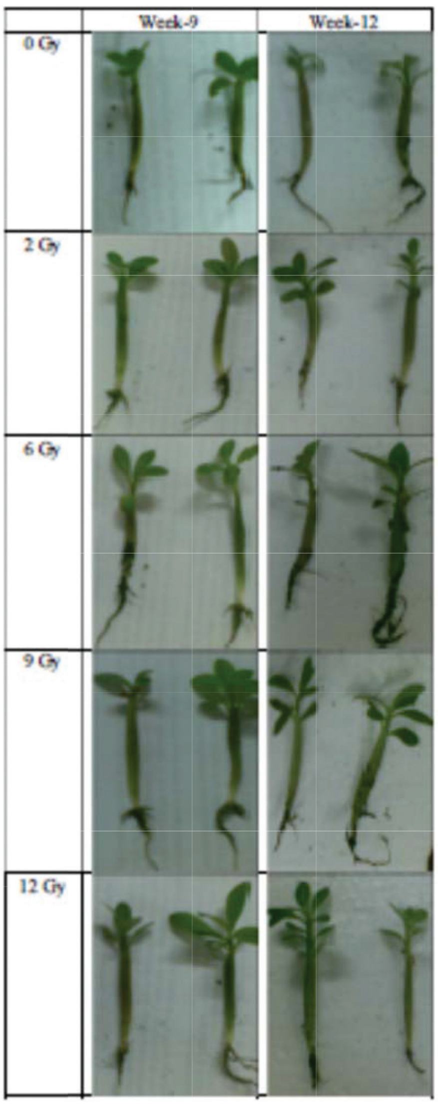


**Fig. 5.** The growth of the *Adenium obesum* plant at observation time week 9 and 12 at various doses of Co-60 gamma ray exposure

The results showed that at 6 Gray doses was the optimum dose to influence the growth conditions of biomass and plant height at any time of observation. These results approach the literature of research results that have been carried out by Acta Hoticulturae 607 on seeds (*Beta vulgaris* L.) irradiated with gamma rays Cobalt-60 with a dose variation of 2.5; 5.0; 7.5 and 10.0 Gy, indicating that the 5 Gy radiation dose is the optimum dose that can cause growth stimulation at the stem length and plant root length. Gamma irradiation of Cobalt-60 causes genetic mutations and gives rise to a variety of physical variations in *Adenium obesum* plants.

## Conclusion

The results showed that the radiation dose treatment significantly affected the growth of biomass and plant height of *Adenium obesum* at the 3rd, 9th and 12th weeks of observation but did not significantly affect the number of leaves that grew. The optimum dose of irradiation to stimulate the growth of biomass and plant height of *Adenium obesum* at the 3rd, 9th and 12th weeks is 6 grays. So, Co-60 gamma ray irradiation affects the growth of biomass and plant height of *Adenium obesum*.

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