The effect of nuclear radiation on mutation chromosomes of shallot cells (*Allium cepa*)

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**Abstract.** Environmental pollution by nuclear radiation has been known to endanger chromosomes. This research aims to analyze the abnormal forms of root shallot chromosomes which are irradiated by nuclear with various levels of radiation. This is a quasi-experimental research. The treatment group was given radiation through nuclear reactors at a dose of 11.88 rad, 8.69 rad, 1.28 rad, 1.15 rad, 0.26 rad, 0.00034 rad, 0.00016 rad, while the control group was without radiation. The staining of the preparations was carried out using Acetocarmine 2%. Reading preparations using a microscope with a magnification of 400x. The results of the study showed the emergence of various forms of chromosomal abnormalities. Abnormal forms that appear are pole to pole arrangement chromosome, unequal separation, binucleate cell, pulverized ball metaphase, sticky anaphase with broken bridge, chromosome rosette, abnormal chromosome fragment, early cell plate formation at late anaphase, sticky metaphase, binucleate cell with a bridge, chromosome fragments, binucleate cells, chromosome gaps, ball anaphases with a diagonal scattered anaphase bridge, and aberrant grouping at anaphases in a giant cell. The novelty of this preliminary study is that chromosomal abnormalities in the onion roots were found even in the very low radiation group. Large-scale research is required and their effects if eaten by animals or humans need further research.

1. **Introduction**

A nuclear leak has been linked to the risk of organ cancer and leukemia [1]. Over the years it has been observed that DNA is the most important target for the interaction of ionizing radiation and the free radicals it produces. Besides, it is accepted that DNA damage occurs instantly after radiation interactions. In the last two decades, the paradigm of the effects of radiation not only impacts the target but also threatens the effects of the non-targeted radiation. This phenomenon has been widely investigated by some scientists, to include the effect of ionizing radiation which is not directly exposed to traces of ionizing radiation. This phenomenon results in systemic DNA damage and also some abnormal changes in cell function after local irradiation [2].

Ionizing radiation has a dangerous and beneficial potential. The effect of ionizing radiation is detrimental if interacting with human tissues or instruments can have a negative impact because it can cause disease or damage [3]. Ionizing radiation has been generally accepted to have the risk of damaging DNA [4]. The International Commission on Radiological Protection estimates that 100 mSv radiation exposure increases the risk of cancer by 0.5%. The central hypothesis from the Linear No Threshold model is that low-dose ionizing radiation can induce carcinogenesis through the so-called "one stroke
action", i.e. one or more strands of deoxyribonucleic acid can be broken down by hitting only one electron particle. Regardless of the radiation dose, radiation exposure increases the risk of cancer. Radiation exposure of 10 rad has been estimated by the International Commission on Radiological Protection to increase cancer risk by 0.5% [5].

Radiation is defined as the transmission or emission of energy through space or object. Radiation can be divided into ionizing radiation and non-ionizing radiation. Nuclear radiation is ionizing radiation. Nuclear material emits ionizing radiation and is measured in signals (Sv) [6]. Nuclear radiation cannot be detected by the human senses because it cannot be seen, touched, or smelled. This radiation affects the atoms when it passes through [7]. This radiation can be generated from various sources both natural and man-made, including accelerators, radioisotopes, and reactors [8]. A study showed nearly 1,000,000 non-elderly adults in the healthcare market throughout the US showed that a large number of patients received doses of up to 0.05 Gy / year. That is a sizable value, considering the reference level for emergencies set by the International Commission for Radiological Protection (ICRP) is from 0.02 to 0.05 Gy / year [9].

Every change in DNA alignment is interpreted as a mutation [10]. A mutation is any change in the DNA sequence that is far from normal. This implies that there are normal alleles that are prevalent in the population and that mutations turn them into rare and abnormal variants [11]. Mutations are defined as any changes from normal in the DNA sequence. This implies that there are normal alleles that are prevalent in the population and mutations turn them into rare and abnormal variants. Polymorphisms, on the other hand, are variations in DNA sequences that are common in populations [12].

Chromosomes are defined as a package of DNA strands that are inside the nucleus of certain cells and organelles that contain noncoding sequences and genetic codes [13]. Generally, mitosis consists of five stages: prophase, prometaphase, metaphase, anaphase, and telophase [14]. In the telophase stage, chromosomes begin to break down when they reach the opposite pole. The spindle thread returns to become a monomer and becomes a cytoskeleton for daughter cells. The nucleus begins to form in each new cell [15].

*Allium cepa* (shallot) was used in this experiment because it has several advantages, namely low cost, easy to use, good chromosome conditions for the study of chromosomal damage or mitotic disorders [16]. *Allium cepa* test can be a practical and sensitive method for detecting genotoxic and environmental mutagens [17]. This test can also show the effect on DNA of organisms exposed to various test agents on DNA. The *Allium cepa* test has a correlation of 82% with the mammalian test system in the carcinogenicity test [18]. This research aims to analyze the abnormal forms of red onion chromosomes which are irradiated by nuclear with various levels of radiation.

2. **Research methods**

2.1. **Research design**

This determination is a quasi-experiment with a pre-posttest design with a control group. The object of the research is 40 shallot’s roots.

2.2. **Research objects**

*Allium cepa* that has arisen roots is stored in a 10ml vial bottle containing water. The shallots are stored at five location points (each location is stored on 5 samples of shallots), namely in the laboratory as a control, greenhouse, and three points with different radiation rates in the TRIGA 2000 reactor area. These three points are the points where there is radiation emission gamma which is sourced from the cobalt reaction and nuclear reaction. Whereas in the greenhouse there is gamma radiation emitted from cesium 134 radiation. The sample is stored for a certain time at that point and is repeated 3 times. In total there were 8 treatment groups. The radiation dose rate at each point was measured using the Inspector 1000 Analyzer. Large data on radiation rate are listed in table 1:
Table 1. The dose of radiations among groups.

| Point    | Duration (Hour) | Total Dose (rad) |
|----------|-----------------|------------------|
| Control  | 24              | 0                |
| Group 1  | 24              | 0.000156         |
| Group 2  | 24              | 0.000336         |
| Group 3  | 24              | 0.264            |
| Group 4  | 66              | 1.14708          |
| Group 5  | 116             | 1.276            |
| Group 6  | 24              | 2.01144          |
| Group 7  | 116             | 8.6884           |
| Group 8  | 66              | 11.88            |

2.3. Shallot root preparation
The irradiated onion sample is harvested by cutting the root tip which has grown more than 2 cm at 00.00 AM. The root was fixed using a farmer solution (70% alcohol mixture: acetic acid in a ratio of 3:1) for 48 hours. The fixed root is rinsed with distilled water three times. Then the roots are soaked in 1N HCL on a watch glass and heated using Bunsen for 5 seconds then allowed to stand for 2 minutes and rinsed again with distilled water 3 times. Furthermore, the roots are stained, some coloring agents that can be used such as aceto-orcein and acetocarmine [19]. In this study the roots were soaked in 1% acetocarmine solution in a watch glass and heated for 5 seconds then allowed to stand for 5-10 minutes. After staining, the roots are transferred to the slide and then dropped with 1 drop of distilled water and squeezed using a squash using a glass cover. The preparations were observed using a light microscope with a magnification of 400x. data processing.

Mitosis index formula:

\[
\text{Mitosis index} = \frac{\text{The number of cells undergoing mitosis in one area of view}}{\text{The total number of cells in one field of view}} \times 100\
\]

2.4. Research location
The study was conducted at the Centre for Applied Nuclear Science and Technology (PSTNT) of the National Nuclear Energy Agency (BATAN) located in Bandung. Samples were given radiation treatment in the area of the TRIGA 2000 Reactor area of the National Nuclear Energy Agency (BATAN) Bandung. Preparations and observations were carried out in the Radiation and Environmental Compound Group field laboratory at BATAN Bandung.

3. Results and discussion
The description of chromosomal abnormalities in all groups is shown in Figure 1, while the normal mitotic process in figure 2. The percentage of cells containing abnormal chromosomes is shown in Figure 3.
Figure 1. Various types of chromosomal abnormalities in *Allium cepa*.

Figure 2. Normal chromosomes in the stages of mitosis *Allium cepa*: a) Prophase; b) Metaphase; c) Anaphase; d) Telophase.
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Figure 3. The percentage of cells containing abnormal chromosomes.

The results of this study indicate that there are various abnormalities in the cleavage process of Allium cepa. The abnormal forms are pole-to-pole arrangement chromosome, unequal separation, binucleate cell, pulverized ball metaphase, sticky anaphase with broken bridge, chromosome rosette, abnormal chromosome fragment, early cell plate formation at late anaphase, sticky metaphase, binucleate cell with a bridge, chromosome fragments, binucleate cells, chromosome gaps, ball anaphases with a diagonal scattered anaphase bridge, and aberrant grouping at anaphases in a giant cell.

During mitosis, cells compress their chromosomes into solid rod-shaped structures to ensure their reliable transmission to daughter cells [20]. In the process of Meiosis, prophase is cell division which is divided into five stages: leptotene, zygotene, pachytene, diplotene, and diakinesis. DNA double-strand break (DSB) formation and initiation of paired interactions occur at the entrance to prophase I, while complete synapses and stabilizing paired interactions are observed at the pachytene stage where DSB is repaired to form crossovers and non-crossovers (gene conversion without crossovers) [17].

The results showed that the greater the radiation, the more chromosomal abnormalities appeared except group-7. In group-7, no mitotic chromosome phases were seen, almost all shallot root cells were damaged. This can be explained by the specificity of mitotic Allium cepa root cells. The time required for Allium to do mitosis is very short. Therefore, the duration of radiation exposure is very important. In group no. 7, the total radiation dose received is smaller than group-8, but the duration is longer (116 hours), as for group no. 8 for 66 hours. In the control group, very rare sticky chromosomes also found. A study revealed sticky chromosomes can also be observed rarely in normal individuals [21].

What's interesting about the results of this study, the Allium cepa which is stored in a nuclear reactor room that is not operating with nuclear radiation levels 0.00156 rad has experienced chromosome abnormalities, namely ball metaphase, binucleate cells, roseate chromosomes, coagulated anaphases, sticky chromosomes at anaphase stage, pulverized ball metaphase, sticky anaphase with the broken bridge, the sticky chromosome with the broken bridge, and unequal separation.

4. Conclusion
Nuclear radiation causes various abnormalities on the chromosome Allium cepa roots. The greater the radiation the greater the abnormality. The novelty of this preliminary study is that chromosomal abnormalities in the onion roots were found even in the very low radiation group. Large-scale research is required and their effects if eaten by animals or humans need further research.

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