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EFFECTS OF CHRONIC GAMMA IRRADIATION ON SHALLOT CHROMOSOMES *(Allium ascalonicum* Linn*)*

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ABSTRACT

Radiation contamination can occur from natural radiation or from man-made sources, such as radiation for medical research or for nuclear weapons manufacture. Radiation contamination can impact living things, the eco-system and the food chain, so several methods have been invented to test the level of radiation contamination. One of those methods is the Allium test, which is simple and cost-effective and makes it easy to clearly detect abnormalities from radiation. The objective of this study was to observe the effect of different levels of chronic gamma irradiation on the chromosomes of root tip cells of the shallot (*Allium ascalonicum* Linn.) (2n=16) and on growth of the shallots. Growing shallot bulbs were exposed to chronic gamma radiation from a Cobalt-60 source at the Nuclear Technology Research Center (NTRC), Kasetsart University, Thailand. They were placed 2 meters from the source and were exposed to 0, 10, 20, 30, 40, 50, 60, 70 and 80 Gy at the dose rate of 0.0078 $Gy.min^{-1}$. Following exposure, cells were fixed either immediately (0 hours) or after a 24-hour recovery period. Root tip squashes were observed by light microscope, 1,000 cells per specimen, to detect chromosome abnormalities. In the cells fixed 0 hours after exposure, chromosome abnormalities were observed in 1.967, 12.01, 0.964, 9.677, 9.417, and 8.923% of the cells of plants exposed to 10, 20, 30, 40, 50 and 60 Gy radiation, respectively, but no abnormalities were observed in the cells of plants exposed to 70 and 80 Gy. In the cells fixed 24 hours after exposure, chromosome abnormalities were observed in 4.216, 2.750, 14.955, 15.15 and 6.932% of the cells of plants exposed to 10, 20, 30, 40, and 50 Gy, respectively but no abnormalities were observed in the cells of plants exposed to 60, 70 or 80 Gy. That means root tip cells of the shallot can use for testing the low dose level of radiation contamination (10-60 Gy). The most commonly observed chromosome abnormality was micronucleus at interphase, followed by fragments and bridges. As for the growth rate following chronic irradiation, the height (leaf length) was recorded after 7 days and it was found that there was no significant different in growth rate among the treatment groups exposed to different doses of gamma irradiation, but the mean height of all the irradiated plants (2-5 cm) was significantly lower than that of the non-irradiated control plants (13 cm).

Keywords: chronic irradiation, gamma ray, *Allium ascalonicum* Linn, Allium test.

INTRODUCTION

At many sites around the world high levels of background radiation occur, where environmental exposure can be significant. Moreover, accidents at nuclear facilities can increase exposure levels of radionuclides in the environment. It is important to understand the risk of low doses of ionizing radiation and the kinds and extent of damage that radiation can cause in an organism. Many studies and experiments have been done to find out the effects of ionizing radiation on living things. The studies often use animals or plants as samples. Higher plants have many advantages for use in such experiments, and are frequently used as indicators for environmental examination, monitoring of toxic chemicals in the environment, or for investigating mutations by toxicity. Not only can plants be used to detect the effects of toxicity from chemicals, metals, heavy metals, etc., but they can also be an indicator to estimate the endpoint of chromosome damage that results in the abnormalities seen in the cells and tissues of their leaves, roots, and pollen (Grant, 1999).

Some of the most favored plants used for such tests are *Allium cepa*, *Viciafaba*, *Zea mays* Linn., *Pisumsativum* L., etc. For example Zaka *et al*. (2002) investigated the induction of chromosome aberrations by low acute irradiation to moderate doses ranging from 0 to 10 Gy from a Cobalt-60 source in root meristem cells of 6-day-old Pisum plantlets. The percentage of root tip meristem cells displaying chromosome aberrations was estimated immediately after irradiation and after 20 h recovery time. The dose-effect curves showed non-linear responses, especially in the low dose range (0–1 Gy), which is of particular interest. After 20 h of recovery, a steep increase of aberrations was observed for cells exposed to 0.4 Gy, followed by a plateau for doses until 1 Gy. There was an irradiation effect on plant growth during the first and second generations, showing the persistence of cell division anomalies as a long-term effect of acute irradiation. The results suggested the induction of a genomic instability. In 2004, Zaka *et al.* studied effects of low doses of short-term gamma irradiation (0 - 60 Gy) on growth and development through two generations of *Pisumsativum* and found that doses higher than 6 Gy significantly inhibited the G1 plant growth and productivity, and no seedling survived irradiation with 40 Gy and above. These effects were transmitted and were even more severe in the next generation, G2. Irradiated G1 (≥10 Gy) and G2 (≥0.4 Gy) plants were significantly smaller than controls. The mean number of pods produced per plant was reduced by at least 20% at all doses in both G1 and G2. In parallel, the mean numbers of ovules and normally developed seeds per pod were significantly

reduced after 10 Gy in G1 and after 0.4 Gy in G2, leading to a significant drop in seed production. This effect was correlated with a linear decrease in male fertility linked to abnormal meiosis (tetrads with micronuclei) as a function of doses from 0 to 10 Gy, in G1 and G2 plants. These long-term changes in plant development demonstrate a genomic instability induced by irradiation. However, there were neither quantitative nor qualitative changes in storage proteins in G1 seeds at any of the irradiation doses tested from 0 to 10 Gy. Alzandi (2012) used Pisumsativum L. as a biological indicator to verify a method of decreasing lead content in soil. The results showed that Pisumsativum L. could be a good plant for phytoextraction, or natural entrapment of toxic substances from the soil.

Allium cepa or onion has been popularly used in many studies because it has large chromosomes and the number of chromosomes is not too much (2n=16). The Allium test was begun in 1938 by Levan, who studied the effect of colchicine on onion chromosomes. Fiskesjö (1983, 1985) used the Allium test for monitoring the harmful effects of chemicals on biological materials in the environment. The Allium test has shown good correlation with other test systems, involving general toxicity (root growth) and genotoxicity (chromosome aberrations). Living material of Allium is easily stored and handled. It is inexpensive and assures very clear chromosomes. An Allium test takes a relatively short time to carry through: 2 days for chromosome preparations and 3-4 days for measurement of the root growth. From the growth curves, EC (effect concentration) values can be obtained and the Allium test should be considered as a warning and also an indication that the tested chemical may be a risk to human health and to our environment. Allium test has been used in radiation contamination monitoring also. For instance, Kovachuk *et al.* (1998) used *Allium cepa* to study the toxicity of soil contamination in the vicinity of the Chernobyl nuclear power plant accident in Ukraine and found that the soil in this area was highly toxic and could be a cause of genetic damage. The study also confirmed that the Allium test was an effective and affordable biological monitoring system for the evaluation of the ecological and genetic risks in the Chernobyl area. Vaijapukar *et al.* (2001) studied the effect of gamma irradiation induced morphological and cytological changes of onions as a biological indicator at low doses (50-2000 cGy), such as could be encountered in a nuclear emergency, and the result showed that the irradiated onions could be identified on the basis of significant changes in mitotic index and percentage of micronuclei at 200 and 400 cGy, respectively, and the study could also be useful to predict accident-incurred gamma doses in case of a nuclear emergency. The morphological studies alone cannot justify accepting the irradiation of onion as a biological indicator for lower gamma dose measurement with confidence, but it can be used for qualitative measurement of gamma dose evaluation.

This study aims to observe the differentiation of *Allium ascalonicum* Linn. Chromosomes after exposure to chronic gamma irradiation (the dose rate 0.0078 $Gy.min^{-1}$) at doses of 0, 10, 20, 30, 40, 50, 60, 70, and 80 Gy. Root cells were fixed at 0 and 24 hours after irradiation. The growth of the shallots was also measured for 7 days.

MATERIALS AND METHODS

The test organism

Seventy-two bulbs of common shallot, *Allium ascalonium* Linn, were prepared for treatments including the control. The roots were grown in tap water on platforms that were floated in a cup (Figure-1) under laboratory conditions. After reaching a length of 3 cm (±0.5 cm), the roots were treated with gamma rays. The set root length was reached after about 2-3 days and the water was changed every day.

Gamma irradiation and root tip fixation

The gamma irradiator using a Cobalt-60 source (dose rate 0.0078 $Gy.min^{-1}$) at the Nuclear Technology Research Center (NTRC), Kasetsart University, Bangkok, Thailand was used in this study. When the shallot roots reached the length of 3 cm, treatments were performed at doses of 0, 10, 20, 30, 40, 50, 60, 70 and 80 Gy as chronic irradiation with the samples placed 2 meters from the source. After irradiation, shallot roots were cut and fixed in Farmer's fixative solution for 2 conditions, 0 hours and 24 hours after exposure.

Figure-1. shallot bulbs for irradiation (left) and gamma room at nuclear technology research center (NTRC), Kasetsart university, Bangkok, Thailand.

Roots staining and Slide preparation

The roots were hydrolyzed by 1N HCL at 60 degree Celsius for 10 minutes. Afterwards, the material was immediately transferred into Feulgen stain (Schiff's reagent) and kept in the dark at room temperature (for at least 30 min or until the tissue stains deep purple). The root tip was then squashed in 45% acetic acid. The color stain was long lasting for 45-60 minutes. The slides were examined by 400x light microscope and chromosome abnormalities were counted for 1000 cells from each bulb.

Plant growth

After irradiation, the shallots were planted in pots for 7 days to observe the growth by measuring the height of onion leaves from base to leaf tip.

Figure-2. Shallots planted in pots for growth observation.

RESULTS AND DISCUSSIONS

Effect of chronic gamma irradiation on shallots chromosome

After being exposed to irradiation at doses of 0, 10, 20, 30, 40, 50, 60, 70 and 80 Gy as chronic irradiation, root tips from half the shallots were fixed immediately after exposure and half were fixed after a recovery period of 24 hours. Of the root tip cells that were fixed immediately after radiation exposure, 12.011% of cells in the 20 Gy treatment group were found to have abnormal chromosomes, while 9.677%, 9.417% and 8.923% of cells from the 40, 50 and 60 Gy treatment groups, respectively, were found to have abnormal chromosomes. The lowest rates of abnormal chromosomes observed were zero per cent in the 70 Gy and 80 Gy treatment groups and 0.661% in the control. Rates of 1.967% and 0.964% were observed in the 10 and 30 Gy treatment groups. The most common abnormalities found were micronucleus at interphase (mean occurrence of 4.756% in all samples), followed by fragments and bridges in 0.080% and 0.011%, respectively. (Table-1, Figure-3).

Table-1. Percentage of chromosome abnormalities observed in shallot following chronic gamma irradiation at doses of 0 - 80 Gy in cells fixed immediately after exposure (0 hours).

Radiation Dose (Gy)	**Percentage of cells with micronucleus**	**Percentage of cells with fragments**	**Percentage of cells with bridges**	**Total percentage of cells with chromosome aberrations**
0	0.331	0.331	0	0.661
10	1.770	0.197	0	1.967
20	11.725	0.191	0.095	12.011
30	0.964	0.000	0	0.964
40	9.677	0.000	0	9.677
50	9.417	0.000	0	9.417
60	8.923	0.000	0	8.923
70	0.000	0.000	0	0.000
80	0.000	0.000	0	0.000
Average	4.756	0.080	0.011	4.847

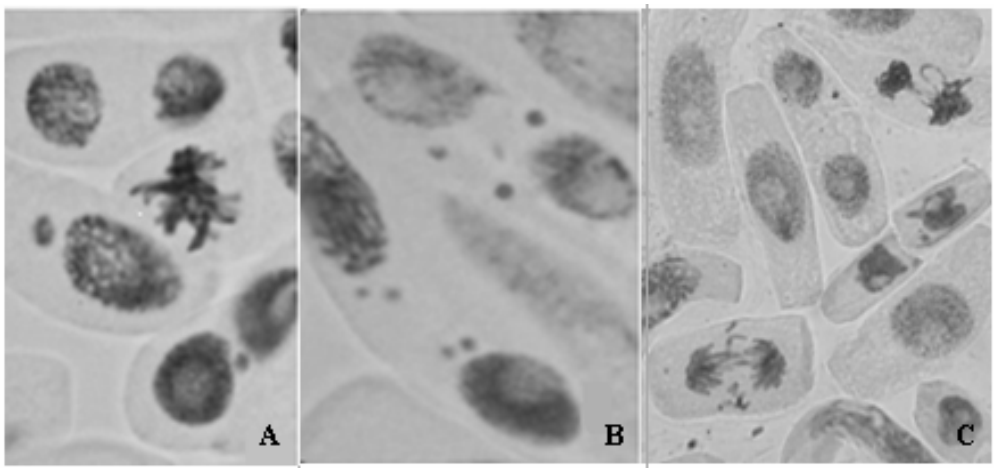

Figure-3. The chromosome abnormalities of gamma irradiated cells at doses of 10, 20, 30, 40, 50, 60, 70 and 80 Gy when cells were fixed immediately after exposure (0 hours). (A-B) Micronucleus in interphase and prophase stage (C) Bridge in anaphase stage.

As for the shallot root cells that were fixed 24 hours after radiation exposure, the highest percentage of abnormal chromosomes was found in the 40 Gy treatment group, at 15.155%. For the 30, 50 and 10 Gy treatment groups the observed abnormality rates were 14.955%, 6.932% and 4.216%, respectively. At 20 and 80 Gy, 2.750% and 0.198% of cells, respectively, displayed abnormal chromosomes. Zero abnormalities were observed in cells from the 60 and 70 Gy treatment group. Again, the most frequently observed abnormality was micronucleus at interphase, in 4.606% of samples, followed by fragments and bridges in 0.323% and 0.033%, respectively. (Table-2, Figure-4).

Table-2. Percentage of chromosome abnormalities observed in shallot following chronic gamma irradiation at doses of 0 - 80 Gy in cells fixed 24 hours after exposure (24 hours).

Radiation Dose (Gy)	**Percentage of cells with micronucleus**	**Percentage of cells with fragments**	**Percentage of cells with bridges**	**Total percentage of cells with chromosome aberrations**
0	0.089	0.357	0	0.446
10	3.137	0.784	0.294	4.216
20	2.750	0.000	0	2.750
30	14.058	0.897	0	14.955
40	14.855	0.299	0	15.155
50	6.562	0.370	0	6.932
60	0.000	0.000	0	0.000
70	0.000	0.000	0	0.000
80	0.000	0.198	0	0.198
Average	4.606	0.323	0.033	4.961

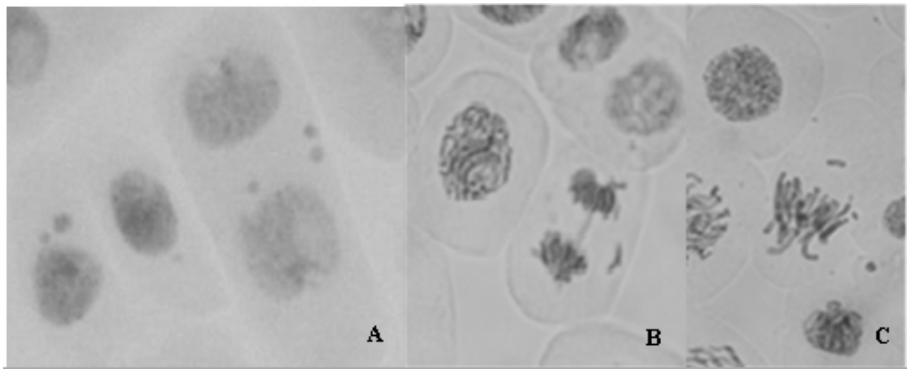

Figure-4. Chromosome abnormalities of gamma irradiated cells at doses of 10, 20, 30, 40, 50, 60, 70 and 80 Gy when cells were fixed 24 hours after exposure (24 hours). (A) Micronucleus in interphase stage (B) Bridge and fragment in anaphase stage (C) Fragment in metaphase stage

Comparing the results from the root tips fixed at different time periods after irradiation, we found that when cells were fixed 24 hours after exposure compared to immediately (0 hours), the percentage of abnormal chromosomes observed was less at the doses of 20, 50, and 60 Gy but was greater at the doses of 10, 30 and 40 Gy. (Figure-5)

When cells are exposed to ionizing radiation it may damage the chromosomes, which are the most important part in living cells. The chromosomes are responsible for growth and cell division. There are mechanisms in cells that allow them to repair certain kinds of damage to DNA or to chromosomes. In cases where damage to chromosomes is not extensive, in many cases cells can perform self-repair, after which their chromosomes will function normally again. In this studied, we may not have been able to detect some chromosome anomalies that occurred because the cells were able to repair them. In other cases, chromosome damage from ionizing radiation cannot be repaired by the cells' internal self-repair mechanisms, and then the cells are unable to function properly. Ordinary processes like cell division may be disrupted, and the cells may divide more slowly or faster than normal, or they may experience damage to other cellular functions that could result in cell death. Whether the damage from irradiation is extensive or is reparable depends mainly on the amount and type of radiation received. In addition, the sensitivity to radiation and responsiveness of each cell is not the same. Some cells display the effects of damage immediately on exposure to radiation. Cells in this group are usually cells in G2 and M phase. Other cells tend to have a slower response and the damage is not immediately apparent because the cells were in the resting phase (IAEA, 2010). The low levels of radiation exposure on living cells, the biological effects are so small they may not be detected. There has repair mechanisms against damage induced by radiation as well as by chemical carcinogens. Consequently, biological effects of radiation on living cells may result in three outcomes: first, injured or damaged cells repair themselves, resulting in no residual damage; second, cells die, much like millions of body cells do every day, being replaced through normal biological processes; or third, cells incorrectly repair themselves resulting in a biophysical change. (U.S.NRC, 2011)

In this study, in the root tip cells of the control group (not exposed to gamma radiation), we still found a small number of abnormalities of chromosomes, i.e. fragments in 0.344% and a micronucleus in the interphase and prophase stage in 0.21%. This could occur due to a malfunction of a chemical reaction or due to structural changes within the cell itself in nature, or could be caused by background radiation, but in either case it is normally not detrimental to the cell. (IAEA, 2010)

In addition, we observed that increasing doses of gamma radiation seemed to have an effect of delaying cell division in shallot. The root tip cells from plants that were exposed to higher doses of radiation tended to have noticeably more cells in interphase. In particular, almost all the cells in plants from the 50 to 70 Gy radiation dose treatments were observed to be in interphase and cells in other stages of cell division were rarely found, or only 1-3 cells per root tip. When a delay in cell division occurred after irradiation, the cells had time to repair the damage incurred to their genetic material before the next stage of growth. During the G1/S stage, the cell could take time to repair any chromosome or DNA damage from radiation before entering S phase and beginning DNA replication; or similarly, cells in G2/M phase could repair the damage that occurred prior to the period of cell division (Sun, nd).

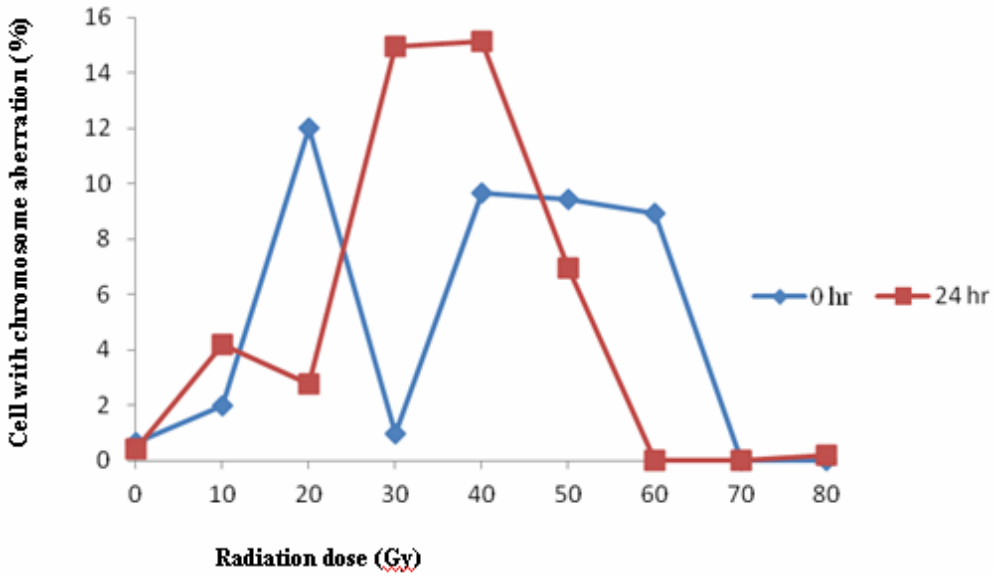

Figure-5. The percentage of cells with chromosome aberrations in shallots exposed to chronic gamma irradiation (dose rate 0.0078 Gy min-1) at doses of 0 - 80 Gy, comparing cells that were fixed immediately (0 hour) after exposure with cells fixed 24 hours after exposure.

Growth data

After radiation and root tip removal for fixing, all shallots were planted in pots, including the control treatment, some of which were dead. The growth rate of the control treatment was faster than that observed in the other treatment groups. Irradiated shallots grew less than the non-irradiated control (Figure-10), and statistical analysis by ANOVA and F-Test at a level of confidence of 95% ($P < 0.05$) showed that it was a significant difference in plant height after 7 days. Comparing the growth of the different treatment groups (exposed to chronic gamma irradiation at doses of 10, 20, 30, 40, 50, 60, 70 and 80 Gy), the mean height across all doses was not significantly different (Table-5), but differed from the mean height of the non-irradiated control, which achieved a height of 13.689 cm. In experiments on shallots irradiated with chronic irradiation the average height was around 1.825 to 5.029 cm. These results were different from the work of Tangpong *et al.* (2009), who studied the effect of acute and chronic gamma irradiation on *Anubias congesis* N.E. Brown grown in tissue culture. The plants were irradiated with a Cobalt-60 gamma source at NTRC, Faculty of Science, Kasetsart University, at a distance of 2 meters from the radiation source, with dose rate of 0.71 Gy/hour, for doses of 0, 14.34, 28.60, 31.24, 42.90, 51.16, 65.55, 82.42, 91.69, 105.99 and 120.30 Gy.The researchers recorded the survival rate and growth rate at 60 days after irradiation and found that the radiation dose did not affect the survival rate but did affect the growth rate such as the leaf length and leaf width decreased as the radiation doses increased. In the present study the radiation doses used were lower, and the plants were observed for only 7 days, so that may explain why no difference in height was observed among the different doses.

Roongtanakiat *et al*. (2012) studied the influence of gamma radiation on the growth of native Thai vetiver 2 ecotype, Kampaengphet 2 and Ratchaburi. Samples were placed 2 meters from the radiation source for doses of 0, 65, 104, 116, 157, 182, and 205 Gy for Kampaengphet 2 and doses of 0, 63, 87, 127, 150, 173 and 213Gy for Ratchaburi. Gamma radiation was found to affect the survival of Kamphaengphet 2 and the LD_{50} at 90 days was 100 Gy. For Ratchaburi, the survival rate was 100% but growth rate was affected. The GR50/90 values for plant height of the Kamphaeng Phet 2 and Ratchaburi were 118 and 109 Gy, respectively. In relation to the biomass, the GR50/90 values for shoot dry weight, root dry weight and total dry weight of the Kamphaeng Phet 2 ecotype were 120, 125 and 121 Gy, respectively, and they were 68, 66 and 67 Gy, respectively, for the Ratchaburi.

In both studies, the growth model was able to confirm the effect of chronic gamma irradiation on decreasing the growth of the plants. Plant height and weight decreased, compared to controls that were not irradiated, as the radiation dose increased. Changes in morphology of the plants were observed as well.

Chromosomal abnormalities in the onion genus Allium may occur due to toxicity, whether chemical or radiation, as has been described in several previous researches. For example, S.G. Vaijapurkar *et al*. (2001) have explained that when onions were exposed to gamma rays in low doses (50 - 2000 cGy), chromosome abnormalities, in the form of reduced cell division and increased percentage of micronuclei within the cell, were observed at doses of 200 and 400 cGy. The morphology study of onion in seed germination and root length could not be used as an indicator of biological confirmation of onions in the study of gamma radiation in low dose, but it could be a measure of quality for evaluating gamma

radiation. Kutsokon *et al.* (2005) initially used cockles, or Tradescantia-SH, and Allium test to determine the toxicity of soil contaminated by radiation from Cs-137 and Am-241 in the area of the Chernobyl power plant and found that increased radiation caused morphological changes to increase. When considering Allium testing of chromosome abnormalities at doses of 1-300 Gy, the statistical reliability of chromosomal abnormalities increases as radiation is increased to 5 Gy, and the reliability would increase to 100% when the dose is increased to 200 Gy. Abnormal chromosomes were found when the radiation dose was 40 Gy or higher, but very little abnormalities were found when radiation was 1-40 Gy. From this finding one could conclude that high doses clearly affect cell damage, while damage is still observable in doses lower than 40 Gy. This is consistent with the work of Kovalchuk *et al.* (1998) who used *Allium cepa* as a biological monitoring indicator to assay radiation contamination exposure from a radiation accident. They found in areas where the amount of radiation contamination was increasing, the percentage of germination and cell division of the onion was down. But the percentage of chromosome abnormalities increased with increased radiation. This test could confirm the reliability of the method of using a light microscope to examine root tips in order to investigate the cytotoxicity and chronic radiation gene damage pattern of plants growing with radiation.

Table-3. To compare the difference in average height the shallots were exposed gamma chronic irradiation at doses 0-80Gy and measure on day 7 after transplantation.

Treatments	**The average height of the shallots** (cm)
0 Gy	13.689[a1/]
10 Gy	2.825[b]
20 Gy	5.029[b]
30 Gy	2.900[b]
40 Gy	2.217[b]
50 Gy	2.157[b]
60 Gy	2.317[b]
70 Gy	1.825[b]
80 Gy	2.389[b]
F-Test	*
C.V.(%)	59.118

*=The difference statistically significant 95% as confidence level.
[1/] = Means followed by the same letter in column is not significantly different at 5 % level by LSD.

CONCLUSIONS

For chronic gamma irradiation (dose rate 0.0078 $Gy.min^{-1}$) at dose of 0, 10, 20, 30, 40, 50, 60, 70 and 80 Gy, in the shallot root tip cells fixed 0 hours after exposure, chromosome abnormalities were observed in the cells of plants exposed to 10, 20, 30, 40, 50 and 60 Gy, but no abnormalities were observed in the cells of plants exposed to 70 and 80 Gy. In the cells fixed 24 hours after exposure, chromosome abnormalities were observed in the cells of plants exposed to 10, 20, 30, 40, and 50 Gy, but no abnormalities were observed in the cells of plants exposed to 60, 70 or 80 Gy. The root tip cells of the shallot can use for monitoring the harmful effects of the low dose level of radiation contamination (10-60 Gy) on biological materials in the environment. As for the growth rate following chronic irradiation, the height (leaf length) was recorded after 7 days only and it was no significant different in growth rate among the treatment groups exposed to different doses of gamma irradiation, but the mean height of all the irradiated plants (2-5 cm) was significantly lower than that of the non-irradiated control plants (13 cm).

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