Effects of gamma irradiation on lipid peroxidation, survival and growth of turmeric in vitro culture

K Chusreeaeom1 and O Khamsuk2

1 Department of Applied Radiation and Isotopes, Faculty of Science, Kasetsart University, Bangkok, Thailand.
2 Department of Botany, Faculty of Science, Kasetsart University, Bangkok, Thailand.
E-mail: fscikac@ku.ac.th

Abstract. Gamma radiation has been widely applied in agriculture for crop improvement; however, it can induce oxidative stress in plant cell. This research aims to study effects of acute and chronic gamma irradiation on lipid peroxidation, plantlets survival and growth rate in M1/V1 generation of turmeric. Plantlets cultured on MS medium were exposed to acute gamma radiation from Cs-137 (dose rate 3.7 Gy/min) at 0, 20, 40, 60 and 80 Gy. For chronic irradiation with a Co-60 source (dose rate 0.0057 Gy/min), turmeric were exposed to gamma radiation at 0, 21, 41, 62 and 82 Gy. Results showed that the content of malondialdehyde (MDA) was gradually increased with radiation doses after chronic irradiation, whereas after acute irradiation, it significantly increased at the dose of 40 Gy compared to non-irradiated plantlets. Differences between acute and chronic exposure to gamma irradiation were observed for MDA at the highest dose. Chronic irradiation at 21, 41 and 62 Gy also influenced plant development, mainly due to the stimulation of seedling survival and growth, while 20 Gy acutely gamma irradiation maximally promoted and decreased afterward. The median lethal dose (LD50) and 50% growth reduction dose (GR50) after acutely irradiated were reported to 74 and 66 Gy, respectively.

1. Introduction
Turmeric (Curcuma longa Linn), the member of family Zingiberaceae, is an economically important spice and medicinal plant cultivated widely in South-East Asian countries. To put the turmeric cultivation as an industry, it is therefore essential to develop turmeric genotypes with improved drug yielding potential, rhizome yield as well as having tolerance to biotic and abiotic stress. Induced mutation by gamma ray is widely used to broaden the genetic variability within a short period of time. Mutation breeding is often used to improve current varieties and to generate new varieties. Examples of favorable traits induced after exposures are higher yields, earlier maturity, non-photoperiod sensitivity, good characteristic, tolerance and resistance to biotic and abiotic stress. Literature has documented the effect of irradiation on various plant species, for examples, curcuma [1], chrysanthemum [2] rice [3] and banana [4]. However, considering the negative effects of irradiation after exposure and radiation dose-response curve is also important.

Gamma radiation induces several cytological, genetic [3], morphogenetic, biochemical [5] and physiological alterations in cells and tissues of plants [6]. One of the effects of gamma radiation is to enhance the production of reactive oxygen species (ROS). High concentration of ROS are toxic, they can damage the membrane lipids, protein, and DNA of the cell. Lipid peroxidation induced by free
radicals, is also important in membrane deterioration. The level of lipid peroxidation, measured as malondialdehyde (MDA) content, has been considered as an indicator of stress induced oxidation in cell membranes [7].

The objective of the current study is to investigate physiological response in terms of malondialdehyde (MDA) and proline contents as well as the morphological response measured by plantlets survival and growth rate in turmeric seedling after exposure to different dose of gamma radiation. We also determined the LD_{50} and GR_{50} dosage for an irradiation mutagenesis.

2. Materials and methods

2.1. Plant materials and gamma irradiation

_In vitro_ culture plantlets of turmeric (_Curcuma longa_ L.) were used as the plant material. Under aseptic conditions, MS basic medium (Murashige and Skoog, 1962) with 2.5% kelcogel®, 0.5 mg/l 6-benzylaminopurine (BA) and 30 g/l sucrose were used for multiplied plantlets several times to obtain enough plants for conducting the experiments. Sixty days growing shoots were cut and transferred to MS medium without hormones before being subject to radiation treatments.

2.2. Gamma irradiation

Turmeric plantlets cultured on MS medium were exposed to acute gamma radiation from Cs-137 (dose rate 3.7 Gy/min) at doses of 0, 20, 40, 60 and 80 Gy. For chronic irradiation with a Co-60 source (dose rate 0.0057 Gy/min), plantlets were exposed to gamma radiation at dose of 0, 21, 41, 62 and 82 Gy. Irradiated treatments were conducted at the Nuclear Technology Research Center, Kasetsart University, Bangkok, Thailand.

2.3. Growth conditions

Irradiated and unirradiated (control) plantlets were then multiplied by sub-culturing on the MS medium without hormones. Leaves were used for lipid peroxidation and proline content analyses. After 60 days, M1V1 generation were observed for the number of surviving plantlets and the number of new shoots. The data were calculated as a percentage of the control. The median lethal dose (LD_{50/60}) and 50% growth reduction dose (GR_{50/60}) were estimated.

2.4. Physiological analysis

Lipid peroxidation, characterized by the MDA content, and proline content were measured. The MDA content was determined using the methodology described by Hodge et al., (1999). A 1-mL portion of a 0.1% tricholoroacetic acid (TCA) solution was added to a 0.1 g sample of fresh leaves, and the mixture was centrifuged at 12,500 rpm for 20 min at 5°C. A 0.5-mL portion of the supernatant was then taken out. After that, a 1-mL portion of 0.5% tiobarbituric acid (TBA) in 20% TCA was added to 0.5-mL of the supernatant and incubated at 98°C for 30 minutes. The absorbances at 532 and 600 nm were measured with the spectrophotometer. The MDA concentration was calculated using the following equation:

\[
\text{MDA (µmol g}^{-1}\text{FW)} = \left[\left(\frac{A532-A600}{0.25}\right) \times (155 \times 10^{-3}) \times (4\times 10^{-3})\right] \times 0.25
\]

The proline assay was based on the method by Bates et al. (1973), which uses 3% sulfosalicylic acid in grinding fresh plant samples. Portions of ninhydrin (1 mL) and glacial acetic acid (1mL) were placed into tubes containing 0.1-g portions of the grinded samples. The tubes were kept in a 100 °C water bath for 30 minutes. After cooling, 2-mL portions of toluene were added to the mixtures. The absorbance was measured at 520 nm and calculated using the standard curve.

2.5. Statistical analysis

A completely randomized design (CRD) was used with three replicates of both the treated samples and controls. The data was subjected to analysis of variance (ANOVA), after which where the main effects
were significant, mean separation was carried out using Duncan’s New Multiple Range Test (DMRT). Standard error (SE) was calculated to test the significance results of three replicate experiments.

3. Results and Discussion

3.1 Effect of gamma irradiation on lipid peroxidation
Different changes in MDA content of turmeric samples were observed after exposure to acute and chronic gamma irradiation. Turmeric exposed to acute gamma radiation at the dose of 40 Gy showed significant increase \( (P < 0.05) \) in MDA content, which was about 52% higher than the MDA level of control (0 Gy), before decreased to same level as control at the highest dose of 80 Gy. By contrast, the MDA content gradually increased with doses when exposed to chronic irradiation, in which the highest MDA concentration was observed after an irradiation at 81 Gy (Table1). MDA is the end product of lipid peroxidation, ordinarily indicating the level of lipid peroxidation and reflecting the membrane deleterious [8]. This process proceeds by a free radical chain-reaction mechanism whereby a major indicator of oxidative stress with overproduction of reactive oxygen species (ROS) such as hydroxyl radicals, superoxide radical and hydrogen peroxide. These ROS molecules react rapidly with almost all structural and functional organic molecules causing an interruption of cellular metabolism during irradiation [9]. The increase of the MDA production in plant material was also observed after an irradiation to 200 Gy of soybean [10], in Z. latifolia seedling exposed to 50 and 100 Gy [11]. However, the decrease of MDA content of plant material was observed in chickpea after the maximum irradiated to 700-1000 Gy [12]. Gamma irradiation at 200 Gy was sufficient to decrease the lipid peroxidation products in irradiated rice [13]. In the present study showed the decrease of MDA level after the maximum accumulation at 40 Gy of acute exposure; however, it demonstrated the comparable levels to those of control (Table1). This decrease in MDA level can be attributed to plant for re-growing, irradiated cells at the high dose and dose rate may evolved various protective mechanism undergo antioxidant enzyme activities, DNA repair or cellular osmotic adjustment for protecting of membrane integrity [13].

Table1. Effect of the acute and chronic gamma radiation dose on the content of MDA (product of lipid peroxidation) in leaves.

| Acute irradiation dose (Gy) | MDA content (µmol/g of fresh tissue) | Increase (%) | Chronic irradiation dose (Gy) | MDA content (µmol/g of fresh tissue) | increase (%) |
|---------------------------|--------------------------------------|--------------|-------------------------------|--------------------------------------|--------------|
| 0 (control)               | 0.75±0.01 b                          |              | 0 (control)                   | 0.75±0.01 c                         |              |
| 20                        | 0.87±0.03 ab                         |              | 21                            | 0.84±0.04 bc                        |              |
| 40                        | 1.14±0.08a                          | 52.0%        | 44                            | 0.99±0.07bc                         |              |
| 60                        | 0.90±0.04ab                         |              | 65                            | 1.00±0.08ab                         |              |
| 80                        | 0.74±0.03 b                          |              | 81                            | 1.09±0.09a                          | 45.3%        |

F-test *
%C.V. 9.4 17.7

The values represent the mean ± S.E. calculated on the basis of results of three replicate experiments.
* Statistically significant difference at \( P < 0.05 \) from the value for 0 Gy.

3.2 Effect of gamma irradiation on proline content
Gamma irradiation has positive effects on the biosynthetic rates of proline in turmeric plantlets. However, it was not statistically significant in the plantlets exposed by acute irradiation compared to non-irradiated. The highest proline content in the leaves was observed after acute irradiation to 40 Gy (32.0% greater than that of the control) and decreased afterwards. On the other hand, proline accumulation slightly increased after chronic irradiation. Chronic exposure to low dose of 21 Gy showed the induction in proline content, and the highest dose of gamma radiation (81 Gy) led to a significant
increase in total proline content by compared to untreated control plantlets. The increase in the proline content was also observed in wheat gamma-irradiated to 100 Gy [14], in P. corylifolia plants gamma-irradiated to 15 and 20 kGy [15], in pigeon peas gamma-irradiated to 250 Gy [16] and in Arabidopsis seedlings gamma-irradiated to 50 Gy. To avoid oxidative damage, plant have evolved various protective mechanism to counteract the effects of reactive oxygen species in cellular compartments. Our data showed that ionizing radiation increased the proline content, suggesting that proline plays an important role in the defense system against gamma rays. Proline is a scavenger of ROS. It can stabilize the structure and function of macromolecules such as DNA, protein and membranes [5].

| Table 2. Effect of the acute and chronic gamma radiation dose on the content of proline in leaves. |
| --- | --- | --- | --- | --- | --- |
| Acute irradiation Dose (Gy) | proline content (mg/g fresh tissue) | Increase (%) | Chronic irradiation Dose (Gy) | proline content (mg/g fresh tissue) | Increase (%) |
| 0 (control) | 0.25±0.01 | 0 (control) | 0.25±0.01b |
| 20 | 0.29±0.05 | 21 | 0.27±0.06ab |
| 40 | 0.33±0.09 | 32.0% | 44 | 0.32±0.05ab |
| 60 | 0.27±0.02 | 65 | 0.31±0.02ab |
| 80 | 0.28±0.05 | 81 | 0.36±0.05a | 44.0% |

F-test | ns |
%C.V. | 22.21 |

The values represent the mean ± S.E. calculated on the basis of results of three replicate experiments.

Table 3. Survival percentage and growth rate percentage of tumeric at 60 days after acute and chronic irradiation.

| Acute irradiation dose (Gy) | Plantlets survival (% of control) | Growth rate (% of control) | Chronic irradiation dose (Gy) | Plantlets survival (% of control) | Growth rate (% of control) |
| --- | --- | --- | --- | --- | --- |
| 0 (control) | 100±0.58a | 100±0.74a | 0 (control) | 100±0.58a | 100±0.74a |
| 20 | 110.32±1.15a | 113.82±0.49a | 21 | 100.8±0.4a | 127.14±1.91a |
| 40 | 103.97±2.52a | 93.97±0.77a | 44 | 109.72±0.58a | 125.88±0.21a |
| 60 | 98.15±0.58a | 65.33±0.46b | 65 | 115.08±1.15a | 128.39±0.56a |
| 80 | 26.19±1.53b | 14.07±0.52c | 81 | 91.71±2a | 90.70±0.81a |

F-test | * | * | ns | ns |
%C.V. | 20.07 | 19.71 | 13.00 | 22.40 |

The values represent the mean ± S.E. calculated on the basis of results of three replicate experiments.

* Statistically significant difference at $P < 0.05$ from the value for 0 Gy.

3.3 Effect of gamma irradiation on survival and growth rate
The correlation between the types of gamma irradiation, plantlets survival and growth rate is presented in Table 3. Following acute irradiation at the dose of 80 Gy was significantly different ($p < 0.05$) on plantlets survival between irradiated plantlets and untreated control. The effect of radiation on growth was estimated by measuring plant height at 60 d after irradiation and calculating the growth rate. The dose of 20 Gy slightly stimulated plant growth, whereas the plant growth at the highest dose (80 Gy) significantly ($p < 0.05$) decreased compared to non-irradiated plantlets. The number of plantlets survival and growth rate of irradiated turmeric decreased as the gamma dose increased. This might referred to the radiation injury and may be noticeable in several form including a reduction in sprouting ability and plant survival. Similar results were observed in Curcuma spp [2] and torch ginger [17].
The effects of chronically exposed in vitro culture plantlets are summarized in Table 3. The plantlets exposed to radiation from 0 to 81 Gy were not statistically significant from the control regarding to the parameters of plantlets survival and growth rate. At the dose of 21, 44 and 65 Gy, the chronical exposure stimulated plant growth. The lower dose rate of radiation probably caused little damage to the plants genetic material, so that the cells could repair themselves. Additionally, plant growth caused by low doses of gamma irradiation may possibly due to stimulation of cell division, cell elongation nor DNA repair mechanism [18]. The long-term exposure of plants to low-dose-rate radiation can increase their radioresistance and had a more effective system of sigle-strand DNA break repair [19].

**Figure 1.** The turmeric plantlets at 60 d after acute (A) and chronic (B) gamma irradiation.

Induced mutation is an important tool for producing genetic variation. Exposure to gamma radiation combining to in vitro culture is known to produce morphological, physiological and biochemical mutants. In this study, the optimize dosages for mutation breeding program were evaluated by the median lethal dose (LD$_{50/60}$) and 50% growth reduction dose (GR$_{50/60}$).

**Figure 2.** Plantlets survival and growth rate (% of control) after acute (A) and chronic (B) gamma irradiation.

In the present study, the exposure by acute irradiation could be estimated the LD$_{50/60}$ from plantlets survival (% of control) was 74 Gy. The GR$_{50/60}$ was assessed from growth rate (% of control) providing approximately 66 Gy. However, the LD$_{50/60}$ and GR$_{50/60}$ after chronically exposure could not be estimated because they were not appreciable difference among irradiated treatments and non-treated plantlets. The effects observed after exposure were intensely influenced by several factors, some related to radiation features for examples dose, dose rate, duration of exposure and some related to plant characteristics such as tissue architecture, genome organization, stage of development, physiological and biochemical processes [20]. Overall, our data on lipid peroxidation, proline content and median lethal dose (LD$_{50}$) after acute irradiation seems to suggest that 40-74 Gy could be a suitable dose for a mutation breeding program for turmeric.
4. Conclusions
Our study showed that radiation stress caused an increase in the MDA content leading to the enhanced lipid peroxidation. The increasing gamma dose initiated proline accumulation that was known to be a scavenger of ROS. In addition, the irradiation at the higher dose and dose rate resulted in decreasing in plantlets survival and growth rate suggesting an inhibition of cell proliferation and cell division whereas the lower dose rate caused a stimulation of the cell. Our data provided the information of appropriated dose for gamma-mutagenesis in turmeric that would be beneficial in breeding program.

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