The Effect of Gamma rays on the Radiosensitivity and Cytological Analysis of *Zingiber officinale* Roscoe Varieties Bentong and Tanjung Sepat

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Abstract - **The radiosensitivity and cytogenic analysis of two *Zingiber Officinale* Roscoe varieties; Bentong and Tanjung Sepat were carried out using Caesium-137 as gamma ray source. Six different doses treatment were applied as follows: 0, 5, 7, 9, 11, 13 and 15 Gray. The LD_{50} (Lethal dose) value was determined based on the rhizomes survival rate. With attention to survival rate data, results indicated that the optimum doses for ginger varieties Bentong and Tanjung Sepat were 8.14 Gy and 9.38 Gy respectively. Gamma rays have reduced significantly the mean survival rate of rhizomes and this reduction was more noticed in Bentong variety. The chromosome behaviors were observed under mitotic stages. The most chromosomal aberration common anomalies observed in root tips are clumping of chromosomes, stickiness and laggards. It was noticed that increased doses level (5, 7, 9, and 11 Gy) resulted in higher occurrence of chromosomal aberrations in cells.**

Keywords—**Cytology, Radiosensitivity, Gamma rays, *Zingiber officinale* Roscoe.**

I. Introduction

Ginger *(Zingiber Officinale* Roscoe) is a monocotyledons plant which belongs to family Zingiberaceae. Rhizomes of the plants are commonly used as spice meanwhile both rhizomes and leaves give important source of medicine [1]. It is one of the herbaceous species with high potential toward of sustainable bio-economy sector [2]. In Malaysia, ginger has been cultivated commercially in Pahang, Selangor, Sabah and Sarawak regions.

The main ginger varieties cultivated in Malaysia are Bentong, Tanjung Sepat, Bara and China [1]. Ginger has gained attention in the aspects of economic importance mainly in the food processing and pharmaceutical sectors [3]. However, its production in comparison to other export crops is relatively low due to its inherent low yields which attributed to the lack of improved varieties [4]. Sterile plant reproduction system with no viable seed, poor flowering and low set of fruit and seeds resulted in low variability which is the main issue in the propagation and productivity of ginger plant [5]–[6].

Normally, ginger is propagated by vegetative means of underground rhizomes; but, this propagation method is limited by slow multiplication rate [2]. The conventional breeding technique is also hard to practice because of their genetic complications of asexually reproductive plant system. Therefore, genetic complications encountered in conventional breeding have lead the breeders to apply induced mutation as alternative crop improvement for vegetatively propagated crops [7].

Induced mutation has been recognized as the most effective technique for induction of morphological and genetic variability in plants mainly in those with limited genetic variation. According to FAO/IAEA database, more than 500 mutant varieties of vegetative propagated plants have been released, resulting of induced mutation as a breeding tool [8]**.** Among physical mutagen, gamma rays are the most efficient and energetic form of such electromagnetic radiation which is more penetrating than other radiation [9]. Therefore, radio-sensitivity study is necessary to obtain optimum gamma rays doses which could cause a higher frequency of mutation in cells [10]. Cytogenetic analysis is also required to access the information regarding the role and effect of gamma rays and in elucidating the response of genotypes to the mutagen.

The present study was conducted to determine the LD_{50} and the effect of gamma rays on the chromosomal behavior of irradiated ginger varieties Bentong and Tanjung Sepat.

II. Procedure

A. Determination of radiosensitivity

Rhizomes samples of ginger varieties Bentong and Tanjung Sepat were obtained from Agricultural Department of Putrajaya, Malaysia. The selected rhizomes were cut into smaller pieces of about 4-5 cm long and contained 2 or 3 point buds. The rhizomes moisture contents were equilibrated prior to irradiation. The irradiation treatments (0 (control), 5, 7, 9, 11, 13 and 15 Gray) at dose rate of 4.31 Gy per minutes were carried out at Malaysia Institute of Nuclear Technology (MINT), Bangi, Selangor, Malaysia. The gamma irradiation was emitted from the Caesium-137 source using Biobeam GM 8000 Germany machine.

Immediately after irradiation, the rhizomes were sown into 12cm x 12cm black polyethylene bags containing the mixture

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of garden loam soil, sand and cocoa peat sowing media at the ratio of 3:2:1. The rhizomes were planted in a side-netted rain shelter of 30 m long x 10 m wide x 4.5 m located in Universiti Teknologi MARA (UiTM) Campus Puncak Alam, Selangor, Malaysia.

Radiosensitivity test of the plant was identified by measuring the survival rate (lethal dose) of the plant, 40 days after irradiation treatments [11].The treatments were laid out in Randomized Complete Block Design (RCBD) with eight replications and were labeled as M_1V_1 generation.

B. Determination of chromosomal behavior

Plant tissue, primarily root cells were used to evaluate the chromosomal aberrations. It is one of the oldest, simplest and reliable method for cytogenetical analysis [12]. The root tips of about 1.0-2.0 cm in length were excised in the mornings. The root tips were fixed in Carnoy's fixative (glacial acetic acid: ethanol) at the ratio of 1:3 for 24 hours. Then the root tips were washed thoroughly in distilled water and kept in 4 % iron alum solution for 3 minutes following iron alum haematoxylin squashes technique [13]. The various types of cells with abnormal chromosomal behavior at various mitosis stages were observed.

III. Result And Discussion

A. Determination of Lethal Dose (LD_{50})

Lethal dose is defined as a dose that cause maximum frequency of mutation with minimum damage occurs in plants. The LD_{50} dose of irradiation was determined based upon sprouting percentage. Based on the plotted graphs (Fig. 1 and Fig. 2), the rhizomes survival percentage decreased with increased doses when compared to control plant (non-irradiated rhizomes). The highest means of survival rate was recorded from control plant (100%). However, increased gamma ray doses caused the survival rate to fell gradually.

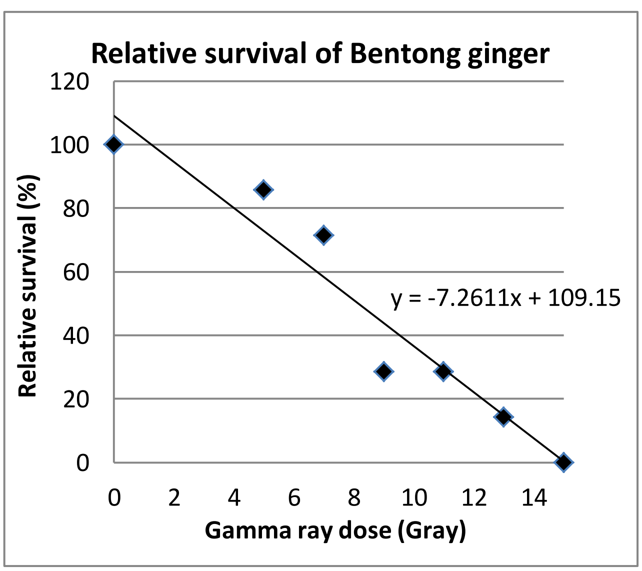

Fig.1: Relative survival percentages in Bentong ginger

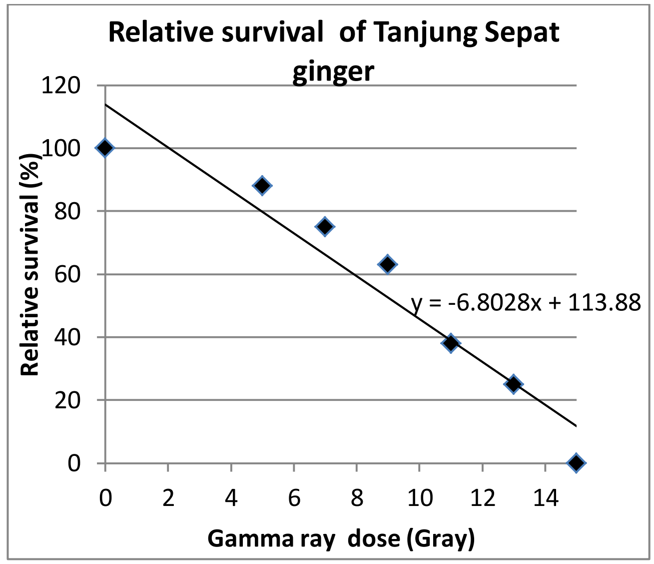

Fig.2: Relative survival percentages in Tanjung Sepat ginger

By referring to Fig. 1, the mean survival rate at 5 Gy and 7 Gy, the survival rates were 85.7% and 71.4% respectively. The mean survival rate fell sharply from 71.4% at 7 Gy to 28.5% at 9 Gy. At 11 Gy, the mean survival was unchanged with 28.5% survival. The reducing pattern was followed by 14.3% survival at 13 Gy. Thus, results indicated that the optimum dose LD_{50} for Bentong ginger was 8.14 Gy.

Meanwhile, the graph in Fig. 2 showed the mean survival rate at 5 Gy was 88% and at 7 Gy was 75%. The mean survival was decreased to 63% at 9 Gy and dropped suddenly to 38% at 11 Gy. At 13 Gy, there was only 25% survival. It was concluded that the optimum dose LD_{50} for Tanjung Sepat ginger was 9.38 Gy.

Based on the observation of mean survival rate, the sensitivity to gamma ray in Bentong was more severe as compared to Tanjung Sepat ginger. Survival of Tanjung Sepat ginger at 5 and 7 Gy doses were higher than Bentong ginger. With attention to the above results, other similar studies have been done on Nigeria ginger varieties in references [14], and the researchers concluded that the optimal gamma ray dose for Nigeria ginger is between average 5 to 9 Gray. Other research on other crops also showed similar finding the rate sensitivity to gamma irradiation depends on the variety [15]–[17].

B. Determination of chromosomal behavior

Cytological analysis in term of mitotic behavior is one the most dependable indices to determine the potency of the mutagen [18]**.** In both varieties, only diploid cells with 2n=22 chromosomes have been observed and there was too much of irregular mitosis abnormalities revealed. Mostly are asymmetrical division, c-metaphase, laggard at metaphase, and clumping of chromosome with laggard as showed in Fig. 3.

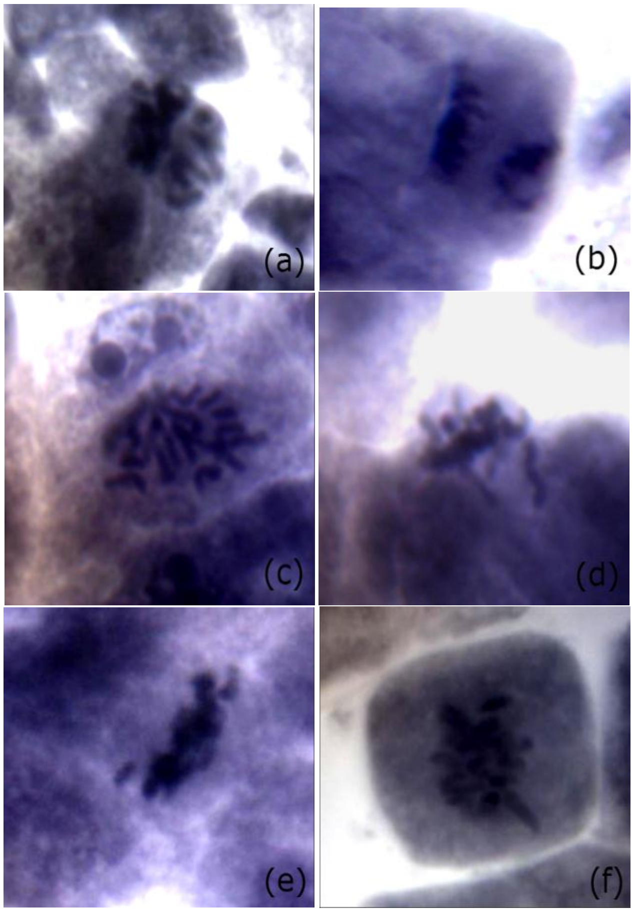

Fig. 3: Mitosis abnormalities in treated ginger plants (2n=22) (a) & (b) Asymmetrical division; (c) C-Metaphase; (d) & (e) Laggard at Metaphase; (f) Clumping of chromosome with laggard;

The irradiated dose seemed well correlated with the amount of chromosomal damage. There are much chromosomal aberrations were observed at dose levels 5, 7, 9 and 11 Gy. In this observation, clumping chromosomes appeared in the prophase, metaphase and telophase. Meanwhile sticky chromosomes were found in the anaphase and also in metaphase phase. This aberration was occurred due to the effect of gamma ray on the nucleic acid polymerization process or due to partial dissociation of the nucleoproteins and adjustment in their organization pattern [19]–[20]. Another founding in references [21] stated that the clumping of chromosomes is due to stickiness between them. The stickiness disturbed the normal arrangement chromosome at the metaphase which leading to their impotence to separate [21]. Then the spindle fiber pulled the chromosome towards the poles thus bridges were broken into fragments. This fragment has to move either towards the poles or formed the laggards [22]–[23]. As the consequence, omission of the arrival of chromatids at the poles caused of the event of fragments at metaphase [24]. It can be concluded, the gamma ray causes complex genetic and physiological damages of the plants.

VI. Conclusion

This research work was conducted to study the effect of gamma rays to the radiosensitivity of two ginger varieties; Bentong and Tanjung Sepat. In establishing an optimum dose, both varieties shows they are sensitive to gamma ray but the rate of their sensitivity differs between varieties. It was noticed, gamma irradiation has effect a broad range of chromosomal aberrations in both varieties ginger and higher proportion has been attributed to clumping, laggards and stickiness of chromosomes.

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