UNIVERSITI PUTRA MALAYSIA

GAMMA RADIOSENSITIVITY STUDY ON TOMATO (LYCOPERSICON ESCULENTUM) AND OKRA (ABELMOSCHUS ESCULENTUS)

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GAMMA RADIOSENSITIVITY STUDY ON TOMATO (*Lycopersicon esculentum*) AND OKRA (*Abelmoschus esculentus*)

By

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ABSTRACT

Gamma irradiation has been found to be very useful technique for crop improvement. Besides, the proper use of induced mutation in plant breeding has become a profitable approached. This investigation was carried out to determine the LD_{50} and effect of gamma irradiation on the germination, plant height, survival and dry weight of plant shoot of irradiated seeds of tomato and okra. This study was not intended to compare differences among the performances of these plants. Seeds of tomato (*Lycopersicon esculentum*) and okra (*Abelmoschus esculentus*) were irradiated with varying doses of 300, 400, 500, 600 and 800 Gy using gamma cell irradiator facility at Malaysia Institute of Nuclear Technology (MINT). For each dose, ninety seeds were used. These treatments as well as ninety seeds of control (unirradiated seeds), were planted in sand beds at Horticulture Unit, University Putra Malaysia Bintulu Campus. This study revealed that the number of germination, plant height, survival and dry weight of plant shoot of tomato and okra decreased with increasing radiation, with 800 Gy having profound effect on these variables. The LD_{50} for survival and germination of the doses for both plants were consistent with literature. Irradiating tomato and okra seeds above 600 Gy caused mortality in these plants.

ABSTRAK

Sinaran gamma telah ditemui sebagai suatu teknik yang sangat berguna untuk pembaikan tanaman. Selain itu, penggunaan rangsangan mutasi yang sesuai pada pembiakbakaan tanaman telah menjadi pendekatan yang menguntungkan. Penyelidikan ini dijalankan untuk menentukan LD_{50} dan kesan sinaran gamma kepada percambahan, ketinggian pokok, ketahanan pokok dan berat kering pucuk bagi benih tomato dan okra yang dikenakan sinaran gamma. Kajian ini tidak bertujuan untuk membandingkan perbezaan antara pokok. Benih tomato (*Lycopersicon esculentum*) dan okra (*Abelmoschus esculentus*) telah didedahkan kepada sinaran gamma dengan 300, 400, 500, 600 dan 800 Gy dos dengan menggunakan kemudahan pemancar sel gamma di MINT. Bagi setiap rawatan ini, sembilan puluh biji benih digunakan. Benih yang telah dirawat ini bersama-sama dengan sembilan puluh benih yang tidak dirawat, telah ditanam di Unit Hortikultur, Universiti Putra Malaysia Kampus Bintulu. Kajian ini mendedahkan bahawa bilangan percambahan, ketinggian pokok, ketahanan pokok dan berat kering pucuk untuk tomato dan okra berkurangan dengan peningkatan sukatan sinaran dengan 800 Gy mengalami kesan yang ketara. LD_{50} untuk ketahanan pokok dan percambahan bagi kedua-dua pokok selaras dengan kajian perpustakaan . Biji benih tomato dan okra yang dikenakan sinaran melebihi 600 Gy menyebabkan kematian kepada tomato dan okra.

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APPROVAL SHEET

I certify that this research project report entitled "gamma radiosensitivity studies on tomato (*Lycopersicon esculentum*) and okra (*Abelmoschus esculentus*)" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences, University Putra Malaysia Bintulu Campus.

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LIST OF ABBREVIATIONS

Gy	Gray
LD	Lethality Dose
MARDI	Malaysian Agricultural Research and Development Institute
MINT	Malaysian Institute of Nuclear Technology
DAP	Day after Planting
SAS	Statistical Analysis System

CHAPTER 1

INTRODUCTION

Mutation breeding is defined as the utilization of induced mutation for the purpose of desired crop improvement. According to Broertjes and Van Harten (1978), mutation breeding makes use of the possibility of altering genes by exposing seeds or other plant parts to chemical or physical mutagens.

According to Chahal and Gosal (2002) the pioneering experiment of mutation breeding began in 1896, where Becquerrel discovered that radio sensitivity induces mutation. In the same year, the use of radioactivity for inducing mutation had been proposed by De Vries but, mutation was known to occur in animals and plants much before this time. For example, a short legged sheep was discovered by an English farmer in the 18th century; this sheep was used to establish a breed named Ancon. After thirty one years, which was in 1927, Muller showed that x-ray induced genetic deviant in drosophila. Meanwhile, in 1928 Stadler reported high mutation rates in x-rayed maize (*Z.mays*) and barley (*H.vulgare*). Mutation experiment in plants started in 1934-35 by Herman Nilsson-Ehle and Ake Gustaffson (Singh, 1983). They reported erectoids mutants in barley with compact head type and stiffcr straw. Currently, induced mutations are considered as an alternative for crop improvement programme and as an alternative to hybridization and recombination in plant breeding.

There are two types of mutation. Mutations occur in natural populations without any treatments by man at low rate are known as spontaneous mutations. While

mutations that are artificially induced by treatment with certain physical (radiation) or chemical agent, are known as induced mutations. These agents that induced mutations are known as mutagens. The physical mutagens includes various kinds of radiation either ionizing radiation or nonionizing radiation (UV radiation). For the chemical mutagens, it may include alkylating agents (e.g. sulphur mustards), acridine dyes (e.g. acriflavin), base analogues (e.g. 5-bromouracil) or others (e.g. sodium azide).

Gamma-rays have the smallest wavelengths and have the most energy of any other wave in the electromagnetic spectrum. These waves are generated by radioactive atoms and in nuclear explosions. Cobalt-60 and caesium-137 are the main sources of gamma rays used in radiobiological work. Caesium-137 is used in many installations since it has a much longer half-life than cobalt-60. These radioisotopes are stored in lead container when not in use and must be operated by remote control mechanisms when needed to irritate plant material.

All kinds of plant parts can be irradiated using physical or chemical mutagens. Besides seeds and pollen grains when produced, (Nybom and Koch, 1965), tuber, bulbs or corms, dormant cuttings, grafts, bud wood, stolons and rhizomes (Broertjes, 1972) can also be used as long as these plant parts are normally used for propagation

The factors that modify response of seeds to ionizing radiation may be group into two major categories; the environmental factors such as atmosphere, seed water content, post irradiation storage and temperature, and the biological

factors such as genetic differences, nuclear and interphase chromosome volumes (IAEA, 1977).

A number of crop varieties have been developed through mutation breeding demonstrating the potential of the technique for crop improvement. The first commercial success with induced mutation was reported in 1934 with the release of a new tobacco cultivar 'Chlorina' through X-ray irradiation. Since then, a large number of mutant cultivars have been developed in various countries in the seed propagated crops. Some important varieties developed through mutation breeding can be found in the work of Chahal and Gosal (2002).

Tomatoes (*Lycopersicon esculentum*) are among the most widely cultivated vegetable crops in the topics. They are grown extensively throughout sub-Saharan Africa, in parts of Central and Latin America, and are of great economic importance in Asia (Vilmorin, 1993). The crop is grown for its fruit, which are cooked as vegetables, eaten raw in salads or used in chutneys. On the other hand, Okra (*Abelmoschus esculentus*), is a popular home garden vegetable in the South of Africa, belonging to the mallow family (Malvaceae). The edible portion of the plant is its immature pods, which are used in soups and stews, or as fried or boiled vegetable. The amount of research of irradiating seeds of a tomato or okra plant and then determining the abnormalities that occur throughout its development is modest (Hawryliak, 2002). Agricultural scientists are also experimenting with increasing the agricultural productivity and quality of certain crops by irradiating seeds, stem cuttings, and detached leaves with

gamma rays or x-rays. By increasing the amount of exposure to these ionizing forms of irradiation, and increasing the intensities of gamma or x-rays, crops will have an increasing mutation rate.

This gamma radio sensitivity study on tomato and okra was carried out in order to meet these objectives:

- To determine LD_{50} of tomato and okra seeds to gamma ray and appropriate doses for mutation induction.
- To evalute changes or improvement on plant height and changes of leaf after gamma radiation.

It was expected that gamma irradiation may induce genetic changes in tomato and okra.

CHAPTER 2

LITERATURE REVIEW

2.1 Plant Breeding

Plant breeding is the act of developing cultivars suitable for man. The basic process is selection from among genetically different plants. These derive their differences from mutation or recombination (Micke, 1984). The type and degree of genetic variation depend upon the parents. The potential result of selection is restricted by the genetic variation existing in the unselected population. However, the selection method (criteria applied, techniques used and intensity of screening), determines the part of the population that will be advanced to the next generation.

2.2 Mutation Breeding

According to studies of Micke (1976), there are different definitions of the term 'mutation'. Definitions range from 'a sudden phenotypic change in a character of an individual, not due to crossing or segregation' up to 'an alteration in the macro-molecule of DNA' (where it remains open, whether the alteration leads to a change in gene function or not). Included under the term 'mutation' is also the augmentation of genetic material through nucleotide or gene copies, through additional individual chromosomes, as well as through the multiplication of whole genomes towards polyploidy.

Mutation breeding is a nonconventional line of genetic science that deals with both permanent heritable genotypic and phenotypic changes (traits) intended to

bring about new and improved varieties among select agricultural crops (Lacandula, 2005).

2.3 Molecular Basis of Gene Mutation

Proteins produced by the genes governing the traits may produce a character of an organism. The type and the sequence of amino acids present in that protein molecule influence the properties of a protein which in turn, is determined by the base sequence of the gene directing production of that protein. An amino acid is encoded by a set of three bases known as codon. Mutation in a character would generally occur due to a change in the amino acid sequence of the protein determining expression of that trait. The change in amino acid sequence of this protein would obviously be due to a change in the base sequence of DNA molecule coding for the protein. The change in base sequence of a DNA molecule may occur either in two ways which is base substitution and base addition or deletion (Singh, 1983).

2.3.1 Base Substitution

Base substitution is when one base in a DNA molecule is replaced by another one. There are two types, transition and transversion.

2.3.1.1 Transition

Occurs when a purine is replaced by another purine (e.g. adenine is replaced by guanine or vice-versa) or a pyrimidine is replaced by another pyrimidine (e.g. thymine is replaced by cytosine or vise-versa). Clearly, one base substitution

affects the base sequence of only one codon. As a result, only one amino acid is altered in the concerned protein (IAEA, 1977).

2.3.1.2 Transversion

In transversion, a pyrimidine (thymine or cytosine) is replaced by a purine (adenine or guanine) or vice-versa. Consequently, transitions and transversions are relatively less deleterious. But sometimes, base substitutions may generate such codons that do not code for any amino acid which is known as nonsense codon. Whenever a nonsense codon is produced, it acts as terminator of the polypeptide chain.

2.3.2 Base Addition and Deletion

Base addition is insertion of one or more bases in a DNA molecule, while base deletion is a loss of one or more bases. Both addition and deletion lead to mutations. If the number of bases added or lost is a multiple of three, one to several amino acids would be either added to or deleted from the concerned protein. But if the number of bases lost or inserted is not a multiple of three, the base sequences of all the codons beyond the point of insertion or deletion are altered. Consequently, all the codons beyond this point will now code for a different amino acid than before (Singh, 1983).

2.4 Types of Mutations

There are two types of mutation, namely spontaneous mutation and artificially mutation.

2.4.1 Spontaneous Mutations

Mutations occur in natural populations without any treatment by man at low rate, known as spontaneous mutations. According to IAEA (1977), almost every species of plant studied, including the very simples, is known to have members arise in its midst which differ from the rest. These are mutants which occurred after a change in the genetic material. The changes and the mutant individuals appeared apparently at random in any population of living things. No cause is now known that explains their appearance. As a result, they have been called spontaneous mutations.

2.4.2 Artificially Mutations/Induced Mutations

Mutations that are artificially induced by treatment with certain physical (radiation) or chemical agents are known as induced mutations. Mutation induction has become a proven way of creating variation within a crop variety. It offers the possibility of inducing desired attributes that either cannot be expressed in nature or have been lost during evolution. More than 1700 mutant cultivars of crop plants with significantly improved attributes such as increased yield, improved quality, disease and stress resistance, have been released worldwide in the last 30 years (Brunner, 1995).

2.5 Mutagens

Mutagens are agent that induces mutations (Allard, 1960). Mutagens fall into either of the two categories, chemical or physical mutagens.

2.5.1 Chemical Mutagens

The number of chemical mutagens is very great and continuously increasing. However, for the purpose of mutation induction, only a few are really useful. Most of these belong to the special class of alkylating agents such as ethyl methanesulphonate (EMS), diethyl sulphate (dES), ethyleneimine (EI), ethyl nitroso urethane (ENU), ethyl nitroso urea (ENH) and methyl nitroso urea (MNH). Azides are also effective mutagens (IAEA, 1977). Beside alkylating agent, acridine dyes (acriflavin, proflavin, acridine orange, acridine yellow, ethidium bromide), base analogues (5-bromouracil, 5-chlorouracil) and antibiotic are also groups of chemical mutagens and commonly used in mutation breeding.

2.5.2 Physical Mutagens

The physical mutagens includes various kinds of radiation either ionizing radiation such as α – rays, β – rays, fast neutron, thermal neutron, X – rays and γ- rays) or nonionizing radiation (UV radiation). Ionizing radiations are effective mutagens and have been used successfully for several years, but their efficiency depends on the plant radiosensitivity (El-Lakany and Sziklai, 1969). Broertjes and Van Harten (1978) also claimed that most mutation breeders prefer ionizing radiation because of some benefits- easily applicable, clean, good penetration and reproducibility (high mutation frequency).

2.6 Gamma - rays

Gamma rays were discovered by the French chemist and physicist, Paul Ulrich Villard in 1900 while he was studying uranium. Working in the chemistry

department of the École Normale in rue d'Ulm, Paris with self-constructed equipment, he found that the rays were not bent by a magnetic field. For a time, it was assumed that gamma rays were particles. The fact that they were rays was demonstrated by the British physicist, William Henry Bragg in 1910 when he showed that the rays ionized gas in a similar way to X-rays. In 1914, Ernest Rutherford and Edward Andrade showed that gamma rays were a form of electromagnetic radiation by measuring their wavelengths using crystal diffraction. The wavelengths were similar to those of X-rays and were very short, in the range 10-11m to 10-14m. It was Rutherford who coined the name 'gamma rays', after naming 'alpha' and 'beta' rays; the nature of the different rays were unknown at that time.

According to IAEA (1970), gamma-rays have the smallest wavelengths and have the most energy of any other wave in the electromagnetic spectrum. These waves are generated by radioactive atoms and in nuclear explosions. Cobalt-60 and caesium-137 are the main sources of gamma rays used in radiobiological work. Caesium-137 is used in many installations since it has a much longer half-life than cobalt-60. These radioisotopes are stored in lead container when not in used and must be operated by remote control mechanisms when needed to irritate plant material.

Gamma rays are often defined to begin at an energy of 10 keV, a frequency of 2.42 EHz, or a wavelength of 124 pm, although electromagnetic radiation from around 10 keV to several hundred keV is also referred to as hard X-rays. There is no physical difference between gamma rays and X-rays of the same energy. Gamma ray is a term for high-energy electromagnetic radiation produced by

nuclear transitions, while X-ray is a term for high-energy electromagnetic radiation produced by energy transitions due to accelerating electrons. Gamma rays are a form of ionizing radiation; they are more penetrating than either alpha or beta radiation (neither of which is electromagnetic radiation), but less ionizing. For instance, a gamma ray will pass through 1 cm of aluminium, while an alpha ray will be stopped by even a single sheet of paper.

2.6 Dose of the Mutagen

The dose for any ionizing radiation is defined as the amount of energy absorbed per mass of irradiated matter at the point of interest. The unit of absorbed dose is rad (radiation absorbed dose) where 1 rad equal 100 erg/g or 10^{-2} joule/kg, and it is expressed as rad per second or minute or per hour. But in mutation breeding experiments, irradiation dose is generally expressed as kR or Gray (Gy) where 1 Gy = 100 rad and 1 kR = 10 Gy (Chahal and Gosal, 2002). When an ionizing particle or photon travels through a medium, it deposits energy in a straight path. The amount of energy deposited or lost by a particle/photon per unit length of its path is described as linear energy transfer (LET). A radiation that produces a few ionizations per micron of its path has low LET value and is called sparsely ionizing radiation e.g., X-rays and gamma-rays (Singh, 1983).

Mutagens generally induce a high frequency of chromosomal changes and mitotic and meiotic irregularities. Usually, the damage increases with the mutagen dose, but it may not necessarily be proportional. An optimum dose is the dose which produces the maximum frequency of mutations and causes the minimum killing. The dose required for high mutagenic efficiency depends on

the properties of the mutagenic agent, the solvent medium and biological system. Many researchers claimed that a dose close to LD_{50} should be the optimum. LD_{50} is the dose of mutagen which would kill 50 per cent of the treated individuals (Allard, 1960). This optimum dose varies with the crop species and with the mutagens used. Generally, a preliminary experiment is conducted to determine the suitable mutagen dose. Overall, an overdose is likely to kill too many treated individuals, while an under dose would produce too few mutations. Mutation breeders should therefore apply doses that generate optimal and not maximal mutation frequencies to achieve a high frequency of useful mutations and minimize the occurrence of drastic and nondesired mutations (Konzak, 1984).

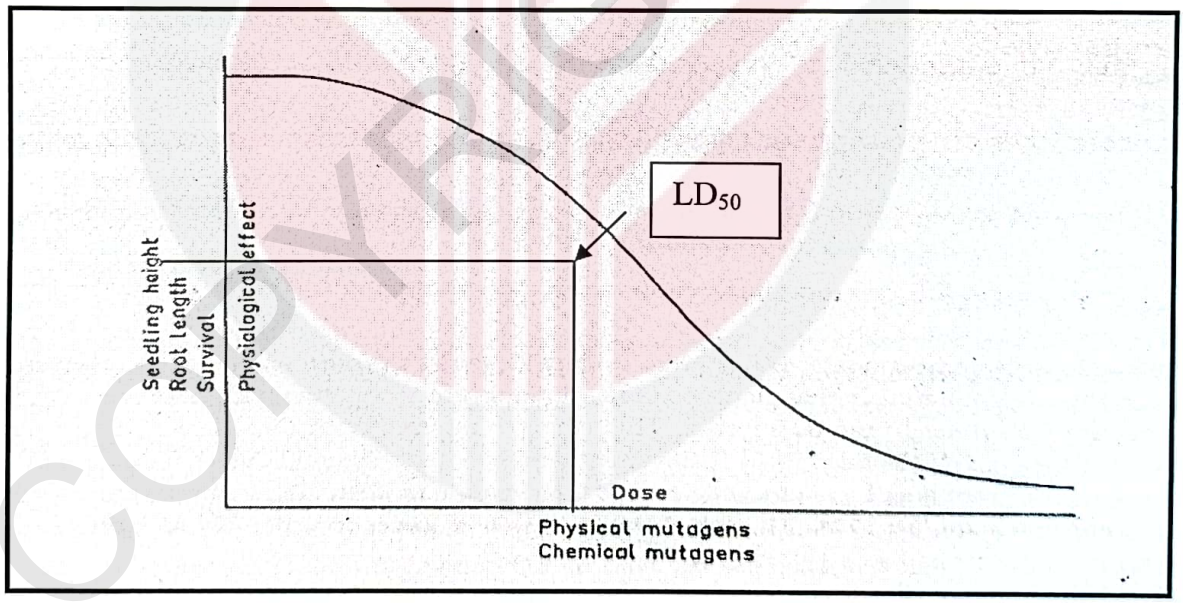

Figure 2.1: Model Curve Illustrating LD_{50}

Source: IAEA (1977)

2.8 Plant Material for Mutagenic

All plant parts can be treated through one or the other method but some are easier to treat than others especially with a particular type of mutagen. A

common practice is to treat the seeds (Chahal and Gosal, 2002) but in vegetatively propagated plants whole plants, dormant cuttings, bulbs, tubers and corms can also be treated (IAEA, 1977).

2.8.1 Seeds

Seed is the most commonly treated material because it can tolerate physical conditions which generally are tolerated only by non-living molecules (IAEA, 1970). They can be desiccated, soaked, heated or frozen. Besides that, seeds are also able to be maintain for extended periods of time under a vacuum almost free of oxygen as well as under high pressures of oxygen or other gasses. When seeds are used, the dose that is sufficient to inhibit the germination of 50 % seeds i.e. LD_{50} is generally applied. The dose and rate i.e. duration of application of a mutagen vary with plant species and should be determined through experimentation (Chahal and Gosal, 2002). Original seed (M_0) is treated after induction of mutation at a locus become heterozygous and thus become M_1 seed and plants so obtained constitute M_1 generation. Seeds produced by M_1 plants give rise to M_2 generation of plants which is expected to show segregation. The M_1 plant usually exhibit reduced vigour and seed sterility. Irrespective of the plant species the M_1 plants are usually chimeric which posses mutated and non mutated branches/tillers. The dominant mutation expresses in the M_1 itself whereas a recessive mutation first expresses only in the M_2 generation.

2.9 Factors Affecting Radiation Effects

The various factors that influence radiation effects may be group into three major categories, which are biological factors such as genetic differences,

nuclear and interphase chromosome volumes, environmental factors such as oxygen, water content, temperature and the last factor being chemical factors.

2.9.1 Biological Factors

2.9.1.1 Genetic Differences

According to the studies of Blixt (1970a), Krausse and Evdokimova (1973), Walter and Haung (1973), there are differences in radiosensitivity among genotypes within a species and in some cases, the differences can be relatively great. However, Wangenhein and Walter (1968) claimed that the differences may due to any of several factors, but do not appear to be related to nuclear or interphase chromosome volumes. The differences in radiosensitivity among genotypes within species are usually much less than between species.

2.9.1.2 Nuclear and Interphase Chromosome Volumes

The most important biological factors governing radiosensitivity of plant species are nuclear volume, interphase chromosome volume and DNA content (Sparrow and Pond, 1956). Radiation effects or radiosensitivity is related inversely to interphase chromosome volume and to a lesser extend DNA content when the oxygen effects are eliminated. The greater resistance of polyploids as compared to closely related diploids results from their reduced interphase chromosome volumes rather than from the protective effect of an increased number of chromosomes (Singh, 1983).

2.9.2 Environmental Factors

2.9.2.1 Oxygen

Oxygen is a major modifying factor of biological, including genetic, damage caused by sparsely ionizing radiations in 'dry' biological systems, such as dormant seeds. Furthermore, its effect may be markedly influenced by various secondary factors such as temperature, seed water content, radiation energy, hydrogen ion concentration etc (IAEA, 1970). Higher mutagenic efficiency (less damage in terms of seedling injury and chromosome aberrations in relation to mutation frequency) can be obtained if seeds or plant parts are irradiated in an atmosphere completely free of oxygen. This can be accomplished by irradiating seeds in an anoxic atmosphere (nitrogen or partial vacuum) or by adjusting the seed water content to 12-14 %. If seed water content is not adjusted, seeds should be soaked in water which is bubble with an inert gas, e.g. argon or nitrogen, after anoxic irradiation.

2.9.2.2 Water Content

Water content of the seed is a critical factor affecting mutation frequency. Seeds with 12-14 % water usually give higher mutation frequency through irradiation (Chahal and Gosal, 2002). Even minor differences in water content can have a very pronounced influence on the end biological effect. For example, it has been shown with barley seeds with small increase in water content from 10.7 to 11.0 % reduced the response to post irradiation oxygen treatment by about 3 fold (Conger *et al.*, 1968a). Dry, dormant seeds are the most commonly used objects for mutation induction. They contain, genetically relevant seed embryos, largely synchronized cell initials in the G1 phase (DNA presynthetic gap 1 during

interphase). Moreover, a seed moisture equilibration over 60 % glycerol to 12-14 % minimizes the effects of modifying factors to low LET radiation and warrants reproducibility of parameters of primary damage within practical limits (Brunner, 1995).

2.9.2.3 Temperature

The temperature of plant material during, before and after irradiation has a distinct effect on the total amount of genetic damage induced by X- or gamma-rays. However, the role of either high or low temperature as a modifying factor of radiation damage is not clearly enough understood to warrant its application (IAEA, 1970). However, it appears that there is a close interrelation of temperature, oxygen and water content in determining the development of radiation damage in barley seeds (Caldecott, 1958, 1961; Conger *et al.*, 1971). A very low temperature ranging from -80 to -96 °C may provide protection against radiation damage in plant seeds (Konzak *et al.*, 1960; Nilan, 1954; Nymbom *et al.*, 1953). Dry-ice temperature applied during radiation to barley seeds reduced the frequency of chromosome aberrations, increased M_1 plant survival and produced the same mutation frequency when compare with material irradiated at room temperature. The protective effect of low temperature may be that of decreasing the mobility of radiation induced free radical and hence their interaction with oxygen. However, when seeds which have been irradiated and stored at dry-ice temperature (-78 °C) are brought to room temperature in the presence of oxygen, a rapid development of damage was observed (Konzak *et al.*, 1960).

In general, both pre- and post- irradiation heat treatments have been found to reduce the radiation induced damage in seeds (Caldecott and Smith, 1952; Konzak *et al*, 1960; Nilan, 1964; Santos, 1965). 'Heat-shock' treatments applied immediately after seed irradiation has been found to reduce damage in terms of M_1 seedling height and chromosome aberration frequencies without decreasing mutation frequencies (Gaul, 1957a and b; Khvostova, 1966; Konzak *et al.*, 1960), related to the water and oxygen content of the seed (Konzak *et al.*, 1961b; Nilan *et al.*, 1962b).

2.9.3 Chemical Factors

There are various chemicals agent which either protects the seeds from radiation damage or increase radiation effect. The protective agent such as cyanides, nitrates, sulphur or sulfhydryl containing compounds, amines, amino acids, peptides, reducing substances and certain chelating agent, reduce radiation damage by several ways such as by reducing oxygen tension in the cells (reducing agents), by acting as traps for free radicals, by combining with biologically important molecules and thereby increasing their radio resistance and by fostering repair mechanisms that reduce primary damage (Singh, 1983). Usually, protective agents act via more than one of the above modes, and they are object-specific, i.e. they may exert protective effects in some biological systems but not in others. However, these agents are virtually ineffective when high LET radiations are employed. Certain chemical such as Mn, Cu, Zn may increase the mutagenic effectiveness depending on the biological target treated; they may act either via increased absorption of photon or through an indirect action on oxygen (Auerbach, 1976).

2.10 Observation of Mutagen Effects in the First Generation after Seed Treatment

2.10.1 Plant Injury and Lethality

When seeds are exposed to ionizing radiation or chemical mutagen, several effects can be observed. Measure and analyze the physical damage and chromosomal aberrations. The physical damage can be determined by cytological analysis and by measuring plant injury in the M_1 generation (Sparrow, 1961). Plant injury can be measured by taking seedling height (determined 10-14 days after), root length, emergence under field or laboratory condition, survival under field or laboratory condition, number of spikes per plant, number of florets per spike, number of seeds per spike and fruits and/or seeds per plant (IAEA,1977). Determination of M_1 injury using seedling height and survival should be a routine procedure in mutation breeding, because it has been established that these characters are correlated in cereals between M_1 seedling height and survival on the one hand and M_1 mutation frequency (Gaul, 1959a).

2.10.1.1 Seedling Height and Root Length

There are three methods of raising seedlings to determine seedling height, namely, Flat Method, Petri-Dish Method and Growing-Rack Method. However, the extensively used is Flat Method (Petry, 1921). In this method, the seeds are sown in boxes or pots filled with a sterile mixture of sand and soil (50:50) or any convenient medium. One of the advantages of this method is that it can certainly be used with ease for any species. The mutagen induced shortening of the germinating plants is measured on the first leaf. A similar test can be performed by sowing the mutagen-treated seeds in a sand bed on greenhouse

benches. With any of the three methods, the plants should be placed in a controlled uniform environment either in a "Greenhouse" or a chamber. Under controlled environmental conditions the number of seeds per treatment should be at least 40 (Mikaelsen, 1966), otherwise several hundred are to be preferred. There should be at least three replications per treatment. Design and analysis of such experiments may fallow the randomized method.

Another rapid method of determining the effect of mutagenic seed treatment is to measure root length. Again, this method is old and was already used by Koernicke (1904). A modern procedure for *Arabidopsis* is described by Muller (1966) and may be used similarly for crop plants. For both tests, seedling height and root length, a statistic of the mean values obtained from the various mutagenic treatments is of interest. Figure 2.2 shows a sigmoid curve for means of seedling height or root length are characteristic of the response to dose of any physical or chemical mutagen and any plant. The actual course, however, varies considerably with different mutagenic treatments and plant used.

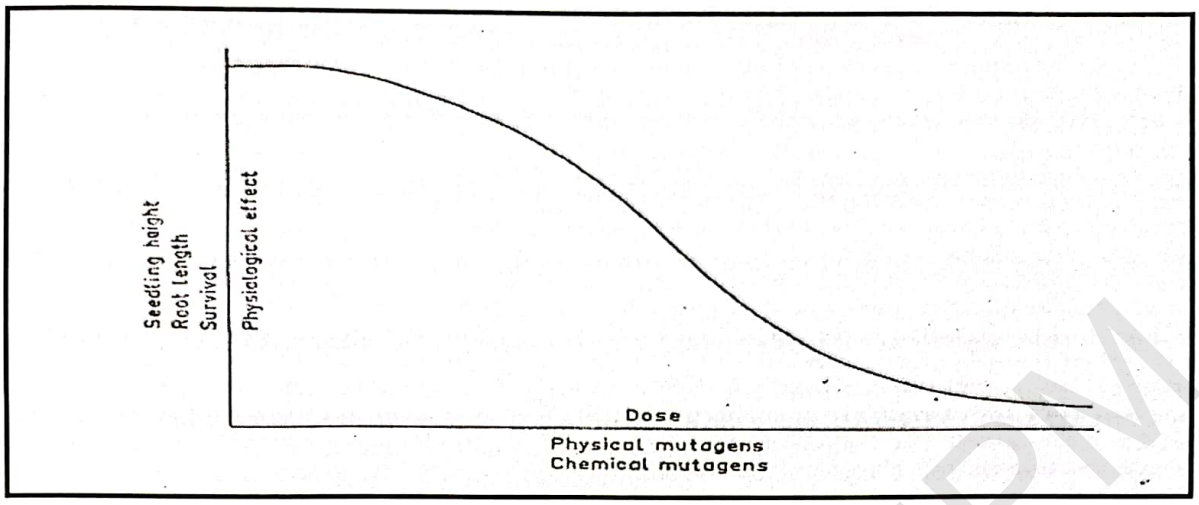

Figure 2.2: Model Curve Illustrating the Effect of Increasing Dose on Physical Damage.

Source: IAEA, (1977).

2.10.1.2 Germination or Survival

The germination ability of normal seeds of most species does not suffer, or only slightly, from radiation even if very heavy doses are applied. Actual plant death might occur at any time between onset of germination and ripening; however, there are critical phases during plant development at which lethal effects are more prominent (Micke and Wohrmann, 1960). The latter can be easily determined quantitatively by counting the number of germinating plants in the field after a given period of time. 'Surviving plants' are usually counted at the time of harvest of the M_1 generation. In many plant species survivors may be defined as those plants that produce at least one spike (inflorescence), regardless of whether seeds are produced (Gaul, 1963). The dose effect curve of survivors has a sigmoid shape similar to that of seedling height and root length.

2.10.1.3 Prediction of Dose Effect

In most of the mutation experiments one is interested to know the lethality produced by a definite dose before the mutagenic treatment is conducted. Because radiation sensitivity depends on many factors, it is difficult to predict a dose effect, e.g. 90 % lethality. A preliminary trial or early test can be conducted before the main treatment in which the effect of a series of different doses on the reduction of seedling height is determined. Then, the desired doses can be chosen accordingly (IAEA, 1977). It is important that the treatment conditions of the pilot experiment and the main experiment are the same.

2.11 Limitations of Mutation Breeding

Singh (1983) claimed that experience with mutation breeding has some limitations and needed to be considered. The frequency of desirable mutation is very low, about 0.1 percent of the total mutation. Therefore, large M_2 and subsequent populations have to be grown and carefully studied. This involves considerable time, labour, and other resources. Besides, efficient, quick and inexpensive selection techniques are required to screen large populations. Mutation breeding is more easily applied to such characters where quick screening techniques are available such as disease resistance. For this reason, mutation breeding has been more successful with those characteristics where the mutant phenotype is distinct and easily detectable.

These limitations are also due to the desirable mutation which is commonly associated with undesirable side effects because of other mutation, chromosomal aberrations and others. Moreover, often mutations produce pleiotropic effects.

The chief procedure for reducing/eliminating pleiotropic effects is to transfer the gene into different genetic backgrounds by hybridizing the mutant with randomly selected range of elite varieties. Brock (1965) concluded that mutations in quantitative traits are usually in the direction away from the selection history of the parent variety. This may tend to limit the degree of improvement attainable in a quantitative trait that has been the object of selection for a long period of time, e.g., yield. Besides, there may be problems in the registration of a mutant variety since it may be difficult to convincingly demonstrate the new variety to be distinct from the parent variety. Lastly, the factor that limits the mutation breeding is that most of the mutations are recessive. Detection of recessive mutations is almost impossible in clonal crops and is difficult in polyploids species. Consequently, in polyploid species, larger populations have to be grown and larger doses of mutagens have to be applied.

2.12 Achievements of Mutation Breeding

A number of crop varieties have been developed through mutation breeding demonstrating the potential of the technique for crop improvement. The first commercial success with induced mutation was reported in 1934 with the release of a new tobacco cultivar 'Chlorina' through X-ray irradiation. The next mutant cultivar released in 1950 was the white mustard cultivar 'Primex' which resulted from X-ray treatment at the Swedish Seed Association at Svalof (Chahal and Gosal, 2002). Since then, a large number of mutant cultivars have been developed in various countries in the seed propagated crops. Some important varieties developed through mutation breeding are listed in Table 2.1 below.

Table 2.1: Some Important Varieties of Selected Field Crops Released in Different Countries through Mutation Breeding

Plant species and variety	Country	Important characters
Allium cepa (onion)		
- Brunette	Netherlands	Early, good quality and high yield.
Lycopersicon esculentum (tomato)		
- Luch 1	USSR	- Early, high yielding
- S 12	India	- Dwarf, 30% more yield than Sioux
Capsicum annuum		
- Krichimsky ran	Bulgaria	Early maturing, high yield
Glycine max (soybean)		
- Raiden	Japan	- Earliness
- Raiko	Japan	- Earliness
- KEX-2	Korea	- Earliness and high yield

Source: Chahal and Gosal (2002).

Micke and Donini (1993) while reviewing the progress of mutation breeding noted that 1363 cultivars have derived valuable characters through mutagenesis. The mutant varieties represent improvements in yield, reduction in height, earliness, increased stress resistance, bolder seed size, improve quality, disease resistance, improved adaptability and others. Recent works by Bansa and Appiah (2003) have also reported the successful use of gamma radiation dose of 120Gy to effectively inhibit sprouting in yams for six months under tropical ambient conditions. Furthermore, higher gamma radiation doses of 2–4 kGy have been used to successfully reduce the infection rate in sugar beet seeds (Rizk and Moussa, 2003) while a dose of approximately 500 Gy has been employed to disinfect and also reduce microbial populations in cocoa beans (Adesuyi, 1996).

Some of the mutant varieties have made valuable economic contributions. According to estimate, one mutant durum wheat variety in Italy has generated an annual benefit of US $ 7 million. Micke (1995) concluded that 'the proper use of induced mutation in plant breeding has become a profitable approach.

2.13 Crop Background

2.13.1 Tomato (*Lycopersicon esculentum*) Description

Tomato (*Lycopersicon esculentum*) is a member of the nightshade family (Solanaceae), along with the pepper, eggplant, and potato. Tomato is said to be a native of Tropical America (Thompson and Kelly, 1957), its original home being probably in Peru or Mexico. It is used in many ways such as cooked, salad, soup, preserves, pickles, ketchups, sauces and many other products and is served baked, fried and as a sauce on various foods. In Malaysia, the area used for tomato production is 700 ha (Swiader and George, 1999).

Tomato is a herbaceous annual plant with bisexual flowers. The fruit is true berry while the leaves are alternate and compound. A number of lateral branches develop from the axil of the leaves on main stem resulting in a bushy plant. Numerous hairs and oil glands are found on the stem and are ruptured when the plant is handled. Generally, the flowers are borne at every third internode separated by three leaves (Dhesi and Nandpuri, 1968).

Tomato is a moderate season crop and does not tolerate frost. High temperatures followed by low humidity and dry winds increase flower drop and there is no fruit set (Smith, 1932). The best seeds germination takes place at 18 to 30 °C but

at 14 °C, the seeds can be germinated (Vilmorin, 1993). The fruit develops good colour and better quality when weather is warm and sunny.

2.13.2 Okra (*Abelmoschus esculentus*) Description

Okra (*Abelmoschus esculentus*), is a popular home garden vegetable in the South of Africa, belonging to the mallow family (Malvaceae). The edible portion of the plant is its immature pods, which are used in soup and stews, or as a fried or boiled vegetable. It is thought to be Asiatic origin, but most likely in Ethiopia and the upper Nile region of Sudan (Swiader and George, 1999).

The plant is a tender annual that grows upright to a height of 3 to 7 feet. It has a half-woody stem and many short branches. Flower buds develop in the axil of each leaf above the six to eight most basal leaves. As the stem elongates, the hibiscus-like flowers open one at a time. The fruit is a pod (one pod per blossom), usually ridged, that grows to 8 inches in length and 1 inch in a diameter. The pods are green or purplish-green when immature, but they develop into dark brown capsules when mature. Okra is a very tender plant that grows best in hot weather (temperature above 85 °F), especially in regions with warm nights (Swiader and George, 1999). Almost any garden soil can be used to grow okra, provided it is well drained since the plant is intolerant of prolonged wet conditions.

2.14 Successful Works on Induced Mutation to Tomato (*Lycopersicon esculentum)* and Okra (*Abelmoschus esculentus*)

Induced mutation approached to tomato (*Lycopersicon esculentum)*, has been mentioned by Yamaguchi (1982). The author claimed, in tomato mutation

breeding, four cultivars were registered totally including two in India and one each in USSR and Japan. Main improved characteristics were earliness, uniform fruit ripening, high yield, disease resistance and dwarf growth type. Gonzales, *et al.* (1999) also reported the successful use of ^{60}Co gamma ray irradiation at doses of 300 and 500 Gy to induce drought tolerant in two Cuban tomato varieties INCA 9-1 and Amalia.

Besides the successful works on irradiated tomato, the study by Mokobia and Anomohanran (2005) on okra also gave a good result. They investigated the effect of gamma irradiation on the subsequent germination and growth of irradiated okra seeds. From the result they obtained, they suggest that for better yields to be obtained, seeds/crops of Okra, meant for planting should be stored using minimal gamma-ray radiation.

The previous studies, led to the prominent conclusion that the process of exposing substances to radiant energy or ionizing radiation such as gamma rays is useful in solving various agricultural problems: reduction of post-harvest losses through suppressing sprouting and contamination, eradication or control of insect pests, reduction of food-borne diseases and in extension of shelf life, and breeding of high-performance well adapted and disease resistant agricultural crop varieties (Andress *et al*, 1994; Emovon, 1996).

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Material

Two varieties were selected for this study. The varieties were MT 1 for tomato and MK Be 1 for Okra. These varieties of tomato and okra were obtained from MARDI. These two varieties were chosen because of their economic value and higher germination percentage (90 %).

3.2 Seed Moisture Content

A 0.102 g tomato and 0.192 g okra seeds were used for moisture content analysis. The moisture contents were analysed using a moisture analyzer (AND, MX – 50) at Agrotechology Laboratory, Universiti Putra Malaysia Bintulu Campus. The moisture contents for okra and tomato were 10.55 % and 9.37 % respectively.

3.3 Sand Bed Preparation

A day before planting the seeds, the sand beds were prepared. All the debris and the grasses were discarded. The sand beds were watered to field capacity. The purpose was to ensure the beds were moist and loose enough for planting.

Figure 3.1: Sand Bed Preparation

3.4 Seed Treatment

Seeds were sent to Malaysian Institute of Nuclear Technology (MINT) for mutagenic treatment using gamma radiation. For the first experiment which was carried out on 27 of September 2006, the seeds were treated with lower doses. The doses were: 0 (control), 50, 100, 150, 200 and 250 Gy. Unfortunately, after planting there were no effects of gamma radiation on tomato and okra. Then, on 27 of December 2006, new seeds of tomato and okra were treated with higher doses at the rates of 0 (control), 300, 400, 500, 600 and 800 Gy. For each dose, 90 seeds were used. Treatments were replicated 3 times.

3.5 Planting Procedure

The planting experiment was done in a greenhouse at Horticulture Unit, Universiti Putra Malaysia, Bintulu Campus (UPMKB). After receiving the treated seeds from MINT, the seeds were sown in sand beds which measured 4.6 m x 0.7 m. Each replicate had six rows with different treatments including control (unirradiated seeds). Each row had 30 seeds. The distance between the seeds was 2 cm and the distance between rows was 5 cm. Markers and chopsticks were used to ensure identification of the doses of the planted seeds. The experimental design was completely randomized design. Water was applied

manually once a day using a watering can to maintain the soil moisture. Weed control was done manually. No application of pesticides, herbicides, and fertilizers was done in order to avoid any effect that will interfere throughout the study.

Figure 3.2: Okra and Tomato Seeds Sown in Sand Bed with Distance 2 cm between Seeds and 5 cm between Rows

3.6 Data Recorded

Records on variables such as germination, plant height, survival and dry weight of plant shoot were noted. The planted seeds were observed daily starting from 1st day of seed germination. At 2 days interval germinating seeds were counted. The criteria for germination were that the radical appeared normal and should exceed the seed length (El-Lakany and Sziklai, 1970). Plant height was measured weekly using a meter rule. Each measurement was carried out five times and the mean height recorded. Measurements of height were taken only when the first leaf had stopped growing (IAEA, 1977).

Survived plants were counted at harvest i.e. 45 days after planting (DAP). Survived plants are defined as those plants that produced at least one spike (inflorences), regardless of whether seeds are produced (Gaul, 1963). For the

shoot dry weight, the survival plants shoot were taken to the Agrotechnology Laboratory, UPMKB and oven dried until constant dry weight was achieved. Changes caused by radiation effect such as leaf wrinkles, light green leaf (chlorophyll mutation) and plant height were recorded throughout the study.

Data on germination, height, survival and dry weight of plants shoot over a period of five weeks were statistically analyzed using the Statistical Analysis System Version 9.1. Analysis of variance was used to detect treatment effect and Tukey's Test used to compare treatment means at $P \leq 0.05$. LD_{50} (Lethal Dose that reduce 50 % of the total population) were determined after the data collection (IAEA, 1977).

Figure 3.3: Plant Height Measurement

CHAPTER 4

RESULTS

4.1 Seeds Germination

4.1.1 Tomato Germination

The effect of irradiation on tomato seed germination with time is presented in Table 4.1.

Table 4.1: Germination of Irradiated Tomato Seed with Time

	Days After Planting (DAP)			
Dose rate	2	4 (%)	6	Dose factor
Control (D0)	88.33	90.00	90.33	89.56[a"]
300 Gy (D3)	85.67	86.33	87.00	86.33[a"]
400 Gy (D4)	74.67	76.67	76.67	76.00[b"]
500 Gy (D5)	65.67	66.00	66.33	66.00[c"]
600 Gy (D6)	58.33	65.33	65.33	63.00[c"]
800 Gy (D8)	39.00	43.67	48.33	43.67[d"]
Time factor	68.61[a']	71.33[a']	72.33[a']	

Note: Same alphabet within column indicates no significant difference between treatment means using Tukey's Test at $p \leq 0.05$.
Same alphabet within row indicates no significant difference between treatment means using Tukey's Test at $p \leq 0.05$.
Means without alphabet (interaction) indicate no significant difference using Tukey's Test at $p \leq 0.05$
' mean of time factor.
" mean of dose factor.

There was no interaction between gamma dose and time. Time as a factor was also not significant. However, different doses affected germination, with control

and the highest dose (D8) having the highest and lowest effects on germination respectively. Generally, the higher the dose, the lower was the germination. Similar observation was made for okra (Table 4.2). LD_{50} for tomato and okra were obtained at 790 and 770 Gy, respectively.

4.1.2 Okra Germination

Table 4.2: Germination of Irradiated Okra Seed with Time

Dose rate	Days After Planting (DAP) 2	4 (%)	6	Dose factor
Control (D0)	90.33	90.67	90.67	$90.57^{a"}$
300 Gy (D3)	81.33	83.33	83.67	$82.78^{b"}$
400 Gy (D4)	70.67	71.00	71.33	$71.00^{c"}$
500 Gy (D5)	61.00	62.00	62.67	$61.89^{d"}$
600 Gy (D6)	56.00	57.33	59.00	$57.44^{d"}$
800 Gy (D8)	40.67	41.67	44.67	$42.33^{e"}$
Day factor	$68.67^{a'}$	$67.67^{a'}$	$66.67^{a'}$	

Note: Same aphabet within column indicates no significant difference between treatment means using Tukey's Test at $p \leq 0.05$.
Different alphabets within row indicate no significant difference between treatment means using Tukey's Test at $p \leq 0.05$.
Means without alphabet (interaction) indicate no significant difference using Tukey's Test at $p \leq 0.05$
' mean of time factor.
" mean of dose factor.

4.2 Plants Height

4.2.1 Tomato Height

Table 4.3 shows that there was significant interaction between dose and time. At 7 and 14 DAP, only the highest dose (800 Gy) was statistically different from the control. At 21 and 28 DAP, doses 600 and 800 Gy significantly reduced tomato height compared to the control while the other doses did not compared to control. At 35 DAP, except for 300 Gy, the other doses significantly reduced tomato heights, indicating that the effects of the doses were pronounced with time. However, the lowest dose showed good effect compared with control.

Table 4.3: Effect of Irradiation on Tomato Height with Time

	Day After Planting (DAP)					
Dose rate	7	14	21 (cm)	28	35	Dose factor
Control (D0)	1.58^{a}	2.66^{a}	3.95^{a}	5.42^{a}	7.81^{a}	$4.28^{a"}$
300 Gy (D3)	1.54^{ab}	2.72^{a}	3.80^{a}	5.02^{ab}	7.20^{ab}	$4.05^{a"}$
400 Gy (D4)	1.58^{a}	2.74^{a}	3.48^{ab}	3.93^{ab}	4.88^{bc}	$3.32^{a"}$
500Gy (D5)	1.43^{ab}	2.45^{ab}	3.29^{ab}	3.86^{ab}	4.68^{c}	$3.14^{a"}$
600 Gy (D6)	1.28^{ab}	2.17^{ab}	2.71^{bc}	3.41^{bc}	4.75^{c}	$2.86^{b"}$
800 Gy (D8)	1.16^{b}	1.86^{b}	2.17^{c}	2.46^{c}	3.5^{c}	$2.23^{b"}$
Time factor	$1.43^{e'}$	$2.43^{d'}$	$3.23^{c'}$	$4.02^{b'}$	$5.47^{a'}$	

Note: Different alphabets within column indicate no significant difference between treatment means using Tukey's Test at $p \leq 0.05$.
Different alphabets within row indicate significant difference between treatment means using Tukey's Test at $p \leq 0.05$.
' mean of time factor.
" mean of dose factor.

4.2.2 Okra Height

As shown in Table 4.4, there was significant interaction between dose and time. At 7 DAP only 300 and 400 Gy did not have effect on okra height compared with control. From DAP 14 to 35, all the doses had significant effect on okra height. Comparison among doses generally indicated that their effects were statistically similar.

Table 4.4: Effect of Irradiation on Okra Height with Time

Dose rate	Day After Planting (DAP) 7	14	21 (cm)	28	35	Dose factor
Control (D0)	4.70^{a}	7.37^{a}	8.38^{a}	9.61^{a}	11.95^{a}	$8.40^{a''}$
300 Gy (D3)	3.72^{ab}	5.34^{b}	6.29^{b}	7.30^{b}	10.69^{ab}	$6.67^{b''}$
400 Gy (D4)	3.61^{abc}	5.09^{bc}	5.61^{b}	6.64^{b}	8.52^{bc}	$5.89^{c''}$
500 Gy (D5)	3.25^{bc}	4.20^{cd}	5.05^{b}	6.28^{b}	6.92^{c}	$5.14^{d''}$
600 Gy (D6)	2.91^{bc}	4.47^{cbd}	5.08^{b}	5.91^{b}	6.59^{c}	$4.99^{d''}$
800 Gy (D8)	2.55^{c}	3.45^{d}	4.98^{b}	5.88^{b}	6.93^{c}	$4.76^{d''}$
Time factor	$3.46^{e'}$	$4.99^{d'}$	$5.89^{c'}$	$6.93^{b'}$	$8.60^{a'}$	

Note: Different alphabets within column indicate no significant difference between treatment means using Tukey's Test at $p \leq 0.05$.
Different alphabets within row indicate significant difference between treatment means using Tukey's Test at $p \leq 0.05$.
' mean of time factor.
" mean of dose factor.

4.3 Plants Survival

4.3.1 Tomato and Okra Survival

The effect of different doses on the survival of tomato is shown in Figure 4.1. Except for D3 where the survival was statistically similar to D0 (Control), those of D4, D5, D6 and D8 were statistically lower, indicating that the higher the gamma dose, the lower was the survival rate of tomato. In the case of okra, similar observation was made (Figure 4.2) except that the survival percentage of D3 and D4 were not different from that of the control. The LD_{50} (survival) for tomato was obtained at 640 Gy. The LD_{50} (survival) value for okra was 580 Gy.

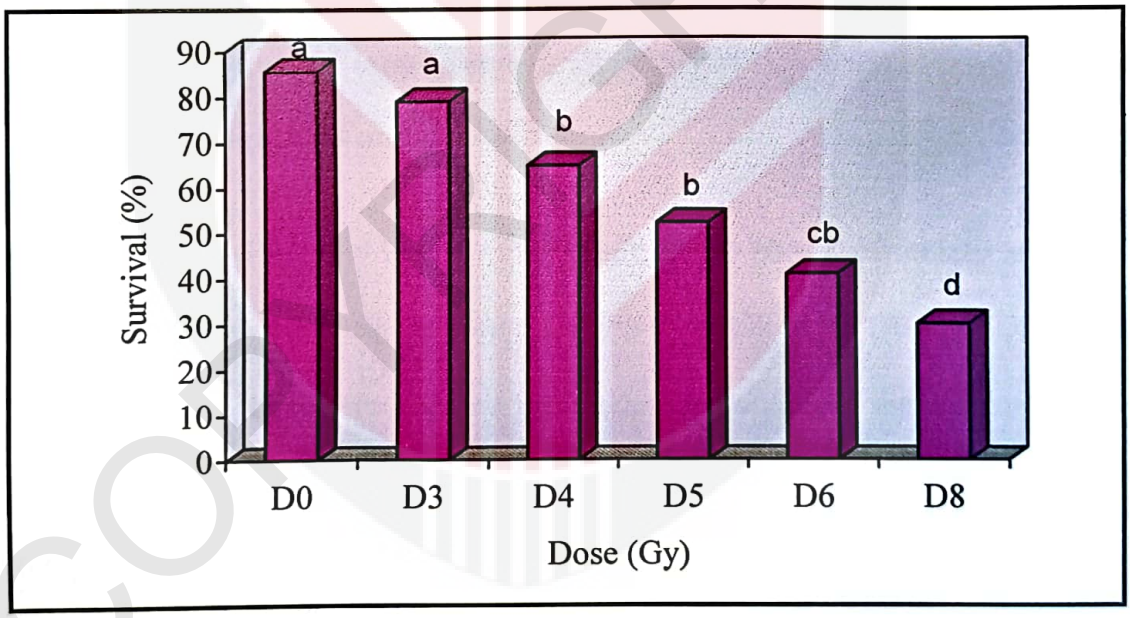

Figure 4.1: Effect of Irradiation on Survival of Tomato

Note: Different alphabets indicate no significant difference between treatment means using Tukey's Test at $p \leq 0.05$

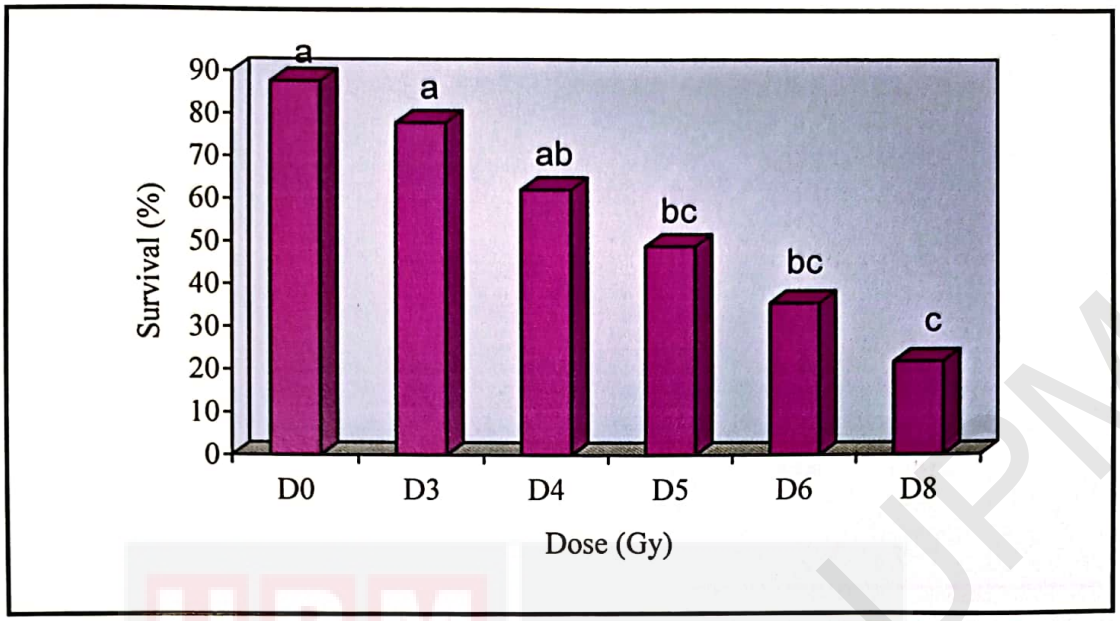

Figure 4.2: Effect of Irradiation on Survival of Okra

Note: Different alphabets indicate no significant difference between treatment means using Tukey's Test at $p \leq 0.05$.

4.4 Dry Weight of Plants Shoot

4.4.1 Dry Weight of Tomato Shoot

Figures 4.3 and 4.4 show that the dry weight of the shoots of tomato and okra compared to D0 were significantly different for D4, D5, D6 and D8. This indicates that higher doses reduced dry matter production of these plants while the opposite was true for the lower dose D3. An attestation of this can be found in Figure 4.5.

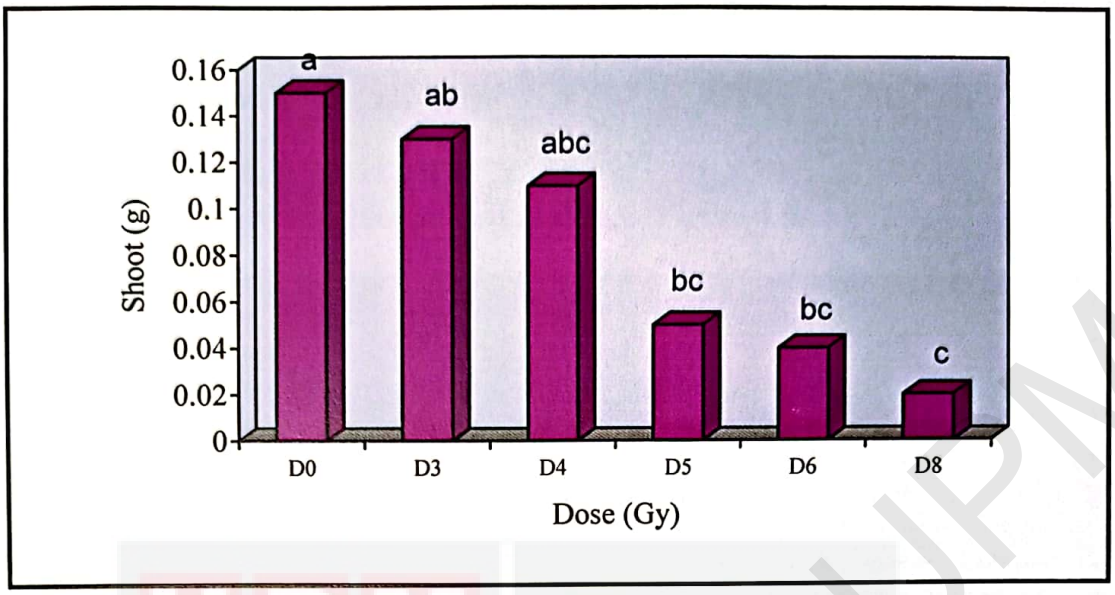

Figure 4.3: Effect of Irradiation on Dry Weight of Tomato Shoot

Note: Different alphabets indicate no significant difference between treatment means using Tukey's Test at $p \leq 0.05$.

4.4.2 Dry Weight of Okra Shoot

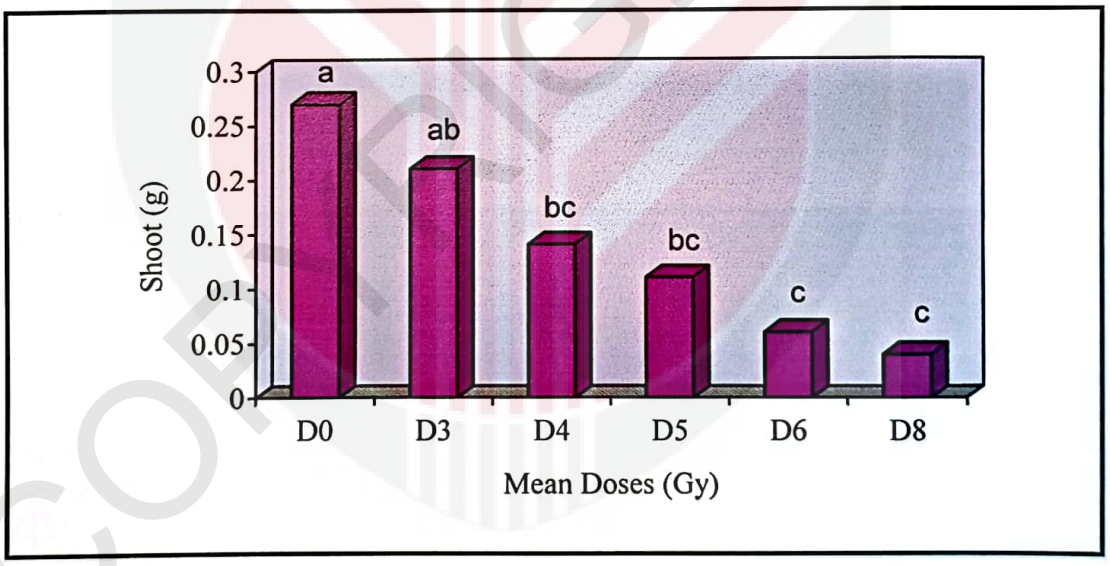

Figure 4.4: Effect of Irradiation on Dry Weight of Okra Shoot

Note: Different alphabets indicate no significant difference between treatment means using Tukey's Test at $p \leq 0.05$.

4.5 Mutant Characters of Plants

4.5.1 Tomato Mutant Character

Plant height decreased with increasing dosage (Figure 4.5).

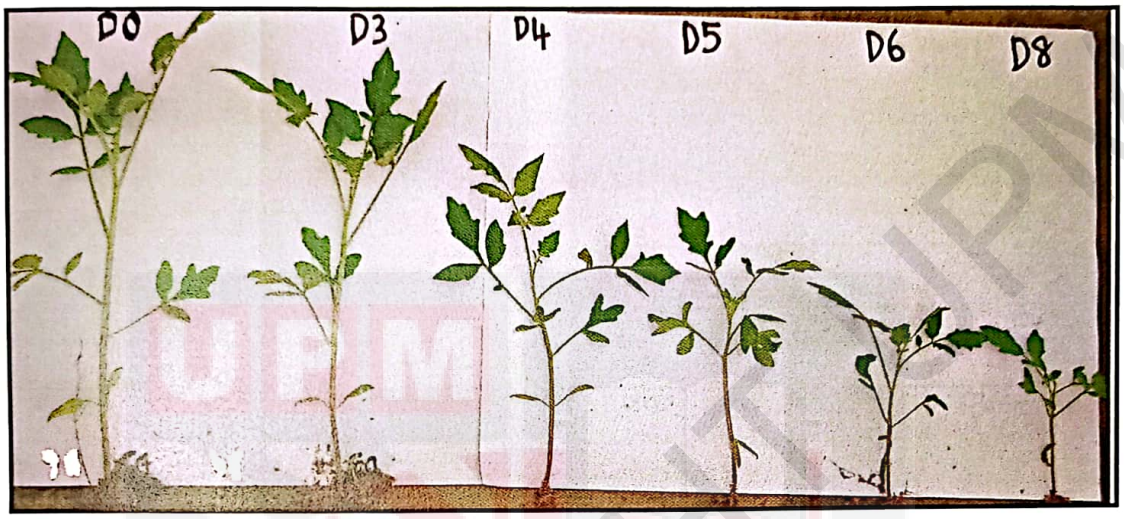

Figure 4.5: Effect of Irradiation on Tomato Growth

4.5.2 Okra Mutant Characters

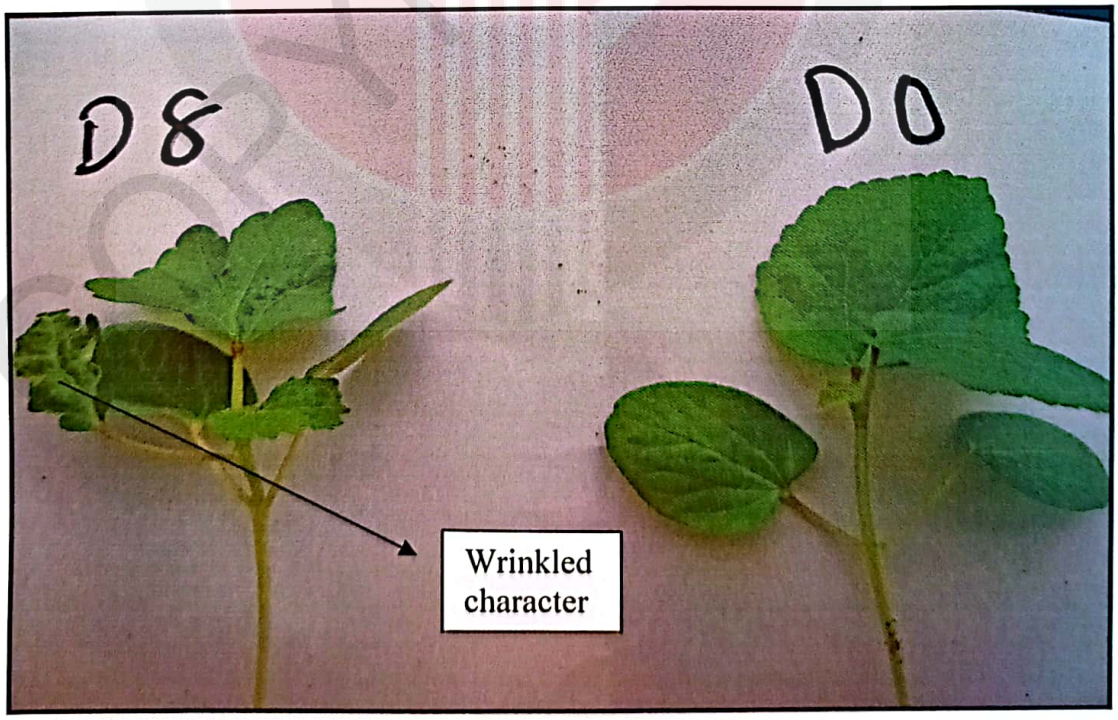

Figure 4.6: Leaflet mutation

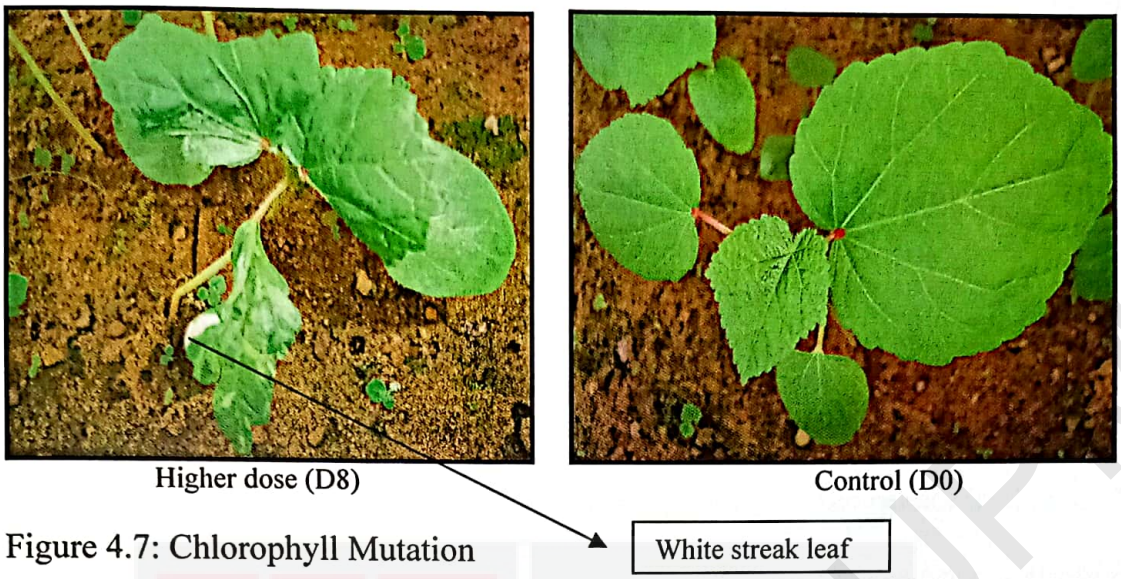

Figure 4.7: Chlorophyll Mutation

Figures 4.6 and 4.7 show some mutations in okra leaf at D8 (highest dosage).

CHAPTER 5

DISCUSSION

5.1 Seeds Moisture Content

The inverse relationship between moisture content and radiosensitivity has already been recognized (Caldecott, 1955; Osborne, *et al.*, 1963). Although seed moisture content might have affected radiosensitivity of the plants in this study to some extent, this effect is regarded as minimal because the radiosensitivity of these crop to moisture.

5.2 Seeds Germination

According to Table 4.1, tomato seed germination showed no significant effect in terms of interaction between doses and DAP. This was probably because during germination phase, the doses applied gave no much impact with time increase. But, among doses it showed significant difference. Unirradiated seed (D0) germination was about 89.56 % which was good enough for germination of this variety (MT 1), and was consistent with MARDI 90 % of germination. However, as dose increased, the germination decreased. This possibly was because higher doses of gamma ray inhibited the germination of seeds. The lethal dose (LD_{50}) for germination that would kill 50 % of living plant for tomato after estimation was found to be about 790 Gy. This means, to achieve 50 % of tomato germination, the seed should be treated with about 790 Gy. But, in this study, dose 800 Gy still can be applied for LD_{50}.

For okra germination (Table 4.2), there was no significant interaction between DAP and dose. As can be observed from the table, germination of higher doses was significantly different compare with control seeds. Germination of the control seeds was 90.57 % and increasing dose decreased seeds germination. The LD_{50} (germination) for okra was about 770 Gy, suggesting that to achieve a germination of 50 % in okra, a gamma radiation dose should be 770 Gy. The purpose of estimating LD_{50} in certain quantities of mutagens are required to cause low plant injury but in high genetic effects (IAEA, 1970).

5.3 Plants Height

The significant reduction in tomato plant height particularly doses D6 and D8 (Table 4.3) might be because the plant is relatively sensitive higher doses (Hawryliak, 2002). By increasing the amount of exposure of irradiation, and increasing the intensities of gamma rays, tomato will have an increasing mutation rate.

Mokobia and Anomoharan (2005) found that the seeds of okra which were irradiated with low gamma radiation grew higher than those irradiated with a high dose. This finding is in agreement with the observation made in this study where D5, D6 and D8 were found to reduce okra height significantly.

5.4 Plants Survival

As presented in Figure 4.1, seeds without gamma radiation survived better than seeds treated with 600 Gy and 800 Gy. The percentage of living plants after harvesting reduced compare to total germination. This was probably because of

rainfall which occurred during growth phase and it increased the mortality of plant. As reported by Amos (2004), environmental requirements for germination are fewer and simpler than those for whole plant development, so germination is relatively independent of the environment for a considerable period of seedling development. Besides, higher doses also increased the rate of plant mortality. LD_{50} for tomato survival after estimation was about 640 Gy, which was lower than LD_{50} for germination. This is because even heavy doses of ionizing radiation, which caused 100 % lethality when counted at the time of harvest, may have little effect upon the onset of germination. This confirms the work of Micke and Wohrmann (1960), who found that actual plant death might occur at any time between onset of germination and ripening; however, there are critical phases during plant development at which lethal effects are more prominent.

According to Figure 4.2, there was significant difference in okra survival according to Tukey's Test. Increasing doses applied, seems to decrease the plant survival. When the result of germination percentage was compared with plant survival, slight differences were recognized. This might be due to environmental stress (e.g. raining season) during plant development and the effect of higher doses applied. The differences between LD_{50} (germination) and LD_{50} (survival) of okra might be because the little effect of doses during germination phase. This also confirms the work by Micke and Wohrmann (1960).

5.5 Dry Weight of Plants Shoot

Figure 4.3, obviously shows that tomato dry weight of shoots for control seeds were higher compare to those treated with 800 Gy. These differences are due to

the differences in plant height of these treatments. In other words, the plant height might have an effect on the dry weight values. Logically, the better the plant grows, the higher will be its dry weight. This is because control seeds developed better and their roots can obsorbed much water from the soil. Once the moisture content was higher, it gave higher value of oven dry weight.

Figure 4.4, also shows that the dry weight of okra decreased with increasing dose. It was assumed that plant height influenced greatly dry weight since the root study was not practical to get from the soil, although no literature was found to confirm this.

From the study of Savaskan and Toker (1991), it was found that shoot and root lengths decreased considerably in rye (*Secale cereale* L.) subjected to gamma irradiations with sharp effects on the development of roots compared to shoots.

5.6 Mutant Characters of Plants

From the study, no mutant characters of tomato plants were found. This might be due to the low rate of doses. Moreover, as claimed by IAEA (1970), factor mutations cannot be recorded in M_1 generation, except when special tester stocks are used or haploid gametes are mutated. However, the only thing that could be observed is shortening plant height as shows in Figure 4.5.

In this study, the mutants found on okra were mainly of leaflet mutation, such as wrinkled leaf. This was due to the fact that the leaf of higher doses (D8) had wrinkled character. Besides, the leaf chlorophyll mutation such as white streak

leaf was found in plants treated with 800 Gy. These mutant characters might be due to over dose. The characters of white streak leaf identified in appearance of white streak from edge to middle vein similar to the study of Chontira *et al,* (2005). These mutant characters were shown in Figure 4.6 and Figure 4.7. These mutants were not found in the control plants. Chontira *et al.* (2005), considered these as real mutants and not the result of recombinant between the parental lines.

CHAPTER 6

CONCLUSION

According to theory, with increasing dose the value obtained for each of these biological criteria (germination, seedling height, survival, root length, number of seeds per spike (inflorescences) and fruits and/or seeds per plant) will decrease. From the study on tomato, it proved that as dose increased, seeds germination, plant height, survival and dry weight shoot decreased. The result from Okra germination, plant height, survival and dry weight of shoot were also agreed with the theory. After estimation, LD_{50} (germination) and LD_{50} (survival) for tomato were found to be about 790 Gy and 640 Gy. For okra plant, LD_{50} (germination) and LD_{50} (survival) were approximately 770 Gy and 580 Gy. From this study, the highest dose of gamma ray applied (800 Gy) for okra induced physiological changes in terms of reduced plant high, chlorophyll mutation and leaflet mutation. Higher doses applied to tomato seeds also induced physiological changes in terms of plant height reduction. Overall, the results for tomato and okra have achieved the objectives of this study.

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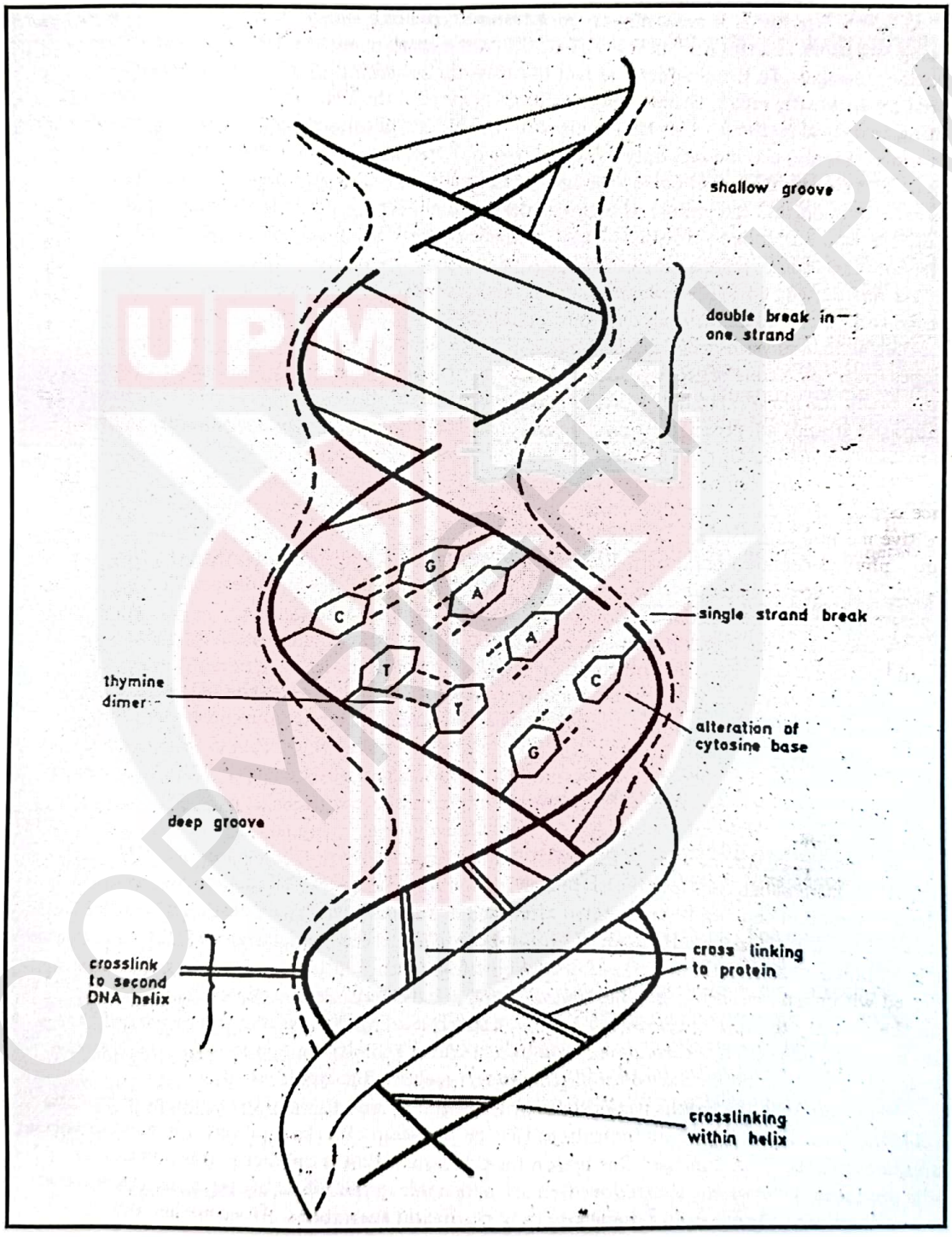
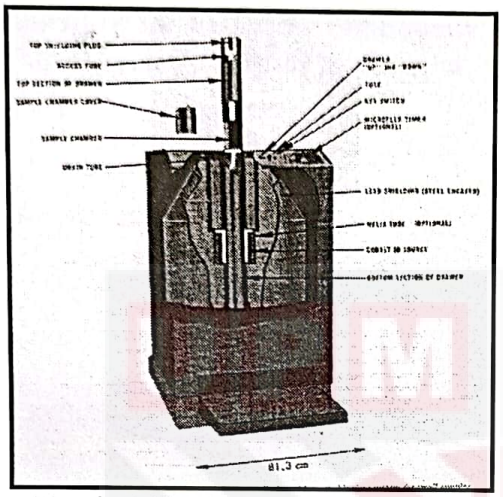
APPENDIX A

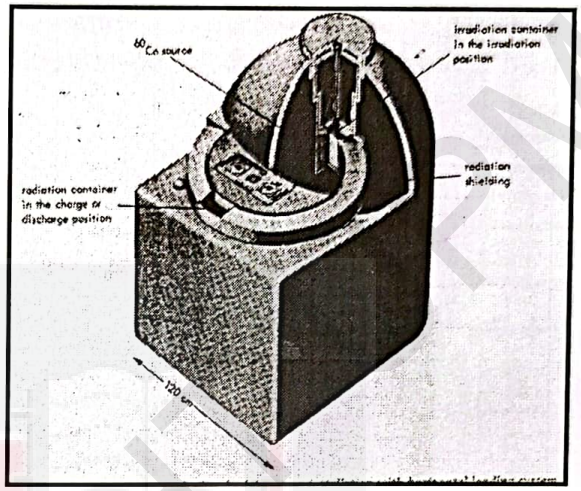


Schematic representation of alterations in DNA caused by radiation

APPENDIX B



Vertical loading system for small samples



Horizontal loading system

Gamma irradiation facility for laboratory installation with vertical and horizontal loading system

PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitle "gamma radiosensitivity studies on tomato (*Lycopersicon esculentum*) and okra (*Abelmoschus esculentus*)" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, University Putra Malaysia Bintulu Campus and published in the form approved by the Faculty.

NORFADZRIN BT FADZIL

Date: 04 MEI 2007.