THE EFFECTS OF RADIATION ON POPCORN (Zea mays var. praecox Sturt)

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DEDICATION

To my wife Rukayyat A. Kasim for all the patience, endurance and inspiration during the course of this study.

and

To my parents Alhaji Adamu A. Nuhu, Zainab Abu Adamu, my step mother Hajara Adamu and the rest members of my family.

D E C L A R A T I O N

I hereby declare that this thesis is a record of my own research work. It has not been presented by me in any previous application for a higher degree.



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C E R T I F I C A T I O N

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ABSTRACT

Air dried seeds of inbred line of popcorn (Zea Mays Var. Praecox Sturt) were irradiated with gamma rays (cobalt 60) at the doses of 10,20,30,40 and 400 Gray (gy) and thermal neutrons at 25,40,50,65 minutes and 12 hours with a flux of 1.5 x 10^{4} $n.cm^{-2}.S^{-1}$ and sown in the field, laboratory and green house.

Result of these treatments, demonstrated a drastic reduction of seed germination, seedling height, root length, and seedling survival. The reduction was positively associated with the dose in both M_1 and M_2 generation. However, there was a slight increase of seed germination in M_2, raised from treatment at higher doses (t400Gy and N12hrs) of M_1 generation.

Among the induced dorminant seedlings mutants, are the occurence of sectors of chloroplyll abnormalities (albina, xantha virescent; lustescent; chlorina and striata) and other morphological changes in appearance; most of which are lethal or unfavourable to growth in both M_1 and M_2 generation. The mean number of mutants per mutation were found to be 7.05 in gamma rays and 6.15 in thermal neutrons.

The capacity of favourable mutants induced include increase in tiller formation, number of cobs per plants; increased earliness; earlength; and seed weight

in most of the treatments. The increase were in direct proportionality with the dose and were more frequent in gamma rays than in thermal neutrons.

The class of dominant mutants observed at maturity, include traits such as tassel seed, male sterility and morphological traits not expressed in the seedling stage. However, most of the classes were not transmitted to M_2 generation and the highest percentage observable was not more than 4% in both gamma rays and thermal neutrons and were mostly confined to higher doses.

There was also no correlation between the group of mutation (Chlorophyll deficient) observed and male sterility; and not only the frequency is independent of the degree of male sterility, but also the spectrum of chlorophyll mutation does not indicate any connection with M_1 and M_2 male sterility.

The frequency of mitotic and meiotic aberrations induced include, formation of multivalents(translocation), non pairing, laggards; fragments; eliminated; non-oriented chromosomes, single and dicentric bridges (inversions), induced deformed and aborted pollen grains at different doses in both M_1 and M_2 generation.

Thus considering the total results on the capacity of favourable mutants; genetics and cytogenetics of

gamma rays and thermal neutrons induced mutations seems to be of practical values. In particular the increase in the number of tillers, cobs, earliness, male sterility, ear length, seed weight are beneficial chracters which could be exploited and utilized for breeding of popcorn.

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ABBREVIATIONS AND DEFINATIONS

1. Mutation/mutant: Is any heritable change in genetic material.
2. Mutagen: any physical,chemical or biological agent that significantly increase mutational events above the spontaneous background level.
3. Irradiation:- Is lthe process of subjecting the seeds to an ionizing radiation.
4. M_o :- is the parental or non irradiated population.
5. M_1 :- Is the first mutant generation, (ie the resultant offspring from the irradiated population).
6. M_2 :- Is the second mutant generation.
7. et al:- and others
8. e.g.:- for example
9. e.t.c:- and so forth.
10. i.e:- that is.

THE EFFECTS OF RADIATION ON POPCORN
(Zea mays VAR. praecox Sturt)

CHAPTER I

1.0 INTRODUCTION

ORIGIN AND GENERAL CHARACTERISTICS OF POPCORN (ZEA MAYS VAR. PREACOX STURT).

The name maize is derived from South-American-Indian - name Mahiz. Maize is also known by the name of Indian corn and in America simply as `corn'. It is food crop of considerable merit in many parts of the world, particularly in Northern Nigeria. U.S.A produces about 50 percent of the World total output.

Margelsdorf and Reeves (1939) visualized maize as a wild podcorn originating from a remote Andropogonaceous ancestor which gave rise on the South American continents, to a single species (Zea mays on the North American-continent, to a more variable genus, Tripsacum. Maize belongs to tribe Tripsaceae, family Poaceae, which contains three genera that are of American origin, Tripsacum, Euchlaena and Zea.

According to Purseglove (1976) quoted sources, Columbus introduced Maize crop into Spain, from where it spreads to Portugal, France, Italy, South Eastern Europe and northern Africa; and that Maize was introduced into West Africa from the north across the Sahara by Arab traders. More over this seems to be supported by the very Hausa name - "Masara" (ie

`Egyptians Corn')

Popcorn (Zea mays Var. Praecox Sturt.) has endosperm with only small proportion of soft starch, the major part being corneous starch. The small size of its seeds and cobs characterizes this group. Small, hard, pointed seeds occur from crosses of maize with Euchlaena and such crosses are believed to be the original source of popcorn (Hayes 1955). The phenomenon of "popping" is due to the formation of steam in the central part of the endosperm where the cells contain a fairly large proportion of water. The steam pressure built-up, results in the exploision. Poor popping is often attributed to an inadequate or excessive amount of moisture in the central soft starchy region of the kernel. Popcorn is used mainly as a staple human food, a feed for livestock and as a raw material for many industrial products.

1.2 INDUCED MUTATION

The idea of inducing mutation by the use of radiation was first proposed by DeVaries (1904). Strict methods of analysis were not formulated until Muller (1927) published his data on Drosophila. Somewhat later Stadler (1928) elaborated similar principles in crop plants. Mutations are the tools that geneticists used to study the nature and function of genes. They also represent the building blocks with which new cultivars are developed by breeding. Since spontaneous mutation occurs at a low frequency and often do not

include the full range of possible variability; mutations are induced in high frequencies by physical or chemical agents. Mutations, either *spontaneous or artificially induced, both in seed* and vegetatively propagated crops, are of scientific and commercial interest. Mutations are the basis of plant evolution and from a scientific point of view, they may help us to better understand plant growth and development. They provide us with the raw material for the genetic improvement of economic crops and occassionally a new cultiver results with only a little further breeding; thus numerous mutatants have been induced and selected strictly for genetic or physiological studies.

Mutagenesis, the artificial inducement of mutations, has been an important research approach since 1928 when Stadler; Muller and Altenburg showed that X-rays and radium cause heritable changes in barley; maize and Drosophila. There followed a *proliferation of experiments designed to determine* the nature of mutation and to find a practical application for this phenomenon. Maize as well, as other plant and animals were treated with a variety of atomic radiations, including those of the Bikini atom bomb tests and gamma rays in the fields irradiated by cobalt ^{60} (^{60}Co) sources. Initially ionizing radiations were thought to have a great potential for producing useful genetic changes. However, it become clear that most of

the changes were not in the basic units of heredity but in the structure and distribution of chromosome, which in most cases were not necessarily conducive to higher yield or better quality. Nonionizing radiation in the ultraviolet spectrum was to be more effecient for producing "Point mutations" but problems with tissue penetration prevented its use as a successful mutagen for higher plants(Cornu and Maizonnier 1983). Chemical agents were successfully used to produce point mutations in the early 1940s but were slow to attain popularity because the germinal nuclei remain difficult to treat directly.

Mutant induction and selection methodology has now progressed to the point, where it provides a viable additional option to plant breeders for creating useful genetic variability. In most situations, mutation induction should be looked upon as a tool, that in combination with other tools such as cross breeding can be used to achieve desired plant breeding goals. Mutation breeding, i.e use of mutants, either directly or indirectly via crosses with other gene sources in the development of plant cultivars, thus represents a complementary method of modern plant improvement (Konzak et al 1984, Lysenkov 1989; Sigurbjornsson and Micke 1974). Genetic variability available in germplasm collections can be supplemented by induced mutations. In some cases the germplasm sources may be inadequate or in a particularly poor genetic background. Mutant

variation can provide genes at previously unknown loci as well as new series of alleles with similar phenotypic expressions; but in better adapted genetic background (Gustafsson 1979; Gustafsson and Lundqvist 1981). Several cultivars derived from direct utilization of induced mutants, have shown that, short straw,earliness and resistance to certain diseases can be introduced in other-wise well adapted varieties without significantly altering their other attributes (Sigurbjornsson and Micke 1974). Induced mutation led to some of the highest quality durum wheats to barley, rice and castor beans with substantially decreased maturity time (Swaminathan 1969, Cheng et al 1990, Benamer, 1990, and Koh 1987) to significant increase in lysine content in barley (Doll, 1975) to disease resistance in the pepper mint plant where no useful source of resistance could be found else where (Murray 1969). A desired mutation can be recovered in a homozygous stage already in the M_2 or M_3 generation as compared with F_6 or F_7 generation in the case of hybridization. The time required for breeding has thus been considerably reduced. It is possible to make use of many mutations that could not manifest themselves in a generation derived from seed propagated. In crops that never bear seeds, the induced-mutation methods offers the only effective way of breeding (Broertjes 1972).

In the process of breeding crop plants, the progress obtainable tends to be limited by the variability present in nature, so that further progress in breeding becomes more difficult. The possiblity offered by mutagenic agents to induced new genetic variation is therefore of extreme interest, it might in many cases be the only answer to problems posed to the practical breeder.

Popcorn, a natural cross-pollinator, is diploid with *ten pairs of chromosomes* (n=10)(*Coe and* Neuffer, 1977); has a vast reservoir of spontaneous mutants, which are systematically removed or reorganized in the process of forming the inbred lines used in the production of hybrid maize. This natural variability is the basis for the presence of well-adapted races and inbred lines of maize in many different habitats.

There has been very little use of irradiation by maize breeders;(Gottschalk and Wolff 1983, Konzak et al. 1984, Pasztor 1979), whereas it has been used extensively in breeding rice (Rutger 1983), barley (Robberlen et al 1983) and other crops (Konzak et al. 1984). Maize being an important crop, in this country deserves equal attention by mutation breeders, as it is the case with conventional plant breeders. It is with the following objectives in view that a systematic mutation studies was started in this crop, by subjecting the seeds to gamma rays and thermal Neutrons radiation.

(i) To induce and analyse the range of mutations/new variability that might be observed in response to different doses of Thermal neutrons and gamma rays radiation.

(ii) To induce male sterility and analyse the way sterility observed in M_1 generation will be reflected in some later generations.

(iii) To analyse statistically how a group of mutations of different origin, observed are correlated with this sterility.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 MUTAGENIC AGENTS

Currently there are four major classes of mutagenic agents being used in induced mutation studies: radiation, chemicals, transposable element and callus culture (Bird and Neuffer 1988). In the early days, nearly all mutagenic treatments, were made with X-rays. Of 95 recorded crop varieties in 1973, the breakdown according to the mutagens used was as follows: - X-rays 42, gamma rays 23, neutrons 19, Chemical and internal isotopes (^{32}P) -2 (Sigurbjornsson and Micke 1974).

2.1.1 IONIZING RADIATION

Treatment of maize with a range of ionizing wave lengths, in the electromagnetic radiation spectrum results in mutations. X-rays and gamma rays are among the wave lengths absorbed by the genome and because they are highly penetrating, they are easy to use. However, they are distruptive to cell organelles, cell chemistry and chromosome integrity, as well as DNA structures. (Bird and Neuffer 1988). Gamma rays and X-rays are the best tools now available for restructuring the genome by deletion, duplication, inversion and translocation of chromosome segments (Neuffer 1982). The

most useful of the chromosomes aberrations produced by X-rays are translocations, either between basic chromosomes or between A (basic) and B (supernumerary) chromosomes. Translocation are important in a program of gene location to chromosome arm (Beckett 1982; Bircheler 1982). Gamma irradiation using ^{137}Ce and ^{60}Co seems to be a somewhat more effecient mutagenic process (Wagner and Plewa 1985). X-rays have been used in studies of maize plant development through formation of mutant sector (Johri and Coe 1983a, b, Poethig et al 1986).

Neutrons, alpha rays and beta rays are also used in mutagenesis (Auerbach 1973 and 1976; Paszfor 1979). Internal sources or chemically incorporated radio isotopes also have been use successfully in trans-mutation (Mashenkov, 1986; Hollaender, 1973; Miller and Miller 1971).

2.2.0 USEFUL MUTANTS IN CEREALS

A wealth of new traits or combinations of traits is readily obtainable in mutagenized populations. Cultivars developed using induced mutants may carry improvements in a wide variety of chracteristics. Mutant genetic variability has been used as a basis for the development of a new conceptual models for crop plants (Adams; 1982; Blixt and Vose 1984). Adams (1973) formulated a concept of plant architecture for field beans and sought to induce mutants to complement the available genetic variation for constructing model

prototypes. Blixt (1976) has demonstrated the use of a computer data bank on mutant gene stocks as an aid in pea breeding. Induced mutant cultivars also have proved to be outstanding parent for further cultivar developments.

Mutation breeding has been most successful in cereals. The record of the joint FAO/IAEA Division shows released figure up to the end of 1975 as follows: wheat 15, rice 16; Barley 40; Oats 5. the largest number of released mutant varieties derived from induced mutations is in Ornamental plants.

2.2.1 GROWTH HABIT MUTANTS

The early determinate bush habit trait from an induced mutant cultivar `Michelite' was incorporated in a series of Michigan's dry bean (Phaseolus vulgaries L) cultivar. The germplasm source of this early maturing bush habit trait was too abnormal for direct use but provided the germplasm necessary for ultimate cultivar improvement (Adams 1982). The succcess of this series of cultivars and especially the wide adaptability led Adams (1973) to use the approach of bean plant architecture to develop a new ideotype with a combination of traits considered necessary for maximizing the physiological effeciency and harvestability of field beans. Infact Shaikh et al (1982b) have recently induced upright, determinate mutant of Mungbean having upright pods and improved

harvest index.

In the tomato, Lycopersicon esculentum, Mill, induced mutants play an important role, along with natural variation and germplasm from alien species, in developing the crop for its many uses (Rick 1976). Mutants have been induced in the cultivated tomato with unique - germplasm previously unavailable in genetic resource collections.

Alexander et al (1971) for example were able to induce mutants for short style length, needed to assume adequate pollination. They also sought and obtained non branching mutants for developing tomatoes better adapted to machine harvesting, staking and with a single uniformly maturing fruit cluster. Such plants could be grown at a higher density than other types, the terminal fruit cluster would hold the fruit away from soil to reduce looses from rot and uniform ripening would permit one-time harvesting.

In the Snapdragon, (Antirrhinum majus L.) the mutant has a strongly dominant apical meristem but the original mutant has abnormal flowers and is a relatively weak plant of no direct use. However, the wealth of induced Antirrhinum mutants described by Stubbe (1974) and Harte (1974) merits fuller evaluation by commercial plant breeders not only for their potential exploitation but also as example of mutant types inducible in modern successful cultivars.

Leaf and plant structure modifications also have been induced in Pea, and a small number of new Pisum sativum L. cultivars have been induced in Pea (Micke and Donini 1982). Extreme mutant traits such as leafless, semileafless and fasciated are now in extensive use in Pisum breeding. The use of these mutants in breeding is expected to result in a mark change in appearance and agronomic performance capabilities of future Pea cultivars (Blixt and Vose 1984; Snoad and Hedley 1981).

The most dramatic recent mutant of maize affecting plant stature is leafy (Lfy) which gives up to eight extra leaves above the ear node (Shaver 1983). It is a dominant subject to modifiers according to the inbred line. Photosynthate potential for the ear may be increased by lfy. Prolifacy is increased, and there are many other potential advantages, especially, if combined with early pollination factors. OlejincZak and Patyna (1981) found great reduction in plant height in M_1 seedlings from treated seed. Most mutant phenotypes were shorter with more tassel branches and fewer leaves.

In Barley (Hordeum vulgare L) the short, stiff-straw of an X-ray induced mutant has been incorporated into several cultivars (Micke 1982). Semidwarfing trait, of the winter barley, induced by chemical mutagen diethyl-sulfate, has been incorporated into many promising advanced breeding lines (Ullrich et al

1982). This novel approach to breeding barley allows rapid and effecient movement of desirable mutations into adaptive background suitable for the best expression of the traits (Ramage 1981 and 1976).

In rice (Oryza sativa L.), the semidwerf trait of the mutant cultivar induced has been exploited (Sato 1980).

The complexity of the quality traits is such that cross breeding to improve the agromomic properties has produced a high-yielding semi-dwarf strain, but with somewhat inferior organoleptic qualities. However, high-yielding, agronomically improved semidwarf mutants of the tall, lodging-susceptible, but high quality have been induced (Gangadharan et al 1976).

Semidwarf mutants also appear to be among the most frequently induced vital mutants in both tetraploid wheat (Triticum turgidum L.) and hexaploid bread wheat, T. aestivum (Konzak 1981, 1982).

In Oats (Avena sativa L) also a new dominant semidwarf mutant has been induced (Brown et al 1980) and the mutant is proving useful in cross breeding (Burrows 1979).

2.3.0 MUTANTS AFFECTING PHYSIOLOGICAL TRAIT

Breeders commonly find mutations controlling the time of flowering and inflorescence in many crops such as soybeans and cotton. Mutations for earliness should allow expansion into new crop maturity zones (Bird and

Neuffer 1988). Although most mutants relate in some way to changes in physiology, certain types are best classified by their altered physilogical traits, such as the commonly induced earliness or lateness mutants (Micke 1979b; and Mutation breeding Newsletters 1979-1980). Mutations to photo period insensitivity have occured in cotton, Gossypium hirsutum L (Raut et al 1971), potato (Updhya et al 1974; Shepard et al 1980), rice (Hseih and Chang 1975; Rutger 1982), Jute (Mia et al 1976) and soybeans (Koo, 1972). Earlines mutants have been frequently induced in a wide range of crop species. Notable among others are Allium cepa, Capsicum annum (Pepper), Glycine max (soybeans) Gossypium hirsutum (Cotton), Hordeum vulgare (barley), Oryza sativa (rice), Triticum aestivum and Zea mays, while the late maturing include: Arachis hypogaea (groundnut), Avena Sativa (Oat) and Brassica juncea (Indian mustard) (Micke 1979b Mutation breeding newsletters 1979-80 and Benamer, 1990).

In barley, a cultivar was developed by direct use of an induced early maturing semidwarf mutant that has the potential for improvement of two row-malting (Molina-Cano 1982, Kivi 1981, Girkontaite and Burneikene 1989).

In rice, early ripening mutants cultivars adapted for multiple cropping practices in the tropics and subtropics have been developed (Rutger 1982, Sajo and Simon 1976). Using X-ray irradiation, Zacharias (1967)

was abled to induced mutants of Soyabeans that matured 5-7 days earlier than the parent population.

In chick pea (Cicer arietinum L.) an induced early mutant with higher pod set and a higher yield than the parent cultivar was produced (Shaik and Majid, 1982). In Lupines, Porsche (1967) induced an abundance of earlier flowering mutants in Lupinus albus L. including mutant that did not respond to increasing temperatures. In cotton, early flowering mutants, permit expansion of good quality locally cotton cultivars (Raut and Jain 1976). These mutants represent exceptionally valuable germplasm.

Another mutant with a remarkably altered vegetative phase has been obtained in Spinach (Spinacia oleraceae L.) (Handke 1980, 1981) which has a high dry matter and four times the Iron content of the original cultivar.

Among other physiological types are those that confer tolerance to herbicides and fungicides (Gressel and Segel 1978; Aviv and Galum 1977). Infact, DDT - resistance barley are now induced. Low level 2, 4-D tolerance occurs in birds - foot trefoil Lotus corniculatus L. (Swanson and Tomes 1980) and tolerance to propane and metribuzin occurs in soybeans (Barrentine et al. 1976).

2.4.0 MUTANTS AFFECTING PROTEIN CONTENT, NUTRITIONAL VALUE OR PROCESSING PROPERTIES

A few genotypes with higher lysine content in the

grains have been found in collections of maize, barley and sorghum; However it was also demonstrated that high lysine mutants could be induced with various mutagens (Doll, 1975). Following the discovery that the grain of certain mutants in maize had a markedly changed amino acid composition (Nelson 1970), other mutants with changed protein content and/or composition were isolated from mutagen-treated barley (Bansal 1974; Hadji Christodoulou and Della 1973), Sorghum (Axtell et al 1979) and wheat (Denic 1978; Dumanovic et al 1973; Parodi and Nebreda, 1982a,b). A number of protein mutants of rice and wheat have been reported (Brunori et al 1980, 1982; Tanaka, 1978). One high-protein wheat mutant was released to commercial growers. The varying rates at which dry matter and nitrogen accumulates in different genotypes suggests greater independence of the two processes than was once thought; other factors, possibly nitrogen availability, but not competition for the supply of assimilates as suggested by Bhati and Rabson (1976), may limit the realization of high-yielding varieties capable of producing high grain protein (Brunori et al 1980, 1981). The range of total protein content, seed size and variation in protein composition detected electrophoretically among collections of wild T dicoccoides offers considerable scope for exploitation (Mansur - Vergara 1983 and Howey et al 1988).

The existence of normal endosperm, high lysine sources in wheat implies that other genetic mechanisms for increasing lysine exist (Favret 1976b). Most mutants with significant changes in the proportion of amino acids in proteins have been confirmed, but many of the most studied mutants of barley are found to have marked pleiotropic" effects on yield and other properties (Doll 1979).

High threonine levels are produced in the grain of maize mutants isolated by Hibbeard and Green (1982) from azide-treated callus cultures, subjected to inhibition by a combination of the amino acids lysine and threonine. Since threonine is the second limiting amino acid in maize, sorghum, barley and wheat, the method holds promise in improving the nutritional quality of these species. (Konzak and Kleinhofs 1984).

With rice, the nutritional quality of the protein is relatively high for a cereal grain,but generally the levels of protein are exceedingly low. Many induced *higher-protein mutants and selections from land races* have been identified (Haq et al. 1973). Only in a few instances has there been an indication that both yield and protein content have been improved in the same mutant line (Bhagwat et al 1979; Harn et al 1973). Most high protein mutants selected in this way retained the desirable agronomic traits, including the yield capacity of the initial cultivar (Haq 1979).

The nutritional quality of animal fodder also is being improved by use of mutants. Brown midrib (bm) mutants have been found to affect the amount of ligni in leaves and stems of maize, resulting in significantly improved digestibility by ruminant animals (Barnes et al 1971). the amylyses in sorghum further showed that the nutritional improvement due to the mutant genes was similar to that obtained in maize and this can be expected to increase animal production with consequent economic benefits from the improvements in digestible energy.

Proanthocyanin-free mutants in barley have been induced to prevent the formation of chill-haze in bear produced from barley malt,(Wettstein et al 1977, Wettsterin-Knowles and Sogard 1980). Use of the mutant barley renders superfluous the requirement of chemical to stabilize bear, resulting in considerable economic savings to brewers and the elimination of a likely pollutant.

Low-alkaloid, nonshattering, early and other types of induced mutants of Lupinus angustifolius, have made possible the resumption of cultivation of these species and the development of genetic improvement of this species as a high-protein and oil seed crop. (Pakendorf and Rensburg 1981).

Induced low-erucic acid mutants of rape species, Brassica napus and B camprestris L. have revitalized the cultivation of these species as edible oil crops

(Axtell et al 1979). In contrast, other breeders are exploiting the available germplasm to achieve high erucic acid strains of rape for production of industrial oil, these mutants, can further improve the efficiency of rape seed oil for use as a renewable vegetable oil substitute for diesel engine fuel (Paterson el al 1981, a b).

A mutant of flax or Linseed, (Linum usitalissimum L; with increased oil content and degree of unsaturation was induced by Srinivasachar et al (1972). The mutant can be used as basic material to convert linseed oil to an edible type, clearly demonstrating that changes in oil content and quality are inducible and have potential for modifying crop quality for almost any intended use.

2.5.0 MUTANT AFFECTING DISEASE AND PEST RESISTANCE

To secure the badly needed high yields, crops must be protected by all possible means from diseases and other pest. Breeding for disease resistance certainly represents the most important way to counteract the pathogen. Thus induced mutations are now being developed as a complementary tool in breeding for disease resistance (Favret 1976a, Micke and Donini 1982. Lu et al 1988).

These mutant disease resistances include resistance to Victoria blight (Helminthosporium victoriae Meeh and Murphy) in Oats, resistance to powdery mildew (Erysiphe

graminis D.C) in barley and resistance to downy mildew (Sclerospora graminicola (Sacc) Schroet) in pearlmillet, (Pennisteum americarum (L) Locke syn; P typhoide Burn). Resistance to Victoria blight may really involve modification of a complex locus or pseudo allelic association rather than a `pleiotropic relation(Lu, et al 1988; Wallace et al 1967;) between resistance to certain races of Crown rust (Puccinia coronata) and susceptibility to Victoria blight (Konzak, 1956, 1959).

Oat mutant cultivars, were isolated. These cultivars carry an induced modification of the gene locus, resulting in blight resistance while retaining useful levels of the crown rust resistance (Collins and Jones (1979). Resistance to downy mildew (Sclerospora gramicola) was induced in the maintainer line, resulting in the pearl millet hybrid variety (Murty 1976).

Resistance to yellow mosaic virus disease has been induced, in two of eight cultivars of Mungbean (Phaseolus aureus Roxb.) by gamma radiation treatments to seeds (Shakoor et al 1977) of which no resistance to the disease was available in the germplasm. In Soybeans (Glycine max (L.) Merr.) Mutagen treatments have been used to induced a moderate level of resistance to rust, Phakopsora pachirhize syd. which is endemic on other plants and of no economic importance until Soybeans were cultivated. The induced field

resistance to rust appears so far to be the only effective source of resistance to the disease, since all natural sources identified by laboratory studies proved susceptible in field trials (SmutKupt et al 1982).

In Jute (Corchorus capsularis) a gamma-radiation induced mutant was found to have resistance to stem rot disease in artificial inoculation tests (Haq et al 1974).

Resistance to powdery mildew in barley was the first induced disease resistance reported in plants. This first single instance of the unusual mutant recessive allele Ml-O was reported by Freisleben and lein (1942), but mutants at the ml-O locus have since been repeatedly induced (Gustafsson 1979). The ml-O locus is unsual in that it confers resistance to all races world wide of the powdery mildew fungus (Jorgensen and Jensen 1979). However, more important for practical purposes, crosses of some of the low necrotic spotting ml-O mutants with modern breeding lines have produced strains that carry ml-o resistance without the associated necrotic spotting(Jorgensen and Jesen 1979). The selection yield well and thus have cultivar potential (Jorgensen 1981).

2.6.0 MUTANTS AFFECTING CROSS OR SELF-FERTILITY (MALE STERILITY)

A number of instances have been reported in which various types of irradiation treatment were used to

advantage in the production of male sterile mutants. For example an induced sterility in rice following X-ray application to the panicles of rice plants and in cotton, mutation to the non dehiscent anther variant has been isolated following X-irradiation of seed; Male sterile water melons and male sterile cucumbers have been obtained following irradiation treatments (Bowers 1960).

Some of the most useful mutants in plants are those that permit greater control of pollination. Hanna and Powell (1974) have induced partially apomitic mutants of Pearl Millet (Pennisetum americanum (L) leeke) and have identified a mutagen-induced apomitic form in Sorghum (Sorghum bicolor (L). Moench) as well. Hanna and Burton (1981) also have hybridized apomitic P setaceous and P orientale with P americanum to obtain apomictic male sterile, interspecific hybrids. While the hybrid plants are highly seed sterile, the back crosses to wheat resulted in recovery of plants exactly like the original F1. These hybrids provides unusually new genetic base material for investigating further the apomixis mechanism and its possible exploitation via a variety of tools including mutagens that are now available for plant genetics and breeding research (Hittalmani and Shivashankar (1987).

A large number of nuclear genetic male sterile mutant genes are available in barley (Hockett and Reid

1981), including some of induced origin. In durum wheat, nuclear genetics male sterile mutants have been induced using radiation by Bozzini (1974) A similar durum wheat male sterile mutant has been induced recently using diethyl sulfate (DES) and is being exploited to develop MSFRS population. (Thomson and Shantz 1978).

In bread wheat, Driscoll and Barlow (1976) induced simply inherited recessive male sterile mutant located on chromosome 4A by X-ray treatments of pollen. This mutant trait is linked to the semidwarfing genes the mutant male sterile gene, is now especially useful for MSFRS population breeding of hexaploid wheats (Driscoll, 1979, 1982, VazQuez and Sanchez -Monge 1987).

Genetic male sterile mutants for use in MSFRS Schemes are available or can be readily induced in most self pollinated plants species. In fact, suitable genetic male-sterile mutants are available in Soybeans Glycine max (L). Merill (Brim and Burton 1979), In safflower, Carthamus tinctorius L.(Heaton and Knowles (1982), in Sesame, Sesamun indica (Chauhan and Singh 1971; Osman and Yermanos 1982), and Pigeon pea, Cajanus cajan (L) Millsp; (Reddy et al 1978).

Cytoplasmic male sterility (CMS) has certain advantages over some forms of nuclear genetic male sterility, especially for hybrid seed production, and in most cases has been discovered in nature or produced via transferring nuclear material by backcrossing into

alien species cytoplasm (Ahokas 1978, 1980a). Few cases of induced cytoplasmic mutations are known but Favret and Ryan (1966) reported induction of a CMS in wild Hordeum spontaneum; and transferred it to cultivated barley. Complete fertility restorers were found in the wild population and partial restorers in cultivated barley (Ahokas 1980b). Ahokas (1980a) also has identified both CMS and Complete fertility restorers in rye (Secale cereale L). Kinoshita (1976) have reported induction of CMS mutants in sugar beets, (Beta vulgaris L). The features of the CMS systems among the mutants suggest that a variety of mechanisms have been induced. Similarly Burton and Hanna, (1982) have induced a CMS mutant in pearl Millet maintainer line. Burnham et al.(1981) have described genetic methods for differentiating between types of nuclear geneticcytoplasmic factor interaction mechanisms controlling CMS and its restoration. As discussed also by Washnock (1972) and Burnham et al. (1981), Cytoplasmic restoration of genetic male sterility is being sought as an alternative to CMS. Such a system seems feasible for wheat especially since chromosome ID carries a gene or genes that restore fertility and vigour to hexaphoid wheats in (Aegilops squarrosa L), (Triticum teruschii (Coss), cytoplasm (Kihara 1973;)

2.7.0 BIOLOGICALLY USEFUL MUTATIONS

In a search for agronomically useful mutants, we

may lose sight of another equally important group, namely mutants that permit the solution of fundamental problems in biology. These have no immediate economic impact but in the long run may be more valuable; because they help in our understanding of all aspects of plant and animal growth. Both artificially induced and naturally occuring. Spontaneous mutants have been useful to many scientists studying the details of such process as photosymthesis, starch and protein deposition, plant development, and disease resitance. The E.M.S mutant collections have been especially productive in this way (Miles 1983; Miles and Metz 1985). A different concept of the role of the host plant in the expression of dominant disease lesion mimic mutants that were produced by E.M.S treatment of Maize pollen (Hoising ton et al 1982; Neuffer et al 1986; Walbot et al 1983). New genes controlling meiosis have been found by using N-methyl-N-nitrosourea (Golubovskaya and Khristolyabova 1985, Mashenkov 1986)). Induced dwarf mutants are being used to determine the steps in biosynthesis of gibberellic acid and related growth hormones.

CHAPTER III

3.0.0 RESEARCH METHODOLOGY

The type of maize used in this study is popcorn (Zea mays Var. Praecox Sturt) (n=10) which has been grown for seven generations and obtained from the department of Biological Sciences, A.B.U. Zaria.

3.1.0 TREATMENT.

3.1.1 GAMMA-RAY

Air dried seed of a highly inbred line of popcorn (Zea mays Var. Praecox Sturt) was irradiated with Gamma cell 220 cobalt 60 (^{60}CO source at the following doses: 0; 10; 20; 30; 40; and 400 Gray (Gy) at the Biological control unit (BICOT) VOM, Plateau State.

3.1.2 THERMAL NEUTRONS

Type; 5 Curie $^{241}AM/Be$ neutron source; flux available at 2.5Cm within a perspex glass medium= $1.5x10^{4}$ $n.cm^{-2}$ $.5^{-1}$ (Thermal). The source is of fast Neutrons (Mean energy 4.46 Mev). The perspex, parraffin moderates these fast neutrons to thermal energy.

Other sets of air dried seeds were irradiated with thermal neutrons, flux $1.5x10^{4}$ $n.cm^{-2}$ $.5^{-1}$ at 25; 40; 50; 60 minutes and 12 hours at the Centre for Engery Research and Training (CERT) A.B.U. Zaria.

3.2.0 - SOWING OF SEEDS

Due to the detrimental effects of mutagens, the sowing was done immediately, in the field; laboratory;

and in the green house.

3.2.1 GREEN HOUSE/LABORATORY

In the green house and the laboratory, the flat method was employed. This involve sowing of the treated seeds in plastic trays containing vermiculites. The filling in the container has a depth of about 6cm. The seeds were sown in rows 1cm deep, embryo upwards; the distance between the row is 3cm and within the row 4cm. Each treatment is replicated five times and analysis followed randomized block design (RBD).

3.2.2 POTS:

Three hundred and thirty pots, in groups of 15 per treatments and with two replications were used in the experiment. Three (3) seeds were sown in each pot. Similarly a control population (ie O treatment) were also sown on the same date.

3.2.3 FIELD:

Field planting involved the use of 3 by 2 metres block in the field plot, at a planting rate of 3 seeds per hole. Each of the eleven treatments was replicated five (5) times. The design and analysis of the experiment followed Randomised block design (R.B.D).

3.3.0 *DATA COLLECTION*

3.3.1 GERMINATION RATE AND PERCENTAGE:

Due to induced injury and delay in germination/emergence of the treated seeds, the estimate was recorded at a time when the control population was considered 50 and 90% emerged (i.e

germination rate was recorded after 5 and 8 days of planting). The percentages of different treatment doses were then compared.

3.3.2 SEEDLING HEIGHT AND ROOT LENGHT

The measure of radiosensitivity by the use of seedling height and root length was simply measured in `cm' by holding the leaves errect and recording the highest point of the highest leaf. The same treatment was applied to the root. The measurements were taken on the 15th day after sowing. The average height was taken for each treatment and comparism made among the different treatment doses in M_1 and M_2 generation.

3.3.3 SEEDLING SURVIVAL

Estimates of seedling survival records provide data on delay effects of the treatment, so as to acertain the relative emergence to seedling survival of the treatment population. Percentage of the surviving plant was taken for both M_1 and M_2 generation thirty (30) days after sowing. Plants were considered dead if no photosynthetic tissue was observable. The percentage survival of different treatments were then compared.

3.3.4 CHIMERA INDUCTION (CHLOROPHYLL MUTANTS)

Data on the occurance of sectors of chlorophyll deficient mutants or morphological changes in the appearance of M_1 plants (ie. different chlorophyll mutations) is useful for estimating the effectiveness

of the treatment. Thus, by analysing material from the field and green house the normal classification was used. The frequency of different mutants (albina; xantha, virescent lustescent, chlorina; and striata) and their percentages were taken in the M_1 and M_2 generations.

3.3.5 DELAYED DEVELOPMENT

The recorded growth of some mutagen treated material was estimated in relation to the time of flowering and maturity of the plants, as well as the variability in plant development within and between treatments. The time of flowering/tasseling, at 50 percent flowering and 90% maturity were noted and recorded in days.

3.4.0 CYTOLOGICAL EFFECTS AND ANALYSIS

3.4.1 MITOTIC ABERRATIONS

After treatment of seeds, the analysis of the first mitotic cycle in root cells offered a quick test to determined the effect of the mutagens. Since individual seeds germinate at different rates. A limited number of seeds (40 per treatment) were used for cytological investigation.

For mitotic studies, 4mm length of root tips from germinating seeds were kept in paradichlorobenzine for 8 minutes and then fixed in 1,3 -acetic acid alcohol (glacial) ethanol-propanoic acid for 24 hours; washed for 3 hours in absolute alcohol and preserved in 70% alcohol at 4^{o} C in a refrigerator until used.

For smear preparation, some root tips were washed in distilled water for 15 minutes, rinsed in cold INHCL and hydrolysed in Warm INHCL at 60°C for 8 - 10 minutes in a water bath. Smears are made from the hydrolysed root tips by staining with a drop of 0.75% aceto-Orcein (2g Orcein dissolved in 300ml of 45% acetic acid at 60°C) on the slide, a coverslip was then applied and pressed gently with a small piece of blotting paper. Photomicrographs were taken from the temporary smeared preparations with wild -20 photomicroscope.

3.4.2 MEIOTIC ABERRATION

When the development of the M_1 and M_2 plants has; proceeded to maturity, anthers are fixed at the begining of anthesis inorder to determine guantitatively chromosome mutations surviving to this stage.

For meiotic investigation some young unopened spikelets are selected for the experiment. With the help of a small pin, anthers are extracted from the tassel and fixed in 1,3-acetic acid alcohol (glacial) for 24 hours; Washed for 3 hours in absolute alcohol; and preserved in 70% alcohol at 4°C until used. Some anthers were washed in distilled water for 15 minutes, rinsed in cold INHCL and hydrolysed in Warm INHCL at 60°C for 8 - 10 minutes in a Water bath. With a sharp razor blade the ends of the anthers were cut and gently pressed in the middle with pointed needles,

forcing the pollen mother cells. (P.M.C) out on to the slide. The P.M.C are then stained with aceto-orcein stain and smeared in the usual manner. Photomicrographs of different stages of meiosis with different chromosomal abnormalities (laggards, fragment, unequal separation; normal and deformed pollen grains; bridges etc) were taken from temporary smeared preparations with wild - 20 photomicroscope, using ILFORD Films.

3.4.3 DETERMINATION OF DELETERIOUS EFFECT OF RADIATION ON POLLEN FORMATION IN POPCORN (POLLEN ANALSYSIS)

Pollen samples were collected from plants of popcorn (Zea- mays Var. Praecox sturt). This was done by breaking off a small branch of a tassel just ready to shed. These were fixed in 1,3 acetic acid alcohol (glacial) ethanol-propanoic acid and stored in 70% alcohol until used. For analysis pollen grains from single anthers were stained in a 0.2% solution of lodine, potassium Iodide. Normal starch- containing grains stained deply, thus making it possible to distinguished them from grains not properly filled. These were classified as deformed and aborted pollen grains and their frequency and percentages were taken in different treatment doses and comparism made within and between the treatment.

3.4.4 TILLER FORMATION

A total of one hundred and fifty (150) plants for each treatment were observed and classified according to the number of tiller produce; namely one tiller, two fillers; three tillers and more than three (3) tillers. in both M_1 and M_2 generation for both gamma rays (^{60}CO) and thermal neutrons, their frequency and percentages were taken and compared within and between treatments.

3.4.5 EAR MUTANTS

The number of cobs produced in M_1 and M_2 generation plants were classified as Zero (0) Cob, one, two; three; four; five and above. Their percentages were taken and compared between and within gamma rays (^{60}CO) and thermal neutrons.

3.4.6 MALE STERILITY:

The degree of M_1 and M_2 sterility varies greatly. Thus M_1 and M_2 sterility was recorded quantitatively interms of pollen abortion and the position or replacement of a tassel. For investigations of pollen abortion common cytological techniques were used. For a record of male sterility, the frequency of completely sterile and partially sterile M_1 and M_2 plants, were taken. Correlation coeficient analysis was used to compare mutation frequency and the degree of M_1 sterility, so as to determine whether the spectrum of chlorophyll mutations in M_1 and M_2 has any connection with M_1 sterility.

3.4.7 SURVIVAL TO MATURITY

Estimates of the number of plants in each treatment at the time of maturity, provided information on the severity of the injury induced by the mutagens, when compared with the number of seeds planted. The treatment doses were then classified into low (LD10), Average (LD 50) and high or lethal dose (LD100).

3.4.8 HEIGHT AT MATURITY:

The measure of height at maturity period was done in `cm' by measuring the whole plant from the ground level to the highest point of the tassel. Plants were then grouped into dwarf; short, or tall plants in all the treatments. The percent reduction in height were also compared with the control.

3.4.9 EAR LENGTH

The measurement of average Ear length were done with a metre rule in `cm'. The measurement was replicated five times. Analysis followed R.B.D and least significant differences (L.S.D) was used to separate the means of individual treatment.

3.5.0 ONE HUNDRED GRAIN (100) SEED WEIGHT:

The measurement/Weighing was done with an electric weighing balanced machine in grams (g) Each treatment was replicated five times, analysis followed R.B.D and the individual mean for each treatment were separated and compared using least significant difference. (L.S.D).

3.5.1 STATISTICAL ANALYSIS

For the analysis of data collected in this study, the following statistical methods were used:- Mean (Average) Percentages, Analysis of variance (Anova) and correlation coefficient (r) analysis.

CHAPTER FOUR

4.0 RESULT

Result obtained in both the M1 and M2 generation of gamma rays cobalt 60 (^{60}Co) and thermal neutron irradiated popcorn (Zea mays Var. Praecox Sturt) shows the following:-

4.1.0 PERCENT GERMINATION

The treated seed were generally slow to germinate in both gamma rays cobalt 60 and thermal neutrons compared with the control in M1 generation and the time lapse between seedling and seed emergency was found to increase with increase in radiation dose, with a severe effect (ie zero germination) in thermal neutrons N40 and N12 hrs of radiation after four (4) days. The percent germination in both laboratory and field condition decreased with an increase in radiation dose, with a much lower percentage germination at the highest doses of both gamma rays cobalt 60 (77.78 and 56%) and thermal neutrons (55.56%) and 55.26%) in the field and laboratory respectively (Table 1, fig. 1 and 2). The dominant lethal mutants are expressed as failure of some seeds to germinate in both gamma rays cobalt 60 and thermal neutrons.

However, in M_2 generation, though there was also slow germination in some treatments X40Gy and N50), there was a general increase of seed germination in the highest doses (X400Gy and N12 HRS) of gamma rays and

thermal neutrons after four days. This differential increase and decrease of seed germination led to lack of proportionality of percent germination with the dose. Inspite of this variation, there was still an element of linearity of a decrease in percent germination with an increase in dose (Table II fig. 3) Similarly analysis of variance (Anova) of the number of germinated seed in the field indicated that the treatment is highly significant at both 1% and 0.5% level of signficance (Appendix 1).

4.2.0 SEEDLING HEIGHT AND ROOT LENGTH

A comparison of the mean values (average seedling height and root length (after 5 and 15 days repectively) of treatment, both within and between gamma rays cobalt 60 and thermal neutrons, indicated the dependance of height and root length of germinating popcorn (Zea mays var. praecox sturt) with the dose of both the M_1 and M_2 generation. Reduction in seeding height and root length was noticed in all treatments and there was a steady decrease in height and root length with the increase in dose (Table 1 fig.4 and 5) and the maximum reduction was observed (18.49 and 5.80cm), (18.51 and 6.87) in the highest doses of gamma rays. ^{60}Co and thermal neutrons for seedling height and root length measurement of M1 generation. However, an apparent increase was observed on seedling height especially at the highest dose (400Gy) recording up to 41.60 cm compared to 33.60cm over that of the control in gamma

rays treated seedlings. Contrary to increase in seedling height, there was a steady decrease in root length, with the maximum reduction recorded in the highest dose (7.25cm compared to 13.52 of the control). (A similar and steady decrease was observed in both the seedling height and root length of thermal neutrons treated seeds. A comparism between gamma rays ^{60}Co and thermal neutrons, revealed that thermal neutrons produce less variability among individual seedlings than did the gamma rays in both M1 and M2 generation (Table 1 and II fig. 4 - 5).

4.3.0 PERCENT SEEDLING SURVIVAL AND LETHALITY AFTER 30DAYS

The relative survival percentage in both gamma rays ^{60}Co and thermal neutrons treated seedlings indicated a decrease in relative survival percentage with an increase in dose in M_1 and M_2 generation. The relative survival decreases steadly with an increase in dose up to the lowest percentage survival of 77.78 and 73.33% was observed for gamma rays and thermal neutrons respectively in M_1 generation and a record of 70.60% and 66.67% was observed for gamma rays and thermal neutrons respectively in M_2 generation, indicating a decrease of survival in M_2 compared with M_1 generation (Table 1 and II fig. 1-4).

Contrary to decrease in the relative percent survival, there was a steady increase in the percent lethality with an increase in radiation dose for both

gamma rays and thermal neutrons irradiated seedlings with the highest value of 22.22% and 26.67% lethality observed at the highest doses of gamma rays (400Gy) and thermal neutrons (12HRS) in M_1 generation. A similar increase in percent lethality was recorded in M_2 generation up to 73.33 in 40Gy treatment dose of gamma rays and 43.33% lethality in N65 treatment of thermal neutrons, clearly indicating an increase of lethality of M_2 seedlings compared to M_1 generation seedlings (fig. 1-4).

TABLE 1: The effect of gamma rays (^{60}Co) and Thermal neutrons radiation on germination, percent germination, seedling height and root length and the relative percentage of seedling survival and lethality of popcorn (Zea mays Var Praecox sturt). In M_1 generation

MUTAGEN	TREATMENT (DOSE)	NO GERMINATED AFTER 5 DAYS	NO GERMINATED AFTER 5 DAYS	PERCENT GERMINATION (%) FIELD	PERCENT GERMINATION (%) LAB	AVERAGE SEEDLING (X) CM HEIGHT	AVERAGE SEEDLING (X) CM ROOT LENGTH	RELATIVE* PERCENT SEEDLING(%) SURVIVAL AFTER 30 DAYS	LETHALITY
Gamma ray	0 Gray	10	45	100.0	100.0	22.00	10.27	100.0	0
	10 Gray	8	43	95.56	89.61	21.42	9.20	97.78	2.22
	20 Gray	5	43	95.56	83.12	19.78	8.53	93.33	6.67
	30 Gray	6	40	88.89	74.03	20.58	7.60	88.88	11.12
	40 Gray	4	39	86.67	60.39	20.31	6.20	82.22	17.78
	400 Gray	2	35	77.78	56.00	18.49	5.80	77.78	22.22
THERMAL NEUTRON	N25 at 1.5 x 10	7	44	97.78	86.84	19.16	10.73	91.11	8.89
	N40 at 1.5 x 10	0	41	91.11	97.36	19.87	9.33	84.44	15.56
	N50 at 1.5 x 10	6	30	60.74	78.95	19.37	8.40	91.11	8.89
	N65 x 1.5 x 10	2	38	84.44	73.68	18.64	7.13	80.00	20.00
	N12HRS at 1.5x10	0	25	55.56	55.26	18.51	6.87	73.33	26.67

* (i) Relative survival percentage was calculated as:

$$\frac{\text{\% survival of dose treatment}}{\text{\% survival of control}} \times 100$$

(ii) Percent Lethality was estimated as:-

100 - Relative survival percentage.

TABLE II: The effect of gamma rays (^{60}Co) and Thermal neutrons radiation on germination, percent germination, seedling height and root length and the relative* percentage seedling survival and lethality of popcorn Zea mays Var Praecox sturt). In M_2 generation

MUTAGEN	TREATMENT (DOSE)	NO GERMINATED AFTER 5 DAYS	NO GERMINATED AFTER 8 DAYS	PERCENT GERMINATION (%) FIELD	PERCENT GERMINATION (%) LAB	AVERAGE SEEDLING (X) CM HEIGHT	AVERAGE SEEDLING (X) CM ROOT LENGTH	RELATIVE* PERCENT SEEDLING SURVIVAL AFTER 30 DAYS (%)	LETHALITY (%)
	0	25	60	100.0	100.0	33.60	13.52	100.0	0
	10 Gray	24	53	88.33	91.20	38.30	14.08	83.33	16.67
Gamma	20 Gray	27	50	83.33	86.40	40.30	10.67	76.67	23.33
ray	30 Gray	23	47	78.33	72.00	34.90	9.03	70.00	30.00
	40 Gray	0	18	23.50	50.86	38.20	7.95	26.67	73.33
	400 Gray	30	48	80.00	30.95	41.60	7.25	70.60	26.40
	N25 at 1.5x104nth/cm^2	18	56	93.33	95.56	41.50	13.83	86.66	13.34
	N40 at 1.5x104nth/cm^2	37	47	78.33	86.60	40.50	12.65	71.67	28.33
THERMAL	N50 at 1.5x104nth/cm^2	10	45	75.00	82.05	36.40	11.05	68.33	31.67
NEUTRONS	N65 at 1.5x104nth/cm^2	29	38	63.33	65.30	37.90	14.21	56.67	43.33
	N12HRS at1.5x104nth/cm^2	30	47	78.33	48.92	37.70	8.34	66.67	33.33

* (i) Relative survival percentage was calculated as:

$$\frac{\text{\% survival of dose treatment}}{\text{\% survival of control}} \times 100$$

(ii) Percent Lethality was estimated as:-

100 - Relative survival percentage.

4.4.0 M1 AND M2 CHIMERA INDUCTION (CHLOROPHYLL DEFICIENT MUTANTS:

About 90% of the gamma rays and thermal Neutrons induced mutation in popcorn were found in the seedling stage. Induced among the dominant seedling mutants are the occurance of sectors of chlorophyll abnormalities (Mutants) and other morphological changes in the appearance of M1 and M2 plants. Most of these mutants characters were lethal or unfavourable to growth. The type of mutants found are albina, which is charactrized by entirely white leaves of the seedlings and it is a lethal mutation, Xantha is also a lethal whose leaf colour varies from orange -yellow to faint yellowish-white (plate 5) Virescent, is characterized by yellow-green colour of the leaves, leaves appear first as yellow green but gradually change to green (plate 6), Lustescent- chlorophyll deficient mutant, appear as green originally, but the pigments gets destroyed, leaves wither and turn yellow, it is a lethal mutation (plate 7). In Chlorina, leaves appear with persistant yellow green colour (plate 8) and it is usually a viable mutation. The leaves of striata mutant, appears with longitudinal stripes of yellow on green or white on green (plate 9) a viable mutation. The spectrum of these chlorophyll deficient morphological mutants in M_1 and M_2 generation were analysed and presented in table III and IV. Among the chlorophyll deficient mutant albinas and xantha are rare while Lustescent and virescent are the most frequent. In M1 generation of

the 274 chlorophyll deficient mutants obtained by gamma rays and thermal neutrons irradiation, 2.73% were albina, 10.38% Xantha, 22.95% lustescent, 34.42% Virescent, 22.13% chlorina, and 7.38% were striata. However, in M_2 generation of 366 chlorophyll deficient mutant 5.11% were albina, 9.85% xantha, 12.41% lustescent,44.16% virescent, 16.42% chlorina, and 12.05% striata. But when the two mutagens are compared in both M_1 and M_2 generation, gamma rays produce higher chlorophyll deficient mutants in M_1 generation than - Thermal neutrons. While in M_2 generation Thermal neutrons produce more chlorophyll deficient mutants than gamma rays cobalt 60 source (Table III and IV).

Other morphological mutants, include dwarf plants, distorted growth of small narrow leaves (plate 10), Frequent also among the mutants are rolled leaves, fusion of 2-3 blades in one leaf (plate II), complete absence of one part or almost both part of the blade (plate 12) and small plants (plate 13). These have proved to be haploids or aneuploids and are often two weak to make an ear. The sizable numbers of sectored dominant mutants provides a unique opportunity to observed dominant lethal mutants which would have died, had they not been sustained and protected by normal tissue to almost maturity.

TABLE III: Spectrum of chlorophyll deficient mutants observed in M_1 generation of gamma ray and thermal neutrons irradiated popcorn Zea mays Var Praecox sturt) seedlings.

MUTAGEN	TREATMENT (DOSE)	TOTAL NO. OF MUTANTS	PERCENTAGE (%) ALBINA	(%) XANTHA	LUTESCENT	VIRESCENT	CHLORINA	STRIATA
	0	0	0	0	0	0	0	0
	10 Gray	10	-	-	20.0	80.0	-	-
Gamma	20 Gray	14	-	21.43	7.14	42.86	-	28.57
ray	30 Gray	35	-	11.43	5.71	28.57	37.14	17.14
	40 Gray	42	4.76	19.05	9.52	30.95	35.71	-
	400 Gray	69	11.59	15.94	8.70	34.78	17.39	11.59
	N25 at 1.5x10^{4}nth/cm^2	7	-	-	16.67	25.05	50.0	8.33
	N40 at 1.5x10^{4}nth/cm^2	2 18	-	12.82	25.64	41.03	20.51	-
THERMAL	N50 at 1.5x10^{4}nth/cm^2	20	-	9.52	38.10	28.57	23.81	-
NEUTRONS	N65 at 1.5x10^{4}nth/cm^2	25	-	15.09	24.53	18.87	26.42	15.09
	12HRS at 1.5x10^{4}nth/cm^2	34	4.84	9.68	12.90	35.48	19.34	17.74
	GRAND TOTAL OF MUTANTS	274	2.73%	10.38%	22.95%	34.42	22.13	7.38

TABLE IV: Spectrum of chlorophyll deficient mutants observed in M_2 generation of gamma ray and thermal neutrons irradiated popcorn Zea mays Var Praecox sturt) seedlings.

MUTAGEN	TREATMENT (DOSE)	TOTAL NO. OF MUTANTS	PERCENTAGE (%) ALBINA	XANTHA	LUTESCENT	VIRESCENT	CHLORINA	STRIATA
	0	0	0	0	0	0	0	0
	10 Gray	14	-	-	42.86	50.0	7.14	-
Gamma	20 Gray	20	-	-	20.0	50.0	25.0	5.0
ray	25 Gray	25	-	40	24.0	48.0	24.0	-
	40 Gray	37	10.81	21.62	37.84	21.62	5.41	-
	400 Gray	62	9.68	16.13	17.74	32.26	17.74	6.45
	N25min at 1.5x104nth/cm^{2}2	12	-	-	28.57	42.85	-	28.57
	N40min at 1.5x104nth/cm^{2}2	39	-	-	11.11	66.67	-	22.22
THERMAL	N50min at 1.5x104nth/cm^{2}2	42	-	-	20.01	70.05	10.0	-
NEUTRONS	N65min at 1.5x104nth/cm^{2}2	53	40	-	24.07	52.05	-	20.00
	12HRS at 1.5x104nth/cm^{2}2	62	8.82	2.94	14.71	52.94	8.82	11.76
	GRAND TOTAL OF MUTANTS	366	5.11%	9.85%	12.41%	44.16	16.42	12.05

4.4.1 MEAN NUMBER OF MUTANTS PER TREATMENT PER IN M_1 AND M_2 GENERATION

Changes of the chlorophyll apparatus was used as the basis of mutations on the assumption that other types of mutation are induced with the frequency parallel to that of the chlorophyll mutations. The result demostrated variability of the relative frequency of various chlorophyll mutants, following treatment with different doses of the same mutagen. the highest frequency of chlorophyll mutation obtained by irradiation with gamma rays and thermal neutrons for all the treatment tested in M_1 and M_2 generation are presented in table V. The mean number of chlorophyll mutations per treatment, never exceed 14 in the average population. Thus in the average population 7.05 mutants/treatment was obtained for gamma irradiation and 6.15 mutants/treatment after treatment with thermal neutrons (Table V). However if the types of chlorophyll mutation are considered separately (ie Albina, Xantha, etc) in M_1 and M_2 generation for both gamma rays and thermal neutrons, the same results were obtained (Table VI and VII). Therefore by multiplication of the known number of mutations by 7.05 or 6.15 the number of mutants can be estimated. Moreso because of the multicellular structure of ear primordia in seed and intervening cell division between the induction of mutations in the germ line cell and the detection of mutations in the M_2 plants, it is not easy to estimate correctly the mutation rate due to seed treatment.

TABLE V: Mean number of mutants/treatment of gamma ray and thermal neutron irradiated popcorn (Zea mays Var Praecox sturt)

VARIETY	YEAR	GAMMA RAY COMBALT 60 GRAY DOSE(Gray)	MUTANTS PER TREATMENT(X)	VARIETY	YEAR	THERMAL NEUTRONS 1.5X104nit/cm2 DOSE(nth/cm2)	MUTANTS PER TREATMENT (X)
	1991	10	4.04		1991	25.1.5x104	3.65
	1991	20	5.62		1991	40min 1.5x104	4.24
	1991	30	7.58		1991	50min 1.5x104	5.51
	1991	40	7.48		1991	65min 1.5x104	7.07
	1991	400	7.28		1991	12HRS 1.5x104	13.03
Praecox	1992	10	4.24	Praecox	1992	25min 1.5x104	4.80
	1992	20	6.75		1992	40min 1.5x104	3.80
	1992	30	7.00		1992	50min 1.5x104	5.41
	1992	40	12.78		1992	65min 1.5x104	5.29
	1992	400	7.74		1992	12HRS 1.5x104	8.73

Mean Value for gamma ray = 7.05

Mean value for Thermal neutrons = 6.15

TABLE VI: Mean number of mutants per chlorophyll mutation in a Treatment of gamma rays (60Co) and thermal neutrons irradiated popcorn (Zea mays Var Praecox sturt), in M_1 generation.

MUTATION	YEAR	TREATMENT DOSE	NUMBER OF MUTANTS/CHLOROPHYLL MUTATION (X) TOTAL (X)	ALBINA (X)	XANTHA (X)	LUTESCENT (X)	VIRESCENT (X)	CHLORINA (X)	STRIATA (X)
GAMMA RAY	1991	10 Gray	4.04	2.70	9.20	5.00	1.25	2.82	3.26
	1991	20 Gray	5.62	3.12	4.67	14.00	2.33	6.12	3.50
	1991	30 Gray	7.58	7.20	8.75	17.50	3.50	2.69	5.83
	1991	40 Gray	7.48	21.00	5.25	10.50	3.23	2.80	2.14
	1991	400 Gray	7.28	8.63	6.27	11.50	2.88	5.75	8.63
THERMAL NEUTRONS	1991	25min $1.5x10^4$nit/$cm^2$2	3.66	7.25	2.60	3.50	2.33	2.70	3.50
	1991	40min $1.5x10^4$nit/cm^2	4.24	3.23	1.98	9.00	1.50	5.25	4.50
	1991	50min $1.5x10^4$nit/cm^2	5.51	2.22	8.20	5.00	1.43	10.00	6.20
	1991	65min $1.5x10^4$nit/cm^2	7.07	25.00	3.60	4.17	1.92	2.70	5.00
	1991	12HRS $1.5x10^4$nit/cm^2	13.03	12.00	36.00	7.20	2.00	12.00	9.00

TABLE VII: Mean number of mutants per chlorophyll mutation in a treatment of gamma rays (^{60}Co) and thermal neutrons irradiated popcorn (Zea mays Var Praecox sturt), in M_2 generation.

MUTATION	YEAR	TREATMENT DOSE	NUMBER OF MUTANTS/CHLOROPHYLL MUTATION (X) TOTAL (X)	ALBINA (X)	XANTHA (X)	LUTESCENT (X)	VIRESCENT (X)	CHLORINA (X)	STRIATA (X)
GAMMA RAY	1992	10 Gray	4.24	2.40	6.03	2.33	2.00	10.00	2.70
	1992	20 Gray	6.75	7.30	2.20	5.00	2.00	4.00	20.00
	1992	30 Gray	7.00	4.20	25.00	4.17	2.08	4.17	2.40
	1992	40 Gray	12.78	37.00	9.25	4.63	2.64	4.63	18.50
	1992	400 Gray	7.74	10.33	6.20	5.64	3.10	5.64	15.50
THERMAL NEUTRONS	1992	25min $1.5x10^4 nit/cm^2$	4.80	-	-	6.00	4.00	2.00	12.00
	1992	40min $1.5x10^4 nit/cm^2$	3.80	-	7.80	3.90	2.44	4.88	-
	1992	50min $1.5x10^4 nit/cm^2$	5.41	-	10.50	2.63	3.50	4.20	-
	1992	65min $1.5x10^4 nit/cm^2$	5.29	-	6.63	4.08	5.30	3.79	6.63
	1992	12HRS $1.5x10^4 nit/cm^2$	8.73	20.67	10.33	7.75	2.02	5.17	5.64

4.5.0 MUTANTS AFFECTING TILLER FORMATION

One of the novelty, partially dominant mutant observed, resulting from gamma rays (^{60}Co) and thermal neutrons irradiated popcorn (Zea mays var. praecox sturt) was the formation of tillers. The result demonstrate variability of the relative frequency of the number of tillers formed following treatment with different doses of the same and different mutagens (Table VII). A total of 150 plants were observed and classified according to the number of tiller produced, namely one tiller (plate 14), two fillers (plate 15) three tillers (plate 16) and more than three (3) tillers (plate 17). The number of tillers formed increases with the dose in both gamma rays and thermal neutrons, with the highest percentages of 40% and 17.33% obtained at the highest doses of 400 Gray (Gamma ray and 12HRS thermal neutrons respectively Table VIII).

However in M_2 generation, less than 1% of the tillers formed in M_1 generation was observed and this was mainly confined to the 400 Gray treatment dose of gamma rays and could not survive up to maturity.

Table VIII Frequency of tiller formation in gamma rays (^{60}Co) and thermal neutrons irradiated popcorn (Zea mays Var praecox sturt)

MUTAGEN	TREATMENT (DOSE)	TOTAL NO. OF PLANTS OBSERVED	NUMBER OF FILLERS AND PERCENTAGE ONE (1)	TWO (2)	THREE AND ABOVE	% TOTAL OF TILLER
	0	150	0	0	0	0
	10Gray	,,	-	-	-	0
GAMMA	20 ,,	,,	5.33%	1.33%	-	6.66%
RAY	30 ,,	,,	8.67%	-	0.67	9.34
	40 ,,	,,	6.67%	2.67%	-	9.34
	400 ,,	,,	14.67%	20.0%	5.33%	40.0%
	25 $1.5X10^4/cm^2/s^{-1}$	150	-	-	0.67%	0.67
THERMAL	40 ,, ,,	,,	5.33%	-	-	5.33
NEUTRONS	50 ,, ,,		2.00%	2.67%	-	4.67
	65 ,, ,,	,,	6.67%	2.00%	0.67%	9.34
	12hrs ,, ,,	,,	10.67%	5.33%	1.33%	17.33

4.5.1 EARLY/DELAYED DEVELOPMENT MUTANTS

Although most mutants relate in some way to changes in physiology, certain types are best classified by their altered physiological traits, such as the commonly induced earliness or lateness mutants and changes in the relative proportion of the vegetative and generative phases in the growth of plants. When M_0, M_1 and M_2 generation are compared, there results, an

induced abundance early tasseling/flowering and maturity mutants especially at the M_2 generation, though a number of mutants at M_1 generation, (Table IX) are later tasseling/flowering (45 days) and maturing (100 days) than the original parental (M_0) plants, that tassels 43 days and matures in 97 days.

However, early tasseling, 19 days, instead of 45 days in M_1 and 43 days in M_0 generation were observed in M_2 generation. In addition to early ripening, M_2 generation matures a month earlier (74 days) instead of 97 and 100 days observed in M_0 and M_1 generation respectively (Table IX).

Table IX Comparative analysis of Mo, M1 and M2 generation of gamma ray (^{60}CO) and thermal neutrons induced early tasseling/flowering and maturity times in Popcorn (Z. mays Var praecox Sturt)

GENERATION	DATE OF PLANTING	NUMBER OF DAYS TO FIRST APPEARANCE OF TASSEL	50% TASSELING	50% MATURITY	90% MATURITY
M_0	24/4/91	43	58	83	97
M_1	27/9/91	45	58	88	100
M_2	15/5/92	19	27	59	74

In the population of M2 about 50% of the agronomically interesting variants affected time of pollination.

4.5.2 FERTILITY COUNT BASED ON THE NUMBER OF COBS PRODUCED PER PLANTS (EAR MUTANTS)

Result obtained indicated an increase in the number of cobs/ears produced per plant in both M_1 and M_2 irradiated population and the increased was in direct proportionality with the dose for both gamma rays and thermal neutrons. The increase in the number of ears/cobs per plants has been more striking in the irradiated population than the control(Table X). The number of cobs produced in M1 generation ranges from zero (none) to five and above, with two cobs as the most frequent and five and above as the rare mutants obtained only at 400Gy dose of gamma rays and accounted for only 4%, and 1.67% 4.0% and 7.67% at 50 min, 65min and 12 hrs thermal neutrons treated dose respectively in the sampled population.

However; in the M_2 generation, the frequency of occurance, of these mutants was relatively low when com pared with the M_1 generation. The number of cobs/ear produced in M_2 ranges from one to five and above with two and three cobs as the most frequent, and five and above as the rare mutant (1.67% and 7.33%) for gamma rays (400Gy) and thermal neutron (65min) irradiated seeds respectively (Table XI). One striking difference that was observed between the M_1 and M_2 generation ear mutants was the production of at least one viable cob/ear per plant in M_2 generation, while in M_1 generation different percentages of cobs sterilities

(zero cobs) was observed in all the treatments, thus there tend to be an increase in the number of cob/ear setting in the M_2 generation, population; more than the number observed in M_1 generation.

TABLE X: Fertility Count (Ear Mutant) based on the number of ear/cobs produced per plants in gamma rays (60CO) and thermal neutrons irradiated popcorn (Zea mays var. praecox sturt) in M1 generation.

MUTATION	TREATMENT DOSE	TOTAL NO OF PLANTS OBSERVED	PERCENT (%) NUMBER OF COBS PRODUCED PER PLANT ZERO (0)	ONE (1)	TWO (2)	THREE (3)	FOUR (4)	FIVE AND ABOVE (5 <)
	0	300	6.67	53.33	40.00	-	-	-
	10 Gray	300	6.67	26.67	53.33	13.33	-	-
	20 Gray	300	5.33	16.33	59.33	11.33	7.67	-
	30 Gray	300	13.33	20.00	46.67	17.33	2.67	-
	40 Gray	300	10.00	8.00	68.67	12.67	1.33	-
	400 Gray	300	4.00	9.33	60.00	20.33	2.33	4.00
	25min $1.5x10^4$nth/cm^2	300	8.00	19.33	53.33	19.33	-	-
	40min $1.5x10^4$nth/cm^2	300	2.67	24.00	60.00	10.67	2.67	-
	50min $1.5x10^4$nth/cm^2	300	6.67	16.00	59.33	16.33	-	1.67
	65min $1.5x10^4$nth/cm^2	300	7.33	22.67	54.67	10.00	1.33	4.00
	12HRS $1.5x10^4$nth/cm^2	300	8.33	21.33	52.67	8.67	1.33	7.67

TABLE XI: Fertility Count (Ear Mutant) based on the number of ear/cobs produced per plants in gamma rays (^{60}CO) and thermal neutrons irradiated popcorn (Zea mays var. praecox sturt) in M_2 generation.

MUTATION	TREATMENT DOSE	TOTAL NO OF PLANTS OBSERVED	PERCENT (%) NUMBER OF COBS PRODUCED PER PLANT					
			ZERO (0)	ONE (1)	TWO (2)	THREE (3)	FOUR (4)	FIVE AND ABOVE (5 <)
	0	300	-	40.00	56.00	4.00	-	-
	10 Gray	300	-	38.33	60.00	1.67	-	-
	20 Gray	300	-	51.67	51.67	46.66	-	-
	30 Gray	300	-	1.67	43.33	53.33	1.67	-
	40 Gray	300	-	11.67	33.33	50.00	5.00	-
	400 Gray	300	-	6.67	45.33	38.00	8.33	1.67
	25min $1.5x10^4$nth/cm^2/S-1	300	-	18.33	76.67	5.00	-	-
	40min $1.5x10^4$nth/cm^2/S-1	300	-	38.67	48.33	9.67	3.33	-
	50min $1.5x10^4$nth/cm^2/S-1	300	-	39.00	47.67	13.33	-	-
	65min $1.5x10^4$nth/cm^2/S-1	300	-	7.33	35.67	42.67	7.00	7.33
	12HRS $1.5x10^4$nth/cm^2/S-1	300	-	3.67	53.67	39.33	3.33	-

4.5.3 MALE STERILE INDUCED MUTANT

The next class of dominant mutants observed include the mature plant traits, such as tassel seed, male sterility and morphological traits not expressed in the seedling stage. In all the treatment,mutants affecting the size and number of tassel branches, male sterility and other aspects, often without affecting plant size were observed. Many variants give fewer or smaller tassel branches on normal size plants. On the basis of the appearance and relative position of tassel in M1 and M2 generation induced mutants, (male sterility) were classified as `highly sterile', `partially sterile', `both' and fertile plants (Table XII and XIII). "Highly sterile" are the plants with tassels been completely erase or turned into female ear or unisexual mutant and these mutants are usually seed sterile, `partially sterile' are plants developing seeds on the tassels, while the plants with `Both' sterilities posses ear/cob at the tassel in addition to the cob on the normal part of the stem and the`fertile' plants, are the normal appearing without any induced morphological alterations.

In M1 generation, the appearance of these different kind of sterilites tend to increase with the dose in both gamma rays and thermal neutrons treatments. The category "Highly sterile" appeared to be more frequent compared with the other classes (Table XII, However, most of the classes were not transmitted

to M_2 generation, and the highest observable percentage was not more than 4% in both gamma rays and thermal neutrons sampled population, and these are mostly confined to higher doses (40 and 400 Gray gamma) and 65min and 12 Hrs x $1.5x10^4$ nth/cm^2 5' thermal neutrons (Table XIII)

In order to analysed how a group of mutations (Chlorophyll deviations) observed in M_1 and M_2 generation are correlated with male sterility, a statistical analysis (correlation coefficient) was conducted between the two characters. Result obtained at M_1 generation, indicated that there is no significant correlation between M_1 chhorophyll mutation and male sterility (r=+0.133 df=8) less than 0.549 and 0.632 at 1% and 0.5% (p= 0.1 and p= 0.05) level of significance, and similar t test conducted (t=0.3796) revealed no significant relationship at P=0.1% (1.860) and 0.05% (2.306) level of significance. Similarly there is no significant correlation between M_2 chlorophyll mutation and male sterility in M_2 generation (r=+0.5476, df=8) at both 1% and 0.5% level of significance and `t' test also indicated lack of association (t=1.8511 less than the table value (see appendix II and III) Moreso, it has also been observed that the rate of M_2 chlorophyll mutation is statistically independent (r=+0.5057 df=8 less than table value 0.632 at 5% level of significance) of the

degree of sterility of the M_1 tassel. Not only the mutation frequency is independent of the degree of M_1 sterilty, but also the spectrum of chlorophyll mutation does not indicate any connection with M_1 tassel sterility (t= 1.6580 df =8 less than table value 1.860 and 2.306 at 1% and 0.5% level of significance (see appendix IV)

TABLE XII: Frequency of plants with varying sterilities (based on the conversion of male part completely to female, Average height and percent survival at maturity in gamma rays and thermal neutrons irradiated popcorn Zea mays Var. Praecox sturt) at M_1 generation.

MUTATION	TREATMENT DOSE	NUMBER OF PLANTS STUDIED	PERCENT (%) STERILITY FERTILE	PARTIALY STERILE	HIGHLY STERILE	BOTH	AVERAGE(X) HEIGHT AT MATURITY(CM)	RHP* (%)	PERCENT SURVIVAL AT MATURITY
	0	200	100	0	0	0	158.20	100%	100.00
	10 Gray	200	90.0	-	10.0	-	162.00	102.4%	97.78
GAMMA RAYS	20 Gray	200	86.0	1.0	10.0	1.0	141.30	89.43%	93.33
	30 Gray	200	73.5	9.0	15.0	2.5	129.40	81.80%	87.88
	40 Gray	200	37.5	16.0	45.0	2.0	124.12	78.46%	82.22
	400 Gray	200	15.0	10.0	60.0	15.0	106.30	67.19%	74.78
	25min $1.5x10^4$nth/cm^2	200	92.0	-	8.0	-	146.32	92.49%	91.11
	40min $1.5x10^4$nth/cm^2	200	94.5	0.5	5.0	-	167.12	105.64%	84.41
	50min $1.5x10^4$nth/cm^2	200	70.0	2.5	20.0	7.5	168.00	106.19%	89.44
	65min $1.5x10^4$nth/cm^2	200	82.0	5.0	10.0	3.0	117.28	74.13%	80.00
	12HRS $1.5x10^4$nth/cm^2	200	94.0	1.0	3.0	1.0	102.56	64.83%	70.33

NOTE: (1) Fertile - These are normal appearing plants with tassel and cobs/ear at their rightful position
(ii) Partially Sterile: - These are plants developing seeds on the tassels
(iii) Highly Sterile: - Plants with tassels been completely erase or turn to female/ear unisexual mutants
(iv) Both: Plants with ear at the tassel plus additional normal cobs/ear on the stem.

* RHP = Relative Height Percentage.

TABLE XIII: Frequency of plants with varying sterilities (based on the conversion of male part completely to female, Average height and percent survival at maturity in gamma rays and thermal neutrons irradiated popcorn Zea mays Var. Praecox sturt) at M_2 generation.

MUTATION	TREATMENT DOSE	NUMBER OF PLANTS STUDIED	PERCENT FERTILE	(%) STERILITY PARTIALY STERILE	HIGHLY STERILE	BOTH	AVERAGE(X) HEIGHT AT MATURITY(CM)	RHP* (%)	PERCENT SURVIVAL AT MATURITY
	0	200	100	0	0	0	200.60	100%	100.00
	10 Gray	200	100	-	-	-	187.00	93.22%	83.33
GAMMA RAYS	20 Gray	200	100	-	-	-	173.90	86.69%	73.67
	30 Gray	200	100	-	-	-	168.40	83.95%	69.00
	40 Gray	200	97	2.0	1.0	-	143.10	71.34%	24.67
	400 Gray	200	94	2.0	4.0	-	124.53	62.08%	86.66
	25min $1.5x10^4$nth/cm^2	200	100	-	-	-	167.00	83.25%	86.66
	40min $1.5x10^4$nth/cm^2	200	100	-	-	-	145.70	72.63%	70.67
	50min $1.5x10^4$nth/cm^2	200	99.0	1.0	-	-	141.00	70.29%	64.38
	65min $1.5x10^4$nth/cm^2	200	98.0	2.0	-	-	129.40	64.51%	52.67
	12HRS $1.5x10^4$nth/cm^2	200	95.0	4.0	-	1.0	178.50	59.07%	48.50

NOTE: (i) Fertile - These are normal appearing plants with tassel and cobs/ear at their rightful position
(ii) Partially Sterile: - These are plants developing seeds on the tassels
(iii) Highly Sterile: - Plants with tassels been completely erase or turn to female/ear unisexual mutants
(iv) Both: Plants with ear at the tassel plus additional normal cobs/ear on the stem.
*RHP = Relative Height Percentage.

4.5.4 SURVIVAL TO MATURITY

Surviving plants are counted at the time of harvest of the M_1 and M_2 generation. In this work survivors were defined as those plants that produce at least one ear, regardless of whether seeds are produced; in addition to the actual plant death. On comparing the results obtained in M_1 and M_2 generation for both gamma rays and thermal neutrons rays, it was evident that, there was a decrease in percent survival with the increase in dose. After gamma irradiation, the number of non surviving plants varies between 2.22 - 25.22% in M1 generation and 16.33 - 75.33% in M2 generation. Comparing the results with those after thermal neutrons treatment one may find that inspite of the nearly the same survival rate in low dose of thermal neutrons and gamma rays, there was a striking difference (8.89 - 29.67% in M_1 and 13.34-51.5% in M_2) in the percent number of non surviving plants. Thus if lethal mutation (non surviving plants) are considered without given consideration to possible non genetic effects, the total percent survival decreased, with increase in dose. (Table XII, and XVIII). Therefore, dose N65. 1.5×10^4 nth/cm^2/S^{-1} of thermal neutrons may be considered as 50% lethal dose (LD50) in M_2 generation, while X40 Gray can as well be considered as lethal dose (LD 100). However for practical mutation breeding and to some extent for mutation research, the values do not give enough

information. They specify only the percentage of mutants in relation to the number of non surviving plants.

4.5.5 HEIGHT AT MATURITY

Result obtained at M_1 and M_2 generation, indicated that over half of the variants induced affected stature, most of them giving short plants or low ear forms. Short plant mutant are defined as those having nearly normal leaf number and area but shorter internodes, which grade into semi dwarf types and small plants as short, but with fewer and smaller leaves. Short plants sustain normal grain yields; but not small plants. Also some tall plant, high eared, thick stalk and wide leaf variants were observed. A fair proportion were completely or partially dominant.

Seeds treated with gamma rays and thermal neutrons radiation results in plants showing a gradual decrease in height with increasing doses (Table XII, and XIII), though this was not regular in M_1 generation. The greatest reduction in height (32.81% and 35.17%) occured with the higher does of 400Gy and 12 HRS. $1.5x10^4$ nth/cm ^{2} /S $^{-1}$ for gamma and thermal neutrons in M1 generation. Similar reduction of 37.92% and 40.93% was observed in the same highest doses of gamma and thermal neutrons in M_2 generation (Table XII and XIII).

Other mutant characters observed, were ageotropism and increased plant height especially at the lower dose (x10Gray, $40x1.5x10^4$ nth/cm ^{2} /S $^{-1}$ and

$50x1.5x10^{4}$ nth/cm^{2})/S^{-1} in comparison with the control. The greatest increased in height (2.4% and 6.2%) was recorded for gamma and thermal neutrons respectively, but these were not reflected in M_2 generation.

4.5.6 MUTAGENIC EFFECT ON EAR LENGHT

Data obtained from the measurement of ear length after gamma rays and thermal neutrons irradiation in M1 and M_2 generation were presented in appendix V and VI.

Analysis of variance (Anova) at M_1 indicated that, the treatment were significant (2.56 at P(0.05) df 10, and 40) statistically, therefore the increase in length was not due to chance alone. On comparing the means of all treatment, using LSD (least significant difference) of 1.7, it was observed that treatment X400Gy dose produced the longest ear (16.10cm) than all the treatment, but this increased in length was not statisfically different from treatment N12HRS 30, 10, 20, 40 and N40, however, these are significantly different statistically from the control (0), N50, N65, and N25, (See appendix V.)

Similar analysis of variance (ANOVA) at M2 generation indicated that the treatments are highly significatnt (4.15 at P(0.05) df 10 and 40) and therefore the increased in ear length compared with the control was not due to chance alone (see appendix VI) On comparing the means of all the treatment using LSD 2.13, it was observed that, the treatment dose 30 produced the longest ear length (19.06cm) though the

increased in length were not statistically different from that of treatment N65, 20, 400, 40, N40, N50, and the control, but statistically defferent from treatment N12, and 10, which were also different statistically from treatment N25 (see appendix VI) The result also indicated an increase in ear length in M_2 (19.06cm) compared with the M_1 (16.10cm) generation.

4.5.7 MUTAGENIC EFFECT ON 100 GRAIN WEIGHT

Data obtained on the mutagenic effect on 100 grain weight in M1 and M2 generation is presented in the appendix VII and VIII. Result from the analysis of variance (ANOVA) at M1 generation indicated that the treatment was highly significant (10.47) at P(0.05) (10.40df). Hence the increased in weight was not due to chance alone. On comparing all the means in the treatments using LSD (least significant difference) of 1.18, it was observed that, the 100 grain seed weight of 400 Gy treatment was the heavist (12.10g than all the remaining treatment. However, this increase in grain weight was not statistically different from treatment N25, and 30 but different - statistically from the control (0) and all the remaining treatments (See appendix VI).

Similar analysis of variance (Anova) in the M1 generation (Factorial uncepts) also indicated a significant effect when the control was compared with treatment. Significant effect was also observed statistically (App. VII) when gamma rays was compared

with thermal neutrons. Also recorded was the significant effect both within gamma rays and thermal neutrons treatment doses.

Result also obtained from the analysis of variance of 100 grain seed weight of M_2 generation, indicated that the treatment was highly significant (Appendix VIII) at (P 0.05) (10, and 40 df). On comparing the means, using LSD).405,it was observed that 400 Gy gamma rays treatment produced the highest (12.42) means than all the remaining treatment and was statistically different from all of them. Treatment 30, N25, 20 are not statistically different, but different and infact more than the control and remaining treatments 40, N12, 10, N65, N50) which are not statistically different among themselves. Analysis of variance, by means of factorial concepts also indicated a signficant effect in all the comparisms except that of the control and treatment. (See Appendix VIII).

4.5.8 CYTOLOGICAL EFFECTS

The role of irradiation in enlarging genetic variability of quantitative characters, thereby creating a scope for selection has been established. In the present investigation, very encouraging results have been obtained for many polygenic characters and it was envisaged to induced more variability in polygenic characters like plant height, tiller number, yield and maturity. Most of these changes resulting from

mutagenic treatment are also cytologically observed and most of the changes were due to chromosomal mutations/ aberration which are recorded in mitosis and meiosis.

4.5.9 INDUCED MITOTIC ABERRATIONS

After treatment of seeds, the analysis of the first mitotic cycle in the root cells provided the level of effectiveness of the two mutagens (gamma rays and thermal neutrons) in inducing mutation. There was a considerable variability in the chromosome mutation induced between the different treatment doses and the two mutagenic agents. Results of the different mutagenic agents indicated that the frequency of aberrant cells, increases with increase in radiation dose.

At the prophase of mitotic division, observations of induced chromosomal abnormalities was not possible because the chromosome are still thin and small, and within the limit of light microscope the pairing is more or less complete (plate 4 fig. 1-2). Most of the observations were made at mitotic metaphase and anaphases for quantitative chromosome mutations. Metaphase stage was characterised by the formation of multivalents, ranging from bivalents, trivalents and quadrivalents, the frequency of which increase with an increase in irradiation dose. Other abnormalities include the non-orientation of chromosome on the metaphase plates, rings chromosomes and univalents (plate 4 fig. 3-7).

At the anaphase stage, chromosomal abnormalities observed include laggards, eliminated chromosomes, fragments and bridges (plate 4 fig. 8-12).

One pacularіaties at this stage was the occurance of high frequency of single and multiple bridges. Most of the bridges occured as double or multiple bridge (plate 4 fig. 8) which may lie separate and more or less parallel and/or apparently touching in which case they frequently lied across each other or occasionally interlocked. Acentric fragment also occur. The size of the fragments ranges from minute dots to rods which appearred to be as long as a whole chromosomes. The cause of bridge formation was due to the fusion of two centromere bearing chromosome fragments. After the effective chromosome spliting into two chromatids, in the anaphase the dicentric chromatids tends to go to opposite poles and a bridge was formed which breaks later. It has been found that the frequency of the bridges is directly proportional to the dose of both gamma and thermal neutrons.

Telophase stage was not characterized by serious chromosomal abnormalities. The only aberration observed was non-separation of the two daughter cells by cell membrane (cytokinesis) and the continous reduplication of the chromosome (plate 4 fig. 13-15).

4.6.0 INDUCED MEIOTIC ABEARRATIONS

The types of meiotic abnormalities/aberrations observed resulting from gamma rays and thermal neutrons treatment in different stages of meiotic division are as follows:-

4.6.1 PROPHASE 1 (LEPTOTENE)

Chromosomes are visible within the nuclear membrane. They appear to be single, longer, thinner and have clear chromomeres. Only short segments of each chromosome can be distinguished at this stage. The nucleolus is clearly visible (plate 1 fig. 1-2). Later the chromosomes, group in a tight compact structure to one side of the nucleus (fig. 2 plate 1). There are no clear visible chromosomal abnormalities induced at this stage.

4.6.2 PROPHASE 1(ZYGOTENE)

The stage was characterized by pairing (synapsis) between the homologous chromosome. When pairing is complete, homologous centromeres are adjacent as telomeres, and the homologous are loosely coiled rationally around each other. However, it was observed that pairing was either complete or partially interrupted as a result of mutagenic treatment (plate 1 fig.3). The frequency of nonpairing is directly dependent on the dose of irradiation and seems to be higher in gamma rays irradiation treatment as compared with thermal neutrons. Thus since chiasma frequency is

genotypically controlled character, the segregates, particularly those affected by radiation showed pairtial or complete failure of pairing and chiasma formation.

4.6.3 PROPHASE 1 (PACHYTENE):

The paired chromosomes are thicker and shorter. The chromosomes are fewer, larger, and more conspicuous. Individual homologues are difficult to distinguish. The two chromosomes of each bivalent presumably replicate, each forming two chromatids (fig 4 plate 1) later, pairs of non-sister chromatid formed chiasmata. The chromosomes are uniformly condensed and evenly distributed within the nucleus (fig 4 plate 1). Pachytene analysis is in most cases not suitable for detail observation or quantitative record of small deficiencies.

4.6.4 PROPHASE 1 (DIPLOTENE):

The chromosomes are diffused, because of the formation of lateral extensions from the main chromosome axis. Chromosomes are visually recognisable. They typically no longer show rational coils of one chromosome around the other. Chromosome separates, and each was observed to consist of two chromatids. The chromosomes are separated throughout their length, except at one or more points, where they are brought together (chiasmata) (fig 5 plate 1). The chiasmata are the basis of genetic exchange which

results in recombination of alleles at different loci on homologous chromosomes. One of the induced chromosomal abnormalities observed was the failure to form a chiasma after pairing (desynapsis), thus the consequent univalents are likely to show irregular disjunction at anaphase (fig. 38 plate 3).

4.6.5 PROPHASE 1 (DIAKINESIS):

Ten bivalents are usually observed in a normal dividing meiotic prophase at diakinesis. The bivalents move towards the periphery of the nucleus and lied well separated on the side of nuclear membrane (fig.6-13 plate 1). Chromosomes further contract and become more regid. The shape assumed by a particular bivalents depends on the position of the centromere and on the number and position of the chiasmata (fig 6-13 plate 1). Bivalents with a single chiasma appeared as open crosses (fig 6 and 7 plate 1). Where two chiasmata are present the bivalent, is a ring shaped (fig 8-13 plate 1). Three or more chiasmata lead to the production of chains like configurations, in which successive loops lied in planes at right angles to one another (fig. 9, 11 and 12 plate 1).

Although 10 bivalents are usually observed at diakinesis, induced quadrivalents and other multivalents in the form of trivalents and univalents (fig 11-13 Plate 1) due to mutagenic effect of gamma rays and thermal neutrons are also of frequent

occurance. The frequency of induced multivalents was dose dependent and increases with an increase in irradiation dose in both gamma rays and thermal neutrons irradiation and was also found to be more in gamma rays, compared to thermal neutrons (Table XIV). Two quadrivalents, 2 trivalents and 2 univalents are shown in fig 6 plate 1; 3 quadrivalent, 2 trivalent and 2 bivalent (fig 7); 7 bivalents, 1 quadrivalent and 2 univalents (fig 8); 3 quadrivalents, 2 trivalents and 2 univalents (fig 11); 4 quadrivalents, 1 trivalents and 1 univalent (fig. 12). The frequency of such observed multivalents was analysed and shown in (table XIV). Other type of chromosome mutation observed at Diakinesis in most of the treatment are translocations. Reciprocal translocations are recognized by ring or chain formation (fig. 9-13 plate 1). Two reciprocal translocations per cell are rare, however rings or chains of 5 to 6 chromosomes were observed (fig. 10 plate 1). Visible deficiencies were not observed in M_1 diakinesis.

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TABLE (XIV):- Cytogenetic effects of gamma rays (Cobalt 60) and thermal neutron irradiation on the frequency of multivalent at diakinesis (prophase 1) in popcorn (Zea mays var. Praecox sturt).

DOSE	CHROMOSOME COMPLIMENT	NO OF P.M.C OBSERVED	FREQ UENCY	PERCENTAGE
0	10 bivalents	250	25	10.00
x10	9 bivalents, 2 univalents	160	71	44.38
x20	7 bivalents, 1 trivalent, 3 univalents	132	80	60.60
x30	4 bivalents, 3 trivalents, 3 univalents	140	87	62.14
x40	2 bivalents, 5 trivalents, 1 univalent	160	94	58.75
x400	2 bivalents, 4 quadriva-lent	210	186	88.57
N25	2 trivalents, 3 guadrivalent 1 bivalent.	182	98	56.38
N40	3 quadrivalents, 2 trivalents, 2 univalents	195	106	29.27
N50	4 quadrivalents, 1 trivalents and 1 univalent	140	89	63.07
N65	2 quadrivalents, 1 trivalents 1 univalent and 1 bivalent	138	90	65.21
N12	3 quadrivalents, 1 trivalents, 2 bivalents, 1 univalent	120	112	93.33
TOTAL	NUMBER OF PMC OBSERVED	1827		

4.6.6 METAPHASE 1

The bivalents at this stage are arranged on the spindle equator (plate 1 fig. 14-16). There was a conspicuous stretching of chromosome (fig 17-22 plate

2). Bivalents with one chiasma are rod like with the chromosomes straight and end to end (fig. 16 and 17). Bivalents with terminalized chiasma in each of the equal arms form an open ring (fig 18 plate 2). Where the bivalent forms a single proximal chiasma, a cross-shaped configuration was formed (fig. 22 plate 2). Most of the univalents are noticeably under contracted and unable to co-orientate on the spindle equator, thus after oscillating from one side to the other, it eventually lies between the equator and one pole (fig 14 -17).

The only type of induced chromosomal aberrations that can easily be recognized in most of the treatments with gamma rays and thermal neutrons at metaphase 1 are translocations. Reciprocal translocation are identify by ring or chain formation at metaphase 1 (fig. 17-22 plate 2). Similarly, there was an occasional mal-orientation of the bivalents (fig 25 plate 2). This mal-orientation is unstable and usually one member re-orients to produce a stable bipolar orientation.

Other induced chromosomal abnormalities observed are the sticky chromosomes (fig 23-28 plate 2) in which the entire chromosomes clumped together as a mass or in a set of two to three groups inform of higher order of multivalents (hexavalent with a fragments (fig. 28).

Still, at this stage, the dicentric chromosome were unpaired in 26.2% of the cells analysed and they lay off the plate as in fig. 14-16 plate 1). In 48

cells or 2.5 percent, lay off the plate and acted like univalents (fig. 17-18 plate 2). In the remaining (72.3 percent) the dicentric were paired lying on the plate with the appearance of a normal bivalent (fig. 18-20 plate 2). The dicentric bivalent most frequently occured as a rod bivalent with one chiasma in the long arm and was easily recognized by its typical shape (fig.19 plate 2) less frequently it was seen as a small ring bivalent.

4.6.7 ANAPHASE 1

At first anaphase, the bivalents and quadrivalents disjoin and moves towards the poles (fig 29-37). During this stage the separation of chromosome ends (fig. 35-37 plate 3). The study of anaphase 1 stage revealed the occurance of both induced univalent bridge (single bridge fig. 38 plate 3) and dicentric bridge (Table XV). The characteristics of dicentric bridge, permit easy identification due to its double structure and can easily be distinguished from univalent dicentric bridge. Only about 26% of the anphases analysed in this material have such univalent bridges. Bridges concerned with other chromosome than the dicentrics occasionally occur, along with some fragments and univalent. Only about 38 percent of the total 1775, studied had dicentric bridges.

The behaviour of univalent at this stage varies. They can either lag during separation (plate 2 fig 32-

33), divide (fig 36) or are eliminated (fig 35). The occurance of fragments at anaphase 1, without chromatid bridge seemed to indicate that the bridge had been disjoined early or carried to one of the poles leaving the fragments between separating groups of chromosomes (fig. 36 and 37 plate 3). The fragments are in the form of small body and usually lies at the equator of the spindle, it lacks movement and therefore appears to be acentric. In some pollen mother cell, the anaphase bridge broke at the point where the two chromatids are associated or at two approximating places one on either side of the point of association (fig. 37). The frequency of chromatid bridges, fragments, laggards and eleminated chromosomes have been analysed at anaphase 1, and the results were shown in (Table XV) the occurance of fragments and laggards lead to unequal separation of daughter chromosomes at the poles; cases of 10-10 (fig. 33), 9-10 (fig. 35), 10-8 (fig 36 plate 2 and 3) are of frequent occurence.

4.6.8 TELOPHASE 1, INTERKINESIS AND PROPHASE II.

Telophase 1 appears to be of very short duration. The chromosome reaching the poles agregate very closely, the laggards and fragments get excluded and probably form micronuclei (fig.37 plate 3) An uneven or unequal number of chromosome reaching the two poles is reflected by the occurance of unequal size of the polar nuclei. The nuclear membrane is formed

(cytothinesis/interkinesis) at telophase 1 to produced two distinct cells called dyads and the nucleus returns to the interphase stage and the second meiotic division followed immediately.

TABLE XV: Cytogenetics effects of gamma rays (^{60}/CO) and thermal neutrons irradiation on the frequency of chromatid bridges, a lagging and eliminated chromosomes at first anaphase in popcorn Zea mays Var. Praecox sturt).

DOSE	NO. OF P.M.C SHOWING CLEAR SEPARATION	NO. OF CHROMATID BRIDGE		NO. OF FRAGMENT	NO.OF LAGGARDS	NO. OF ELIMINATED CHROMOSOMES	FREQUENCY	PERCENTAGE
		SINGLE	DOUBLE					
0	300	-	-	-	-	-	300	16.90
10	280	1	-	-	2	-	200	11.27
20	250	-	-	2	1	-	180	10.14
30	240	-	2	-	2	1	150	8.45
40	210	1	1	3	-	4	120	6.76
400	180	3	4	1	1	3	100	5.63
N25	270	-	-	2	-	2	160	9.02
N40	240	-	-	4	2	1	145	8.17
N50	190	1	-	2	2	-	130	7.32
N65	160	2	4	1	-	2	120	6.75
N12HRS	180	3	2	-	4	1	170	9.58
TOTAL							1775	100%

4.6.9 METAPHASE II

At metaphase II, each daughter group from meiosis I formed a separate spindle, in a distinct dyad (fig 43 plate 3). At this stage 10 chromosomes are observed at each pole of the second metaphase plate when there is a clear separation. However instances of unequal distribution of chromosomes due to radiation damage were also noticed. Similarly induced multivalents (translocation) in the form of trivalents and quadrivalents were also of frequent occurance (fig 39-42 plate 3) and most of these were found at the higher doses of irradiation in both gamma rays and thermal neutrons, in comparism with the control and lower treatment doses. The frequency of different chromosomes distribution on second metaphase plates, number of chromosomes oriented, number of eliminated chromosomes, and fragments were analysed and presented in table XVI.

TABLE XVI: Cytogentic effect of gamma rays and thermal neutrons radiation on the frequency of different chromosome distribution, number of non-oriented, eliminated chromosomes and fragments on the second metaphase plate in popcorn (Z mays var praecox sturt).

DOSE	NO. OF CHROMOSOMES NO SECOND META-PHASE PLATE	NO OF NON-ORIENTED CHROMOSOMES	NO OF ELIMINATED CHROMOSOMES	FRAGMENTS	NO OF P.M.C OBSERVED	FRE-QUEN CY.	PERCENTAGE (%)
0	10 - 10	-	-	-	150	0	0.00
X10	10 - 9	-	1	-	160	58	36.25
X20	10 - 8	1	-	1	120	52	43.33
X30	9 - 8	1	2	-	160	65	40.62
X40	8 - 8	2	1	1	170	72	42.35
X400	8 - 7	-	2	3	140	105	75.00
N25	9 - 10	1	-	-	125	49	39.40
N40	8 - 10	2	-	-	170	68	40.00
N50	7 - 8	1	3	1	180	74	41.11
N65	8 - 6	2	1	3	130	83	63.84
N12HRS	5 - 5	2	3	5	115	95	82.60
TOTAL					1620		

P.M.C. POLLEN MOTHER CELL

4.7.0 ANAPHASE II

Anaphase II, was also studied to determine the different kinds of chromosomal aberration, resulting from the treatment doses of gamma and thermal neutrons irradiation. At this stage the centromere of the aligned chromosome divides and the daughter chromosome separate to the opposite ends of the spindle. However,

induced non-disjunction, delayed separation of anaphase chromosomes was observed. The Chromosome distribution was unequal due to high frequency of induced eleminated chromosomes, coupled with preponderance of laggards (fig. 44 plate 3). Fragments were also observed in most of the cells without bridges.

Other classes of induced chromosomal aberration are single and double bridges. In this study, anaphase II, configuration could be subdivided into the following classes - single bridges, double bridges, and absence of bridge but occurance of fragments, laggards or eliminated chromosomes. It is apparent from table XVII that single bridges in Anaphase II are much more frequent than double bridges. The high frequency of single bridges in anaphase II may probably be accounted for by sister-strand crossing over. The frequency of chromatid bridges, lagging, eliminated chromosomes and fragments were analysed and the result is shown on Table XVII.

4.7.1 TELOPHASE II

At telophase II, four (4) groups of daughter chromosomes which are haploid in number and unreplicated are observed. The daughter chromosomes are enclosed by nuclear envelops (fig. 45 plate 3). Micronuclei are observed in the majority of the cells at this stage. The micronuclei represent the consequences of the proceedings induced abnormal meiotic events and also

the alignment and transformation of laggards, fragments, and eliminated chromosomes. Other forms of abnormality observed was the presence of empty cells, out of the four daughter cells (fig 46-47 plate 3).

TABLE XVII:- Cytogenetic effect of gamma rays and thermal neutrons irradiation on the frequency of chromatid bridges, fragments, lagging and eliminated chromosomes at second anaphase in popcorn (Z mays var. praecox sturt)

DOSE	NO. OF P.M.C. SHOWING CLEAR SEPARATION	NO OF CHROMATID BRIDGE SINGLE	NO OF CHROMATID BRIDGE DOUBLE	NO OF FRAGMENTS	NO OF LAGGARDS CHROMOSOMES	NO. OF ELIMINAED	FREQUENCY	PERCENTAGE
0	250	-	-	-	-	-	250	12.92
X10	260	-	-	2	-	1	230	11.89
X20	200	2	1	1	2	-	180	9.30
X30	230	1	-	1	-	2	160	8.27
X40	180	-	1	2	1	1	190	9.82
X400	210	2	3	2	1	2	200	10.34
N25	215	-	1	1	2	-	150	7.75
N40	190	1	-	3	-	2	140	7.24
N50	200	1	2	1	-	1	120	6.20
N65	160	3	2	-	1	3	130	6.72
N12HRS	170	2	2	2	2	2	185	9.55
TOTAL							1935	100%

4.7.2 INDUCED POLLEN STERILITY:-

Pollen formation in the irradiated popcorn was found not be regular, this was due to formation of

deformed and aborted pollen grains in most of the treatment doses (figure 49-52 plate 3) in comparism with morphologically good pollen (47-48 plate 3) in non-irradiated materials (control). The degree of pollen deformity and abortion/sterility is directly proportional to the dose of the radiation and seems to be higher (62.54%) in gamma rays (^{60}Co) than in thermal neutrons (37.46%). Figure 49 is a deformed pollen grain from gamma rays (40Gy) treatment. Figure 50 is a deformed pollen grain at N65 minutes thermal neutrons treatment. Figure 51 is an aborted gamate/pollen from N12 HRS thermal neutrons treatment. While figure 52 is an aborted pollen grain at the chronic treatment dose (x400Gy) of gamma rays cobalt-60.

The frequency of morphologically good pollen, deformed and aborted (sterile) pollen were analysed and the result presented in table XVIII.

TABLE XXIII:- Frequency of pollen fertility (morphologically good pollen), deformed and aborted pollen (sterile) in irradiated popcorn (Z mays var. praecox sturt)

DOSE	MORPHOGICALLY GOOD POLLEN	DEFORMED POLLEN	ABORTED POLLEN	FREQUENCY	PERCENTAGE
0	1000	0	0	0	0
X10	850	40	16	56	1.79
X20	816	64	32	96	3.08
X30	652	85	52	137	4.39
X40	470	264	105	369	11.83
X400	260	480	208	688	22.05
N25	870	52	20	72	2.31
N40	681	70	71	141	4.52
N50	604	84	89	173	5.54
N65	586	186	182	368	11.79
N12HRS	245	350	302	1020	32.70
TOTAL				3120	100%

CHAPTER FIVE

5.0.0 DISCUSSION

5.1.0 PERCENT GERMINATION, SEEDLING HEIGHT, ROOT LENGHT AND SURVIVAL

In general, treatment with mutagens (gamma rays and thermal neutrons) reduced germination, seedling height, root length and seedling survival; the reduction was positively associated with the dose. These observation are in agreement with earlier findings (Ramulu, 1971 Bird and Neuffer 1988). However, irradiation with 10Gy gamma-rays resulted in increased of survival. Low doses of irradiation are known to result in beneficial effects, such as increase in plant height, stem and leaf thickness etc. In several plant species (Sheppard and Hawkins 1990).

Popcorn Zea Mays Var. praecox sturt) showed a marked differences in their response to gamma-rays and thermal neutrons. There was a high sensitivity with resfect to germination and seedling injury. However, the seeds treated with the highest dose showed the highest reduction in percent germination, seeding height and survival which may be due to a high frequency of induced deletions or translocations (Laxmi et al 1975a). The genetic architecture of the organism is a potent factor in determining its response to mutagens (Goud et al 1970).

Treatment with gamma rays cobalt 60 caused more biological damage than thermal neutrons in this study, which is contrary to some other reports, which indicated that thermal neutrons is known to be more effective in producing biological damage than gamma rays in a number of plant species (Hoebee et al 1988; Oda and Thomas 1987). The apparent increase in height and germination in some treatment doses especially in the M_2 generation may be due to duplication of chromosome in addition to combined effect of favourable environmental factors.

5.2.0 CHLOROPHYLL DEFICIENT MUTANTS:- Six different types of chlorophyll deficient mutations, albina, xantha, lutescent, virescent, chlorina, and striata were observed in both M_1 and M_2 generation. Chlorophyll mutation are of common occurance after mutagenic treatments (Goud et al 1970; Reddy and Gupta 1989; Ranchyalis et al 1988). The mutagenic agents (gamma and thermal neutrons differ with respect to the spectrum and the frequency of chlorophyll mutations induced (Bird and Neuffer 1988). Differential behaviour of the mutagens to produce specific types of chlorophyll mutants, may be ascribed to their mutagenic effectiveness. Zacharias and Ehrenberg (1962) reported that X-ray produced leaf spots, is proportional to the square of the dose. In the first leaves, the size of spots is much smaller than initial later formed leaves,

since different number of initial cells are responsible for development of the consective different leaves. It is believed that chlorophyll and carotenoids are two groups of pigment present in maize chloroplastids; any deficiency in either or both group would result in chlorophyll mutation expression. Obviously the albino lacks both pigments. Light or pale green (xantha) mutants may be deficient in carotenoids and yellow (virescent) mutants may be deficient in chlorophyll. the other colour mutants, lutescent, chlorina and striata, that were also observed may result from the breakdown or shortage in various degrees of certain pigment(s). Thus, the whole range of seedling chlorophyll mutants may result from deficiency of either chlorophyll or carotenoids or both and each of these types may result from a mutation in a number of loci.

In general induced chlorophyll deficient mutants increase with an increased in radiation dose (Table V) However, the spectrum of chlorophyll mutation produced by gamma radiation was different from that of the neutrons (Table III and IV). Gamma rays produced chlorina type more frequently as compared to thermal Neutrons. It is likely that the observed differences in spectrum, in the present study are due to differential action of mutagens on the genetic materials. The possibility of selective elimination of some of the mutant types however, cannot be rule out,

because mutation observed in M_2 generation represent only a fraction of the originally induced mutational lessions. Many of which are possibly eliminated during ontogeny.

When expressed as percent M_2 plants, the frequency of chlorophyll mutations increase with an increased in the dose of mutagen irradiation and this is at variance with the result obtained by Singh et al (1976) who reported a decrease in chlorophyll mutation frequency with increase in dose of mutagen, but agree with those of Ramulu (1971). In this work the biological damage was considerable and increase with the dose. It is probable that the induced chlorophyll mutations would have simultaneously been mutants for other survival characters, and thus all the chlorophyll mutants which were likely induced would not be recovered in the M_2 generation (Table IV). A number of background gene mutations are likely to occur along with a macromutation (Micke, 1969). Such cases would be expected to increase with an increase in the dose of the mutagen. Furthermore the frequency of gene mutation would tend to decrease at higher doses of the mutagen because of chromosome damage and somatic selection (Carlson 1978; Girkontaite and Burneikene 1989).

The average frequency of chlorophyll mutations was much higher in the case of gamma rays than thermal

neutrons. Infact some of the treatment of thermal neutrons, did not produced an appreciable amount of chlorophyll mutation. Thermal neutrons has been reported to induced high frequency of chlorophyll mutations (Natrajan and Upadhya, 1964). The lower effectiveness of thermal neutrons compared to gamma rays in the present study was due to low recovery of mutants probably because of greater biological damage in thermal neutrons, treated population. This contention was supported by the fact that, lower doses induced damages, showed higher frequency of certain category of chlorophyll mutation (lutescent and virescent) than with gamma rays. The highest frequency of chlorophyll mutation was recorded in the X400 Gy dose of gamma ray treatment. A comparism of the frequency of chlorophyll mutations at different doses within and between treatments show degree of differential response.

5.3.0 MEAN NUMBER OF MUTANTS PER MUTATION

Changes in the chlorophyll contents (mutants) from a given mutagen may provide an indication of the mutability of a given genotype for other specific traits. It is commonly assumed, althoguh not rigorously proven, that induced mutations, represent the same kinds of changes in the genes as occur from the natural causes, they show normal inheritance and consequently perform similar, to genetic variations that breeders routinely exploit (Konzak 1976). Most induced

mutants show recessive inheritance, but more rarely occuring dominant mutant have been isolated from mutagenized plant populations (Brown et al 1980; Franckowiak et al 1976; Konzak 1981, 1982). Even lethal mutants may contribute to vigour when heterozygous (Salerno et al 1981).

A reduced frequency of mean mutant recovery in this study especially in the M_2 generation may suggest that the mutants were due to a large deletion. However, according to Gustaffson and Lundqvist (1981) the greater number of mutants isolated from seed treatment in higher plants cannot be due to large deletions, since many different functional alleles at specific loci have been identified. Smaller sized chromosome segment alterations base pair deletions and duplications as well as simple base transition and transversions may be, the possible cause (plate 2 and 3). The most extensive analysis of induced mutant allelism have been made in barley Gustaffosn 1972; 1979; Gustaffsson and Lundqvist 1981) But a typical recombination data for some mutants suggest other causes (Amano 1979; and Rosichan 1979). However, the range, frequency and mean spectrum of mutation produced in gamma rays, differ significantly from that produced in thermal neutrons. This is in agreement with earlier reported work of Nilan (1972).(also see table V and VI).

Induced genetic variation sometimes may include rare, valuable mutants previously unknown or unlikely to survive in natural population (Gustaffson 1979; Gustaffson and Lundqvist 1981) Phenotypically similar mutants may be controlled by one or more alleles at one or more loci. Thus Wettstein et al (1971) reported in barley 31 gene loci for 33 albina mutants, examined, and 20 gene loci and alleles controlled the xantha phenotype. For the virescent phenotype, 14 gene loci were found from analysis of 20 mutants and 16 gene loci controlled the tigrina and Zonata phenotype of 27 mutants. Five gene loci with more than one allele were responsible for the phenotype of 49 viridis mutants (Konzak and Kleinhofs 1984). These examples indicate the very great potential for genetic variability stored within the genomes of plant species, a potential that is being increasingly realized through the induction of mutations using artificial agents.

The decrease in the mean number of mutants, in the M_2 generation especially in thermal neutrons treatment might be due to large deletion and subsequent elimination of lethal mutations that could not survive the M1 generation (Table V and VI.

5.4.0 MUTANTS AFFECTING TILLER FORMATION:-

Tiller formation and number varied widely in the treated population compared to the control, but the range was much wider in irradiated populations in both

gamma rays and thermal neutrons compared to their respective controls (Table VII). The result is in agreement with the findings of Dhowukshe and Bhowal (1976). The high prevalent occurance of tillers (1-4) in the M_1 generation and the transmission of less than 1% in the M_2 generation, might be attributed to physiological stress as a result of irradiation coupled with cytological effects (probably duplication) in M_1 generation and the resultant recovery from the stress and eliminantion of some mutant in M_1 may account for the low (1%) expression of tillers in the M_2 generation. Induced tiller formation was also reported in wheats by Konzak and Kleinhofs (1984). More so Birds and Neuffer (1988) used chemical mutagen to induced tillers in Zea mays:

Tiller formation is relatively high in gamma rays treatments compared with the thermal neutrons in both M_1 and M_2 generation (see Table VIII), this might be due to differential effect of gamma rays and thermal neutrons in inducing chromosomal and gene/point mutation as reported by, Hoebee et al (1988), VazQuez and Sanchez - monge (1987) and Prokof'eva et al (1989), Reddy 1988).

5.5.0 EARLY DEVELOPMENT MUTANTS:- The reported induced early flowering and maturity in this work especially in the M2 generation may be due to changes in genetics and physiology resulting from the irradiation treatment

with gamma rays and thermal neutrons treatments. This view is supported by the work of Konzak and Kleinhofs (1984). However, when the generations (M_0 - M_2) time of flowering and maturity were compared (Table IX) there was an increase in earliness and maturity period from M_0 to M_2 generation. When the two mutagens gamma rays and thermal neutrons were compared, gamma rays produced relatively early flowering and maturity mutants especially in the doses of 20Gy, 40Gy and 400Gy, more than the thermal neutrons. Such commonly induced earliness and lateness mutants with altered physiological traits, have been reported in cotton (Raut et al 1971), Potato (Shephard et al 1980), rice (Hseih and Chang 1975; Rutger 1982); Jute (Mia et al 1976) and Soybeans (Koo et al 1970; Koo 1972). Earliness mutants have been frequently induced in a wide range of crops species (Konzak and Kleinhofs 1984).

Ukai and Yamashita (1981) analysed 61 induced early mutants of barley. Four of the mutants carried genes controlling photo period response, two of which are allelic to the earliness locus and three mutants carried genes controlling vernalization effects. They found that the early mutants had varying degrees of changes in response to photoperiod or vernalization. Changes in the relative proportion of the vegetative and generative phases in the growth of plants was also induced in wheat (Kar et al 1978). Lawrance (1968)

analysis of flowering times mutants in Arabidopsis thaliana showed that the flowering time variation was predominantly due to additive effect of recessive genes.

5.6.0 FERTILITY COUNT (EAR MUTANTS) BASE ON THE NUMBER OF COBS PRODUCED PER PLANTS

The increase in the number of ears (1-5) has been more striking in the irradiated population than the control (Table X and XI). Irradiation has either induced favourable mutations for number of ears or resulted in a physiological stress, such that multiple eared genotypes were produced in the early generations (M_1 and M_2). Theoretically, this should be a good base for a general increase in yield (Shaver 1983; Said et al 1988, Benamer, 1990). However, though a clear increase in the number of Cobs per plant was established, this increase was regularly accompanied by a decrease in the number of seeds per Cobs. Therefore the mean value for the total yield of the mutants types are in general equivalent to or higher than the values of the control (Table X and XI). There is no doubt that the positive character of this genotype can be used for practical breeding, it is only necessary to increase the number of cobs/seeds per plant by carrying out-suitable hybridizations. Thus, it should be possible to combine the positive characters, high number of seeds per cobs and high number of cobs per plant by crossing these genotype to obtain a new pure line which is homozygous for two mutant recessive

genes. Furthermore, mutants induce in this research could be used for practical breeding after having transferred the mutant genes into different genomes. It has been shown in the previous works (schmitz 1988; Barriga 1989). That an important percentage of mutant genes becomes detactable primarily in the M_2 generation. This is not only valid for popcorn (Zea mays var praecox Sturt) but all species having relatively low seed production under normal conditions and a strongly reduced fertility after application of mutagens, thus it is not enough to cultivate the progenies of M_2 generation in which segregation of mutant genes occurs (Barriga et al 1989)

5.7.0 INDUCED MALE STERILE MUTANTS

The mutagenic agents (gamma rays and thermal neutrons) used in the present work induced a varying degree of male sterility in M_1 and M_2 generation (Table XII and XIII). The results have demonstrated that the capacity of an agent to induced mutations may not have a similar bearing on plant fertility or other morphological abnormalities (appendix II to IV). This is not unexpected since radiations produced more chromosomal mutations which are sieved off during meiosis (Kivi 1965; Davidenk et al 1988 and Koh 1987).

The plant of maize are monoecious; megaspores are produced in female flowers and micropores are produced in male flowers (tassel) located at the stem apex.

Plants which are homozygous for the receissive gene SK are cobless and therefore unisexual. Plants which are homozygous for the receissive gene ts are tassel-seeded (ie have a cob in place of the tassel and are therefore unisexual). Plants which are homozygous for both recessive gene (sk, sk, ts ts) are unisexual and produce only megaspores. On the other hand, plants of genotypes Sk sk, Ts Ts, and SK SK TS ts produced only microspores and are unisexual. SK SK ts ts, SK SK ts ts and SK SK ts ts plants too are unisexual and produced only megaspores, but they are tassel seeded, because ts ts is epistatic to SK SK (Sinha and Sunita - Sinha 1980) see table XII & XIII).

Although it is generally assumed, that chromosome mutations are the main source of sterility, translocation (plate 2 and 3) are in most cases the only type of mutation that could readily be recognized in meiosis. In gamma ray treated populations, pollen sterility would have been mainly due to translocations and other cytological anomalies which were induced in much higher frequency by gamma rays than by thermal neutrons (Table XII and XIII). The fact that treatment with gamma 400Gy showed the highest pollen sterility as well as the highest frequency of translocation, supports this suggestion. The pollen sterility induced by thermal neutrons appeared to be due to gene mutations, invisible deficiencies or both (Sato and Gaul 1967).

The present work demonstrated that malesterility in M_1 generation were partially transmitted in M_2 generation (Table XII). These findings was supported by the evidence presented by Gaul and Mittelstencheid (1960) that the radiation induced M1 sterility is partly transferred into later generations. A large part of the M1 sterility is caused by physiological damage and consequently not transfered to M_2 generation.

The developmental breakdown resulting in male sterility has been studied in a number of cases at various stage of pollen abortion, from early stages of meiosis thorough to almost normal pollen production (plate 3 fig.47-52). In the majority of cases breakdown involves tapetal malfunctions, whether it be delayed degeneration as in flax (Pande and Raghuvanshi 1987). And Pearl Millet(Reddy and Reddi 1970, Mayers and Gritton 1988)and breakdown before tetrad stage (plate 3 fig.46) as in wheat (Howey et al 1988) or tapetal hypertrophy as in onion, corn, and Barley (Kaul and Singh 1966, Hittalmani and shivashanikar 1987; Strel'Nikova 1979). Some cases of meiotic irregularity (plate 1) have also been observed in cotton at prophase 1 (Murthi and Weaver, 1974) and in lettuce where a high degree of asynapsis occurs (Raj et al 1988).

Another example of male sterility is governed by a number of mechanism at different stages of pollen abortion can be seen (plate 3 fig. 49-52). An

interesting class of chromosomal mutants that involved normal pollen formation, however, the anthers fail to dehisce has been detected in Sorghum, where it is cytoplasmically inherited (Choudhury et al 1968).

Correlation coefficient analysis between M_1 and M_2 chlorophyll deficient multants; M_1 sterility and M_1 chlorophyll deficient mutants demostrated no relationship between the M_1 sterility and chlorophyll deficient mutantions in M_2 (appendix II to IV).

However, several attempts have been made to established correlations between M_1 sterility and M_2 mutation frequency. Caldecots (1961) showed a close relationship between the decrease in M_1 sterility and chlorophyll mutation frequency in M_2. Gaul (1964) studying the effect of X-rays on seed fertility and mutation frequency proposed that the frequency of factor mutation is completely independent of the degree of sterility. Kivi (1965) however, demostrated that the highest chlorophyll mutation was obtained among the descendent of moderately damage (medium fertility) M_1 individuals.

5.8.0 SURVIVAL AND HEIGHT AT MATURITY

A drastic reduction (31.02%) of survival at maturity observed in gamma rays 400Gy treatment dose was not unexpected because chronic doses of gamma rays were known to caused a severe reduction of survival at maturity. The same idea was supported by the work of

Venkateswarlu et al (1978); Zagorcheva and Mariya (1987); Khamankar and Singh (1988). However irradiation with gamma rays 10Gy did not noticeably reduce survival, but higher doses of gamma rays, further reduced the survival. The survival was very poor (24.70% of the control) at 40Gy (Table XIII). This was supported by the work of sheppard and Hawkins (1990) that indicated hormesis; - the increase in growth resulting from low doses of gamma rays and thermal neutrons were as a result of stress factor and was approached as a stochastic phenomenon. Similar reasons could be attributed to the increase in survival in the low doses of thermal neutrons. The increase in percent lethality in the highest dose of thermal neutrons, especially in the M_2 generation may be due to large deletions resulting from the chromosomal aberrations, which was significantly higher after all the treatments than the control (Lichu and Yachevskaya 1987).

Height at maturity in general decreased with the increase dose of gamma rays and thermal neutrons. The reduction in height was only slightly induced at the low doses of the treatments (Table XIII) and this conforms with the work of Lysenkov (1989) that induced a wide range of viable mutants at a frequency, including forms with a combination of useful traits of breeding value. The height was reduced drastically (62 and 59% of the control) at 400Gy and 12HRS treatments.

Additional reduction of height at the higher doses, may have resulted from other causes, such as chromosomal aberration and gene mutations. These findings do not agree with others (Mohmed et al 1973) where only slight reduction (10-15%) in height was observed even in the highest dose of irradiation.

5.9.0 MUTAGENIC EFFECT ON EAR LENGHT:-

Considering the results on the capacity of positive mutants; in particular, the increase in ear length are beneficial characters induced, which result from irradiation treatment with gamma rays and thermal neutrons. There is no proportionality between the dose and the increase in ear length. However, analysis of variances of the result obtained both in M_1 and M_2 generations (Appendix VI and V) indicated that the treatment were significant at P> 0.05 and P> 0.1%. This conformed with the earlier findings of Lysenkov (1989) who is able to induced mutants with a combination of useful traits of breeding value. Similar result was obtained by Zagor-Cheva and Mariya (1987) who proves the presence of genetic control. However, when the means of the treatments are compared and separated using LSD (1.7 in M_1 and 2.3 in M_2), the result indicated a significant increase in the induced ear length in some treatments X400Gy and N12 HRS) in M_1 generation and (X30, N65.) in M_2 generation more than their respective control. This may probably be

interpreted that some doses of both gamma and thermal neutrons are capable of inducing beneficial mutation. But when the induced ear length are compared in both M1 (16.10 cm) and (19.06cm) in M_2 generation, the result shows that the highest ear length mutant, was obtained at the M_2 generation and this might be due to heterosis effect in the M_2 generation (Reddy 1988; Barriga et al 1989).

5.9.1 MUTAGENIC EFFECT ON 100 GRAIN WEIGHT

Though all the treatments responded in increasing the variability of quantitative characters, thereby creating a scope for selection in positive direction, different trend was observed in the case of yield (100 grain seed weight). The increase in yield is not directly proportional to the dose in both gamma rays and thermal neutrons treatments. However, analysis of variance (Anova) of the result obtained in M1 and M2 generation (appendix VII and VIII) indicated that the treatments are highly significant at P>0.05 and 0.1%. But when the means are compared using LSD (1.18) in M_1 and (0.4) in M_2 generation, the result demonstrated an increase in yield in some treatments in both M_1 (12.10) and M_2 (12.42) generation better than their respective controls. The result was similar to the earlier findings of Benamer (1990). Rai et al (1987) Kumar and Yadava (1988) who reported an increase in yield in irradiated population compared to the control. Said et

al (1988) report demostrated a greater reduction in 100 grain weight occuring in the highest doses of gamma-radiation. In this view Gregory (1968) consider that mutation affecting yield in peanuts are predominantly of small effects and therefore not easily detectable in the earlier generation. The increase in grain weight may be due duplication of chromosomes.

5.9.2 CYTOLOGICAL EFFECTS

The frequency of mitotic and meiotic chromosome aberrations induced by gamma rays and thermal neutrons under different doses differ to a considerable extent (plate 1-4, Table.14-17). From comparism it was evident that gamma rays were associated with relatively low frequency of chromosome aberrations followed by a high amount of sterility (plate 3 fig 49-52) and mutation rate; while in thermal neutrons the reverse situation was found. Several workers have reported high mutation rates induced by gamma and thermal neutrons (Lichu and Yachevskaya 1987) with few or no mitotic and meiotic chromosome aberrations. The high incidence of the translocation observed may be attributed to causes other than breakage (Feveret 1960; Konzak et al 1961; Froese-Gertzen et al 1964 Oda and Thomas 1987). In such studies cytologically detectable structural alteration of chromosomes are rare. The occurance of breaks in the centromeric region has been observed (Natarajan and Upadhya, 1964). In the present research appreciable amount of breakeages and exchanges at

centromeric regions, involving both homologous and non-homologous chromosome have been observed. (plate 2). Occurance of such breakages of chromosomes at meiosis obviously leads to duplications and deletion for the whole arm of the chromosome at the time of gamate formation. Thus, it is clear that though the frequency of translocation may include a considerable degree of chromosomal sterility, in the case of gamma rays, a high incidence of mitotic and meiotic aberrations, mostly translocations are recorded. Though the chromosome aberrations set a serious limitation for the recovery of mutations, appreciably high rates were observed in the case of thermal neutrons. The predominant type of meiotic aberrations, were translocation as evidenced by the occurance of multivalents at mataphase I (plate 1 and 2). Moreso, it was noted that majority of induced mutants and most especially those examined cytologically all resulted from chromosomal alteration. This is in agreement with early work of Longley (1950) who reported in maize, by using X-ray and Bikini treated material, the prevalence of breaks in regions adjacent to centromere.

Cytological analysis of both mitotic and meiotic stages (plate 1-4) gives evidence that most of the mutants resulting from gamma-irradiation are the result of chromosomal alterations which results in the loss of the locus concerned (Prokof'eva et al 1989), VazQuez and Sanchez-Monge (1987). In so much as the largest

number of mutants possessed a ring chromosome or lost the entire chromosome (plate 1 and 2) one may predict that mutation produced under similar circumstances which results in the simultaneous loss of several linked genes, may in most cases be due to similar cytological events. The dominant vissible mutations which are often lethal when homozygous, are usually associated with strutural alterations of the chromosomes (plate 1 and 2) They may involve large inversions, small inversion, small duplications or small deficiencies. Most of the phenotypic changes are probably position effects, although this has not been proven in all cases, in the present study, the induced dominant phenotypes appeared to be similar to that previously described by Nataka et al 1989).

It is known that, the process of inducing mutations with radiation, constitute diverse types of genetic phenomenon and phenotypic changes, ranging from mortality to subtle changes in the physiology of the plants. Mutations may not only be due to changes in genetic materials per se but also to changes in timing of DNA regulated processes or in the context of DNA. Deficiencies, duplication, rearrangements, giving position effect, transposition and recombination products of genetic elements, following both normal and unequal crossing over have all been shown to simulate true gene mutation (Proklof'Eva et al 1989) Indeed it may be questioned if true gene mutation has been

established experimentally, although it is believed that such mutation do occur and constitute a basis for Mutant observed. It has not been possible with my present techniques to demonstrate unequivocally that any specific mutational changes does involve gene and not an extra genic alteration. A deficiency or chromosomal rearrangement so minutes as to be Cytologically undetectable may be responsible for the mutant phenotype. Thus, it is not easy to descriminate between point mutation and gene mutations; although it could be demonstrated that the mutant phenotype is inseparably associated with some chromosomal alterations (plate 1 Fig. 5-12).

On the other hand, certain investigations in maize as reported by Lichu and Yachevskaya (1987) indicated that X-ray induced mutation may consist of solely extragenic alterations, while spontaneous mutations include many mutational changes, which have not been associated with chromosomal rearragments. Since there is no way by which mutations induced at miscellenous loci can be classified as to the type of genetic changes; it is only apparent that detailed study mutations at selected loci, enables one to attempt separating `true' gene mutations from extrageneic-alterations simulating gene mutations. In their study of X-rays induced mutation at the A1 locus in maize, stadler and Roman (1948) found that the induced mutations were all associated with deficiencies; some

of these were so minute as to be cytologically undetectable but could be demonstrated by genetic test.

CHAPTER SIX

6.0.0 SUMMARY AND CONCLUSION

Irradiation of popcorn (*Zea mays* var. *praecox* sturt) with gamma rays (^{60}CO) and thermal neutrons drastically decreased percent germination, seedling height, root length and seedling survival in M_1 and M_2 generation, with a slight increase of seed germination in the higher doses (X400Gy and N12HRS) in M2. The decrease was positively associated with the dose. Among the induced dorminant seedling mutants are the occurance of sectors of chlorophyll abormalities and other morphological alteration, which include albina, xantha, virescent, lustescent, chlorina and striata. Most of these mutants were lethal or unfavourable to growth.

The result also demonstrated induced tillering habit; increased number of cobs per plant; increased seed weight; earliness and earlength. The increase is in direct proportionality with the dose and occured more frequently in gamma rays than in thermal neutrons. The class of dominant mutants observed at maturity; included traits such as tassel seed; male sterility and morphological traits not expressed in the seddling stage. In an effort to correlate the group of mutation observed in M_1 and M_2 generation, with male sterility the result indicated that, not only the frequency is independant of the degree of M_1 and M_2

sterility, but also the spectrum of chlorophyll mutation does not indicate any association with this sterility.

The frequency of mitotic and meiotic aberrations induced in different doses differed to a considerable extent. Included among the abnormalities were non pairing, translocations, duplication, fragments, laggards; eliminated; non-oriented chromosomes, single and dicentric bridges, deformed and aborted pollen grains.

It was however, concluded that, the use of gamma rays and thermal neutrons radiations have proved to be capable of inducing beneficial and nonbeneficial mutation; thus could serve as a valuable tool for genetic improvement; thereby widening the variability of agro-economic characteristics, producing factors with a genetical behaviour different from the usual one and provide genes which are not, or only exceptionally found in germplasm collections.

The total results on the capacity of favourable mutant; gamma rays and thermal neutrons strains seems to be of practical values; in particular, the increase in the number of cobs per plant; seed weight earliness; earlength and tillers are beneficial characters, which deserves further attention so as to be exploited or utilized for popcorn breeding.

Moreso genetics and cytogenetics analysis of the induced mutation could contribute much, to a deeper

understanding of the mutation events in higher plants especially our knowledge of the genetic contents of the chromosome of crops. This also offer very valuable material for physiological investigations of a single gene effects and for investigation of the mutation process and thus deserve more attention.

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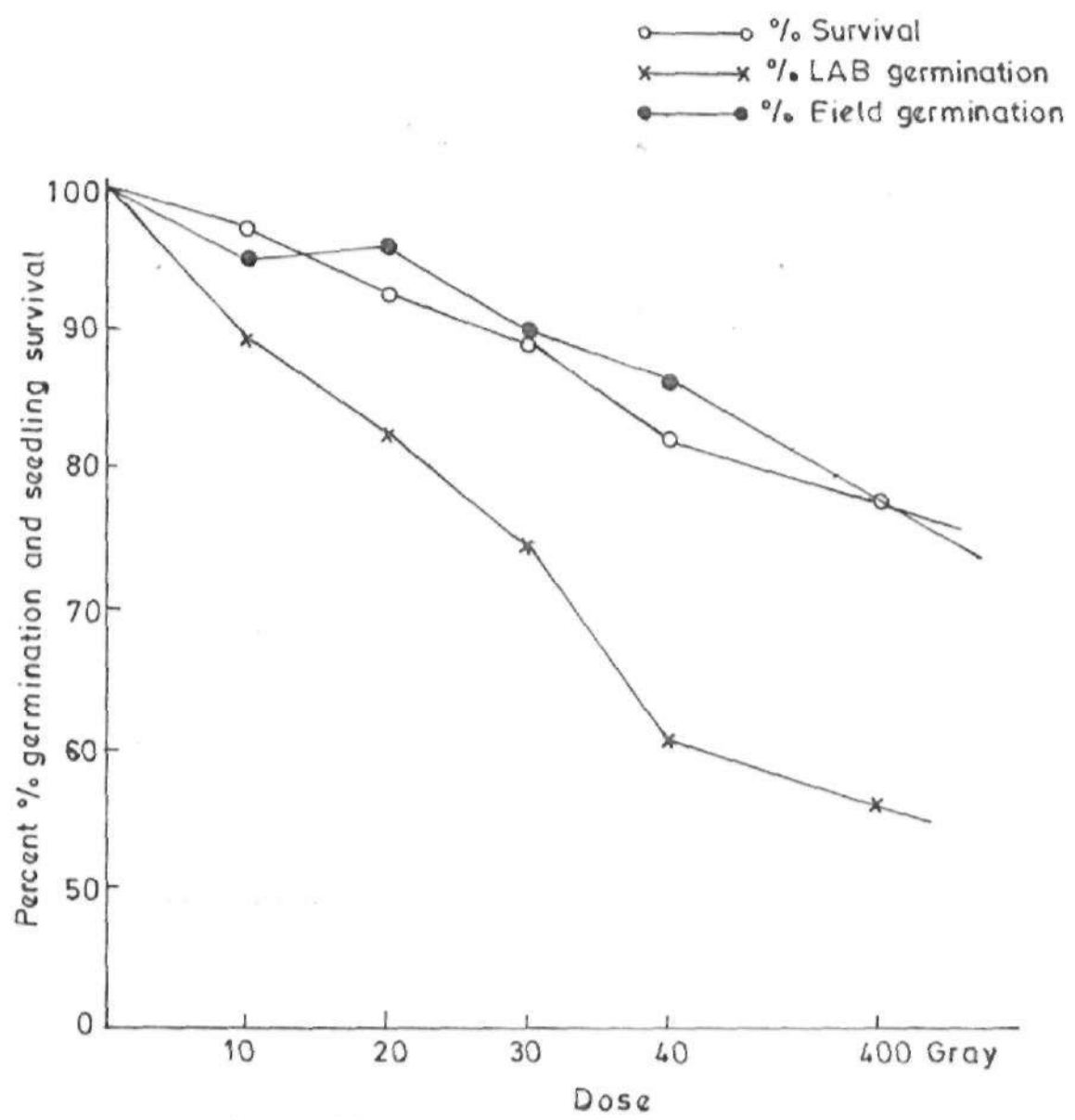


FIGURE 1: THE EFFECT OF GAMMA RAYS (^{60}Co) IRRADIATION ON PERCENT GERMINATION AND SEEDLING SURVIVAL IN POPCORN (*Z. MAYS* VAR. *PREACOX* STURT) IN THE M_1 GENERATION

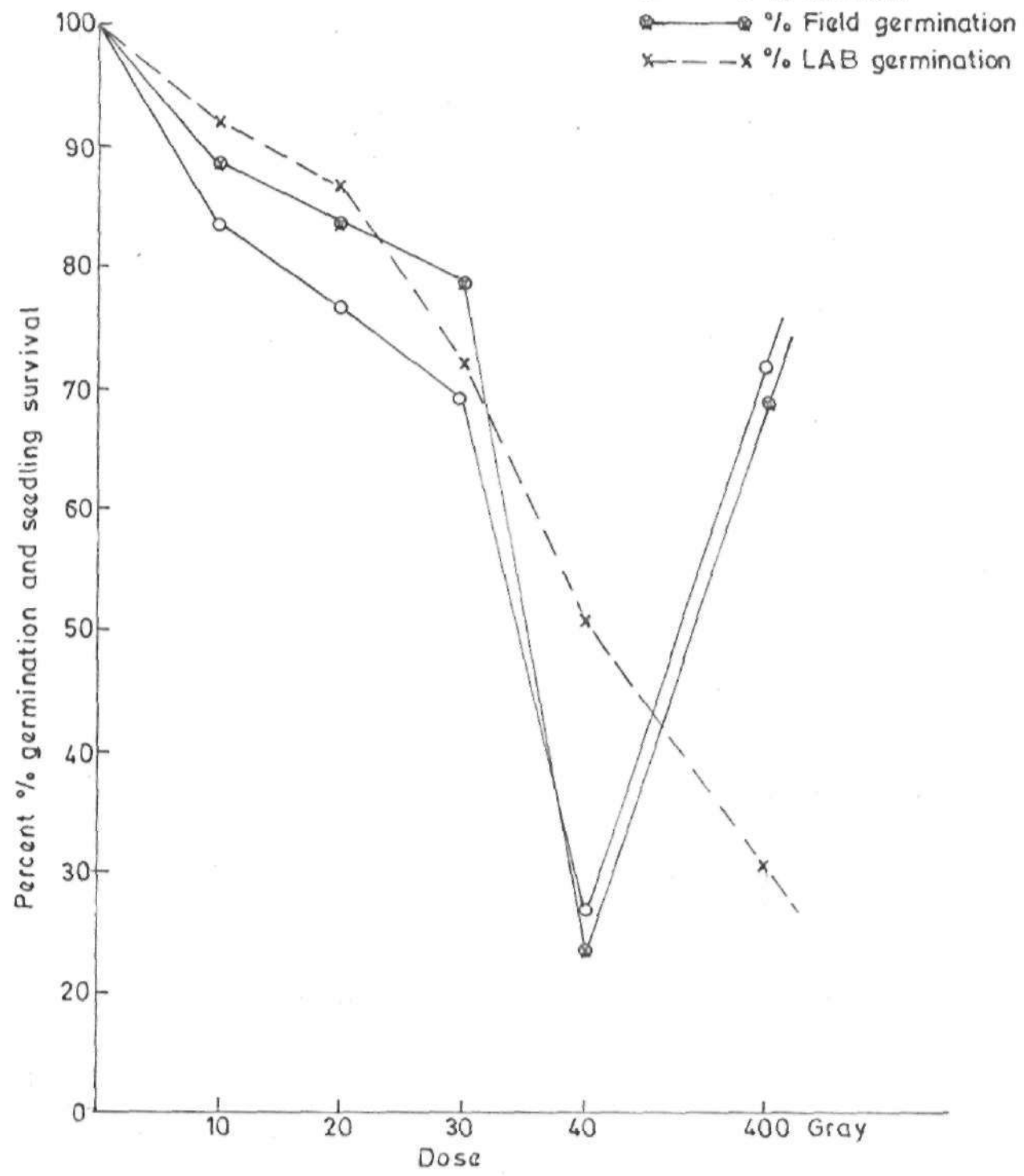


FIGURE 2: THE EFFECT OF GAMMA RAYS (^{60}Co) IRRADIATION ON PERCENT GERMINATION AND SEEDLING SURVIVAL IN POPCORN (*Z. MAYS* VAR PREACOX STURT) IN THE M_2 GENERATION

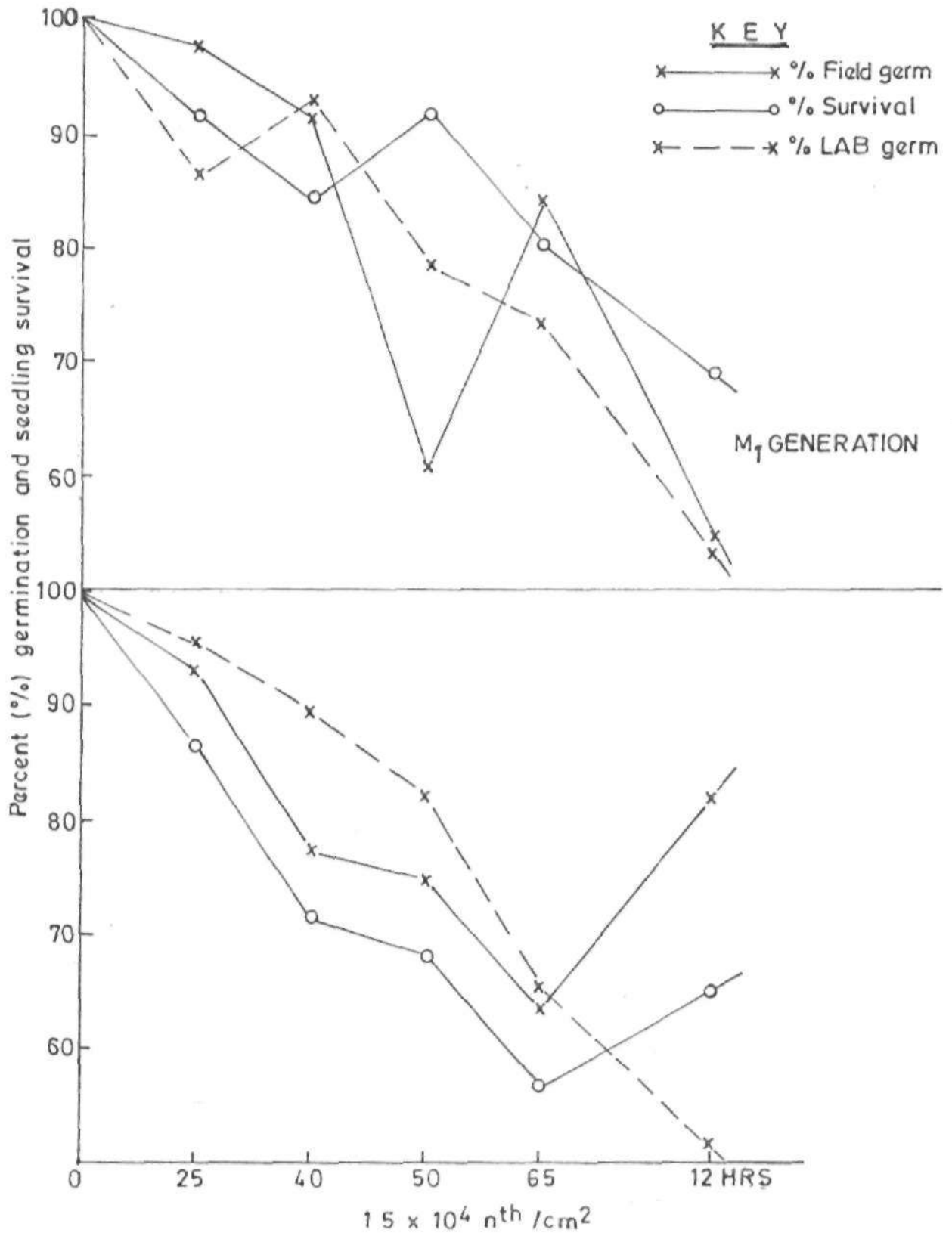


FIGURE 3: THE EFFECT OF THERMAL NEUTRONS IRRADIATION ON PERCENT GERMINATION AND SURVIVAL IN POPCORN (Z. MAYS VAR. PREACOX STURT) IN THE M_1 AND M_2 GENERATION

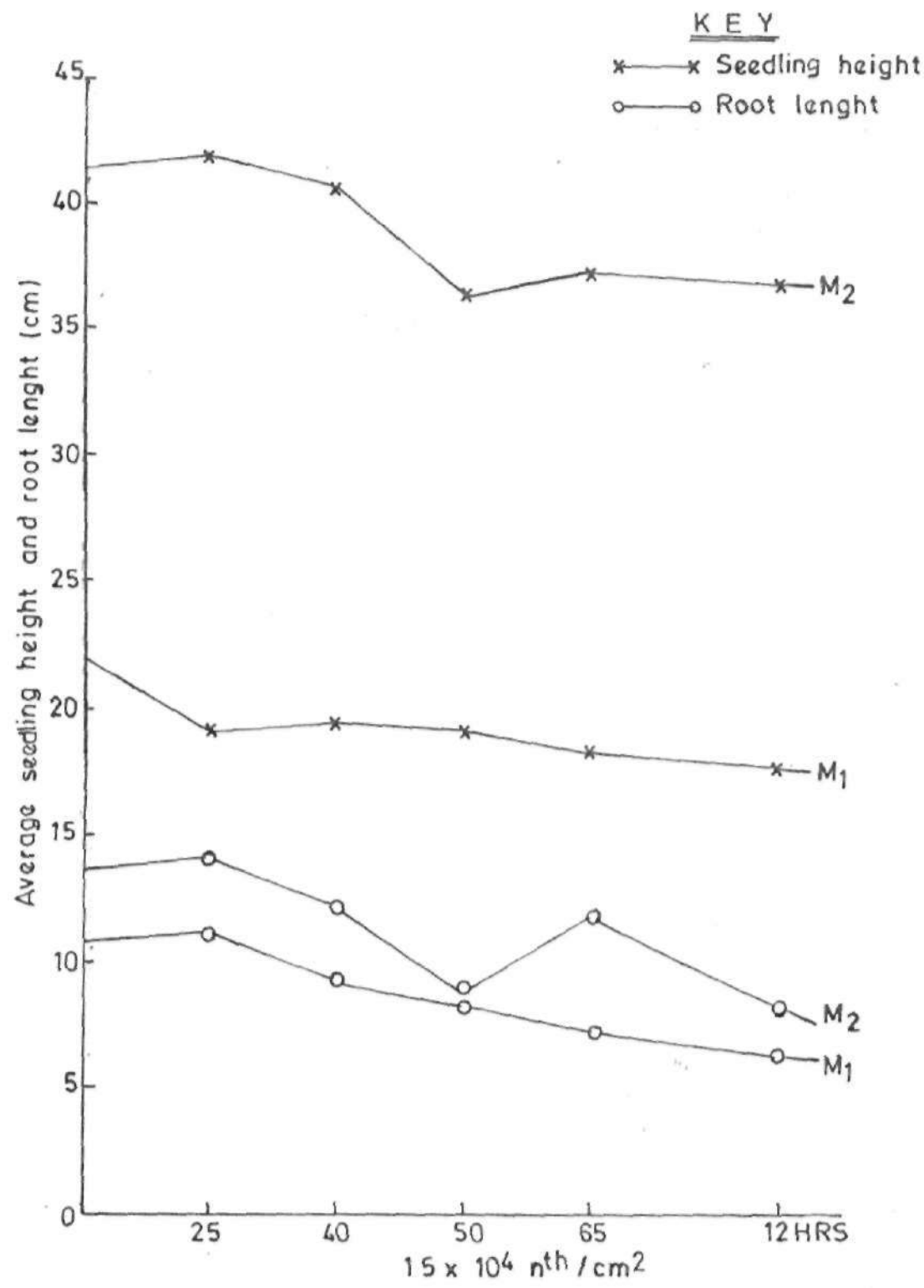


FIGURE 5: THE EFFECT OF THERMAL NEUTRONS IRRADIATION ON SEEDLING HEIGHT AND ROOT LENGHT IN POPCORN (Z. MAYS VAR. PREACOX STURT) IN THE M_1 AND M_2 GENERATION

KEY TO PLATES

PLATE

1 - 2 PROPHASE I (LEPTOTENE) - Chromosomes group in a tight - compact structure to one side of the nucleus.

3. PROPHASE I (ZYGOTENE) - With an interrupted pairing.

4. PROPHASE I (PACHYTENE) - The chromosomes are uniformly condensed and evenly distributed within the nucleus.

5. PROPHASE I (DIPLOTENE) - Chromosomes are separated throughout their length, except at one or points, where they form chiasmata.

6-13 PROPHASE I (DIAKINESIS): - Chromosomes contract and assumed a particular shape - Bivalents, open crosses, a ring and a chain configurations (induced multivalents).

14-22 METAPHASE I: The bivalents are arranged on the spindle equator, with different abnormalities (multivalents) and mal-orientation.

23-28 METAPHASE I - Sticky chromosomes, the chromosomes are clumped together in a set of two to three groups of higher order - multivalents with fragments.

29-39 ANAPHASE I: Bivalents and quadrivalent disjoined and moves towards the poles. The abnormalities induced include, univalent dicentric bridges, laggards, fragments and eliminated chromosomes.

37 TELOPHASE I: The chromosome reaching the poles agregate very closely.

39-42 METAPHASE II: Induced multivalents (translocation) in the form of trivalents and quadrivalents.

43 METAPHASE II: A distinct dyad is formed, representing two daughter cells from meioses I.

44. ANAPHASE II: The daughter chromosomes separate to the opposite ends of the spindle

45. TELOPHASE II: Four groups of daughter cells (tetrad), enclosed by nuclear envelope.

46 TELOPHASE II: Tetrads with two empty daughter cells

47-48 MOCPHOLOGICALLY good pollen grains

49 A deformed pollen grain from 40Gy gamma rays irradiation treatment

50 A deformed pollen grain from N65 thermal neutrons irradiation treatment.

51 An aborted pollen grain resulting from chronic irradiation of thermal neutrons (12HRS $1.5x10^{-10}$ nth. $cm^{2} . S^{-1}$)

52. An aborted pollen grain resulting from chronic irradiation of gamma rays (400 Gray).

KEY TO PLATE 4

1-2 PROPHASE I: Chromosomes appeared thin and small

3-7 METAPHASE: Multivalent and univalent chromosomes are arranged on the spindle equator.

8-12 ANAPHASE: Chromosomes separates towards the poles (9) and the presence of multiple bridges (8) which are interlocked.

13-15 TELOPHASE: Complete separation and aggregation of daughter chromosomes without the formation of cells membrane.

PLATES

PLATE 1: Cytogenetic effect of gamma-rays and thermal neutrons irradiation and meiotic abnormalities in Popcorn (Zea mays Var. Preacox Sturt).

PLATE 1

Cytogenetics effect of gammc-rays and thermal neutrons irradiation and meiotic abnormalities in Popcorn(Z. mays var. Praecox Sturt.).

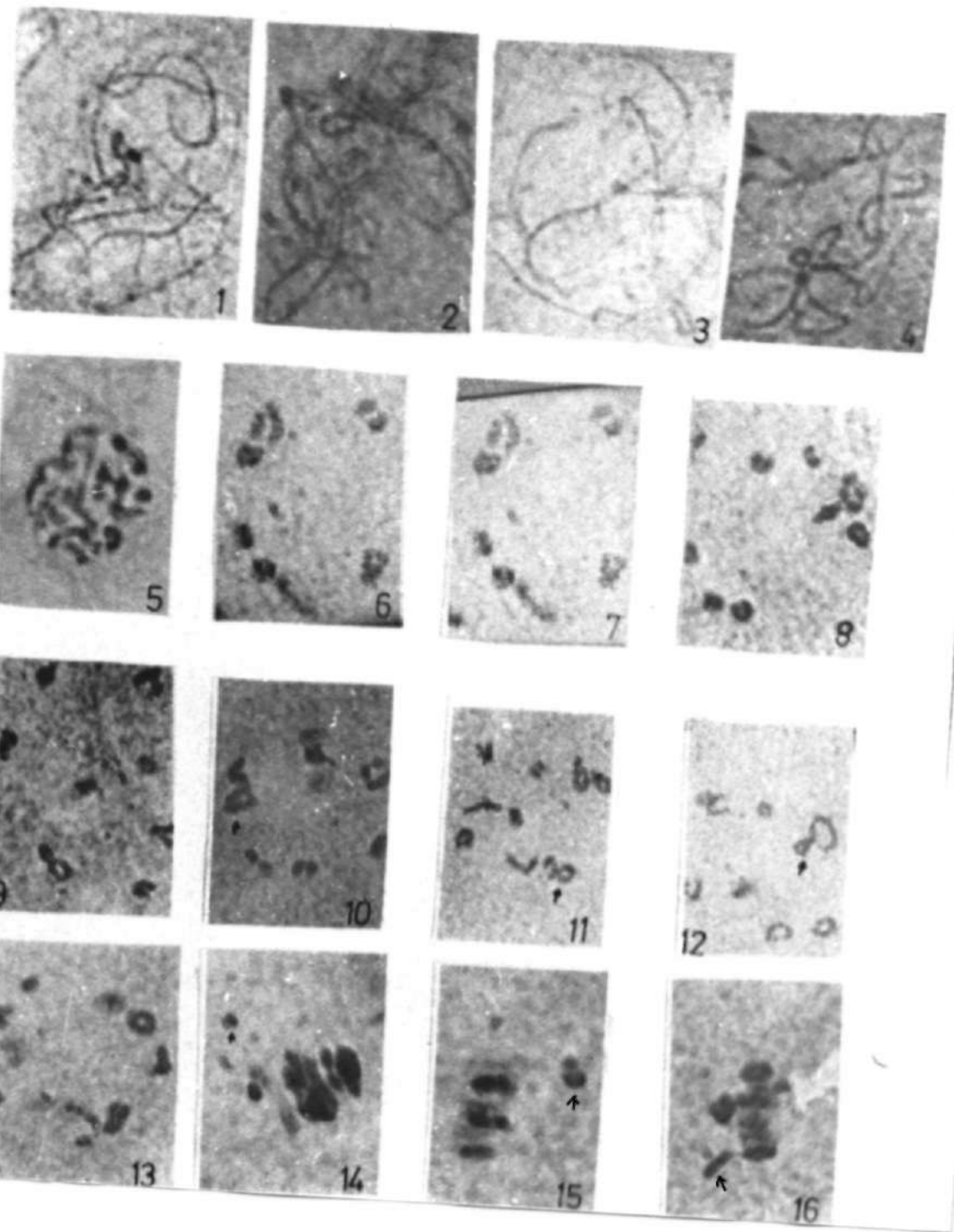


PLATE 2: Cytogenetic effect of gamma-rays and thermal neutrons irradiation and meiotic abnormalities in Popcorn (Zea mays Var. Preacox Sturt).

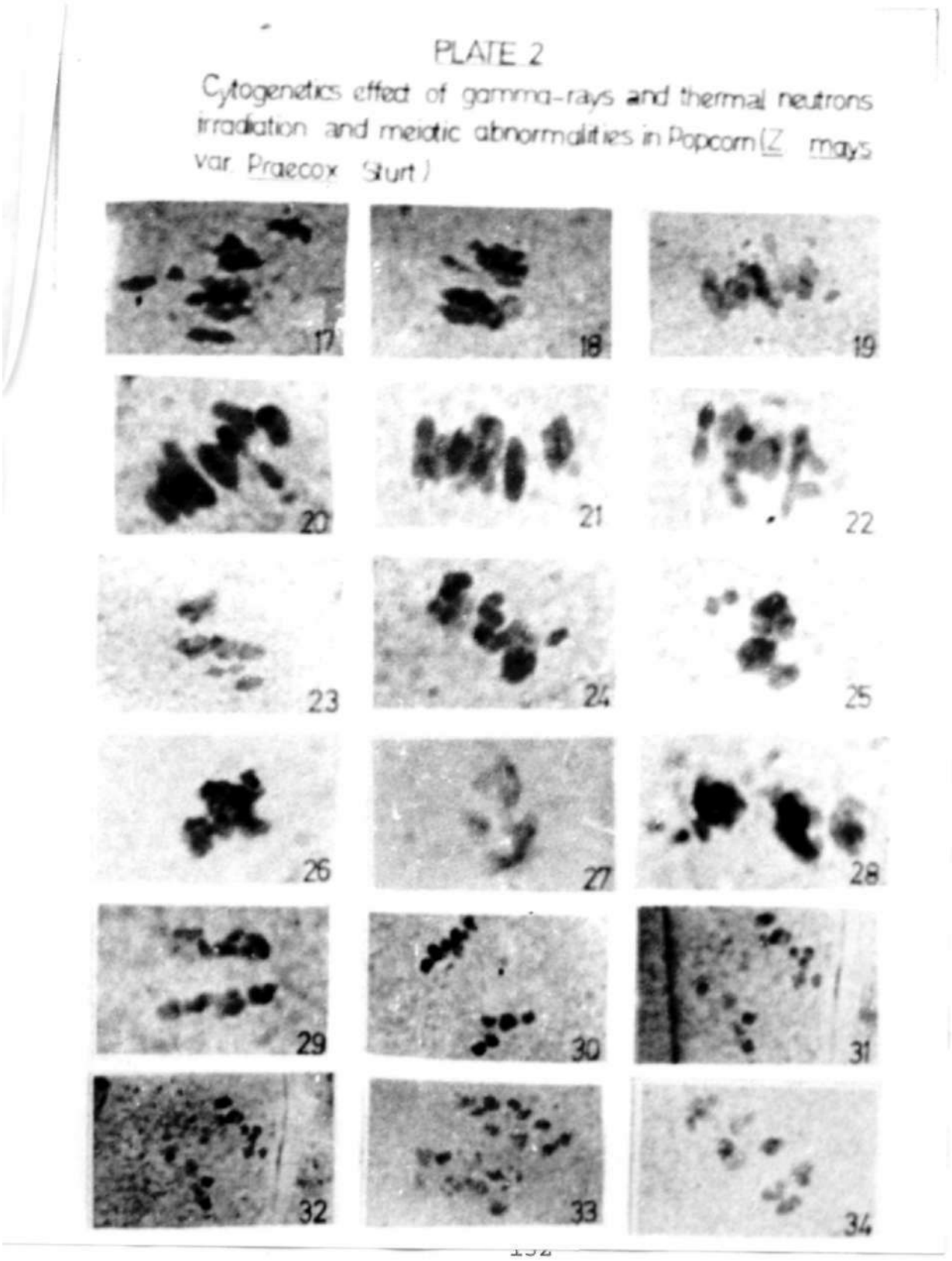


PLATE 3: Cytogenetic effect of gamma-rays and thermal neutrons irradiation and meiotic abnormalities in Popcorn (Zea mays Var. Preacox Sturt).

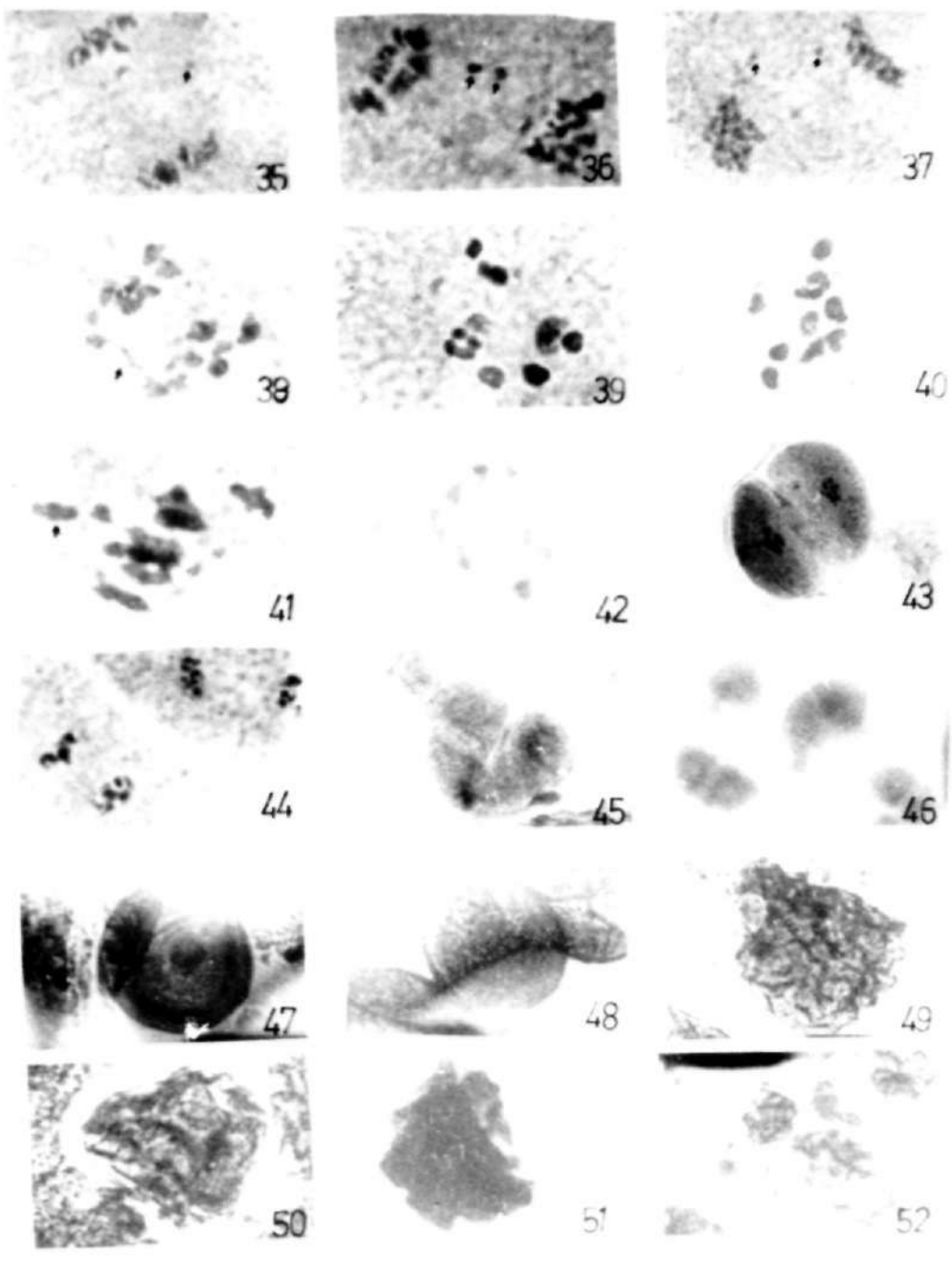


PLATE 4 Cytogenetic effect of gamma-rays and thermal neutrons irradiation and mitotic abnormalities in Popcorn (Zea mays Var. Preacox Sturt).

PLATE 4

Cytogenetics effect of gamma-rays and thermal neutrons irradiation and mitotic abnormalities in Popcorn (Z mays var. Praecox Sturt.).

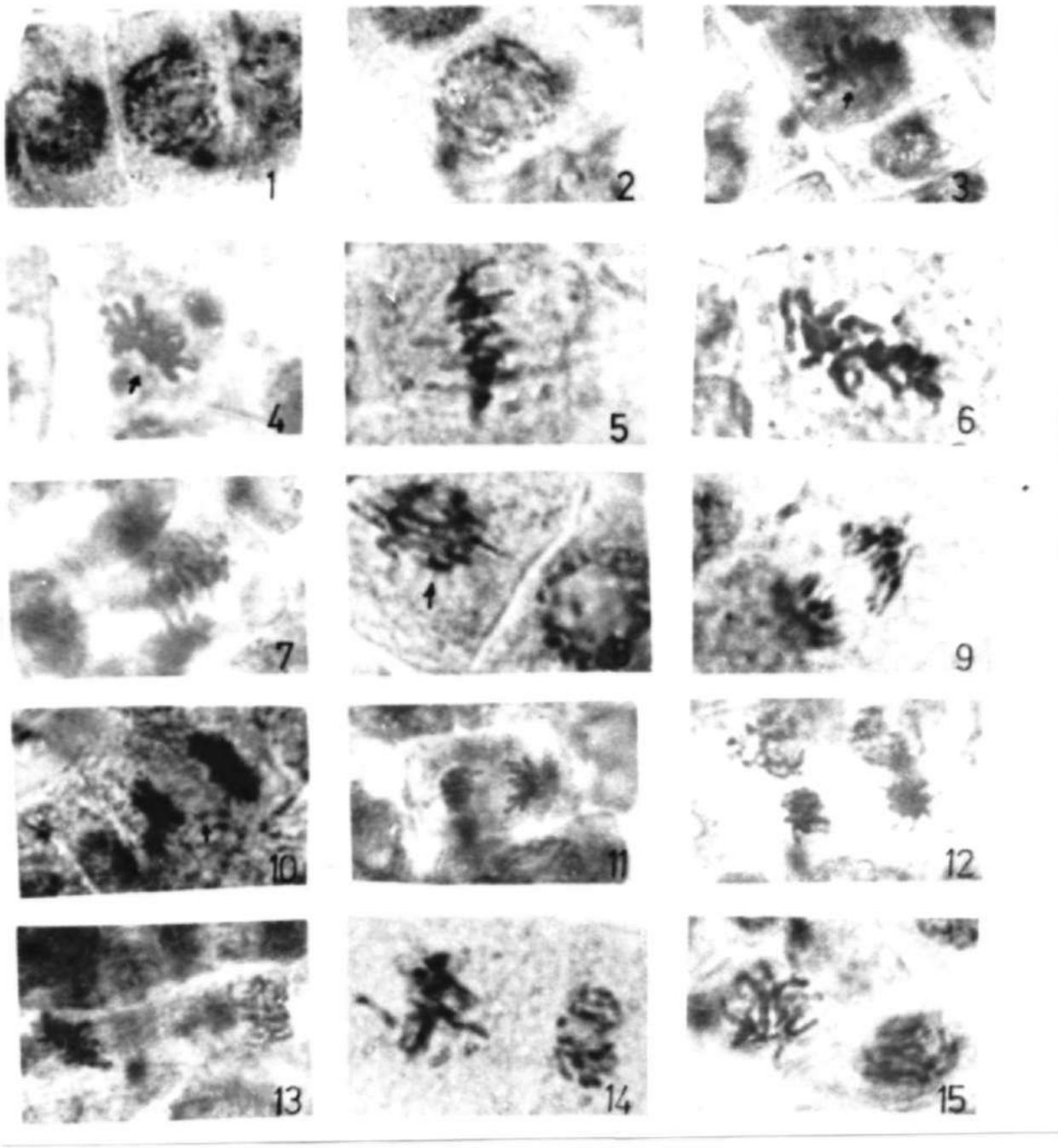




PLATE 5 Xantha - A chlorophyll deficient mutant, leaf colour from orange yellow to faint yellowish white.



PLATE 6 Virescent - A chlorophyll deficient mutant. Yellow-green colour of the leaves, leaves appear first as yellow-green but gradually change to green.



PLATE 7 Lustescent - A chlorophyll deficient mutant, appear as green but the pigments gets destroyed and turn yellow.



PLATE 8 Chlorina - A chlorophyll deficient mutant, with persistant yellow and green colour.



PLATE 9 Striata - A chlorophyll deficient mutant, with longitudinal stripes of yellow on green or white on green.



PLATE 10 A morphological mutant with distorted growth and narrow leaves.



PLATE 11 A morphological mutant with rolled leaves and blades fused in a single leaf 2-3



PLATE 12 A morphological mutant with complete absence of one part or almost both part of the blade.



PLATE 13 A growth mutant (small plant) in the same pot with a vigrous plant



PLATE 14 A tiller mutant with a single tiller.



PLATE 15 A tiller mutant-with two (2) tillers



PLATE 16 A tiller mutant-with three (3) tillers.



PLATE 17 A tiller mutant - with more than three (>3) tillers

APPENDIX

APPENIDX I: The effect of gamma and thermal neutrons irradiation of Popcorn (Zea mays Var. Praecox Sturt) on the rate of germination in the field.

BLOCKS

	I	II	III	IV	V	ETRET(X)	$\bar{X}$
0	30	22	29	29	20	190	38.0
10	31	11	32	21	19	114	22.8
20	20	13	21	18	4	76	15.2
30	28	19	34	16	43	140	28.0
40	16	8	29	13	29	95	19.0
400	30	20	28	6	13	970	19.4
N25	27	26	25	24	10	112	22.4
N40	31	10	27	21	20	109	21.8
N50	18	9	16	17	11	71	14.2
N65	15	8	19	18	7	67	13.4
N12HRS	28	5	20	9	10	72	14.4
EBLOCKS	274	151	28[illegible]	192	186	1083	

ANOVA

Source:	DF	SS	M.S	F
Block	(b-1)=4	1196.00	299.0	5.45 *
Treatment	(E-1)=10	5083.75	508.38	9.27 **
Error	(b-1)(t-1)=40	-2194.30	54.86	
		4085.45		

* Significant at both 1 and 5%

LSD = 9.4804

Separation of Mean ($\bar{X}$)

0	30	10	N25	N40	400	40	20	12	N50	N65
38.0	28.0	22.8	22.4	21.8	19.4	19.0	15.2	14.4	14.2	13.2
<--------->			<----------							
					b					

APPENDIX II: The correlation Coefficient Analysis between Chlorophyll mutation and male sterility in M_1 generation of gamma rays (Cobalt 60) and thermal neutrons irradiated Popcorn (Zea mays Var. Preacox Sturt).

	MUTANTS/MUTATION		STERILITY/PLANTS		
	X	X^2	Y	Y^2	XY
1.	4.04	16.32	0.10	0.0101	0.404
2.	5.62	31.58	0.12	0.0144	0.674
3.	7.58	57.46	0.27	0.0729	2.020
4.	7.48	55.95	0.43	0.1849	3.216
5.	7.28	53.00	0.70	0.4900	5.096
6.	3.65	13.32	0.08	0.0064	0.292
7.	4.24	17.98	0.06	0.0036	0.254
8.	5.51	30.36	0.26	0.0676	1.433
9.	7.07	49.98	0.18	0.0324	1.273
10	13.03	169.78	0.06	0.0036	0.782
	65.50	495.74	2.26	0.8900	15.47

r = +0.133. The calculated r = value (+0.133) is less than the table value (1.860 and 2.306) at 0.1% and 0.05% level of significant and thus the correlation is not significant. Similar result was obtained when `t' test was conducted (t = 0.3796) compared to table value (1.8600 and 2.306) at P = 0.1% and 0.5% level of significant.

APPENDIX III: The correlation coefficient analysis between chlorophyll mutation and male sterility in M_2 generation of gamma rays (^{60}Co) and thermal neutrons irradiated Popcorn (*Zea mays* Var. *Praecox* Sturt).

MUTANTS/MUTATION ($\bar{X}$)		STERILITY/PLANTS (Y)		
X	X^2	Y	Y^2	XY
4.24	17.98	0.0	0.00	0.00
6.75	45.56	0.0	0.00	0.00
7.00	49.00	0.0	0.00	0.00
12.78	163.33	0.03	0.0009	0.383
7.74	59.91	0.06	0.0036	0.464
4.80	23.04	0.0	0.00	0.00
3.80	14.44	0.0	0.00	0.00
5.41	29.27	0.01	0.0001	0.054
5.29	27.98	0.02	0.0044	0.106
8.733	76.21	0.05	0.0025	0.437
66.54	506.72	0.17	0.008	1.444

r = 0.5476. The calculated r value (+0.5476) is less than the table value (0.549 and 0.632 df = 8) at P = 0.1 and 0.5%. Similar result was obtained with t test. (t = 01.8511 less than Table value 1.860 and 2.306 df 8) at P = 0.1 and 0.05%.

APPENDIX IV: The correlation coefficient analysis between chlorophyll mutation in M_2 generation and male sterility in M_1 generation of gamma-rays (^{60}Co) and thermal neutrons irradiated Popcorn (Zea mays Var Praecox Sturt).

	MUTANTS/MUTATION ($\bar{X}$)		STERILITY/PLANTS		
	X	X^2	Y	Y^2	XY
1.	4.24	17.98	0.10	0.0100	0.424
2.	6.75	45.56	0.12	0.0144	0.810
3.	7.00	49.00	0.27	0.0729	1.890
4.	12.78	163.33	0.43	0.1849	3.216
5.	7.24	59.91	0.70	0.4900	5.418
6.	4.80	23.04	0.08	0.0064	0.384
7.	3.80	14.44	0.06	0.0036	0.228
8.	5.41	29.27	0.26	0.0676	1.407
9.	5.29	27.98	0.18	0.0324	0.952
10	8.73	76.21	0.06	0.0036	0.524
	66.54	506.72	2.26	0.8900	17.53

r = +0.5057. The calculated r value (r = +0.5057) is less than the table value 0.549 and 0.632 df = 8) at P = 0.1 and 0.05% and thus the correlation was not significant. Similar result was obtained with a `t' test (t = +1.8511) which is less than the table value (1.860 and 2.306 df=8) at p = 0.1% and 0.05% level.

APPENIDX VIII: The effect of gamma rays (60Co) and thermal neutrons irradiation on 100 grain seed weight of M2 generation of Popcorn Zea mays Var. Praecox Sturt).

BLOCKS

Treatment	I	II	III	IV	V	TOTAL	MEAN
0	11.03	11.00	10.95	11.04	10.85	54.87	10.97
10	10.65	10.40	11.05	10.85	10.70	53.65	10.73
20	11.50	10.65	11.50	11.30	10.88	55.83	11.17
30	11.65	11.50	11.75	11.55	11.45	57.90	11.58
40	11.35	10.75	10.50	11.20	10.75	54.55	10.91
400	12.85	13.15	11.65	12.50	11.95	62.10	12.42
N25	11.60	11.35	11.75	12.25	11.65	57.60	11.52
N40	10.00	10.20	10.45	10.35	10.30	51.30	10.26
N50	10.20	11.00	10.00	10.65	10.20	52.05	10.41
N65	10.40	10.85	10.45	10.20	10.70	52.60	10.52
N12HRS	10.65	10.95	10.75	10.65	10.65	53.65	10.75
EBLOCKS	121.88	121.80	120.80	121.54	120.08	606.10	

ANOVA

Source:	DF	SS	M.S	F
Block	4	0.22	0.05	0.50NS
Treatment	10	19.64	1.96	19.60**
Error	40	4.00	0.10	
Total	= 54	23.86	* P = 1 and 5%	

LSD = 0.405

Separation of Mean (X)

400	30	N25	20	0	40	N12	10	N65	N50	N40
12.42	11.58	11.52	11.17	10.97	10.91	10.75	10.73	10.52	10.41	10.26
<---->		<---------->		<---------->				<---------->		
a		b		c				d		

FACTORIAL CONCEPTS (ANOVA)

Source:	DF	SS	M.S	P
Control Vs Treatement	1	0.014	0.014	0.146NS
Gamma Vs Ther.Neutrons	1	5.660	5.660	58.958**
Within gamma	4	9.05	2.263	23.573**
Within Thermal neutron	4	4.920	1.229	12.802**
Error	44	4.216	0.096	
Total	54	23.860	*P = 1 and 5%	