CYTOLOGICAL STUDIES ON POPCORN
(Zea mays var. praecox Sturt.)

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A THESIS SUBMITTED TO THE POST GRADUATE SCHOOL IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE AWARD OF THE MASTER OF SCIENCE DEGREE IN BIOLOGY OF THE AHMADU BELLO UNIVERSITY, ZARIA.

DIGITIZED

M.Sc. BIOLOGY

OCTOBER, 2000.

DEDICATION

This piece of work is dedicated to my parents, my family and all those that have been showing concern in my progress be it directly or indirectly.

DECLARATION

I hereby declare that this thesis is a record of my research work. It has not been presented by me in any previous application of a higher degree. All the references cited in this thesis are specially acknowledged.

Abdu Muhammad Gali — Date: 30.10.2022

CERTIFICATION

This thesis entitled "Cytological studies on popcorn (*Zea mays var. praecox Sturt.*) by Abdu M. Gali meets the regulations governing the award of the degree of Master in Science, i.e. M.Sc. in Biology of Ahmadu Bello University, and it approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Two strains (A and B) of inbred line of popcorn seeds (Zea mays var. **praecox** Sturt.) were used in the first trial and another strain (C) of popcorn was used in the second trial and raised to maturity, in the departmental garden. Inflorescences were collected from the first two strains for cytological study of meiosis on the 53rd, 55th, 57th and 59th days after sowing and at 6.00am, 9.00am. 12.00noon, 3.00pm and 6.00pm each day. In second trial, anthers from **the** young unopened spikelets were selected and pretrealed with various concentrations of colchicine at 0.05, 0.1 and 0.15 per cent, for time periods of 1, 2 and 4 hours respectively.

Results of the investigation demonstrated that behaviour at meiosis in both the two strain was normal. Meiosis advanced linearly with the plant-ages. There was no correlation between interphase stage and all the other stages of meiotic divisions. Stages of the first division are highly correlated with one another but are less correlated with the stages of the second division while the stages of the second division are also highly correlated with one another. Similarly, the stages of the second division were influenced by the times of fixation and the ages of the plants. The proportions of the number of the pollen mother cells (PMCs) at different times of fixation has shown that the frequency of the interphase cells and the stages of the first division were more in the morning and the stages of the second division were more predominant in the evening which indicated that the interphase cells undergo meiotic division as

the day passes and by the evening time most would have completed the second meiotic division.

The data obtained on the effects of colchicine in arresting cell division at metaphase using another strain of popcorn (C) showed that the frequency of the chromosomal abnormalities was directly proportional to dose and duration of treatment. Treatments led to accumulation of more cells in meiosis I than meiosis II. Arrest of cells at metaphase I and metaphase II was another significant effect observed. Several meiotic irregularities with variable frequencies observed at metaphase include sticky chromosomes, sticky disoriented/non-oriented chromosomes, multivalents, bivalents as well as univalents and laggards. The most frequent aberration observed at anaphase were chromosome bridges. Other meiotic irregularities include unequal separation, laggards and multivalents which were observed at anaphase. In the control plants, meiosis had normal stages with insignificant deviations.

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CHAPTER ONE

INTRODUCTION

Maize – *Zea mays L.*, (also known as corn), common name for a cereal grass widely grown for food and livestock fodder (Parker, 1998). It is cultivated throughout the tropical and sub-tropical regions and a very important food for man and his animals (Howes, 1974).

Maize is the most important cereal in the world after wheat and rice. It is also the most widely distributed (Purseglove, 1976). Maize is used for three main purposes:

1) staple human food, particularly in the tropics
2) feed for livestock, particularly in temperate and advanced countries, providing over two – thirds of the total trade in feed grains;
3) raw material for many industrial products. It also has a number of subsidiary uses.

Worldwide, wheat, maize and rice are produced in greater quantities than any other crop. Of these crops, maize has the highest average yield per hectare (Obi, 1991; Okoruwa and Kling, 1996). This high yield of maize is produced with a relatively small expense on seeds, labour and other inputs and the produce (harvest) is realized within a short period of time 90 to 120 days (Obi, 1991).

Maize is an excellent source of carbohydrates, but because it is low in total protein and the protein is of poor quality (deficient in lysine and tryptophan) maize diet must be supplemented with proteinaceous foods for a satisfactory growth, however, consumption with a legume improves protein quality in the diet (Okoruwa and Kling, 1996; Parker, 1998). Varieties differ in physical and chemical properties of the grain, which affects their suitability for various end uses (Okoruwa and Kling, 1996). The grain is rich in thiamine but low in niacin. The low content of niacin and tryptophan can lead to pellagra, a deficiency disease, common in South Africa. Maize is unsuitable for bread making as it is deficient in gluten (Purseglove, 1976).

The composition of the grain on a dry weight basis is about: starch 77 per cent; sugar 2 per cent; protein 9 per cent; fat 5 per cent; pentosan 5 per cent; ash 2 per cent. The protein content varies from 6 – 15 per cent of which zein predominates; and is deficient in tryptophan and lysine. More than 80 per cent of the fat is in the embryo or germ. Yellow maize is fairly rich in vitamin A due to cryptoxanthin (Purseglove, 1976). The maize kernel is made up of endosperm 83 per cent of total dry weight; germ 11 per cent; pericarp 5 per cent and tip cap (pedicel) 1 per cent (Okoruwa and Kling, 1996).

In Africa, maize consumption accounts for about 64 per cent of the total daily calorie intake of the rural dwellers especially during the hunger period (Obi, 1991). Maize is a crop of increasing importance in West and Central Africa. It is the most widely cultivated crop in the region (Fajemisin, 1992). Maize is the most commonly grown cereal in Nigeria with annual production of around 6.2 millions

tonnes for use as a major food item, as livestock feed and other industrial uses (Jose *et al*., 1999).

Maize is native to the Americas and was the staple grain of the region for many centuries before Europeans reached the New World. The origin of maize remains a mystery. Conclusive evidence exists, from archaeological and palaeobotanical discoveries, that cultivated maize has existed in south-western North America for atleast 3,000 years (Parker, 1998). Wild maize was once thought to have grown in the Tehuacan Valley of southern Mexico 7,000 years ago. More recent evidence puts the appearance of maize in that region at a much later date, about 4,600 years ago. Early wild maize was not much different in fundamental botanical characteristics from the modern maize plant (Parker, 1998).

The name maize is derived from an Arawak-Carib word, 'mahiz'. It is also known as 'Indian corn' and in America simply as 'corn' (Purseglove, 1976). According to Schrimpf, 1965; in 1737, Linnaeus (Linn.) gave maize the name *Zea mays*. The generic name, of Greek origin was used buy the Greeks to describe other kinds of cereals; which was derived from the word 'life', while the species name orginated from 'mahiz'.

Indian corn or maize (*Zea mays L.)* is a member of the tribe *Tripasaceae* or *Maydeae* of the grass family *Poaceae* (Leonard and Martin, 1963). The *Zea*-genus has only one single species, which is however so abundant in forms (Schrimpf, 1965).

Zea mays is a highly variable naturally cross pollinated, markedly heterogeneous, complex species, which hybridize freely (Purseglove, 1976). Cultivars are sub-divided into groups according to the structure of the grain which is dependent upon one or a few genetical characters. This classification is useful agriculturally, but has little meaning botanically, although the groups are given the status of botanical varieties.

Popcorn is a variant of flint corn with small kernels of great hardness (Parker, 1998). The grains have a high proportion of very hard corneous endosperm with a little soft starch in the centre. When heated, the moisture in the kernels expands, causing the kernels to pop open (explode) the endosperm becoming everted about the embryo and hull to produce a palatable white fluffy mass (Parker, 1998; Purseglove, 1976). Two types are recognized:

(a) rice popcorn in which the grains are pointed and tend to be imbricated.

(b) Pearl popcorn in which the grains are rounded and very compact. It is unimporant outside America, but it is grown in small quantities in the United States and Mexico and is the basis of popcorn confections (Purseglove, 1976).

A cytological study of meiosis in hybrids is a useful corollary to a genetical study, since it may give some indication of the overall genetic divergence between the parents (Callan and Spurway, 1951). It is also of value in the analysis of chromosome problems since exceptional behaviour of chromosomes sheds light on the sequence of events leading to the normal behaviour.

Expression of agronomic characters in crop plants are the results of environmental interactions with genetically controlled developmental patterns (Siemer, *et. al.*, 1969). Knowledge of the mode of inheritance of agronomic characters is one of the important objective of research in plant breeding (Leng, 1951).

The comparatively small number of relatively large chromosomes in maize permits easy cytological study (Jugenhiemer, 1958); and simple means of obtaining sequential stages in microspore development (Skvarla and Larson, 1966). In 1950, Rhoades described some important meiotic stages in maize.

Flowering is usually prolific in nature; however, in some localities and in some years flowering may be sperse (Youngner *et. al.*, 1968).:

The objectives of this study has been:

(a) To determine the age at which meiotic activity could be observed.

(b) To determine the right time of fixation to obtain the different stages of meiosis; and

(c) To determine the efficacy of a chemical (colchicine) at different concentrations and periods of treatment in the arrest of meiotic metaphase.

CHAPTER TWO

LITERATURE REVIEW

2.1 Meiosis

Meiosis is the nuclear process, typical of sexually reproducing eukaryotes, which precedes the formation of gametes or spores (D'Amato, 1977). It consists o of two successive nuclear divisions. The first division is a reduction division while the second division is an equational division (Lam, 1972). The events of meiosis in terms of morphologicaly distinguishable stages are quite similar in a wide variety of eukaryotic organisms, both plants and animals (Stubble-field, 1973).

The development of the microsporocyte and the anther containing it are central in the life cycle of all higher plants, including maize. The events occuring in the micropsorocyte itself, from meiosis through differentiation of mature pollen grains are crucial to the development of male fertility (Bouchard and Walden, 1990).

The switch from the vegetative to the reproductive pathway of development in flowering plants requires the commitment of the subepidermal cells of the ovules and the anthers to enter the meiotic pathway. The hypodermal cells and the archesporal cells differentiate into a megasporocytes and microsporocytes (Sheridan *et. al.,* 1996). The initiation of flowering, the conversion of shoot apical meristems from vegetative to reproductive development, is a critical event in the life cycle of higher plants. Factors

controlling the onset of flowering vary among plant species. In some species, photoperiodism has a major influence on flowering time, allowing the plant to co-ordinate flowering with specific seasons (Lee *et. al.*, 1994).

2.1.1 Duration of Meiosis

Knowledge of the time taken by meiosis is rather fragmentary and is sometimes given without specifying the temperature. The following published results on the duration of meiosis demonstrated that, : *Lilium longiflorum* and *Gasteria spp.* – 4 – days, *Vicia faba*, 3 to 4 days; *Antirrhinum majus*, from the last premeiotic mitosis to tetrad 30 to 34 hours (Wilson, 1959); *Tradescantia reflexa*, at room temperature, 18 to 23°C, 2 days *T. Paludosa*, at unspecified room temperature, 52 hours, *Oenothera spp.* at 10°C, 12 days but from leptotene to metaphase 1, only 6 days as reported by Wilson (1959).

The rate of meiotic development in higher plants is profoundly affected by temperature. It has been noted that meiosis takes longer time at low temperature than at high temperatures. For instance, Sax (1938) stated that the duration of meiosis in *Tradescantia* may be twice as long in winter as during the summer. It was only comparatively recently, however, that experiments to measure the absolute effects of temperature on the duration of meiosis in plants grown under carefully controlled conditions have been made. Wilson (1959); estimated the duration of meiosis in *Endymion nonscriptus* at each successive 5°C temperature interval over the range 0-30°C. He found that over this temperature range the duration of meiosis decreased with increasing temperature. A comparison of the estimates for meiotic duration in *Trillium erectum* pollen mother cells at various

temperatures (Hotta and Stern, 1963; Kemp, 1964; Bennett *et. al.*, 1972) reveals a similar relationship between meiotic duration and temperature demonstrated in *Endymion.*

The duration of pollen maturation is also temperature dependent; thus, Beatty and Beatty (1953) noted that development from tetrad stage to pollen grain mitosis in *Tradescantia* lasted about 6-days in summer, but about 14-days in winter. Some species normally complete pollen development at low mean temperature, for instance, *Trillium eractum* and *Larix deciduas*. (Ekberg and Eriksson, 1967). Other species complete pollen development within a relatively short period even at 5°C, for instance, *Stellaria media.*

Temperature effects on meiotic behaviour have also been recognized. These include variation in the coilings of chromosomes, the amount of chromosome pairing, and the number and position of chiasmata (Barber, 1941 and 1942; Elliot, 1955). Thus, a number of authors have indicated that temperature variations applied before the initiation or at early stages of meiosis may affect chromosome synapsis in higher plants (Bennett *et. al.*, 1972). Changes in temperature can affect the general pattern of meiosis as well as the form and behaviour of the chromosomes. Chromosomes may exhibit three major effects from changes in temperature. These are alterations in the frequency and changes in the position of chiasmata and variations in internal coiling (Dowrick, 1957). All these effects may be caused by changes in the duration of meiosis or its stages.

The duration of meiosis and its stages has been intensively studied in several important cereal species including wheat, rye and barley (Bennett and

Kaltsikes, 1973). Comparison of data for unrelated diploid plant species, including those obtained using cereal species, has shown that the duration of meiosis is positively correlated with nuclear DNA content. Similarly, there is a positive correlation between nuclear DNA content and meiotic duration for tetraploid species, but the slope of the regression for tetraploids is about twice that of the slope for diploids. In addition, comparison of closely related diploid and polyploid cereal species showed that the duration of meiosis decreased as ploidy level increased. This phenomenon has been recorded for autopolyploids and polyhaploids (Bennett and Kaltsikes, 1973).

In diploids the time spent in meiotic and pollen development is a function of the nuclear DNA content of the species (Bennett *et. al,* 1972). Consequently, plants with high take nuclear DNA content, such as *Trillium* and *Larix species* take much longer time to complete anther development at low temperatures than do species with low nuclear DNA content, such as *Stellaria media.*

The duration of meiosis and pollen development in several cereal genotypes all grown at 20°C has been investigated (Bennett *et. al.,* 1972). The possible development of hybrid cereals using natural and induced male sterile lines pollinated with intersown male-fertile lines has led to an increased interest in the duration of stages of anther development in different environments.

2.2 Duration of the Mitotic Cycle

A considerable amount of work has been reported on the duration of the cell cycles in cereal root-tip meristem cells (Ayonoadu and Rees, 1968; Avenzi and Deri, 1969; Yang and Dodson, 1970). Bennett and Finch, 1972 studied a

second barley variety, *Moris otter* and compared the rates of development in winter and spring variety. They observed that these estimates are in good agreement with previous measurements of cell cycle time made for other cereal species. As in other cereal species, the duration of the division stages during mitosis in barley are much shorter than the durations of the corresponding stages of meiosis.

The duration of the mitotic cycle varies considerably even within one species. Bennett *et. al.,* 1972, reported variation in mitotic cycle duration between cells in one plant tissue. Clowes, 1965, showed that the duration of the mitotic cycle in *Zea mays* root tip meristems varies from 14-hours in cap initial cells to about 174 hours in the quiescent center. In addition to naturally occuring variation in mitotic cycle times, variation may be induced in response to various factors including temperature (Wimber, 1966; Sisken *et. al.,* 1965); ionising radiation as reported by Bennett *et. al.,* (1972) and supply of mineral nutrients (Morimota and James, 1969).

The rate of chromosome movement at anaphase in staminal hairs of *Tradescantia* at various temperatures was studied and data on plant and animal materials have been provided (Wilson, 1959). In addition, the duration of various stages of cell division in root-tips of *Pisum* at temperatures of 15, 20, 25 and 30°C were determined and found that , the mean duration of mitosis in *Pisum* at various temperatures were as follows: 15°C, 2.95 hours; 20°C, 1.83 hours; 25°C, 1.38 hours and 30°C, 1.09 hours. Thus meiosis in *Endymion* anthers takes about 20 times as long as mitosis in *Pisum* at the same temperature.

Mitotic cycle duration is also correlated with various nuclear characters. Van't Hof and Sparrow, 1963 demonstrated a positive relationship between DNA content per cell and minimum mitotic cycle time in several diploid plant species and Ayonoadu and Rees, 1968 reported a similar correlation in *Secale cereal* with different numbers of supernumerary chromososmes (B. chromosomes). A positive linear correlation between interphase nuclear volume and DNA count per cell has also been demonstrated (Van't Hof and Sparrow, 1963).

Bennett and Rees, (1969) and Bennett (1970) found a large natural and induced variations in chromosome volume, nuclear volume and nuclear dry mass in meristems of *Secale cereale* and *Viciafaba*. While these characters showed variation up to four hundred per cent, the 4C DNA amount remained constant. The total volume of C metaphase chromosomes in the main root-tip of four day old *Vicia faba* seedlings grown on sand was about 30 per cent greater than in internal root-tip meristems of 14 day seedlings and the mean minimum and maximum volumes of nuclear was about 15 per cent grater in the former than in the latter meristem. Bennett *et. al.*, 1972 worked on the mitotic cycle duration in *Vicia faba* root-tip meristems, with the same 4C DNA content but a different nuclear volume and dry mass. They found that the results are consistent with the hypothesis that the metabolic activity and mitotic rate of *Vicia faba* meristematic cells are positively correlated with their mean nuclear volume, mean C – metaphase, chromosome volume and nuclear protein content. Possible mechanisms are suggested whereby cell time could be directly determined by variation in total nuclear RNA and protein content.

2.3 Major Developmental Events In Maize *(Zea mays L.)*

The timing and correlation of major developmental events in maize was reported by Siemer *et. al.*, (1969). Number of days from planting to a given event was greatly influenced by genotype. Hybrids developed faster than corresponding inbred lines in all respects except the full development of kernels.

The period between planting and sexual maturity was shown to actually consists of two distinct phases in the developmental cycle of the maize plant (Leng, 1951). The first phase is differentiation of vegetative parts which terminated by elongation of the growing point and marks the beginning of tassel differentiation. The second phase commenced with tassel initiation and the development of reproductive structures that is completed when sexual maturity is reached.

Developmental differences attributable to planting date or growing season were affected by temperature, soil moisture or both. Low temperature and moisture deficiency retard development (Jugenheimer, 1976). Internode elongation, axillary bud development and cessasion of the ear elongation proceeded acropetally, while ear initiation was basipetal.

Four pairs of events were closely related in time:

1. Tassel initiation and the beginning of stalk elongation.
2. Top ear initiation and the beginning of rapid stalk elongation.
3. Accelerated tassel peduncle elongation and tassel emergence and
4. Accelerated top ear elongation and attainment of maximum top ear kernels per raw potential.

Tassel initiation is the first visible sign that a plant has shifted from the vegetative to the reproductive stage of development (Leng, 1951; Siemer et. al., 1969). Thus determination of tassel initiation would be important in the modelling of maize growth and in the studies of the inheritance of maturity.

The time interval separating anthesis of the upper and lower florets of the same spikelets as well as the progression of shedding in tassel of maize was determined (Hsu and Peterson, 1992). Spikelets were sampled in 4 – sectors of the tassel (1 top to 4 bottom of tassel) in 25 genotypes (15 inbred lines, 3 hybrids, 3 tetraploids and 4-Mexican varieties). The period of anthesis within a tassel was statistically similar among 3 of the types (inbred, 6.68 days; tetraploids, 6.89 days and hybrids, 7.00 days), but was significantly longer in Mexican varieties 10.17 days.

Photoperiod and temperature are known to affect the timing of tassel initiation in maize (*Zea mays L.*). Furthermore, differences among cultivars in sensitivity to photoperiod have been documented (Russell and Stuber, 1985). At one extreme the race Tehua (collection chs 234) averaged 228.0 more leaves under a long day than a short day photoperiod; in contrast, the existence of photoperiod insensitive cultivars have also been reported (Russell and Stuber, 1985).

2.4 Meiotic Indices in Maize

An index to allow identification of meiotic stages in maize without excision of a portion of the tassel was sought by Mock and Frey (1970). They measured association of meiotic stage with several morphological measurements

including expanded leaf number, days after emergence, plant height and stem diameter. They found that expanded leaf number proved to be a better index of meiosis than days after emergence, plant height or stem diameter.

Sarvella and Grogan (1965) used number of nodes and internode elongation as indices of meiotic activity in maize. It was suggested that leaf number was a satisfactory criterion for defining growth phases of maize before silking as reported by Mock and Frey (1970). Chase and Nanda (1967) showed that leaf number was a reliable measure of maturity in maize hybrids. Internode elongation began when approximately the eighth leaf was expanded, an event that signaled the initiation of tassel differentiation and microsporogenesis (Mock and Frey 1970). Leng (1951) found that visible leaf number ranged from seven to nine at tassel initiation and he concluded that within maize genotypes, leaf number was a suitable index of internal development.

2.5 Induced Mutations

In broad sense, the term mutation according to Spencer, 1947 may include any change in the genetic constitution of an individual or part of an individual, not due simply to recombinations of pre-existing genotypes. Mutations in plants have been induced by treatment of pollen, young embyos or seeds. Treatments include the use of X-rays, ultraviolet ray, radium, temperature, electricity, mustard gas, chemicals, gamma radition and aging of seeds (Jugenheimer, 1976). 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).

Exposure of cells to a mutagen results in the formation of different types of chromosomal aberrations which is in itself a major heritable genetic damage besides some of them being cell lethal as a consequence either of a mechanical inhibition (bridges) or the loss of acentric fragments (Prasad, 1983) An understanding, therefore, of what can actually happened to cells carrying chromosome aberration (bridges, fragments and micronucle) becomes important from the fact that it provides an insight into the organization of tissues involved in the formation of seeds for future generation. Chromosomal aberrations may be used elegantly to assess qualitatively and quantitatively the efficacy of a mutagen (Anantha-Reddy, 1983). Chromosomal aberrations have also been considered as reliable indicators of mutagenic activity (Badr, 1983). Different types of mutagens are useful in inducing variability and became potential tools in plant breeding programmes (Madhusudana-Rao and Manmada-Rao, 1983). Mutagens have remarkable possibilities of improving plants with regard to their quantitative as well as qualitative characters (Laxmi *et. al.*, 1983; Adamu, 1997). As a result of progress in understanding the roll of induced mutations, a number of economically useful mutant varieties have been commercially released.

2.6 Chemical Mutagenesis

2.6.1 History of Induced Chemical Agents

Mutations can be induced by chemical agents both in plants and in animals (D'Amato and Hoffman-Ostenhof, 1956).

The substances whose mutagenic action is to be tested are usually administered in aqueous solutions. On the influence of chemicals on plant

chromosomes at meiosis, the solutions were introduced through the vessel system of excised inflorescences. In order to obtain mutated pollen grains for genetic experiments, this method has been modified. An injection method for the study of mutagenic influences on meiotic chromosomes has also been devised. The use of chemical mutagenesis either synthetic or of biological origin and tested for mutagenic activity have also been reported (D'Amato and Hoffman-Ostenhof, 1956). Various alkaloids (morphine, narceine, atropine, scopolamine and colchicine) exert a remarkable chromosome breakage action at meiosis in the hybrid *Oenothera (sauveolens X hookeri) X flavens*.

The majority of workers, however, prefers the use of root meristems for cytological studies of this kind (D'Amato and Hoffman – Ostenhof, 1956).

Several chemicals have the ability to inhibit cell division and induce chromosomal abnormalities (Badr, 1983). Chemicals are well known source of damage to hereditary material (Walker, 1973). Their effects on chromosomes are primarily to cause breakage with subsequent reunion and/or loss of fragments. Such damage may bring about aberration leading to invariability of the cell, or the outcome may be one of the chromosome mutation (inversion, translocation etc.). With the identification of different potential chemical mutagens and with better understanding of numerous parameters which influence the sensitivity to mutagens, the scope for increasing the frequency and spectrum has broadened. The relative ease of application and the comparative low cost of chemical mutagens has made an unprecedented surge of interest in the artificial induction of mutations with these agents (Madhusudana-Rao and Manmada-Rao, 1983).

Practical methods of chemical mutagenesis are examined. Examples are given of the successful use of induced mutations in maize to analyse the genetic control of electron transport in photosynthesis, of embryo development, of the appearance of disease symptoms and of the steps in the tryptophan pathway (Neuffer and Chang, 1990). The use of chemical mutagens to eliminate undersirable genes from the elite lines, to produce commercially useful types and to produce breeding material is also mentioned. Studies on the action of chemicals on germ cells are of paramount importance since anomalies produced in them may be transmitted to the next generation (Anantha-Reddy and Subramaniyan, 1983).

2.6.2 Morphology and Metaphase Arrest

A series of chemicals are used for metaphase arrest, by inhibition of spindle formation which bring about chromosome contraction and separation of the chromatid arms (Walker, 1973). The inhibition of spindle formation and the chromosome contraction facilitate chromosome spreading. These chemicals are therefore most useful for general morphology study of the chromosomes and for detailed study of the karyotype.

2.6.3 Induction of Polyploidy

A number of chemical agents induce polyploidy. Among them are chloral hydrate, Acenaphthene, Sulfanil-amide, Ethyl-mercury-chloride, Hexachlorocyclohexane and Colchicine (SRB *et. al.*, 1965). Of these colchicine has been most widely and effectively used. The most frequently employed technique for inducing a doubling of the number of chromosomes involves the use

of specific chemicals which either suppress the formation of the spindle or stimulate an extrareplication of the chromosomes (Dawson, 1962).

The properties of arresting metaphase and the induction of polyploidy are interrelated. Polyploidising chemicals like colchicine inhibit the formation of the spindle within the two poles and confine the chromosomes within one nucleus. *colchicine action has been classified under narcosis, as the narcotic action allows* the tissue to recover as soon as the influence of the chemical is removed (Sharma and Sharma 1980).. Evidently polyploid cells, which are formed by colchicine action, divide normally and give rise to polyploid shoots.

Polyploidy plays an important role in the evolution and differentiation of plant and animal tissues (Ilgren *et. al.*, 1983). Polyploidy has long been *recognized as very important factor in plant evolution.*

Induction of polyploidy, though associated with reduced fertility, has assumed great significance as chromosome doubling is usually associated with the changes in the morphological and physiological behaviour apart from chromosomal instability owing to cytological irregularities (Mercykutty and Kumar, 1983).

The importance of polyploidy in agricultural and horticultural practices is well known. The increase in gene dosage resulting from multiplication of chromosome sets brings about gigantism in all characters in general. Enhancement of tolerance and adaptability are also added characteristics of polyploids (Sharma and Sharma, 1980). Moreover, several interspecific and intergeneric crosses have been made fertile through polyploidy. All these facts

taken together have made the induction of polyloidy an effective tool in the hands of agriculturists and horticulturists.

2.7 Colchicine (An Alkaloid Chemical)

Colchicine, an alkaloid extracted from the autumn crocus (*Colchicum autumnale*) – (Paarker, 1998). It was first isolated in 1883, the alkaloid being isolated from the roots. The empirical formula of colchicine is $C_{22}\,0_6\,N$ (Sharma and Sharma, 1980).

Colchicine is soluble in water. It is remarkable in the sense that, though highly water soluble, it is very active at an extremely low concentration.

Originally the role of colchicine was considered to be that of a catalyst (Sharma and Sharma, 1980). It brings about a change in the colloidal stage of the cytoplasm, causing spindle distrubance. It is a common herb in Europe, S. Africa and Asia (Hussaini, 2000).

C. luteum is found in India from Muree Hills to Kashmir (Hussaini, 2000).

Colcemid (Ciba) is de-acetyl methyl colchicine and is used more extensively in *in vitro* studies of mammalian chromosomes since it does not show certain toxic effects attributed to colchicine. Its use in certain rapidly dividing tissues, like ascitic fluids, has occasionally been considered to be superfluous (Sharma and Sharma, 1980). It or even colchicine itself, has no effect on cells entering division or in prophase stages.

There is a great number of compounds among which colchicine remains the foremost, that inhibit the spindle mechanism completely or in part and prevent separation of daughter chromosomes into two parts (Deysson, 1968).

Colchicine, an alkaloid is a universal mitotic inhibitor, effective in plant and animal cells both in *vitro* and *in vivo* (Gelfant, 1963). In addition, its effects are not rapidly removed (Izumi *et. al.*, 1983). It inhibits mitosis in metaphase by disorienting the structural organization of the asters and the spindle. The chromosomes are thus unable to move and may be arrested in metaphase for as long as 10 hours, after which the cell either degenerates or recovers from the effects of colchicine (Gelfant, 1963).

Colchicine is most commonly used for metaphase arrest because of its high effectiveness over a wide range of concentrations on both plant and animal tissues (Walker, 1973). Effective spindle arrest, resulting in a high frequency of metaphase plates, with the chromosomes well clarified and straightened has been obtained in meristematic cells of roots, shoot apex and in both 1st and 2nd divisions of the pollen grain nucleus (Sharma and Sharma, 1980). Colchicine is water soluble and the duration of treatment is inversely proportional to the concentration (Walker, 1973). Generally suitable for the study of chromosome structure and metaphase arrest, in low concentration and short period of treatment. Both mitotic and meiotic tissues respond well to colchicine treatment. Colchicine is applied on seeds and young seedlings, just germinating seeds, on mature seedlings and young inflorescences (Sharma and Sharma, 1980). Both mitotic and meiotic tissues respond well to colchicines treatment (Walker,1973).

Colchicine affects a number of processes in cells. In addition to its effects on mitosis, i.e. suppression of anaphase and lengthening the duration of metaphase, this drag also reduces cell cycle duration (Davidson, 1979). It also

shortened the mitotic cycle of cells in interphase at the time of treatment and stimulated cells in G_1 or S to complete interphase faster than untreated cells (Webster and Davidson, 1970).

The production of polyploidy cells in plant tissue is effected most readily by prolonged pre-treatment of actively dividing cells in colchicine (Walker, 1973). As an agent of inducing polyploidy, colchicine is more active in plants than in animals (Sharma and Sharma, 1980). When spindle function is inhibited, nuclear and cell division are also as a rule. The chromatids separate but are included within a common nuclear envelope so that the number of chromosomes per mitotic cycle in each cell is doubled. Beside the interference of spindle formation and cell division, colchicine also tends to shorten chromosomes, although in this instance the greater degree of contraction apparently results from a more protracted prophase period during which contraction continues beyond the usual state reached by metaphase (Swanson, 1965). It was also shown that in *Tradescantia* when colchicine prevents cell division by an inhibition of the spindle without reducing the rate of chromosome division, the chromosomes become smaller and smaller as the polyploidy of the cells increased (Swanson, 1965).

In order to induce polyploidy, seeds and seedlings were treated with various concentrations of aqueous colchicine (0.1 per cent to 0.5 percent). The seeds were treated for 18 to 24 hours while the treatments given to seedlings were of 4 to 6 hours (Dnyansagar and Nadkharni, 1983). According to Thomas (1963), colchicine induces polyploidy in plants. Also colchicines retard growth and that

the retardation was proportional to its dose. Low doses of colchicine not only promoted the germination of seeds of *Petunia axillaris* but also shortened the time required for germination.

Colchicine induced tetraploids and triploids (Atwood, 1947). The tetraploids from pure sativa (*Medicago sativa*) showed increase growth compared with the diploids, but tetraploids from *Ultana alfalfa*, derived from the cross M. *sativa X M. falcatae* did not show increase growth. Both triploids and tetraploids were derived. The triploids showed very luxuriant growth and pollen fertility in both 3n and 4n plants was high but seed setting in both was low. Induction of tetraploidy by treatment with colchicine (0.5 percent solution for 9 hours) in *Rauwolfia serpentina* has been reported (Banerjee and Sharma, 1983). The induced tetraploid plants were shorter, slow growing, having larger stomata but lower stomatal frequency. The tetraploids did not show the characteristic gigantism. The pollen grains had greater variation in size and shape in comparison to the diploid plants. Tetraploids were also induced through colchicine treatment of 1.5 – 2.0cm long seedling in 4 Pea cultivars (Mercykutty and Kumar, 1983). Yudin and Khvatova (1985), indicated that the 0.1 to 0.2 per cent colchicine concentrations normally used to produce tetraploids from diploid maize proved ineffective or poorly effective in work with 38 chromosome maize *X Tripsacum* hybrids.

Colchicine derived tetraploid lines were in most cases unstable, with a high percentage of aneuploidy. Colchicine treatment of seedlings also induced mutations, some of them of breeding value. Individual lines and hybrids showed

specificity of response to treatment and environmental conditions, especially at the start of growth, affected the percentage of tetraploids obtained. The tetraploids were shorter in stature than the diploids from which they were derived (Genov, 1991).

Addition of colchicine to the root medium has led to enhanced nodulation which is thought to be either due to production of more polyploid cells in the superficial layers of the root tissues or it could be that colchicine has induced more number of deformed root-hairs (Kabi and Bhaduri, 1978).

The development of the use of colchicine to produce polyploids freely has made possible re-examination of experimentally controlled material of great practical as well as theoretical importance (Stebbins, 1947). The more is known of how polyploidy has operated in the past to produce new species and races of plants, the better be able to use experimental polyploidy as a means of improving useful cultivated plants.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Source of Materials

The type of maize seeds used in this study is popcorn (*Zea mays var. praecox Sturt.*) (n = 10) which were provided by Dr. S. Mu'Azu of the department of Biological Sciences, A.B.U., Zaria who in bred them for seven generations. The seeds of two (2) strains (A and B) in the first trial and another strain (C) in the second trial were sown in pots and raised to maturity in the departmental garden.

3.2 Cytological Techniques

3.2.1. Inflorescences for cytological meiotic study

Inflorescences were used from strains (A and B) for cytological meiotic study which were taken immediately after teassling (from young unopened spikelets) and subsequent collections were made from tassels at different ages (before and after emergence of the tassels) upto the day when the anthers became fully differentiated into mature pollen grains. Sample collections were made five times at 6.00a.m., 9.00a.m., 12.00noon, 3.00p.m. and 6.00p.m. each day on the 53^{rd}, 55^{th}, 57^{th}, and 59^{th} days after sowing. Pre-trail collections were made right from 25^{th}, 30^{th}, 35^{th}, 40^{th}, 45^{th}, 47^{th}, 49^{th}, 51^{st}, and 52^{nd} days after sowing.

3.2.2. Anthers for the colchicine studies

For the colchcine studies, anthers from young unopened spikelets of strain C were selected and pretreated with colchicine. The anthers were soaked by

placing them directly into the various aqueous colchicine solutions of 0.05 per cent, 0.1 per cent and 0.15 per cent concentrations for time periods of 1, 2 and 4 hours respectively for all the three different concentrations (Walker, 1973). The control anthers were not pretreated with colchicine.

After pretreatment, the anthers were thoroughly washed to free them of colchicine before fixation (Sharma and Sharma, 1980; Love and Love, 1975). Treatment is followed immediately by fixation (Walker, 1973).

3..3 Fixation/Squashing

Inflorescences taken before and after the emergence of the tassels were fixed in modified fresh Carnoy's fluid (6:3:1 of absolute alcohol: chloroform: glacial acetic acid) for 24 hours and stored in 70 per cent alcohol in the refrigerator until when needed (Singh *et.al.*, 1977). The pretreated anthers after being thoroughly washed with tap water were also fixed in modified fresh Carnoy's fluid for 24 hours and stored in 70-percent alcohol in the refrigerator until when needed as well.

One or two anthers were placed on a clean slide and a drop of 1 – per cent aceto-orcein was added (Love and Love, 1975). It was allowed to stay for 2-minutes in order to allow the anthers to pick up the stain. A clean cover slip was placed on the top of it and the anthers were crushed by gentle tapping. The pollen mother cells spread thinly with a light pressing of the cover slip. The slides

placed on the top of it and the anthers were crushed by gentle tapping. The pollen mother cells spread thinly with a light pressing of the cover slip. The slides prepared were viewed under a microscope. Temporary smear showing good dividing stages with abnormalities were sealed with nail varnish.

For meiotic divisions, pollen mother cells were studied. Different stages shown by the pollen mother cells were counted from ten (10) prepared good slides from each sample taken and the counting was made by selecting five suitable portions from each slide and the results were recorded.

Different abnormal chromosomal behaviours shown by the pollen mother cells resulting from the colchicine pretreatment were studied from the preparations of anther squashes. The frequency of the different kinds of chromosomal aberrations were recorded from ten (10) prepared good slides from each sample taken.

Photomicrographs of the suitable meiotic stages and the different kinds of chromosomal aberrations observed were taken for the purpose of illustration.

All the photomicrographs were prepared from Aceto-orcein stain smear preparations.

3.4 Statistical Analysis

The results were analysed using correlation coefficient(r) analysis and analysis of variance (Anova) procedure. Means were calculated by analysis of variance.

In the first trial, correlation coefficients of the 12-characters (meiotic stages) in the two strains of popcorn; mean square estimates of time, day, strain

and their various interactions as well as the mean values and times of fixation in the two strains were considered.

In the second trial, correlation coefficients of the abnormalities at metaphase and that of the abnormalities at anaphase; their mean square estimates; their mean values as well as the mean estimates of their treatments were considered in popcorn treated with colchicine. These were compared using Least Significant Differences (LSD) at > 0.05 level.

CHAPTER FOUR

RESULTS

The results obtained indicated that meiosis was normal in the two strains (A and B) of popcorn used in the first trial and almost all the stages were found in the two strains at one time or the other. Similarly, only premeiosis (interphase) occurred fifty two days after sowing in both the two strains. In the second trial, seeds of strain C popcorn were treated with colchicine and meiotic chromosome behaviour was studied from the treated and untreated anthers of this strain.

4.1 Strain A Samples taken on the Fifty Third Day

For strain A samples taken on the fifty third day, pollen mother cells (PMCs) observed at 6.00a.m. (2,515 cells), 9.00a.m. (3,017), 12.00noon (2,657) and at 3.00p.m. (3,168) showed no stages of meiosis, all the cells being in the interphase. However, at 6.00p.m. all the various stages of meiosis were observed (Table 1).

TABLE 1: NUMBER OF POLLEN MOTHER CELLS AND MEIOTIC STAGES ANALYSED FOR FIFTY THIRD DAY OLD STRAIN A POPCORN (*Zea mays var. praecox Sturt.*) PERCENTAGES ARE GIVEN IN PARENTHESES

Time of fixation	Interphase	Leptotene	Zygotene	Pachytene	Diplotene	Diakinesis	Met. I	Ana. I	Telo. I	Met. II	Ana. II	Telo. II	TOTALS
6.00pm	71 (3.39)	84 (4.01)	97 (4.63)	68 (3.25)	96 (4.59)	125 (5.97)	125 (5.97)	203 (.9.70)	356 (17.02)	156 (7.45)	300 (14.34)	412 (19.68)	2,093 (100.00)

4.2 Strain B Samples taken on the Fifty Third Day

The samples collected from strain B on the fifty third day showed all the various stages of meiosis only at 6.00pm and the stages of the first meiotic division (Leptotene to telophase 1) were observed in the case of the sample taken at 9.00am. All the samples taken at 6.00am, 12.00noon and 3.00pm showed only premeiotic pollen mother cells (interphase), (Table 2).

TABLE 2 NUMBER OF POLLEN MOTHER CELLS AND MEIOTIC STAGES ANALYSED FOR FIFTY THIRD DAY OLD STRAIN B POPCORN (*Zea mays var. praecox Sturt*). PERCENTAGES ARE GIVEN IN PARENTHESES.

Times of fixation	Interphase	Leptotene	Zygotene	Pachytene	Diplotene	Diakinesis	Met. I	Ana. I	Telo. I	Met. II	Ana. II	Telo. II	TOTALS
6.00am	3,015	0	0	0	0	0	0	0	0	0	0	0	3,015
	(100.00)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(100.00)
9.00am	174	185	425	521	801	715	105	45	95	0	0	0	3,066
	(5.68)	(6.03)	(13.86)	(16.99)	(26.13)	(23.32)	(3.42)	(1.47)	(3.10)	(0)	(0)	(0)	(100.00)
12.00	2,923	0	0	0	0	0	0	0	0	0	0	0	2,923
noon	(100.00)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(100.00)
3.00pm	2,597	0	0	0	0	0	0	0	0	0	0	0	2,597
	(100.00)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(100.00)
6.00pm	29	84	97	217	415	529	57	96	417	98	216	721	3,027
	(0.96)	(2.78)	(3.20)	(7.17)	(13.71)	(17.48)	(1.88)	(3.16)	(13.78)	(3.24)	(7.14)	(25.50)	(100.00)

4.3 Strain-A-Samples taken on the Fifty Fifth Day

For strain A samples taken on the fifty fifth day, pollen mother cells (PMCs) observed at 6.00am (3,025 cells), 9.00am (2,927), 12.00 noon (2,569) and 3.00pm (3,150) showed no stages of meiosis, all the cells being in the interphase. However at 6.00pm, all the various stages of meiosis were observed (Table 3).

TABLE 3: NUMBER OF POLLEN MOTHER CELLS AND MEIOTIC STAGES ANALYSED FOR FIFTY FIFTH DAY OLD STRAIN A POPCORN (*Zea mays var. praecox Sturt*). PERCENTAGES ARE GIVEN IN PARENTHESES.

Time of fixation	Interphase	Leptotene	Zygotene	Pachytene	Diplotene	Diakine-sis	Met. I	Ana. I	Telo. I	Met. II	Ana. II	Telo. II	TOTALS
6.00pm	109	132	212	150	240	193	143	189	282	216	312	415	2,593
	(4.20)	(5.09)	(8.18)	(5.78)	(9.26)	(7.44)	(5.52)	(7.29)	(10.88)	(8.33)	(12.03)	(16.00)	(100.00)

TABLE 4: NUMBER OF POLLEN MOTHER CELLS AND MEIOTIC STAGES ANALYSED FOR FIFTY FIFTH DAY OLD STRAIN B POPCORN (*Zea mays var. praecox Sturt.*) PERCENTAGES ARE GIVEN IN PARENTHESES

Times of fixation	Interphase	Leptotene	Zygotene	Pachytene	Diplotene	Diakine-sis	Met. I	Ana. I	Telo. I	Met. II	Ana. II	Telo. II	TOTALS
6.00am	669	215	168	136	515	435	75	51	124	68	57	107	2,620
	(25.53)	(8.21)	(6.41)	(5.19)	(19.66)	(16.60)	(2.86)	(1.95)	(4.73)	(2.60)	(2.18)	(4.08)	(100.00)
9.00am	1,269	565	235	728	0	0	0	0	0	0	0	0	2,797
	(45.37)	(20.20)	(8.40)	(26.03)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(100.00)
12.00	639	125	96	312	210	98	0	0	0	0	0	0	1,480
noon	(43.18)	(8.45)	(6.49)	(21.08)	(14.18)	(6.62)	(0)	(0)	(0)	(0)	(0)	(0)	(100.00)
3.00pm	446	115	123	221	727	829	106	215	567	0	0	0	3,349
	(13.32)	(3.43)	(3.67)	(6.60)	(21.71)	(24.75)	(3.17)	(6.42)	(16.93)	(0)	(0)	(0)	(100.00)
6.00pm	1,528	120	132	315	00	0	0	0	0	0	0	0	2,095
	(72.94)	(5.72)	(6.30)	(15.04)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(100.00)

4.5 Strain A Samples taken on the Fifty Seventh Day

In the case of strain A samples taken on the fifty seventh day, all the various meiotic stages were observed throughout all the five different periods (times) of this day during which the samples were taken (Table 5). Anthers showing pollen grains were also observed from these same samples as the first appearance of inflorescences (on the plants) were noticed on this same day.

TABLE 5: NUMBER OF POLLEN MOTHER CELLS AND MEIOTIC STAGES ANALYSED FOR FIFTY SEVENTH DAY OLD STRAIN A POPCORN (*Zea mays var. praecox Sturt.*) PERCENTAGES ARE GIVEN IN PARENTHESES

Times of fixation	Interphase	Leptotene	Zygotene	Pachytene	Diplotene	Diakinesis	Met. I	Ana. I	Telo. I	Met. II	Ana. II	Telo. II	TOTALS
6.00am	652	420	385	312	285	236	80	45	66	32	28	25	2,666
	(28.21)	(15.75)	(14.440	(11.75)	(10.69)	(8.85)	(3.00)	(1.69)	(2.480	(1.20)	(1.05)	(0.94)	(100.00)
9.00am	385	306	502	169	535	615	770	256	769	47	40	68	4,462
	(8.63)	96.85)	(11.25)	(3.79)	(12.00)	(13.78)	(17.26)	(5.74)	(17.23)	(1.05)	(0.90)	(1.52)	(100.00)
12.00 noon	132	45	316	138	93	124	431	112	314	255	135	195	2,290
	(5.76)	(1.97)	(13.80)	(6.03)	(4.06)	(5.40)	(18.82)	(4.89)	(13.71)	(11.14)	(5.90)	(8.52)	(100.00)
3.00pm	25	299	713	842	492	753	278	197	441	263	250	718	5,271
	(0.47)	(5.67)	(13.53)	(15.97)	(9.33)	(14.29)	(5.27)	(3.74)	(8.37)	(4.99)	(4.74)	(13.63)	(100.00)
6.00pm	120	305	325	414	502	346	569	415	480	360	280	689	4,805
	(2.50)	(6.35)	(6.76)	(8.62)	(10.45)	(7.20)	(11.84)	(8.64)	(9.99)	(7.49)	(5.83)	(14.34)	(100.00)

4.6 Strain B Samples taken on the Fifty Seventh Day

The samples taken on the fifty seventh (57) day had all the various meiotic stages that were observed except in the case of the sample taken at 6.00p.m. in which interphase; leptotene, zygotene, pachytene, diplotene and diakinesis (Prophase-1 substages) were observed (Table 6). Anthers that showed pollen grains were also observed from all these samples taken at all the five different periods of this day.

TABLE 6: NUMBER OF POLLEN MOTHER CELLS AND MEIOTIC STAGES ANALYSED FOR FIFTY SEVENTH DAY OLD STRAIN B POPCORN (*Zea mays var. praecox Sturt.*) PERCENTAGES ARE GIVEN IN PARENTHESES

Times of fixation	Interphase	Leptotene	Zygotene	Pachytene	Diplotene	Diakinesis	Met. I	Ana. I	Telo. I	Met. II	Ana. II	Telo II	TOTALS
6.00am	523	216	168	97	82	77	60	41	57	27	23	46	1,417
	(36.91)	(15.24)	(11.85)	(6.84)	(5.79)	(5.43)	(4.23)	(2.89)	(4.03)	(1.91)	(1.62)	(3.26)	(100.00)
9.00am	312	150	365	400	212	183	226	101	116	53	33	252	2,403
	(12.98)	(6.24)	(15.19)	(16.64)	(8.82)	(7.62)	(9.40)	(4.20)	(4.84)	(2.22)	(1.37)	(10.48)	(100.00)
12.00 noon	36	57	40	106	178	160	76	180	169	260	325	216	1,803
	(2.00)	(3.16)	(2.22)	(5.88)	(9.87)	(8.87)	(4.22)	(9.98)	(9.37)	(14.42)	(18.03)	(11.98)	(100.00)
3.00pm	68	37	55	105	125	94	35	41	169	265	273	328	1,595
	(4.26)	(2.32)	(3.45)	(6.58)	(7.84)	(5.89)	(2.19)	(2.57)	(10.60)	(16.62)	(17.12)	(20.56)	(100.00)
6.00pm	27	60	83	97	235	207	0	0	0	0	0	0	709
	(3.81)	(8.46)	(11.71)	(13.68)	(33.14)	(29.20	(0)	(0)	(0)	(0)	(0)	(0)	(100.00)

4.7 Strain A Samples taken on the Fifty Ninth Day

The last set of samples collected in the case of strain A in which meiotic stages were observed was on the fifty ninth (59^{th}) day. All the various meiotic stages were observed only from the first two samples taken at 6.00am and 9.00am while the sample taken at 12.00noon of the same day showed premeiotic pollen mother cells (interphase stage) and also leptotene, zygotene and pachytene substages of prophase-1. However, anthers from the samples taken at 3.00pm and 6.00pm showed only pollen grains (Table 7). Anthers that showed pollen grains were observed from the first three samples of this same day as well.

TABLE 7: NUMBER OF POLLEN MOTHER CELLS AND MEIOTIC STAGES ANALYSED FOR FIFTY NINTH DAY OLD STRAIN A POPCORN (*Zea mays var. praecox Sturt.*) PERCENTAGES ARE GIVEN IN PARENTHESES

Times of fixation	Interphase	Leptotene	Zygotene	Pachytene	Diplotene	Diakinesis	Met. I	Ana. I	Telo. I	Met. II	Ana. II	Telo. II	TOTALS
6.00am	222	155	145	168	226	168	38	36	28	35	26	37	1,284
	(17.29)	(12.08)	(11.29)	(13.08)	(17.60)	(13.08)	(2.96)	(2.80)	(2.18)	(2.73)	(2.03)	(2.88)	(100.00)
9.00am	215	209	260	150	147	105	36	29	19	8	10	15	1,203
	(17.87)	(17.37)	(21.61)	(12.47)	(12.22)	(8.73)	(2.99)	(2.41)	(1.58)	(0.67)	(0.83)	(1.25)	(100.00)
12.00 noon	269	164	321	164	0	0	0	0	0	0	0	0	918
	(29.30)	(17.86)	(34.98)	(17.86)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(100.00)

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4.8 Cytological Effects

Meiotic chromosome behaviour was studied from the anthers that were treated with an alkaloid chemical (colchicine) and from the control anthers (untreated) of strain C popcorn.

The changes resulting from the mutagenic treatment were cytologically observed as induced chromosomal aberrations which were recorded in meiosis. The types of meiotic abnormalities/aberrations observed resulting from colchicine treatment in different stages of meiotic divisions were as follows:

Prophase substages:

Meiotic chromosome behaviour was normal for all the treated and untreated anthers observed in he case of these substages.

4.9 Metaphase I

The types of induced chromosomal aberrations that can easily be recognized in most of the treatments with colchicine are sticky chromosomes in which the entire chromosomes clumped together as a mass and sticky disoriented chromosomes in which the chromosomes clumped together but not having a normal pattern of orientation of the chromosomes (Fig.17, plate 5). Other chromosomal abnormalities such as chromosomes with multivalents, bivalents, univalents and laggards were as well observed.

The frequency of these chromosomal aberrations which largely was dependent on dose and duration of treatment is shown in Table 8.

TABLE 8: METAPHASE 1 - COMPARATIVE CYTOLOGICAL EFFECTS OF COLCHICINE ON POPCORN (*Zea mays var. praecox Sturt.*) PERCENTAGES ARE GIVEN IN PARENTHESES.

Concen-tration	Treat-ment period	Total No. of PMCs observed	Total No. of PMCs showing clear separation (normal orientation)	Total abnormal PMCs	No of sticky chromo-somes	No. of sticky disoriented/ non-oriented chromo-somes	Multiva-lents	Bivallents	Univa-lents	Laggards
0.15%	4-hours	2,164	24 (1.11)	2,140 (98.89)	1,295 (60.51)	703 (32.85)	79 (3.69)	23 (1.07)	15 (0.70)	25 (1.17)
0.15%	2-hours	1,964	32 (1.63)	1,932 (98.37)	1,121 (58.02)	667 (34.52)	54 (2.80)	41 (2.12)	31 (1.60)	18 (0.93)
0.15%	1-hour	1,560	76 (4.87)	1,484 (95.13)	751 (50.64)	618 (41.64)	34 (2.29)	36 (2.43)	38 (2.56)	15 (1.01)
0.1%	4-hours	1,728	105 (6.08)	1,623 (96.92)	883 (54.40)	624 (38.45)	56 (3.45)	26 (1.60)	22 (1.36)	22 (1.36)
0.1%	2-hours	1,645	197 (11.98)	1,448 (88.02)	753 (52.00)	851 (40.12)	47 (3.25)	27 (3.25)	28 (1.93)	13 (0.83)
0.1%	1-hour	1,506	234 (15.54)	1,272 (84.46)	627 (49.29)	547 (43.00)	35 (2.75)	26 (2.04)	24 (1.89)	13 (1.02)
0.05%	4-hours	1,689	153 (9.06)	1,536 (90.94)	759 (49.41)	658 (42.84)	34 (2.21)	26 (1.69)	32 (2.08)	27 (1.76)
0.05%	2-hours	1,692	242 (14.30)	1,450 (85.70)	705 (48.62)	669 (46.14)	33 (2.27)	23 (1.59)	10 (0.69)	10 (0.69)
0.05%	1-hour	1,607	315 (19.06)	1,292 (80.40)	571 (44.20)	660 (51.08)	22 (1.70)	24 (1.86)	6 (0.46)	9 (0.70)
0	0	307	289 (94.14)	18 (5.86)	0 (0)	10 (3.27)	2 (0.65)	0 (0)	3 (0.98)	3 (0.98)

4.10 Anaphase I

Anaphase 1 was greatly disturbed in the treated anthers in comparison with the untreated anthers. The treated anthers had a large number of pollen mother cells with multiple bridges with or without fragments (Fig. 23, plate 7). Single and double bridges were also observed (Fig21, plate 7).

Other chromosomal abnormalities such as unequal separation, laggards, multivalents were as well observed. The frequency of all these chromosomal abnormalities have been analysed and the results were shown in Table 9.

TABLE 9: ANAPHASE 1 - COMPARATIVE CYTOLOGICAL EFFECTS OF COLCHICINE ON POPCORN (*Zea mays var. praecox Sturt.*) PERCENTAGES ARE GIVEN IN PARENTHESIS.

Concent-ration	Treatment period	Total No. of PMCs observed	Total No. of PMCs showing clear separation (normal orientation)	Total abnormal PMCs	No of chromatid bridges (single)	No of Dicentric (multiple) bridges	No. of unequal separation	No. of Laggards	No. of multivalents
0.15%	4-hours	90	0 (0)	90 (100)	3 (3.33)	58 (64.44)	8 (8.89)	3 (3.33)	18 (20.00)
0.15%	2-hours	96	10 (10.42)	86 (89.58)	4 (4.65)	48 (55.81)	12 (13.95)	8 (9.30)	14 (16.28)
0.15%	1-hour	90	13 (14.44)	77 (85.56)	10 (12.99)	36 (46.75)	10 (12.99)	12 (15.58)	9 (11.69)
0.1%	4-hours	108	14 (12.96)	93 (86.11)	8 (8.60)	47 (50.54)	12 (12.90)	11 (11.83)	15 (16.13)
0.1%	2-hours	94	14 (12.96)	80 (85.11)	10 (12.50)	41 (51.25)	10 (12.50)	13 (16.25)	6 (7.50)
0.1%	1-hour	93	14 (14.89)	77 (82.80)	13 (16.88)	34 (44.16)	8 (10.39)	16 (20.78)	6 (7.78)
0.05%	4-hours	85	16 (17.20)	70 (82.35)	12 (17.14)	24 (34.290	10 (14.29)	14 (20.00)	10 (14.29
0.05%	2-hours	87	15 (17.65)	69 (79.31)	20 (28.99)	21 (30.43)	8 (11.59)	16 (23.19)	4 (5.80)
0.05%	1-hour	93	20 (21.51)	73 (78.49)	32 (43.84)	13 (17.81)	8 (10.96)	18 (24.66)	2 (2.74)
0	0	177	169 (95.48))	8 (4.52)	5 (2.82)	0 (0)	3 (1.69)	0 (0)	0 (0)

4.11 Dyads

At this stage, normal 2 (dyads) group of daughter chromosomes were enclosed by nuclear envolopes.

Empty cells were observed from the daughter cells which was probably due to certain induced abnormal meiotic events such as laggards, delayed and eliminated chromosomes which are earlier observed.

4.12 **Metaphase II**

At this stage, 10 chromosomes were observed at each pole of the second metaphase plate when there is a clear separation. However, instances of sticky chromosomes, disoriented metaphase were mostly noticed among the chromosomal abnormalities observed (Fig.24, 25 plate 8) at metaphase II and most of these were found at the higher concentrations of colchicines and also in the anthers that had a longer duration of treatment in comparison with the control and lower concentration of treatments. Other chromosomal abnormalities include multivalents and laggads were also observed.

The frequency of these different chromosomal abnormalities on second metaphase plate were analysed in Table 10.

TABLE 10: METAPHASE II – COMPARATIVE CTYOLOGICAL EFFECTS OF COLCHICINE ON POPCORN (*Zea mays var praecox Sturt.*). PERCENTAGES ARE GIVEN IN PARENTHESES

Concentration	Treatment period	Total no. of PMCs observed	Total no. of PMCs showing clear separation	Total abnormal PMCs	No. of sticky chromosomes	No. of sticky disoriented/non-oriented chromosomes	Multivalents	Laggards
0.15%	4 hours	1512	21 (1.39)	1481 (97.95)	895 (60.43)	536 (36.19)	39 (2.63)	21 (1.42)
0.15%	2 hours	1364	23 (1.69)	1341 (98.31)	689 (51.38)	615 (45.86)	25 (1.86)	12 (0.89
0.15%	1 hour	1129	55 (4.87)	1074 (95.13)	531 (49.44)	509 (47.39)	12 (1.12)	14 (1.30)
0.1%	4 hours	1098	82 (7.47)	1016 (92.53)	540 (53.15)	435 (42.81)	24 (2.36)	17 (1.67)
0.1%	2 hours	1130	151 (13.36)	979 (86.64)	500 (51.07)	440 (44.94)	26 (2.66)	13 (1.33)
0.1%	1 hour	1055	191 (18.10)	864 (81.90)	429 (49.65)	409 (47.33)	16 (1.85)	10 (1.16)
0.05%	4 hours	913	154 (16.87)	759 (83.13)	386 (50.86)	342 (45.06)	15 (1.98)	16 (2.11)
0.05%	2 hours	933	263 (28.19)	670 (71.81)	324 (48.36)	318 (47.46)	14 (2.09)	14 (2.09)
0.05%	1 hour	1028	348 (33.85)	680 (66.15)	285 (41.91)	370 (54.41)	12 (1.76)	13 (1.91)
0	0	215	211 (98.14)	4 (1.86)	0 (0)	3 (1.40)	1 (0.47)	0 (0)

4.13 Anaphase II

This stage was also studied to determine the different kinds of chromosomal aberrations resulting from the treatment of anthers with colchicine. At this stage induced delayed separation of anaphase chromosomes and unequal separation coupled with laggards and multivalents were observed.

The frequency of these different chromosomal aberrations have been analysed at anaphase II and the results were shown in Table 11.

TABLE 11: ANAPHASE II – COMPARATIVE CTYOLOGICAL EFFECTS OF COLCHICINE ON POPCORN (*Zea mays var. praecox Sturt.*). PERCENTAGES ARE GIVEN IN PARENTHESES

Concentration	Treatment period	Total no. of PMCs observed	Total no. of PMCs showing clear separation	Total abnormal PMCs	No. of chromatid bridges (single)	No. of chromatid bridges (Dicentric)	No. of unequal separation	No. of Laggards	No. of multivalents
0.15%	4 hours	58	4 (6.90)	54 (93.10)	2 (3.70)	43 (79.63)	6 (11.11)	6 (11.11)	7 (12.96)
0.15%	2 hours	67	12 (17.91)	55 (82.09)	8 (14.55)	32 (58.18)	7 (12.73)	5 (9.09)	3. (5.45)
0.15%	1 hour	63	13 (20.63)	50 (79.37)	10 (20.00)	21 (42.00)	8 (16.00)	9 (18.00)	2 (4.00)
0.1%	4 hours	82	13 (15.85)	69 (84.15)	10 (14.49)	34 (49.28)	10 (14.49	10 (14.49)	5 (7.25)
0.1%	2 hours	73	13 (17.81)	60 (82.19)	12 (20.00)	20 (33.33)	12 (20.00)	12 (20.00)	4 (6.67)
0.1%	1 hour	71	15 (21.13)	56 (78.87)	12 (21.43)	16 (28.57)	12 (21.43)	13 (23.21)	3 (5.39)
0.05%	4 hours	68	14 (20.59)	54 (79.41)	13 (24.07)	14 (29.93)	12 (22.22)	12 (22.22)	3 (5.56)
0.05%	2 hours	77	17 (22.08)	60 (77.92)	15 (25.00)	13 (21.67)	15 (25.00)	15 (25.00)	2 (3.33)
0.05%	1 hour	89	21 (23.60)	68 (76.40)	18 (26.47)	12 (17.65)	18 (26.47)	18 (26.47)	2 (2.94)
0	0	138	135 (97.83)	3 (2.17)	2 (1.45)	0 (0)	1 (0.72)	0 (0)	0 (0)

4.14 **Tetrads**

At this state, normal 4 (tetrads) groups of daughter chromosomes were observed. The daughter chromosomes were enclosed by nuclear envelopes.

Empty cells were observed out of the four daughter cells. This was also probably due to certain induced abnormal meiotic events such as laggards, delayed and eliminated chromosomes which were earlier observed.

4.15: **Correlations of the 12 characters (meiotic stages) in Two Strains of Popcorn (proportions of the Pollen Mother Cells (PMCs) at each stage).**

Correlations of the interphase stage (V_1) with the correlations of all the remaining meiotic stages showed no significant correlations with all the other characters. Correlations of the leptotene stage (V_2) with zygotene, pachytene, diplotene, diakinesis, metaphase 1 and anaphase 1 showed highly significant correlations at 1-per cent level while that with telophase I showed significant correlation at 5-per cent level. Correlations of the zygotene stage (V_3) with pachytene, diplotene, diakinesis, metaphase 1, anaphase1, telolphse 1, metaphase II and telophase II showed significant correlations at 5-per cent level. Correlations of the pachytene stage (V_4) with diplotene, diakinesis, anaphase I and telophase II showed highly significant correlations at 1-per cent level while that with metaphase I, telophase I and metaphase II showed significant correlations at 5-per cent level. Correlations of the diplotene stage (V_5) with diakinesis, metaphaseI, anaphase I, telophase 1 and telophase II showed highly significant correlations at 1-per cent level while that with metaphase II showed significant correlation at 5-per cent level. Correlations of the diakinetic stage (V_6) with metaphase I, anaphase I, telophase I and telophase II showed highly significant correlations at 1-per cent level while that with metaphase II and anaphase II were significantly correlated at 5-per cent level. Correlations of the metaphase 1 (V_7) with anaphase

I, telophase II, metaphase II , anaphase II and telophase II showed highly significant correlations at 1-per cent level. Correlations of the anaphase I (V_8) with telophasel, metaphase II, anaphase II and telophase II showed highly significant correlations at 1-per cent level. Correlations of the telophase I stage (V_9) with metaphase II, anaphase II and telophase II showed highly significant correlations at 1-per cent level. Correlations of the metaphase II stage (V_{10}) with anaphase II and telophase II showed highly significant correlations at 1-per cent. Correlation of the anaphase II stage (V_{11}) with telophase II (V_{12}) showed a highly significant correlation at 1-per cent level. The correlation coefficients is shown in Table 12.

TABLE 12: CORRELATION COEFFICIENTS OF THE 12-CAHRACTERS (Meiotic stages) IN TWO STRAINS OF POPCORN (*Zea mays var. praecox Sturt.*)

	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
V_1	1.000	0.081^{ns}	-0.072^{ns}	0.031^{ns}	-0.123^{ns}	-0.129^{ns}	-0.114^{ns}	-0.166^{ns}	-0.151^{ns}	-0.203^{ns}	-0.214^{ns}	-0.201^{ns}
V_2		1.000	0.753^{xx}	0.801^{xx}	0.488^{xx}	0.452^{xx}	0.430^{xx}	0.410^{xx}	0.372^{x}	0.236^{ns}	0.176^{ns}	0.263^{ns}
V_3			1.000	0.800^{xx}	0.644^{xx}	0.672^{xx}	0.673^{xx}	0.550^{xx}	0.574^{xx}	0.418^{xx}	0.299^{ns}	0.446^{xx}
V_4			1.000	0.564^{xx}	0.580^{xx}	0.359^{x}	0.404^{xx}	0.370^{x}	0.348^{x}	0.273^{ns}	0.452^{xx}	
V_5				1.000	0.961^{xx}	0.539^{xx}	0.604^{xx}	0.704^{xx}	0.337^{x}	0.309^{ns}	0.423^{xx}	
V_6					1.000	0.501^{xx}	0.633^{xx}	0.773^{xx}	0.331^{x}	0.323^{x}	0.476^{xx}	
V_7						1.000	0.807^{xx}	0.821^{xx}	0.567^{xx}	0.403^{xx}	0.469^{xx}	
V_8							1.000	0.881^{xx}	0.751^{xx}	0.710^{xx}	0.703^{xx}	
V_9								1.000	0.586^{xx}	0.578^{xx}	0.640^{xx}	
V_{10}									1.000	0.920^{xx}	0.806^{xx}	
V_{11}										1.000	0.849^{xx}	
V_{12}											1.000	

KEY: x – significant at 5% level; xx – significant at 1% level, ns – not significant

4.16: Mean square estimates of Time, Day, Strain and their various interactions of the 12-characters (Meiotic stages) in two strains of Popcorn,.

The results indicated that the times of fixation for metaphase II, anaphase II and telophase II were highly significant. Similarly, day of sample collection (age of the plants) for zygotene, metaphase I, metaphase II and anaphase II were highly significant at 1-per cent level while telophase II was significant at 5-per cent level. As regards to the strain, metaphase I was highly significant at 1-per cent level while interphase was significant at 5-per cent level in the case of the two strains used.

The interaction of the strain and age of the plant indicated that zygotene, pachytene and metaphase I were highly significant at 1-per cent level while interphase, leptotene and telophase I were significant at 5-per cent level as regards to the interactions between the strains and the days the samples were taken. The interaction between the age of the plants and times of fixation indicated that metaphase II and anaphase II were significant at 5-per cent level. The interactions between the strain used and times of fixation indicated that pachytene, metaphae II and anaphase II were significant at 5-per cent level. The mean square estimates of the meiotic stages is shown in Table 13.

TABLE 13: MEAN SQUARE ESTIMATES OF TIME, DAY, STRAIN AND THEIR VARIOUS INTERACTIONS OF THE 12-CHARACTERS (MEIOTIC STAGES) IN TWO STRAINS OF POPCORN (*Zea mays var. praecox Sturt.*)

Source/ DF	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
Time 4	217222.94^{ns}	22172.10^{ns}	21556.97^{ns}	33011.19^{ns}	27232.46^{ns}	37220.85^{ns}	16493.88^{ns}	9602.23^{ns}	32939.90^{ns}	11010.23^{x}	21008.29^{x}	92719.56^{x}
Day 3	1231997.03^{xx}	50527.53^{x}	121096.83^{xx}	101139.30^{x}	95803.13^{ns}	106757.47^{ns}	133080.70^{xx}	32941.80^{x}	112364.43^{x}	47958.43^{xx}	33192.20^{xx}	116721.89^{x}
Strain 1	3573050.63^{x}	902.50^{ns}	41538.03^{ns}	11560.00^{ns}	19536.40^{ns}	10956.10^{ns}	74822.50^{xx}	12673.60^{ns}	27092.03^{ns}	9030.03^{ns}	5152.90^{ns}	18190.23^{ns}
S.D. 3	1993896.23^{x}	68369.10^{x}	97566.16^{xx}	138345.60^{xx}	127424.47^{ns}	147143.30^{ns}	75214.17^{xx}	10850.20^{ns}	78446.23^{x}	2024.03^{ns}	936.30^{ns}	25762.49^{ns}
D.T. 12	386182.75^{ns}	7664.78^{ns}	12191.05^{ns}	19971.40^{ns}	37412.78^{ns}	41943.38^{ns}	13706.41^{ns}	4126.84^{ns}	30080.30^{ns}	8280.01^{x}	12730.05^{x}	44495.16^{ns}
S.T. 4	143586.56^{ns}	7816.50^{ns}	12981.59^{ns}	63035.94^{x}	6644.84^{ns}	241.85^{ns}	11891.50^{ns}	13489.98^{ns}	22600.40^{ns}	10329.15^{x}	14167.09^{x}	18668.66^{ns}
Error 12	378187.75	10145.18	14488.39	19997.45	50560.99	58212.72	6867.00	5292.33	19854.77	3032.73	3927.82	26505.10

KEY: x – significant at 5% level; xx – highly significant at 1% level; ns – not significant.
V_1 to V_{12} represents meiotic stages (interphase, leptotene, zygotene, pachytene, diplotene, diakinesis, metaphase, anaphase I, telophase I, metaphase II, anaphase II and telophase II

4.17: Mean values and Times of fixation of the 12 – characters (Meiotic stages) in two strains of Popcorn.

The results of the mean – values and times of fixation indicated that the proportions of the number of pollen mother cells (PMCs) at interphase stage (V_1) was highest at 6.00am, then followed by 12.00noon but the lowest was observed at 6.00pm. The mean – values at different times of fixation were significantly different in the case of the leptotene stage (V_2). The proportions of the number of PMCs at this stage was highest at 9.00am, then followed by that at 6.00am but the lowest was observed at 12.00noon. The proportions of the number of PMCs at zygotene stage (V_3) was highest at 9.00am, then followed by that at 6.00am but the lowest was observed at 12.00noon. The proportions of the number of PMCs at pachytne stage (V_4) was highest at 9.00am, then followed by that at 6.00am but the lowest was observed at 6.00pm. Similarly, the proportions of the number of PMCs at diplotene stage (V_5) was highest at 9.00am, then followed by that at 6.00pm but the lowest was observed at 12.00noon. The proportions of the number of PMCs at diakinetic stage (V_6) was highest at 3.00pm, then followed by that at 9.00am but the lowest was observed at 12.00noon. In the case of the metaphase I stage (V_7), the mean-values at different times of fixation were significantly different and the proportions of the number of PMCs was highest at 9.00am, then followed by that at 6.00pm but the lowest was observed at 6.00pm. The mean-values at different times of fixation were also significantly different at anaphase I (V_8) and the proportions of the number of PMCs was highest at 6.00pm, then followed by that at 3.00pm but the lowest was observed at 6.00am. The

proportions of the number of PMCs at telophase I (V_9) was highest at 6.00pm, then followed by that at 3.00pm but the lowest was observed at 6.00am. The mean-values at different times of fixation for metaphase II (V_{10}), anaphase II (V_{11}) and telophase II (V_{12}) were all significantly different and the proportions of their number of PMCs were highest at 6.00pm, then followed by 3.00pm but the lowest were observed at 9.00am in the case of metaphase II and anaphase II while the lowest was observed at 6.00am in the case of telophase II. The mean values and times of fixation of the meiotic stages in shown in Table 14.

TABLE 14: MEAN – VALUES AND TIMES OF FIXATION OF THE 12-CHARACTERS (meiotic stages) IN TWO STRAINS OF POPCORN (*Zea mays var. praecox Sturt.*)

	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}	V_{12}
Times of fixation	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
6.00am	647.6^{a}	125.75^{ab}	108.25^{a}	89.12^{a}	138.5^{a}	114.5^{a}	31.62^{a}	21.62^{a}	34.38^{a}	20.25^{b}	16.75^{b}	26.87^{b}
9.00am	294.4^{a}	176.87^{a}	223.37^{a}	246.00^{a}	21.9^{a}	202.3^{a}	142.12^{a}	53.88^{ab}	124.87^{a}	13.50^{b}	10.38^{b}	41.88^{b}
12.00 noon	499.9^{a}	48.88^{b}	96.62^{a}	90.00^{a}	60.1^{a}	47.7^{a}	63.38^{ab}	36.50^{ab}	60.38^{a}	64.37^{ab}	57.50^{b}	51.38^{b}
3.00pm	392.0^{a}	56.38^{b}	111.37^{a}	146.00^{a}	168.0^{a}	209.5^{a}	52.38^{ab}	56.63^{ab}	147.12^{a}	66.00^{ab}	65.37^{b}	130.75^{b}
6.00pm	235.5^{a}	98.12^{ab}	118.25^{a}	157.62^{a}	186.0^{a}	175.0^{a}	111.75^{ab}	112.87^{a}	191.87^{a}	103.75^{a}	138.50^{a}	286.00^{a}

Means with the same letter or same superscripts under the same variable are not significantly different, otherwise they are different using Duncan Multiple Range Test (DMRT).

4.18: **Correlations of the abnormalities at Metaphase in Popcorn treated with Colchicine**

Correlation of the total number of pollen mother cells (PMCs) observed (V_1) with the correlations of all the different types of abnormalities observed showed highly significant correlations at 1-per cent level except in the case of the PMCs that showed clear separation of the chromosomes. The correlations of the total number of PMCs showing clear separation (V_2) with the total abnormal PMCs, number of sticky chromosomes and the number of laggards observed showed highly significant correlations at 1-per cent level while that with multivalents showed significant correlations at 5-per cent level. Correlations of the total abnormal PMCs (V_3) with all the types of abnormalities observed showed highly significant correlations at 1-per cent level.

Similarly, the correlations of the number of sticky chromosomes (V_4) with all the types of abnormalities observed showed highly significant correlations at 1 per cent level. Correlations of the number of sticky disoriented/non-oriented chromosomes (V_5) with multivalents, bivalents and laggards showed highly significant correlations at 1-per cent level while that with univalents showed significant correlation at 5-per cent level. Correlations of the number of multivalents observed (V_6) with all the types of abnormalities observed showed highly significant correlations at 1-per cent level. Correlation of the number of bivalent (V_7) with the number of univalents (V_8) showed highly significant correlation. The correlation coefficients of the abnormalities observed at metaphase is shown in Table 15.

Table 15: CORRELATION COEFFICIENTS OF THE ABNORMALITIES AT METAPHASE IN POPCORN (*Zea mays var. praecox Sturt.*) TREATED WITH COLCHICINE

	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9
V_1	1.000	-0.431^{ns}	0.986^{xx}	0.951^{xx}	0.959^{xx}	0.890^{xx}	0.751^{xx}	0.627^{xx}	0.714^{xx}
V_2		1.000	-0.577^{xx}	-0.655^{xx}	-0.432^{ns}	-0.528^{x}	-0.139^{ns}	-0.201^{ns}	-0.561^{xx}
V_3			1.000	0.983^{xx}	0.949^{xx}	0.904^{xx}	0.707^{xx}	0.606^{xx}	0.751^{xx}
V_4				1.000	0.880^{xx}	0.940^{xx}	0.658^{xx}	0.567^{xx}	0.766^{xx}
V_5					1.000	0.753^{xx}	0.664^{xx}	0.542^{x}	0.670^{xx}
V_6						1.000	0.685^{xx}	0.610^{xx}	0.707^{xx}
V_7							1.000	0.921^{xx}	0.355^{ns}
V_8								1.000	0.413^{ns}
V_9									1.000

KEY: x – significant at 5% level; xx – significant at 1% level, ns – not significant
V_1. V_9 represents different types of abnormalities observed at Metaphase.

4.19 Mean square estimates of the abnormalities at Metaphase in Popcorn treated with Colchicine.

The results indicated that the mean square estimates of both the metaphase and the treatments were highly significant at 1-per cent level in the case of the total number of pollen mother cells (PMCs) (V_1) observed. The mean square estimates of the treatment was highly significant at 1-per cent level in the case of the total number of PMCs showing clear separation (V_2). The mean square estimates of the metaphase and the treatments were highly significant at 1-per cent level in the case of the total abnormal PMCs (V_3) observed. Similarly, as regards to the number of sticky chromosomes (V_4), the mean square estimates of the metaphase and the treatments were highly significant at 1-per cent level. The mean square estimates of the metaphase and the treatments in the case of the number of sticky disoriented/non-oriented chromosomes (V_5) were highly significant at 1-per cent level. The mean square estimates of the metaphase and the treatments were highly significant at 1-per cent level in the case of the number of multivalents (V_6) observed. In addition, the mean square estimates of the metaphase was highly significant at 1-per cent level in the case of both the number of bivalents (V_7) and the number of univalents (V_8) observed. The mean square estimates of the treatments was highly significant at 1-per cent level in the case of the number of laggards (V_9) observed. The mean square estimates of the abnormalities at metaphase is shown in Table 16.

TABLE 16: MEAN SQUARE ESTIMATES OF THE ABNORMALITIES AT METAPHASE IN POPCORN (*Zea may var. praecox Sturt.*) TREATED WITH COLCHICINE

Source/ DF	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9
ANAP 1	1504261.25^{xx}	1411.20^{ns}	1418846.45^{xx}	416449.80^{xx}	154880.00^{xx}	2247.20^{xx}	3175.20^{xx}	2184.05^{xx}	28.80^{ns}
TRTS 9	338507.16^{xx}	21966.47^{xx}	458065.34^{xx}	167922.58	62538.36^{xx}	485.89^{xx}	56.53^{xx}	70.85^{ns}	76.91^{xx}
Error 9	19435.03	545.42	25317.34	8281.80	6364.78	59.98	56.53	70.83	10.69
Corrected									
Total 19									

KEY: x – significant at 5% level; xx-highly significant at 1% level; ns – not significant.

4.20 Mean values of the abnormalities at Metaphase observed in Popcorn treated with Colchicine

The mean values of the total number of pollen mother cells (PMCs)-(V_1), total number of abnormal PMCs (V_3), number of sticky chromosomes (V_4), number of sticky disoriented/non-oriented chromosomes (V_5), multivalents (V_6), bivalents (V_7) and univalents (V_8) observed were significant at 5-per cent level. The proportions of the number of the mean values were higher in the case of metaphase I throughout all the variables used. The mean values of the abnormalities at metaphase is shown in Table 17.

TABLE 17: MEAN VALUES OF ABNORMALITIES AT METAPHASE IN POPCORN (*Zea mays. var. praecox Sturt.*) TREATED WITH COLCHICINE

	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Meta.1	1586.20[a]	166.70[a]	1419.50[a]	746.50[a]	573.70[a]	39.60[a]	25.20[a]	20.90[a]	15.40[a]
Meta. 2	1037.70[b]	149.90[a]	886.80[b]	457.90[b]	397.70[b]	18.40b	0.00[b]	0.00[b]	13.00[a]

Means with the same letter or same superscripts under the same variable are not significantly different, otherwise they are different using Duncan Multiple range Test (DMRT).

4.21 Mean estimates of abnormalities at Metaphase observed in Popcorn treated with Colchicine.

The mean estimate values of the total number of PMCs (V_1), total number of PMCs showing clear separation (V_2), total abnormal PMCs (V_3), number of sticky chromosomes (V_4), number of multivalents (V_6), number of bivalents (V_7) and the number of laggards (V_9) observed were significant at 5-per cent level while the number of sticky disoriented/non-oriented chromosomes (V_5) was significant at 5-per cent level only with the control (0-treatment). The mean estimates of the abnormalities at metaphase is shown in Table 18.

TABLE 18: MEAN ESTIMATES OF ABNORMALITIES AT METAPHASE IN POPCORN (*Zea mays var. praecox Sturt.*) TREATED WITH COLCHICINE

	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9
Treatments	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
0.15% 4-hours	1838.0^{a}	22.5f	1810.5^{a}	1095.0^{a}	619.5^{a}	59.0^{a}	11.5^{ab}	7.5^{a}	23.0^{a}
0.15% 2-hours	1664.0^{ab}	27.5^{f}	1636.5^{ab}	905.0^{ab}	641.0^{a}	39.5^{b}	20.5^{a}	15.5^{a}	15.0^{bcd}
0.15% 1-hour	1344.5^{bc}	65.5^{ef}	1279.0^{bc}	641.0^{cd}	563.5^{a}	39.0^{bc}	18.0^{ab}	19.0^{a}	14.5^{bcd}
0.1% 4-hours	1413.0^{bc}	93.5^{e}	1319.5^{bc}	711.5^{bc}	529.5^{a}	40.0^{b}	13.0^{ab}	11.0^{a}	19.5^{abc}
0.1% 2-hours	1387.5^{bc}	174.0^{cd}	1213.5^{c}	626.5^{cd}	510.5^{a}	36.5^{b}	13.5^{ab}	14.0^{a}	12.5^{abc}
0.1% 1-hour	1280.5^{c}	212.5^{bc}	1068.0^{c}	528.0^{cd}	478.0^{a}	25.5^{bc}	13.0^{ab}	12.0^{a}	11.5^{cd}
0.05% 4 hours	1301.0^{c}	153.5^{d}	1147.5^{c}	572.5^{cd}	500.0^{a}	24.5^{bc}	13.0^{ab}	16.0^{a}	21.5^{ab}
0.05% 2 hours	1312.5^{c}	252.5^{b}	1060.0^{c}	514.5^{cd}	493.5^{a}	23.5^{bc}	11.5^{ab}	5.0^{a}	12.0^{cd}
0.05% 1 hour	1317.5^{c}	331.5^{a}	986.0^{c}	428.0^{d}	515.0^{a}	17.0^{cd}	12.0^{ab}	3.0^{a}	11.0^{d}
0 (control)	261.0^{d}	250.0^{b}	11.0^{d}	0.0^{e}	6.5^{b}	1.5^{d}	0.0^{b}	1.5^{a}	1.5^{e}

4.22 Correlations of the abnormalities at Anaphase in Popcorn treated with Colchicine

Correlations of the total number of pollen mother cells (PMCs) – (V_1) with the total number of PMCs showing clear separation showed highly significant correlation at 1-per cent level while that with the total number of abnormal PMCs, number of unequal separation and the number of laggards showed significant correlations at 5-per cent level. Correlations of the total number of PMCs showing clear separation (V_2) with the total abnormal PMCs, number of dicentric bridges, number of unequal separation and the number of multivalents showed highly significant correlations at 1-per cent level while that with the number of multivalents showed significant correlation at 5-per cent level. Correlations of the total abnormal PMCs (V_3) with dicentric bridges, number of unequal separation and the number of multivalents showed highly significant correlations at 1-per cent level while that with the number of laggards showed significant correlation at 5-per cent level. Correlation of the number of single chromatid bridges (V_4) with the number of laggards showed highly significant correlation at 1-per cent level. Correlation of the total number of dicentric bridges (V_5) with the number of multivalents showed highly significant correlation at 1-per cent level. Correlation of the number of unequal separation (V_6) with the number of laggards (V_7) showed highly significant correlation at 1-per cent level. The correlation coefficients of the abnormalities at anaphase is shown in Table 19.

TABLE 19: CORRELATION COEFFICIENTS OF THE ABNORMALITIES AT ANAPHASE IN POPCORN (*Zea mays var. praecox Sturt.*) TREATED WITH COLCHICINE

	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
V_1	1.000	0.882^{xx}	-0.479^{x}	-0.212^{ns}	-0.337^{ns}	-0.475^{x}	-0.452^{x}	-0.100^{ns}
V_2		1.000	-0.837^{xx}	-0.267^{ns}	-0.620^{xx}	-0.611^{xx}	-0.580^{xx}	-0.448^{x}
V_3			1.000	0.253^{ns}	0.762^{xx}	0.585^{xx}	0.555^{x}	0.716^{xx}
V_4				1.000	-0.362^{ns}	0.364^{ns}	0.813^{xx}	-0.357^{ns}
V_5					1.000	0.130^{ns}	-0.058^{ns}	0.867^{xx}
V_6						1.000	0.691^{xx}	0.164
V_7							1.000	0.089^{ns}
V_8								1.000

KEY: x – significant at 5% level; xx – significant at 1% level; ns – not significant. (V_8) – number of mulivalents.
V_1 - V_8 represents different types of abnormalities observed at anaphase.

4.23 Mean square estimates of the abnormalities at Anaphase in Popcorn treated with Colchicine.

The results indicated that the mean square estimates of both the anaphase and the treatments were highly significant at 1-per cent level in the case of the total number of PMCs (V_1) observed. The mean square estimate of the treatments was highly significant at 1-per cent level in the case of the total number of PMCs showing clear separation (V_2). The mean square estimates of both the anaphase and that of the treatments were highly significant at 1-per cent level in the case of the total abnormal PMCs (V_3) observed. In the case of the number of single bridges (V_4), the mean square estimate of the treatments was highly significant at 1-per cent level. The mean square estimates of both the anaphase and that of the treatments were highly significant at 1-per cent level in the case of the dicentric bridges (V_5). As regards to the number of laggards (V_7), the mean square estimate of the treatments was highly significant at 1-per cent level. The mean square estimate of the anaphase was highly significant at 1-per cent level in the case of the number of multivalents (V_8). The mean square estimates is shown in Table 20.

TABLE 20: MEAN SQUARE ESTIMATES OF THE ABNORMALITIES AT ANAPHASE IN POPCORN (*Zea may var. praecox Sturt.*) TREATED WITH COLCHICINE

Source DF	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
ANAP 1	2576.45^{xx}	51.20^{ns}	1881.80^{xx}	11.25^{ns}	684.45^{xx}	7.20^{ns}	6.05^{ns}	140.45^{xx}
TRTS 9	1210.05^{xx}	3885.91^{xx}	863.76^{xx}	89.16^{xx}	449.12^{xx}	19.11^{ns}	59.94^{xx}	28.58^{ns}
Error 9	53.78	59.98	57.02	13.03	24.23	11.09	1.72	9.78
Corrected Total 19								

KEY: x – significant at 5% level; xx – significant at 1% level; ns – not significant

4.24: **Mean values of the abnormalities at Anaphase observed in Popcorn treated with Colchicine.**

The mean values of the total number of PMCs (V_1), total abnormal PMCs (V_3), number of dicentric chromatid bridges (V_5) and the number of the multivalents (V_8) were significant at 5-per cent level. The proportions of the number of the mean-values were highest in the case of anaphase I in all the types of abnormalities considered except in the case of the number of unequal separation (V_6) observed where the mean-value for anaphase II was higher than in anaphase I. The mean – values of the abnormalities at anaphase is shown in Table 21.

TABLE 21: MEAN VALUES OF ABNORMALITIES AT ANAPHASE IN POPCORN (*Zea mays var. praecox Sturt.*) TREATED WITH COLCHICINE.

	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Anap.I	101.30 [a]	28.90 [a]	72.30 [a]	11.70 [a]	32.20 [a]	8.90 [a]	11.10 [a]	8.40 [a]
Anap.2	78.60 [b]	25.70 [a]	52.90 [b]	10.20 [a]	20.50 [b]	10.10 [a]	10.00 [a]	3.10 [b]

Means with the same letter or same superscripts under the same variable are not significant, different, otherwise they are different using Duncan Multiple Range Test (DMT).

4.25: **Mean estimates of abnormalities at Anaphase observed in Popcorn treated with Colchicine.**

The mean estimate values for all the types of abnormalities considered were significant at 5-per cent level except in the case of the total number of PMCs showing clear separation (V_2) where all the different treatments were not significantly different but was significantly different with the control (0-treatment). The mean estimate values of abnormalities at anaphase is shown in Table 22.

Means with the letter or same superscripts under the same variable are not significantly different, otherwise they are different using Duncan Multiple Range Test (DMRT).

TABLE 22: MEAN ESTIMATES OF ABNORMALITIES AT ANAPHASE IN POPCORN (*Zea mays var. praecox Sturt.*) TREATED WITH COLCHICINE

	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8
Treatments	Mean	Mean	Mean	Mean	Mean	Mean	Mean	Mean
0.15% 4-hours	74.00 ^{c}	2.00 ^{b}	72.00 ^{ab}	2.50 ^{d}	50.50 ^{a}	7.00 ^{ab}	4.50 ^{d}	12.50 ^{a}
0.15% 2-hours	81.50 ^{bc}	11.00 ^{b}	70.50 ^{ab}	6.00 ^{cd}	40.00 ^{abc}	9.50 ^{ab}	5.60 ^{d}	8.50 ^{abc}
0.15% 1-hour	76.50 ^{c}	13.00 ^{b}	63.50 ^{ab}	10.00 ^{bcd}	28.50 ^{cde}	9.00 ^{ab}	10.50 ^{c}	5.50 ^{abcd}
0.1% 4-hours	95.00 ^{b}	13.50 ^{b}	81.00 ^{a}	9.00 ^{bcd}	40.50 ^{ab}	11.00 ^{a}	10.50 ^{c}	10.00 ^{ab}
0.1% 2-hours	83.50 ^{bc}	13.50 ^{b}	70.00 ^{ab}	11.00 ^{bcd}	30.50 ^{abcd}	11.00 ^{a}	12.50 ^{bc}	5.00 ^{abcd}
0.1% 1-hour	82.00 ^{bc}	15.50 ^{b}	66.50 ^{ab}	12.50 ^{bc}	25.00 ^{de}	10.00 ^{ab}	14.50 ^{b}	4.50 ^{abcd}
0.05% 4 hours	76.50 ^{c}	14.50 ^{b}	62.00 ^{b}	12.50 ^{bc}	19.00 ^{def}	11.00 ^{a}	13.00 ^{bc}	6.50 ^{abcd}
0.05% 2 hours	82.00 ^{bc}	17.50 ^{b}	64.50 ^{ab}	17.50 ^{ab}	17.00 ^{ef}	11.50 ^{a}	15.50 ^{ab}	3.00 ^{bcd}
0.05% 1 hour	91.00 ^{bc}	20.50 ^{b}	70.50 ^{ab}	25.00 ^{a}	12.50 ^{f}	13.00 ^{a}	18.00 ^{a}	2.00 ^{cd}
0 (control)	157.50 ^{a}	152.00 ^{a}	5.50 ^{c}	3.50 ^{d}	0.00 ^{g}	2.00 ^{b}	0.00 ^{e}	0.00 ^{d}

CHAPTER FIVE

DISCUSSION

Results of the investigation showed that the behaviour of the two inbred line strains (A and B) of popcorn in terms of growth and development looked similar. Most of the seeds from both the two strains germinated 3-days after sowing while the remaining seeds germinated 4-days after sowing and at the same time they exhibit definite time patterns in their developmental cycles including tassel development. This is in line with the observation made by Leng (1951) who concluded that corn exhibit definite time patterns in tassel development. Behaviour at meiosis in both strains used in the first trial was also completely normal.

In general, cytokinesis between the daughter nuclei at the end of the first meiotic division was observed in the two strains. At the end of telophase II, cytokinesis resulted into 4-daughter nuclei. This is in agreement with the findings of Rhoades (1950) who stated that the cell plate arising at telophase, divides the mother cell into two daughter cells. A cell plate is formed between the daughter nuclei, and the second meiotic division is now completed. Ghidoni (1975) stated that additional information on the cell plate formation comes from other workers who reported that once meiosis is completed, the four spores of each quartet remain together for sometimes and the two division planes can be identified.

The data obtained on the effects of colchicine in arresting cell division at metaphase using strain C of popcorn at various concentrations (0.05, 0.1 and 0.15 per cent) and for different periods of treatment (1, 2 and 4 hours) showed

that the effects varied mostly with the concentration and duration of treatment. High manifestation effects were obtained with high concentration and high duration of treatment but using the three range of concentrations and at different treatment periods, it was observed that effective metaphase arrest was obtained from all the samples used. This is in agreement with the statement of Walker (1973) who stated that concentrations for metaphase arrest range from 0.05 to 1 per cent aqueous colchicine solutions for the time periods of 1-4 hours.

The results indicated in Table 1 presents data showing meiosis only at 6.00pm. This probably showed that meiosis have just commenced in the inflorescence of that plant around that period. This is in agreement with the findings of Love and Love (1975) who stated that in many plants meiosis commences in the afternoon or evening and is completed next morning.

The results given in Table 2 presents data on meiosis at different stages of divisions for different periods of fixation. Most samples showed only pre-meiotic pollen mother cells (interphase). While anthers taken at 9.00am showed pollen mother cells with the stages upto the end of the first meiotic division (leptotene to telophase I), the anthers from the sample taken at 6.00pm showed pollen mother cells with all the various meiotic stages upto the end of the second division. This was probably due to the full commencement of meiosis around that period which is also in agreement to the statement of Love and Love (1975) who stated that meiosis commences in many plants in the afternoon or evening.

As the results in Table 3 showed, meiotic stages have taken place only at 6.00pm. An unexpected feature of this result is the occurrence of meiosis only at 6.00pm on the 55th day after sowing as it was indicated in the case of the results that occurred on the 53rd day after sowing. This result disagree with the observation of Mock and Frey, 1970, who reported that meiosis advance linearly as days after emergence increased. This result was obtained probably because in the case of this particular plant from which the anthers were obtained, meiosis have just began only on this 55th day after sowing since from what was observed some seeds germinated 3-days after sowing while others 4-days after sowing.

The data obtained on meiotic stages in Table 4 for different periods of fixation showed that in all the samples examined, most were still in early phases of division. The differences in the linear advancement of meiosis between the two results shown in Tables 2 and.4 may be due to the differences in the plant-ages (anther developmental stages). This is in line with the findings of Bouchard and Walden (1990) who reported a fair degree of consistency in the relationship of anther developmental stage to microsporocyte stages. Similarly, Mock and Frey (1970) reported meiosis to advance linearly with days after emergence.

The results given in Table 5 presents data on meiosis for all the different periods fixation was made. The anthers collected from all these different plants were at their right developmental stage of meiotic activity. This showed that meiotic activity advance regardless of the period of the day fixations were

made. These results are comparable to those of Mock Frey (1970) who stated that meiosis advance linearly as days after emergence increased.

The results given in Table 6 presents data on meiosis which showed all the various stages of meiotic divisions throughout all the periods of the day fixations were made except in the case of the sample taken at 6.00pm. In the case of this sample taken at 6.00pm, the anthers examined showed only pollen mother cells with meiotic stages upto diakinetic stage (interphase and prophase I substages). No meiotic activity was observed from the anthers in the case of this B-strain after the 57th day. Anthers at different levels of maturity examined on this day showed pollen grains along with others that were still undergoing meiotic activity from the same samples which were taken after the tassels emerged. This is in agreement with the findings of Bouchard and Walden (1990) who stated that there is lack of synchrony among developing anthers in maize.

As the results in Table 7 showed, meiotic activity ended on this fifty ninth (59th) day after sowing in the case of stain A. The data presented on this table showed pollen mother cells with meiotic stages whose proportions decreased linearly upto the last sample taken at 12.00noon. This indicated that as the duration of the meiotic activity comes to an end, more and more anthers became fully differentiated into mature pollen grains.

A special aspect of the results which tables 5 and 7 showed is that meiotic activity advance linearly as the ages of the plants increased on

subsequent days throughout the different periods of the days examined until when all the micropsorocytes in the anthers became fully differentiated to mature pollen grains. This showed that once the anthers reached the right developmental stage, meiotic activity advanced regardless of the periods of the day fixations were made. This is comparable to the statement that meiosis advanced linearly with plant-ages (Mock and Frey, 1970). Similarly, as it was observed in the case of strain B, anthers at different levels of maturity examined showed pollen grains along with others that were still undergoing meiotic activity from the same samples which were taken after the tassels emerged. This may also be due to the same reason which is the lack of the synchrony among the developing anthers in maize. The result is in agreement with the findings of Bouchard and Walden (1990) who reported that the chief difficulty in preparing staged material from maize is the lack of synchrony among developing anthers. In a single tassel, one may find anthers in spikelets of the lowest lateral branches containing microsporocytes in early meiotic phase, while microsporocytes of spikelets midway up the main spike will have already reached the uninucleate microspore stage. Spikelets of a particular portion of the tassel, such as the central spike, are in closer synchrony with one another. However, even at the level of the individual spikelet, the existence of two florets at different points of growth means that two different stages of anther and microsporocytes development are represented (Bouchard and Walden, 1990).

Correlations of the meiotic stages has shown that interphase (V_1) stage has no significant correlation with all the stages of the meiotic divisions. Stages of the first division are highly significantly correlated at 1% level with one another but are less correlated with the stages of the second division. Similarly, stages of the second division are also highly correlated with one another (Table 12). As regards the times of fixation, it was seen that interphase and stages of the first division were not significant but it was significant in the case of the stages of the second division. In the same manner, regarding the age of the plants, it was also seen that it was significant in the case of the stages of the second division but not significant in the cases of the both the interphase and the stages of the first division (Table 13). Thus, the stages of the second division are more influenced by the times of fixation and the ages of the plants.

The proportions of the number of the PMCs at different times of fixation has shown that the frequency of the interphase cells and the stages of the first division were more in the morning and the stages of the second division were more predominant in the evening (Table 14). This indicated that these interphase cells were more in the early morning of the day, then went meiotic division as the day passes and by the evening time most (interphase cells) would have completed the second meiotic division.

In general, although the events occurring in the microsporocytes from meiosis through differentiation of mature pollen grains shown by the two strains of popcorn used looked similar, but certain significant differences were observed between the two strains:

In terms of comparing their meiotic activities with their ages, it was observed that strain B was more linearly advanced than strain A (Tables 2, 4 and 4.6 compared with Tables 1, 3 and 7).

Besides, the duration of meiosis (number of days) taken was longer in the case of strain A (7-days) than in the case of strain B (5-days).

In addition, complete meiotic stages/divisions were examined in the case of strain A from all the samples that meiotic activity were observed right from the first day (53rd day after sowing) the actual meiotic activity were observed to have started to the last day (59th day after sowing) when it was brought to an end while in the case of strain B samples that have started meiotic activity showed stages/divisions at different phases: some only prohase sub-stages (leptotene to diakinesis), some stages up to the end of the first division (leptotene to telophase I) and in some cases complete meiotic divisions were observed (all the various stages of the first and second divisions) – (see Tables 2, 4 and 6).

Similarly, this aspect of the study was conducted to compare the effectiveness of the applicability of colchicine to metaphase arrest in relation to dose and duration of treatment as well as to study the chromosome behaviour at various stages of meiosis.

Treatment of anthers with 0.05, 0.1 and 0.15 per cent colchicine solutions for the time periods of 1, 2 and 4 hours as indicated in used Tables 8 and 10 resulted in the accumulation of metaphase cells in both the meiotic divisions (metaphase I and metaphase II) was an important effect observed. This is in agreement with the statement that the favoured concentration for metaphase arrest range from 0.05 to 1 per cent aqueous colchicines solution being used for time periods between 1-4 hours (Walker, 1973). Similar observation was made by Izumi *et. al.,* 1983 in relation to the accumulation of metaphase cells as a result of treatment of the colchicine treatment. The mean values of the number of PMCs were higher in the case of metaphase I than in metaphase II as indicated in Table 4.17 as most of the PMCs were arrested at metaphase I. The metaphase chromosomes were more contracted than normal with stickiness manifestation as was also stated by Poggio *et. al.,* (1990). The frequency of the chromosomal abnormalities varied in different treatments. The mean estimate values in both the total abnormal PMCs (V_3) and the number of sticky chromosomes (V_4) observed indicated that the treatments of 0.15% 4-hours were the highest in both the two cases while the mean estimate values for the control were not at all comparable with all the treatments used showed that both the concentration and the period of the treatment influences the effectiveness of the chemical (Table 18). Thus, the effects of the chromosomal abnormalities on pollen mother cells revealed that the frequency was directly proportional to dose and duration of treatment as stated by Kumar *et. al.,* (1997).

Cytological analysis of anaphase I and anaphase II as indicated in Tables 9 and 11 revealed that bridge formation (mostly multiple bridges) and chromosome separation which was not always complete were often seen in anaphase and telophase (Dayal, 1979). From the number of the mean estimate values obtained for all the treatments, it was observed that the number of the dicentric chromatied bridges (V_5) were the highest in the case of 0.15% 4-hours (Table 22). The dicentric bridges were observed to be the commonest abnormality with the anaphase stage. This has shown the influence of both the concentration and the period of treatment considered. In the case of the control, there was no even single dicentric bridge observed although there were very few PMCs which were observed to have shown other abnormal conditions. The anaphase bridges probably arise due to splitting error or to local disturbances in chromosome duplication or to primary structural changes in chromosomes (Dayal, 1979). Occasionally laggards were also observed (Kumar and Sachan, 1993).

In the control plants (untreated), meiosis had normal stages with insignificant deviations (Table 10, 18 and 22). They are characterized by the regularity of tetrads. Cytological examination revealed no changes in chromosome behaviour at all stages.

Generally, these anthers (treated differently) differ among themselves as well as from the control population in spectrum of the meiotic abnormalities. Such meiotic abnormalities probably were responsible for the blockage of meiotic stages. This is in agreement with the findings of D'Amato (1977) who

stated that an abnormality of the meiotic process as expected, the result is a block of the meiotic stage controlled by the gene(s) involved. It appears that the division process in general has probably been affected by such treatments due to disturbances in normal progression of the nuclear cycle and/or disturbances in the spindle apparatus.

Summary and Conclusion

This work attempts to study meiotic activity in two strains (A and B) inbred lines of popcorn (*Zea mays var. praecox Sturt.*) which were used in the first trial and in the second trial another strain (C) of popcorn was pretreated with various concentrations of colchicine solutions at different periods of treatment. Inflorescences at different plant ages and times of fixation in the first trial and selected pretreated anthers from the young unopened spikelets were fixed in Carnoy's fluid and stored in 70% alcohol in the refrigerator until when needed. Slides were prepared using 1% aceto-orcien and sealed with nail varnish.

The result demonstrated that meiotic activity of the pollen mother cells (PMCs) was normal in the two strains of the first trial from the interphase stage to the formation of four haploid microspores. Two cell plates were formed following the first and second meiotic divisions. Fifty two days after sowing pre-meiosis (interphase) stage was seen from the anthers observed in both the two strains used. In strain A, stages of meiosis were observed from fifty third to fifty ninth day after sowing (duration of meiosis was seven days) while in the

case of strain B, stages of meiosis were observe from fifty third to fifty seventh day after sowing (duration of meiosis was five days).

There was no significant correlation between the interphase stage and all the stages of meiotic divisions. Stages of the first division were highly significantly correlated at 1-per cent level with one another ($P<0.01$). Also the stages of the second division were highly significantly correlated at 1-per cent level with one another ($P<0.01$). The proportions of the number of the PMCs at different times of fixation have shown that the frequency of the interphase cell and the stages of the first division were more in the morning and the stages of the second division were more predominant in the evening. Similarly, the stages of the second division were more influenced by the times of fixation and the ages of the plants.

Regarding the applicability of colchicine to metaphase arrest, the result demonstrated that the frequency of the chromosomal abnormalities varied in different treatments. The frequency was directly proportional to dose and duration of treatment. Treatments led to accumulation of more cells in meiosis I than meiosis II and also the arrest of cells in metaphase I and metaphase II. At metaphase stages, the most frequent aberrations were chromosomes with stickiness manifestation and also sticky disoriented/non-oriented chromosomes while at anaphase the most frequent aberration observed were chromosome bridges (mostly dicentric). Also unequal separation of chromosomes, multivalents as well as chromosme lagging were quite frequent. In contrast to

the treated, plants of the original population (untreated) demonstrated a normal meiosis, although few cells in them, too, were aberrant.

Correlations of the total abnormal PMCs at metaphase with all the types of abnormalities observed showed highly significant correlations at 1-per cent level ($P<0.01$) but there was no significant correlation between the total abnormal PMCs and the PMCs showing clear separation. The mean estimate values of the number of sticky chromosomes observed at different treatments indicated that the treatment of 0.15% 4-hours was the highest while the control was not all comparable with all the treatments used. These sticky chromosomes were the most frequent aberration observed at metaphase. In the same manner, the mean estimate values of the number of dicentric bridges observed for different treatments at anaphase also indicated that the treatment of 0.15% 4 hours was the highest while in the control there was no even single dicentric bridge observed. These have shown the influence of both the concentration and the period of treatment used.

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Key To Plate 1

Figure 1: Leptotene – chromosomes are long and in the form of fine single threads.

Figure 2: Zygotene – Homologous chromosomes become closely associated in pairs (aligned together end to end to form a bivalent).

Figure 3: Pachytene – Chromosomes thicker than at earlier stages and are associated in pairs throughout their length.

Figure 4: Diplotene – The chromosomes contract greatly at this stage. The homologous chromosomes seem to repel each other but remain attached at points called Chiasmata.

Plate 1



FIG.1 – LEPTOTENE

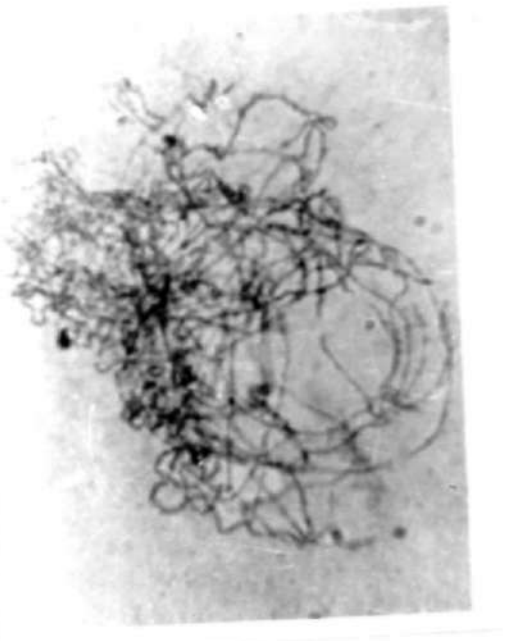


FIG. 2 – ZYGOTENE

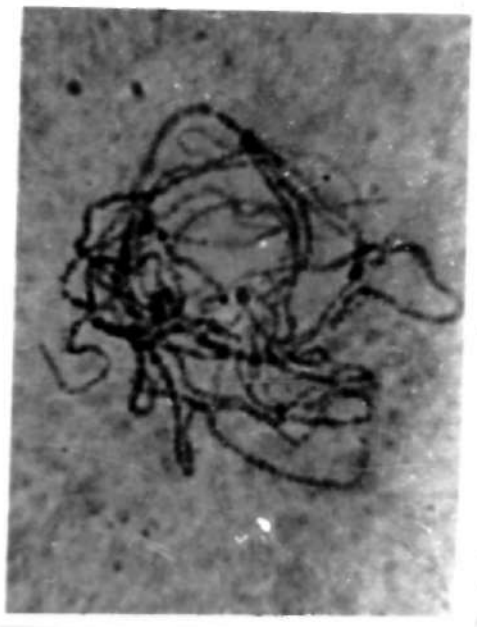


FIG.3 – PACHYTENE

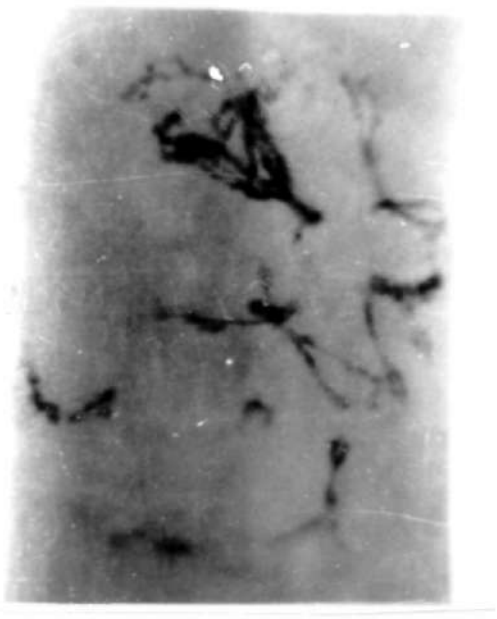


FIG.4 – DIPLOTENE

EARLY STAGES OF MEIOSIS IN POPCRON (*ZEA MAYS VAR. PRAECOX STURT.*)

Key To Plate 2

Figure 5: Diakinesis – The bivalent continue to thicken and move toward the nuclear memberane, distributing themselves evenly. The chromosomes in each bivalent are usually joined by two terminal chiasmata at either end of the chromosomes resulting in a ring formation.

Figure 6: Metaphase I – The members of the bivalents are highly condensed and arranged equidistant on opposite sides of the equatorial plate of the spindle.

Figure 7: Early anaphase I – The members of the homologous chromosome pair move toward opposite poles of the spindle.

Figure 8: Late anaphase I – The two groups of homologous now become completely separated.

Plate 2



FIG.5 – DIAKINESIS

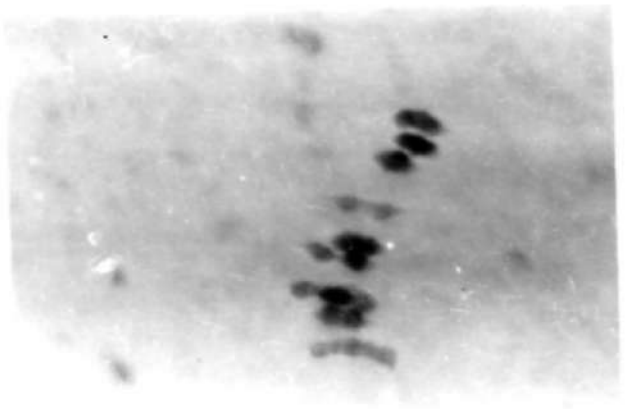


FIG. 6 – METAPHASE I



FIG.7 – EARLY ANAPHASE I

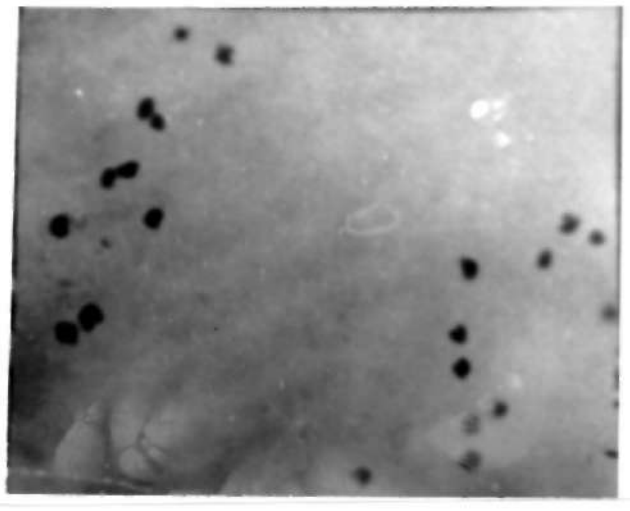


FIG.8 – LATE ANAPHASE I

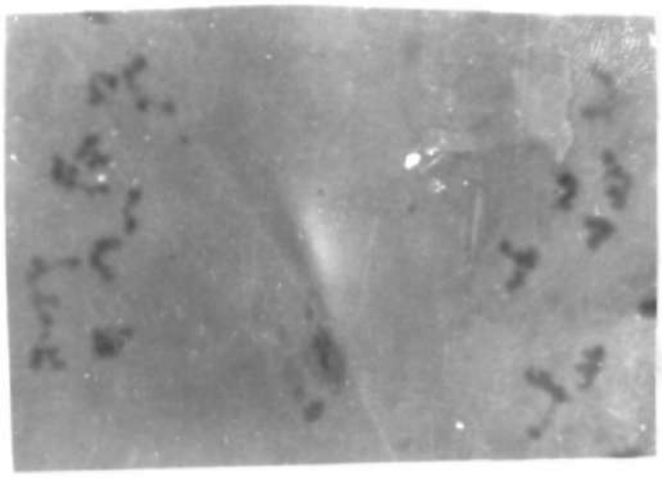
MIDDLESTAGES OF THE FIRST MEIOTIC DIVISION IN POPCORN
(ZEA MAYS VAR. PRAECOX STURT.)

Key To Plate 3

Figure 9: Telophase I with interkinesis – The homologous chromosome pairs have reached opposite poles and each group of chromosomes possesses the haploid number of centromeres.

Figure 10: Dyad formation – Two groups of aggregated chromosomes are formed with a cell plate between the 2-daughter nuclei. This stage concludes the first meiotic division.

Plate 3



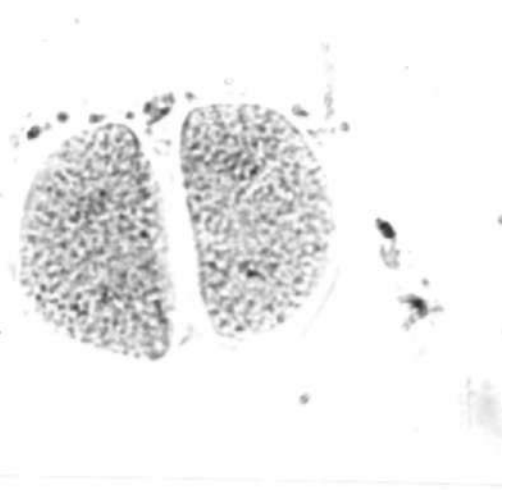


FIG. 9 – TELOPHASE I

FIG.10 – DYAD FORMATION

END OF FIRST MEIOTIC DIVISION IN POPCORN *(ZEA MAYS VAR. PREACOX STURT.)*

Key To Plate 4

Figure 11: Metaphase II – The chromosomes line up on the equatorial plate.

Figure 12: anaphase II – The daughter centromere of each chromosome pull apart toward their respective poles. At this stage, the chromatids of the individual chromosomes separate and go to their respective poles.

Figure 13: Telophase II – Chromosomes stop moving and become diffused.

Figure 14: Tetrad formation (Quartet) – Four microspores with 2-distinct cross – walls (cell-plates) and the second meiotic division is completed.

Plate 4

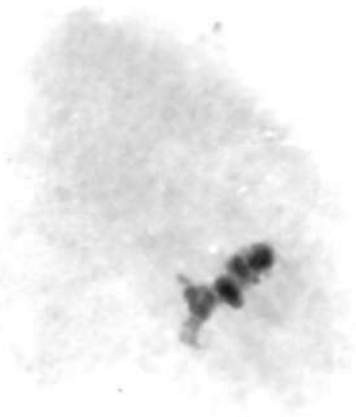


FIG.11 – METAPHASE II



FIG.12 – ANAPHASE II

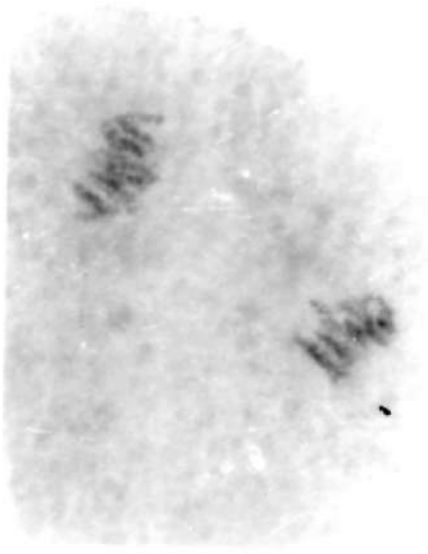


FIG.13 – TELOPHASE II



FIG.14 – TETRAD FORMATION

STAGES OF SECOND DIVISION IN POPCORN (*ZEA MAYS VAR. PRAECOX STURT.*)

Key To Plate 5

Figure 15: Multivalent formations – Contracted and scattered metaphase chromosomes with multivalent formations.

Figure 16: Multivalent formations – Metaphase chromosomes more contracted than normal with multivalent formations.

Figure 17: *Non-oriented chromosomes – Scattered non-oriented* metaphase chromosomes.

Plate 5

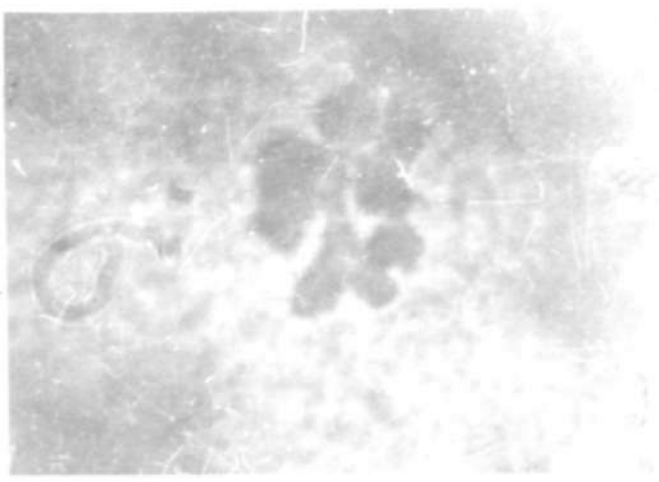


FIG.15 – MULTIVALENT FORMATION

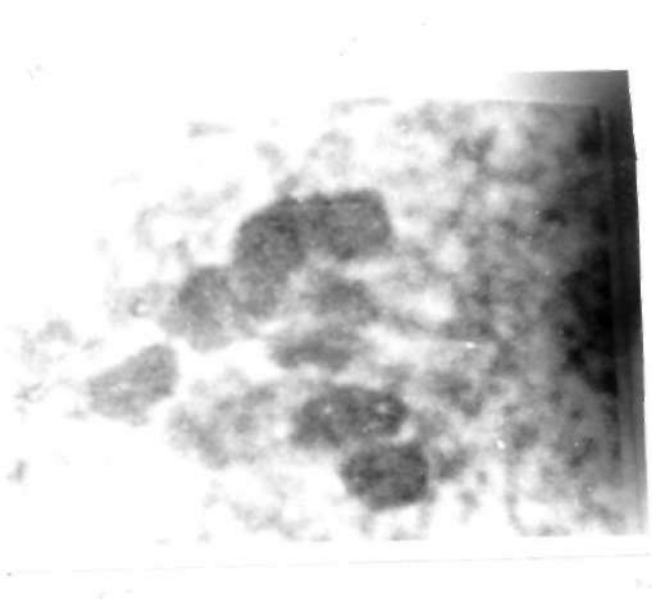


FIG.16 – MULTIVALENTS FORMATIONFIG. 17 – NON-ORIENTEDCHROMOSOMES

ABNORMALITIES AT METAPHASE I IN POPCORN
(*ZEA MAYS VAR. PRAECOX STURT.*) TREATED WITH COLCHICINE

Key To Plate 6

Figure 18: Sticky chromosomes with univalents – Metaphase chromosomes with stickiness manifestation and the presence of univalent chromosomes.

Figure 19: Non-oriented chromosomes – Scattered non-oriented metaphase chromosomes.

Figure 20: Non-oriented chromosomes with univalents – Scattered non-oriented metaphase chromosomes with the presence of 2-univalent chromosomes.

Plate 6



FIG.18 – STICKY CHROMOSOMES WITH UNIVALENT

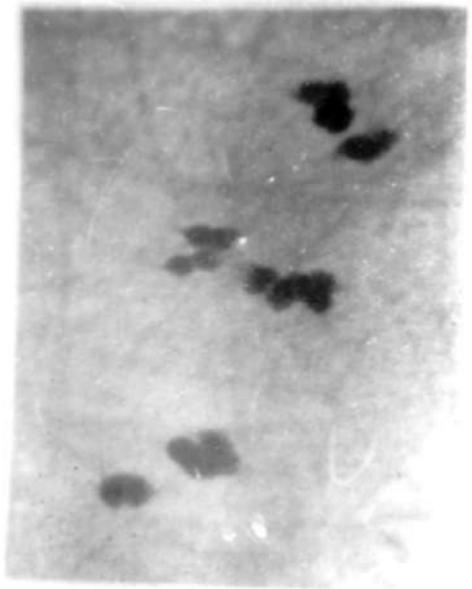


FIG.19 – NON-ORIENTED CHROMOSOMES



FIG.20 – 2-UNIVALENTS AND NON-ORIENTED CHROMOSOMES

ABNORMALITIES AT METAPHASE I IN POPCORN (*ZEA MAYS VAR. PRAECOX STURT.*) TREATED WITH COLCHICINE.

Key To Plate 7

Figure 21: Multivalents with a bridge formation – Multivalent anaphase chromosomes with the presence of a single bridge formation.

Figure 22: Sticky chromosomes – Anaphase chromosomes with a high stickiness manifestation.

Figure 23: Sticky chromosomes with dicentric bridges – Anaphase chromosomes with stickiness manifestation, unequal separation and the presence of dicentric bridges.

Plate 7



FIG.21 – MULTIVALENTS WITH A SINGLE BRIDGE FORMATION

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FIG.22 – STICKY CHROMOSOMES

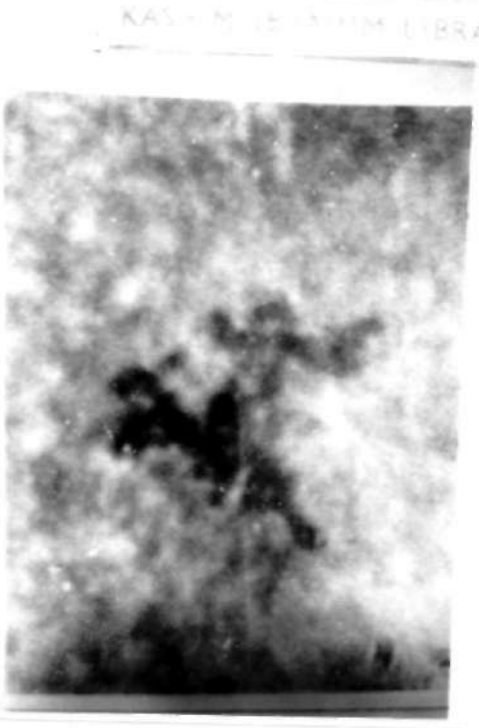


FIG.23– STICKY CHROMOSOMES WITH DICENTRIC BRIDGES

ABNORMALITIES AT ANAPHASE I IN POPCORN (*ZEA MAYS VAR. PRAECOX STURT.*) TREATED WITH COLCHICINE

Arrow in Fig.22 is pointing to dirt on the eyepiece of the microscope

Key To Plate 8

Figure 24: Sticky chromosomes – Metaphase II chromosomes with stickiness manifestation.

Figure 25: Sticky non-oriented chromosomes with multivalents – Metaphase II disoriented chromosomes with the presence of multivalents.

Plate 8

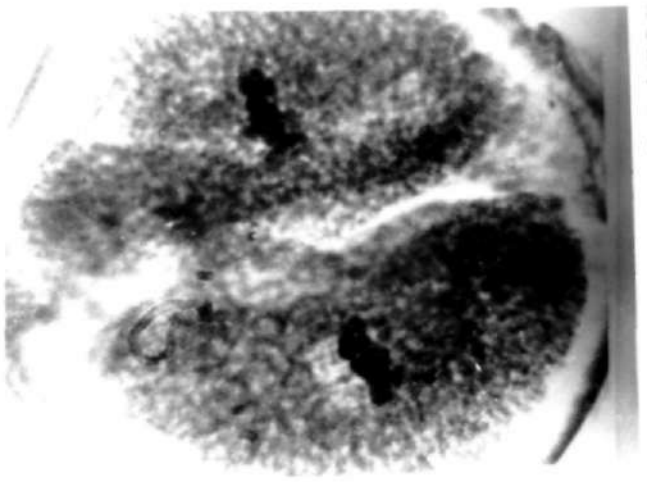


FIG.24 – STICKY CHROMOSOMES

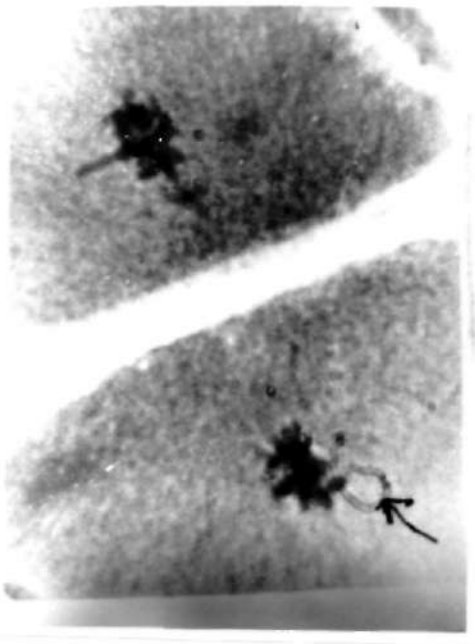


FIG.25 – STICKY NON-ORIENTED CHROMOSOMES WITH MULTIVALENTS

ABNORMALITIES AT METAPHASE II IN POPCORN (*ZEA MAYS VAR. PRAECOX STURT.*) TREATED WITH COLCHICINE.

Arrow in Fig.25 is pointing to dirt on the eyepiece of the microscope