CHROMOSOMAL ABERRATIONS AND VARIATIONS IN CHROMOSOME NUMBER, IN *SORGHUM VULGARE* TREATED WITH X-RAYS AND THERMAL NEUTRONS

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AND THERMAL NEUTRONS

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CHAPTER I

INTRODUCTION

Mutations and chromosomal aberrations have been considered to play an important role in the study of the mechanisms of heredity and evolution. These mutations and chromosomal aberrations may arise spontaneously in nature; however, Muller (1927) and Stadler (1928) independently discovered that X-rays can increase the mutation rate in animals and plants, respectively. Since then a considerable amount of effort has been devoted to the study of radiation-induced mutations and chromosomal aberrations. Recently, a large proportion of the work on chromosomal aberrations has been directed toward studies of both biophysical and biochemical aspects of aberrations.

Change in chromosome structure and organization following the exposure of cells to different mutagenic agents can be viewed from a number of aspects. The important mutagenic agents which have been discovered are physical agents such as ionizing radiations and chemical agents such as nitrogen mustard and certain organic peroxides. These mutagenic agents usually differ in efficiency in inducing chromosomal aberrations.

Generally, the breakage and rejoining of the chromosomes caused by mutagenic agents result in four types of chromosomal aberrations, i.e., deletions (deficiencies), inversions, duplications, and translocations showing the different cytological configurations such as rods (fragments), chains, rings, and crosses. Translocations are of special value in genetic studies in determining the correspondence of loci on the linkage maps and as a means of marking or controlling chromosomes or parts of chromosomes which are being studied.

In the past thirty years, many different plants and animals have been used for such studies. In plants, Zea, Hordeum, Oryza, Triticum and Tradescantia, have been used extensively because of certain individual advantages. However, sorghum with small chromosomes has rarely been used for such studies; thus, very few genetic markers have been studied so far. The present study was conducted for the purpose of providing further information on sorghum cytogenetics. X-ray and thermal neutron radiations which have been used widely in plants were employed. The main objectives of this experiment, therefore, are: (1) to identify cytologically any chromosomal aberrations; (2) to determine the suitable dosages of both types of radiations for production of chromosomal aberrations, particularly translocations; and (3) to establish homozygous translocation stocks for genetic studies.

In previous studies with the sorghum variety, Wheatland, frequent abnormalities in chromosome number including polyploidy and aneuploidy, were observed in microsporocytes. These were also observed in this study.

In addition, although some cytological studies of chromosome morphology of sorghum have been conducted in recent years, no complete account of meiosis, so far as is known, has been available. A series of photomicrographs of the different meiotic stages are presented in the Appendix.

CHAPTER II

REVIEW OF LITERATURE

Studies of the effects of radiations on organisms began when Muller (1927) in Texas showed in *Drosophila* that X-rays were able to induce mutations indistinguishable from those that appeared spontaneously. The notable feature of the discovery was that X-rays gave an induced mutation rate many times greater than the natural one. Muller (1928) designed the ClB method which enabled him to detect a great increase in mutation frequency in *Drosophila* after using X-rays. The following year, Stadler in Missouri obtained the same effects of X-rays on seedlings of barley and on the endosperm and seedlings of maize. Since then a variety of mutagenic agents have been discovered for the induction of mutations and chromosomal aberrations.

Various Mutagenic Agents For Inducing Chromosomal Aberrations

In general, the cytologically and genetically effective radiations are of two kinds: ionizing and non-ionizing. The ionizing radiations are the α-, β-, and γ- radiations of radioactive substances, X-rays, protons and neutrons. The only effective non-ionizing radiation is ultraviolet light (Swanson, 1957). In addition to radiations, the important discovery of mutagenicity of nitrogen mustard (Auerbach and Robson, 1946) opened up a new field and stimulated considerable research on the important roles played by various chemicals in induction of chromosomal aberrations. More recently, vegetable oil was also found to be effective as a mutagenic agent (Swaminathan and Natarajan, 1956, 1959; Thomas, 1961). Although the modes of action of the various types of radiations and of chemical mutagenic agents may be quite different, they have a common feature in producing morphological changes in chromosome structure. Some of them may interact and give increased aberration yields (Swanson, 1944). It was also found that the efficiency of the mutagenic agents was influenced by factors such as temperature, the water content of treated materials, and oxygen tension in the air (Swanson, 1957).

The general idea of the biological action of radiation in inducing mutation and breakage of chromosomes has been established; it is most plausibly attributed to chemical change resulting from the ionization. Since the chemical bonds which hold a molecule together are constituted by electrons shared between the atoms joined by the bond, the removal of a bonding electron is likely to lead to its dissociation or to other chemical change. The removal of electrons other than bonding ones is also likely to lead to chemical changes, since the energy involved in ionization greatly exceeds that required to remove an atom from the molecule. The chemical change is mainly the cause of the mutation or chromosome breakage.

For the production of structural changes in chromosomes it is necessary to irradiate during the resting stage, or early prophase (Evans, 1962). According to Sax (1938), using irradiated Tradescantia, the production of the greatest frequency of chromosomal aberrations is associated with the period of greatest chromosomal activity -- meiotic and mitotic prophase. Many investigators, studying chromosomal aberrations, irradiated seeds or flower buds and studied division figures in the developing pollen or root tip.

Types Of Chromosomal Aberrations Induced By Irradiations

After irradiation, the chromosomes are broken at one or several points. Following breakage, they may rejoin like the original chromosome and function normally (King, 1962) or may rejoin in new ways. Such union will lead either to a single chromosome in which a portion between the breakage points is in reverse order and forms an inversion as in maize (Faberge, 1951) and barley (Burnham et al., 1954) or to two chromosome structures, one a ring formed by the joining of the ends of the segment between the breakage points, and the other a rod formed by the joining of the two remaining fragments. One of these two chromosome structures lacks a centromere, and may be lost at cell division. Thus the result is a deletion, as observed in Triticum (Smith, 1948). If two different chromosomes are broken, the non-homologous union between the four broken ends leads to an interchange (reciprocal translocation). This is the most frequent configuration observed after irradiation.

Of the various types of chromosomal aberrations, interchanges are most interesting to both cytologists and geneticists since the configurations of interchange can be easily identified cytologically and are useful in genetic studies. It is, therefore, necessary to discuss in detail the various cytological configurations of interchange and their orientation during meiosis. The value of interchange in genetics will be discussed later. When chromosome interchanges occur, different features may result depending on the segments interchanged. If the interchange of portions of chromosomes occurs between non-homologous chromosomes, and the interchanged pieces are long, the configuration at the pachytene stage is 4-armed or cross-shaped. If a crossover has occurred in each of the 4-arms, the configuration at diakinesis and metaphase I will be a ring of four chromosomes. If one long piece has exchanged with a very short one, the ring will be rare or non-existent, the pachytene configurations being T-shaped, the metaphase I ones being chain, as in maize (Burnham 1932; Brink and Cooper, 1932; Clarke and Anderson, 1935). If both pieces are short, rings may not occur or may be rare; chains may be most frequent but two "pairs" will be common also, as in maize (Clarke and Anderson, 1935). If two translocation types have one chromosome in common, a ring of six chromosomes will be formed in the hybrid. If segmental relations in the two reciprocal translocation types are quite different, two rings of four chromosomes will be produced (Yamashita, 1951). When a third reciprocal translocation type is crossed with the above type of hybrid (two rings of four chromosomes), three rings of four chromosomes will be produced, if the third reciprocal translocation is quite independent of the first two. By the crossing experiments of reciprocal translocation types, Yamashita (1951) obtained rings of fourteen chromosomes in rice.

In addition, pseudo-isochromosomes are also obtained in X-ray- or neutron-irradiated barley (Caldecott and Smith, 1952; Caldecott et al., 1954; Caldecott, 1955); or maize (Morris, 1955). The pseudo-isochromosome is the result of a reciprocal translocation between major portions of non-homologous arms of homologous chromosomes. The breaks occurred close to the centromeres. In most cases, pseudo-isochromosomes show no tendency to associate with each other or with other chromosomes. Thus, a pseudo-isochromosome consists of a complete centromere and two arms which are identical except for a short interstitial segment (Morris, 1955).

The Value Of Translocations In Genetic Studies

As already mentioned, interchanges of chromosomes are valuable in genetic studies. Anderson (1935) has summarized the value and uses of interchanges in his general statement that they are of special value in building up a knowledge "of such items as the location of given genes on the observed chromosome thread, and the linkage relationships of conspicuous markers on the chromosomes; also to make available means of marking or of controlling desired portions of chromosomes". Reviewing the information on interchanges of chromosomes in plants, Burnham (1962) listed 12 items of value in the uses of interchanges. Some important ones were: (1) to furnish information on chromosome behavior which resulted in a marked advance in cytology; (2) to determine the factors affecting chromosome segregation in interchange complexes as in maize (Burnham, 1950a); (3) to determine: (a) the linkage group carried by each chromosome and the orientation of the linkage map within the chromosome, (b) the linkage group to which new genes belong, and (c) gene positions relative to cytological markers within the chromosomes including the centromere (McClintock, 1931); (4) to study the inheritance of complex characters such as oil content in corn kernel (Miller, 1951); and (5) if the translocation is very unequal a duplication plus deficiency type may survive and be useful in linkage tests (*Zea Mays* and *Triticum Monococcum*, Smith, 1948).

Efficiency Of Radiations In Inducing Chromosomal Aberrations

Radiations usually differ in the efficiency with which they produce a given effect compared on the basis of equal total tissue dose of ionization. According to Evans (1962), the different efficiencies of various radiations are not due to qualitative differences between the radiations, but rather to differences in rate of energy dissipation along the tracks of the ionizing particles in the tissues. The specific ionizing density or the rate of energy dissipation along a particle track is the important factor in determining the relative biological efficiency for chromosome breakage (Giles and Tobias, 1954). In some experiments with Tradescantia the results showed that radiations having similar linear energy transfer gave similar aberration yields, whereas radiations having different linear energy transfer gave different aberration frequencies (Evan, 1962). Aranson and Morrison (1955) stated that experimental results reported by many investigators indicated that, with any ionizing radiation of particular energy, the frequency of chromosome breaks was directly proportional to the radiation dose. Giles (1943) found that protons of lower average energies are more efficient in producing aberrations in Tradescantia than are those of higher average energies. This greater efficiency of lower average energy protons was due to differences in density of ionization. The density with which ion pairs are produced by a proton along its path is a function of its energy, the two being inversely related. Consequently, protons of lower average energy produce more dense ionization and are more efficient in producing chromosome breaks. On the basis of equal dose as measured in roentgens (r), Arnason et al. (1955) also found that rays of high energy produce fewer breaks than did those of lower energy. Comparative efficiencies as judged from one experiment were 1.0 : 0.82 : 0.74 for 200-kev X-rays, 1.25-Mev r-rays, and 23-Mev X-rays, respectively. In another experiment, the efficiencies were 1.0 : 0.73 : 0.68 for 140-kev X-rays, 1.25-Mev r-rays, and 23-Mev X-rays, respectively. The same conclusion was presented also in reports by other investigators. Catcheside et al. (1946) studied Tradescantia and found radium r-rays to be only 77% as efficient as 150-kev X-rays in producing chromatid interchanges. Kirby-Smith and Daniels (1953) reported that X-rays of 60-kev mean-energy were twice as effective as Co^{60} r-rays or 400-kev mean-energy β-rays.

Using X-rays (16,000 r) and atomic bombs (equivalent 16,000 r of X-rays), Smith (1950) indicated that there were no striking differences in the kinds of mutants produced. Rings of four and six chromosomes were found in both irradiated barley and wheat. The frequency of interchanges was considerably higher in the tetraploid (durum) wheat than in the diploid barley. There was a considerably higher frequency of interchanges from the X-rayed than from the atomic bomb-irradiated materials. Compared with the effect of high intensity X-rays, Takabe (1960) found P^{32} treatment was about 1/3 or 1/4 less effective in inducing chromosomal aberrations in pollen mitosis and in root tip of Tradescantia. Lea and Catcheside (1942) found that a minimum of 15 to 20 ionizations were required for the possibility of breakage of a chromatid thread.

A number of researchers have studied the relative efficiency of X-rays and neutrons in inducing chromosomal aberrations. Thoday (1942) reported that thermal neutrons were more efficient than X-rays in inducing chromosomal interchanges of Tradescantia. The same conclusion was drawn from physiological effects such as inhibition of growth (Aebersold and Lawrence, 1942). Results from Sax (1940, 1941) and Thoday (1942), in studying Tradescantia irradiated by X-rays and neutrons, indicated that rings are even more frequent with neutrons than with X-rays. By using barley, Caldecott (1955), Caldecott et al. (1952, 1954) found different rings with different numbers of chromosomes from X-ray and thermal neutron treatment and the data showed that thermal neutrons were more effective than X-rays. They also indicated that for both irradiations there was a linear relationship between the frequency of interchange and the dose of radiation. A similar experiment on rice was conducted by Shastry et al. (1961) and many multivalent configurations were obtained. In irradiated Tradescantia, Sax (1940, 1941), Rick (1940), Marinelli et al. (1942) found that X-ray-induced translocations increase approximately as the square of the dose when the time of irradiation is kept constant. For neutrons, however, the relationship between dosage and frequency of translocations has been found to be approximately linear (Giles, 1940; Thoday, 1942). From these experiments, the greater efficiency of neutrons over X-rays indicated that more than one ionization was necessary to break a chromosome in Tradescantia (Giles, 1940; Thoday, 1942), Vicia faba, and Pisum sativum (Marshak, 1939).

Seed Set Percentage Of Plants With Chromosomal Interchanges

Some chromosomal aberrations, particularly deficiencies, result in the pollen or embryo sac being nonfunctional. In general, seed set percentage is used as an indication of pollen or embryo sac abortion. Theoretically, in the meiosis of translocation plants, if crossing over is ignored, there are three possible types of segregation, i.e., adjacent I, adjacent II, and alternate. Alternate segregation results in zigzag or N configurations and adjacent segregation results in open rings. Chromosome disjunction from the zigzag configuration yields normal spores with a complete chromosomal complement; chromosome disjunction from the open ring results in spores with a deficiency and leads to pollen abortion. Since the adjacent II disjunction where the homologous centromeres go to the same pole appears to be relatively rare, only two types of segregation probably occur with equal frequency and thus account for the observed semisterility of the pollen or ovule. This pattern, since it was first found in maize (Brink, 1927; Burnham, 1932) is known as the Zea pattern. The same pattern was shown later in rice (Soriano, 1959) and in sorghum (Garber, 1948, Webster, 1961). However, some plants with interchanges showed very low sterility such as 25% in barley (White et al., 1948)* and from 11 to 16% in different interchanged lines of wheat (Burnham et al., 1954). Hanson and Kramer (1949) indicated that in barley there was a higher proportion of alternate segregation of the chromosomes in the rings. It was noticed also that a certain plant produces different degrees of sterility in different types of aberrations. When both pieces of interchanged chromosomes in maize were long, the usual configurations being a ring of four chromosomes, spore abortion was about 50%, while in maize having a chain configuration, spore abortion was 20 to 25%, (Burnham, 1932, 1950). Likewise, wheat plants with one complex of four chromosomes showed only 5 to 10% sterility; those with two complexes of four, 10 to 20%; those with one complex of six chromosomes, 20 to 30%. Completely random segregation in such types should result in 66.6, 88.8 and 99% sterility, respectively, while with no adjacent II disjunction occurring should result in 50, 70, and 75% respectively (Thompson and Hutcheson, 1942). Those complexes are probably due to special condition, such as interstitial chiasmata, or early opening of the complex. In some cases, irregular 3-1 segration from the ring of four chromosomes also occurs, resulting in (n-1) and (n+1) combinations. In higher plants the (n-1) combinations usually abort (Burnham, 1956).

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*According to Burnham (1956)

From these data, we may conclude that seed set and pollen abortion percentage could be used to indicate certain plants with heterozygous reciprocal translocations but they are not a critical test.

General Procedure For Establishment Of Homozygous Interchanges

Burnham (1956) has pointed out the general procedure for identifying plants with homozygous interchanges used by most workers. The heterozygous interchanges crossed with normal gave the ratio of 1 normal : 1 heterozygous interchange. Selfs of the heterozygous plants or sib crosses between them gave the same 1 : 1 ratio but the normal (fertile) are of two types, either normal or homozygous interchange. The homozygous interchanges have been identified by the fact that when crossed with a normal the F_1 has an association of four chromosomes and is partially sterile in species without completely directed segregation. This test has been the standard procedure in isolating lines homozygous for the interchanges in Zea, Triticum, Hordeum, Datura, and Nicotiana. Also some workers have identified chromosomal aberrations cytologically from progenies of selfed plants obtained from irradiated seeds (Clarke and Anderson, 1935, in Zea; Smith, 1950, in Hordeum and Triticum; and Soriano, 1959, in Oryza).

Chromosomal Aberrations Induced By Radiations

Some species of plants with certain advantages for cytological studies such as small numbers of large chromosomes have been extensively used. The results of these experiments will be briefly discussed as follows:

Maize:

X-rays and other radiations have been used to produce chromosome aberrations in maize (Anderson et al., 1934; Anderson, 1935; Randolph et al., 1948; Longley, 1950; Morris, 1955). Following X-ray treatments, interchanges were observed as rings or chains at diakinesis (Anderson and Clokey, 1934; Anderson, 1935). It was found that the frequencies of interchange were closely proportional to the length of the different chromosomes. Randolph et al. (1948), in materials treated with X-rays with a dosage of 15,000 r, found translocations, inversions and deletions. Reciprocal translocations were the most frequent type of induced aberrations. Morris (1955) found 18 interchanges involving opposite arms of homologous chromosomes among a total of 1,636 maize plants from X-ray- or thermal neutron-irradiated seeds. From analysis of the pachytene stage, Longley (1950) listed the chromosome identification and the break points in 588 translocations. Those included 179 from known dosages of X-rays, 172 from the Test Able A-bomb, those from earlier X-ray treatments and other sources. Comparing ultraviolet radiation and hard X-rays on the chromosomes of maize endosperm, Faberge (1951) found no consistent differences in inducing chromosomal aberrations. Both radiations produced rings, dicentric translocations and inversions. The most common effect was the production of breakage-fusion-bridge cycles.

Barley:

In partially sterile plants from barley seeds irradiated by X-rays at a dosage of 20,000 r, Burnham et al. (1954) found rings or chains of four chromosomes. From normal-appearing parent heads, however, only rings of four chromosomes were observed. Among these progenies, bridge plus fragment formation was found at metaphase I in one partially sterile line, suggesting the presence of an inversion.. At the same dosage of X-rays, in the progeny of treated seeds of a recessive short chromosome stock, Moh and Nilan (1954) identified three types of aberrations: (1) a reciprocal translocation between pairs of homologous chromosomes; (2) a "buckle" on a homologous pair indicating a possible deficiency and (3) chromosome rings. In addition to ring configurations, Caldecott and Smith (1952) also found some isochromosomes in irradiated barley. In 3,509 spikes, the frequencies of a ring of four chromosomes, 2 rings of four, pseudo-isochromosomes, a ring of six, and true isochromosomes were 371, 32, 29, 18, and 3 spikes, respectively. The spikes with pseudo-isochromosomes had aberrations which were considered to be the result of interchanges between homologous chromosomes. The same types of interchanges were also observed by Caldecott (1955) following treatment with X-rays and thermal neutrons. He found the frequencies of rings of four, rings of six, two rings of four, two pseudo-isochromosomes, rings of four and rings of six, rings of eight, to be 13.5, 0.6, 1.0, 0.6, 0.0, 0.2 per 100 spikes for X-rays and 27.9, 3.8, 3.0, 1.2, 0.6, 0.7 per 100 spikes for thermal neutrons, respectively. It was also found that there was a linear relationship between the frequency of aberrations and dose of both radiations. The same conclusion was obtained in other experiments (Caldecott et al. 1954). From materials irradiated by X-rays and the atomic bomb, Smith (1950) observed rings of four and rings of six in both barley and wheat.

Wheat:

Triticum monococcum ($2\underline{n} = 14$) was frequently used in these studies. Rings of different number of chromosomes have been found by Smith (1936, 1939, 1948), Thompson and Hutcheson (1942) and Yamashita (1951, 1953) in the progeny of X-rayed seeds of Triticum monococcum. Yamashita (1951, 1953) reported 9 translocations in _T_. _monococcum_ and 7 in _T_. _aegilopoides_ from X-irradiation. One homozygous stock which produced a ring of six when crossed with normal plants probably came from a crossover in differential segments in the chromosome common to two different translocations. The other stocks established produced separate rings of four when crossed with normal. Smith (1948) reported that plants heterozygous for a reciprocal translocation induced by X-rays had chains of four chromosomes in 56% and rings of four chromosomes in 44% of 275 pollen mother cells which suggested that one of the translocation segments was relatively short. In the selfed progeny of plants heterozygous for a reciprocal translocation, about 93% of the plants appeared normal and about 7%, abnormal. The abnormal plants carried a duplication and deficiency. When this latter type of plant was crossed with a normal female, 31% of the progeny were of the duplication plus deficiency type. When selfed, 39% had the duplication plus deficiency. By using durum wheat irradiated by both atom bomb and X-rays, Smith (1950) found rings of four and rings of six chromosomes.

In wheat, most workers have devoted their studies to the per cent sterility of the plants with ring configurations. Pollen abortion in plants with a ring of four chromosomes is very low. The reports of Thompson and Hutcheson (1942) and Smith (1950) have indicated that sterility in plants with ring configuration was only slightly increased over that in normal plants. Pollen abortion was greater in the plants with large rings (Smith, 1938, 1939; Thompson and Hutcheson, 1942; Yamashita, 1951). A high proportion of zigzag orientation in wheat was noticed by Smith (1939) and Thompson and Hutcheson (1942). The former found 73% in the ring of four chromosomes and the latter found 88% in the ring of four chromosomes and 75% in the ring of six chromosomes. Yamashita (1951) computed an average of 82.7% of zigzag orientation for plants with a ring of four but only 22% for a ring of 12 chromosomes. Thompson and Hutcheson (1942) observed the configurations at pachytene stage and determined the relative length of the arms of cross-shaped pachytene configurations in plants with a ring of four and in plants with different kinds of rings of six. They also established fully fertile homozygous translocation lines.

The results mentioned above suggest that the segregation of chromosomes in wheat plants with reciprocal translocations are directed and not entirely random.

Rice:

Cytological studies of rice, Oryza sativa ($2\underline{n} = 24$), irradiated by X-rays have been carried out by a number of workers such as Oka et al. (1953), Parthasarathy (1958), Soriano (1959), Shastry and Ramaiah, (1961). From some partially sterile plants a ring or chain of four chromsomes was identified at metaphase I indicating that heterozygous reciprocal translocation occurred. Parthasarathy (1938) found that 16% of semi-sterile plants from irradiated seeds showed these configurations. With a dosage of 4,000 r, Soriano (1959) found that reciprocal translocations occurred in the first generation after irradiation, indicating the formation of breaks in two non-homologous chromosomes. All the reciprocal translocations displayed an interchange complex, and the frequency of rings ranged from 6.7% to 33%; of chains, from 25.8% to 46.2%. Oka et al. (1953) and Soriano (1959) stated that the progeny of semi-sterile plants with heterozygous reciprocal translocations gave a ratio of 1 normal : 1 partially sterile. These data indicated that there was no directed orientation in an interchange complex at metaphase I, as mentioned before in maize. However, plants bearing interchange complexes did not give 50% but low seed set and showed great variation, e.g., ranging from 16 to 50% (Soriano, 1959; normal plants ranging from 70 to 93%), or from 40 to 50% (Oka et al., 1953). By using fertile and partially sterile plants from irradiated seeds crossed with the original variety, Oka et al. (1953) found that the former produced either fertile or sterile plants, i.e., no segregation was seen within the same cross, and the latter produced both fertile and sterile plants in the same manner as the selfing of a semi-sterile plant. It was noticed that certain strains showed sterility but no quadrivalents were found. Oka et al. (1953) concluded that this strain may have a reciprocal translocation; probably the translocated segment was very short and may not be detectable cytologically.

Recently, Shastry and Ramaiah (1961) studied rice treated by X-rays, thermal neutrons and β -particles. They found multivalents frequently occurred following X-irradiation. The highest association observed was a hexavalent and the number of quadrivalents ranged from 1 to 3 per pollen mother cell. Following thermal neutron-irradiation, quadrivalents were observed in 5 out of 98 pollen mother cells studied in only 1 out of 7 treated plants. A single quadrivalent pollen mother cell was recorded in 2 out of 15 plants studied in β -particle treatments.

Tradescantia:

For the species discussed above, either dry or wet seeds were treated with different radiations for inducing chromosomal aberrations. In Tradescantia, data are available both for microspores irradiated in the resting stages following meiosis, and for microspores irradiated in the prophase of the first pollen grain mitosis. These studies of Tradescantia revealed two types of chromosomal aberrations: chromosome breaks and chromatid breaks. Tradescantia was extensively used in this study because its meiotic cycle is well known, the chromosomes are large and certain species flower throughout the year in the greenhouse. The meiotic cycle covered about one week during the summer and approximately two weeks during the winter. In the past several decades the results of studies by a number of workers with microspores of Tradescantia subjected to various dosages of X-rays may be summarized as follows: (1) Either one or both of the sister chromatids at prophase can be broken by a single "hit"; (2) exchanges or fusion between chromatids of different chromosomes are the result of two independent "hits" as are the ring and dicentric chromosome induced during the resting stage; (3) when the time of irradiation is constant for different X-ray doses the aberration frequency increases as the square of the dosage, and when X-ray intensity is constant, the exponent of the dosage curve equation is less than two; (4) the relation between dosage and frequency of one hit aberrations is linear regardless of the time-intensity factor; (5) most breaks undergo either restitution or produce chromosome aberrations within about an hour after irradiation; and (6) single chromatid deletions are most frequent shortly after exposure at late prophase, while double deletions are most frequent at early prophase. These results were obtained by Sax (1940, 1941), Lea and Catcheside (1942), Thoday (1942), and Catcheside et al. (1945, 1946). By using Tradescantia irradiated by X-rays and neutrons, Thoday (1942) found neutrons to be more effective per ionization than X-rays in producing all types of aberrations, but the relative efficiencies of the two radiations are different for different types of aberrations. Three types of aberrations were evidenced in material fixed 5 days after irradiation. They were a short chromosome and an acentric fragment due to failure to rejoin the broken ends; a ring chromosome and an acentric fragment; and a dicentric chromosome and acentric chromosome or even polycentric. In the material fixed 24 hours after irradiation, the change may affect either one or both chromatids.

Sorghum:

As far as is know, sorghum has been rarely used for inducing chromosomal aberrations by irradiation since cytological studies, compared with those on maize, barley and Tradescantia, are relatively difficult. Garber (1948) is the only worker thus far known to use X-rays on Sorghum versicolor ($2n = 10$). He found chromosomal aberration configurations of rings of four chromosomes in pollen mother cells indicating a reciprocal translocation had resulted. From progenies of 49 plants, 27 semi-sterile plants had a ring of four chromosomes and the remainder were normal. This approximated the 1 : 1 ratio observed in the Zea translocations. As for the seed set percentage, the semi-sterile plants were 34% fertile while normal plants were 86% fertile. Garber (1948) interpreted the difference between the observed (34%) and the expected (43%) seed set to be due to a low frequency of zigzag configurations in the ovule. Garber (1948) also found two types of microspore quartets in the irradiated materials, one in which each microspore contained a single nucleolus, and the other in which two of the four microspores contained diffuse nucleolar bodies. The latter would be expected from non-disjunction of the chromosomes bearing the nucleolar organizing region.

In addition to the plants mentioned above, some other plants are also used for similar studies, e.g., Crepis, Gossypium and Datura. From the progeny of X-rayed Crepis, Levitsky (1940) found that the most common types of aberrations were translocations, inversions, and duplications. By using cotton seeds irradiated with gamma rays, Brown (1950) found all types of quadrivalent associations such as the zigzag and U-shaped configurations and figure-eights.

Chromosomal Aberrations In Sorghum From Sources Other Than Radiation

Although radiation has been used to induce chromosomal aberrations in sorghum, some chromosomal aberrations produced from other sources have been observed. From the derivatives of the haploid sporocyte of *Sorghum vulgare*, Endrizzi and Morgan (1955) found a heterozygous translocation. Rings of four chromosomes were found in 105 of the 106 microsporocytes examined at metaphase I; a chain of four was observed in one pollen mother cell. Trisomic plants were also found in one of the F_1 derivatives of the haploid. Three chromosomal interchanges in *Sorghum vulgare* have been established by Webster (1961) in Nebraska. Rings of four chromosomes have been observed in these materials. By crossing the translocation plants with both normal and other translocation plants, a ring of four and 8 bivalents in the F_1 of the three crosses of normal X translocation stocks were obtained. In three intercrosses between translocations, two of them showed a ring of four and 8 bivalents, the other a ring of six and 7 bivalents. In the intercrosses, a ring of six chromosomes occurred when two interchanges had one chromosome in common. In these intercrosses, two rings of four chromosomes were expected since the three translocations were different interchanges. Webster explained the failure of finding two rings of four in the two interchanges as possibly due to separation of one of the rings of four into two bivalents.

All these chromosomal aberrations in *Sorghum vulgare* were observed at the diakinesis stage or at metaphase I. As far as is known, there are no reports of chromosomal aberrations observed at the pachytene stage.

A great number of cytological studies on sorghum have been carried out in recent years. Garber (1950); Sharma and Bhattachatjee (1957) and Celarier (1958) worked on the cytotaxonomy of sorghum. Brown (1943), Garber (1947), Endrizzi and Morgan (1955), Venkateswarlu et al. (1956), Endrizzi (1957) and Magoon and Shambhulinfappa (1960) worked on the chromosome morphology of sorghum. More recently, Magoon et al. (1961) gave a detailed analysis of the chromosomes at pachytene of 5 different species of Eu-sorghum ($2n = 20$). These were depicted on the basis of relative length, centromeric position, arm ration, and number of chromosomes. In these cytological studies, Eu-sorghum which is a group with glabrous or lightly pubescent nodes and whorled and divided panicle branches (Snowden, 1935), has been extensively used. Sorghum vulgare ($2n = 20$) and S. Halepense ($2n = 40$) have also been used in cytological studies, particularly in cytotaxonomy or for evaluating their position in evolution by crosses between them (Hadley, 1953, 1956; Endrizzi and Morgan, 1955; Krishnaswamy et al. 1956; Endrizzi, 1957). According to Hadley (1959), S. vulgare is favorable for study at nearly every stage of meiosis. Thus, this species is worthy of more intensive cytogenetic study in the future.

Variations In Chromosome Number In Sorghum

In the study of chromosome association in Sorghum vulgare, Chin (1946), Hadley (1953) and Huskins and Smith (1934) stated that 10 bivalents were most common in S. vulgare but that multivalent associations were found. However, a great number of variations in chromosome number have been observed in S. vulgare, particularly in male sterile lines, by other workers. When checking the chromosome number of colchicine-treated and untreated sorghum, Damon (1961) occasionally found polyploid and aneuploid microsporocytes in untreated plants, but the frequency differed from one variety to another; cytoplasmic male sterile Wheatland, Westland and Martin varieties produced cells with irregular chromosome number but Redlan and Combine Kafir-60 did not exhibit such abnormalities. Singh and Hadley (1962) reported that no abnormalities at meiosis in male sterile Combine Kafir-60. In Wheatland materials, Damon (1961) found that the abnormal cells occurred at a frequency of 4.8%. Among abnormal cells, the frequency of cells with $2\underline{n} = 40$ was relatively high (31%) and one cell had approximately $2\underline{n} = 170$. Of aneuploid cells, $2\underline{n} = 6$, 12, and 18 were observed. In cytoplasmic male sterile materials, Damon found that microsporocytes showed an absence of cell walls at metaphase I. Much of the sporogenous materials did not separate into distinct individual sporocytes, but remained as a plasmodial mass of cytoplasm containing a few to several metaphase plates. In the progeny plants of colchicine-induced, nontrue-breeding mutants derived from the sorghum, 'Experimental 3', Sanders and Franzke (1962) found cells with different chromosome numbers. The abnormal cells were also found in barley (Hordeum vulgare, $2\underline{n} = 14$) by Smith (1942) who found that the number of bivalents in different metaphase plates varied from fewer than 7 to more than 100. In some groups, quadrivalents were present, indicating that chromosomes from different cells were proximity at early stage and were able to synapse. In cytoplasmic male sterile sugar beets, Artschwager (1947) found that the breakdown of boundaries occurred between tapetal cells and not pollen mother cells.

The migration of chromosomes from one mother cell to another, or nuclear fusion, or cytomixis, was actually observed long ago.

According to Maheshwari (1950 p. 41), this phenomena evidenced in Oenothera gigas as early as 1911 by Gates. Since then it has been reported in several plants. The most frequent stages of origin of cytomixis were between the zygotene and the diakinesis stage (Levan, 1941; Smith, 1942; Damon, 1961). It may sometimes occur even during the interkinesis stage, as in Lathraea (Gates et al. 1927)*, or at the close of the meiotic divisions (Gelin, 1934)* as in Careopsis tripteris. Damon (1961) also pointed out that in sorghum the high frequency of tetraploid cells and cells with two or three nuclei was possibly due to the failure of cell wall formation at the last mitotic division. Sanders et al. (1962) explained that one possibility for the result of abnormal chromosome numbers was resulted either from a uniform cellar make-up with the same chromosome complement in each cell but with varying pairing relationships, or from a chimeral make-up with the complement varying between the cells with normal and abnormal pairing.

However, Levan (1941) stated that the syncytium formation (a mass of cytoplasm containing many nuclei but not into distinct cells by cell wall or partition of the intervening cytoplasm) or fusion of pollen mother cells in Phleum was limited to the meiotic prophase and no nuclear fusion prior to zygotene occurred. Some observations revealed that the failure of cell wall formation occurred following second division. For example, Yeh et al. (1962), studying a hybrid in rice, reported that the absence of a cell wall was observed at telophase I of first meiotic division and most stages of second meiotic division. Chromosome movement may be caused by slide preparation, for example, Woodworth (1931)* stated that apparent cytomixis was common when a little extra pressure was used in squeezing out the microspore mother cells during smear preparations of anthers.

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*According to Maheshwari, 1950, pp 41-42

The absence of a cell wall between adjacent microsporocytes might be explained in two ways: one is that the cell wall did not form during cell division as reported by most of workers mentioned above; the other is that the cell wall dissolved after formation, as indicated by Matsura (1935)* and Damon (1961).

In some cases, cytomixis would appear to be a symptom of deficient cell wall formation. This may lead to the origin of binucleate or multinucleate pollen mother cells which may be important in the origin of polyploidy (Matsura, 1935*; Levan, 1941; Smith, 1942; Damon, 1961). The fusion of as many as 30 pollen mother cells in a large plasmodium or syncytium was observed in two haploid plants of Phleum pratense by Lean (1941) and in Placelanthus by Matsura (1935)*. Similar behavior has been reported by Stern (1946) in sugar suspensions of pollen mother cells of Trillium erectum, showing 32 nuclei in a cell. In sorghum, Damon (1961) occasionally found some microsporocytes that had two or three nuclei.

Another possibility giving rise to the multiploid microsporocytes is the failure of cytokinesis in which case the cytoplasm fails to divide following the nuclear division. This was reported in sorghum by Damon (1961) and Schertz (1962).

The third possibility giving rise to the multiploid microsporocytes is, as mentioned by Stern (1946), fixation, chemical treatment or heat, which may result in a breakdown of cell structure due to the dispersion of the cytoplasm. The examination of such fixed and stained preparations revealed multinucleated plasmodial masses.

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*According to Maheshwari, 1950, pp. 41-42

According to Levan (1941), in most cases, where fusion of a great number of cells occurred, the spindle at metaphase I was reported to behave rather irregularly. Sometimes each nucleus had a spindle of its own, sometimes different spindles interfere with each other and fuse. In large syncytium of haploid Triticum compactum, Gaines and Aase (1926) reported that the chromosomes of each nucleus of the syncytia may become oriented on an independent spindle, or the chromosomes of contiguous nuclei may become distributed over a single giant spindle. The latter case was also reported by Levan (1941). Levan (1941), in haploid Phleum syncytia, pointed out that no more than one spindle was ever formed, irrespective of the number of nuclei present in the syncytia and that this spindle was normal and bipolar. Smith (1942) had mentioned that in large groups of chromosomes in a giant "cell", the spindle was many times wider than normal, but the length was not greatly changed. Damon (1961) explained that one spindle may be formed for all the bivalents from one large "cell" with two or three nuclei, giving rise to the bivalents on a single metaphase plate.

Aneuploid cells were reported in sorghum by Damon (1961) and Sanders and Franzke (1962) and in barley by Smith (1942). Damon (1961) explained the formation of such cells as the mingling of chromosomes from separate nuclei due to failure of formation of cell walls. A regeneration of the destroyed cell wall may occur not in the original position; aneuploid cells resulted. Sanders and Franzke (1962) stated that aneuploid cells might occur either if the original zygotes of these plants had contained a gain and/or loss of individual chromosomes and, by the time sporogeneous tissue developed in the anthers, or if the original zygotes contained a normal complement which was irregular in mitotic division.

Meiosis In _Sorghum Vulgare_

Many cytological studies in sorghum have been made in recent years. _Sorghum vulgare_ includes many forms of subspecific rank. Its meiotic chromosomes are favorable for study at almost every stage (Hadley, 1959). This species is, therefore, worthy of intensive cytogenetic study and a complete photomicrographic study of the various stages of meiosis would be helpful to workers in this field.

CHAPTER III

MATERIALS AND METHODS

Seeds of Wheatland (Sorghum vulgare) were used for this entire study, since this variety was found previously to be more useful for cytological studies. Seeds were treated by X-rays and thermal neutrons at the Brookhaven National Laboratory, New York, in 1956. Following the treatment, steps were taken in the Botany and Plant Pathology Department at Oklahoma State University to obtain the materials for cytological identification of possibly induced chromosomal aberrations. The field parts of this experiment were conducted at the Agronomy Farm of Oklahoma State University and the cytological study in the Botany and Plant Pathology Department. The irradiated materials were handled by Dr. Margaret Brooks in 1958 and 1959. The procedures in handling these materials are described as follows:

1. In 1956, Wheatland seeds were treated by X-rays and thermal neutrons with dosages of 10,000, 15,000, 20,000, 25,000, and 35,000 roentgen units (r). Before treatment, the seeds were stored in a room at 60% relative humidity which insured a definite uniform water content in all seeds. Untreated materials were used as checks.
2. The seeds were grown under field conditions in 1958. The field arrangement was in three replications for each treatment and consisted of 36 plots in total. Each plot was grown in a single row. Plants were selected in the field on the basis of pollen abortion. Each plant tested for the accumulation of starch by

the standard iodine technique. Pollen with abundant starch shows a dark-blue stain and is usually fertile. Plants showing more than 50% aborted pollen were considered as possibly carrying heterozygous chromosomal aberrations, these were selfed and seeds collected. The treatments with extreme dosages, i.e., the lowest dosage (10,000 r) and the highest dosage (35,000 r) in both X-rays and thermal neutrons, and 25,000 r thermal neutrons, were discarded since the lowest dosage showed no effect and the highest dosage completely inhibited germination.

3. In 1959, the field arrangement consisted of 72 plots, each sown with sufficient seeds from a single selfed head to produce 12 progeny plants. These plots alternated with rows of cytoplasmic male sterile Wheatland. Flowering materials were collected from each plant and tested again by the standard iodine technique. If some plant or plants in that plot showed more than 50% aborted pollen, the plant showing normal fertility were crossed to cytoplasmic male sterile plants and simultaneously selfed. The selfed seeds supposedly were homozygous normal or homozygous for an aberration. Since the original 1958 selections were presumed to carry heterozygous aberrations and were selfed, their 12 progenies might be expected to segregate in a ratio of 1 homozygous normal : 2 heterozygous aberration : 1 homozygous aberration, the last class being the one desired to form the basis of homozygous aberration lines. Since these cannot be distinguished from the homozygous normal lines, crosses to the male sterile lines were made. By these crosses, the chromosomal aberrations are obtained in a heterozygous condition, in the embryo of the crossed seeds, i.e., one normal chromosome from the cytoplasmic male sterile plant associated

with an abnormal chromosome obtained through irradiation. Among the 72 plots, crosses were made in 70 plots. The number of plants crossed or selfed within each plot ranged from 1 to 8. Two hundred sixty-three plants were collected in total. The seed set on some crosses was so low that there was not enough for replanting.

4. In 1960, seeds obtained from the crosses made the previous year were planted. The field arrangement consisted of 263 plots. The seeds for each plot were from a single plant crossed with cytoplasmic male sterile Wheatland. Most of the plots consisted of 10 to 12 plants. The bud (inflorescence) materials were collected and fixed for meiotic studies in a solution of 4 : 3 : 1 of absolute alcohol, chloroform, and glacial acetic acid, respectively. An average of 6 specimens was collected from each plot. One thousand four hundred forty-six specimens were collected and kept in a cool room for cytological study.
5. Cytological study was started in the fall of 1960. The standard aceto-carmine smear technique (Smith, 1947) and Feulgen stain (Darlington and La Cour, 1960) were used. Mechanical vibrations were applied to aid in spreading chromosomes (Boyle, 1961). In an attempt to improve the staining technique, some modified staining methods were used. For example, 45% acetic acid was allowed to run under the cover slip to spread the chromosomes (MacDonald, 1961). Both pachytene and metaphase stages were utilized in chromosome identifications.
6. Because of the failure to identify any chromosomal aberrations, the crossed seeds that were used for producing cytological study materials in 1960, were grown again in 1962. Seed set percentages were recorded. Normal Wheatland was alternately planted with treated

materials in the ratio of 1 : 4 rows in the field for producing pollen. The cytoplasmic male sterile lines were also grown alternately as a check for pollination. The field arrangement consisted of 390 plots including 222 plots of treated materials, 142 plots of normal Wheatland and 26 plots of cytoplasmic male sterile lines.

7. The seed set of all plants was classified into seven classes ranging from 1 for full seed set (100%) to 7 for no seed produced. Seed set of each plant was recorded and the average fertility percentage was calculated for each plot. Twenty plants of treated materials, showing very low seed set (less than 5% or no seed produced), were chosen and transplanted to the greenhouse for bud production for cytological study. For convenience of comparison of chromosomal morphology, some normal Wheatland with full seed set and cytoplasmic male sterile lines with very low seed set were also transplanted. The buds of the tillers were collected and fixed in the same solution used previously. The materials collected in 1960, which had seed set less than 30% (based on head row average) in 1962 were cytologically identified again.

During the cytological search for chromosomal aberrations, a considerable variation in chromosome number was observed. Since the cells with irregular chromosome numbers usually appeared in groups having no boundaries between the sporocytes, the individual chromosomes, or groups of chromosomes, or the whole spindles possibly migrated from one place to another. This made the counting of cells with abnormal chromosome numbers difficult. In this study, the cells with abnormal chromosome numbers were all counted whether they occurred as single cells or cells in groups. The cells with abnormal chromosome numbers were recorded throughout the study for chromosomalaberrations. Most of these cells were observed at metaphase I and anaphase I.

Photomicrographs of various stages of meiosis in Sorghum vulgare are presented in the Appendix. These pictures were taken during the search for chromosomal aberrations.

CHAPTER IV

RESULTS AND DISCUSSION

Identification Of Chromosomal Aberrations

Materials for the study of chromosomal aberrations were collected from the head rows of crossed seeds grown in the summer of 1960. In some rows samples were not collected since either one or few crossed seeds were planted and failed to germinate or the proper stage of meiosis was missed during collecting. The number of samples collected for different dosages for both X-rays and thermal neutrons is given in Table I.

All the samples of meiotic inflorescences collected for cytological study have two chromosome complements from two different sources: one, normal complement from the cytoplasmic male sterile plants and the other from irradiated materials. Chromosomal aberrations, particularly interchanges, in the irradiated materials should be evident during meiosis. If chromosomal interchanges occur, the cross-like or 4 arms configurations might be expected to be present at pachytene stage due to failure of complete synapsis along the length of the chromosome pair and chain or ring figures to be evident at diakinesis or metaphase I. If a paracentric inversion occurs, a bridge and a fragment would be present at metaphase I or pre-anaphase I. These figures of chromosomal aberrations have been identified in most plants studied, e.g., Zea mays (Burnham, 1932; Brink and Cooper, 1932; Clarke and Anderson, 1935;

Longley, 1950); *Oryza sativa* (Parthasarathy, 1938; Shastry et al., 1961); *Triticum monococcum* (Yamashita, 1951, 1953; Smith, 1948); *Hordeum vulgare* (Caldecott and Smith, 1952; Moh and Nilan, 1954; Caldecott, 1955); and *Sorghum versicolor* (Garber, 1948).

TABLE I

SAMPLES COLLECTED FOR CYTOLOGICAL IDENTIFICATION IN 1960

Dosages(r)	Number		
	Head Rows	Samples Collected	Head Rows Without Samples
		X-ray	
10,000	--	--	--
15,000	56	312	0
20,000	67	395	3
25,000	38	179	1
35,000	--	--	--
Total	161	886	4
		Neutron	
10,000	--	--	--
15,000	61	354	0
20,000	41	206	4
25,000	--	--	--
35,000	--	--	--
Total	102	560	4
Grand Total	263	1,446	8

Meiotic stages from pachytene to anaphase during the first meiotic division have been observed in current studies. At the beginning of the cytological study, pachytene was emphasized as being the optimal stage for identification of the cross-shaped figures. As the study proceeded and cells with different chromosome numbers were found;

metaphase I and anaphase I were also observed. Since chromosomes in these stages contract and arrange themselves regularly on the metaphase plate at metaphase I or separate toward the two poles at pre-anaphase I, these stages are more convenient for counting the chromosome number. Compared with the cross-shaped configuration at pachytene stage, the chain or ring of four chromosomes is relatively easy to identify.

Because of the procedures followed after irradiation all samples from the same head row should consist of the same genetic materials. In other words, if any chromosomal aberration was identified in one sample, it would, theoretically, be found in other samples from the same head row. Based on this assumption, only one or two samples were chosen at random from each head row for cytological identification. Each sample was examined in one or two of the following meiotic stages: pachytene, diakinesis, metaphase I and/or anaphase I, since the proper stage was difficult to find in some samples. Two to five slides, with an average of 3, were prepared for each sample by using the standard aceto-carmine smear technique. After two or three days, the prepared slides, were observed under the microscope to search for chromosomal aberrations. The number of samples and cells observed for different treatments are listed in Table II. One hundred eighty-eight samples from 124 head rows for X-ray treatments and one hundred ten samples from 61 head rows for neutrons were studied. From the total of 298 samples observed, no chromosomal aberration was identified. The chromosome number was counted and recorded from the observed cells at metaphase I and anaphase I. A total of 1,664 cells observed at metaphase I or anaphase I was analyzed. In addition to the absence of chromosomal aberrations, variation in chromosome number was observed. The variation in chromosome number will be discussed in the next section.

TABLE II

RESULTS OF IDENTIFICATION OF CHROMOSOMAL ABERRATIONS (MATERIALS GROWN IN 1960)

Dosages(r)	Number Head Rows Observed	Samples Observed	Cells Observed*	Cells With Chromosome Aberrations
			X-ray	
15,000	38	56	409	0
20,000	57	85	437	0
25,000	29	47	268	0
Total	124	188	1,114	0
			Neutron	
15,000	38	62	279	0
20,000	23	48	271	0
Total	61	110	550	0
Grand Total	185	298	1,664	0

*The number of cells does not include cells observed at pachytene.

After handling the irradiated seeds treated by several different dosages as outlined above and examining a number of slides considered to represent a sufficient sample, translocation configurations, either a chain or ring of four chromosomes, were not observed. Seed set usually indicates whether the chromosomes are normal or abnormal but more information on this phase of the problem as well as reconsideration and improvement of the cytological technique used seems indicated.

Technique for cytological study:

The aceto-carmine smear technique (Smith, 1947) used in this study is the standard method employed by most cytologists for the study of different plant materials. The Feulgen stain (Darlington and La Cour, 1960) was also employed for some samples. For improving the technique, some possible modified methods for both staining and spreading chromosomes in slide preparation were employed, i.e., applying a vibrator (Boyle, 1961) and running 45% acetic acid under the cover glass (MacDonald, 1961). The Feulgen stain has proved to be particularly useful because of its specificity for DNA. Since DNA is an important chemical component of chromosomes, the Feulgen stain sometimes can be used for identification of chromosomal aberrations. Compared with the aceto-carmine technique, the cytoplasm is not stained at all but some parts of chromosomes are not stained darkly because of the low concentration of DNA. The period for staining is relatively sensitive and difficult to control. Applying a vibrator to the cover glass after staining spreads the chromosomes. According to Boyle (1961), a Burgess Vibra-Tool or another similar tool is most effective if the steel bit is replaced with a short wooden shaft, which may be easily whittled from a match stick and held at an angle of approximately 45° to the slide. This technique is sometimes helpful in chromosome spreading particularly of tight metaphase plates. The disadvantages are that too much pressure with the tool obliterates everything and too little has no appreciable effect. MacDonald (1961), studying barley pachytene chromosomes, used Boyle's modified aceto-carmine technique. This procedure may help in spreading chromosomes but slide preparation is more time consuming. These modified techniques were only used for part of the samples. The interchanged configurations were not identifiable even after using these techniques for either staining or spreading chromosomes.

To be sure that the failure to identify chromosomal aberrations was due to the materials themselves or to cytological technique, three samples which were collected from plants with translocations were obtained from Dr. O. J. Webster, University of Nebraska. In these materials some translocation configurations, such as a ring of four chromosomes at metaphase I, were observed without any difficulty. This identification indicated that the technique employed in the cytological study was satisfactory and also proved that translocation figures do occur in Sorghum vulgare. Since the technique used in the cytological study was acceptable, the occurrence of aberrations in these materials should be questioned. Materials with chromosomal aberrations usually show partial sterility. The standard method for measuring the degree of partial sterility is by counting the percentage of aborted pollen or observing seed set.

Seed set percentage of materials for identifying chromosomal aberrations

Plants heterozygous for translocations generally show partial sterility. In barley translocation heterozygotes gave 25% sterile pollen (White et al., 1948)*. Maize with rings of four chromosomes gave 50% but with chains, 23% sterility (Brunham, 1932, 1950). Wheat with rings of four chromosomes gave 73% (Smith, 1939), 88% (Thompson et al., 1942) or 83% seed set (Yamashita, 1951). Sorghum with translocations gave 34% sterility (Garber, 1948). The percentage of sterility is calculated from aborted pollen grains and failure to set seed. Thus the percentage of sterility is inversely proportional to the percentage of seed set. The different percentages of sterility or seed set in different plants are due mainly to the relative frequency of the three types of possible segregation from the ring of four chromosomes at anaphase I. In general, maize or sorghum with rings of four chromosomes show approximately 50% sterility, but wheat or barley show less than 50%. In order to check sterility percentages of the materials used in this cytological work, the crossed seeds, part of which were grown for collecting samples for cytological identification in 1960, were planted again in the summer of 1962. Since the crossed seeds were from the crosses of treated materials with normal cytoplasmic male sterile Wheatland, plants from these seeds showed male sterility. To insure pollination, they were grown alternately with normal Wheatland. For checking the amount of pollen produced by normal Wheatland, normal cytoplasmic male sterile Wheatland was also planted. Compared with the planting in 1960, some entries of treated materials were absent due to insufficient crossed seeds. The number of plants in each plot ranged from 1 to 10.

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*According to Burnham, 1956.

In the field, all three different kinds of plants looked the same except that the treated materials and cytoplasmic male sterile Wheatland were sterile. During the later period of growth, the treated materials and cytoplasmic male sterile lines produced more tillers than normal Wheatland. This may be explained if nutrients present in the plants are used for seed production by normal Wheatland but are used for producing tillers by cytoplasmic male sterile lines and treated materials. During the course of plant growth, it was difficult to tell the difference between treated materials and cytoplasmic male sterile lines. After seed maturation, the seed set for each plant was recorded based on 7 classes from 1 (100% seed set) to 7 (no seed produced). Average number of seeds in class 1 heads was calculated from the average of 10 heads which were considered to be in the same class 1. The same manner was used to calculate the seed number for other classes. Based on the seed number of class 1 as 100% seed set, the seed set percentages for classes 2 to 6 were calculated. Based on the individual plants and their seed set percentage within the same plot, the average seed set percentage for each plot was calculated. The resulting seed set percentages for different dosages are given in Table III.

TABLE III

SEED SET PERCENTAGE FOR IRRADIATED AND CHECK MATERIALS

Dosages(r)	No. of Plots	No. of Plants	Mean and SE of the Seed Set %	Range of Seed Set %
X-ray				
15,000	48	385	39.46 ± 2.1	14.3 - 67.7
20,000	59	479	35.37 ± 1.3	18.1 - 58.2
25,000	29	206	33.69 ± 1.5	23.0 - 54.8
Total	136	1,070	36.17	14.3 - 67.7
Neutron				
15,000	52	419	45.58 ± 2.0	17.8 - 70.0
20,000	34	254	37.40 ± 1.7	20.4 - 54.8
Total	86	673	41.49	17.8 - 70.0
Grand Total	222	1,743	38.30	14.3 - 70.0
Untreated Cytoplasmic ms Lines				
	26	328	43.24 ± 2.2	27.3 - 64.5
Normal Wheatland				
	142	1,549	90.06 ± 0.8	45.3 - 100.0

It was surprising to see that the seed set percentage for the cytoplasmic male sterile Wheatland was relatively low (43.25%). It was expected to be close to normal Wheatland (90.06%) since the ovules of cytoplasmic male sterile lines are supposed to be normal. With adequate pollen, the seed set percentage of cytoplasmic male sterile Wheatland might be expected to be as high as that of normal Wheatland. Compared with cytoplasmic male sterile Wheatland, the seed set percentage of most treated materials was slightly low except under one treatment, neutron with a dosage of 15,000 r (45.58%). The average for X-rays was 36.17%; for thermal neutrons, 41.49%; both were lower than that of cytoplasmic male sterile Wheatland. For both irradiations there was a common tendency for materials with lower dosages to show a higher seed set percentage.

It was noticed that plants within the same plot or plots within the same treatment showed variation in seed set percentage. The ranges and standard error of the mean are listed in Table III. For example, in the materials treated by X-rays with a dosage of 15,000 r, the average seed set of the different plots ranged from 14.3 to 67.7%. It was obvious that the range of seed set percentage became wider when the dosages decreased. This situation holds true for both X-rays and thermal neutrons. Statistically, the standard error of a mean usually measures the variability of a sample mean compared to the population mean. As the range of seed set percentage, the standard error of the mean decreased as the dosages increased. That is, the higher dosages gave less variability of seed set. The standard error for normal Wheatland was 0.8 which was lower than for any other treatment. The poor seed set for cytoplasmic male sterile Wheatland and the variability may be caused by one or more of the following possibilities.

A. According to Quinby et al. (1958), the most common practice in commercial production of hybrid seed has been to use a ratio of 3 : 1 with either 6 : 2 or 12 : 4 seed rows to pollen rows, depending on whether 2-row or 4-row harvesting equipment was available. In this experiment, the ratio of 4 : 1 was used. Occasionally 5 : 1 was used. The former (5 seed rows) included 4 rows for treated materials and 1 row for cytoplasmic male sterile Wheatland. Considering the experiment as a whole, however, the ratio of seed rows and pollen rows were approximately 1.5 : 1 or 198 : 142, since many rows with treated materials failed to grow and were replaced by normal Wheatland. Based on the plant number, the same ratio, 1.5 : 1, or 2071 : 1549, prevailed (Table III).

All cytoplasmic male sterile Wheatland was grown adjacent to normal Wheatland. If the poor seed set of the plants was due to insufficient pollen production, the male sterile Wheatland, compared with treated materials, should have received more pollen from the adjacent rows. However, seed set percentages of male sterile Wheatland (43.25%) were not strikingly higher than that of treated materials. To the contrary, the seed set of the male sterile Wheatland was lower than that of neutron treatment with a dosage of 15,000 r (45.58%). As in treated materials, great variation in seed set percentage exists in normal Wheatland. Some rows showed as low as 45% and others showed as high as 100% seed set. Of 142 plots of normal Wheatland, 8 plots had less than 75% seed set including 3 plots with less than 50%.

In view of these factors, there must be something other than shortage of pollen, possibly environment, which causes the abnormal seed set.

B. Seed production may be influenced by factors such as temperature, planting date, blooming date, different locations, direction of wind, size of land, and number of insects. Temperature is related to planting date; in this location, the suitable planting date is in late May or early June. The planting date of this experiment, June 22, was somewhat later than usual. Late planting possibly influences plant growth and delays pollination. On the other hand, the blooming date of cytoplasmic male sterile Wheatland is usually one or two days earlier than that of its fertile counterpart. This could reduce the seed set of the male sterile lines, should no pollen be available. Thus, splitting the planting, or mutilating part of the plants by cutting the stalk to force out tillers or branches is usually employed to increase the pollen production and to extend the period of pollen distribution (Quinby et al., 1958). The size of the land for this experiment was relatively small. Generally, the larger size of the land for an experiment, the more pollen produced and the better chance that cytoplasmic male sterile Wheatland could get the pollen from different directions and at different times. Sometimes the weather was extremely hot or dry and serving to decrease effective pollination.

C. Under some conditions Wheatland produces poorer seed set than other varieties, such as Redlan, Martin, and Combine Kafir-60. Wheatland male sterile plants have relatively short stigmas and may fail to receive pollen during pollination. According to Quinby et al. (1958), sometimes sorghum heads may not be fully exserted; spikelets may open only partially and then close without extruding anthers and stigmas; pollen grains may be empty or anthers fail to dehisce; stigmas may be wilted and non-receptive. Some of these conditions possibly existed in this experiment.

The comparison of seed set of male sterile plants with that of treated materials may be critical. If the seed set of treated materials is the same as that of cytoplasmic male sterile Wheatland, it may be that no chromosomal aberrations were produced by the irradiation.

Based on the average seed set percentage per row, the analysis of variance of the different treatments including both irradiated treatments and cytoplasmic male sterile Wheatland was calculated and showed a highly significant difference (Table IV).

TABLE IV

ANALYSIS OF VARIANCE FOR SEED SET PERCENTAGE

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F
Total	245	37742.27		
Treatment	5	4367.68	873.54	6.28**
Error	240	33374.59	139.06	

**Significant difference at 1 per cent level.

Compared with cytoplasmic male sterile Wheatland, these data indicated that some chromosomal aberrations were probably present in certain irradiated materials. On the other hand, the significant differences may be caused by the factors mentioned above. As already mentioned, two types of crosses were expected: one was normal from the cross of cytoplasmic male sterile lines with homozygous normal, and the other was heterozygous for aberrations from the cross of cytoplasmic male sterile lines with the homozygous aberrations. Theoretically, the plants within the row of either cross should have similar seed set percentage. The head row of the type in which plants are heterozygous for aberrations were expected to have only half as many seed set per head as that in the head row of the type in which plants are homozygous normal. However, these were not found. Only some individual plants showed relatively low seed set percentage, less than 5% or even no seed produced. Plants with very low seed set may be the result of ovule abortion. For further evidence, these materials were studied cytologically.

Cytological study of irradiated materials showing a seed set less than 5%

Twenty plants which showed less than 5% in seed set or no seed set at all in the field, were transplanted to the greenhouse for bud production for cytological study. For convenience of comparison of chromosome morphology, two cytoplasmic male sterile plants which showed very low seed set and one normal Wheatland with full seed set were also transplanted. For transplanting, the chosen plants were dug up with soil and set in a one gallon tin can and arranged in the greenhouse. The main stalk was cut off at a height of about 3 inches above the crown in order to force the tillers to grow out. All the samples were collected from either new branches or tillers. The technique for sample collection, handling and cytological identification employed were the same as before. Of the 20 plants, four died in the greenhouse and some grew abnormally, the leaves turned yellow and good samples could not be obtained. Thus only ten plants were studied. The samples collected and the results observed are given in Table V.

In this group of materials, 731 cells were analyzed. Some figures like chains of four chromosomes were identified from some samples (Fig. 1, See Page 73), but the frequency was relatively low. The highest frequency of chains was found in entry No. 102, which was 4 out of 67, approximately 6% (Table V). Compared with these data of frequency of chromosomal aberrations induced by irradiations from other plants such as *Triticum monococcum* (Smith, 1948), *Oryza sativa* (Shastry and Ramaiah, 1961) and *Sorghum versicolor* (Garber, 1948), the frequency of chains in present materials was too low to draw the conclusion that the chains were induced by the irradiations. On the other hand, no cells showed a ring.

TABLE V

RESULTS OF IDENTIFICATION OF CHROMOSOMAL ABERRATIONS

(MATERIALS FROM GREENHOUSE)

			Number					
Dosage(r)	Entry No. (1962)	Seed Set %	Samples Obs.	Slides Obs.	Cells Obs.	Cells with Chain	9II-2I	8II-4I
X-ray								
15,000	102	2	1	7	67	4		
15,000	147	1	1	6	54			
15,000	177	0	1	4	112			
20,000	262	3	1	4	69			
20,000	268	1	1	4	87			2
Total			5	25	389	4		2
Neutron								
15,000	26	1	1	8	66		2	
15,000	57	1	2	8	276	1	10	
Total			3	16	342	1	12	
Grand Total			8	41	731	5	12	2
Untreated Cytoplasmic ms Lines								
	128	2	1	6	55			
	194	3	1	5	36			
Total			2	11	91			
Normal Wheatland								
	131	100	1	3	27			

Among the cells with chains, two cells had a chromosome number $2\underline{n} = 40$ and were tetraploid, In tetraploid cells, the chain of four chromosomes may be explained as a tetravalent association. A great number of tetravalent associations such as chains of four chromosomes, have been observed in tetraploid sorghum materials (Endrizzi, 1957). Some of these associations are also possible bivalents which happened to lie in contact with each other.

A large number of cells with univalents were identified in some samples. Two univalents and 9 bivalents or 4 univalents and 8 bivalents (Fig. 3, See Page 73) per cell were found. In general, univalents are more often found in hybrids due to the failure of non-homologous or partial homologous chromosomes to synapse during meiosis. The question of whether the univalents in these materials were due to irradiation or due to the cross of irradiated materials X cytoplasmic male sterile lines is unknown.

Cytological study of materials with seed set less than 30% in head rows

Some head rows (plots) showed low seed set on the average. The low seed set was possibly caused by some chromosomal aberrations. It can be determined that the samples collected in 1960 were those which showed low seed set in 1962. Based on the head row seed set, 60 head rows showed less than 30% seed set. Four samples from 4 different head rows in 1960 were chosen from each treatment for both irradiations. A total of 21 samples was chosen for cytological identification again. Samples from this study were different from those examined before, even in the same row. More cells were analyzed and more information was recorded. A great variation in chromosome number was found which will be discussed in the next section. The samples observed and the number of cells analyzed are presented in Table VI.

TABLE VI

RESULTS OF IDENTIFICATION OF CHROMOSOMAL ABERRATION

(MATERIALS FROM 1960, WHICH SHOWED LOW SEED SET IN 1962)

Dosages(r)	Entry No. (Head Row 1960)	Seed Set % (Head Row 1962)	Number			
			Samples Obs.	Slides Obs.	Cells Obs.	Cells with Chromosome Aberrations
			X-ray			
15,000	58	18.1	1	3	39	0
15,000	64	27.2	1	4	155	0
15,000	215	23.0	1	3	30	0
15,000	232	29.8	1	4	27	0
20,000	175	24.3	1	4	76	0
20,000	178	24.2	1	3	345	0
20,000	180	24.9	1	4	44	0
20,000	185	26.5	1	2	27	0
25,000	105	23.0	1	4	78	0
25,000	106	26.5	1	5	27	0
25,000	202	29.3	1	3	37	0
25,000	234	24.3	1	6	65	0
Total			12	45	950	0
			Neutron			
15,000	129	17.8	1	10	156	0
15,000	138	30.0	2	6	448	0
15,000	205	17.8	1	3	85	0
15,000	125	30.0	1	3	224	0
20,000	35	23.0	1	4	63	0
20,000	145	20.0	1	2	75	0
20,000	230	28.7	1	4	44	0
20,000	238	23.0	1	4	31	0
Total			9	36	1125	0
Grand Total			21	81	2076	0

Of 2,076 cells analyzed, no cell showed a chromosomal aberration, whether a chain, ring of four chromosomes, dicentric, or fragment.

Using the standard method of identification of chromosomal aberrations, aberrations have still not been detected. However, some phenomena showed that the plants from irradiated seeds grew abnormally. Most plants growing from the original irradiated seeds gave partial sterility and indicated that irradiation caused disturbance of the seed tissue. This also can be measured from the degree of sterility obtained at different dosages. The higher the dosages the more the damage to the seeds. The plants from the seeds irradiated with lower dosages for both irradiations, i.e., 10,000 r, grew normally. Occasionally some were found to have aborted pollen but these were less than 50% of the total. However, the plants from highest dosages, i.e., 35,000 r, were inhibited in germination or normal growth. The abnormal growth or death of the plants is probably due to the effects of irradiation. These phenomena have been reported by many investigators in other plants (Aebersold and Lawrence, 1942; Marshak, 1939).

In previous researches, either X-rays or thermal neutrons or both with the same dosages as used in this experiment were found to induce chromosomal aberrations or physiological disturbance such as inhibition of germination, seedling growth, and chlorophyll production. Chromosomal aberrations were identified by Smith (1950); Caldecott and Smith (1952); Burnham et al. (1954); Moh and Nilan (1956); Caldecott et al. (1954); Caldecott (1955) in Hordeum; Shastry and Ramaiah (1961) in Oryza; Smith (1950) in Triticum; Giles (1940); Thoday (1942) in Tradescantia; Anderson and Clokey (1934); Anderson (1935); Longley (1950); Morris (1955) in Zea. Furthermore, some chromosomal aberrations were found in materials treated with lower dosages than those in this experiment.

For example, in rice irradiated by X-rays with dosages of 4,000 r, Soriano (1959) evidenced the ring and chain configurations indicating that the reciprocal translocation occurred.

As mentioned above the pollen abortion was supposedly produced by irradiation and the percentage of abortion was proportional to the dosages of both irradiations. These results are also supported by previous researches in other plants by use of irradiations. These phenomena suggested that irradiations have done some damage in the materials. In the current study no observable aberrations in the materials used for cytological study were observed. The question is how this result is obtained. To explain this discrepancy the following points may be suggestive:

First, the fact that genetic abnormality occurred in the procedures but was not identified by cytological study, means that irradiation may have produced an effect but the plants with chromosomal aberrations were lost during handling of the treated materials. Burnham et al. (1956), reviewing chromosomal interchanges in plants, pointed out that homozygous interchanges can be identified by crossing with a standard normal; the F_1 shows an association of four chromosomes and is partially sterile without completely directed segregation. He indicated that this is the standard procedure in isolating lines homozygous for the interchanges in other plants, such as maize, wheat, barley, and Datura. These procedures were followed in this experiment. The only possible step where chromosomal aberrations could have been lost was in choosing of the male parents (treated materials) which were used to cross with cytoplasmic male sterile lines. In 1959, selfed seeds from irradiated materials were grown in head rows. Each head row (plot) included 12 plants. Based on genetic theory, the 12 plants from the selfed seeds were expected in 3 classes with 1 : 2 : 1 ratio, the classes being 1 homozygous normal, 2 heterozygous aberration and 1 homozygous aberration. Theoretically, half of these plants, i.e., 6 plants, have heterozygous aberrations which would cause partial sterility. The other 6 plants, i.e., with either homozygous aberrations or homozygous normal, should show full fertility. During blooming time, the pollen of some plants from each plot were tested by standard iodine technique. If some plant or plants in that plot showed more than 50% pollen abortion, which were supposed to be heterozygous aberration, the plants showing normal fertility were crossed to cytoplasmic male sterile lines. These plants showing full fertility are, theoretically, partly homozygous normal or partly homozygous aberration. They cannot be distinguished from each other on the basis of plant morphology or fertility. By this procedure, one would expect to obtain some plant heads with hybrid seeds from the crosses of homozygous aberration X cytoplasmic male sterile lines. Plants from these seeds would show cross-shaped figures at pachytene or rings or chains of four chromosomes at metaphase I during meiotic division. Theoretically, there is a 50 : 50 chance to obtain these translocated samples. In this procedure, it is possible that all plants crossed with cytoplasmic male sterile lines are homozygous normal since they definitely show full fertility. If this assumption is true, the samples from the plants grown from such crossed seeds could have no chromosomal aberration. The fact that the average percentages of seed set from the treated materials are close to the normal cytoplasmic male sterile also supports this conclusion. In general, the seed set percentage of sorghum carrying translocation gives about half of the normal, i.e., cytoplasmic male sterile plants in this experiment. The overall average of seed set of treated materials is 38.3% (ranging from 33.7 to 45.6%); the normal, 43.25% (cytoplasmic male sterile lines). The difference of seed set is only 5% which is too low to conclude that there is any chromosomal aberration in these materials.

Secondly, in general, seeds treated with irradiations, either X-rays or thermal neutrons, may contain abnormalities in chromosomes resulting from (1) a change in the gene from one allele to the other, such as mutation; (2) a re-arrangement of chromosome material, such as a translocation, inversion, etc.; or (3) a loss or duplication of chromosome segments. All of these are possibly harmful or deleterious to the treated materials and result in partial sterility. Through the steps used in handling the treated materials in this experiment, only the case of re-arrangement of chromosome material such as an interchange, may be easy to identify cytologically. The partial sterility caused by either mutation, deficiency or duplication would be difficult to identify in this material by cytological study.

Thirdly, it is also possible that interchange of chromosomes occurred but both pieces of interchanges were too short to identify. In this case, though a ring of four chromosomes may not be present at metaphase I, possible chains or two "pairs" will be common. This has been found in maize by Clarke and Anderson (1935) and in rice by Oka et al. (1953). The former using the irradiated material of maize, found that from the selfed progenies one line of partially sterile plants carrying interchange showed no rings at meiosis and only occasionally chains of chromosomes. A study of pachytene figures showed that the satellite chromosome and the third chromosome were involved. The large terminal chromosome of the normal satellite is never synapsed with its interchanged mate. In many cases the end portions of the third chromosome also failed to synapse. The ten chromosome pairs were entirely separate at diakinesis; rings were never found. Oka et al. (1953), using the irradiated material of rice, found that a certain strain showed sterility but no quadrivalents were found. This strain was interpreted as possibly having a reciprocal translocation but the translocated segment was possibly very short and not detectable cytologically. This may have happened in the present material.

Fourthly, during the period of cytological identification, some cells were found with a variation in chromosome number, either polyploid or aneuploid. In polyploids, chromosome numbers with 2n = 30, 40, 50, 60, 70, even as high as more than 100 were found. In aneuploids, chromosome numbers with 2n = 12, 14, 16, 18, 22, 24, 26, 28, 34, and 38 were occasionally observed. This situation is possibly related to irradiation effects. However, the frequency of abnormality, other than normal (2n = 20), is not correlated with the different dosages of both irradiations. These abnormalities may have resulted from some other causes and will be discussed in detail in the next section.

Some or all the possibilities mentioned above may explain the cause of the partial sterility from irradiation. Moreover, this may not be all the possible explanation. Further study is, therefore, indicated. For this purpose, several methods may be considered.

1. Some of the plants from selfed seeds showed more than 50% sterility in the 1959 planting. The remaining seeds could be planted again. These plants which showed more than 50% sterility could be transplanted to the greenhouse for collecting the samples for cytological study. Since these plants showing more than 50% sterility are expected to carry translocations.
2. Since the low seed set occurred not only in treated materials but also in cytoplasmic male sterile lines, the type of cytoplasm may play a role in the result. For checking this, sweet seeded tan sorgo

could be employed to cross with fully fertile plants from rows in which some plants show 50% sterility or more. Hybrid seeds can be identified without emasculation by selecting the plump seeds from the shrunken self pollination.

3. The irradiation of sorghum seeds could be repeated and the irradiated materials of the first generation could be checked cytologically. If chromosome aberrations of some plants are observed, these plants should be kept in the greenhouse for further study, either selfing or crossing with normal. This may prevent any chance of losing the plants with the chromosome aberrations. This procedure has been done in some plants such as Zea mays (Clarke and Anderson, 1935) and Oryza sativa (Soriano, 1959).

Variations in Chromosome Number

In the course of cytological study of the irradiated materials, variation in chromosome number including polyploid cells and aneuploid cells was observed. The regular chromosome number in these materials (Sorghum vulgare) is $2\underline{n} = 20$*, i.e., 10 bivalents at metaphase I (See Appendix Fig. 5, Page 86). In polyploid cells, various chromosome numbers were found. Most of these aneuploid counts were observed within a group of pollen mother cells (hereafter referred to as PMCs) which were difficult to distinguish one from another. Some of them, however, such as $2\underline{n} = 12$, 14, 16, and 18 were found in a single cell at metaphase I or anaphase I.

For the sake of convenience, the present materials, studied cytologically, may be classified into three groups as mentioned in the last section. The three groups are:

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*For convenience, $\underline{n} = 10$ is considered as the basic number of Sorghum vulgare, although some researchers have indicated that this species is the allotetraploid, i.e., $4\underline{n} = 20$, so that the basic number is 5 (Endrizzi, 1955; Hadley, 1959).

Group I: Materials collected from the field in the summer of 1960 and listed in Table II.

Group II: Materials collected from the greenhouse in the spring of 1963 which showed less than 5% seed set in 1962 and listed in Table V.

Group III: Materials collected from the field in the summer of 1960 which had seed set less than 30% (based on head row average) in 1962 and listed in Table VI.

The frequencies and percentages of the cells with different chromosome numbers including normal and abnormal (polyploid and aneuploid) for different dosages for both X-rays and thermal neutrons are listed in Tables VII, VIII and IX for Groups I, II, and III, respectively.

TABLE VII

IRREGULARITIES IN CHROMOSOME NUMBER (GROUP I)

	Cells	Normal Cell	Cells with Irregular Chromosome No. (2n)										
Dosage(r)	Obs.	2n = 20	40	10	30	50	60	>100	18	22	24	Total	Other*
					X-ray								
15,000	409	323	68		6	1	1	1				77	9
20,000	437	363	60	5	2		1		3	1		72	2
25,000	268	237	26		1		1	1		1		30	1
Total	1,114	923	154	5	9	1	3	2	3	2		179	12
					Neutron								
15,000	279	266	10	1					1		1	13	
20,000	271	247	23									23	1
Total	550	513	33	1					1		1	36	1
Grand Total	1,664	1,436	187	6	9	1	3	2	4	2	1	215	13

*Cells with two or more nuclei or some bivalents not arranged on the metaphase plate.

TABLE VIII

IRREGULARITIES IN CHROMOSOME NUMBER (GROUP II)

Dosage (r)	Plant No.	Cells Obs.	Normal Cell 2n = 20	Cells with Irregular Chromosome No. (2n)											
				40	30	60	>100	14	16	18	24	26	38	Total	Other*
						X-ray									
15,000	102	67	52	2	2	1				4				9	2
15,000	147	54	53											0	1
15,000	177	112	98	2	1			1	2	4		2		12	2
20,000	262	69	65								4			4	
20,000	268	87	78	2			1			1				4	3
Total		389	346	6	3	1	1	1	2	9	4	2		29	8
						Neutron									
15,000	26	66	59	3							1			4	1
15,000	57	276	237	19					1	2	1		1	24	4
Total		342	296	22					1	2	2		1	28	5
Grand Total		731	642	28	3	1	1	1	3	11	6	2	1	57	13
						Untreated Cytoplasmic ms Lines									
	128	55	52	2											
	194	36	31				3	1	1					5	1
Total		91	83	2			3	1	1					5	1
						Normal Wheatland									
	131	27	24	1											2

*Cells with two or more nuclei or some bivalents not arranged on the metaphase plate.

TABLE IX

IRREGULARITIES IN CHROMOSOME NUMBER (GROUP III)

Dosage(r)	Seed Set %	Cells Obs.	Normal Cell 2n = 20	Cells with Irregular Chromosome No. (2n) 40	10	30	60	14	16	18	22	24	26	28	34	Total	Other*
X-ray																	
15,000	18.1	39	33	4			1			1						6	
15,000	27.2	155	153	2												2	
15,000	23.0	30	30													0	
15,000	29.8	27	23	3												3	1
20,000	24.3	76	70	5												5	1
20,000	24.2	169	148	14		2	2					1		1	1	21	
20,000	24.9	44	42	2												2	1
20,000	30.0	203	181	6	2	1	1	2	2	6						20	1
25,000	23.0	78	74	3		1										4	
25,000	26.5	27	27													0	
25,000	29.3	37	32	2		1				1						4	1
25,000	24.3	65	63	2												2	
Total		950	876	43	2	5	4	2	2	8		1		1	1	69	5
Neutron																	
15,000	17.8	156	133	12		1	1		1	4		1				20	3
15,000	30.0	448	424	17		1			2				1		1	22	2
15,000	17.8	85	72	8			2				1					11	2
15,000	30.0	224	192	30												30	2
20,000	23.0	63	55	8												8	
20,000	20.0	75	73	1		1										2	
20,000	28.7	44	40	2					1							3	1
20,000	23.0	31	29	1												1	1
Total		1126	1018	79		3	3		4	4	1	1	1		1	97	11
Grand Total		2076	1894	122	2	8	7	2	6	12	1	2	1	1	2	166	16

*Cells with two or more nuclei or some bivalents not arranged on the metaphase plate.

The data for the three groups including number of samples observed, frequencies of cells with regular and irregular chromosome numbers were condensed together and are presented in Table X.

In the first group, the frequencies of cells with irregular chromosome number were recorded occasionally during the search for chromosomal aberrations, since, at that time, most samples were observed at the pachytene stage. Cells at metaphase or anaphase stages were occasionally observed; sometimes the cells were counted but sometimes not. Thus, the frequencies of cells with different chromosome numbers in this group might not accurately represent their actual frequencies. The materials of the second group were collected from the branches or tillers of the plants in the greenhouse. They were not as favorable as that from the field for cytological study and the samples studied totaled only 8 for treated materials, 2 for cytoplasmic male sterile plants and 1 for normal Wheatland (Table VIII). The materials of the third group were collected in the field and the frequencies of cells with different chromosome numbers were recorded from all the cells analyzed (Table IX). Thus, the data in this group were more complete than that in the other two groups. In addition to the percentage of cells with different chromosome numbers for the three groups, based on the total cells observed, the percentage for the third group was also calculated on the basis of the head row (Table X). The results from different calculations were relatively close. The cells with irregular chromosome number were also observed in materials of untreated cytoplasmic male sterile plants and normal Wheatland (Table VIII), but any conclusion as to the frequencies of cells with irregular chromosome number from these three different materials could not be drawn from the small number of cells analyzed. The irregularities in chromosome

TABLE X

SUMMARY OF CHROMOSOMAL ABERRATIONS AND VARIATIONS IN CHROMOSOME NUMBER

Group	Radiation	Dosage (r)	Number			Normal Cells 2n = 20	Cells with Irregular Chromosome No.					Others*
							Polyploid		Aneuploid			
			Samples Obs.	Cells Obs.	Cells with Chain		40	>40	18	<or>18	Total and Percentage	
I	X-ray	15,000	56	409		323	68	9	0	0	77 (18.8)	9
	X-ray	20,000	85	437		363	60	8	3	1	72 (16.5)	2
	X-ray	25,000	47	268		237	26	3	0	1	30 (11.2)	1
	Neutron	15,000	62	279		266	10	1	1	1	13 (4.7)	0
	Neutron	20,000	48	271		247	23	0	0	0	23 (8.5)	1
	Total		298	1664		1436	187	21	4	3	215 (12.9)	13
	Percentage			100.0		86.3	11.3	1.3	0.3	0.2	12.9	0.8
							208(12.6%)		7(0.5%)			
II	X-ray	15,000	3	233	4	203	4	4	8	5	21 (9.0)	5
	X-ray	20,000	2	156	0	143	2	1	1	4	8 (5.1)	5
	Neutron	15,000	3	342	1	296	22	0	2	4	28 (8.2)	17
	Total		8	731	5	642	28	5	11	13	57 (7.8)	27
	Percentage			100.0	0.7	87.8	3.8	0.7	1.5	1.8	7.8	3.7
							33(4.5%)		24(3.3%)			
III	X-ray	15,000	4	251		239	9	1	1	0	11 (4.4)	1
	X-ray	20,000	4	492		441	27	8	6	7	48 (9.8)	3
	X-ray	25,000	4	207		196	7	2	1	0	10 (4.8)	1
	Neutron	15,000	5	913		821	67	5	4	7	83 (9.1)	9
	Neutron	20,000	4	213		197	12	1	0	1	14 (6.6)	2
	Total		21	2076		1894	122	17	12	15	166 (8.0)	16
	Percentage			100.0		91.2	5.9	0.8	0.6	0.7	8.0	0.7
							139(6.7%)		27(1.3%)			
	Percentage**			100.0		91.7	5.6	0.7	0.5	0.8		
Grand Total			327	4471	5	3972	337	43	27	31	438	56
Percentage				100.0	0.11	88.83	7.53	0.96	0.60	0.69	9.8	1.25

*Cells with two or more nuclei, some bivalents not arranged on the metaphase plate or cells with univalent chromosomes.

**Percentage calculated on the basis of head row.

number in cytoplasmic male sterile Wheatland and its counterpart were also observed by Damon (1961).

The percentage of the irregularities in chromosome number for the three groups were 12.9 (215 out of 1664 cells), 7.8 (59 out of 731 cells) and 8.0 (166 out of 2076 cells) for Groups I, II, and III, respectively (Table X). These percentages were close to the results obtained by Damon (1961) from normal Wheatland. He found that the percentages were 9.7 and 8.3 for the cells at metaphase I and anaphase I, respectively. These irregularities may be classified into two groups: one is polyploid cells, which covers the situations where the total chromosome numbers in a single cell involves complete genomes; the other group is aneuploid cells, which refers to cells containing chromosomes which do not have true multiples of the basic number. The different groups of materials had different percentages of seed set in the field which did not influence the percentages of cells with irregular chromosome number. It is generally expected that plants with lower percentage of seed set may have had irregularities in their chromosome number. That is, cells with more irregularities in chromosome number were expected in the greenhouse materials (Group II) than in the field. However, the data did not agree with this expectation. Smith (1942) observed irregular chromosome numbers in barley (Hordeum vulgare, $2\underline{n} = 14$), and their frequencies were influenced by environment, indicating that meiosis in the central florets was less abnormal in plants grown in the greenhouse than those grown in the field. This might be true with the three groups of materials which had different percentages of seed set but almost the same frequencies of irregularities in chromosome number. It is also possible that the low percentage of seed set was due to adverse environmental conditions affecting pollination and development of the seeds (see last section), and not due to cytological abnormalities.

The irregularities in chromosome number vary considerably within the samples of the three groups. Some samples had no abnormal cells at all (Tables VIII and IX). Comparisons between the two sources as well as between different dosages of irradiations revealed that there are not correlated with the frequencies of irregularities in chromosome number (Tables VII, VIII, IX, and X). This might indirectly indicate that chromosomal aberrations do not exist in these plants. For convenience, the irregularities in chromosome number will be described and discussed in two parts, i.e., polyploidy and aneuploidy.

Polyploidy

Among the cells with polyploid chromosome numbers, the tetraploid cell ($2\underline{n} = 40$, Fig. 2), as compared to other polyploid cells was found in a relatively high percentage in the three groups. Of the total cells observed, percentages of tetraploid cells for the three groups varied greatly, i.e., 11.3, 3.8, and 5.9 for Groups I, II, and III, respectively (Table X). In addition to tetraploid cells, PMCs with $2\underline{n} = 30$ (Figs. 4 and 15), 50 (Fig. 5), and 60 (Fig. 6), were occasionally observed. In some samples, PMCs with $2\underline{n} = 70$, 80, and even more than 100 chromosomes clumped together at metaphase I were observed. Figure 7 is one of the examples showing a "cell" with approximately $2\underline{n} = 160$. In most cases, a group of PMCs were aggregated together and the boundaries of individual cells were not clear. It seems that the PMCs in the anther sac tend to form plasmodial masses of various sizes in which the chromosomes lie in groups. Sometimes it is difficult to recognize how many cells were really involved in that plasmodial mass (Fig. 8). Groups of chromosomes in a plasmodial mass may occasionally form more than one metaphase plate at metaphase I within a single mass of PMC materials. Similar cases were also observed by Damon (1961) and Sanders et al. (1962) in sorghum, Smith (1942) in barley, and Levan (1941) in haploid Phleum pratense.

Using fertile Wheatland, Damon (1961) found cells with $2n = 40$, 60, 80, 130, and 170. The percentage of tetraploid cells was 2.2% (45 out of 2015 cells) which was lower than that observed in the present materials, (7.5%, Table X). The difference in percentages may be due to different materials. In barley ($2n = 14$), Smith (1942) reported that polyploid cells with chromosome numbers of 14, 21, 28, 35, 42 and as high as 112 bivalents were counted on a single metaphase plate. The different environments (as in the field or in the greenhouse) and different parts of the spike gave different frequencies of the large groups of chromosomes; more large groups of chromosomes were observed from lateral florets of the spike than that from central florets (Smith, 1942). Levan (1941) in haploid Phleum pratense observed a single syncytium having 50 to 80 even up to 150 bivalents at metaphase I.

Aneuploidy

Of the cells analyzed, 58 cells (1.3%) were aneuploid with a total chromosome number either more or less than 20. Most were found in groups of PMCs. For the three group materials in this study and based on the total cells observed, their percentages were 0.5% (7 out of 1664 cells), 3.3% (24 out of 731 cells) and 1.3% (27 out of 2076 cells) for Groups I, II, and III, respectively (Table X). Frequency of aneuploid cells was low as compared to that of polyploid cells. In such aneuploid cells, the number of bivalents that lie together in a single group is variable.

Observations from a group of PMCs revealed that the chromosomes seemed relatively free to move within the pollen mother cell material of an anther sac. This phenomenon is probably closely related to the occurrence of aneuploid cells. Single cells with aneuploid chromosome numbers of 6, 7, 8, and 9 bivalents at metaphase I (Figs. 9, 11, and 13) were observed. Cells at metaphase I or anaphase I with chromosome numbersof more than normal, i.e., 22, 24 (Fig. 10), 26, 28, 34, 36, and 38 (Fig. 12) were found. Of these aneuploid cells, nullisomic type, 9 bivalents (Fig. 13), had a higher percentage (0.6%) than others (Table X). Cells with more than 10 bivalents were very rare (Tables VII, VIII, and IX). In fertile Wheatland, Damon (1961) found a percentage of aneuploid cells of 2.9% (59 out of 2105 cells) including cells with 6, 12, and 18 chromosomes. This percentage of aneuploid cells was almost five times that found in the present materials. Smith (1942) in barley reported only a few cells with chromosome numbers 4, 9, 16, and 17. In the progenies of haploid sorghum, Schertz (1962) reported, among 394 plants, the most frequent type was diploid ($2\underline{n} = 20$) but five plants were trisomic with 21 chromosomes, one had 22 chromosomes, one had a translocation and one was haploid.

At anaphase I, chromosomes in all the cells observed were equally distributed as far as normal or abnormal cells are concerned. All cells with $2\underline{n} = 20$, were in a 10-10 distribution; cells with $2\underline{n} = 40$ in a 20-20 distribution (Fig. 14). A few cells with $2\underline{n} = 30$ in a 15-15 distribution (Fig. 15), with $2\underline{n} = 50$ in a 25-25 distribution (Fig. 5), and one cell with $2\underline{n} = 16$ in an 8-8 distribution (Fig. 16), were observed. No cell with unequal distribution was evidenced.

One cell from the greenhouse materials was found with 9 chromosomes moving to each pole and four chromosomes (two divided univalents) as laggards (Fig. 19).

The chromosomes of polyploid cells were arranged in a regular equatorial plate at metaphase I (Figs. 2, 4, 6, and 7). During the prophase stage, in some cases, different nuclei in a mass of cytoplasm were observed. They, however, usually maintained their individuality. During the pachytene stage, chromosomes pair regularly; one nucleolus with more than 10 pairs was not observed, although the figures at this stage were not clear enough to count the number of chromosomes. However, at the diakinesis stage, cells having one nucleolus with 20 pairs of chromosomes or having two nucleoli with 30 pairs of chromosomes were occasionally observed. Under the case of one nucleolus with 20 pair chromosomes two pairs of chromosomes attached on one nucleolus were found. This phenomenon suggested that the tetraploid cell originated from two cells, possibly two nuclei fusing together at early prophase. In some instances, at late diakinesis, 20 pairs of chromosomes scattered about two connected nucleoli was observed (Fig. 17). This suggested that the cell wall failed to form or that cytokinesis failed to occur or cytomixis has taken place during early stage of cell division.

Observations of different stages of meiosis revealed that tetraploid cells exist in the diploid plants. Forty chromosomes can be counted in a single cell at diakinesis (Fig. 17), metaphase I (Fig. 2) and anaphase I (Fig. 14). Furthermore, as Figure 18 shows two cells may line up side by side at anaphase I, one with 10 chromosomes at each pole, and the other with 20 chromosomes at each pole.

The polyploid cells with a higher chromosome number at metaphase I is possibly the result of cytomixis. Cytomixis is usually considered to be the result of the absence of cell wall formation. In the absence of the cell wall, either the chromosomes or the nuclei may migrate from one cell to another. Figure 20 shows four bivalents of a cell migrated to the other cell, giving rise to two aneuploid cells, one with six bivalents and the other with fourteen bivalents. Sometimes chromosomes of a whole spindle may migrate from one cell to another.

As already mentioned, tetraploid cells had a high frequency among the cells with irregular chromosome numbers. This is possibly closely related to ctyomixis. According to Levan (1941), cytomixis leads to the origin of binucleate PMCs, which may be of importance for the origin of polyploidy. Cytomixis was observed in many plants. In some plants, individual chromosomes or groups of chromosomes or even whole spindles were said to be carried from one cell into another (Maheshwari, 1950). If a part of the chromosomes in a normal cell migrated to another cell, aneuploid cells with more or less than normal chromosome number would result (Fig. 20). If the whole spindle of chromosomes of one, two or more normal cells migrate to another normal cell, polyploid cells would result. The occurrence of cytomixis has been observed in sorghum (Damon, 1961), barley (Smith, 1942), Oenothera (Gates, 1911)*, Lathraea (Gates et al., 1927)*, Coreopsis (Gelin, 1934)*, Phleum (Levan, 1941).

According to Levan (1941), the earlier stages of meiotic prophase were considered to be especially favorable for the origin of cytomixis, it may occur even close to metaphase I. Polyploid cells were frequently observed at metaphase I and anaphase I. Cytomixis may, therefore, take place after the last division of the archesporium. Levan (1941) reported that no nuclear fusion occurred prior to zygotene. Damon (1961), studying the same variety of sorghum as in the present experiment, reported that fusion of chromosome groups did not occur previous to early metaphase I. In other plants, it is reported that the migration of chromosomes is frequent between zygotene and diakinesis, or even during the interkinesis stage of meiosis (Maheshwari, 1950). A description of how cytomixis takes place and the manner of the chromosome migration from one cell to another, was offered by Smith (1942). A chromosome from one cell crosses a connecting bridge and joins those chromosomes in another cell. The connecting bridge was so narrow that all bivalents could not have traveled it simultaneously. Thus the spindle of the merged plate has a pole near the center of the two cells. The latter case was also observed by Levan (1941).

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*According to Maheshwari (1950) pp 41-42.

Some observations revealed that chromosome movement was due to the mechanics of smear preparation. In groups of PMCs any number of bivalents can be found. In some cases, one or two or more bivalents separated from the other bivalents on the same plate. Ten bivalents of a normal cell may be separated in several groups. The chromosomes can apparently move through considerable distances. In some cases, chromosomes from different cells may mingle together; one, two or more bivalents migrating beyond the metaphase plate. This would more likely happen in case of the absence of the cell wall. Therefore, counting the number of aneuploid cells from a group of PMCs should be done with caution. The migration of chromosomes due to the mechanics of smear preparation has been observed by Damon (1961), Smith (1942) and Woodworth (1931, see Maheshwari 1950, p.41). Of course, this may happen where groups of cells are present and no boundary exists between them. Since in the standard aceto-carmine technique in order to obtain good spreading of the chromosomes, the cover glass must be pressed forcibly, the cytoplasm will spread out in all direction. This could transport a single or several chromosomes of normal cells to the spindle area of another cell.

As for the single cell with an aneuploid chromosome number, it is possibly due to cytomixis in which one or two or more chromosomes may migrate from one cell to another during early prophase. One spindle will be formed in each cell, so the chromosomes will orient themselves more or less on one plate at metaphase I (Figs. 9, 10, and 13). For the single cell with aneuploid chromosome number, Damon (1961) gave another explanation. He thought that chromosomes from a "cell" with two or more nuclei, may mingle at late diakinesis or early metaphase I. A regeneration of the destroyed cell wall may occur after this mingling takes place, so that aneuploid cells resulted. Since the frequency of aneuploid cells was relatively low, it may be due to the cells in which some chromosomes were lost during the mitotic division previous to meiosis as in the case of laggards, univalents.

The polyploid cells with higher chromosome numbers are possibly due to the failure of formation of the cell wall, or to the fusion of the nuclei. In the former case, if the cell wall fails to form at the last mitotic division of archesporium, a tetraploid cell resulted, giving 20 bivalents at metaphase I of meiosis. If the cell wall fails to form in a series of successive mitosises previous to meiosis, the polyploid cells with higher chromosome number should result, such as 40, 60, 80 even up to more than 100 chromosomes. The failure of cell wall formation giving rise to polyploid cells has been reported by many workers in plants, such as Phleum (Levan, 1941), Hordeum (Smith, 1942), Sorghum (Damon, 1961). Another possibility for the origin of polyploid cells is fusion of entire cells or nuclei of the sporogenous tissue. Occasionally, a number of nuclei present in a mass of cytoplasm was observed, but the actual fusion was not clearly observed. Undoubtedly, the fusion of cells or nuclei could occur in the absence of a cell wall. The absence of a cell wall may be due to a failure of its initial formation or it may become dissolved following formation (Matsura, 1935*; Stern, 1946). The maximum number of nuclei in a giant "cell" resulting from fusion was 30 in Phacellanthus (Matsura, 1935)*, Phleum pratense (Levan, 1941) and 32 in Trillium erectum (Stern, 1946).

Another possible explanation for the origin of polyploid cells is the failure of cytokinesis. Two or three nuclei in one cell were occasionally observed although the number of such cells was rare. The possible stage of the failure of cytokinesis may be at the last mitotic division previous to the onset of meiosis of the microsporcyte. Since most PMCs observed were at metaphase I, polyploid cells, particularly tetraploid cells, should form before meiosis starts. The failure of cytokinesis in sorghum has been reported by Damon (1961) and Schertz (1962).

One more possibility for the origin of polyploid cells or aneuploid cells may be due to the methods or chemical applied in such a study. It has been reported that some fixations and stains may result in a breakdown of cell structure due to a dispersion of the cytoplasm giving rise to multinucleated plasmodial masses (Levan, 1941). Strong concentrations of acid, including acidic stain such as aceto-carmine, may be intimately associated with the breakdown of cell structure of meiotic cells (Stern, 1946).

The chromosomes of most of the cells were counted at metaphase I. The bivalents in polyploid cells or aneuploid cells with more than 10 bivalents were arranged on one metaphase plate (Figs. 2, 4, 6, 7, and 10). If these cells originated from either the absence of cell wall or the failure of cytokinesis how did these bivalents from different nuclei become arranged on one equatorial plate irrespective of the chromosome number present in the giant cells. Generally, the arrangement of chromosomes (bivalents) on the equatorial plate at metaphase I depends on the spindle formation in which the fibers attach to the chromosomes. In normal cases, chromosomes from one nucleus are arranged on one plate by one spindle. However, one cell with 20 bivalents arranged on two separate plates, was rarely found. Unfortunately, in the process of meiosis in sorghum, at least in the present material, the fibers of the spindle could not be usually observed.

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*According to Maheshwari (1950) pp. 41-42

On the other hand, as already mentioned, among the irregularities in chromosome number, tetraploid cells appeared with high frequency. If these cells are formed due to the absence of a cell wall between two adjacent microsporocytes, they should be autotetraploid, i.e., four homologous chromosomes should be present in each cell. Quadrivalent association should be frequently observed as meiosis proceeds. However, quadrivalent association was never observed. Observations on the tetraploid sorghum species reveal that quadrivalent association occurs with high frequency (Garber, 1944; Hadley, 1953; Huskins and Smith, 1934; Endrizzi, 1957). Some quadrivalent association was observed in a sorghum hybrid with $2\underline{n} = 40$ (Endrizzi, 1957). The most plausible explanation for these phenomena is that, in the giant cell, nuclei maintain their individualities as the chromosomes synapse at the pachytene stage; but only one spindle develops for all the bivalents in the giant cell when the nuclear membrances disappear in the late prophase and the bivalents mingle together from different nuclei. This explanation agrees with the statements from Levan (1941) and Gaines and Aase (1926). Levan (1941) reported that in the *Phleum* syncytia, no more than one spindle was ever formed, and that the spindle was normal and bipolar.

The polyploid cells with $2\underline{n} = 30$ (Figs. 4 and 15), 50 (Fig. 5), and 70, are not reasonably explained on the assumption of the absence of the cell wall or the failure of cytokinesis. The cells with chromosome numbers $2\underline{n} = 30$ and 50 had 15 and 25 bivalents arranged on one metaphase plate, respectively. At the anaphase I they showed a 15-15 and 25-25 equal distribution. Trivalents, univalents, or laggards were never observed in such cells. The origin of these polyploid cells is unknown.

To obtain a satisfactory answer to these questions a further study is necessary. Since this is a complex problem, a further study might be conducted along the following lines:

1. Different varieties of Sorghum vulgare may have different frequencies of irregularities in chromosome number. The initial study has been reported by Damon (1961), who found that cytoplasmic male sterile lines of Wheatland, Westland, and Martin exhibited a large connected mass of cytoplasm but in cytoplasmic male sterile lines of Redlan and Combine Kafir-60 no clear cases of multiploid sporocytes or cell wall destruction were observed. Fertile lines of these varieties may also show some difference in these irregularities.
2. Techniques of handling these materials including fixation and staining, may also be related to the abnormalities. Possibly some techniques other than those used may reduce or prevent the occurrence of such abnormalities. The living cell culture may be taken under consideration. This has been done on Trillium erectum by Stern (1946).
3. To be sure in which stage the cell wall fails to form or dissolves after formation, the certain stages of mitosis of the archesporium and meiosis of sporocytes need to be carefully analyzed.

The irregularities in chromosome number are the basic problem in *S. vulgare* and its use for cytological study. Before using this plant for cytogenetic study, this basic problem should be solved.

CHAPTER V

SUMMARY

In order to establish homozygous chromosomal aberrations, particularly translocation, for genetic studies in Sorghum vulgare ($2\underline{n} = 20$), seeds of the variety, Wheatland, were treated with X-rays and thermal neutrons at dosages of 10,000, 15,000, 20,000, 25,000, and 35,000 r units. Plants from the treated seeds showing 50% or more pollen abortion were selfed. Selfed seeds were planted in head rows. If any plant showed 50% or more pollen abortion in a row the fertile plants within the same row were crossed with cytoplasmic male sterile lines and simultaneously selfed. Crossed seeds were planted and buds were collected for identification of chromosomal aberrations. Two hundred ninety-eight samples were studied cytologically and no chromosomal aberrations were identified.

The seed set percentage of cytological materials varied greatly among the plants (14.3-70.0%). The average seed set percentages of treated materials, cytoplasmic male sterile lines (checks) and normal Wheatland (pollen producer) were 38.3, 43.3 and 90.1%, respectively. Since the seed set percentage of treated materials was close to that of untreated cytoplasmic male sterile lines, the low seed set is possibly caused by some factors other than chromosomal aberrations, such as insufficient pollen, late planting, different blooming date, the particular variety or weather conditions.

Plants from treated materials having less than 5% seed set were transplanted to the greenhouse for cytological study. In two plants, chains of four chromosomes were observed in very low frequency.

During the search for chromosomal aberrations, approximately 10% of the cells studied had irregularities in chromosome number including polyploid and aneuploid cells. Among polyploid cells, chromosome numbers of 30, 40, 50, 60, 80, and up to 180 were observed, and of these, the tetraploid cells (2n = 40) had the highest frequency. Among aneuploid cells, chromosome numbers of 12, 14, 16, 18, 22, 24, 26, 34, 36, and 38 were observed, and of these, the nullisomic (2n = 18) occurred in the highest frequency. The possible origin of the cells with irregular chromosome numbers is discussed.

LEGEND FOR PLATE I

Chromosomal Aberrations and Irregularities in Chromosome Number

Fig. 1. Metaphase I showing chain of four chromosomes (arrow).

Fig. 2. Tetraploid cell with 20 bivalents at metaphase I.

Fig. 3. Metaphase I showing 2 univalents (arrow) and 9 bivalents.

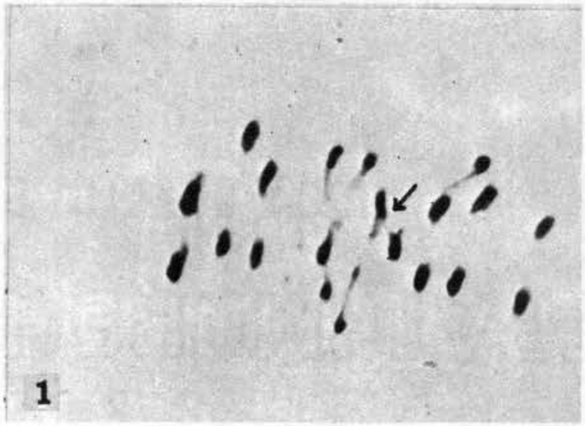
Fig. 4. Cell with chromosomes $2\underline{n} = 30$ at late metaphase I.

Fig. 5. Anaphase I showing 25-25 equal distribution.

Fig. 6. Cell with chromosomes $2\underline{n} = 60$ at late metaphase I.

Fig. 7. "Cell" with chromosomes $2\underline{n} = 160$.

PLATE I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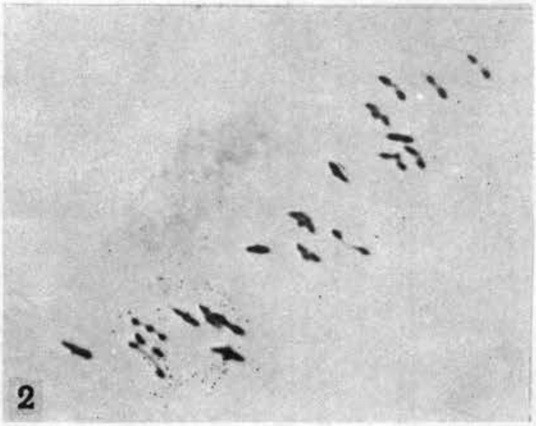
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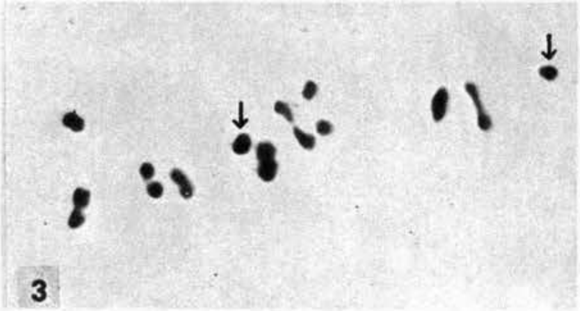
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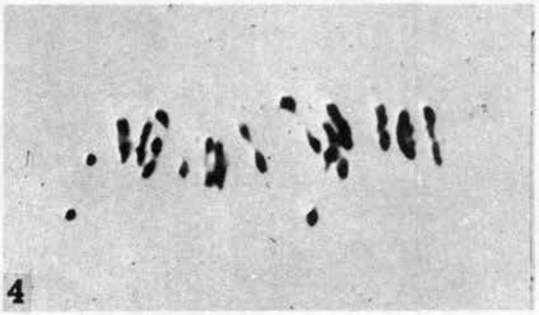
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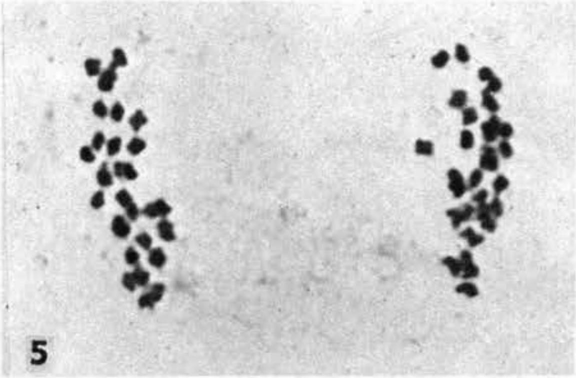
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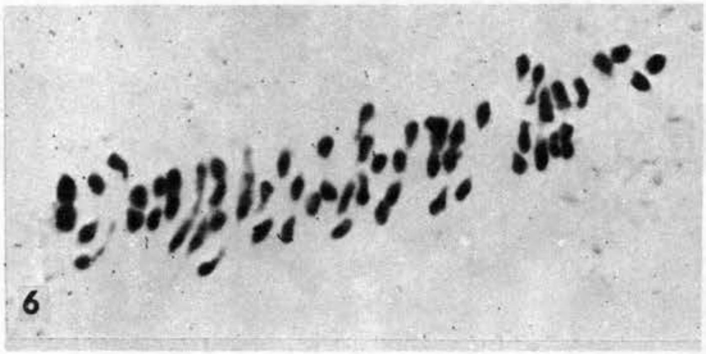
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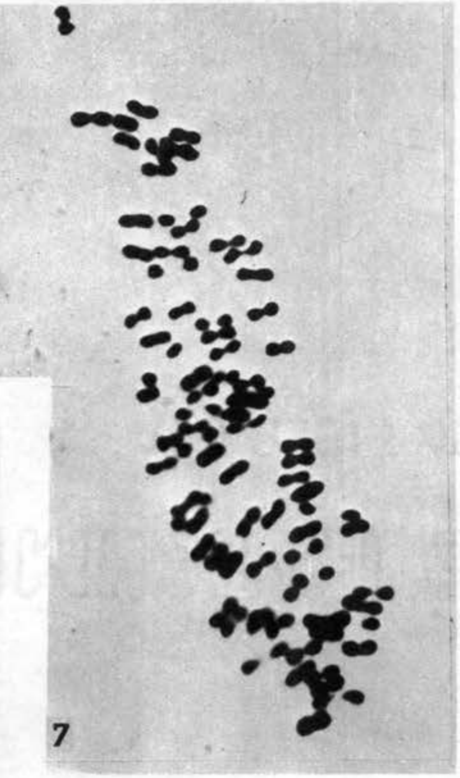
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LEGEND FOR PLATE II

Irregularities in Chromosome Number

Fig. 8. Metaphase I in multiploid sporocytes. No clear boundaries are present between the cells and some groups of chromosomes are in proximity.

Fig. 9. Cell with 6 bivalents at metaphase I.

Fig. 10. Cell with 12 bivalents at metaphase I.

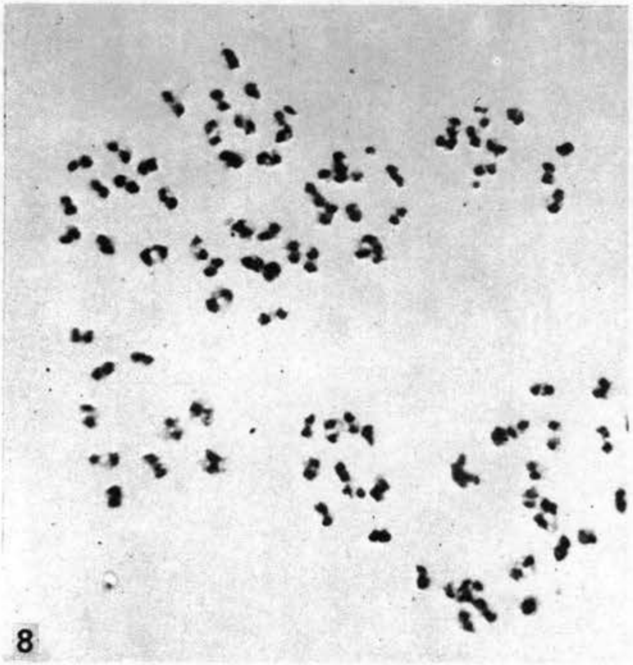
Fig. 11. Cell with 7 bivalents at metaphase I.

Fig. 12. Cell with 19 bivalents at metaphase I.

Fig. 13. Cell with 9 bivalents at metaphase I.

Fig. 14. Anaphase I showing 20-20 equal distribution.

PLATE II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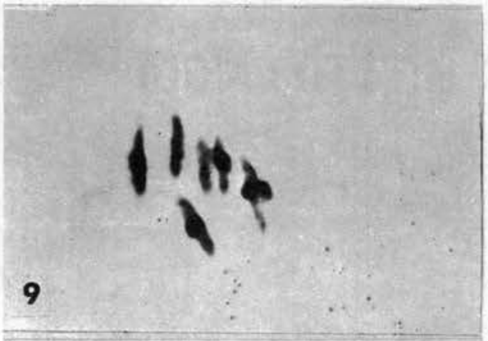
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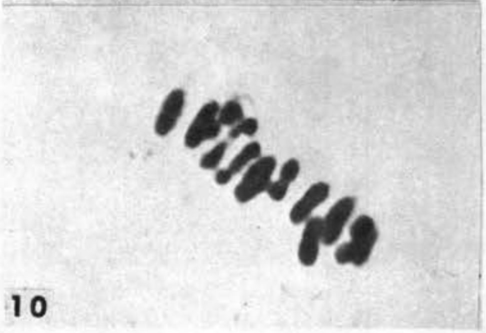
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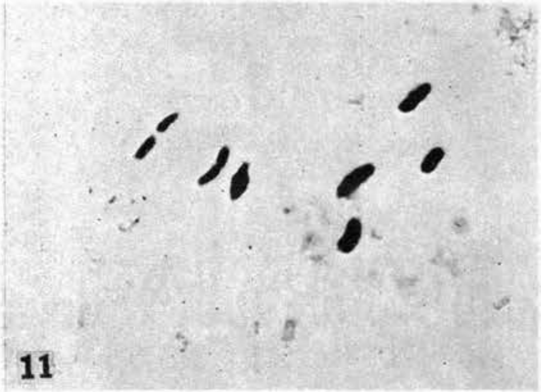
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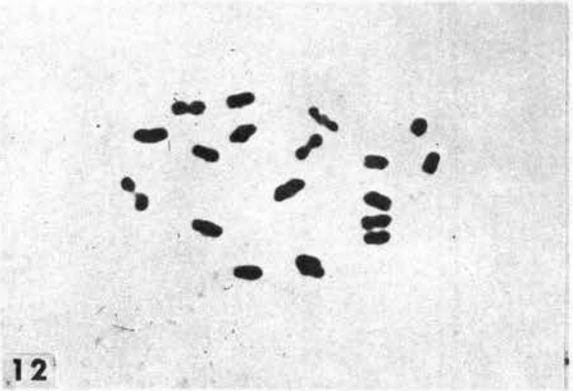
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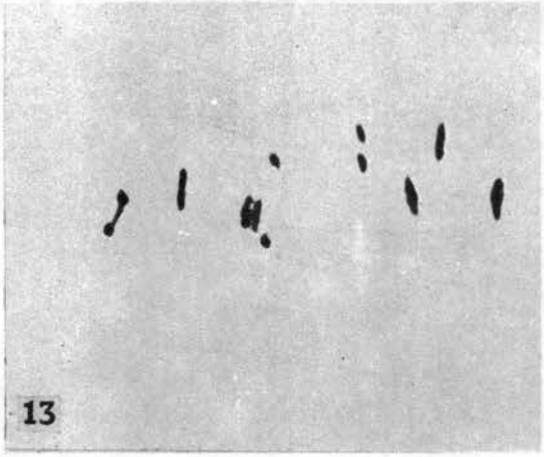
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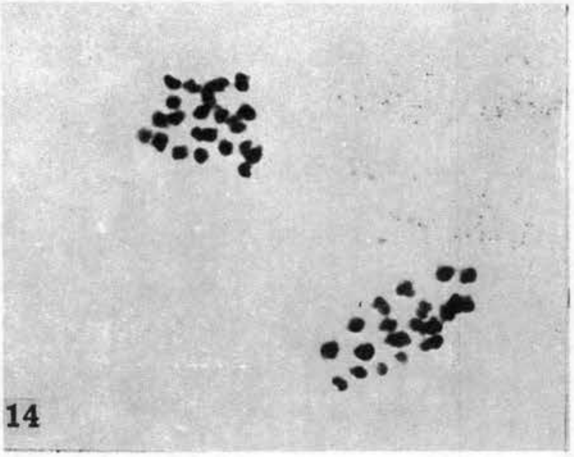
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LEGEND FOR PLATE III

Irregularities in Chromosome Number

Fig. 15. Anaphase I showing 15-15 equal distribution

Fig. 16. Anaphase I showing 8-8 equal distribution

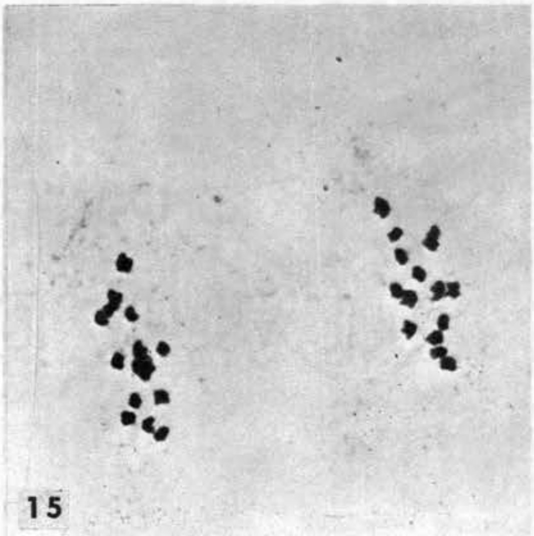
Fig. 17. Late diakinesis showing two nucleoli clumped together and with 20 pairs of chromosomes scattered around them. One pair of chromosomes clumped with nucleoli.

Fig. 18. Two cells side by side, one with 10 chromosomes at each pole and the other 20 chromosomes at each pole at anaphase I.

Fig. 19. Anaphase I showing 9 chromosomes moving to each pole and two divided univalents as laggards.

Fig. 20. Metaphase I showing four chromosome pairs migrated (arrow) from one pollen mother cell to another and resulting in one cell with 6 bivalents and other with 14 bivalents.

PLATE III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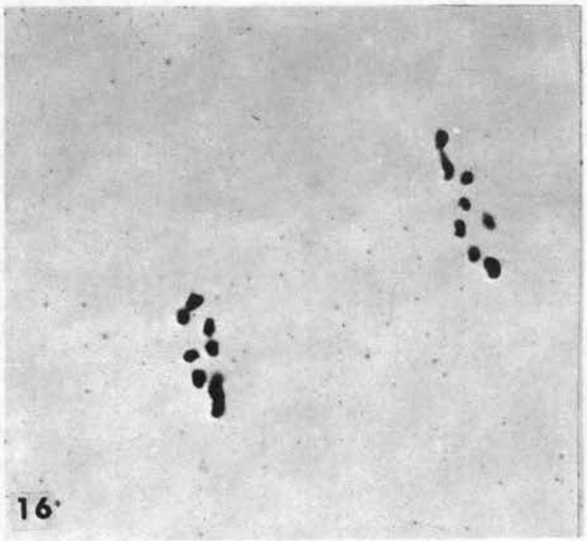
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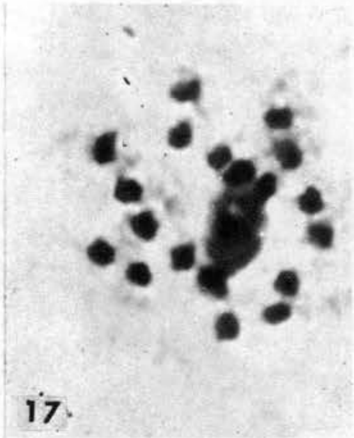
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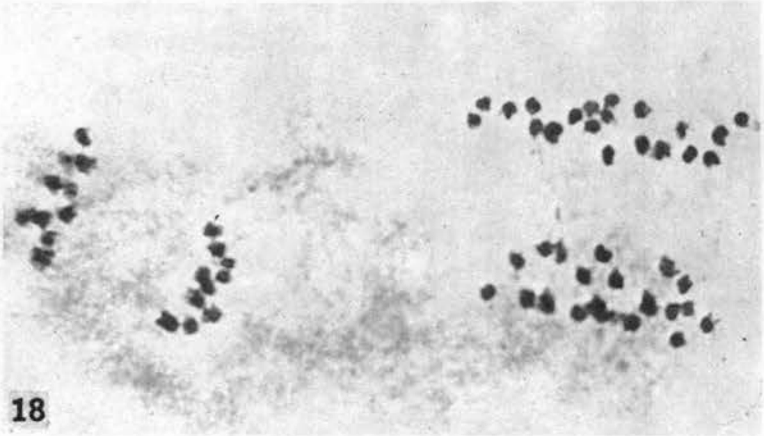
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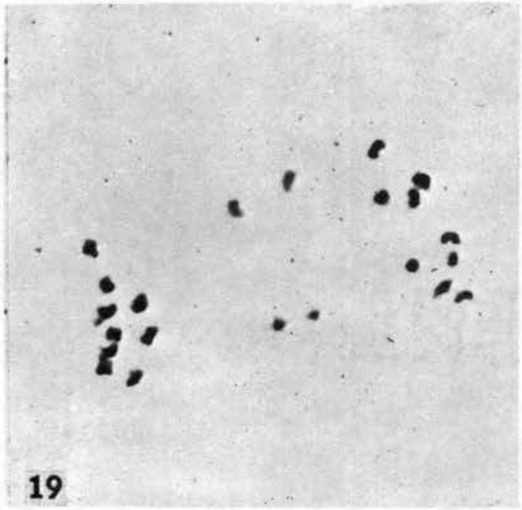
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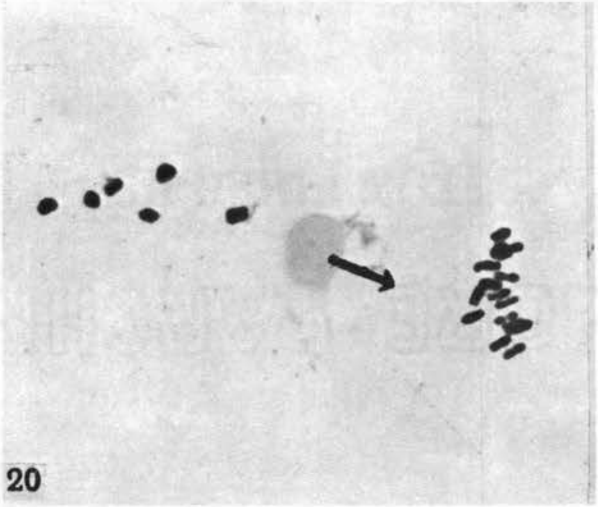
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APPENDIX

Meiosis in Sorghum

Meiosis is a rather complicated type of cell division. It consists essentially of two nuclear divisions which follow each other in rapid sequence. The first involves the separation of chromosomes and leads to the formation of haploid nuclei. The second division involves the longitudinal separation of chromatids in each of these two haploid nuclei, with the result that four haploid nuclei--gametes--are produced. Observations of meiosis are extensively made by most cytologists in studying chromosome morphology, chromosome aberrations, chromosome number, cytotaxonomy and evolution.

In recent years, Sorghum vulgare has been used extensively in cytological studies. Unfortunately, no complete account of meiosis for such plants is available for beginners working on sorghum cytology. A series of photomicrographs of sorghum meiosis, as Rhoades (1950) produced for maize, is necessary. However, excellent pictures are difficult to obtain since sorghum chromosomes are relatively small. Thus some photomicrographs of certain stages are absent in this series presented here.

The processes of meiosis is a continuous one. For the sake of convenience, it is separated into a sequence of steps which possess certain chracteristics permitting easy recognition. Generally, the states of meiosis may be divided as follows:

Division I:

Prophase I

Leptotene

The chromosomes are long, thin, single threads which contain a series of chromatic "beads" called chromomeres.

Zygotene

Homologous chromosomes begin to pair. General pairing is following by a closer chromosome-to-chromosome association or synapsis.

Pachytene (Fig. 1)

The chromosomes and the attachment of the nucleolus to a particular chromosome is visible. Since the chromosomes have shortened and thickened they are more readily distinguished one from the other.

Diplotene (Fig. 2)

Contraction as well as the opening out between homologus and the chromosomes tend to clump in the center of the cell.

Diakinesis (Fig. 3)

The nucleolus becomes detached from its special bivalent. Pairs of homologous chromosomes are still held together at chiasmata but are elsewhere separated.

Metaphase I (Figs. 4 and 5)

The bivalents orient themselves on the spindle. Each bivalent is located such that the centromeres lie on either side of and equidistant from the plate.

Anaphase I (Fig. 6)

Homologous centromeres move toward opposite poles. Each centromere is attached to two chromatids. Chiasmata are fully resolved.

Telophase I (Fig. 7)

Two nuclei form.

Interphase (Fig. 8)

The cytoplasm is separated into two parts. Two cells are formed. The two cells are known as dyad.

Division II:

The chromosomes in each of the two haploid cells enter the second division of meiosis.

Prophase II (Fig. 9)

This stage appears to be rapid and is essentially a contraction.

Metaphase II (Fig. 10)

The centromeres which have been holding the two chromatids together line up on the metaphase plate and divide. A spindle forms in each of the two cells.

Anaphase II

The chromosomes move to the pole.

Telophase II (Figs. 11 and 12)

This stage involves the reconstitution of the interphase nuclei and give rise to four cells, known as a tetrad (Fig. 12). Each cell has a complete chromosome set and half the somatic number. The microspores, one derived from each member of the tetrad, are usually arranged in a isobilateral fashion.

LEGEND FOR PLATE IV

Meiosis in Sorghum vulgare

Fig. 1. Pachytene

Fig. 2. Diplotene

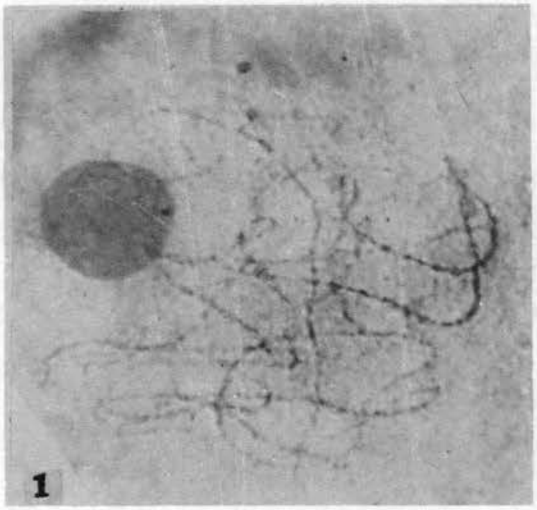
Fig. 3. Diakinesis

Fig. 4. Pre-metaphase I

Fig. 5. Metaphase I

Fig. 6. Anaphase I

PLATE IV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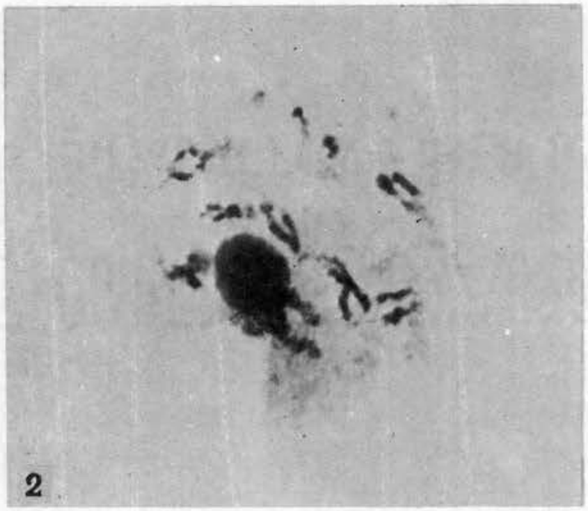
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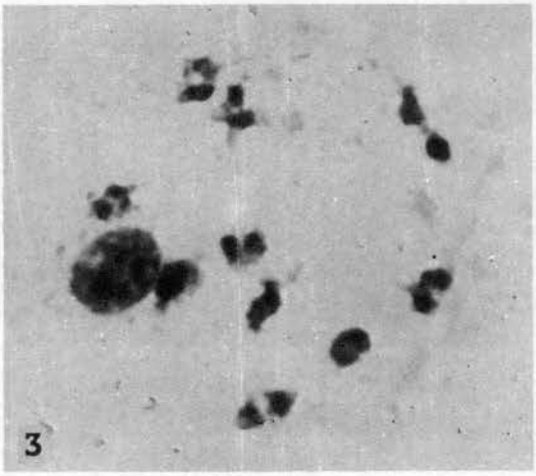
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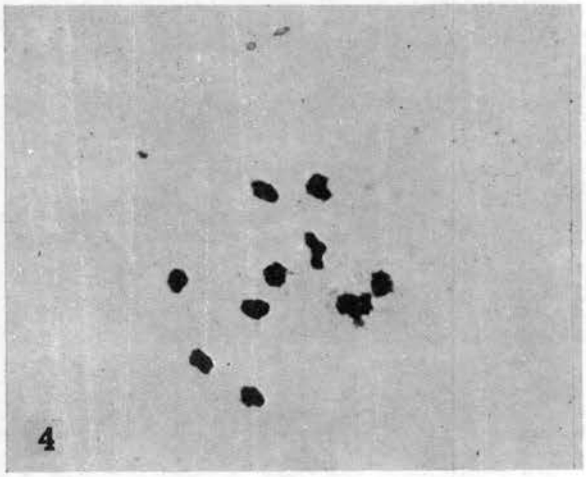
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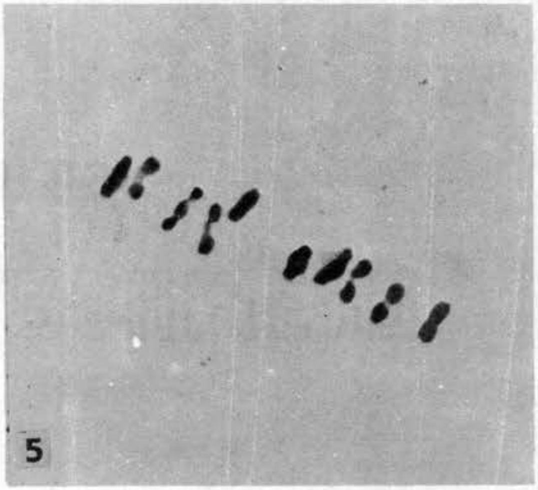
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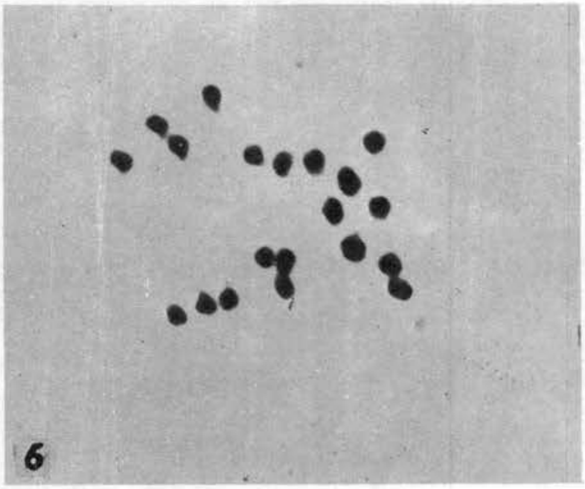
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LEGEND FOR PLATE V

Meiosis in *Sorghum vulgare*

Fig. 7. Telophase I

Fig. 8. Interphase

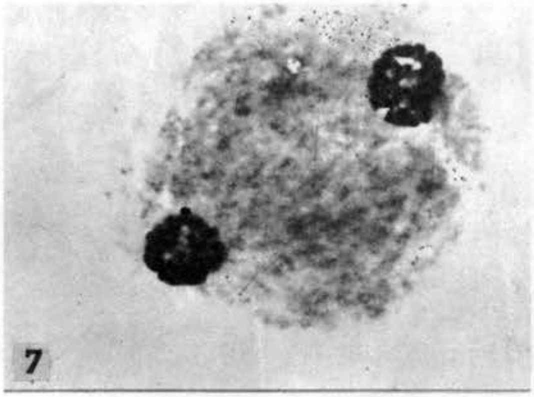
Fig. 9. Early prophase II

Fig. 10 Pre-metaphase II

Fig. 11 Telophase II

Fig. 12 Tetrad

P L A T E V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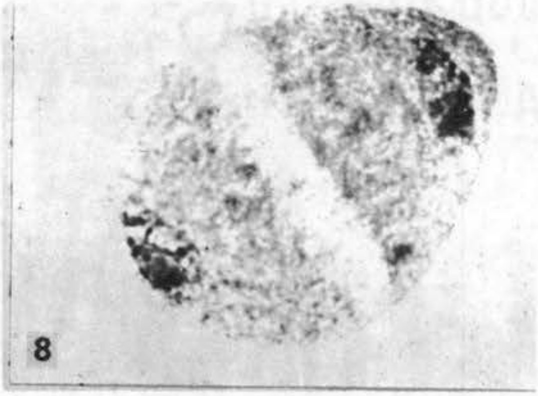
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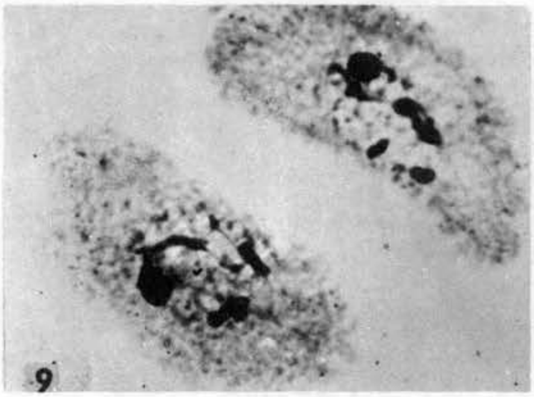
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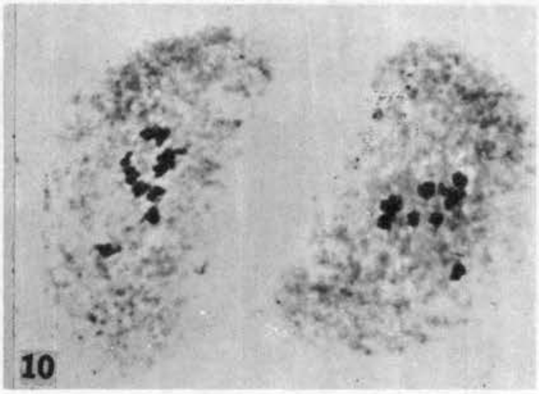
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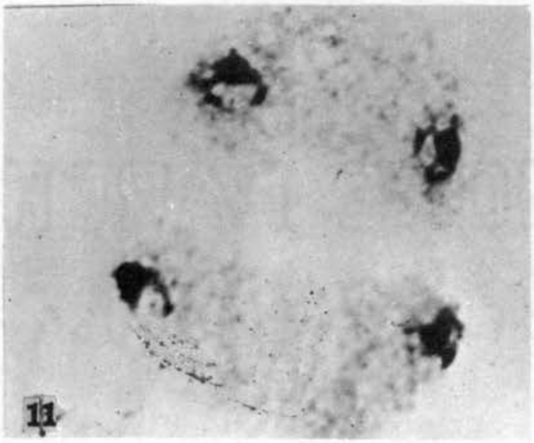
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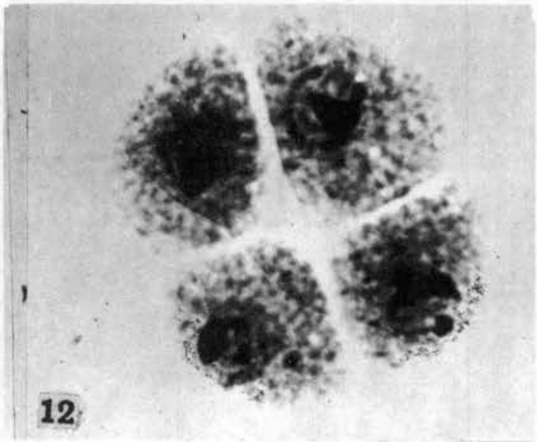
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VITA

Wei-ning Fu

Candidate for the Degree of

Doctor of Philosophy

Thesis: CHROMOSOMAL ABERRATIONS AND VARIATIONS IN CHROMOSOME NUMBER IN *SORGHUM VULGARE* TREATED WITH X-RAYS AND THERMAL NEUTRONS

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Date of Final Examination: May, 1963.