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Somatic Chromosome Reduction in Sorghum After Colchicine Treatment

Gerald Milo Simantel

SOMATIC CHROMOSOME REDUCTION IN SORGHUM AFTER COLCHICINE TREATMENT

BY

GERALD MILO SIMANTEL

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SOMATIC CHROMOSOME REDUCTION IN SORGHUM
AFTER COLCHICINE TREATMENT
Abstract

GERALD MILO SIMANTEL

Under the supervision of Professor James G. Ross

Diploid mutant plants that breed true in subsequent generations arise following colchicine treatment of sorghum seedlings of the variety Experimental 3. The production of diploid mutants was found to be dependent upon the genotype as well as the presence of certain environmental conditions after colchicine treatment. To explain the homozygosity of the mutants, it has been proposed that a reduction of chromosomes to a haploid condition occurred followed by doubling to restore the diploid number. To test this hypothesis, seedlings heterozygous for chromosome markers (one and two reciprocal translocations) were treated.

After treatment of 320 seedlings heterozygous for one reciprocal translocation (two marked chromosome pairs) four immediately true-breeding diploid mutants were observed among the 188 survivors. Two true-breeding mutants among 90 surviving plants were obtained following treatment of 124 seedlings heterozygous for two reciprocal translocations (four marked chromosome pairs). Examination of the chromosomes of the six mutants at diakinesis showed no configuration of four chromosomes indicating that they were homozygous for the marked chromosomes. The mutants also had uniformly high seed set in comparison to the sterility observed in the unmutated plants. To

identify the structural chromosomes of the mutant plants, they were backcrossed to each parent and to the original structural homozygote in the case of mutants from seedlings heterozygous for two translocations. Examination at diakinesis of the F_1 progenies showed a ring or chain configuration of four chromosomes when the mutants were backcrossed to the homozygous translocation parent. When the mutants were crossed to plants having the homozygous normal structure, no associations of four chromosomes were present. Therefore, the identifiable chromosomes of the six mutants were the same as the normal Experimental 3. Four progenies which segregated for mutant and normal plants were also observed, indicating probable chimaeral make up of the treated plants. Further investigations will be necessary to determine the nature of the putative mutant sectors.

The occurrence of mutants with homozygous normal chromosome structure arising from colchicine-treated seedlings heterozygous for two reciprocal translocations removes the possibility of selfing and androgenesis, since in this case the mutants had a different structure than either parent.

The higher proportion of mutants from plants with the original chromosome structure (18.4%) than from those heterozygous for one translocation (4.3%) and for two translocations (2.2%) may perhaps be explained in one of two ways. Irradiation used to obtain the translocation may have caused mutations affecting response to colchicine. Secondly, duplications and deficiencies may occur as expected in any reduction division and since no translocation

homozygotes were found among the mutants, it would appear that these were not as viable and were at a competitive disadvantage.

The homozygosity of the chromosomes in only the true-breeding mutant plants and not in any of the unmutated plants may indicate that the somatic reduction phenomenon is associated in some way with the mutational phenomenon.

The presence of homozygous normal chromosomes identified in the mutants obtained after colchicine treatment constitutes incontrovertible evidence for the occurrence of somatic chromosome reduction followed by doubling of the chromosomes of at least the four marked chromosomes followed in this study. It would be expected that the complete chromosome complement would be involved in such a somatic reduction to explain the phenomenon of the appearance of true-breeding mutants after colchicine treatment involving many mutations, none of which have been found to be linked.

SOMATIC CHROMOSOME REDUCTION IN SORGHUM
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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

James G. Ross
Thesis Adviser

L. O. Fine
Head of the Major Department

W. L. Butcher
Representative, Graduate Faculty

Clem J. Martin

Melvin D. Rumbaugh

David J. Holden

Eugene Whitehead

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INTRODUCTION

True-breeding diploid mutant plants arising after colchicine treatment of sorghum seedlings of the homozygous lines Experimental 1 and Experimental 3 have been reported previously by Franzke and Ross and Ross et al. (20, 21, 59). Analysis of the pairing relationships at pachytene of these mutants, of untreated plants of the lines from which they were derived, and of F_1 hybrids between the two showed ten normal bivalents with no chromosomal irregularities (32). Genetic studies of colchicine-induced mutants indicated that a large number of loci probably on different chromosomes within the one plant were involved and that none of the eight characters studied were linked (18). From these findings it was proposed that as a result of colchicine treatment, somatic reduction of the chromosome number has taken place with concurrent point mutations and subsequent restoration to the diploid number (59).

Atkinson (2) and Huang (33) previously attempted to test the hypothesis of somatic chromosome reduction followed by doubling of the chromosomes. In order to test this hypothesis it was necessary to obtain some type of chromosome or gene marker. A homozygous reciprocal translocation line was obtained in a variety other than Experimental 3. The homozygous translocation line was crossed to Experimental 3 and the F_1 seedlings heterozygous for the translocation were treated with 0.5% colchicine in lanolin. This chromosome marker serves to indicate whether chromosomal homozygosity was induced. This attempt to test the hypothesis failed to produce any

diploid mutants. It has since been shown that the production of diploid mutants depends upon the presence of certain environmental conditions after colchicine treatment (22) and also upon the genotype of the seedlings which were treated (3).

It was then necessary to obtain a chromosomal or gene marker in Experimental 3, since this variety was known to produce diploid true-breeding mutants under certain environmental conditions following colchicine treatment (3, 20, 21, 59). Haensel (30) irradiated the variety Experimental 3 with gamma rays from a cobalt 60 source at anthesis. A number of translocations were identified when the progenies of the irradiated plants were grown in the field. Huang (33, 34) selected three of these lines because of their consistency in the formation of rings and chains at diakinesis and metaphase I. These were obtained in the homozygous condition.

Plants heterozygous for the reciprocal translocations are easily identified since they are 50% fertile and can also be readily recognized by the presence of rings or chains of four chromosomes at diakinesis and metaphase I.

The objective of this study was to determine whether somatic reduction actually is responsible for the true-breeding nature of diploid mutants obtained after colchicine treatment. The homozygous reciprocal translocation lines in a genotype known to react to colchicine treatment by forming immediately true-breeding mutants were crossed to Experimental 3. The occurrence of somatic reduction was tested by treating seedlings heterozygous for these chromosomal

markers with colchicine under the laboratory conditions shown to ensure the appearance of true-breeding mutants by Franzke et al. (22).

LITERATURE REVIEW

True-Breeding Mutants in Sorghum after Colchicine Treatment

Franzke and Ross (20) reported that colchicine treatment of full sibs of a homozygous variety of sorghum, Experimental 3, derived from a cross of Day, Black Amber Cane and Sudan grass, gave diploid mutants possessing a number of ancestral characteristics of which some bred true immediately. Untreated full sibs of the treated plants did not segregate in subsequent generations. Cytological examination of meiosis in untreated Experimental 3 and in the mutants indicated the presence of 20 chromosomes, the diploid number in all cases. The authors proposed that

> Such variant plants could originate through reductional groupings of the somatic chromosomes so that a concentration of chromosomes containing gene blocks originating from one of the ancestors of the polyploid species might occur in one cell. This cell, by virtue of its inherent and perhaps environmental competitive advantage, could form a new growing point and produce a plant with a genotype entirely different from that of the original zygote, in fact homozygous diploidy may thus be induced.

Ross, Franzke and Schuh (59) on further studies of the colchicine-induced mutants treated seven seedlings of a true-breeding variety of grain sorghum, Experimental 3 with 0.5% colchicine in lanolin. Three of these plants became mutants with unchanged chromosome numbers after treatment with colchicine. Progenies of these three mutant plants possessed characteristics differing significantly from those of the untreated plants which were full sibs of the treated plants. The progenies of two of the mutants bred true immediately, the progeny of the other mutant segregated.

Harpstead, Ross and Franzke (32) investigated the nature of chromatin changes after colchicine treatment. The mutant plants, their untreated sibs, and the F_1 hybrids between treated and untreated plants were examined cytologically. Pairing relationships at pachytene in each of these showed that no detectable irregularity or rearrangement of chromatin had occurred. Observation of the F_1 hybrid seedlings demonstrated that the green coleoptile color of two mutant lines was due to a simple recessive gene in contrast to the red color of untreated full sibs. From this investigation it was concluded that the changes in the chromatin were multiple point mutations and not the result of concentration of gene blocks from one of the ancestors as previously proposed.

Foster et al. (18) studied the inheritance of F_2 and F_3 populations resulting from crosses involving colchicine-induced mutants and normal plants. Eight characters were studied and no linkage was found between any of these characters. Two of the mutant characters, presence of awns and coleoptile color were found to be simply inherited, while other characters were influenced by five or six genes. One mutant plant had been changed for at least 12 genes and probably more (19). These investigations gave further evidence substantiating the hypothesis that the colchicine treatment induced multiple point mutations.

Other Effects of Colchicine

Blakeslee and Avery (6) reported that colchicine treatment results in a doubling of the chromosome numbers in various plant

species. Pseudodivision of the nucleus continues in several plant species, and prolonged periods resembling metaphase alternate with periods in which the chromosomes become vesiculated forming abnormal resting nuclei, due to the colchicine's inhibition of spindle formation (55). Brunes and Cohen (9) reported that no single group on the colchicine molecule could be found which is essential for the action of colchicine on mitosis.

Levan (43) reports that tumors which characteristically appear after colchicine treatment result from increased chromosome number and increased cell volume, while the formation of new cells is completely suppressed. He states that colchicine induced mitosis, designated c-mitosis, can be referred to as an inactivation of the spindle apparatus along with a delay of the division of the centromere. This produces what may be expressed as a completion of chromosome mitosis without nuclear or cellular mitosis, thus resulting in polyploidy.

The effect of colchicine in producing polyploids and investigations on the mode of action of c-mitosis have been reported by numerous workers (7, 39, 43, 54).

Abnormalities other than polyploidy arising after colchicine treatment have been reported. Bergner et al. (5) reported that after colchicine treatment of over 2000 Datura seeds, 88 plants with chromosome deficiencies were identified. Seven plants were diploid deficiencies (2n-1) and 81 were tetraploid deficiencies (4n-1). Diploid and tetraploid types with extra chromosomes were also found among

plants grown from seed following colchicine treatment. One haploid plant was observed after colchicine treatment. Nicotiana (2n=18) seeds treated with aqueous colchicine produced 25% variant plants with abnormal chromosome numbers other than tetraploid. The somatic chromosome numbers of these variant plants ranged from nine, a haploid, to 72, an octoploid (62). A haploid sugar beet was observed along with diploid, triploid and tetraploid plants after colchicine treatment (44).

Dunham and Banta (15) treated parthenogenetic eggs of Daphnia longispina with dilute solutions of colchicine at a late stage of ovarian development or during first cleavage stages in the brood chambers of the mothers. They found no atypical individuals among the 469 controls while 60 unusual cases appeared among the 1748 from treated eggs. Of these 60 unusual females, 33 showed no ovarian activity, 20 produced parthenogenetic eggs which failed to hatch and seven were decidedly subnormal reproductively but gave rise to mutant clones. It was concluded that gene mutations or chromosomal aberrations were responsible for the hereditary changes.

Gilbert (unpublished) has observed 26 mutant plants among 253 survivors after treating Decatur barley. Mutations were found for awnlessness, maturity, erectoides, shorter height, mildew resistance, few stigma hairs and albino. Some of these characters were true-breeding immediately after colchicine treatment. Porter and Weiss (57) reported a dwarf true-breeding diploid mutant in soybeans arising from the variety Lincoln after treatment with colchicine.

The dwarf mutant crossed readily with normal diploid plants, and the F_2 generation segregated only for height with a ratio of 3 normal:1 dwarf indicating a single gene had mutated to the recessive. This was similar to the segregation found by Foster (17) for the "rat-tail" mutation in sorghum. Franzke (unpublished) has obtained sunflowers with different patterns of seed color after colchicine treatment. This array of evidence indicates very strongly that colchicine may effect gene mutations.

Colchicine treatment of a different sorghum variety, Experimental 1 was reported by Franzke and Ross (21). From colchicine treatment a slightly taller, true-breeding line was obtained, which upon retreatment gave two dwarf mutants which also bred true immediately and which were distinctly different from any types known to be in the pedigree. These two mutants upon retreatment gave new true-breeding types, some of which resembled the original untreated variety. Thus, a lineal series of mutants, each of which bred true immediately, was obtained. These results provide further evidence that colchicine is an agent for multiple gene mutations. The immediately true-breeding nature of these lines is explained by a reductional division of the chromosomes in the somatic tissue with subsequent restoration to the diploid number.

Evidences for Somatic Chromosome Reduction

The results obtained by Dirks et al. (14) from flax which was treated with colchicine gave further evidence for somatic chromosome reduction. Branches from an F_1 flax plant treated with colchicine

exhibited recessive characteristics for flower and seed color originally present in the heterozygous condition. The progenies of these branches were immediately true-breeding and contained the normal diploid chromosome number. Some other branches bred true for the dominant characteristics, hence it was concluded that the plant had been a chimaera. Abnormal segregation in the progenies of other treated F_1 plants was explained by assuming chimaeral sectors within single branches. From this evidence it was concluded that colchicine effects included somatic reduction with subsequent doubling of chromosomes, or point mutations, or both.

Corn seedlings heterozygous for a translocation were treated with colchicine by Ross (unpublished) to test the hypothesis of somatic reduction. Nine heterozygous plants were treated, and after treatment three of these were found to be homozygous for the translocation. One of the three plants had two tillers, one of which was homozygous while the other tiller was heterozygous for the translocation. These results fit the hypothesis that colchicine induces somatic reduction followed by doubling of the chromosome number.

Atkinson et al. (3) investigated the differential reaction of two varieties of sorghum to colchicine treatment. It was found that colchicine treatment of Experimental 3 resulted in the mutation of 24 out of 43 surviving plants, however, only four out of 54 plants were mutants after colchicine treatment of the variety Norghum. Cytological study of 30 treated plants of each variety indicated only diploids in Experimental 3, but three tetraploid plants were found in

Norghum. These varieties differ in the rate of mutation and in propensity to polyploidy in response to colchicine treatment. The authors concluded that such differences may explain the inconsistencies in results of treating different genotypes.

Sanders et al. (61) studied the environmental factors involved in the induction of true-breeding diploid mutants following colchicine treatment. They concluded that improved growing conditions for treated seedlings decreased the colchicine effect of induction of true-breeding diploid mutants as well as the detrimental effect of colchicine on seedling growth and decreased survival. Franzke, Sanders and Ross (22) defined a laboratory method for the induction of true-breeding mutants.

Villax and Mota (67) reported results from colchicine treatment of a wheat (2n=42) x rye (2n=14) F_1 hybrid which may be considered as an effect of somatic reduction. When culms were treated with colchicine, c-tumors formed but no spikes developed, however, tillers on the same plant showed reduced growth and gave fertile spikes while the untreated F_1 produced sterile spikes. Colchicine had apparently passed from treated culms to untreated ones. Progenies grown from the fertile spikes appeared identical to the wheat plant and contained the 42 chromosomes of the wheat. The authors concluded that the derived wheat plants are homozygous since they resulted from the duplication of a gametic set of chromosomes.

Huskins (35) states that a major factor in Bateson's reluctance to accept the chromosome theory was his conviction that "though

segregation is commonly effected at the reduction-division, evidence steadily accumulates showing that at least in plants of many kinds comparable segregations occur in somatic divisions also." He also affirmed "somehow a somatic cell is evidently able to divide in such a way as to produce cells dissimilar either from the parent cell or from each other or both."

Somatic reduction has been defined as a regular part of metamorphosis for a number of insect species by various authors (10, 38, 51, 52). Berger (4) and Grell (29) have confirmed that somatic reduction is a regular feature in the ileum of the mosquito larvae. The substitution of somatic synapsis for chromosome reduplication in the resting stage constitutes the essence of somatic reduction in the ileum of the mosquito larvae (29). Within the gonads of hermaphrodites of the cushiony scale insect, Icerya perchesi, a somatic reduction gives rise to a haploid cell which divides to form haploid tissue. The nuclei contained within this tissue were found to contain the complete haploid chromosome complement (38).

Kemp (41) reported a gradual disappearance of tetraploid cells from pea root tips after chloral hydrate induced tetraploidy. He concluded that the disappearance of these tetraploid cells is probably due to their division into several smaller cells. Nemec, cited from Kemp (41), adopted the explanation for this phenomenon "that by a sudden automatic process of reduction, very similar to the heterotype reduction occurring in spore mother cells the tetraploid number of chromosomes is reduced to the diploid number." Similar reduction of

chromosome numbers in somatic tissues have been reported in Oenothera by Gates (23), in Fragaria by East (16), and in wheat by Love (47, 48).

Reduction divisions influenced by external factors have been observed in the integumental cells of Hieraceum hoppeanum, in which plants with 45 chromosomes arose from a plant with 90 chromosomes (12). Vaarama (66) found chromosome numbers ranging from 4 to 32 in the progeny of tetraploid Ribes nigurm. All numbers divisible by four were more frequent than might be expected on random distribution, with the maximum number lying at the diploid number (2n=16). Reduction in chromosome numbers has been reported for tetraploid hybrids in several Primula species (65).

Huskins (35) found the chromosomes of Allium cepa (2n=16) separated into two groups of eight in a number of cells after growing the bulbs in an aqueous solution of sodium nucleate. This phenomenon was termed somatic meiosis by Huskins because of the possible implications of the separation of chromosomes, but was later called reductional grouping since neither a mechanism governing it nor its consequences could be defined. Allen et al. (1) later found that colchicine as well as sodium nucleate induced reductional groupings in Allium and Tradescantia, although separations induced by sodium nucleate showed a significantly higher proportion of equal groupings than was effected by colchicine. Reductional groupings of chromosomes in root tips of sorghum in aqueous solution of colchicine has been observed by Atkinson et al. (3).

Huskins and Cheng (36) increased the number of reductional groupings in Allium by cold treatments. They found reductional groupings in 1 to 2% of the controls and in 5% of the plants treated. There are many resemblances between the reductional groupings induced by cold or sodium nucleate on the one hand and a range of chemicals, chloral hydrate, ethylene glycol, inorganic phosphates, colchicine and other alkaloids on the other.

Homologous chromosomes which separated into each of the two groups at reduction division in greater frequencies than would occur at random were observed by Wilson and Cheng (68) in Trillium and by Huskins and Chouinard (37) in tetraploid Rheo. Patau (56) concluded that the group separation is closely determined by what may be called, without physical implications, "homologous repulsion." Therefore, reduced nuclei derived from reductional groupings may be expected to have a more or less complete genome.

Levan and Lotfy (45) observed after treatment of Allium with naphthalene acetic acid and colchicine that there was a mixture of polyploid and diploid cells. The diploid cells regained predominance within the meristem because of their greater viability and more rapid division. It was concluded from this study that the chromosomes may become segregated into several groups, but no separation of sister chromatids into two different nuclei takes place. The products of this segregation are unbalanced and ultimately degenerate. The authors concluded that the segregation into two groups occurs as a result of c-mitosis, wherein the chromosomes are pushed bodily to the

boundary of the cell.

These authors maintain that reduction division is a result of "distributed c-mitosis" where the chromosomes are not scattered throughout the cell, but only to the two ends of an elongate cell. The distribution of about half of the chromosomes to each group is explained by the original position of the prophase nucleus being located near the center of the cell. Kihlman and Levan (unpublished) were not able to find that a "second" normal division should follow upon the distributive c-mitosis.

The conclusions drawn by Levan were in direct contrast to those drawn by Huskins. Levan presents an explanation for the reductional groupings of plants treated with chemicals, but ignores the reductional groupings observed in control plants, and those which resulted spontaneously from a large number of plants.

A number of workers have recently observed tissues containing reduced chromosome numbers which appear to have resulted from somatic chromosome reduction. The occurrence of somatic tissue containing cells with a reduced number of chromosomes has been reported in unstable allopolyploids in cotton (8, 49, 50). The somatic reductions resulted in a halving of the chromosome number which was observed in both tetraploid and hexaploid hybrids. Knott (42) found a case of somatic reduction at first meiotic metaphase in an anther of a wheat plant.

Davis (13) reports evidence for somatic reduction occurring in Allium. A cross was made between onions having a semi-glossy and

glossy foliage. The F_1 hybrid is semi-glossy and the F_2 segregates in the ratio 3 semi-glossy to 1 glossy. In one instance both types appeared on the same plant. Progeny from the glossy seed stem bred true for the glossy character and progeny for the semi-glossy seed stem bred true for the semi-glossy character. Only true-breeding individuals were observed.

Sanders et al. (60) present further evidence of the occurrence of somatic reduction. After colchicine treatment of 20 tetraploid seedlings of sorghum, four plants were observed to be diploid mutants with obvious changes in many characters. A fifth mutant for many characters was a mixoploid made up of cells with diploid and different polyploid numbers. Chen (unpublished) has also obtained diploid mutants after colchicine treatment of tetraploid sorghum seedlings.

Gottschalk (28) demonstrated that a tetraploid Lycopersicon esculentum plant was cytologically instable and contained variable chromosome numbers in different inflorescences. From his findings on the study of premitosis and mitosis, it was concluded that a complete genome separation had occurred similar to that previously reported by Wilson et al. (68) and Patau (56). Glass (25, 26, 27) has identified complete genome complements separated from the rest of the chromosomes within liver cells of the rat. Further studies of this phenomenon in root tips of Bellevalia romana demonstrated a more than random association into the genomic number, but members of chromosome pairs were not separated in greater frequency than would be expected according to chance (27).

MATERIALS AND METHODS

Plant Material

The plant materials used in this study consisted of the grain sorghum variety Experimental 3, three homozygous translocation lines in this variety and translocation heterozygotes obtained by crossing each translocation line with Experimental 3 and two translocation lines crossed together.

The sorghum variety, Experimental 3, resulted from crosses made in 1932 of the Day variety, a late maturing dwarf grain sorghum with Black Amber Cane, a forage variety, and with Sudan grass. An early, dwarf, grain sorghum line resembling Day was selected from each of these crosses. In 1939, these were crossed and from their progeny, through continued selection and selfing, the true-breeding variety "Experimental 3" was produced.

Haensel (30) obtained a number of plants which gave evidence of being heterozygous for translocations following radiation of Experimental 3 panicles in the preanthesis stage with gamma rays. Huang (33) selected three of these, which were designated as plant numbers 165, 231, and 369 (33, 34). These translocations were selected because of the consistency of the rings and chains present at diakinesis in the heterozygotes. In order to obtain plants homozygous for these translocations the three plants were selfed and the progeny examined. The pollen of the progeny was examined since plants containing the translocation in the heterozygous condition have only 50% viable pollen. The plants were also examined by

cytological means. The expected segregation of 1 homozygous:1 heterozygous for the translocation was observed. Since the homozygotes consisted of both homozygous normal and homozygous translocated plants, it was necessary to cross these to Experimental 3 and again observe the progeny. The plants from which heterozygous progenies resulted were thus demonstrated to be homozygous for the translocation. These lines homozygous for the translocation were then designated as T165/T165, T231/T231 and T396/T396, and were retained for use in this study.

Translocation heterozygotes from crosses of the three homozygous translocation lines with normal Experimental 3 and the translocation lines crossed with each other were then obtained by hand emasculation for use in this study.

Methods

The translocation lines were used as females and were emasculated by hand. Crosses were made by placing panicles of the pollen parent above those of the emasculated plants and covering the two with a parchment bag. Experimental 3 was always used as the pollen parent with the exception of cases when two translocation lines were crossed.

The seeds were placed in petri dishes between filter papers and water added. When the coleoptiles of the germinating seeds were approximately equal to one-third the diameter of the seed, the coleoptiles were coated with 0.5% colchicine in lanolin. Care was taken that the coleoptile was completely covered and that the

colchicine-lanolin mixture did not come in contact with the roots. The seedlings were then planted in moist sand in a jar covered with a glass plate and kept under red light from a 250 W Heat Ray lamp with the temperature maintained at 68°F for 5 days. At this time the red light was replaced with fluorescent and incandescent lighting (10 hours) alternating with dark periods (5 hours) for 10 days and then removed to the greenhouse. After approximately 12 days, the seedlings were transplanted into jars containing soil with four plants to the pot.

The resulting plants were then selfed; the seed was threshed by hand, and progenies from the surviving plants were grown in the field where notes on fertility and uniformity were taken. The plants that survived were also transplanted into the field, and plants that produced no seed in the greenhouse were selfed. The progenies from these plants were grown in the field the following year.

This same procedure was followed on all four of the experiments which were performed, two during the winter of 1960, and two during the winter of 1961.

Every mutant resulting from treatment of seedlings containing the structural marker, the heterozygous translocation, was backcrossed to each parent. In cases of mutants from seedlings obtained by crossing two translocation parents, the mutant was backcrossed to both parents and also the normal. In this manner, it was possible to identify the chromosome structure of the mutant plants.

Immature inflorescences for the study of meiosis were collected from the field whenever possible, and from the greenhouse otherwise. These were fixed in 3 ethyl alcohol:1 glacial acetic

acid. Squashes of pollen mother cells were examined after staining with propionic carmine.

The symbols used have been adapted from those in common usage in translocation studies. Experimental 3, or the homozygous normal chromosome structure, is denoted by +/+. Since the chromosomes in sorghum have not been identified, each translocation has been given an arbitrary number. The translocation homozygotes are designated as T396/T396, T231/T231 and T165/T165, and likewise the heterozygotes as T396/+, T231/+ and T165/+. Plants heterozygous for two reciprocal translocations are indicated as T396+/+T231, T396+/+T165 and T231+/+T165.

Mutant plants obtained after colchicine treatment of material heterozygous for translocations were identified numerically in the chronological order in which they were observed, beginning with number one.

EXPERIMENTAL RESULTS

Experiment I

Induction of plants homozygous for structural chromosome markers was attempted by treating with colchicine seedlings heterozygous for reciprocal translocations. The results in Experiment I are shown in Table 1. No induced homozygosity was apparent in any of the plants treated in this experiment. There were, however, two apparently normal plants whose progenies segregated for normal and mutated individuals. These segregating mutants designated as mutants 1 and 2 are illustrated in Figure I and Figure II, C and D. The parent in each instance was of the constitution T165/+.

In the progeny of mutant 1, three changed plants, all of which were fertile, were observed. In addition this progeny contained 17 normal plants which segregated 11 fertile to 6 semisterile (Figure I). One of the changed plants (Figure I, A and A^1) was taller and had lighter colored seeds than the original genotype, Experimental 3. The plant had a fine stem and fine leaves which were purple spotted, an open panicle and tillered considerably more than the original genotype. The second changed plant (Figure I, B and B^1) in this progeny was taller, with fine stems and leaves, had lighter colored seeds and was more fertile than the original. This plant had a large open panicle, long awns and large glumes enclosing the seeds. This plant also tillered more than the original genotype. The third changed plant in this progeny (Figure I, C and C^1) was taller with fine stems and leaves which were purple spotted and tillered considerably. This

Table 1. Experiment I, Induction of Plants Homozygous for Structural Chromosome Markers after Colchicine Treatment of Plants Heterozygous for Structural Markers on Two Chromosome Pairs (One Reciprocal Translocation)

	No. planted	No. survived	Condition of markers as shown in progenies*: Normal genotype, heterozygous for markers	Normal genotype, homozygous for markers	Mutant genotype, heterozygous for markers	Mutant genotype, homozygous for markers
Marker homozygotes						
+/+						
Treated	16	5	--	2	--	3†
Untreated	4	4	--	4	--	--
Marker heterozygotes						
T396/+						
Treated	28	9	9#	--	--	--
Untreated	11	4	4	--	--	--
T165/+						
Treated	24	8	6	--	2##	--
Untreated	9	5	5	--	--	--
T231/+						
Treated	10	2	2	--	--	--
Untreated	4	4	4	--	--	--

*Each progeny contained approximately 35 plants. Translocation heterozygotes (heterozygous for marker) segregate 1 fertile:1 semisterile; homozygotes, all fertile.

†All true-breeding.

#One progeny contained tetraploid plants.

##Two progenies contained normal and mutant plants (mutants 1 and 2, putative chimaera of T165/+ and undetermined homozygotes).

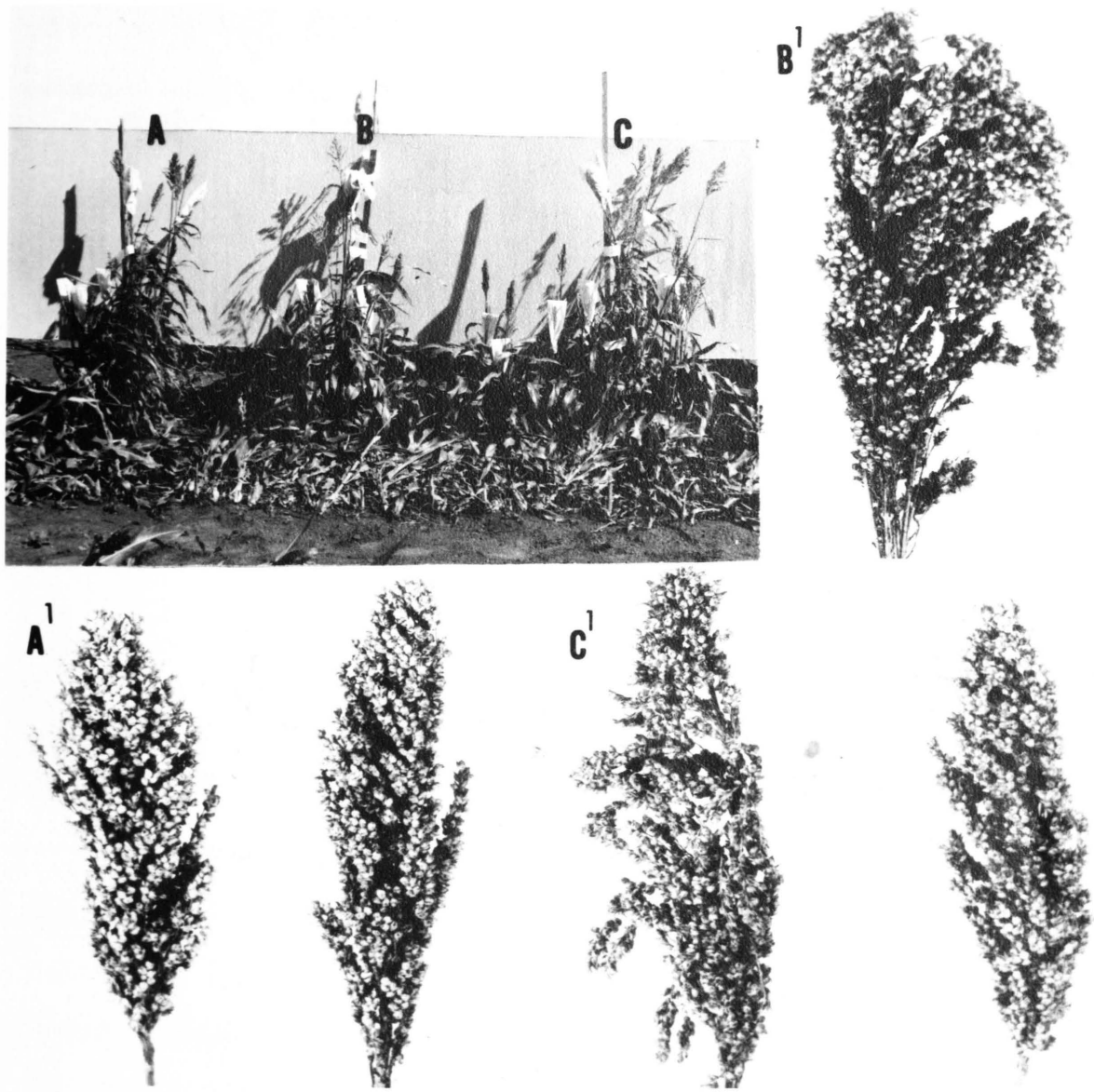

Figure I. Segregating progeny from mutant 1 obtained from T165/+ showing normal plants and three mutant segregants A, B, and C, whose panicle types are shown in A^1, B^1, and C^1

plant had a large slightly open panicle with long internodes in the panicle. The seeds and glumes were similar to those of the original. Cytological examination showed these three plants to be structurally homozygous.

The progeny from mutant 2 (Figure II, C) contained an abnormal plant along with seven normal plants. The normal plants segregated 4 fertile:3 semisterile. The mutated plant (Figure II, D) was fertile, very tall with weak culms which broke off easily and tillered profusely. It had large and loose panicles, long awns, and large glumes which tightly enclosed the seed, so the seed threshed free with difficulty. The glumes were black, the seeds were brown and matured very late. Cytological examination at diakinesis showed the absence of rings or chains of four chromosomes indicating the structural homozygosity of this segregant.

The presence of the structural marker (translocation) in the heterozygous state in the treated plants was ascertained by determining segregation ratios in the progenies. A ratio of 1 fertile:1 semisterile in the progeny indicated that the parent was heterozygous for the marker. No variance in plant type was noted in the progenies of any of the treated or untreated plants with the exception of the two previously described.

Three of the plants which survived after treatment of Experimental 3 were diploid mutants (Figure III, A, C, and D). The head type of Experimental 3 is illustrated in Figure IV, A, and its homozygous nature is illustrated by the uniformity shown in Figure IV, B.

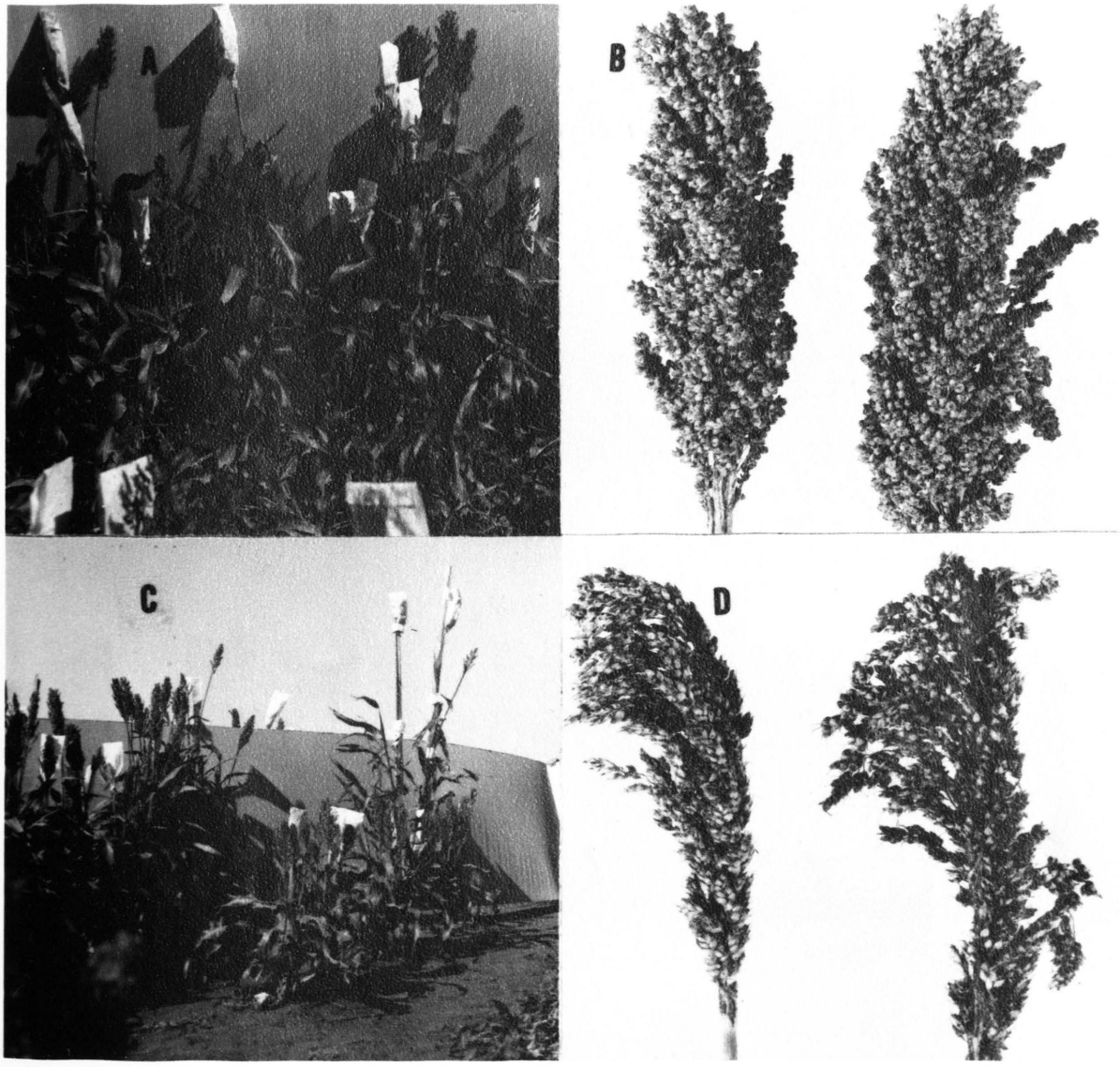

Figure II. Progeny from mutants 3 and 4 obtained from T396/+ (A) showing segregation for normal and mutated plants. (B) Panicles of mutated plants from mutants 3 and 4, respectively. (C) Progeny of mutant 2 obtained from T165/+ showing segregation for normal and one mutated plant. (D) Panicle of the mutated plant from mutant 2

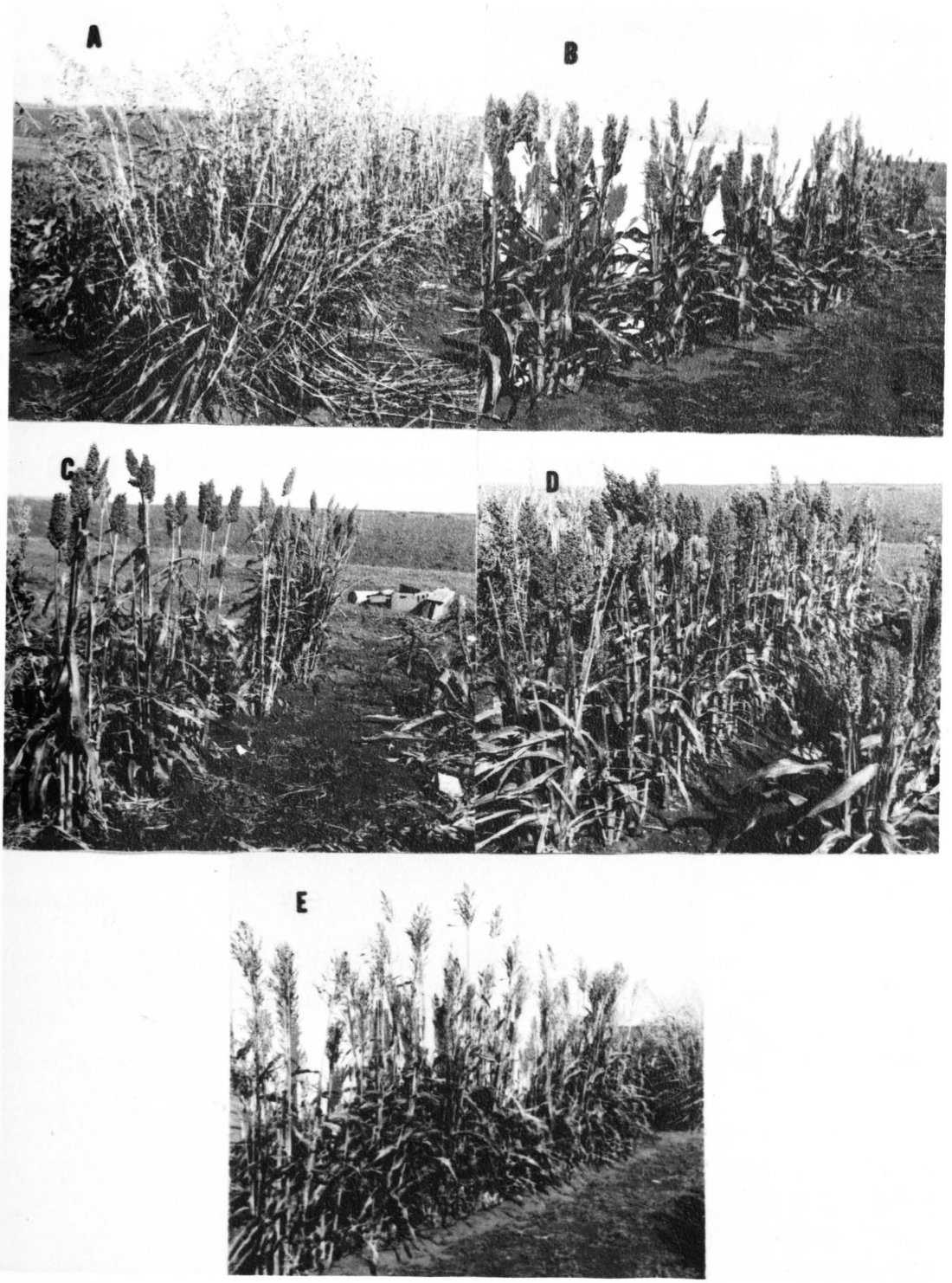

Figure III. Five mutant progenies from Experimental 3 (+/+). A, C, and D were immediately true-breeding. (B) Male sterile true-breeding. (E) Segregating for several characters

One of the mutants obtained from Experimental 3 after treatment was a grass type (Figure III, A) which arises frequently from treatment with colchicine. This plant differed from the original in a number of characters. The mutant was taller, had fine stems and leaves which were purple spotted, tended to lodge easily and tillered profusely. It had a large open panicle, increased fertility, long awns, and the glumes were larger and enclosed the seeds. The progeny from this mutant bred true immediately.

The second mutant (Figure III, C) was taller, had more coarse leaves and stems, and had a larger, more compact panicle than the original. This mutant produced very few tillers, the glumes were larger and the glumes and seed were a red color. The third mutant (Figure III, D) was taller, and had finer leaves than the original genotype. The panicles were also larger and bushier with larger glumes and seeds, both of which were darker in color than Experimental 3. Both of these mutants also produced progeny which bred true immediately.

Experiment II

Induction of plants homozygous for structural chromosome markers (reciprocal translocations) was again attempted by treating seedlings heterozygous for such markers with colchicine in Experiment II. The results in Experiment II are shown in Table 2. Two progenies from apparently unchanged treated plants, mutants 3 and 4 from T396/+, segregated for mutant plants which appeared identical (Figure II, A and B). Both progenies contained normal plants which segregated 1 fertile:1 semisterile, and each progeny contained a number of mutant plants which also segregated 1 fertile:1 semisterile. The two progenies contained a total of 90 plants of which 12 were uniformly changed. Six of the mutants were homozygous and six were heterozygous for the reciprocal translocation. These mutants were identified by pollen counts, seed set and cytological examinations. Mutants 3 and 4 were taller and tillered more than the normal plants growing in the progeny (Figure II, B) and matured earlier than the normal plants.

Three diploid mutant plants, (mutants 5, 6, and 7) homozygous for the structural marker and differing from the original genotype (T396/+) in a number of characters, were observed after colchicine treatment. The presence of translocation homozygotes was ascertained by examination of pollen and by observation of the ratio of fertile and semisterile plants in the progenies. No variance from the expected plant type or semisterility was observed in the surviving non-mutant treated plants or the 45 untreated plants. A number of plants which showed polyploid characteristics were observed in the progeny

Table 2. Experiment II, Induction of Plants Homozygous for Structural Chromosome Markers after Colchicine Treatment of Plants Heterozygous for Structural Marker on Two Chromosome Pairs (One Reciprocal Translocation)

	No. planted	No. survived	Condition of markers as shown in progenies*			
			Normal genotype		Mutant genotype	
			heterozygous for markers	homozygous for markers	heterozygous for markers	homozygous for markers
Marker homozygotes +/+						
Treated	20	5	--	1	--	4[†]
Untreated	5	5	--	5	--	--
Marker heterozygotes T396/+						
Treated	80	55	50	--	2[##]	3[#]
Untreated	52	45	45	--	--	--

*Each progeny contained approximately 35 plants. Translocation heterozygotes (heterozygous for marker) segregate 1 fertile:1 semisterile; homozygotes, all fertile.

[†]One true-breeding.
One true-breeding male sterile.
One segregating for several characters.
One haploid mutant.

[#]Two true-breeding (mutants 5 and 7, +/+).
One true-breeding for all characters except seedling base color (mutant 6, +/+).

[##]Two progenies containing apparently identical mutants and normal plants (mutants 3 and 4, putative chimaera of T396/+ and identical mutant tissue of undetermined nature).

of one treated plant, however, no changes that would appear to be caused by actual gene mutations were noted in this progeny.

The panicle type and progeny of Experimental 3, +/+, of the translocation heterozygote, T396/+, and of mutant 5 are shown in Figure IV, A and D, B and E, and C and F, respectively. The lack of awns and homozygosity of Experimental 3 and the semisterility of T396/+ and segregation for semisterility in its progeny are illustrated. The restored fertility, presence of awns, larger and more compact panicle type, taller nature and apparent complete homozygosity of mutant 5 are evident in C and F. The progeny of mutant 5 also differed from the unmutated form by maturing two weeks earlier and by tillering more. Mutant 5, the panicle of which is shown in Figure IV, C, was induced immediately from a seedling of the constitution T396/+ as in Figure IV, B following colchicine treatment. Similarly the two other mutants (mutant 6 and 7) were transformed directly by colchicine treatment into diploid mutants apparently homozygous for the structural marker. Mutants 5 and 7 bred true for all changed characters and mutant 6 for all but one.

Mutant 6 (Figure V, A and B) was immediately fertile with a larger, more compact panicle (Figure V, A) than the original genotype. The mutant was taller with longer and wider leaves and tillered more than either of its parents. The homozygous nature of the progeny of this mutant is evident in Figure V, B. The progeny matured approximately two weeks later and, although true-breeding for all observable mature plant characters, it segregated for seedling base color, 3 red:1 green.

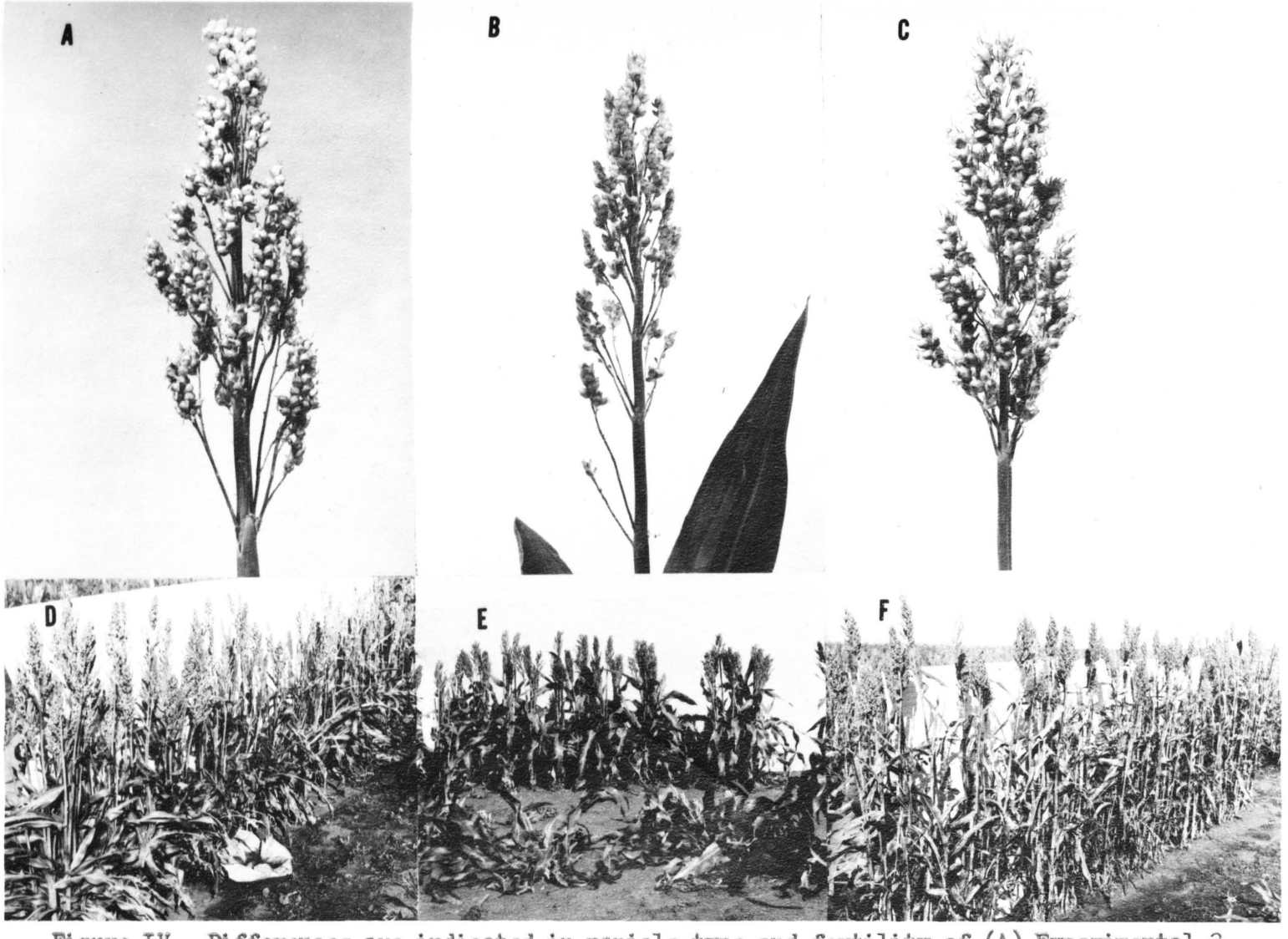

Figure IV. Differences are indicated in panicle type and fertility of (A) Experimental 3, (B) T396/+ and (C) mutant 5 induced from T396/+. The uniformity of the progenies are shown for (D) Experimental 3, (E) T396/+ and (F) mutant 5

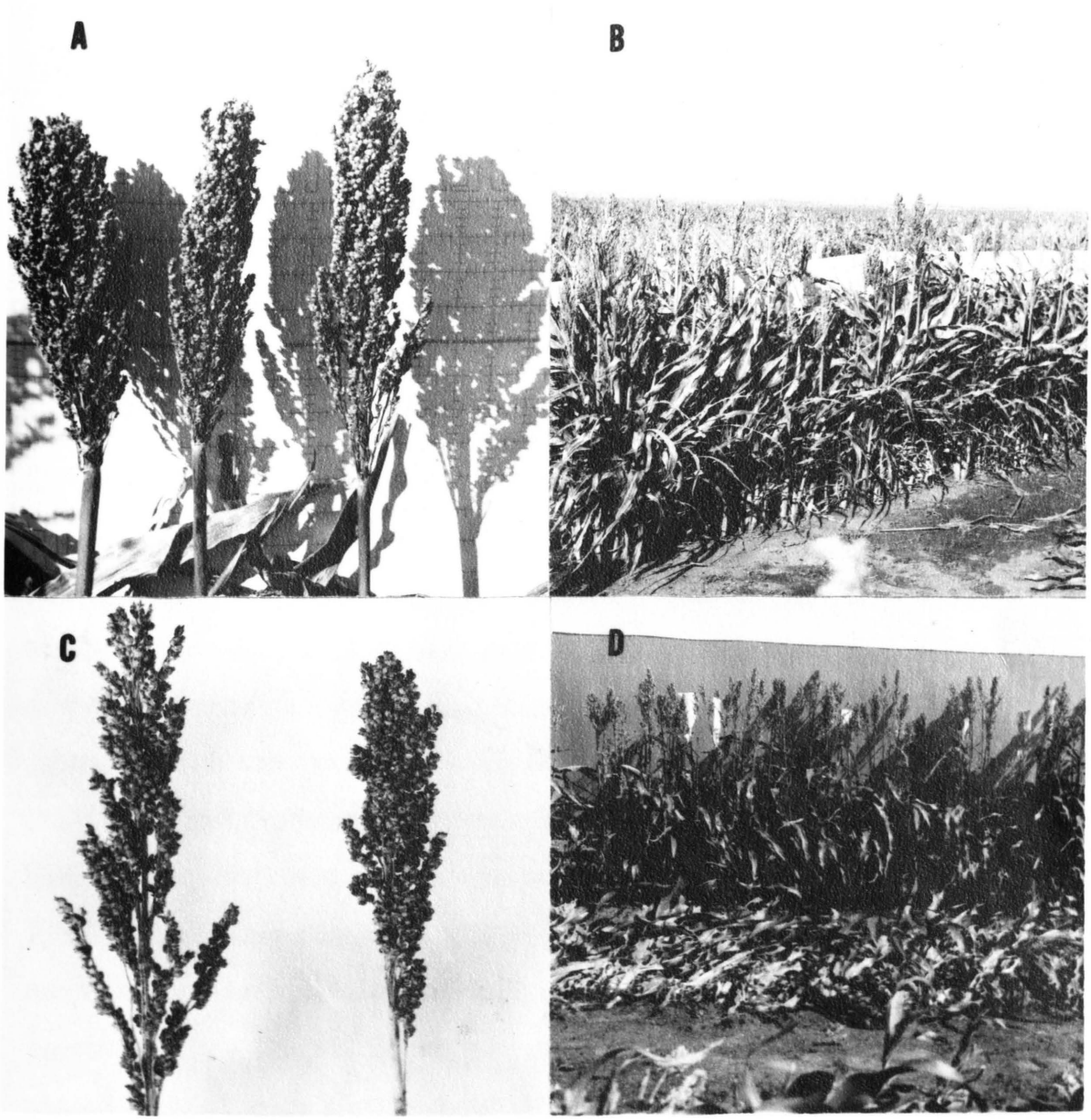

Figure V. The restored fertility and changed panicle types are illustrated for (A) mutant 6 and (C) mutant 7, both mutants induced from T396/+. The uniformity of the progenies of (B) mutant 6 and (D) mutant 7 are shown

Mutant 7 (Figure V, C and D) was similar to Experimental 3 except for a loose-appearing panicle caused by longer rays and shorter axis internodes. The internode length of the panicles of mutant 7 varied considerably (Figure V, C). This progeny tillered more and had a more fragile culm than the original genotype. The homozygous nature of the progeny and the restored fertility are evident in Figure V, D.

Cytological examinations of the meiotic behavior at diakinesis of Experimental 3, +/+, T396/T396 and the F_1 (T396/+) between T396/T396 and +/+ are illustrated in Figure VI, A, B, and C, respectively. This figure illustrates the normal chromosome behavior of both +/+ (Figure VI, A) and T396/T396 (Figure VI, B). Chromosomes of mutant 6 were examined at diakinesis (Figure VI, D). No evidence of a chain or ring was noted at diakinesis or metaphase I which, in conjunction with the uniformly high fertility, indicated that the mutant must have been homozygous for the two pairs of marked chromosomes. When the mutant plant was backcrossed as the male to T396/T396, the F_1 plants were semisterile. These plants were examined at diakinesis and a chain configuration involving the nucleolar chromosome was observed (Figure VI, E). When the mutant plant was backcrossed as the male to +/+, the F_1 plants were fully fertile and did not show any evidence of associations between pairs (Figure VI, F). Therefore, the two pairs of identifiable chromosomes in this mutant are structurally the same as in the normal parent (+/+).

Since mutant 5 produced only one panicle, which was not sampled for cytological study, the meiotic behavior was examined in the

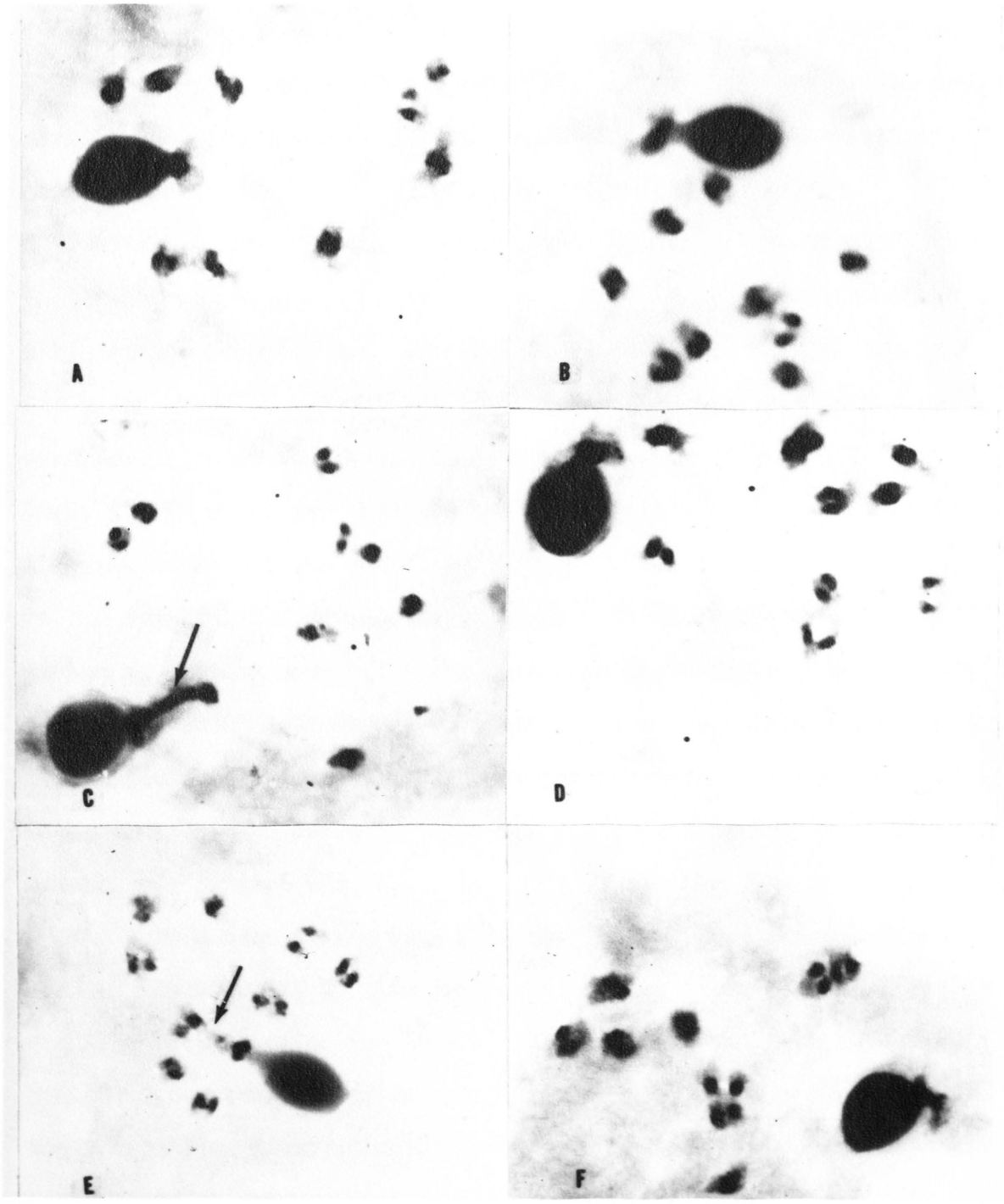

Figure VI. Diakinesis configurations illustrating structural homozygosity induced by treatment. (A) Untreated Experimental 3, +/+. (B) Untreated T396/T396. (C) Untreated T396/+. (D) Treated T396/+, mutant 6. (E) F_1 of mutant 6 X T396/T396. (F) F_1 of mutant 6 X Experimental 3, +/+, ten bivalents

members of its progeny which were true-breeding. Since no configuration of four chromosomes was observed, the original mutant was presumably homozygous for the two chromosomes involved in the reciprocal translocation. When mutant 5 was backcrossed as the male to T396/T396, the progeny was semisterile. Cytological examination indicated that the plants were heterozygous for the translocation since a chain of four chromosomes was observed at diakinesis. When mutant 5 was backcrossed as the male to +/+, the progeny was fertile and no configurations of four chromosomes were observed (Figure VII, A). Thus, it was concluded that the two marked pairs of chromosomes were the same as the normal parent (+/+).

Cytological examination of members of the progeny of mutant 7 showed no associations of four chromosomes at diakinesis and, therefore, indicated that they were homozygous for the marked chromosomes. It was found by backcrossing to both parents in the manner described above that the two pairs of marked chromosomes were homozygous normal, +/+ (Figure VII, B).

Four mutant plants were observed following colchicine treatment of Experimental 3. The progeny of one of these mutants (Figure III, E) segregated for several plant characters, such as height, panicle type, presence of awns and type of growth. One of the mutants appearing after colchicine treatment was male sterile; but when backcrossed to Experimental 3, the progeny bred true (Figure III, B). The male sterile mutant was taller with thicker leaves and culm and had a larger panicle than Experimental 3.

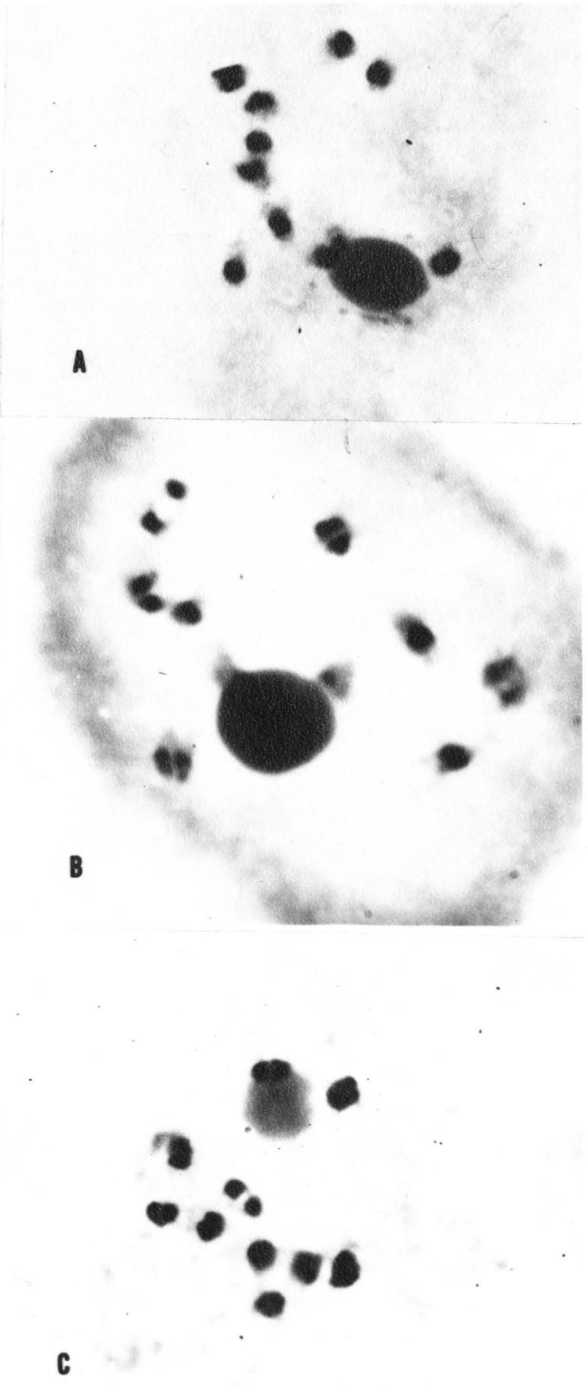

Figure VII. Diakinesis configurations illustrating the homozygous normal structure (+/+) of mutants 5, 7, and 8. (A) F_1 of mutant 5 X Experimental 3, +/+. (B) F_1 of mutant 7 X Experimental 3, +/+. (C) F_1 of mutant 8 X Experimental 3, +/+

One of the mutant plants was a haploid (Figure VIII, A and B) as indicated by cytological examination. This mutant was short with very fine red-tinged leaves and culm. It had an open panicle, very fine glumes, tillered profusely, was awned and was sterile. Since this haploid mutant appeared similar to the variety Winner, which is a colchicine mutant, the two were crossed using the haploid as the female. The five F_1 plants were uniform in appearance. Plants and their panicles are illustrated in Figure VIII, C and D. The F_2 was grown in the field during the summer of 1962, and showed no segregation (Figure IX, A and B). It appeared identical to the variety Winner (Figure IX, C).

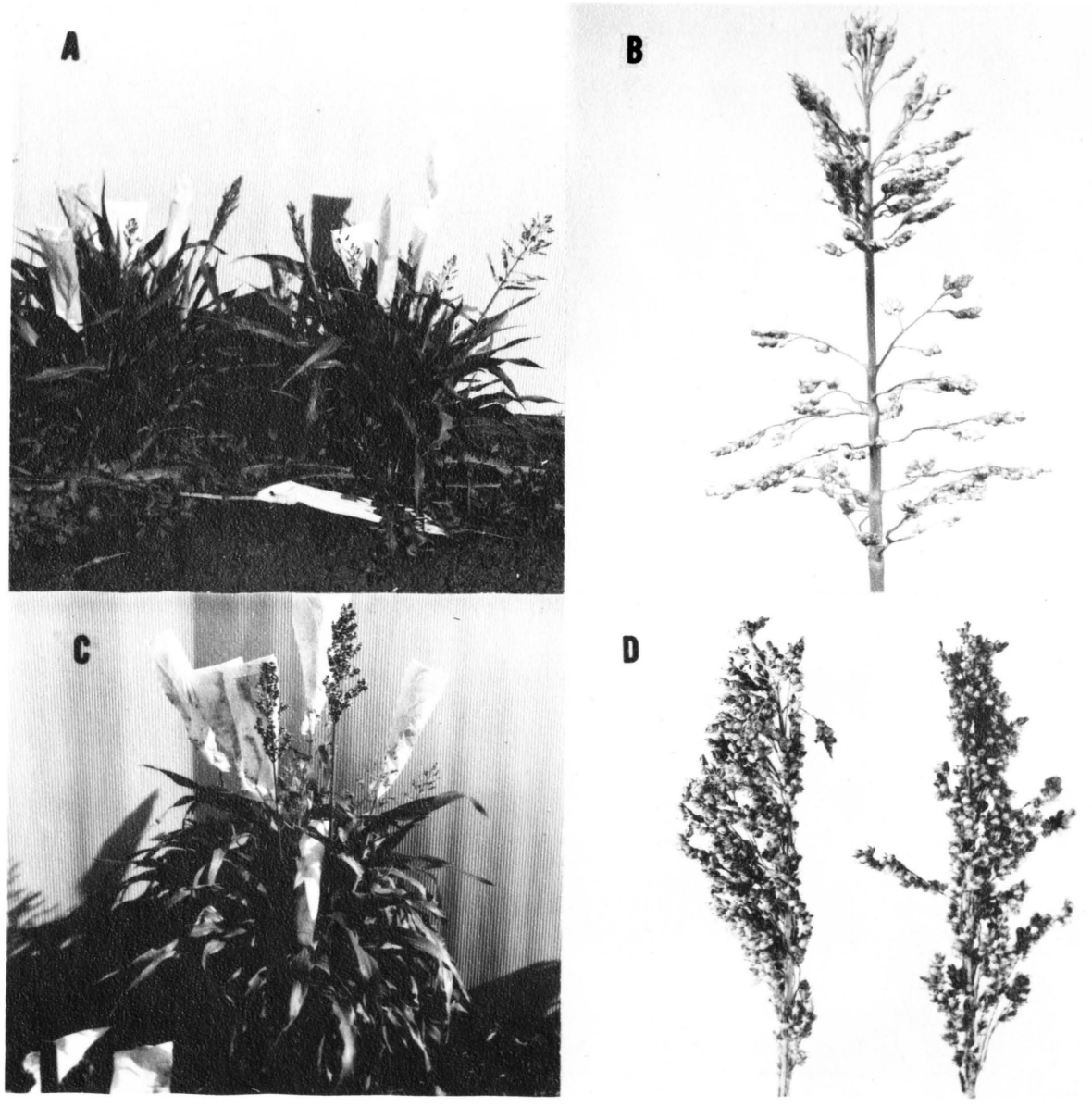

Figure VIII. Haploid mutant plant obtained from Experimental 3. (A) Two plants showing growth habit. (B) Panicle type illustrating sterility. (C) F_1 plants of the haploid mutant X Winner. (D) Panicle type and fertility of F_1 are shown. The F_1 population was indistinguishable from Winner

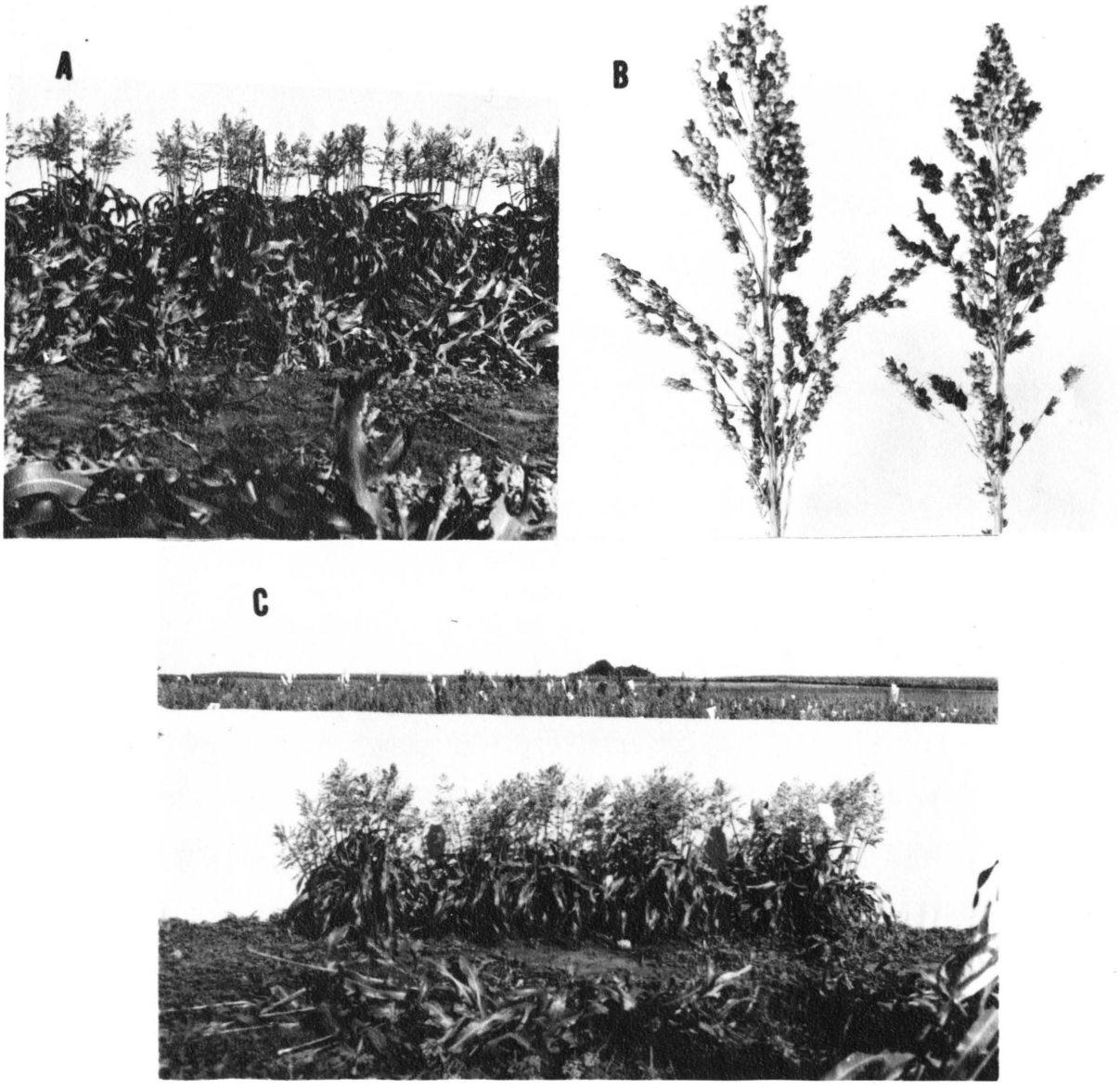

Figure IX. (A) F_2 of haploid mutant X Winner showing no segregation. (B) Panicle type of F_2 plants. (C) The variety Winner which was indistinguishable from the F_2 population

Experiment III

Further attempts to obtain plants homozygous for structural chromosome markers were made by treating seedlings heterozygous for markers in two chromosome pairs and also for markers in four chromosome pairs. Plants heterozygous for one reciprocal translocation have two structural chromosome markers and plants heterozygous for two translocations have four chromosomes marked. The results from Experiment III are in Table 3.

One immediately true-breeding mutant, homozygous for the structural chromosome marker, was induced by colchicine treatment of a seedling of the constitution T396/+. This mutant plant was designated mutant 8. The restored fertility and the larger more compact panicle type of this mutant are illustrated in Figure X, A. Mutant 8 was taller with wider and longer leaves than the original genotype. It had very large glumes and seeds which were darker in color, and the progeny matured two to three weeks later than Experimental 3. The homozygous nature of mutant 8 is illustrated in Figure X, B.

Cytological examination of mutant 8 illustrated the absence of a ring or chain of four chromosomes at diakinesis and metaphase I. This indicated that mutant 8 was homozygous for the two pairs of marked chromosomes. When mutant 8 was backcrossed as the male to the T396/T396 parent, the progeny was semisterile. A cytological examination demonstrated the presence of a chain of four chromosomes involving the nucleolar chromosome at diakinesis. When mutant 8 was backcrossed as the male to the normal parent, +/+, the progeny was

fertile. No evidence of any chromosome configuration involving four chromosomes (Figure VII, C) was indicated. Therefore, the two pair of identifiable chromosomes in mutant 8 were structurally the same as in the normal parent, +/+.

No variance from the normal plant type or semisterility was observed in the surviving nonmutant treated plants, or the untreated plants heterozygous for one reciprocal translocation. All the progenies from unchanged parents heterozygous for one reciprocal translocation were observed to segregate 1 fertile:1 semisterile.

No progenies of plants heterozygous for two reciprocal translocations were grown, since the treated and untreated plants were sterile in the greenhouse and in the field (Figure XI, A and B). No variance in plant type was noted for any of the treated plants.

No variance in plant type or fertility was observed following colchicine treatment of plants T396/T396. The progenies of these plants were fertile, since they were homozygous for the structural chromosome marker.

One immediately true-breeding diploid mutant was observed after colchicine treatment of Experimental 3. It appeared identical to a mutant obtained from treatment of this genotype in Experiment I. This mutant, known as the grass-type mutant, is described in Experiment I and is illustrated in Figure III, A.

Table 3. Experiment III, Induction of Plants Homozygous for Structural Chromosome Markers after Colchicine Treatment of Plants Heterozygous for Structural Markers on Two (One Reciprocal Translocation) or Four (Two Reciprocal Translocations) Chromosome Pairs

	No. planted	No.## survived	Condition of markers as shown in progenies*			
			Normal genotype		Mutant genotype	
			heterozygous for markers	homozygous for markers	heterozygous for markers	homozygous for markers
Marker homozygotes						
+/+						
Treated	20	19	--	18	--	1†
Untreated	6	4	--	4	--	--
T396/T396						
Treated	20	19	--	19	--	--
Untreated	6	4	--	4	--	--
Marker heterozygotes						
T396/+						
Treated	60	56	55	--	--	1#
Untreated	18	11	11	--	--	--
T231/+						
Treated	20	20	20	--	--	--
Untreated	6	2	2	--	--	--
T165/+						
Treated	18	13	13	--	--	--
Untreated	3	2	2	--	--	--

Table 3. (continued)

	No. planted	No. survived	Condition of markers as shown in progenies*			
			Normal genotype		Mutant genotype	
			heterozygous for markers	homozygous for markers	heterozygous for markers	homozygous for markers
T396+/+T165						
Treated	40	34	--	--	--	--
Untreated	12	6	--	--	--	--
T396+/+T231						
Treated	20	20	--	--	--	--
Untreated	6	2	--	--	--	--

*Each progeny contained approximately 35 plants. Translocation heterozygotes (heterozygous for marker) segregate 1 fertile:1 semisterile; homozygotes, all fertile.

†True-breeding.

#True-breeding (mutant 8, +/+).

##Plants for which no progenies are indicated were sterile.

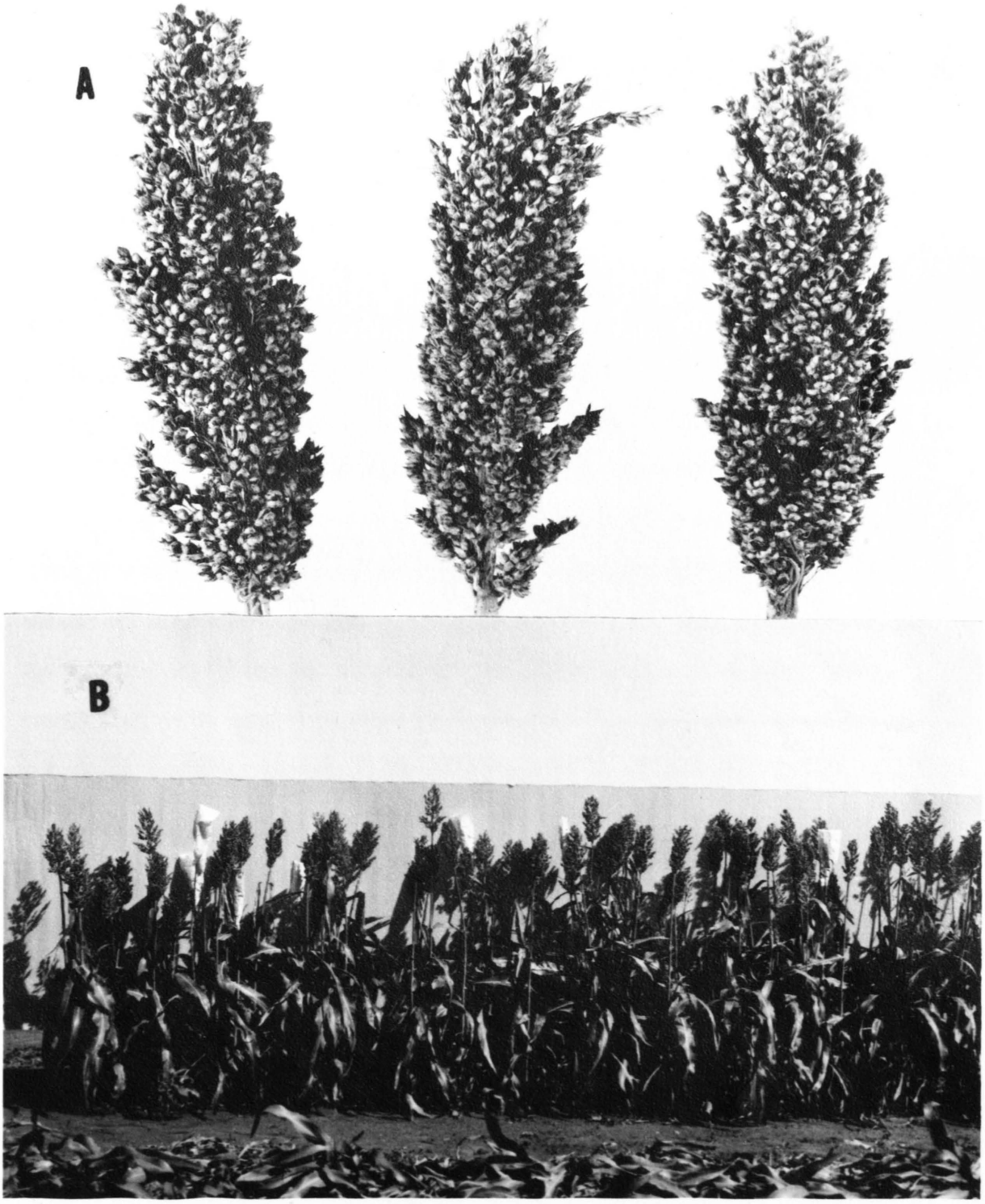

Figure X. Mutant 8 induced from T396/+. (A) Panicle type showing restored fertility. (B) True-breeding progeny

Experiment IV

In a fourth experiment, induction of plants homozygous for structural chromosome markers was attempted again by treating seedlings heterozygous for one and two reciprocal translocations with colchicine. The results of Experiment IV are shown in Table 4.

One immediately true-breeding diploid mutant (mutant 9) was observed following colchicine treatment of a plant with the constitution T396+/+T231. Plants heterozygous for two reciprocal translocations have four pair of marked chromosomes. The panicle type and sterility of such plants are illustrated in Figure XI, A and B.

The restored fertility and large compact panicle type of mutant 9 are illustrated in Figure XI, C. Mutant 9 had large glumes which held the seed tightly, and the glumes and seed were red-tinged in color. The progeny tillered less and was approximately three weeks later in maturity than Experimental 3. The homozygous nature of the progeny of mutant 9 is illustrated in Figure XI, D.

Mutant 9 was the only plant of the original constitution T396+/+T231 which showed variance from the original genotype or produced seed. Cytological examination of two untreated and three treated but unmutated plants indicated the presence of two heterozygous reciprocal translocations (Figure XII, B). The T396/+ is indicated by the presence of the nucleolar chromosome involved in one of the configurations. The two pair of chromosomes involved in T231/+ are not identifiable. Cytological examination of mutant 9, induced from T396+/+T231 following colchicine treatment, indicated the presence of no ring or chain configurations (Figure XII, C). Therefore,

Table 4. Experiment IV, Induction of Plants Homozygous for Structural Chromosome Markers after Colchicine Treatment of Plants Heterozygous for Structural Markers on Two (One Reciprocal Translocation) or Four (Two Reciprocal Translocations) Chromosome Pairs

	No. planted	No.## survived	Condition of markers as shown in progenies*			
			Normal genotype		Mutant genotype	
			heterozygous for markers	homozygous for markers	heterozygous for markers	homozygous for markers
Marker homozygotes						
+/+						
Treated	20	20	--	19#	--	1†
Untreated	6	5	--	5	--	--
Marker heterozygotes						
T396/+						
Treated	80	25	25*†	--	--	--
Untreated	24	19	19	--	--	--
T396+/+T231						
Treated	10	8	--	--	--	1*#
Untreated	2	2	--	--	--	--
T396+/+165						
Treated	36	16†#	--	--	--	1*#
Untreated	12	6	--	--	--	--

Table 4. (continued)

	No. planted	No.## survived	Condition of markers as shown in progenies* Normal genotype: heterozygous for markers	Normal genotype: homozygous for markers	Mutant genotype: heterozygous for markers	Mutant genotype: homozygous for markers
T165+/+231						
Treated	18	12	--	--	--	--
Untreated	6	4	--	--	--	--

*Each progeny contained approximately 35 plants. Translocation heterozygotes (heterozygous for marker) segregate 1 fertile:1 semisterile; homozygotes, all fertile.

+True-breeding.

#Two exceptional progenies contained a number of tetraploid plants.

*+One progeny contained a number of tetraploid plants.

##Plants for which no progenies are indicated were sterile.

*#True-breeding (mutants 9 and 10, +/+).

+##One plant produced 2 seeds, both of which were tetraploid.

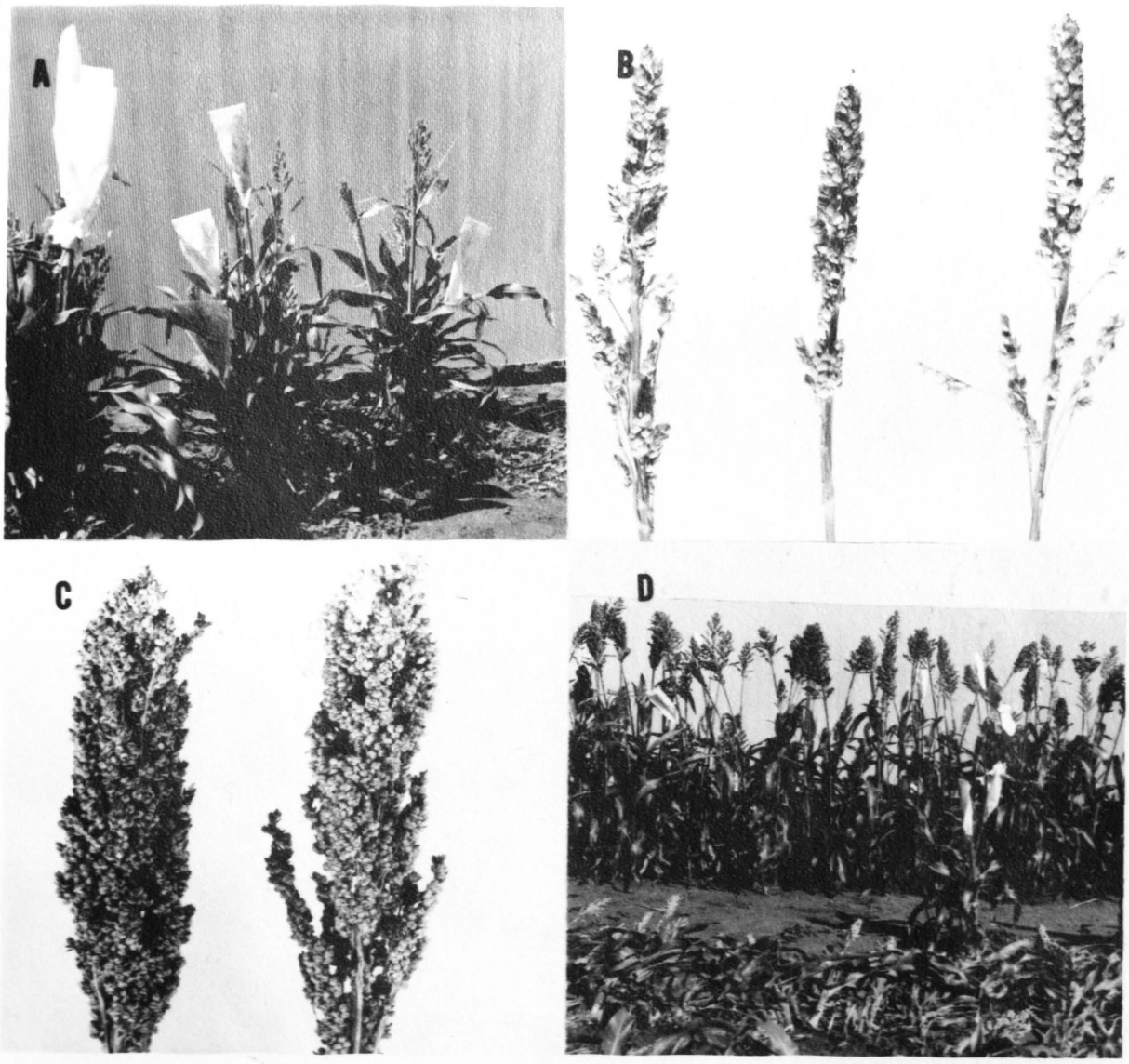

Figure XI. Induction of a true-breeding mutant from a seedling heterozygous for markers on four chromosome pairs. (A) Untreated T396+/+T231 plants. (B) Panicles completely sterile. (C) Panicles of mutant 9 showing restored fertility. (D) True-breeding progeny from mutant 9

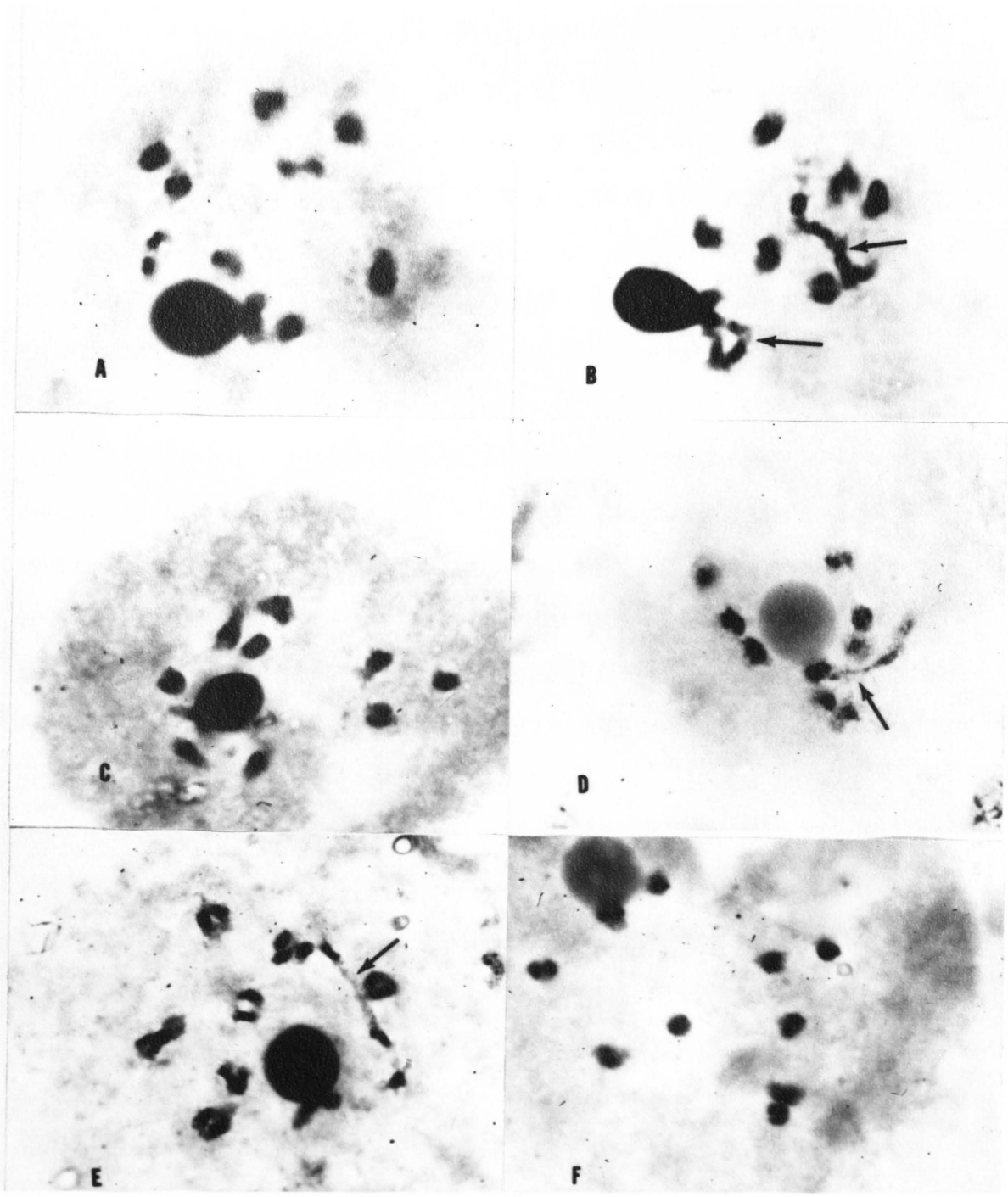

Figure XII. Diakinesis configurations illustrating structural homozygosity induced by treatment. (A) Untreated T231/T231. (B) Untreated T396+/+T231. (C) Treated T396+/+T231, mutant 9. (D) F_1 of mutant 9 X T396/T396. (E) F_1 of mutant 9 X T231/T231. (F) Mutant 9 X Experimental 3, +/+, ten bivalents

mutant 9 is homozygous for the four pair of marked chromosomes.

In order to determine the nature of the chromosome structure, mutant 9 was backcrossed as male to the T396/T396 parent. The progeny was semisterile and cytological examination demonstrated the presence of a chain of four chromosomes involving the nucleolar chromosome at diakinesis (Figure XII, D). When mutant 9 was backcrossed as male to T231/T231 (Figure XII, A), the progeny was semisterile. Cytological examination demonstrated these plants to be T231/+ since a chain of four chromosomes was observed at diakinesis (Figure XII, E), and the nucleolar chromosome was not involved in the chain. When mutant 9 was crossed as male to +/+, the progeny was fertile. Cytological examination indicated the presence of ten bivalents with no configurations of four chromosomes (Figure XII, F). It may, therefore, be concluded that the four marked chromosome pairs are structurally the same as +/+.

Another apparently true-breeding diploid mutant, mutant 10, homozygous for the structural chromosome marker as indicated by examination at diakinesis, was identified following colchicine treatment of plants with the constitution T396+/+T165. Plants of such a constitution also have four pair of marked chromosomes. The treated, unmutated plants and the untreated plants showed no variance from the original genotype and are identical to those illustrated in Figure XI, A and B. These plants are sterile; however, one parent produced two tetraploid seeds. Cytological examination of four untreated plants demonstrated the presence of two heterozygous reciprocal translocations

(Figure XIV, B). The T396/+ can be identified since it involves the nucleolar chromosome, but the two pair involved in the T165/+ are not identifiable.

Figure XIII, A, illustrates the restored fertility and the large, loose panicle with large glumes which enclose the seed, and the presence of awns of mutant 10. This plant is tall with fine leaves which are purple spotted and tillers profusely (Figure XIII, B). Mutant 10 was later in maturing, consequently, no progeny was grown in the field. However, 16 plants were grown in the greenhouse during the fall of 1962, and no variance among the 16 plants was noted. Therefore, mutant 10 apparently is of true-breeding nature.

The normal chromosome behavior, the absence of any ring or chain configurations of T396/T396 and T165/T165, is illustrated in Figure VI, B, and Figure XIV, A, respectively. The progeny (T396+/+T165) resulting from a cross of T396/T396 and T165/T165 can be identified by the presence of two ring or chain configurations of four chromosomes at diakinesis (Figure XIV, B). Cytological examination of mutant 10 showed normal chromosome behavior with the presence of ten bivalents (Figure XIV, C). Therefore, mutant 10 was homozygous for the four pair of marked chromosomes. To determine the nature of the chromosome structure of mutant 10, it was backcrossed as male to the T396/T396 parent. Cytological examination of the progeny illustrated a chain of four chromosomes at diakinesis involving the nucleolar chromosome (Figure XIV, D). When mutant 10 was backcrossed as male to the T165/T165 parent, the progeny was semisterile. Cytological

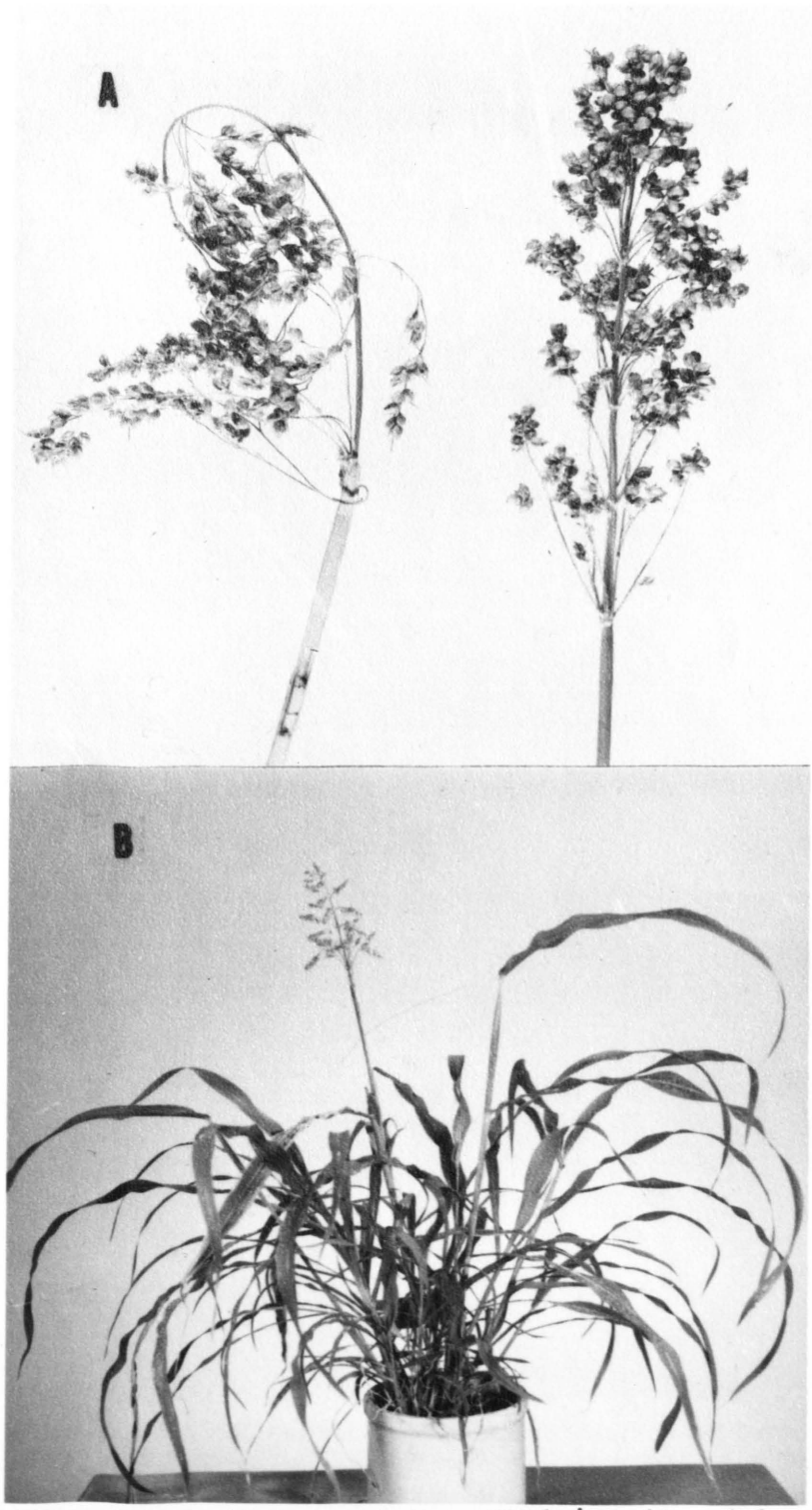

Figure XIII. Mutant 10 induced from T396+/+T165. (A) Panicles showing changed type and restored fertility. (B) Mutant 10 plant showing grass-like growth

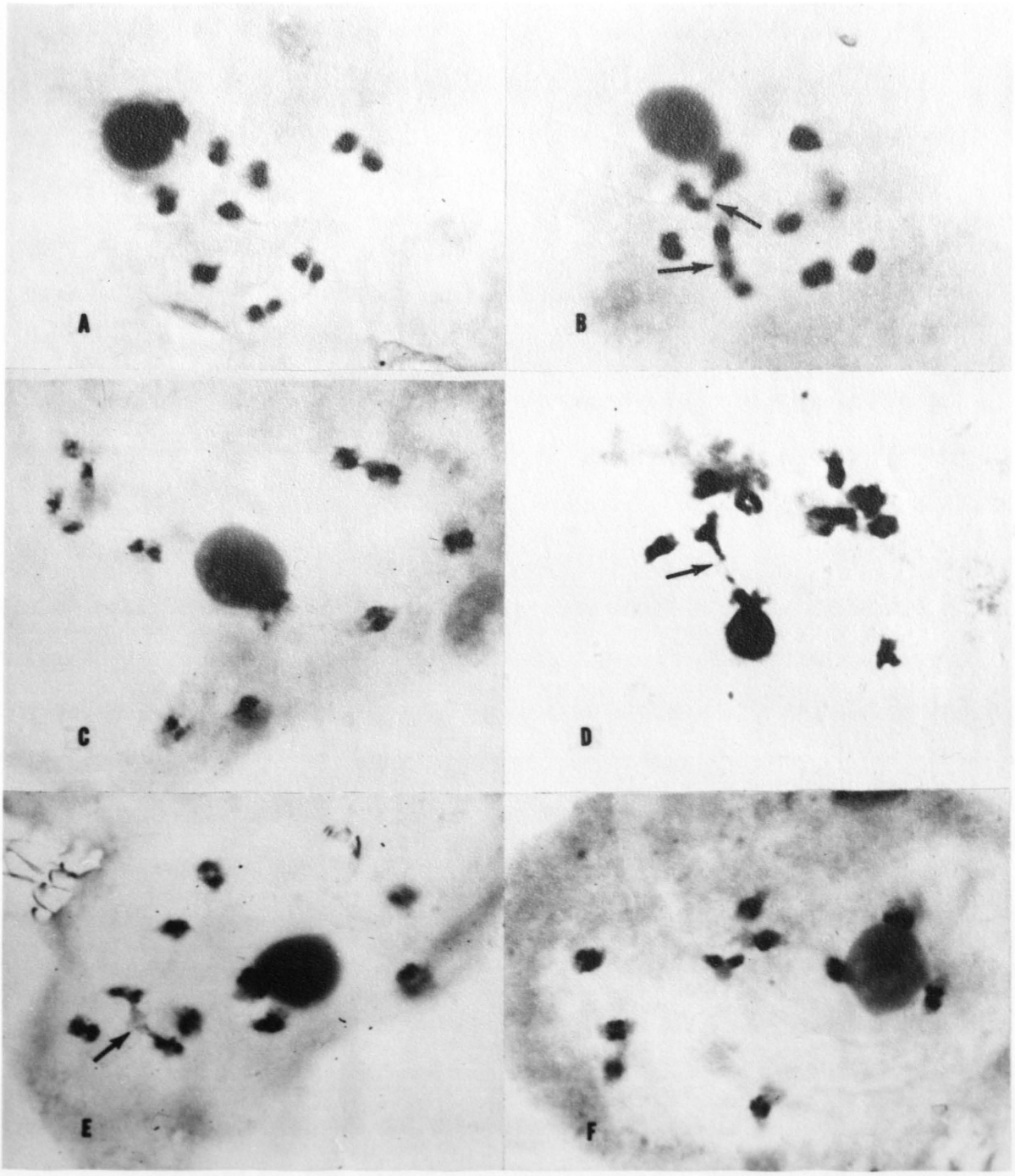

Figure XIV. Diakinesis configurations illustrating structural homozygosity of mutant 10. (A) Untreated T165/T165. (B) Untreated T396+/+T165. (C) Treated T396+/+T165, mutant 10. (D) F_1 of mutant 10 X T396/T396. (E) F_1 of mutant 10 X T231/T231. (F) F_1 of mutant 10 X Experimental 3, (+/+), ten bivalents

examination of the progeny showed a chain of four chromosomes at diakinesis (Figure IV, E). When mutant 10 was crossed as male to +/+, the progeny was fertile. Cytological examination of the progeny illustrated the presence of ten bivalents with no ring or chain of four associations present (Figure XIV, F). Therefore, the four pair of identifiable chromosomes are identical to +/+.

No induced homozygosity was apparent in any of the plants with the constitution T165+/+T231 and T396/+ treated in this experiment. No variance was observed in plant type of the treated and untreated plants. The T165+/+T231 plants were sterile. Cytological examination of four untreated plants T165+/+T231 showed a chain configuration of six chromosomes involved. Therefore, the T165/T165 and T231/T231 lines must involve a mutual chromosome. The T396/+ progenies segregated 1 fertile:1 semisterile, with the exception of one progeny which contained a number of sterile tetraploid plants.

One immediately true-breeding diploid mutant was observed following colchicine treatment of Experimental 3, +/+. This mutant was identical in appearance to the one discussed under Experiment I and illustrated in Figure III, D. Two treated plants produced progenies which contained a number of tetraploid plants which showed no evidence of gene mutation. No variance was noted between the remaining treated and untreated plants, all of which were fertile.

A summary of the results of Experiments I through IV is indicated in Table 5. A total of nine mutant plants was observed after colchicine treatment of plants with homozygous normal chromosome

complements (+/+). No mutants were observed after colchicine treatment of plants homozygous for one reciprocal translocation (T396/T396). A total of eight mutants was noted after colchicine treatment of seedlings heterozygous for one reciprocal translocation (T396/+, T231/+, and T165/+). Four of these mutants were immediately true-breeding, and the same number appeared in segregating progenies. Two immediately true-breeding mutants were observed after colchicine treatment of plants heterozygous for two reciprocal translocations (T396+/+T231, T396+/+T165, and T165+/+T231). All of the immediately true-breeding mutants induced from plants heterozygous for one or two reciprocal translocations were shown to have a homozygous normal chromosome structure (+/+).

Table 5. Summary of Results of Experiments I through IV

Total	Colchicine treated			Untreated		
	No. treated	No. survived	% mutants of survivors	No. untreated	No. survived	% mutants of survivors
Normal homozygotes	76	49	18.4	21	18	0
Translocation homozygotes	20	19	0	6	4	0
One translocation heterozygote	320	188	4.3	127	92	0
Two translocation heterozygotes	124	90	2.2	38	20	0

DISCUSSION of Seed Mixture

The presence of homozygous normal chromosomes identified in the mutants obtained after colchicine treatment constitutes incontrovertible evidence for the occurrence of somatic chromosome reduction followed by doubling of the chromosomes (mutants 5, 6, 7, 8, 9, and 10). This is true of at least the two marked chromosomes when plants heterozygous for one translocation were mutated, and for four marked chromosomes when plants heterozygous for two translocations were mutated. It would be expected that the whole chromosome complement would be involved in such a somatic reduction as was postulated by Franzke and Ross (20) and Ross et al. (59) to explain the phenomenon of the appearance of true-breeding mutants after colchicine treatment.

Since normal Experimental 3 was used as the male parent in all crosses involving plants heterozygous for one reciprocal translocation, it is impossible that the resulting mutants containing homozygous normal chromosome complements after colchicine treatment could have arisen by selfing since the females used in the cross were homozygous for the translocation. The mutants with homozygous normal chromosome structure arising from colchicine treated seedlings heterozygous for two reciprocal translocations rules out the possibility of androgenesis, since, in this case, both parents involved in the cross were homozygous for one reciprocal translocation.

A schematic presentation of the proof for the occurrence of somatic reduction of the four marked chromosome pairs is illustrated in Figure XV. This figure illustrates the two homozygous reciprocal

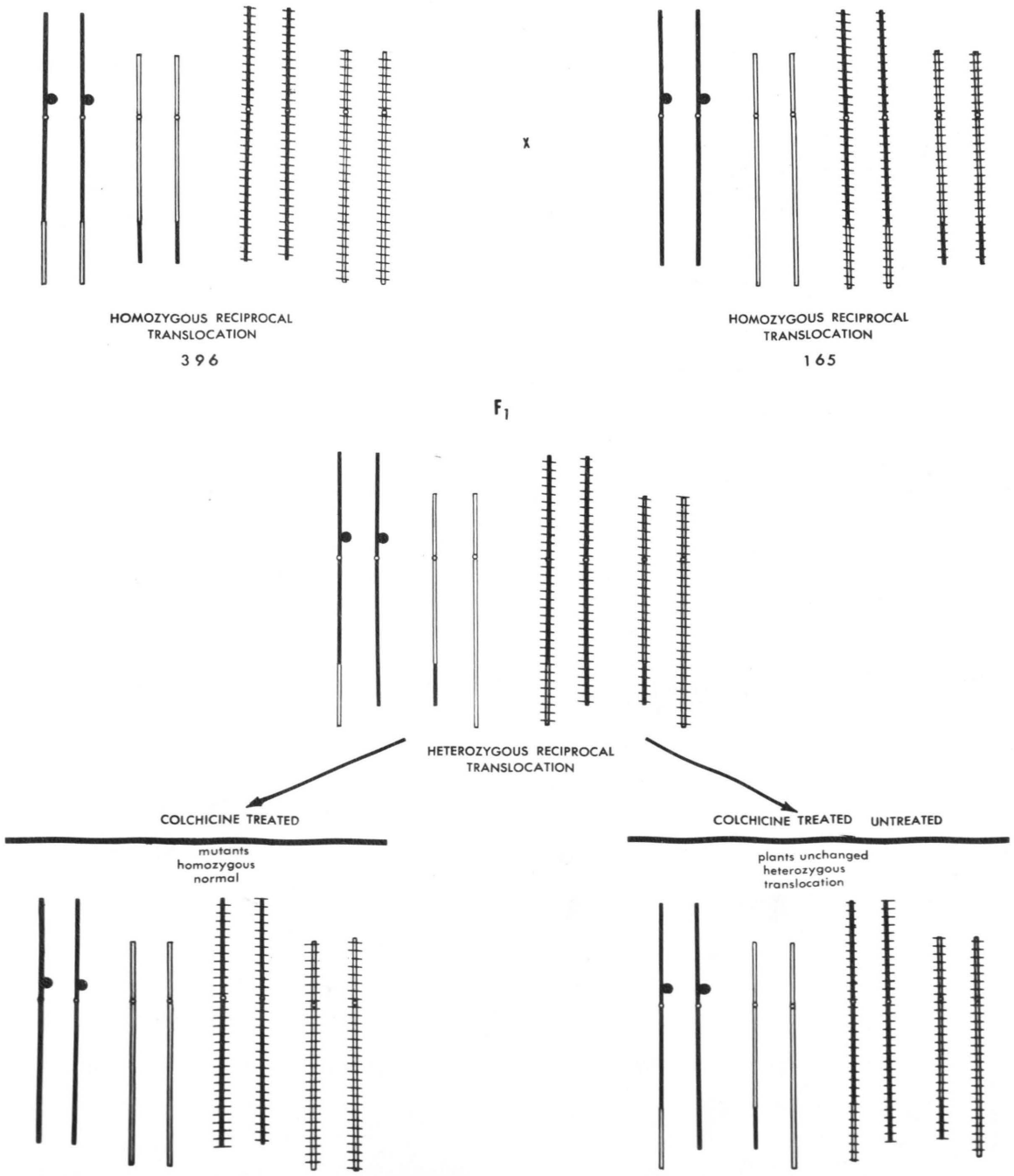

Figure XV. Diagram illustrating the induction by colchicine treatment of homozygosity in seedlings originally heterozygous for structural markers in four chromosome pairs

translocations which were crossed, and the resulting F_1 progeny which was obtained from this cross. The F_1 seedlings which were heterozygous for the two translocations were then treated. The untreated and treated but unmutated plants are represented by the chromosome complement which is heterozygous for the two reciprocal translocations. These plants were sterile and did not produce any progeny. The four pair of untranslocated chromosomes found in each of the two mutant plants 9 and 10, resulting from colchicine treatment of the seedlings heterozygous for the two reciprocal translocations, are illustrated. These mutant plants were immediately fertile and produced abundant seed and were changed for many characters.

The only way that the normal chromosomes could be reconstituted in the homozygous condition in the mutants derived directly from colchicine treatment of seedlings heterozygous for one and two reciprocal translocations would be for a reduction division to have taken place within the colchicine-induced swelling, so that the two or four normal chromosomes would be separated from the translocated chromosomes. Since these single chromosomes consist of two chromatids, it would only be necessary for these to separate, perhaps by an endomitotic process, to restore the original diploid number. That this may not always occur is demonstrated by the appearance of the haploid mutant in this investigation. Sanders and Franzke (60) have presented further evidence of somatic reduction after colchicine treatment. These authors observed four diploid mutants with many changed characters after colchicine treatment of 20 tetraploid seedlings of the sorghum variety Experimental 3. Chen (unpublished) has

repeated this experiment using genetic material related by mutation to that used above and has obtained similar results.

There appears to be little likelihood that the haploid found in this investigation occurred other than by somatic reduction after colchicine treatment, since selection for large seed and vigorous seedlings was made at the time of treatment. Haploids would have been excluded because of their small seed size and lack of vigor. The severity of the treatment causes the death of any weak seedlings such as a haploid. This haploid mutant plant is identical to a diploid mutant which previously has been obtained a number of times after colchicine treatment of Experimental 3 and has been released as the variety Winner. The occurrence of the haploid, therefore, would seem to be a result of treatment and to constitute additional evidence for the occurrence of somatic reduction, which is usually but not always followed by doubling of the chromosome complement. Previous to this investigation no haploid mutant plants had been identified after colchicine treatment of sorghum. This may be due to the very slow growth of the seedling followed by death, since, prior to this investigation, it was the practice to transplant the surviving plants soon after their removal to the greenhouse.

Haploid plants resulting from colchicine treatment have been reported by several authors. Levan (44), after colchicine treatment, obtained a haploid sugar beet plant which had a typical pachytene stage with rather complete pairing at times. Smith (62) observed a haploid Nicotiana plant in addition to a number of heteroploids after

treatment with colchicine. Kehr (40) with Nicotiana and Bergner et al. (5) in Datura observed haploid plants after colchicine treatment. None of these authors attributed the appearance of the haploids to the treatment.

The nature of the mutational effect which involves many genes simultaneously, as reported by Foster et al. (19), is still a matter of speculation but appears to be an effect brought about by conditions within the tissue after colchicine treatment rather than by the effect of the colchicine itself. This may be postulated, since under certain conditions mutations do not occur after colchicine treatment (22, 61).

The reason for previous failures to prove somatic reduction with similar chromosome markers in barley by Ross (unpublished), in corn by Hanson (31), and in sorghum by Atkinson (2) and Huang (33) would appear to be both the unsuitability of the genotype and the environment. The success of this investigation may be attributed to the observation of a differential reaction between genotypes by Atkinson et al. (3) and to the definition of a laboratory method by Franzke et al. (22) by which the appearance of mutants is ensured to a greater degree.

A new growing point may be formed from a single cell which has undergone somatic reduction followed by doubling of the chromosomes. This cell, by virtue of its inherent and perhaps environmental competitive advantage, could then form a new growing point and produce a plant with an entirely different and homozygous genotype. This may possibly indicate a process by which a single cell may show totipotency and form "embryo-like" features similar to those demonstrated

in Haplopappus by Mitra et al. (53). They also found in these cultures somatic pairing and haploid cells, as well as polyploidy, pseudochiasmata and chromosome breaks. It is possible that the situation resulting in the colchicine-swelling is somewhat similar to that within the tissue culture of carrot cells where Steward et al. (63, 64) obtained complete plants from single cells. The process by which the diploid mutant plants arose is presently being investigated by histological studies of colchicine-swellings in tetraploid seedlings (Chen, unpublished). The smaller nuclear size of the diploid cell, as compared to the tetraploid, provides a means of recognizing at an early stage the diploid cells which are endowed with totipotency.

In Table 5, the occurrence of a much higher proportion of mutants is indicated in the homozygous normal material (18.4%) than in the material carrying one heterozygous reciprocal translocation (4.3%) or two heterozygous reciprocal translocations (2.2%). This reduction in the heterozygous translocation plants may have occurred as a result of one or a combination of several factors. The irradiation with gamma rays used to obtain the chromosome translocations may have been responsible for mutations affecting the response to colchicine so that such plants would not form as high a proportion of mutants. At anaphase I of meiosis, when a heterozygous translocation is present, duplications and deficiencies occur in one-half of the gametes as a result of alternate and adjacent segregation. These gametes possessing duplications and deficiencies are nonviable. If it may be assumed that in the somatic reduction process duplications and deficiencies occurred in a similar fashion as at anaphase I of meiosis

when a heterozygous reciprocal translocation is present, then the proportion of true-breeding mutants may be reduced by one-half, since only cells containing the two normal chromosomes or two translocated chromosomes would have been viable. This assumption is based on random segregation of four chromosomes in twos. Consequently, in plants which were heterozygous for two reciprocal translocations, only one-fourth of the cells would be viable, since three-fourths of the cells would contain duplications and deficiencies.

Six true-breeding mutant plants (mutants 5, 6, 7, 8, 9 and 10) were obtained after colchicine treatment of seedlings heterozygous for reciprocal translocations. All of the true-breeding mutants were homozygous for the normal chromosome structure (+/+) identical to that of the original Experimental 3. This may be accounted for by assuming that the homozygous translocation complement is not as viable as is that of the homozygous normal chromosome complement. If this assumption is correct, and assuming random segregation of the chromosomes during the somatic reduction process, then only one-fourth as many mutants should result from treatment of plants heterozygous for one translocation as are obtained from treatment of plants with the homozygous normal chromosome complement. This occurs since one-half of the cells will contain duplications and deficiencies, and one-half of the viable cells will be homozygous for the translocation. Therefore, only one-fourth of the cells will possess the homozygous normal chromosome complement and will be the most viable. In the same fashion, only one-sixteenth as many mutants should result from treatment of plants heterozygous for the two translocations as are

obtained from treated plants with the homozygous normal chromosome complement. This occurs because three-fourths of the cells will contain duplications and deficiencies and will be nonviable. Of the remaining one-fourth of the cells, one-half will be homozygous for one translocation and one-fourth will be homozygous for both translocations. Consequently, one-fourth times one-fourth, or one-sixteenth of the cells, will contain the homozygous normal chromosome complement and theoretically will be the most viable. This corresponds to what was observed in this investigation (Table 5) if the progenies which segregated for normal and mutant plants (mutants 1, 2, 3, and 4) are considered to have arisen from homozygous tissue, as will be discussed later. Treatment of seedlings heterozygous for one translocation resulted in 4.3% mutants, approximately one-fourth of those in homozygous normal plants. The treated seedlings heterozygous for two translocations gave 2.2% mutants, approximately one-eighth of those in homozygous normal plants.

It would seem from the results obtained in this investigation that the homozygous translocation complement is not as viable as the homozygous normal complement. This differential viability may be the result of irradiation with gamma rays causing slight damage to the translocated chromosomes, such as small deletions or insignificant gene mutations. From an evolutionary standpoint, the normal chromosome complement is more viable. Those changes must be very slight, as no phenotypic differences between the three homozygous translocation lines and Experimental 3 were noted.

The mutated plants, mutants 1, 2, 3, and 4, (Figures I and II) which appeared in progenies with unmutated plants may have been produced from chimaeral sectors. Since seed from harvested panicles was bulked, the location of the sectors cannot be known. No obvious difference between tillers or the main culm was noted except that the main culm was sterile. The four mutant plants (mutants 1 and 2) from two segregating progenies of T165/+ treated in Experiment I are homozygous for the structural marker. It has not been determined if these plants are homozygous for the reciprocal translocation or homozygous for the normal chromosome structure. This could be determined by crossing the mutated plants to each parent.

The two progenies (T396/+) discussed in Experiment II (Figure II, A and B) contained 12 apparently identical mutants (mutants 3 and 4). The two parent plants which gave rise to these segregating progenies were adjacent as treated seedlings in the sand jars, were transplanted into the same pot in the greenhouse, and were later transplanted into the field next to one another. Therefore, the environment which gave rise to these mutant plants must have been almost identical. The heads which produced the mutant plants were in all probability produced on tillers; however, no difference between the tillers and the main culm were noted except that the main culm was sterile. The original treated plants or certain sectors of these plants were still heterozygous for the translocation, since their progenies segregated 1 fertile:1 semisterile.

The appearance of these apparently identical mutants in two progenies, which segregated for fertility, may be explained in several ways. These plants may have arisen from chimaeral sectors which mutated and were heterozygous for the translocation and, consequently, the progeny segregated for fertility. For this to occur would require a mutation to the dominant or else simultaneous mutations occurring at both homologues for a number of characters. Since a number of panicles were selfed and the seeds bulked, the mutant seeds and normal seeds became mixed, producing rows which then contained normal and mutant plants. It could be possible that the panicle which gave rise to the mutant plants was heterozygous not only for the translocation but also genotypically; that is, part of the panicle producing mutant plants and part of the panicle producing the original genotype. Another possibility is that somatic reduction occurred in part of the tissue which produced the panicle. Therefore, that part of the panicle had a homozygous mutant complement, and part of the panicle was heterozygous for the translocation and contained the original genotype. If the sector of the panicle which had undergone somatic reduction and mutation was crossed with the sector of the panicle which was heterozygous, then a 1:1 segregation for fertility may be expected in the mutants. The resulting progeny would then be genotypically heterozygous and the progenies from the mutants would be expected to segregate. This possibility will be tested in the field this summer by growing the progenies of the mutated plants. The F_2 progenies of mutant 3 crossed to mutant 4 and mutants 3 and 4

crossed to Experimental 3 will also be grown to determine whether the genotypes of the mutated plants are identical. This will be determined by observing the segregation of the F_2 progenies. It would appear rather improbable that these mutants arose from cross-pollination within a sector, since sorghum is normally self-fertilized and since the homozygous mutant plants have a high seed set.

The appearance of these two identical mutations produced from exactly the same environmental conditions, after colchicine treatment, demonstrates that under certain conditions it may be possible to produce a given type of mutation. The frequent occurrence of other identical mutants such as the grass mutants (Chen, 11) and the white-seeded mutants (the variety Winner) (Geise, unpublished) obtained after colchicine treatment of Experimental 3 presents further evidence of directed mutation. Franzke (unpublished) has recently obtained apparently identical mutant plants from colchicine treatment of diploid and tetraploid seedlings.

The restoration of fertility and homozygosity of the chromosomes in only the true-breeding mutant plants, and not in any of the unmutated plants, may indicate that the somatic reduction phenomenon is associated in some way with the mutational phenomenon. The type or kind of association is unknown. The appearance of progenies containing mutant plants which segregated for fertility and with the translocation in the heterozygous condition in half the plants may indicate that the mutation phenomenon may occur without the occurrence of somatic reduction. The identification of these mutants may

provide further evidence on this subject. If plants, with the original genotype and homozygosity for the normal or translocation chromosome complement, could be obtained from seedlings heterozygous for the translocation after colchicine treatment, this would provide incontrovertible evidence for the separation of these two phenomena. In the case of true-breeding mutants the mutational effect appears to be simultaneous upon many genes causing them to mutate prior to somatic reduction or in an effectively haploid stage, since it would seem improbable that identical mutations could simultaneously occur on both homologues after restoration to the diploid. A mechanism for obtaining identically mutated chromatids in the same chromosome must also be operative. It may be possible that in a colchicine swelling a mutation occurs and is followed by endomitosis resulting in a cell which is tetraploid. This cell may then undergo somatic reduction. In this way the cell would be homozygous for the mutation and be diploid.

The demonstration of the occurrence of a separation of members of four pairs of chromosomes followed by doubling to restore the original number, and to give homozygosity for all the genes located on these chromosomes in somatic tissue, has far-reaching implications. Since colchicine-induced mutants show homozygosity for a number of unlinked genes, as shown by Foster et al. (18, 19), it is probable that in such instances all members of chromosome pairs may be involved in this phenomenon.

It might be expected under certain circumstances within an organism that somatic reduction of chromosomes could occur under natural conditions. The process of evolution in plants may possibly occur more rapidly than previously thought possible, if a mutational effect followed by somatic reduction were to occur, and a new shoot were to form from a cell originating in this manner. If the natural occurrence of this phenomenon can be established, its implication on interpretations of evolutionary process in plants may force the acceptance of concepts of selection not only among individuals as at present but also among cell populations within individuals.

The application of somatic chromosome reduction to plant breeding when control of the occurrence of the phenomenon is determined for any particular species would enable immediate fixation of genotypes. The immediate recognition of desirable genotypes should be facilitated since they would be immediately homozygous. This application may perhaps prove to be a valuable tool in plant breeding. Professor C. J. Franzke, during the last 16 years, has applied this technique in the sorghum breeding program at the South Dakota Experimental Station.

The possible implications of somatic reduction in the origin of cancer cells has been discussed by Ross (58). The evolution of a cancer cell would seem to involve mutational effects which may not be observable as variations in chromosome number or structure. If somatic reduction were to occur in association with mutation, as may be indicated by this investigation, its effect during the initiation of

carcinogenesis might not be detected since immediate restoration to the diploid number could occur endomitotically. Therefore, its occurrence could provide an effective mechanism by which segregation of mutated genes would give rise to cells endowed with a predominance of genes for vigor so that rapid division could take place at the expense of normal body cells.

Explanation of the mechanism of somatic reduction and the definition of its role in growth and development can be expected to fill large gaps in the biological concepts concerning growth and development. The importance of chromosome reduction in somatic tissues in biological processes may perhaps be very great.

SUMMARY

Following the treatment of sorghum seedlings of the variety Experimental 3 with colchicine, diploid mutants arise that breed true in subsequent generations. The production of immediately true-breeding diploid mutants was found to be dependent upon the genotype as well as the presence of certain environmental conditions after colchicine application. To explain the homozygosity of the mutants, it was proposed that a reduction of chromosomes to a haploid condition has occurred followed by doubling to restore the diploid number. This hypothesis was tested by treating seedlings heterozygous for chromosome markers (one and two reciprocal translocations). The reciprocal translocation is a useful marker since it may be easily identified in the heterozygous condition. Plants heterozygous for reciprocal translocations may be recognized since one-half of the gametes contain duplications and deficiencies and are usually nonviable. Therefore, it is possible to identify translocation heterozygotes by pollen examinations since, except in unusual instances, 50% of the pollen and 50% of the ovules are nonviable. They may also be identified by the presence of rings or chains of four chromosomes at diakinesis and metaphase I. The progeny of plants heterozygous for a reciprocal translocation segregate 1 normal homozygote:2 translocation heterozygotes:1 translocation homozygote.

After treatment of 320 seedlings heterozygous for one chromosome marker (two chromosome pairs marked), four immediately true-breeding diploid mutants were observed among the 188 survivors.

Chromosomes of the four mutant plants were examined at diakinesis. Since no configuration of four chromosomes was observed in any of the mutant plants at diakinesis in the prophase of meiosis, the original mutants must have been homozygous for the two pairs of marked chromosomes. The mutant plants also had uniformly high seed set in comparison to the semisterility observed in the unmutated plants. To identify the structural chromosomes of the four mutants, they were backcrossed to the homozygous normal and homozygous translocation parents. Examination at diakinesis in each of the F_1 progenies showed a chain configuration when crossed to the translocation parent. Therefore, the two pairs of identifiable chromosomes in the four mutants are the same as the normal parent.

Four normal appearing plants were obtained after treatment whose progenies segregated for normal and mutated plants. This appears to indicate that the treated plants were chimaeras with mutated sectors. An explanation regarding the nature of the markers in these sectors is not at present possible.

After treatment of 124 seedlings heterozygous for two chromosome markers (four chromosome pairs marked), two fertile immediately true-breeding diploid mutants were observed among the 90 plants which survived. The chromosomes of these two mutants were examined at diakinesis and were structurally homozygous for the four pair of marked chromosomes. The two mutants were backcrossed as males to both homozygous translocation parents and also crossed to normal Experimental 3 to identify the structural chromosomes. Cytological examination of

the F_1 progenies of each mutant backcrossed to both homozygous translocation parents showed a chain of four chromosomes at diakinesis. When the two mutants were crossed to normal Experimental 3, no configurations of four chromosomes were evident. Therefore, the four pair of identifiable chromosomes in these mutants are the same as the normal parent.

Mutants with homozygous normal chromosome structure arising from colchicine treated seedlings heterozygous for two reciprocal translocations removes the possibility of selfing and androgenesis, since the mutants had a different structure than either parent.

After treatment of 76 seedlings of the variety Experimental 3, 49 plants survived, nine of which were mutants. Six of the mutants were immediately true-breeding, one segregated for a number of characters, one was male sterile and one was a haploid mutant.

The much higher proportion of mutants from plants with the original chromosome structure (18.4%) than from those heterozygous for one translocation (4.3%) and for two translocations (2.2%) may perhaps be explained in one of two ways. Irradiation used to obtain the translocations may have caused mutations affecting response to colchicine of such plants. Secondly, deficiencies and duplications that would be expected to occur in any reduction division would cause one-half the genomes to be nonviable in the case of one translocation, and three-fourths in the case of two translocations. Since no translocation homozygotes were found among the mutants, it would appear that these were not as viable and at a competitive disadvantage.

Therefore, the above proportions may be assumed to be reduced in the case of one translocation to one-quarter or 4.6% in the above instance and in the case of two translocations to one-sixteenth or 1.2%.

The restoration of fertility and homozygosity of the chromosomes in only the true-breeding mutant plants, and not in any of the unmutated plants, may indicate that the somatic reduction phenomenon is associated in some ways with the mutational phenomenon.

The presence of homozygous normal chromosomes identified in the mutants obtained after colchicine treatment constitutes incontrovertible evidence for the occurrence of somatic chromosome reduction followed by doubling of the chromosomes. This is true of at least the four marked chromosomes followed in this study. It would be expected that the whole chromosome complement would be involved in such a somatic reduction to explain the phenomenon of the appearance of true-breeding mutants after colchicine treatment involving many mutations, none of which have been found to be linked.

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