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Differential Reaction of Two Varieties of Sorghum to Colchicine Treatment

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DIFFERENTIAL REACTION OF TWO VARIETIES OF SORGHUM TO COLCHICINE TREATMENT

By

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This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and 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.

Thesis Advisor

Head of the Major Department

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INTRODUCTION

The induction of diploid true-breeding mutants by colchicine treatment of a true-breeding variety of Sorghum vulgare Pers. has been reported by Franzke and Ross (9); Ross, Franzke and Schuh (22); and others (7, 10). It has been suggested that such mutant plants might result from point mutations made homozygous by a somatic reduction of the chromosomes followed by a doubling of the chromosome number in a cell which organized a growing point after the original one had been inactivated during c- tumor formation. Observations made during the course of routine work with sorghum indicated that after colchicine treatment some varieties produced tetraploids with few or no gene mutations. The present studies were undertaken during the summer of 1955 and the winter of 1955-56, to demonstrate differences between varieties of sorghum in their reaction to colchicine treatment and to investigate the bases for such differences.

The material used in this study consisted of Experimental 3, an unreleased variety, and Norghum, a released variety, both produced by the South Dakota State College Agricultural Experiment Station, and two translocation stocks obtained from the University of Nebraska.

REVIEW OF LITERATURE

Franzke and Ross (9) reported that treatment of sorghum seedlings with 0.5% colchicine in lanolin induced variants in the true-breeding variety Experimental 3. These variant plants possessed a number of characters which resembled Sudan grass, one of the ancestors of the variety. Some of these variants bred true immediately, suggesting that homozygosity had been induced, while others segregated. The induction of

homozygosity by colchicine treatment was given further support by the results of treatment of F_1 sorghum seedlings. The progenies from treated F_1 plants were significantly more uniform than progenies from untreated F_1 plants. Cytological examination of meiosis in untreated Experimental 3 and in the variants gave no indication of irregularities, nor were any chromosome numbers other than the normal diploid number found. Reductional groupings, as observed by Huskins (11), were found in root tips treated for one hour in a 0.5% aqueous solution of colchicine. They stated:

> "Though the numbers of chromosomes were not necessarily equal on either side, the formation of telophase nuclei proceeded...with the probable subsequent formation of homozygous nuclei. The splitting of the C-chromosomes after the formation of haploid cells would restore the diploid number, perhaps resulting in viable combinations of chromosomes from different ancestors which conceivably could, if the proper combination of chromosomes were present, form tissues... Since sorghum is probably of polyploid origin, it would not be illogical to explain the occurrence of these lines by assuming a concentration of chromosomes containing blocks of genes of Sudan grass in one cell after somatic reduction, such that the inhibition of ancestral characters was no longer exercised... The occurrence of a fortuitous combination of chromosomes could give an advantage to such a cell over those surrounding it, and result in the growth of homozygous tissue from which a new growing point could emerge."

Studies of characters of agronomic importance made on progenies of treated and untreated plants of two varieties of sorghum were reported by Ross, Franke and Schuh (22). In one variety, Experimental 3, plants from three of seven treated seedlings were quite different from untreated full sibs while the remaining four closely resembled untreated plants. Progenies of the variant plants differed significantly from the progenies of untreated plants. The progenies of two of the variant plants were uniform and bred true for four generations, the progeny of the other variant appeared to be segregating. During the same period, progenies of

untreated plants showed no segregation. A very unusual variant was obtained by retreatment of a variant obtained by colchicine treatment of the variety Experimental 1. This variant, which was brachytic with a cylindrical head, thus prompting the name "rat-tail", was unlike any known ancestor of the variety. The authors also noted that treatment of the variety Norghum gave little perceptible differences between progenies. It was found later that Norghum sometimes gave tetraploids after colchicine treatment (Ross and Schuh, unpublished).

The nature of chromatin changes after colchicine treatment was reported by Harpstead, Ross and Franzke (10) in a cytological study of meiosis in the variant plants, their untreated full sibs, and the F_1 hybrids between treated and untreated plants. No chromosomal irregularities of any kind could be detected. The absence in the variants of any association between chromosome pairs, and the regularity of pairing in the F_1 hybrids made it unlikely that any large segments of chromatin had been duplicated or that any other gross rearrangement of the chromatin had been induced by the colchicine treatment. Observations of the F_1 hybrid seedlings indicated that the green coleoptile color of two treated lines was recessive to the red color of the untreated plants of Experimental 3. The F_1 plants were extremely vigorous. Cytoplasmic inheritance was not considered probable since characteristics of the variants occurred in the F_1 when untreated plants were used as female parents. It was concluded that the changes in the chromatin must have been of the nature of multiple point mutations.

A genetic study of F_2 populations of crosses between mutant and untreated plants was made by Foster, (7). He confirmed the previous

observation that green seedling color of the mutant plants was recessive to the red color of untreated plants. He also found that presence of awns, which occurred in mutant plants, was recessive to absence of awns. Seedling color and awns were found to be simply inherited and not linked. Correlations between various quantitative characters and these two qualitative characters gave no evidence of linkage. In one cross, involving the "rat-tail" mutant, correlations between mutant plant characters gave significant values in all cases which could mean that all the mutant characters were due to a mutation at a single locus or in a restricted region of one chromosome. Progenies of two of the mutant plants studied appeared to be heterozygous for several characters indicating that colchicine may cause mutations without inducing homozygous diploidy. The author concluded that colchicine may cause mutations at a large number of loci on different chromosomes.

The results of colchicine treatment of F_1 seedlings of flax has been reported by Dirks, Ross and Harpstead (6). A plant was obtained which had branches differing in the color of flowers and seed produced. Progenies of each of these branches bred true, the branches falling into two groups suggesting that the plant had been a chimera of two sectors, each of which bred true. Anomalous segregation in the progenies of other treated F_1 plants was explained by supposing chimeral sectors within single branches. Evidence of both dominant and recessive mutations was obtained. It was concluded that the colchicine treatment caused either somatic reduction with subsequent doubling of the chromosomes or point mutations or both.

Corn stocks containing heterozygous translocations were treated

with colchicine and examined by Roes (unpublished). Among nine, treated, heterozygous, F_1 plants, three were found to be homozygous for the translocation; one of these was homozygous for the chromosomes from the male parent. One plant had two tillers, one of which was heterozygous, the other homozygous. Fifteen untreated F_1 plants examined were all found to be heterozygous. These results seem to confirm the hypothesis that colchicine causes somatic reduction of the chromosomes but it has been impossible, so far, to repeat these results.

Villax and Nota (31) have reported what they consider to be a case of somatic reduction in a Triticum-Secale hybrid. In an attempt to double the chromosome number in a plant from a cross of winter wheat and rye, colchicine was injected into the culms. An untreated early tiller was sterile and appeared to be hybrid. The treated culms developed o- tumors and died. Adjacent untreated tillers showed reduced growth and gave fertile heads. Plants grown from these appeared identical to the winter wheat parent of the hybrid. The plants had 42 chromosomes; the rye chromosomes had apparently been lost as the characteristic knobs were not observed. The authors stated: "...we are led to conclude that the derived wheat plant is completely homozygous since it has resulted from the duplication of a gametic set of chromosomes".

Porter and Weiss (21) reported the occurrence of a dwarf strain in soybeans which arose in the progeny of a colchicine-treated seedling. The dwarf was found to contain the normal diploid complement of chromosomes. The dwarf character was conditioned by a single recessive factor.

Other reports of the production of gene mutations by colchicine have not been found in the literature. However, Levan and Ostergren (17)

state:

> "Anyone who has worked for some time with colchicine treatments on different plant materials has undoubtedly observed that, besides polyploids which appear, also aberrant types with unchanged chromosome number emerge."

The authors considered these aberrant types to be dauermodifications. Discussing the use of colchicine to induce polyploidy, Becker and Skiebe (2) caution against the use of high concentrations which have been found, they state, to yield diploid adventitious shoots.

The first report of reductional groupings in somatic tissue was by Huskins (11). In Allium cepa root tips treated with 1-4% sodium nucleate, chromosome segregation and/or reduction of the chromosome number was induced. Segregation of long prophase-like chromosomes was found to be the most frequent type. Reductional groupings were also found in the root tips of onion bulbs that were flaccid after several months storage. Huskins and Cheng (12) found reductional groupings in onion roots grown at low temperatures. Separation of chromosomes into two groups was more frequent in prophase than at later stages.

In root tips of Trillium spp. treated with sodium nucleate, Wilson and Cheng (28) found that separation of chromosomes into two numerically equal groups and segregation of homologous chromosomes occurred with much greater than random frequency.

Patau (20) measured chromosome lengths within groups and distances between groups in cells showing reductional groupings in Rhoeo and Allium. He found that, in cells with reductional groupings at prophase, above-random length-agreement between groups was not correlated with distance between groups. At metaphase and anaphase, however, there was a highly

significant positive correlation between these two measurements. It was concluded that the reductional groupings originated at prophase as random irregularities and, later, only those were kept apart which had a high degree of homologous segregation. Reduced nuclei derived from reductional groupings may, therefore, be expected to have a more or less complete genome.

From tetraploid plants of Rhoeo discolor, Huskins and Chouinard (13) obtained 168 tetraploid, 13 triploid and 6 diploid main roots and one diploid shoot. Many roots which were predominately tetraploid had patches of triploid and diploid cells. Somatic reduction of chromosomes was studied in tetraploid and triploid roots. Groupings of 12:12 and 6:18 occurred in the tetraploid and of 9:9 and 6:12 in the triploid with greater than random frequency. The excess of equal distributions might have been explained by mere mechanical causes but some type of repulsion between homologues and/or selective survival was suggested by the excess of 6:18 and 6:12 groupings.

Sharma and Sen (25) grew onion bulbs in solutions of nucleic acid. They found some tetraploid cells in the root tips and reductional groupings in both diploid and tetraploid cells. Cells were observed with micronuclei and tripolar divisions and certain cells had two large telophase nuclei.

Distribution of chromosomes into groups after colchicine treatment was noted by Levan and Lofty (16). They called this "distributive mitosis." They also noted that these groups of chromosomes often formed separate nuclei within one cell. It was noted that the most embryonic cells of the root tip were less affected by c-mitotic substances than

were more specialized cells.

Comparisons of the effects of colchicine and sodium nucleate on Tradescantia and Allium were made by Allen, Wilson and Powell (1). They found that reductional groupings were induced in frequencies of 2-35% by sodium nucleate and 6-20% by colchicine. After colchicine treatment, there was found no clear-cut case of prophase separation similar to that observed after treatment with sodium nucleate.

Variations in mitosis due to treatment with gama-cyclochlorohexane and colchicine have been reported by Wilson, Tsou and Hyypio (29). Cells with micronuclei, and binucleate and tetranucleate cells were observed. The multinucleate cells, it was concluded, were due either to a split anaphase or to "segregational grouping" (reductional grouping). Chromosome reduction is possible by either of these mechanisms.

The presence of "exploded c-mitosis" in colchicine-treated root tips of Commelina communis L. has been reported by Berger, LaFleur and Withus (3). The chromosomes were found to be segregated into two or more groups. Separation into two groups was followed by formation of two nuclei within one cell. In the recovery period, the two nuclei within one cell were observed to divide simultaneously. Two new cell walls were formed so that a binucleate cell and two uninucleate cells resulted from the division.

In a number of colchicine-induced tetraploid bushes of Ribes nigrum and their c_2 progenies raised from seed, Vaarama (30) found that the chromosome number of somatic cells varied from 4 to 32. The frequencies of different somatic chromosome numbers formed a binomial distribution with the highest frequency at the diploid number, 16. However all

numbers divisible by four were more frequent than would be expected due to random distribution. The author concluded that four was the basic chromosome number in Ribes. In a c_2 progeny from a tetraploid plant three diploids were found among 139 plants studied. The author stated that the reduction of chromosome number was due to the division of the spindle into two parts which acted independently. The excess of chromosome numbers divisible by four was thought to be due to selective survival of the balanced genomes.

Variations in chromosome numbers at meiosis in amphidiploids involving Triticum, Aegilops and Agropyron were reported by Sachs (23). In some amphidiploid plants, anthers were found which contained, besides cells having the expected chromosome number, others with reduced chromosome numbers. In one instance, the proportion of such reduced cells reached 14.5%. It was found that the presence of chromosome mosaics depended on the parent species of the amphidiploid and that the tendency to form mosaics persisted to later generations. Cells with reduced chromosome numbers functioned as gametes and their progeny could again produce chromosome mosaics. Reduced chromosome numbers were not found in the root tips. It was concluded that the reduced cells represented an almost random assortment of chromosomes. Mosaics were thought to arise by gene-controlled spindle abnormalities just before meiosis.

Nuclei with reduced chromosome numbers were found in "plasmodial" (multinucleate) pollen mother cells of Helianthemum by Snoad (26). The author concluded that the plasmodia were due to split spindles in several premeiotic divisions associated with a failure of cell wall formation.

The fact that normal pairing took place in the plasmodia nuclei indicated some regularity in the chromosome reduction process.

Chromosome counts in the root tips of Hymenocallis calathium were found by Snoad (27) to give numbers ranging from 23 to 83 with the higher numbers most frequent. Colchicine treatment gave shortened chromosomes often segregated into two or three groups. Spindle abnormalities were found in untreated material, some cells having two distinct spindles. Micronuclei were also observed. In pollen mother cells chromosome numbers ranged from 69 to 86. The low number of univalents observed at meiosis was evidence that there was some regularity in the reduction of chromosome number.

Somatic reduction of chromosome number in cotton was reported by Brown (5). In an abnormal plant of Gossypium hirsutum with approximately 100 chromosomes, produced by pollinating cotton with okra pollen, a sector was obtained with a different growth habit and 51 chromosomes.

Mensel and Brown (19) reported the occurrence of color mosaics in the petals and leaves in polygenomic hybrid cotton plants. From the nature of the mosaics it was concluded that they could be the result of some form of segregation during somatic mitosis. From a polygenomic hexaploid plant having 78 chromosomes a bud was obtained with 39 chromosomes. Meiotic analysis suggested a deficiency of chromosomes of one of the genomes. Some sort of atypical mitosis was indicated as the simplest cytological basis; it would explain the observed mosaics as well as the reduction of chromosome number.

In a three-species-hexaploid cotton hybrid with 78 chromosomes, Mensel (18) has reported the occurrence of two branches with 69 chromo-

somes. Analysis of meiotic pairing indicated a preferential loss of one genome. It was suggested that in chromosomally unbalanced plants, somatic reduction may provide a mechanism whereby the plant can eliminate incompatible chromosomes and so convert to more viable or more fertile forms.

The somatic chromosomes of sorghum have been studied by Huskins and Smith (14).. They noted one pair of chromosomes which could be recognized in all the species studied. It was described as having "... one long portion with a prominent sub-terminal attachment constriction, and a shorter portion which is connected to the longer one by only a fine thread of chromatin." In their figures this pair generally appears longer than the other chromosomes.

Counts of the number of heterochromatic bodies in energic cells of Rhoeo roots were used by Huskins and Steinitz (15) as an indirect method determining chromosome number. From results of their own work and a review of previously reported investigations by other workers, they concluded that, while the number of heterochromatic bodies in a cell did not necessarily equal the number of chromosomes, the number of heterochromatic bodies was proportional to the number of chromosomes. Counts of the heterochromatic bodies of energic cells might therefore be used to estimate their ploidy.

In studies of Nicotiana, Bradley (4) found that the sizes of nuclei at prophase and telophase fell into distinct groups according to chromosome number. Nuclear sizes of energic cells also fell into three relatively distinct classes. She concluded that nuclear size could be useful in estimating the approximate percentages of energic cells of different degrees of polyploidy in populations of cells of a single histological type.

Sizes of nuclei and of cells were used by Satina, Blakeslee and Avery (24) as an indication of polyploidy in different cell layers of periclinal chimeras in Datura.

It was suggested by R. E. Duncan, University of Wisconsin (private communication) that nuclear volume might be used as an estimate of the degree of polyploidy in the c-tumors.

MATERIAL AND METHODS

Plant Material

Two varieties of sorghum, Experimental 3 and Norghum, were used in the main portion of this study. Both are grain sorghums which were produced at the South Dakota State College Agricultural Experiment Station by C. J. Franzke.

Experimental 3 resulted from crosses made in 1932 of Day, a late-maturing dwarf grain sorghum, with Black Amber Cane, a forage variety, and with Sudan grass, Sorghum sudanense (Piper) Stapf. An early, dwarf, grain sorghum line resembling Day was selected from each of these crosses. These were crossed in 1939 and from the progeny, by selfing and selection, the variety Experimental 3 was produced. Seed of Experimental 3 used in this study came from a stock that had been self-fertilized for more than ten generations (9).

Norghum is a selection from the cross (Dwarf Feterita x Dwarf Freed) x Yellow Kafir made in 1939. The parents were obtained from the Branch Experiment Station, Hays, Kansas (8).

Two lines with homozygous translocations were obtained from O. J. Webster, University of Nebraska, Lincoln. The lines were SS 110 from

the variety Sooner and SS 1186 from the variety Cody. Both are dwarf grain sorghums.

Procedure

Method of treating seedlings

For the treatment of seedlings a mixture of 0.5% colchicine in lanolin was used. Seeds were treated with Arasan and germinated on blotters in Petri plates. When the coleoptile was about 2-3 mm. in length a drop of melted colchicine-lanolin was applied. Care was taken to be sure that the coleoptile was completely covered and that the colchicine-lanolin mixture did not come in contact with the roots. Treated seedlings were placed on moist sand in a glased crock covered with a sheet of glass and placed in the greenhouse at a temperature of approximately 80° F. The seedlings were left in the sand until active growth started when they were transferred to soil. Generally, seedlings remained in the sand for 7 to 9 days.

Studies of plants from treated seedlings

As observations indicated that sorghum varieties differed in their reaction to colchicine treatment, some produced diploid mutants while others produced tetraploids, an experiment was undertaken to demonstrate and study such differences.

In the spring of 1955, sixty seedlings of each of the sorghum varieties Experimental 3 and Norghum were treated with colchicine. Surviving plants were transplanted to the field with untreated checks of each variety. Heads were taken from both treated and untreated material and fixed in 3:1 alcohol-acetic acid for chromosome counts. Chromosome counts were made on aceto-carmine smears of the pollen mother cells.

Notes were taken on plant height, number of tillers, presence or absence of awns and aberrant plant characters. Treated and untreated plants were selfed and the seed planted in the field in 1956 to determine breeding behavior.

Studies of treated root tips

The difference in response of the varieties Experimental 3 and Norghum to colchicine treatment in the seedling stage might be due to different reactions of the chromosomes of the two varieties to colchicine. To search for such differences, untreated and colchicine-treated root tips of the two varieties were examined cytologically.

Seeds of the two varieties were germinated on blotters in Petri dishes and the roots cut off when they were about one centimeter long. Half the roots were placed in a 0.5% aqueous solution of colchicine and half were placed in tap water as a check. Three lots were treated, two for one hour and one for two hours. After treatment, the roots were fixed in hot (60° C.) 3:1 alcohol-acetic acid for 15 minutes. They were rinsed in water and stained with Feulgen stain. The root tips were then squashed in aceto-carmine and examined cytologically. Metaphases were recorded as being normal or showing reductional groupings. Where reductional groupings were found the number of chromosomes in each group was recorded.

Studies of c-tumors

Anatomical studies of colchicine-induced tumors of sorghum seedlings were undertaken with two objectives: (1) to study the development of the tumor and events leading to the origin of the new growth; (2) to search for differences between tumors of Experimental 3 and Norghum

which might account for their different reactions to colchicine treatment.

Germinating seedlings of Experimental 3 and Norghum were treated with colchicine in the manner described above. At the time of treatment and each subsequent day for 13 days, four randomly selected seedlings of each variety were scored for development, photographed and fixed in Craf solution. Two seedlings of each variety for each day were selected which were most representative of that day's collection. These were embedded in paraffin, and sectioned with a microtome. The sections were stained with Feulgen stain and counterstained with Fast Green in 95% alcohol. Counts of the heterochromatic areas in nuclei of the apical meristems of the tumors were made. The diameters of nuclei in the apical meristems of the tumors were measured with an optical micrometer and the volumes of the nuclei calculated by treating them as spheres or as ellipsoids.

At the time tumors were being collected for sectioning, twenty-four treated seedlings of each variety were observed and notes were taken on tumor development with the intention of trying to correlate tumor development with the occurrence of mutant characters in plants from the treated seedlings.

Studies of translocation material

Somatic reduction of the chromosomes would be indicated if colchicine treatment of seedlings heterozygous for marked chromosomes gave shoots which were homozygous for the marked or the normal chromosomes. The translocation stocks SS 110 and SS 1186 were crossed with Experimental 3 and P20, a colchicine-induced variant from Experimental 3, in the greenhouse using the translocation stocks as the male parent. F_1 seedlings

from these crosses were treated with colchicine and transplanted to the field together with untreated F_1 plants. Heads were collected for cytological examination and fixed in 3:1 alcohol-acetic acid. Aceto-carmine smears of the pollen mother cells were made and examined to determine whether or not the meiotic configurations expected in translocation heterozygotes were present.

EXPERIMENTAL RESULTS

Studies of Plants from Treated Seedlings

Of the sixty seedlings of each variety treated in the spring of 1955, fifty-two Experimental 3 and fifty-four Norghum survived and were transplanted to the field, also thirty-five untreated plants of each variety were planted for comparison. Forty-three treated Experimental 3 and fifty-four treated Norghum plants survived to maturity and were studied. Photographs of untreated Experimental 3, treated Experimental 3, untreated Norghum, and treated Norghum are shown in Figures 1, 2, 3, and 4, respectively. The variability of the treated Experimental 3 plants as compared to the other groups is clearly evident.

All chromosome counts were made at meiosis of the pollen mother cells. Thirty treated Experimental 3 plants examined were all found to be diploid. Three treated Norghum plants, out of thirty examined, proved to be tetraploid. Ten untreated Experimental 3 and fifteen untreated Norghum plants were examined; all were found to be diploid. Although a detailed search for irregularities at meiosis was not made, none were noted in any of the figures examined while chromosome counts were being made.

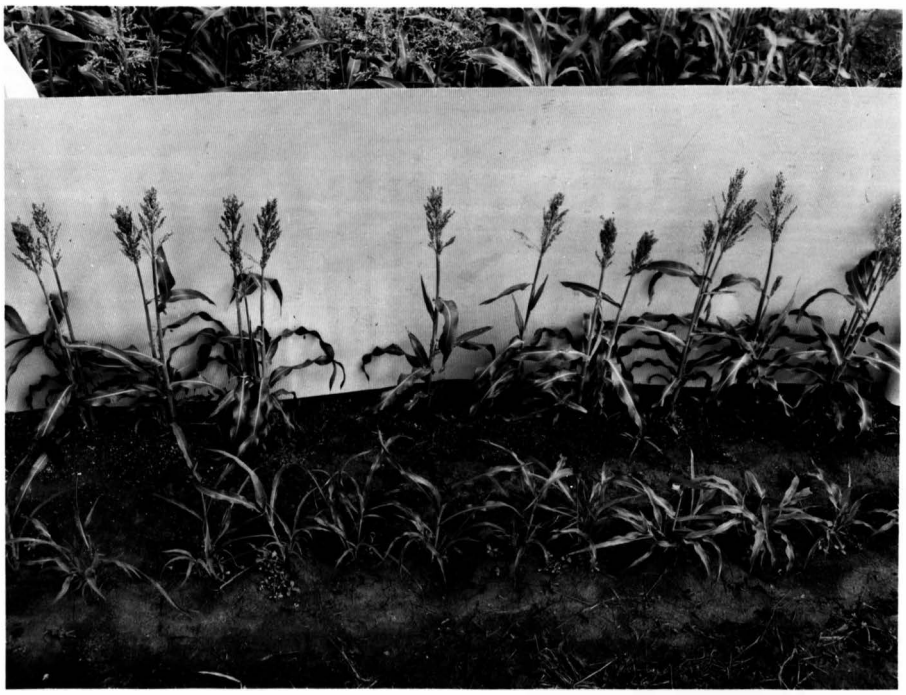

Figure 1. Untreated Experimental 3. Note uniformity of plant characters.

Figure 2. Colchicine-treated Experimental 3 (background) showing the variability induced by treatment.

Figure 3. Untreated Norghum.

Figure 4. Colchicine-treated Norgham.

Data on presence or absence of awns, height and number of tillers of untreated and treated Experimental 3 and Norghum are summarized in Table 1. The fact that the t test reveals no significant difference between untreated and treated Experimental 3 for height and number of tillers, despite the great apparent differences, is due to the extremely high variance of the treated plants. The significant differences between the means of untreated and treated Norghum is undoubtedly due to the adverse effects of colchicine on the treated plants in the seedling stage; the untreated plants were taller and had more tillers than the treated plants. The F test shows a highly significant difference between the variances of untreated and treated Experimental 3 but no significant difference in variability between the two groups of Norghum plants.

Among the treated Experimental 3, a number of plants could be distinguished by their resemblance to forage sorghums. These plants, which were distinguished by having slender culms with narrow leaves, many tillers and a blue-green color, were designated as "forage type." Other plants, distinguished by their tall sturdy culms, broad leaves and dark green color, were designated "vigorous type." The remainder of the plants resembled untreated Experimental 3 in general plant type, though some plants were aberrant for minor characters. The number of plants, number of awned plants, plant height and number of tillers of each of these classes of treated Experimental 3 plants and of untreated Experimental 3 are given in Table 2. Statistical comparisons, made between untreated Experimental 3 and each of the three classes of treated plants, of the means and variances of height and number of tillers are shown in Tables 3 and 4.

Table 1. Number of awned plants, height and number of tillers of untreated and colchicine-treated plants of Experimental 3 and Norghum.

	No. of plants	No. of awned plants	Height in inches			Number of tillers		
			Mean	Range	Variance	Mean	Range	Variance
Experimental 3								
Untreated	26	0	24.6	20-38	24.63	2.5	1-4	1.06
Treated	43	14	37.5	21-57	81.30	7.5	0-25	63.83
t test			1.60			1.51		
F test					3.30**			60.22**
Norghum								
Untreated	34	34	32.5	26-40	13.65	5.1	3-9	1.88
Treated	54	54	29.1	22-36	13.26	3.8	1-8	3.59
t test			4.28**			3.21**		
F test					0.97			1.91

**significant at the 1% point

Table 2. Number of awned plants, height and number of tillers of untreated Experimental 3 and of the three classes of plants of treated Experimental 3.

	No. of plants	No. of awned plants	Height in inches			Number of tillers		
			Mean	Range	Variance	Mean	Range	Variance
Untreated Experimental 3	26	0	24.6	20-38	24.63	2.5	1-4	1.06
Treated Experimental 3								
Normal-type plants	29	2	32.8	21-44	33.08	3.0	0-7	2.90
Forage-type plants	11	11	46.4	34-56	36.06	20.4	14-25	14.06
Vigorous-type plants	3	1	50.7	45-57	36.34	3.7	2-5	2.34

Table 3. t values between means of untreated Experimental 3 and of the three classes of plants of treated Experimental 3 for the characters height and number of tillers.

	Untreated Experimental 3 vs.		
	Normal type	Forage type	Vigorous type
Height	1.20	8.05**	6.36**
Number of tillers	1.41	22.83**	1.61

**significant at the 1% point

The t values between the means of untreated Experimental 3 and of the three classes of treated plants for the characters plant height and number of tillers are shown in Table 3. Differences between the normal type and untreated Experimental 3 are not significant for either character; the difference between the forage type and the untreated are highly significant for both characters while the vigorous type and the untreated are highly significantly different for plant height but not significantly different for number of tillers.

The F values calculated for the same characters between untreated Experimental 3 and each of the three classes of treated plants are shown in Table 4. The classes of treated plants are no more variable than untreated Experimental 3 for plant height but the normal type was significantly more variable and the forage type highly significantly more variable than untreated Experimental 3 for number of tillers.

Although the treated Experimental 3 plants could be conveniently grouped according to plant type, the F test indicates that these groups were not completely uniform. In addition to the variability in number of tillers, other differences within the groups were noted. In the normal type, two plants were awned and twenty-eight were awnless (Table 2);

Table 4. F values between variances of untreated Experimental 3 and of the three classes of plants of treated Experimental 3 for the characters height and number of tillers.

	Untreated Experimental 3 vs.		
	Normal type	Forage type	Vigorous type
Height	1.34	1.46	1.47
Number of tillers	2.74*	13.26**	2.20

* significant at the 5% point
**significant at the 1% point

one plant had pubescent glumes in contrast to the glabrous glumes of the untreated; two plants appeared to be dwarfs. In the forage type, two plants had more compact heads than was typical of the group. One plant of the vigorous type was awned while the other two were awnless (Table 2).

Among the treated Experimental 3 plants, seventeen were distinct mutants. No distinct mutants could be distinguished among the treated Norghum plants.

Although the progenies of the treated plants are not sufficiently mature at the time of writing for a detailed study, the progenies of forage type plants appear to be uniform for the mutant leaf type.

Studies of Treated Root Tips

It was found that chromosomes of sorghum root tips were difficult to stain satisfactorily. Although the procedure adopted was chosen after trying a variety of different fixatives and stains, it did not prove completely satisfactory, and a number of slides had to be discarded.

In the untreated material, cells in division were very infrequent. It seems likely that this was due partly to a low frequency of divisions in the root tips and partly to a failure of cells to begin division after

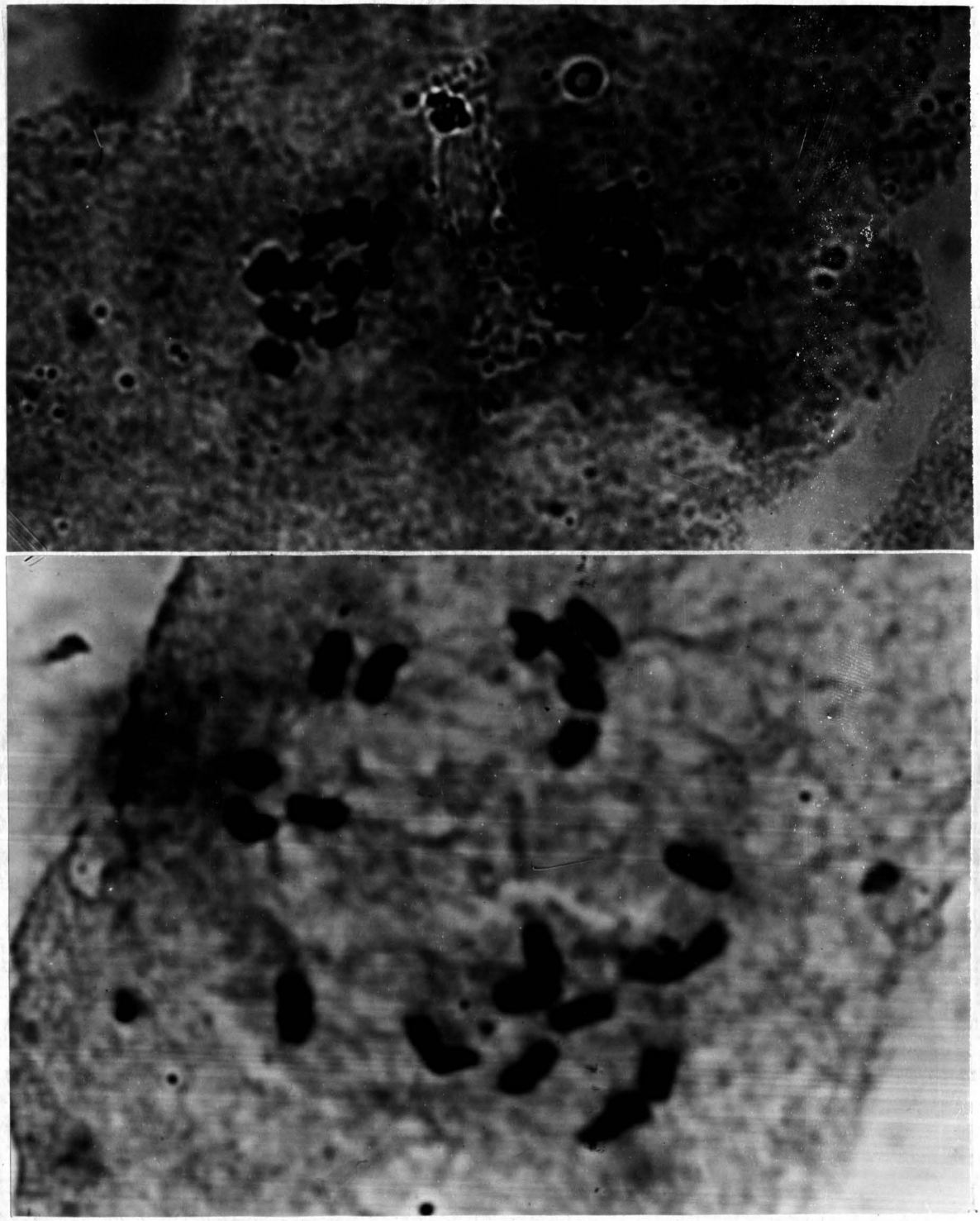

Figure 5. Reductional groupings of chromosomes from colchicine-treated root tips of sorghum. Upper: Experimental 3. Lower: Norghum. The differences shown were not typical of the varieties. (both x2400)

the root tips were placed in water, while cells which had begun to divide completed division during the period of the treatment. The larger number of divisions in colchicine-treated material was no doubt due to stalling of division at metaphase, the expected action of colchicine.

Table 5. Frequency of reductional groupings in untreated and colchicine-treated root tips of Experimental 3 and Norghum.

	No. of roots	Total No. of divisions	Number of reductional groupings	Percent reductional groupings
Experimental 3				
Untreated	24	128	2	1.6
Treated	29	273	25	8.4
Norghum				
Untreated	21	78	0	0
Treated	23	189	15	7.9

Reductional groupings, typical examples of which are shown in Figure 5, were found in the treated roots of both varieties. The double nature of the c-chromosomes is evident. Table 5 shows the number of cells with reductional groupings in relation to the total number of divisions. The slight difference in frequency of reductional groupings between Experimental 3 and Norghum does not appear to be great enough to be significant. Great variations in the number of reductional groupings between root tips were found in both the varieties. In both treated Experimental 3 and Norghum, five of the reductional groupings found occurred in one root.

In most of the cells with reductional groupings, there were two

groups of chromosomes, but in a number of cases, there were more than two distinct groups; these are included in the class "multiple groups" in Table 6. Where only two groups were present, there was considerable variation in the exactness of the reduction. Reductional groupings where there were two groups were therefore subdivided according to the number of chromosomes in each group.

Table 6. Distribution of types of reductional groupings in untreated and colchicine-treated root tips of Experimental 3 and Norghum.

	Type of Reductional Grouping								Total
	Multiple groups	Two groups							
		4:16	5:15	6:14	7:13	8:12	9:11	10:10	
Experimental 3									
Untreated				1	1				2
Treated	4	1		1	2	6	4	7	25
Norghum									
Untreated									0
Treated	2	1	1	1	2	3	2	3	15

In both the treated Experimental 3 and the treated Norghum, there appears to be a concentration of reductional groupings in the classes which are equational or nearly so (10:10, 9:11, and 8:12 classes).

In the majority of cases it was found impossible to distinguish any of the homologous chromosomes, even the long pair called the A-pair by Huskins and Smith (14). In one reductional division of the 10:10 class in a treated Norghum root tip, the members of the A-pair could both be distinguished; they were both in the same group. Since, except for this

one case, homologous chromosomes could not be identified, no estimate of the degree of homologous segregation in the reductional groupings could be made.

Studies of c-tumors

When the tumors were being collected, it was noted that root development was more rapid and more extensive in Norghum than in Experimental 3. Also, there was considerable variation in tumor development among the tumors collected on any day after the beginning of treatment.

Sections of untreated seedlings of Experimental 3 and Norghum are shown in Figure 6, and sections of colchicine-induced tumors of the two varieties are shown in Figures 7 to 13. Insets on the photographs of the sections show the untreated seedlings and the tumors before fixation. From examinations of the sections, it appears that the tumor was due primarily to an increase in cell size in the coleoptile and young leaves rather than to an increase in the number of cells. The increase in cell size was greater in the differentiated cells than in the meristematic cells of the growing point. A clear illustration of this is seen in Figure 9. In the sections of tumors from the first two days after the beginning of treatment, many c-mitotic nuclei were observed; these appeared as dense masses of chromatin in which the individual chromosomes could not be distinguished. In a few cells, two such masses of chromatin could be seen; these may have resulted from reductional groupings but counts of the chromosomes were not possible. After the third day of treatment, c-mitotic nuclei were very rare. Many multinucleate cells were observed in the differentiated tissues of tumors from the second day of treatment and later; typical multinucleate cells from c-tumors of

Experimental 3 are shown in Figure 14. In Figure 14 C, dark staining bodies which appear to be nucleoli can be distinguished in four of the nuclei. Careful examination of the apical meristems revealed very few multinucleate cells in this region, only seven were found in thirty-three tumors examined.

Cells with distinct heterochromatic areas in the nuclei were present in the apical meristems of many of the tumors. This suggested that counts of these might indicate the number of chromosomes within the nuclei. However, the very small size of these bodies made counting very difficult. As it was found impossible to make reproducible counts in many of the nuclei, this method was abandoned.

The distributions of nuclear volumes in the apical meristems of untreated and colchicine-treated seedlings of Experimental 3 and Norghum are shown in Figures 15 to 19. Because of the differences in the numbers of nuclei in the apical meristems of different tumors, the frequencies of nuclei of different size classes is shown as percent of the total number of nuclei measured within the one meristem. Each curve represents the distribution of nuclear volumes in the apical meristem of a single tumor. The number of nuclei upon which each curve is based is indicated on each graph. The great variations between nuclear volumes in tumors collected on the same day and the great differences between successive days indicates that, under the conditions of treatment, development of the tumors was not uniform. Comparisons of the range of nuclear volume of Experimental 3 and of Norghum from the same day of treatment showed that the range in Experimental 3 was greater than that in Norghum in almost every case. In a small number of cases the distribution curves

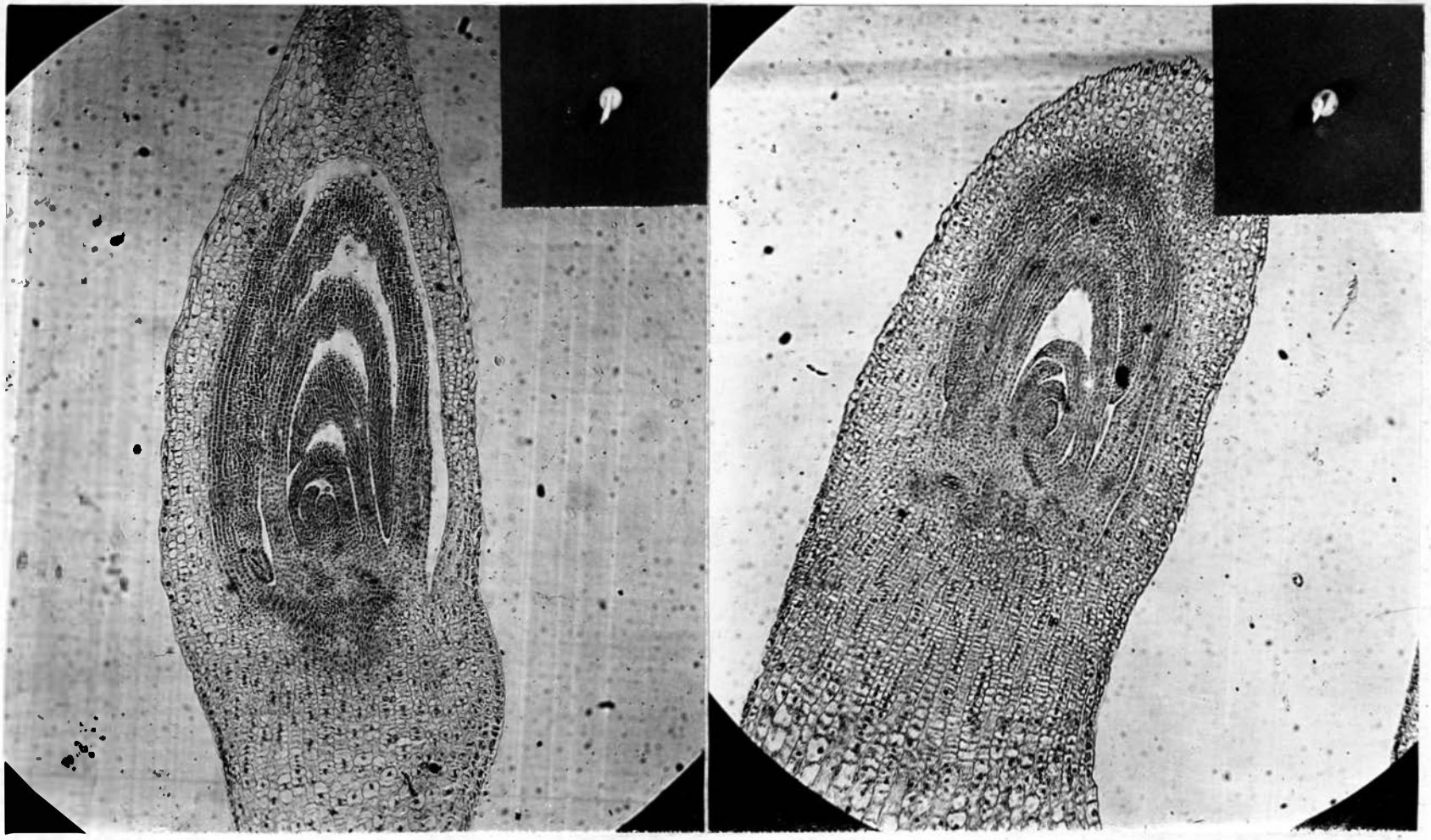

Figure 6. Sections of sorghum seedlings at the time of application of colchicine. Insets show the seedlings before fixation. Left: Experimental 3. Right: Norghum. (sections x76, seedlings x1)

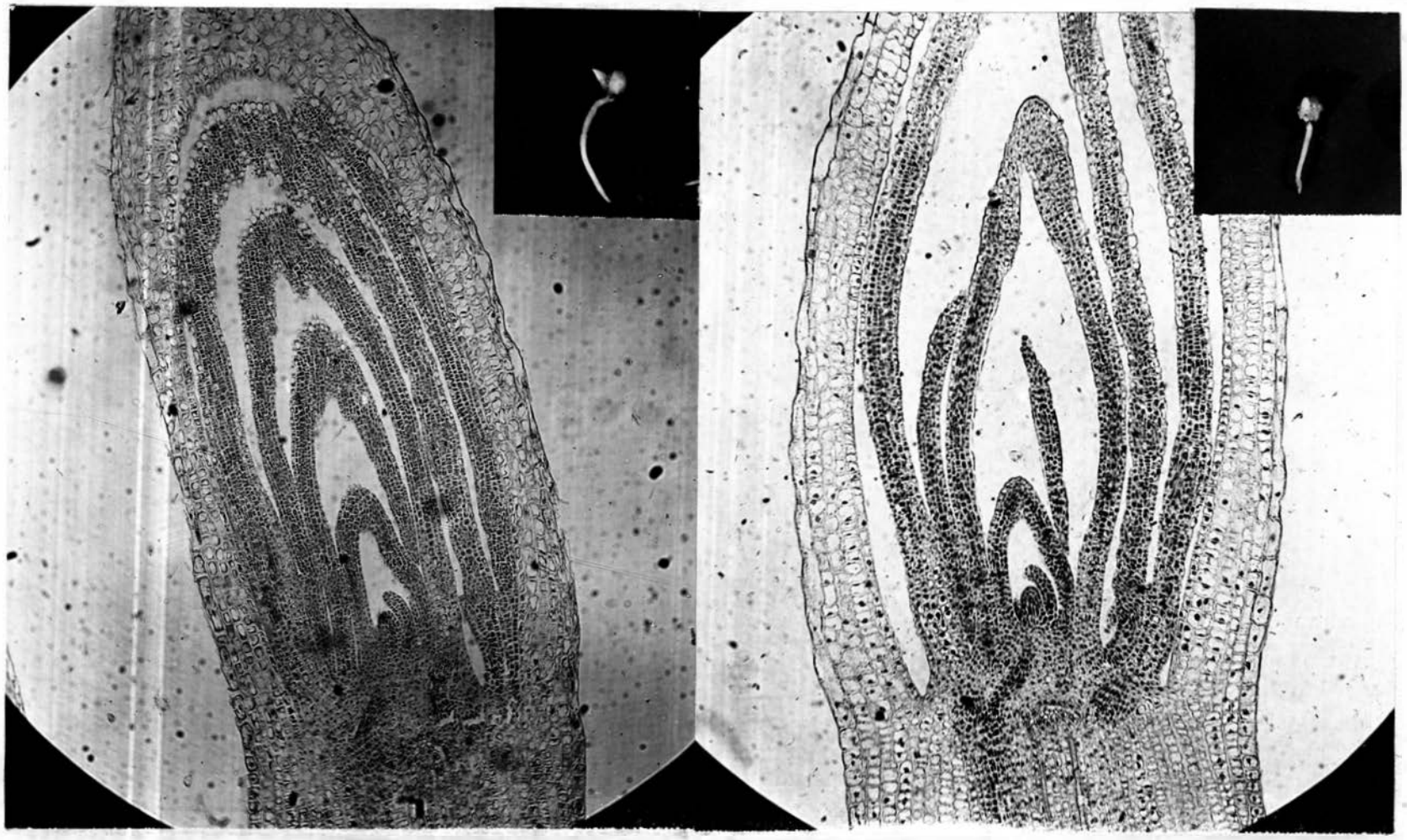

Figure 7. Sections of colchicine-induced tumors of sorghum after one day of treatment. Insets show the tumor before fixation. Left: Experimental 3. Right: Norghum. (sections x76, tumors x1)

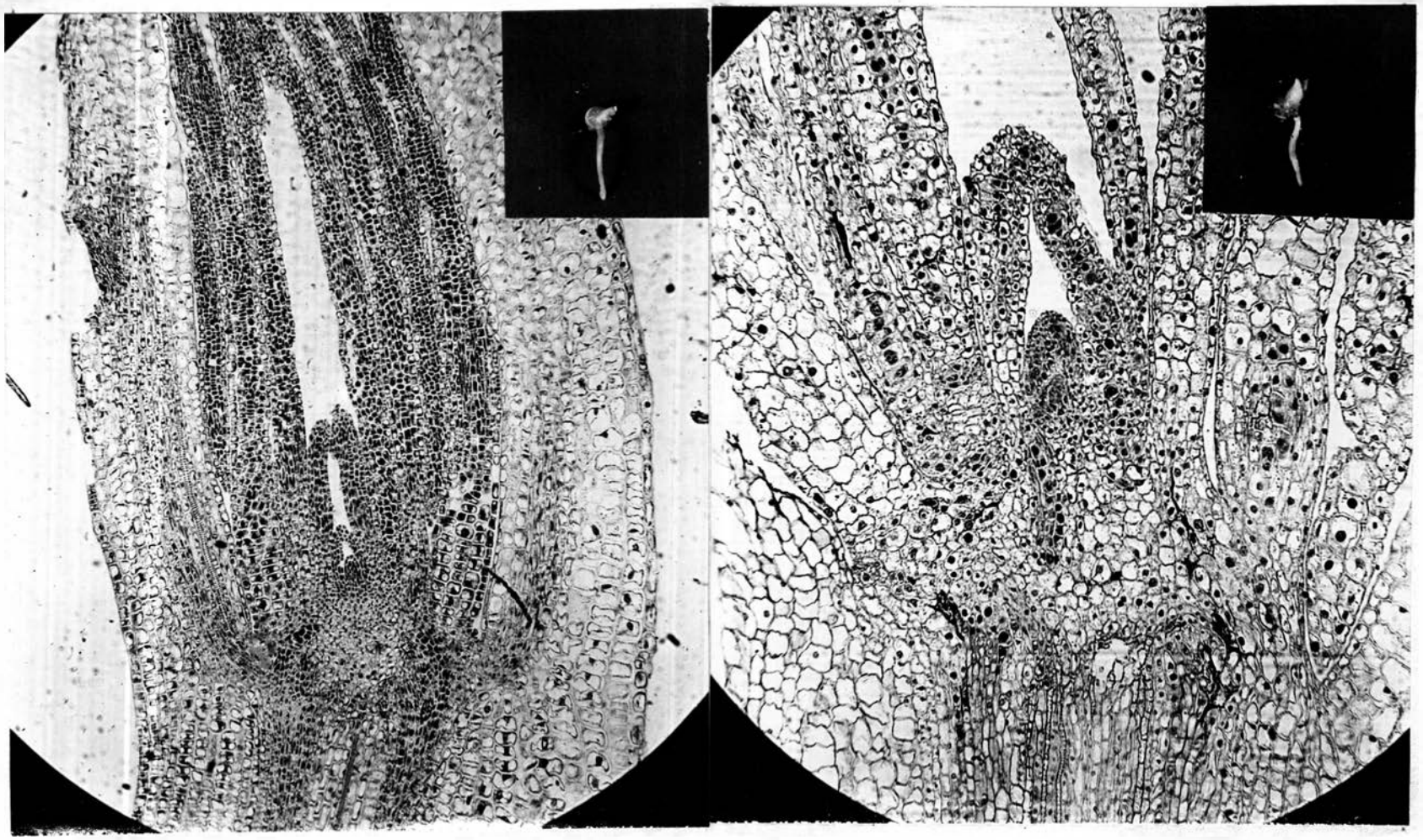

Figure 8. Sections of colchicine-induced tumors of sorghum after three days of treatment. Insets show the tumors before fixation. Left: Experimental 3. Right: Norghum. (sections x76, tumors x1)

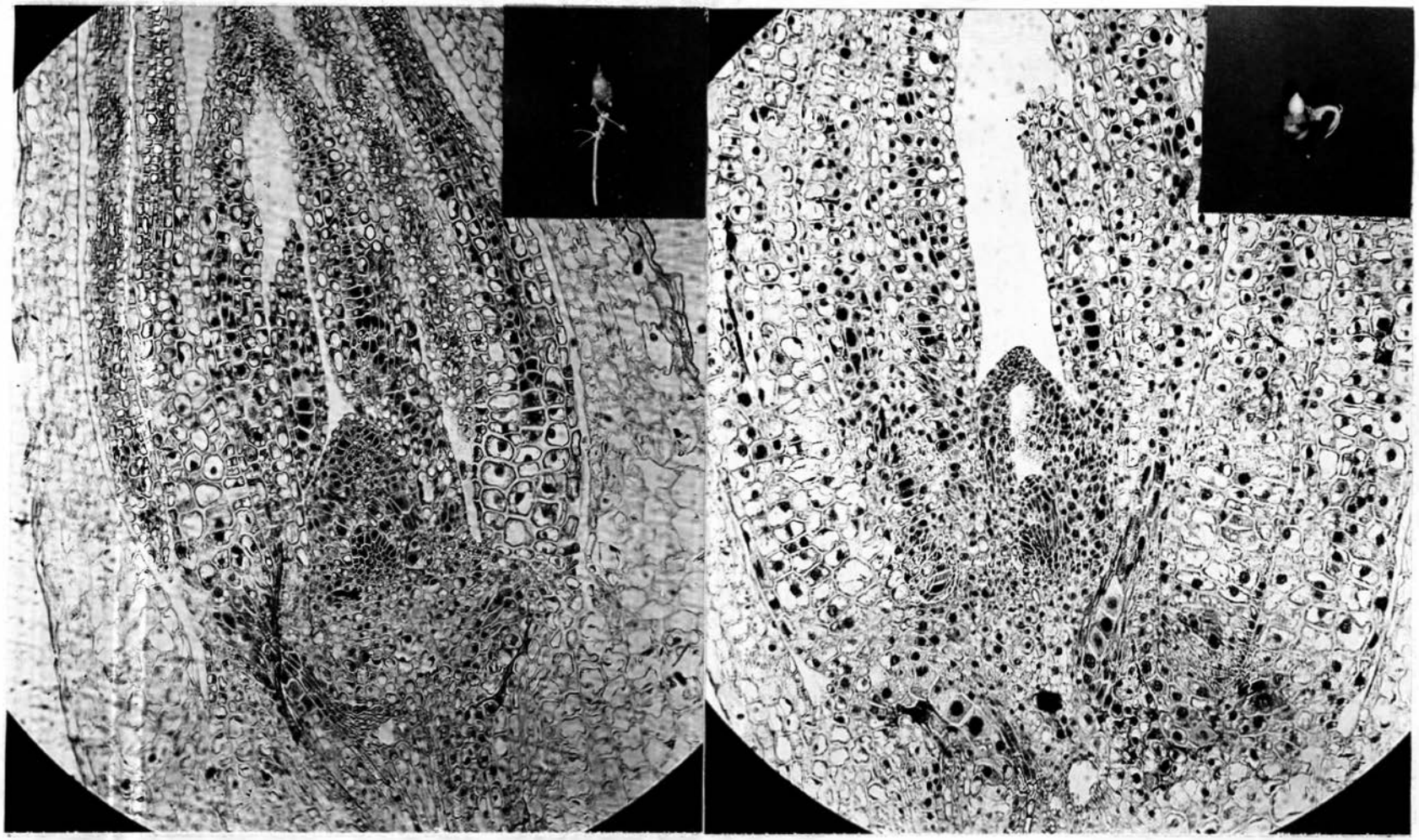

Figure 9. Sections of colchicine-induced tumors of sorghum after five days of treatment. Insets show the tumors before fixation. Left: Experimental 3. Right: Norghum. (sections x76, tumors x1)

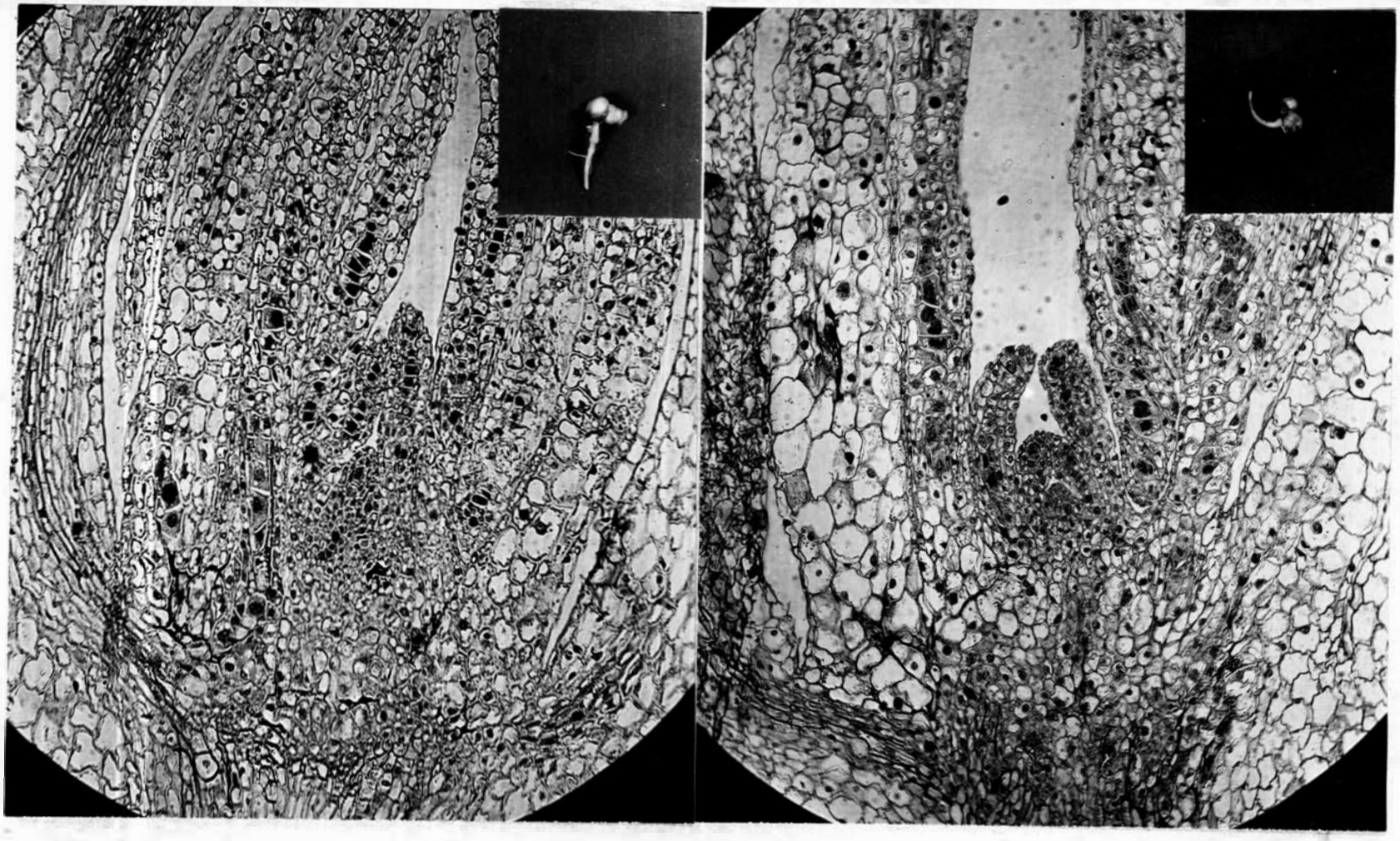

Figure 10. Sections of colchicine-induced tumors of sorghum after seven days of treatment. Insets show the tumors before fixation. Left: Experimental 3. Right: Norghum. (sections x76, tumors x1)

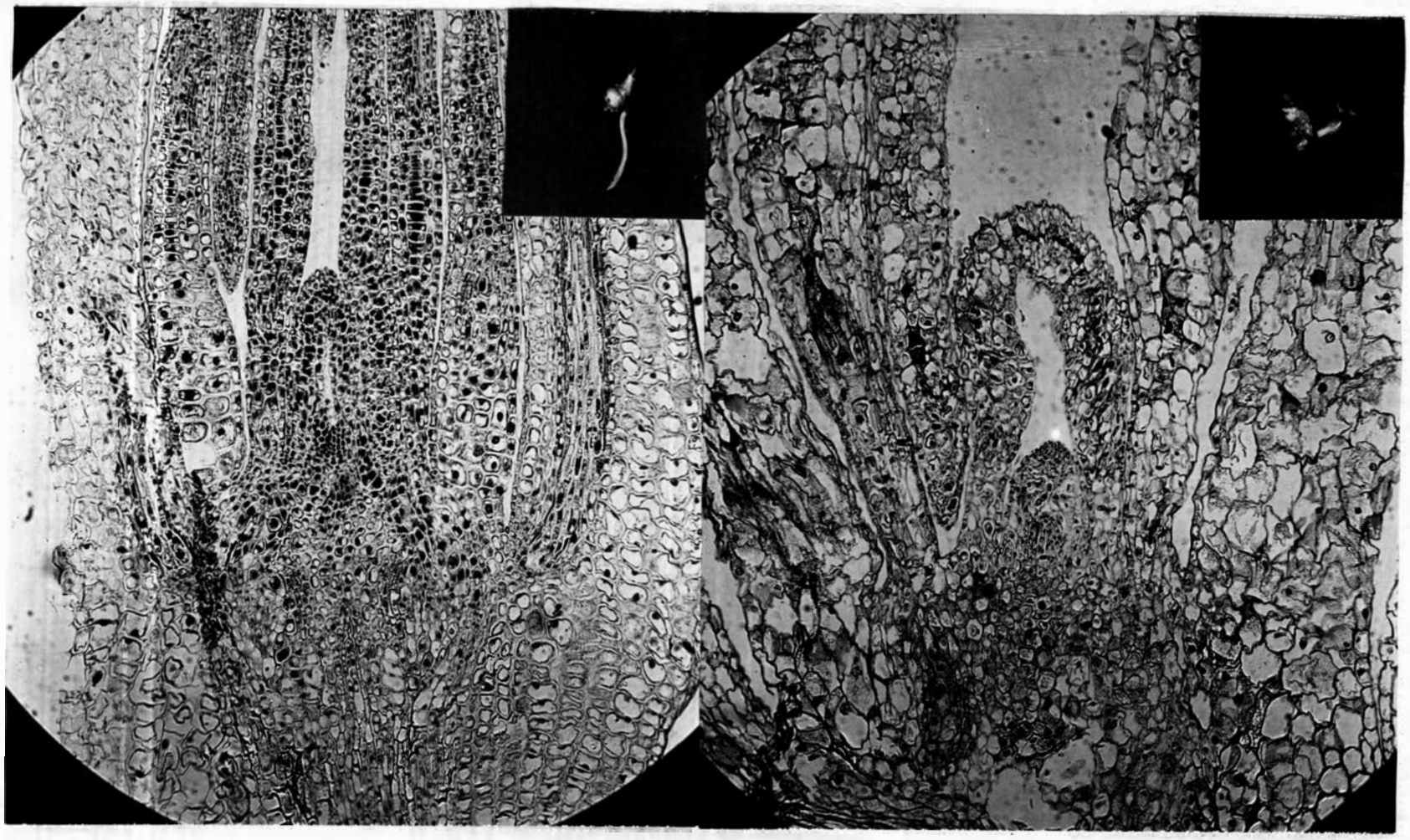

Figure 11. Sections of colchicine-induced tumors of sorghum after nine days of treatment. Insets show the tumors before fixation. Left: Experimental 3. Right: Norghum. (sections x76, tumors x1)

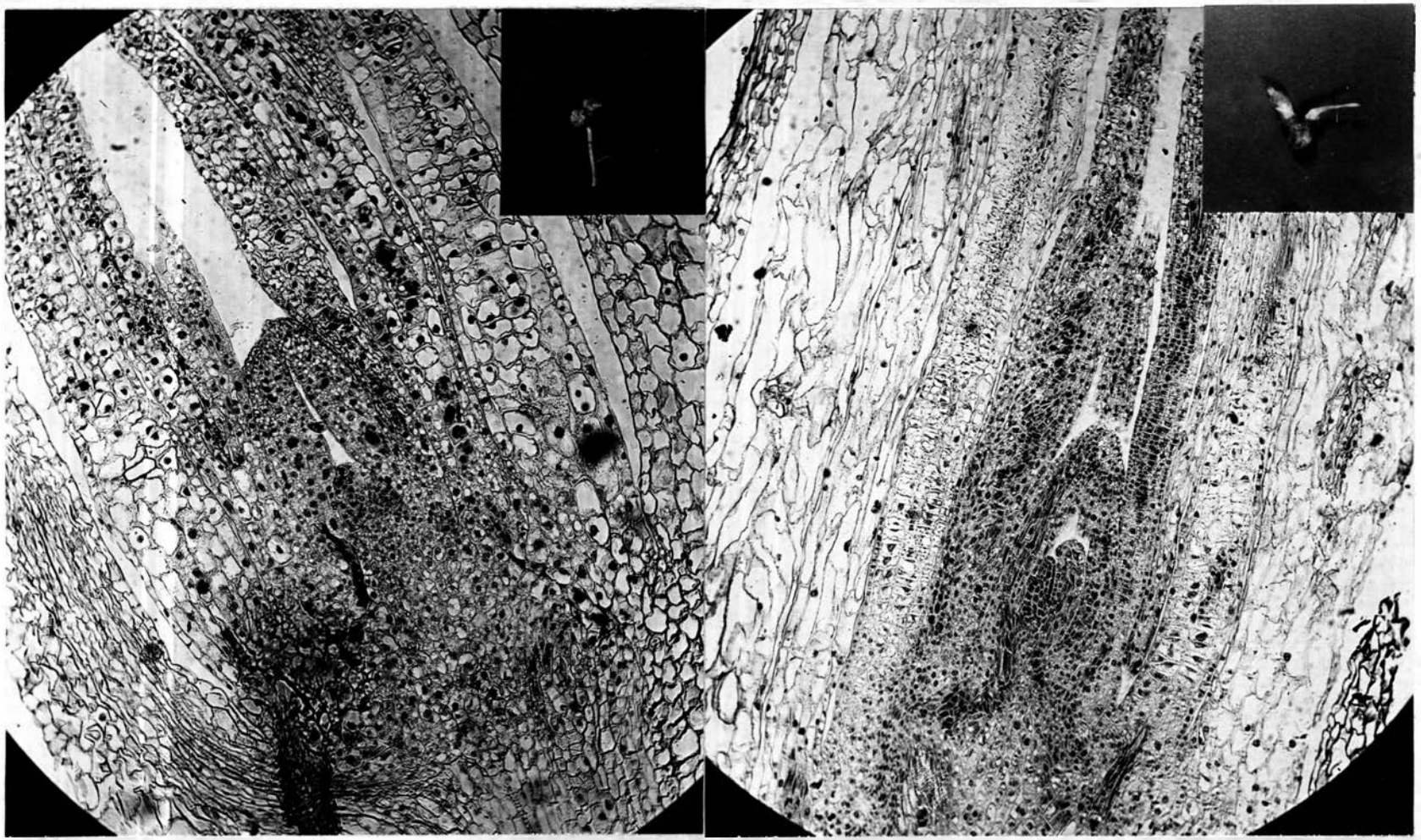

Figure 12. Sections of colchicine-induced tumors of sorghum after eleven days of treatment. Insets show the tumors before fixation. Left: Experimental 3. Right: Norghum. (sections x76, tumors x1)

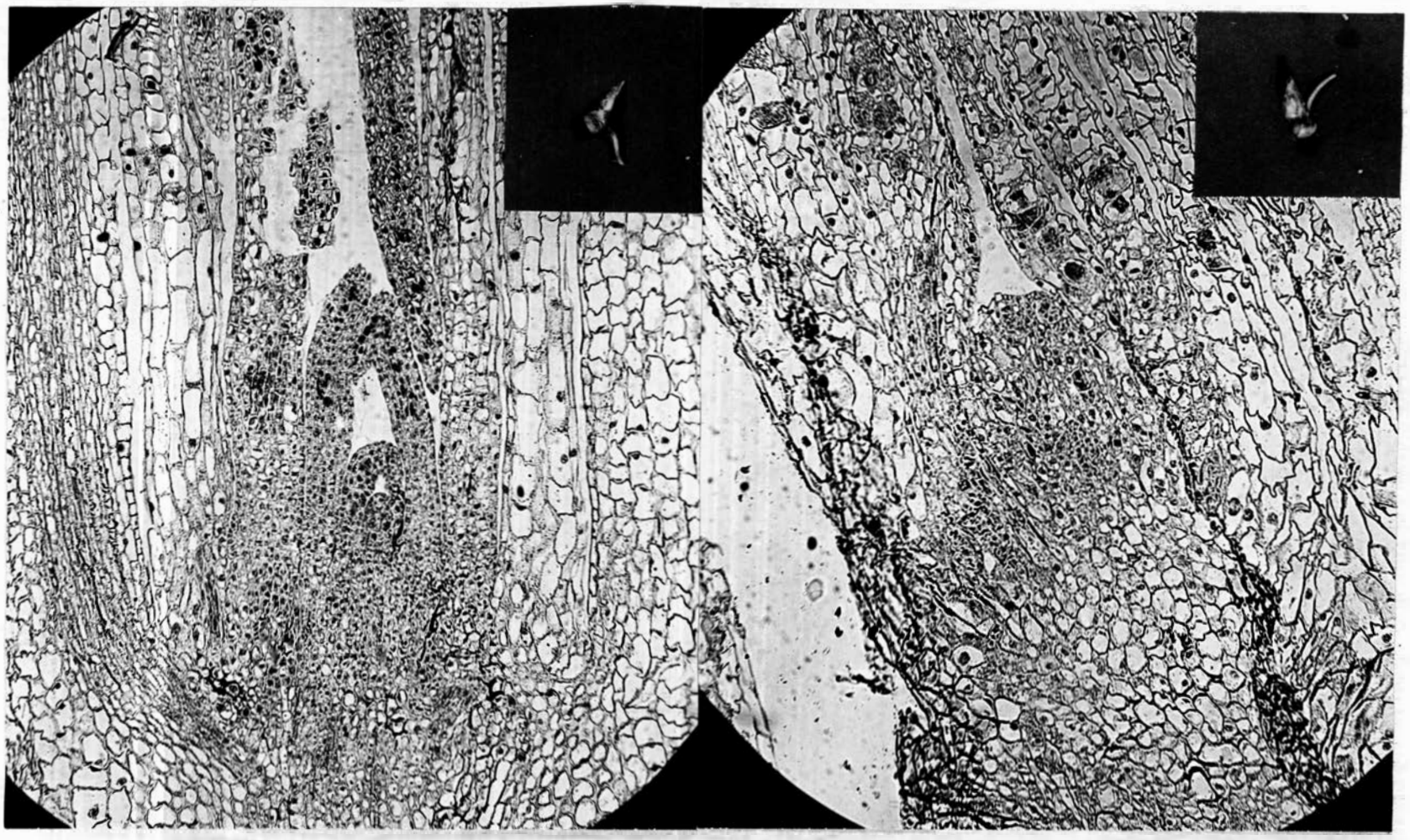

Figure 13. Sections of colchicine-induced tumors of sorghum after thirteen days of treatment. Insets show the tumors before fixation. Left: Experimental 3. Right: Norghum. (sections x76, tumors x1).

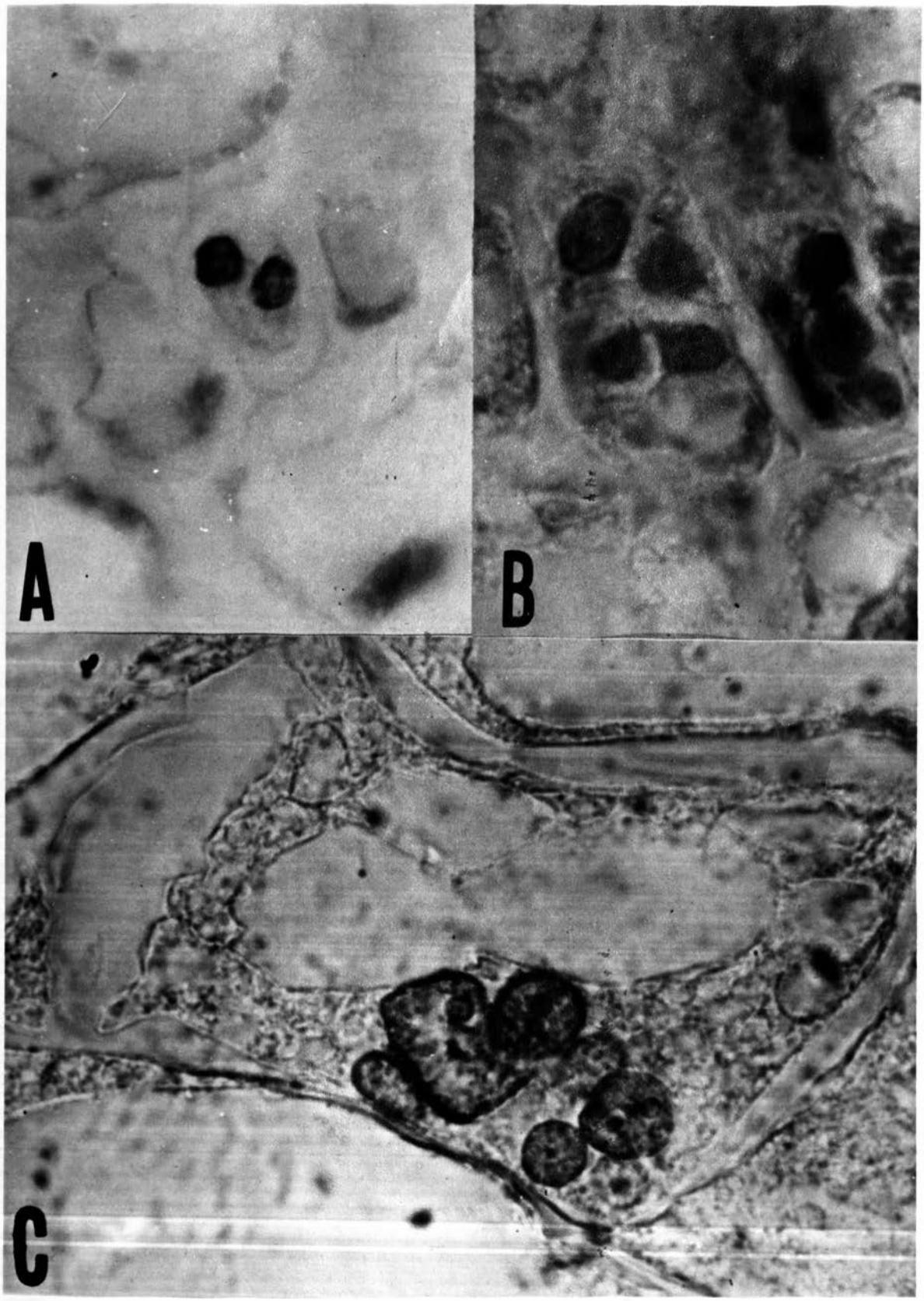

Figure 14. Multinucleate cells from colchicine-induced tumors of Experimental 3. A and B after two days of treatment, C after five days of treatment. (A and B x2400, C x1700)

indicated concentrations of nuclear volumes in more than one distinct size class. In Norghum at the tenth day and in Experimental 3 at the fifth day, the distributions appears to be definitely bimodal (Figures 16 and 19). The distributions for Experimental 3 at the second, ninth and eleventh days (Figures 15 and 17) appear trimodal. Less well marked secondary modes are present in some of the other distributions. Distributions with more than one mode may indicate the presence of nuclei of different ploidy within the apical meristem of a single tumor, but there is no indication that nuclei of higher ploidy are more frequent in Norghum than in Experimental 3.

The average volume of nuclei of the apical meristems of treated Experimental 3 and Norghum were plotted and a curve sketched through the array as shown in Figure 20. In Experimental 3, there is a definite trend of increasing size up to the fifth day followed by a decrease in size from the sixth to ninth day; after the tenth day, nuclear volumes are larger but erratic. In Norghum, a similar trend is evident but less clear; size increases up to the third day and decreases between the third and ninth days. Again the volumes at the later days are erratic.

In treated Norghum two tumors were found which were very unusual. The first of these, shown in Figure 21, was collected on the eighth day of treatment. In the middle of what appears to be the growing point, a sharply delimited area of small cells with small dense nuclei was found. It is possible that this represents a new growing point which originated from a cell within the original growing point. The other unueual tumor, collected on the thirteenth day of treatment, is shown in Figure 22. A mass of small dense cells with what appears to be an apical meristem and

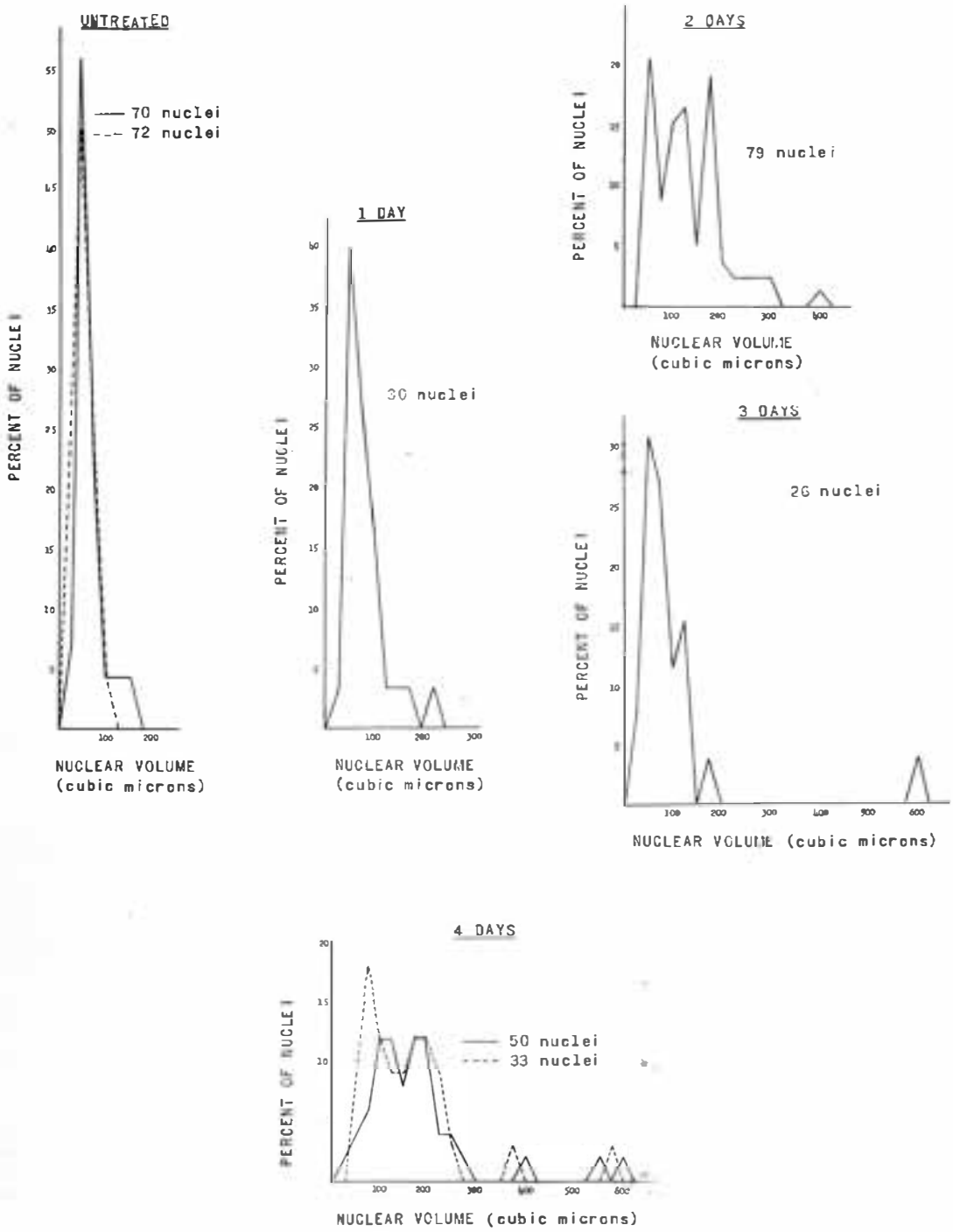

Figure 15. Distributions of nuclear volumes in the apical meristems of untreated Experimental 3 and of colchicine-treated Experimental 3 one, two, three and four days after the beginning of treatment.

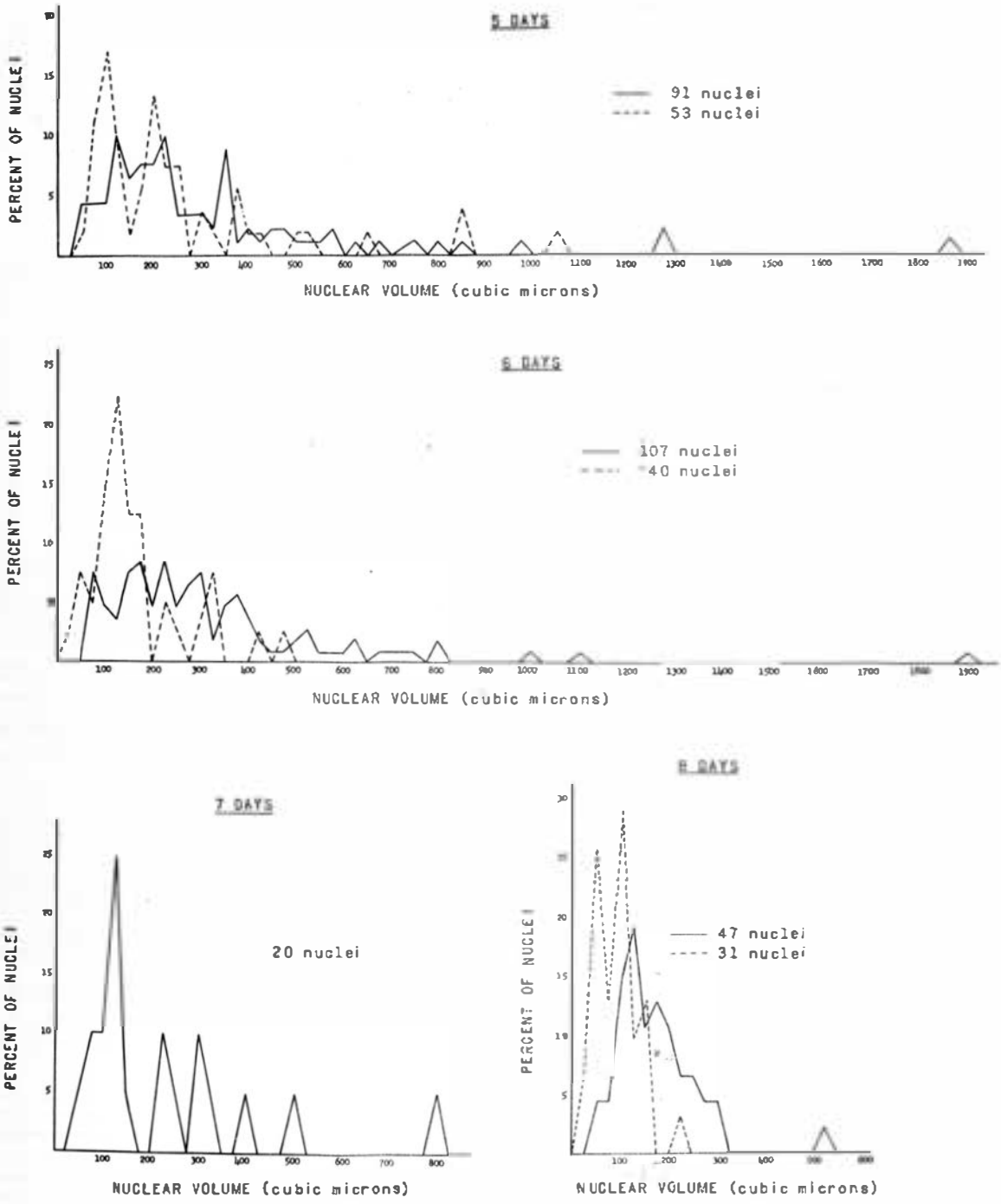

Figure 16. Distributions of nuclear volumes in the apical meristems of colchicine-treated Experimental 3 five, six, seven and eight days after the beginning of treatment.

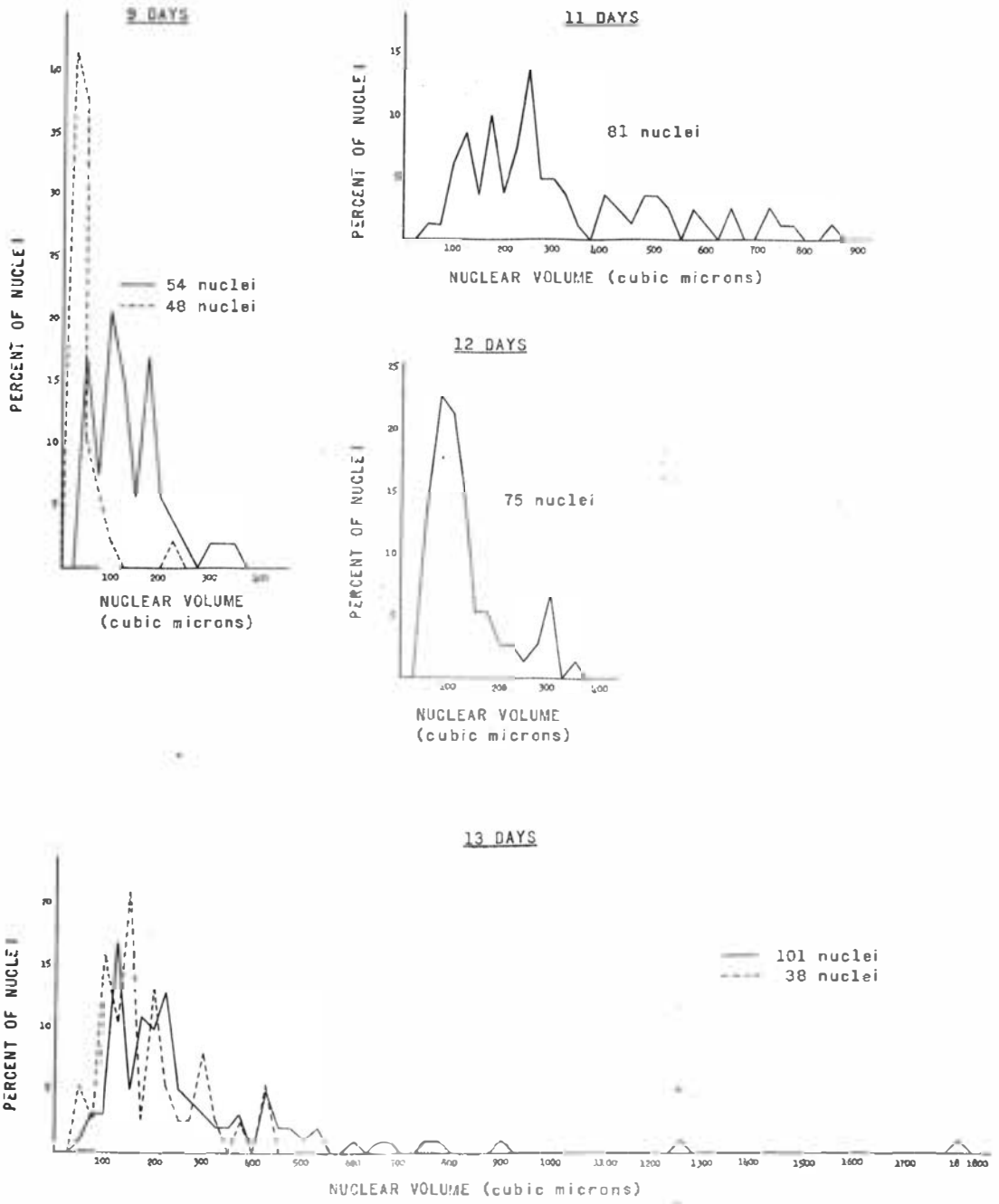

Figure 17. Distributions of nuclear volumes in the apical meristems of colchicine-treated Experimental 3 nine, eleven, twelve and thirteen days after the beginning of treatment.

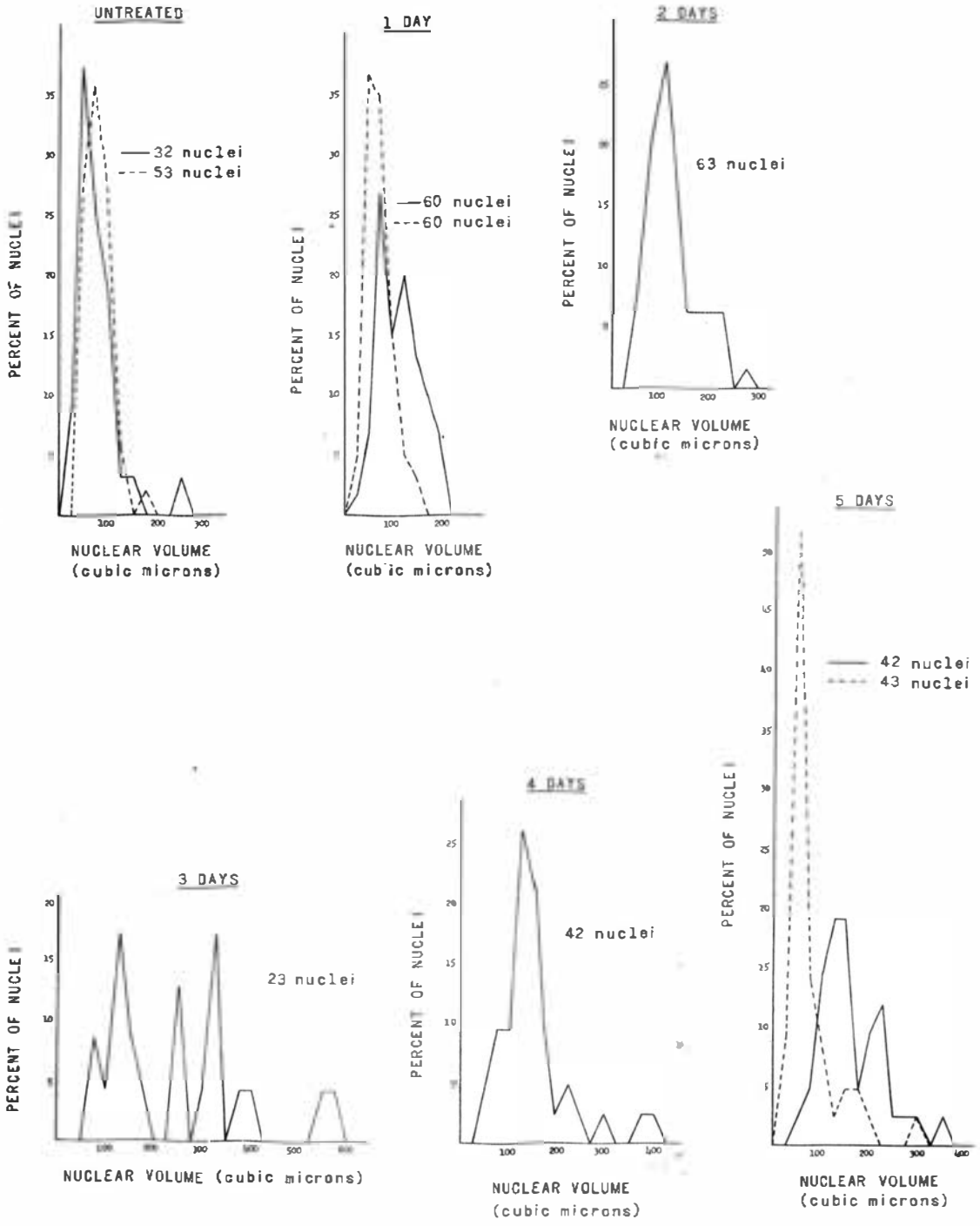

Figure 18. Distributions of nuclear volumes in the apical meristems of untreated Norghum and of colchicine-treated Norghum one, two, three, four and five days after the beginning of treatment.

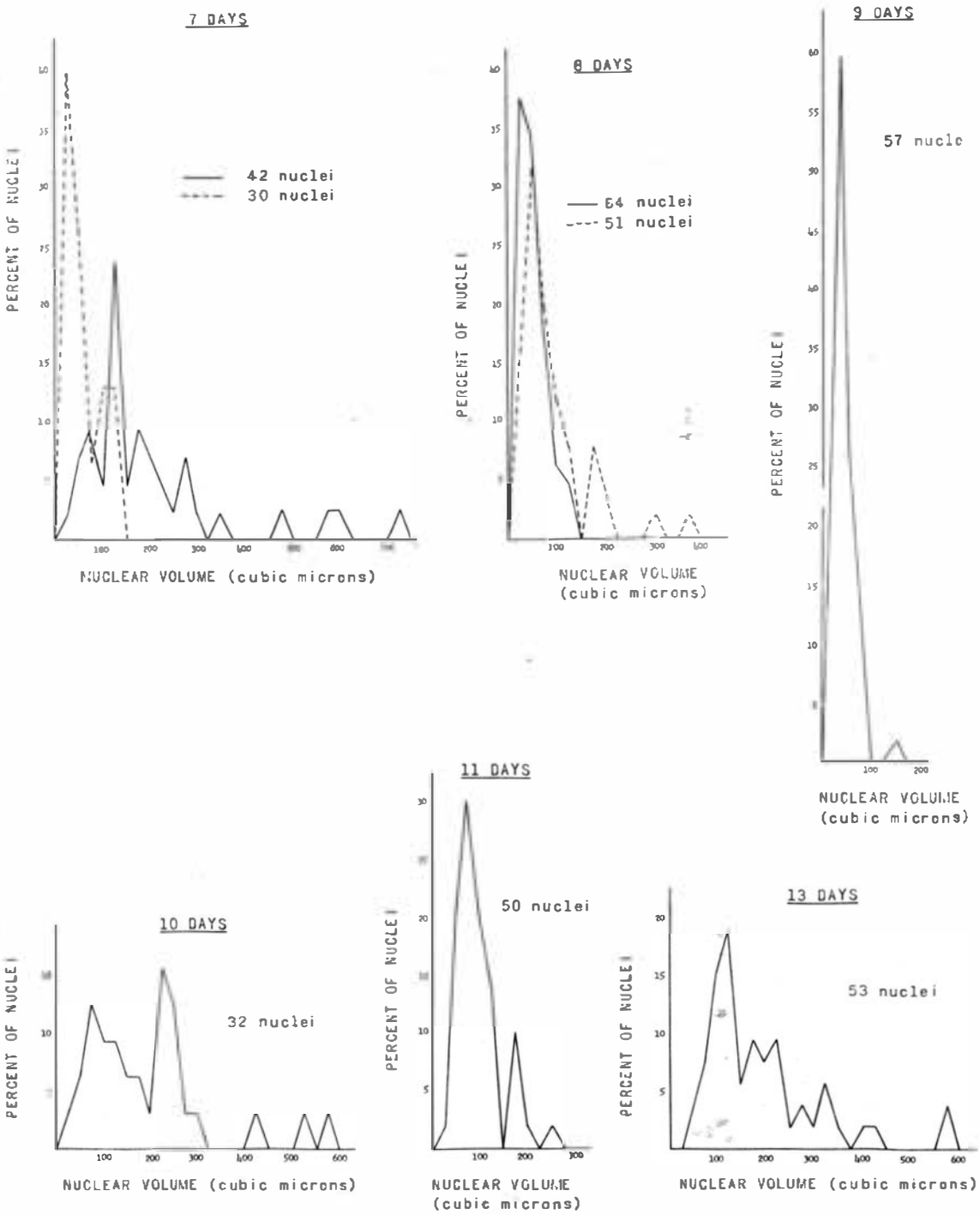

Figure 19. Distributions of nuclear volumes in the apical meristems of colchicine-treated Norghum seven, eight, nine, ten, eleven and thirteen days after the beginning of treatment.

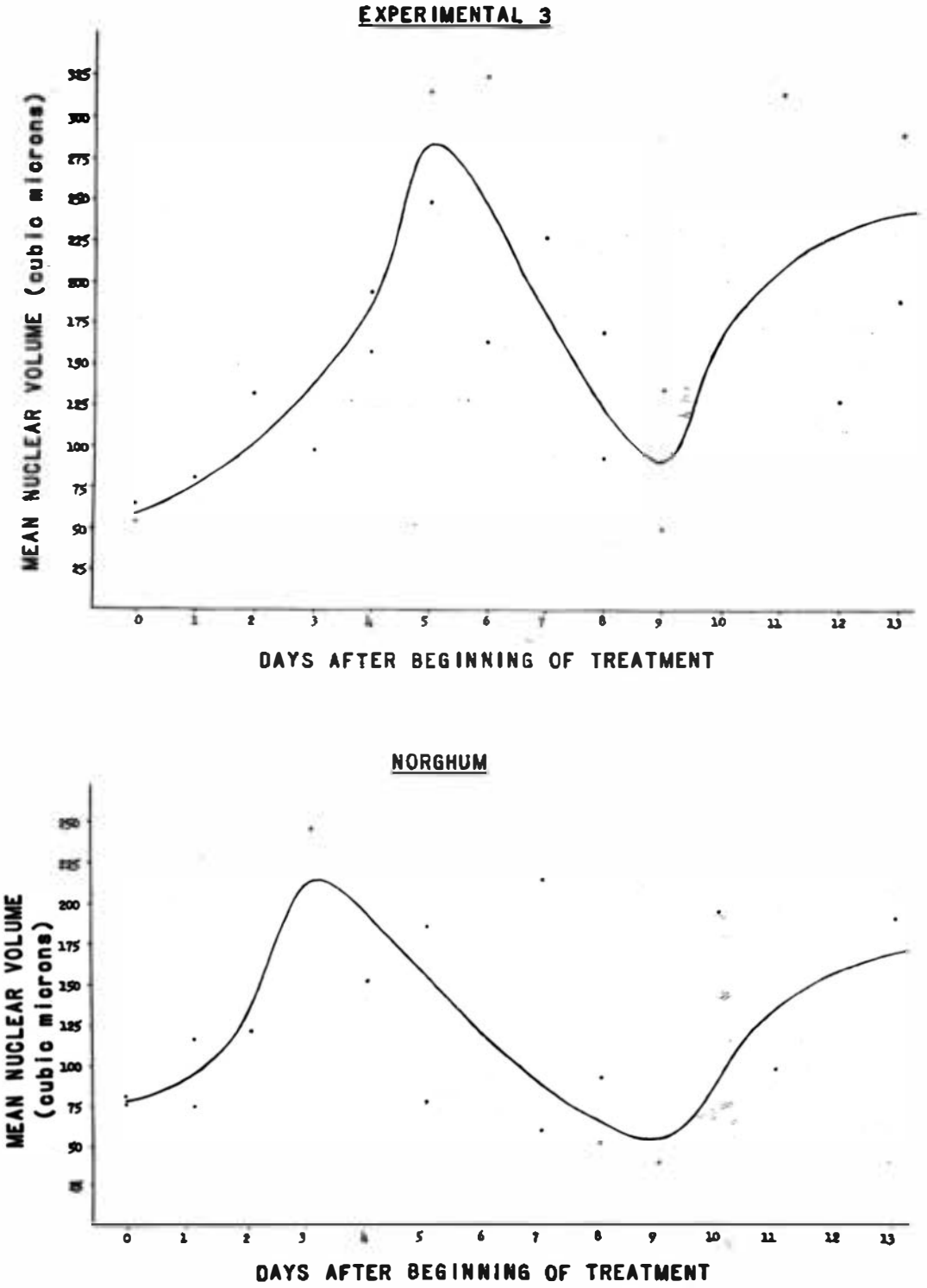

Figure 20. Mean nuclear volumes in the apical meristems of untreated and colchicine-treated Experimental 3 and Norghum.

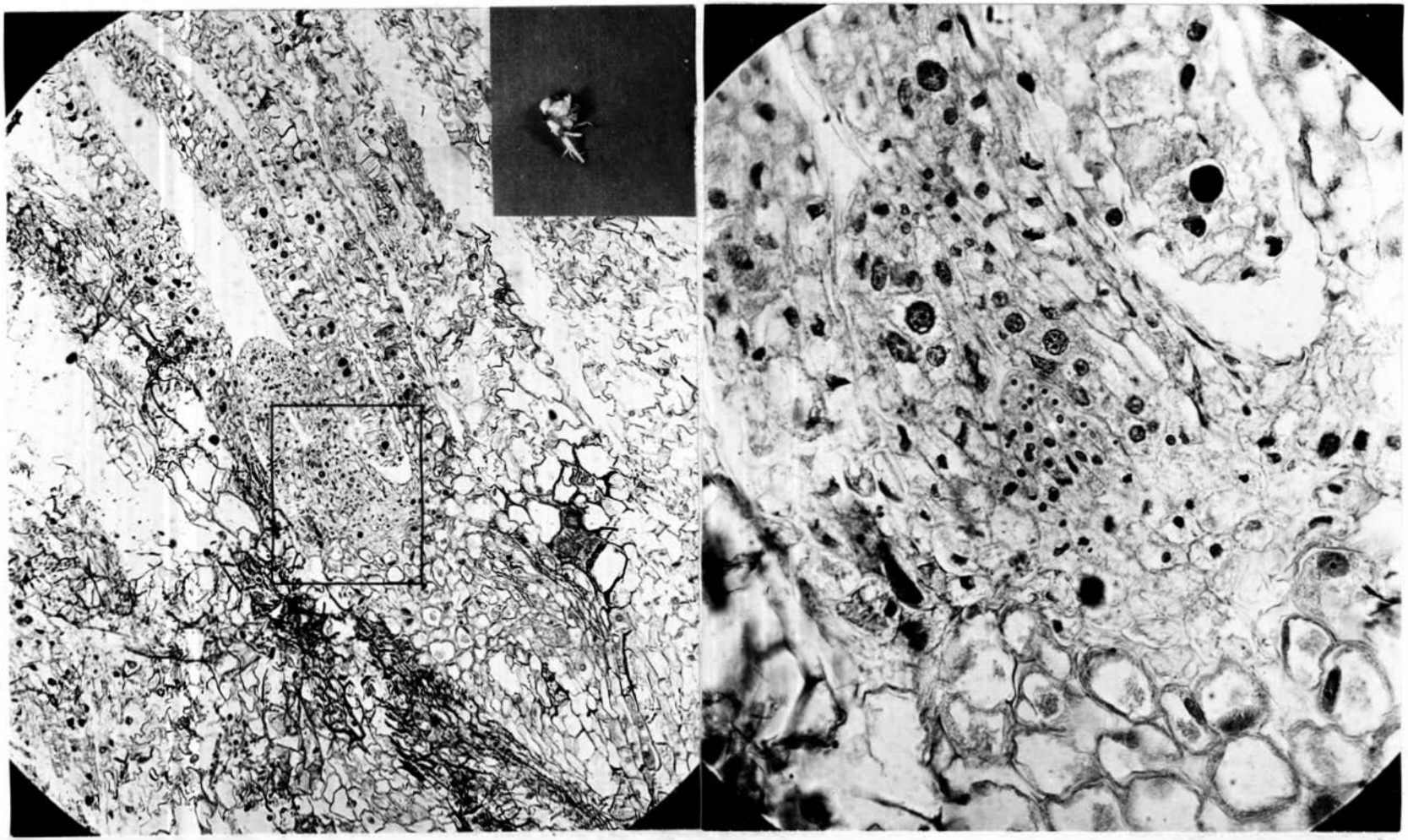

Figure 21. Section of a colchicine-induced tumor of Sorghum after eight days of treatment. Right: an enlargement of the area marked at the left. The inset shows the tumor before fixation. (left x76, right x350)

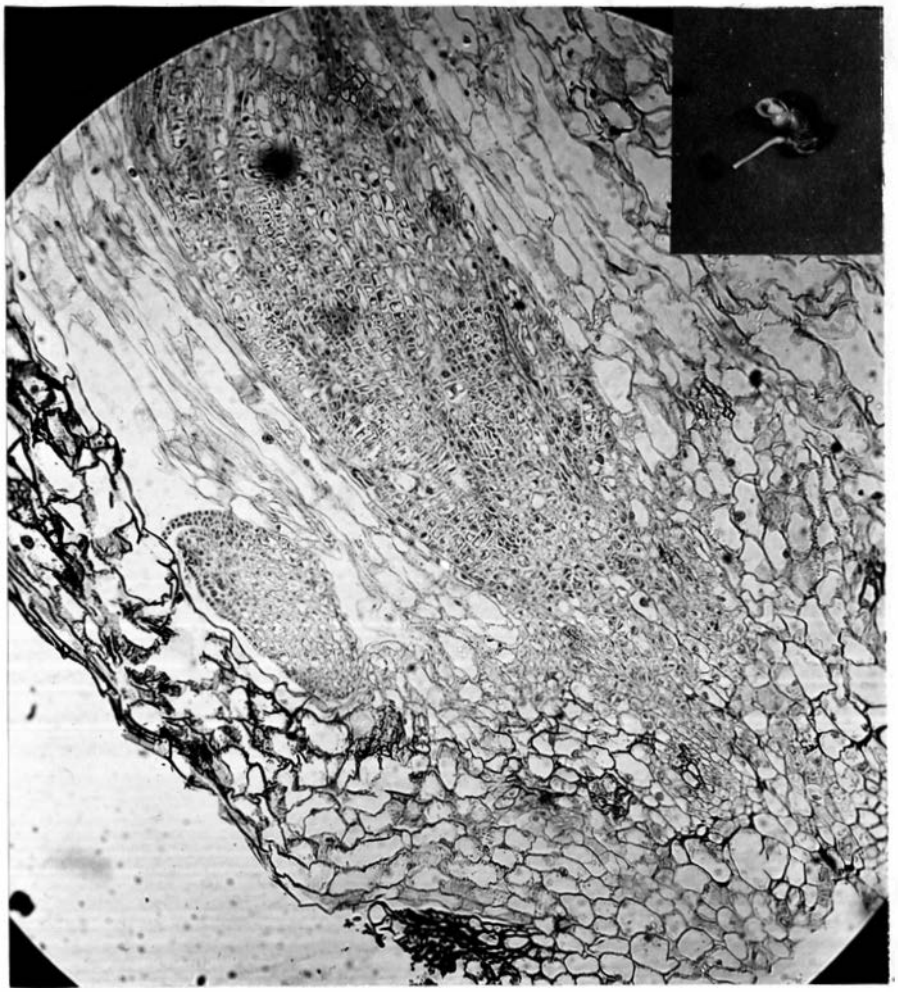

Figure 22. Section of a colchicine-induced tumor of Sorghum after thirteen days of treatment. What appears to be an axillary bud has formed in the axil of the coleoptile. Inset shows the tumor before fixation. (section x76, tumor x1)

one young leaf was found in the axil of the coleoptile. In a group of seedlings, treated to study tumor development, a Norghum tumor developed two shoots, one arising from the center of the tumor and the second apparently from the axil of the first leaf. This plant is shown in Figure 23. The main shoot died shortly after the photograph was taken but the axillary shoot developed into a mature plant. It appears probable that the tumor shown in Figure 22 could have developed in a similar manner.

From the seedlings treated to study tumor development and characters of the mature plants, only two Experimental 3 and three Norghum survived out of twenty-four of each variety treated. None of these plants showed changes.

Studies of translocation material

Survival after treatment was very poor in F_1 seedlings from crosses involving either of the translocation stocks. From crosses with SS 110 as the translocation parent only fifteen plants survived out of eighty-nine treated; from the one cross using SS 1186, only seven plants survived out of twenty-eight treated. None of the treated plants were markedly different from the untreated plants. Cytological examination of untreated F_1 plants from crosses involving SS 110 revealed no associations of chromosome pairs such as would be expected in a translocation heterozygote, consequently cytological examination of treated plants from these crosses was not made. In the cross involving SS 1186, the expected associations at diakineais were easily identified in all the untreated plants. A ring of four and eight bivalents at this stage of meiosis from an untreated plant of this cross are shown in Figure 24. Since a ring of four was found at diakinesis in all the treated plants no evidence for induced

Figure 23. A colchicine-treated Norghum seedling which developed two shoots. The smaller shoot on the left arose from the center of the tumor and the larger one on the right apparently arose from the axil of the first leaf.

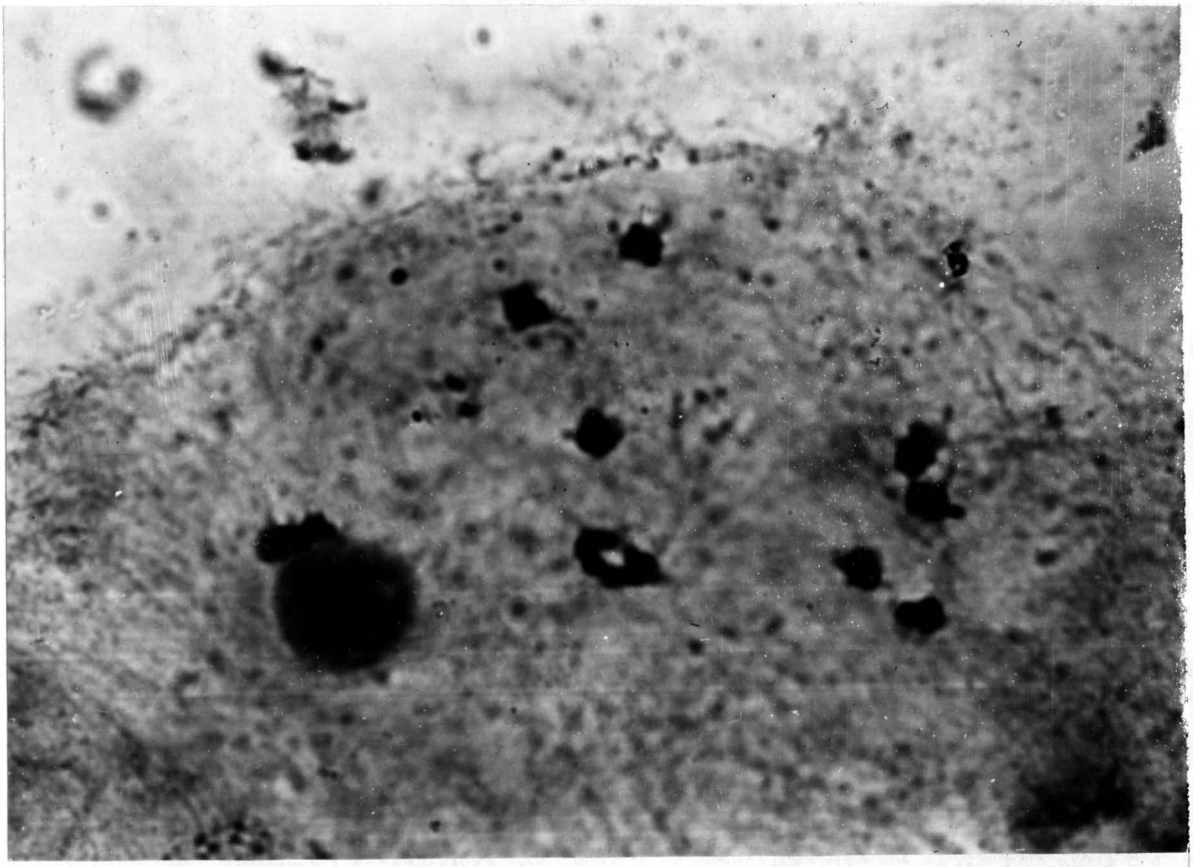

Figure 24. Chromosomes at diakinesis in the untreated translocation heterozygote showing a ring of four and eight bivalents. (x1400)

chromosomal homozygosity was obtained.

DISCUSSION

The induction of diploid mutants by colchicine treatment of the sorghum variety Experimental 3 is in agreement with previously reported work (9, 22, 10, 7) and production of tetraploids in Norghum confirms previous unpublished observations. It appears likely that the difference in response is a characteristic of the variety since the varieties were treated at the same time under conditions as similar as possible. Although the breeding behavior of the mutant plants has not been adequately determined to know whether they are true-breeding or segregating, progenies of forage-type plants of treated Experimental 3 appear uniform for leaf characters. Since presence of awns has been shown to be recessive to absence of awns (7), the treated plants which were awned must have been homozygous at least for that gene. These observations indicate that some of the mutant plants of treated Experimental 3 may be true-breeding for some of the changed characters.

The different reaction of the two varieties to colchicine treatment could not be directly related to the response of the chromosomes to colchicine since reductional groupings were found in colchicine-treated root tips of both Experimental 3 and Norghum with very nearly the same frequency. There appeared to be a tendency for the reductional groupings to be equational or nearly so, as has been reported by other workers (13, 28).

In the sections of c-tumors, little difference could be detected between Experimental 3 and Norghum. Multinucleate cells found in the

tumors may be the result of reductional groupings as has been suggested by Wilson et al.(29). The presence of what appear to be nucleoli in many of the nuclei of multinucleate cells indicates some regularity to the distribution of chromosomes in the formation of nuclei. The greater variability of nuclear volume in the apical meristems of tumors of Experimental 3 may be due to greater sensitivity of that variety to colchicine.

While studies of colchicine-treated translocation heterozygotes failed to provide evidence of induced homozygosity, this may be due to absence in the translocation stocks of the factors necessary for the production of mutations, to the small number of treated plants, or to unfavorable conditions during treatment.

The occurrence of eleven forage-type plants out of seventeen mutants from treated Experimental 3 seedlings may be significant. These plants closely resemble a mutant plant (P15) previously described by Ross et al.(22), and subsequently studied cytologically by Harpstead et al. (10), and genetically by Foster (7). The presence of Sudan grass in the pedigree of Experimental 3 leads to the assumption that these forage-type plants arose through uncovering of ancestral genes of Sudan grass. This could have taken place in three ways: by chromatin rearrangements resulting in a concentration of gene blocks from the ancestral variety, by mutation of suppressor genes to allow expression of ancestral genes present in the variety, or by homozygosity of ancestral genes whose expression had been prevented by residual heterozygosity. However, Harpstead et al.(10) were not able to find any evidence of irregularities or rearrangements of the chromatin, Foster (7) found that the variant characters of P15 were due to mutations at a large number

of loci and no variants of this type have arisen in untreated Experimental 3. It appears unlikely that the above explanations can account for the origin of the forage-type plants. In addition, the occurrence of other types of mutant plants after colchicine treatment leads to the conclusion that treatment has caused true gene mutations. The similarity and number of forage-type plants may be due to the presence in Experimental 3 of particular mutable genes or to some specific effect of colchicine.

The reduction of chromosome numbers in somatic tissues has been reported by a number of workers both as occurring naturally (5, 13, 18, 19, 23, 26, 27, 30), and following certain treatments (1, 9, 11, 12, 28, 29, 31). The reduction has commonly been attributed to abnormalities in mitosis. Chromosome reductions occurring naturally have been found most often in polyploid plants; in most cases some regularity has been found in the reduction process with all or a large part of a genome apparently lost as a unit (18, 19, 26, 27, 30, 31). It has been suggested that the reduction process is random but that only cells with balanced genomes survive (20, 30).

In sorghum, reductional groupings caused by colchicine treatment may result in the formation of multinucleate cells. The tendency of reductional groupings to be equational, combined with selective survival of a balanced genome would favor nuclei which contain a complete set of chromosomes. The diploid number would be restored by splitting of the c-chromosomes. Division of binucleate cells as reported by Berger et al (3) could then result in the formation of uninucleate diploid cells. Gene mutations occurring before splitting of the c-chromosomes would be made homozygous in this manner. Mutations occurring after splitting of the c-chromosomes

would probably occur in only one member of a pair of chromosomes thus giving rise to nuclei heterozygous for the mutation. Mutations occurring at different times would result in nuclei homozygous for some characters and heterozygous for others. A diploid mutant cell, because of genotypic or positional advantage might organize a new growing point; resulting plants might be true-breeding or heterozygous. While this hypothesis can account for the appearance of diploid mutants in Experimental 3, there is as yet no explanation for the lack of such mutants in Norghum or for the lack of tetraploids in Experimental 3. That specific mutations have not been observed in Norghum may be due to their being concealed by the induction of tetraploidy or to a varietal difference in susceptibility to colchicine.

The results of this study suggest additional lines of investigation which might be undertaken. Reasons for poor survival of seedlings after treatment should be investigated. Definition of the conditions during treatment which give rise to diploid mutants in Experimental 3 should lead to better reproduction of results. Studies of the results of treatment of Experimental 3 and Norghum under varying conditions might provide some information regarding their differential response. Chromosomally marked stocks of Experimental 3 and Norghum should be developed. These could be used to discover whether the origin of diploid mutants is due to somatic reductional divisions, to point mutations, to a combination of these effects, or to some other mechanism. They could also be used for further studies of the differential response of the two varieties to colchicine.

SUMMARY

Studies were made of plants from colchicine-treated seedlings, of colchicine-treated root tips, and of sections of colchicine-induced tumors of the sorghum varieties, Experimental 3 and Norghum; and of F_1 sorghum plants heterozygous for a translocation which were treated with colchicine.

Colchicine treatment of seedlings induced diploid mutants in Experimental 3 and tetraploids in Norghum.

Reductional groupings were found in colchicine-treated root tips of both varieties. A high proportion of the reductional groupings appeared to be equational or nearly so. No difference was discovered between varieties in the reaction of their root tip chromosomes to colchicine.

Colchicine-induced tumors of sorghum are formed primarily by enlargement of cells in differentiated tissues in the terminal bud of the seedling. Multinucleate cells were observed in sections of c-tumors of both Experimental 3 and Norghum. Measurements of nuclear volumes in the apical meristems of the tumors gave no evidence of higher frequencies of polyploid cells in Norghum than in Experimental 3.

Studies of meiosis in plants from treated seedlings of an F_1 hybrid heterozygous for a translocation gave no evidence of induced homozygosity.

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