FREQUENCY OF MULTIVALENTS IN AUTOTETRAPLOID SORGHUM, *SORGHUM VULGARE* PERS.

by

Willie James Washington

A Thesis Submitted to the Faculty of the

DEPARTMENT OF PLANT BREEDING

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

1 9 6 6

STATEMENT BY AUTHOR

This thesis has been submitted in partial fulfillment of requirements for an advanced degree at The University of Arizona and is deposited in the University Library to be made available to borrowers under rules of the Library.

Brief quotations from this thesis are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Willie James Washington

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Robert L. Voigt

ROBERT L. VOIGT
Associate Professor of Plant Breeding

August 19, 1966
Date

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude and appreciation to Dr. Robert L. Voigt, the major professor and thesis advisor for his assistance and advice in the conduct of this study, and for the helpful suggestions in the preparation of this thesis. Special appreciation goes to Dr. John E. Endrizzi, Head of the Department of Plant Breeding, for his critical evaluation of this thesis and helpful suggestions.

Gratitude is extended to the entire staff of the Department and to all others who helped to make this study possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

ABSTRACT

Sorghum seedlings of the variety Combine Kafir 60 were treated with three concentrations of an aqueous solution of colchicine, 0.05, 0.1, and 0.2 per cent. A cytological analysis was made on some of the treated plants; five plants were found to be tetraploids. The cytological analysis was made to study the pairing behavior of the chromosomes with emphasis on multivalent association.

Interpretation of statistical data shows that pairing of chromosomes in sorghum is on a random basis, and can be expected to follow a binomial distribution $(a + b)^{10}$ where a equals bivalent frequency, b equals multivalent frequency, and the power 10 is the number of groups of homologous chromosomes.

The majority of the chromosomes were associated as bivalents and multivalents with the frequency of chromosomes associated as multivalents exceeding 50 per cent. These results coupled with data reported on heterozygous reciprocal translocations in sorghum, in which gametes are duplicate and deficient for part of a chromosome resulting in sterility, seems to invalidate the hypothesis of chromosome substitution proposed by Sanders and Franzke as being

the origin of colchicine-induced diploid sorghum mutants where no multivalents are formed.

The results in this study indicate that there is no preference for bivalent pairing in autotetraploid sorghum, in fact, there seems to be a preference for multivalent formation.

INTRODUCTION

Seedlings of diploid sorghum, Sorghum vulgare Pers., treated with colchicine yield both diploid true-breeding and nontrue-breeding mutants (1, 9, 11, 12, 13, 14, 16, 24, 26, 27, 29, 31, 32). Two proposals have been given to explain the occurrence of the observed diploid mutants following colchicine treatment (11, 24). The first proposal was based on a phenomenon known as reductional grouping of the somatic chromosomes which would result in the chromosomes containing gene blocks from one ancestor of the polypooid species occurring in one cell (11). A plant originating from this phenomenon would contain duplications and deficiencies for whole chromosomes even though the 2n number has not been changed. Consequently, at meiosis in such a plant multivalents would be expected. However, detailed cytological studies by Harpstead, Ross, and Franzke (17) revealed no detectable irregularities in chromosome arrangement and pairing. Consequently, the original hypothesis was abandoned and a new hypothesis was proposed by Harpstead et al. (17). To obtain true and nontrue-breeding colchicine-induced mutations in sorghum, this hypothesis states that ". . . the changes in the chromatin must be of the nature of multiple point mutations perhaps involving minute structural changes . . . ."

Recently, Sanders and Franzke (29) and Franzke and Sanders (15), after considering certain kinds of cytological data, resurrected the original proposal as the most probable means for the origin of the colchicine-induced mutants. The proposal states that "Colchicine-induced diploid mutants arise from the substitution of chromosomes of similar phylogenetic origin (homeologous or analogous chromosomes), and that substitutions have not been detected cytologically because there is a tendency for bivalent rather than multivalent pairing to occur in sorghums with $2n = 20$, and because pairing may occur between analogous chromosomes."

If both reductional grouping and chromosome substitution occur, one would expect to see multivalent associations at meiosis, but they were not seen. The failure to observe multivalents in the diploid mutants was explained on the basis of preferential pairing. However, there are published data that are not in agreement with this which indicates that this interpretation may be incorrect.

It was the purpose of this study to review the literature to check the validity of the original hypothesis of reductional grouping, and to make a careful cytological analysis of chromosome pairing in colchicine-induced autotetraploid sorghum.

REVIEW OF LITERATURE

Chromosome behavior in autotetraploids has received considerable attention since polyploidy in plants was first recognized. Much information has been published on autotetraploids; however, this review will cover only that part that is directly related to the present study.

Morrison and Rajhathy (20, 21) made a cytological survey of diploids and their autotetraploids in several species of plants. The plants differed as to chromosome size (small, medium, and large) and included plants with large numbers of chromosomes per cell. It is generally assumed by cytologists and geneticists that quadrivalent frequencies in autotetraploids are related to (a) chiasma formation or frequency, (b) chromosome size, (c) the existence of localized rather than random chiasmata, (d) the existence of a high number of chromosomes within a cell, which would interfere with the physical processes of pairing, (e) environment, and (f) genetical control.

As a result of their studies, Morrison and Rajhathy came to several conslusions in relation to autotetraploids which are as follows:

1. All species having either small, medium, or large chromosomes had about two-thirds of their chromosomes united as quadrivalents.

2. Plants with small chromosomes had over 70% of their chromosomes associated as quadrivalents, i.e., plants with small chromosomes formed as many quadrivalents as plants with medium or large chromosomes. Thus, chromosome size has no influence on quadrivalent frequency.
3. Plants with many chromosomes regardless of their size showed the same percentage of chromosomes in quadrivalents as plants with few chromosomes; therefore, crowding in the cell does not interfere with the physical process of pairing.
4. Chiasma frequency of autotetraploids was less than twice that of diploids, but no definite correlation relating chiasma frequency to the number of quadrivalents could be established.
5. Localization of chiasmata was regarded as not exercising major control over quadrivalent formation.
6. The effect of environment was not found to be significant on chromosome associations.
7. There was no evidence for a progression toward diploidization in any of the autotetraploids.
8. The results of this study do not deny the existence of a gene which controls quadrivalent frequency, but it does give some evidence against the acceptance of autotetraploids as those plants with twice

the expected chromosome number in which no quadrivalents are found.

Briefly restating the above conclusions, their study clearly showed that there are no major differences in regard to the percentage of quadrivalents among autotetraploids of different species with different numbers and sizes of chromosomes. All tetraploids are expected to show approximately two-thirds of their chromosomes paired as quadrivalents.

Sanders and Franzke (29) and Franzke and Sanders (15) in their recent proposal in which they hypothesized that the colchicine-induced diploid mutants in sorghum arise from substitution of chromosomes of similar phylogenetic origin apparently ignored the studies on chromosome behavior in autotetraploids by Morrison and Rajhathy (20, 21). Furthermore, there are other pertinent data reported on sorghum that Sanders and Franzke failed to consider in formulating their hypothesis. It is believed that these data, when properly considered, invalidates their hypothesis.

Translocations have been reported in sorghum by Endrizzi and Morgan (8), Harpstead et al. (17), Huang, Ross, and Hansel (18), Simantel and Ross (32), and Simantel et al. (31). It is known that adjacent disjunction of the chromosomes in a heterozygous interchange results in

gametes that are duplicate and deficient for segments of chromosomes involved in the translocation. In diploids such gametes generally abort. The ring forming translocation reported by Endrizzi and Morgan (8) showed that pollen abortion (81.6%) was essentially identical to the frequency of adjacent disjunction (83.8%).

In the translocation studies by Harpstead et al. (17), Huang et al. (18), and Simantel et al. (31) it was reported that when one interchange is heterozygous approximately 50% viable pollen and approximately 50% seed set was obtained. Whereas Simantel and Ross (32) reported that individuals that contained two heterozygous translocations, both pollen viability and seed set was zero. Also, Endrizzi and Morgan (8) reported that a diploid sorghum plant which contained, in addition to a centric fragment (for one arm of one chromosome), a deficiency for a short segment of an arm of another chromosome was completely sterile.

It is evident in all these cases that duplication and deficiency for parts of single chromosomes in sorghum results in lethality of essentially all cells carrying them.

Chromosome pairing in both autotriploids and autotetraploids in sorghum have been reported. In addition chromosome pairing has also been reported in triploid and tetraploid sorghum plants whose chromosome constitution was

essentially that of autoploids. These data are shown in Table 1.

With the exception of results reported by Chin (3) and Schertz (30), it can be noted in Table 1 that in pure autotetraploids of sorghum about 30 to 60 per cent of the chromosomes are joined as quadrivalents. Apparently the percentage of chromosomes pairing as quadrivalents in sorghum is somewhat lower than the two-thirds that Morrison and Rajhathy (20, 21) reported to be characteristic of autotetraploids in general. Nevertheless, it can be concluded that for all practical purposes pairing in autotetraploid sorghum behaves in the fashion characteristic of that described for autotetraploids in general by Morrison and Rajhathy.

In Table 1 it can be seen that pairing in autotetraploid sorghum is almost wholly II and IV, with III and I occurring with a very low frequency. This phenomenon is characteristic of nearly all autotetraploids reported in many species (20), and therefore bears little or no relationship to the preferential pairing phenomenon in Sorghum vulgare as suggested by Sanders and Franzke (29).

It has been reported that polyploidy is often associated with a decrease in chiasma frequency as a result of greater competition in chromosome pairing blocks (34). However, Chin (3) has shown that in sorghum there is no significant change in chiasma frequency as chromosome

Table 1. Frequency of Chromosome Association at Diakinesis and Metaphase I.

Materials	No. of Cells	Average No. Per Cell of Uni-valents	Bi-valents	Tri-valents	Quadri-valents	Source
AUTOTETRAPLOIDS						
Experimental 3	50	0.9	9.9	0.06	4.7	Ross & Chen (25)
Experimental 3	53	1.2	12.8	0.04	3.3	Sanders & Franzke (28)
Experimental 3 x M 15	110	0.1	11.3	0.00	4.3	Ross & Chen (25)
M 15	52	0.3	11.0	0.05	4.4	Ross & Chen (25)
SA 403	70	0-3.0	14-18	0.08	0.5-3.0	Schertz (30)
Hegari		0.22	13.83	0.11	2.94	Chin (3)
$IDX_{25}/A/8/3\ BC_2$	25		11.44		4.28	Doggett (6)
$IDX_{35}/A/3/1\ BC_2$	25		11.12		4.44	Doggett (6)
F_6(27 U. T. x Wiru x Msumbiji)	420	0.15	7.46	0.09	6.17	Doggett (5)
Combine Kafir 60	263	0.57	9.02	0.25	5.11	Present Paper
AUTOTETRAPLOID-LIKE						
Sorghum almum	77	0.32	11.52	0.08	4.10	Endrizzi (7)

Table 1--Continued

Sorghum almum 16/5/7 P.13	25		13.40		3.48	Doggett (6)
Sorghum almum 9/1/B.P. 4	25		14.44		2.76	Doggett (6)
Kafir x *S. almum*	75	1.01	10.44	0.55	3.88	Endrizzi (7)
Kafir x *S. almum*	72	0.35	10.69	0.12	4.47	Endrizzi (7)
Kafir x *S. almum*	81	0.81	12.61	0.15	3.32	Endrizzi (7)
Common Sudan x *S. almum*	94	0.36	11.52	0.08	5.50	Endrizzi (7)
AUTOTRIPLOIDS						
White Collier Sorgo	24	1.79	1.79	8.20		Price & Ross (23)
F_2 (MS#2 Dwarf Tan Kafir x Redlan F_1)	24	1.58	1.46	8.50		Kidd (19)
S.D. 100	141	2.76	2.80	7.00		Erichsen & Ross (10)
AUTOTRIPLOID-LIKE						
Kafir x *S. almum*	85	1.65	1.65	8.35		Endrizzi (7)
Kafir x *S. halepense*	133	4.34	4.34	5.66		Hadley (16)

number is increased. He states that, "the correlation ($r = 0.22 \pm 0.22$) between chiasma frequency and the number of quadrivalents per cell indicates that quadrivalent formation has little effect on chiasma frequency." Chin listed the range and mean of chiasmata per cell found in the different levels of ploidy in Sorghum vulgare variety Hegari. These were as follows: in autooctavalents the chiasma frequency ranged from 79 to 93 with a mean of 84 ± 0.95, the chiasma frequency in quadrivalents ranged from 37 to 47 with a mean of 40.7 ± 0.67, and the chiasma frequency in diploids ranged from 20 to 25 with a mean of 21.7 ± 0.43. Therefore, based on the level of ploidy, the chiasmata frequency per chromosome is essentially the same in the 2n, 4n, and 8n forms of sorghum.

Sanders and Franzke (29) and Franzke and Sanders explained the origin of colchicine-induced diploid mutants in sorghum on the basis of chromosome substitution. They concluded that chromosome substitution would not be observed cytologically because there is a tendency toward bivalent rather than multivalent pairing to occur in diploid sorghum (2n = 20).

In a cytological study by Sanders and Franzke (28) of a tetraploid plant from the variety "Experimental-3," they reported means of 3.3 quadrivalents, 0.04 trivalents, 12.8 bivalents, and 1.2 univalents per cell. In this case the multivalent mean is lower than the means reported for

autotetraploids in most studies including the present one (Table 1). On the basis of their data and certain other data on tetraploid, triploid, and diploid sorghums, it can be pointed out that there are indications of a bivalent pairing mechanism which may operate at certain chromosome levels in sorghum and suppress formation of multivalents where they would otherwise be expected. However, these indications are not always verified in the literature.

They suggest that the lack of multivalent formation of the duplicated chromosome in the supposed substitution lines may be due largely to the following: (1) "pairing of analogous chromosomes may be limited chiefly to cases where a homologue is missing. Such phenomenon could be designated as preferential pairing . . . (2) the tendency for bivalent pairing may be positively correlated with the presence of an even number genome, i.e., diploids and tetraploids . . . ."

It is upon such evidence that the hypothesis of analogous chromosome was proposed to explain the formation of true-breeding mutants from true-breeding seedlings in sorghum.

MATERIALS AND METHODS

A cultivated grain sorghum, variety Combine Kafir 60, was used in this study. The seeds were disinfected with a ten per cent chlorox solution. The seeds were then germinated on a two per cent bacto-streptomycin water agar (4).

The procedure used in applying colchicine to the diploid sorghum seedlings to produce autotetraploids involved a modification of the procedure used by Nielsen and Drolsom (22). Disinfected seeds were germinated on a 1/4 to 1/2 inch layer of two per cent bacto-streptomycin water agar in petri dishes. When the coleoptiles reached a length of 1/2 to 3/4 inch, an additional layer of cool (semi-fluid) water agar was added to protect the base of the coleoptiles and roots from colchicine. Between 16 and 24 hours after the addition of the second layer of agar, the exposed coleoptile tips were decapitated with fine scissors. The seedlings were divided into three lots and treated with 0.05, 0.1, and 0.2 per cent aqueous solution of colchicine which was poured over the agar surface until the cut coleoptiles were submerged. The dishes were then placed in a vacuum desiccator and aspirated. The vacuum used was that created by pulling a column of mercury to a height of 20 inches in a glass tube. The vacuum was then

released slowly and the same procedure was repeated a second time. This method enabled the colchicine solution to be drawn inside the coleoptile to make contact with the shoot primordium. After the second evacuation the colchicine solution was poured off and the seedlings were rinsed with distilled water. The dishes were covered and the seedlings remained in the laboratory for 24 hours before being transplanted to individual plant pots in the greenhouse.

A mixture containing one part peat moss and one part perlite was used to grow the seedlings in the greenhouse. This mixture was placed in plant pots and moistened thoroughly before transplanting the seedlings. The seedlings were grown in the greenhouse at as near normal growth conditions as possible for a period of four weeks, and those that survived were transplanted to the field eight to twelve inches apart in 40 inch rows. Observations were made periodically during the growing season to detect plants that possessed certain gigas characters. When a plant was observed that was different from the normal or control, it was tagged for further observations. All tagged plants were studied cytologically and selfed by placing a glycine bag over their inflorescence. The tagged plants that were found to be tetraploids are referred to in this study as plants A, B, C, D, and E. For cytological studies panicle branches were collected and fixed in a 3:1

ethyl alcohol-glacial acetic mixture. Twenty-four hours after fixation they were placed in a 70% alcohol solution for storage until cytological examination. The standard propionic carmin-squash technique was used for staining of pollen mother cells for cytological studies. Cytological analyses were made at metaphase-I which is the best stage for critical analyses of chromosome conjugation.

A preliminary cytological analysis was conducted on the raw autotetraploids for the purpose of learning how to differentiate the various multiple and bivalent configurations so as to be fairly well acquainted with shapes and forms of univalents, bivalents, trivalents, and quadrivalents chromosome configurations in sorghum when making the final analyses.

In the final analysis, counts were made from slides containing well-spread cells. This allows the scoring of nearly all cells on a slide. Whenever there was a question whether a cell contained, for example, 4 or 5 quadrivalents, the lesser number was recorded for that cell. This was done to avoid as much as possible any bias toward a higher frequency of quadrivalents per cell.

The plants that were found to be tetraploids from cytological examination were transferred in the fall to the greenhouse for additional cytological and morphological studies.

OBSERVATIONS AND RESULTS

A total of 589 seedlings were treated with three different concentrations of anaqueous colchicine solution (0.05, 0.1, and 0.2 per cent). The number of seedlings per treatment, the number that reached maturity, and the number of tetraploids produced are presented in Table 2.

Table 2. Results of Colchicine Treatment.

Treatments	Total Number of Seedlings Treated	Number of Seedlings Reaching Maturity	Per Cent Maturing	Number of Tetra-ploids
0.05% Colchicine	186	141	75.80	0
0.1% Colchicine	191	131	68.58	2
0.2% Colchicine	212	98	46.20	3
TOTALS	589	370		5

Of the 589 seedlings treated with colchicine only 370 survived to maturity. Of these only five were identified morphologically and cytologically as tetraploids. In the population that survived the 0.05 per cent concentration, no tetraploids were detected. The five tetraploids were recovered from the 0.1% and 0.2% treated material, two in the 0.1% treatment and three in the 0.2% treatment.

In the treated seedlings, one of the most notable effects of the colchicine was swelling of the coleoptile. Many seedlings exhibited retarded growth or rosette formation for two to four weeks after transplanting. These characteristics were most common in the 0.1% and 0.2% treatments. The rosette stage was followed by a rapid surge of growth.

At maturity, the autotetraploid plants were characteristically different from all other plants. They possessed certain gigas characters, particularly in their leaves which were thicker and coarser-textured than leaves of diploid plants. The autotetraploids were also slightly shorter, and had many tillers. Stomata of both diploids and tetraploids were examined microscopically but no observable differences were noted.

Meiosis was studied in approximately fifty pollen mother cells of normal, non-treated plants and no ireegularities were found; all chromosomes were paired as bivalents.

Cytological studies of meiosis were made on the five autotetraploid sorghum plants. Several meiotic stages were observed but all chromosome counts were made at metaphase I. Two hundred and sixty-three cells were analyzed. Although there was a certain amount of variation in chromosome pairing between plants, the mean number of configurations per cell per plant, however, was very

similar (Table 3). The mean number of univalents was higher in plant C than in the other plants. In plant A, one cell had eight univalents.

Since the mean number of univalents per cell was approximately one or less than one, the number of lagging chromosomes would be expected to be at a minimum at anaphase I in all plants. This was observed to be the case.

The quadrivalent frequency observed in the present study is higher than that reported by most workers but lower than that reported by Doggett (5) for one particular auto-tetraploid sorghum (Table 1).

The quadrivalent configurations observed at metaphase I were rings and chains exhibiting alternate or adjacent co-orientation (Figure 1 A, B, C, D). There were nine cells with an association of 4 II and 8 IV. The maximum association observed was 2 II and 9 IV (Figure 1 A), which was found in one cell in the preliminary study and therefore not recorded in Table 3.

Figures 1 A through 1 D are camera lucida drawings of metaphase I of the tetraploid plants and contain respectively, 2 II + 9 IV, 1 I + 6 II + 1 III + 6 IV, 8 II + 6 IV, and 6 II + 7 IV. Figures 2 A through 2 D are photomicrographs of the 4n plants showing respectively, 1 I + 6 II + 1 III + 6 IV, 8 II + 6 IV, 12 II + 4 IV, and 2 I + 9 II + 5 IV.

Table 3. Association of Chromosomes at Metaphase I in Colchicine-Induced Autotetraploid Sorghum vulgare.

4n Plants*	Chromosome no.	No. of Cells	Univalents		Bivalents		Trivalents		Quadrivalents		Percentage of Chromosomes in Multivalents
			Range	Mean	Range	Mean	Range	Mean	Range	Mean	
A	40	27	0-8	0.777	6-14	9.074	0-3	0.407	3-7	4.936	52.68
B	40	103	0-3	0.437	4-16	9.311	0-3	0.262	2-8	5.010	52.06
C	40	67	0-6	1.134	3-16	8.433	0-3	0.327	2-8	5.135	53.80
D	40	19	0-2	0.316	6-16	9.210	0-3	0.210	2-7	5.158	53.15
E	40	47	0-2	0.191	4-14	9.042	0-2	0.149	3-8	5.319	54.30
Combined		263		0.597		9.008		0.270		5.103	53.75

*Plants B and D resulted from 0.1% colchicine treatment and plants A, C, and E resulted from 0.2% colchicine treatment.

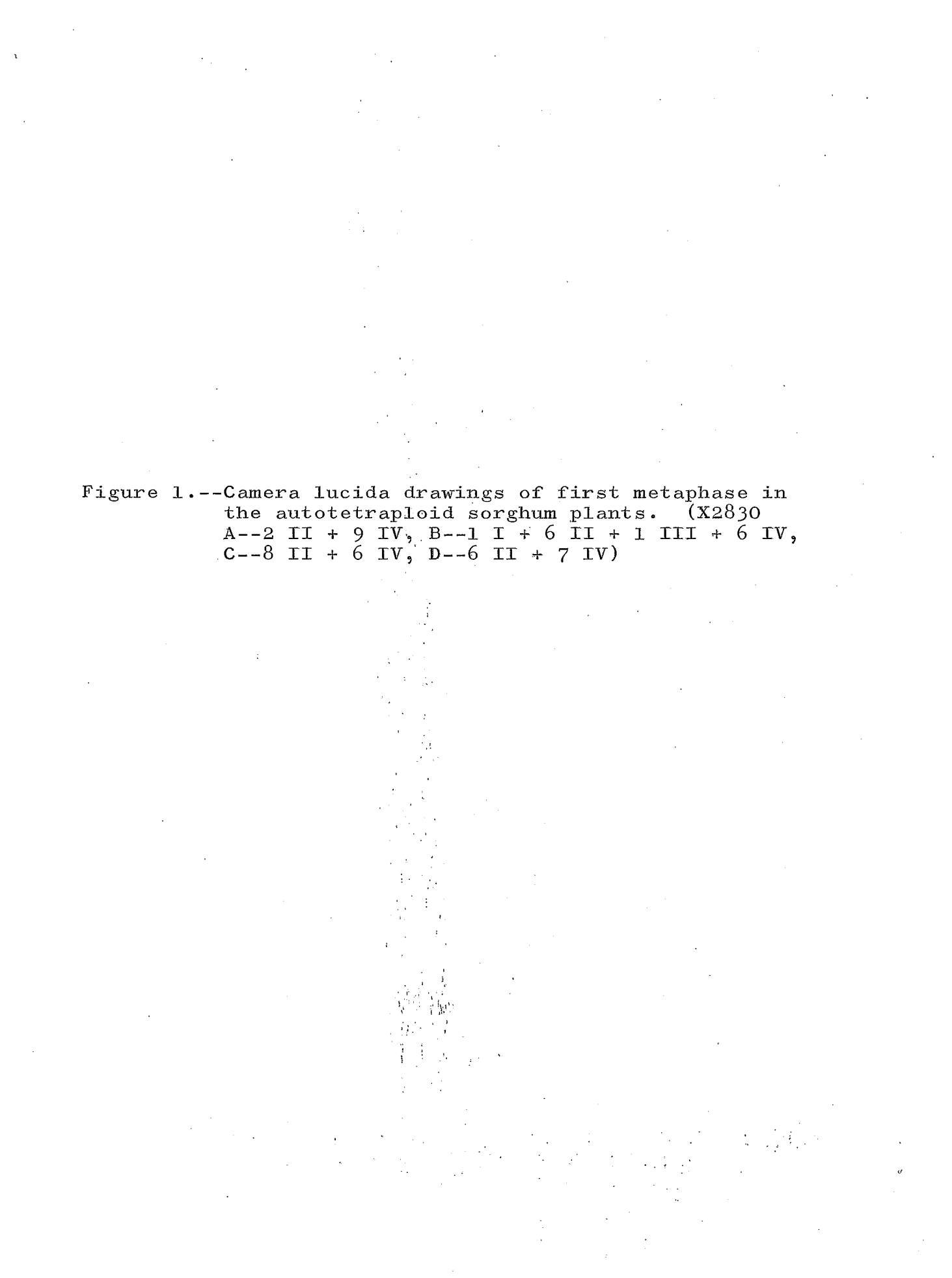

Figure 1.--Camera lucida drawings of first metaphase in the autotetraploid sorghum plants. (X2830 A--2 II + 9 IV, B--1 I + 6 II + 1 III + 6 IV, C--8 II + 6 IV, D--6 II + 7 IV)

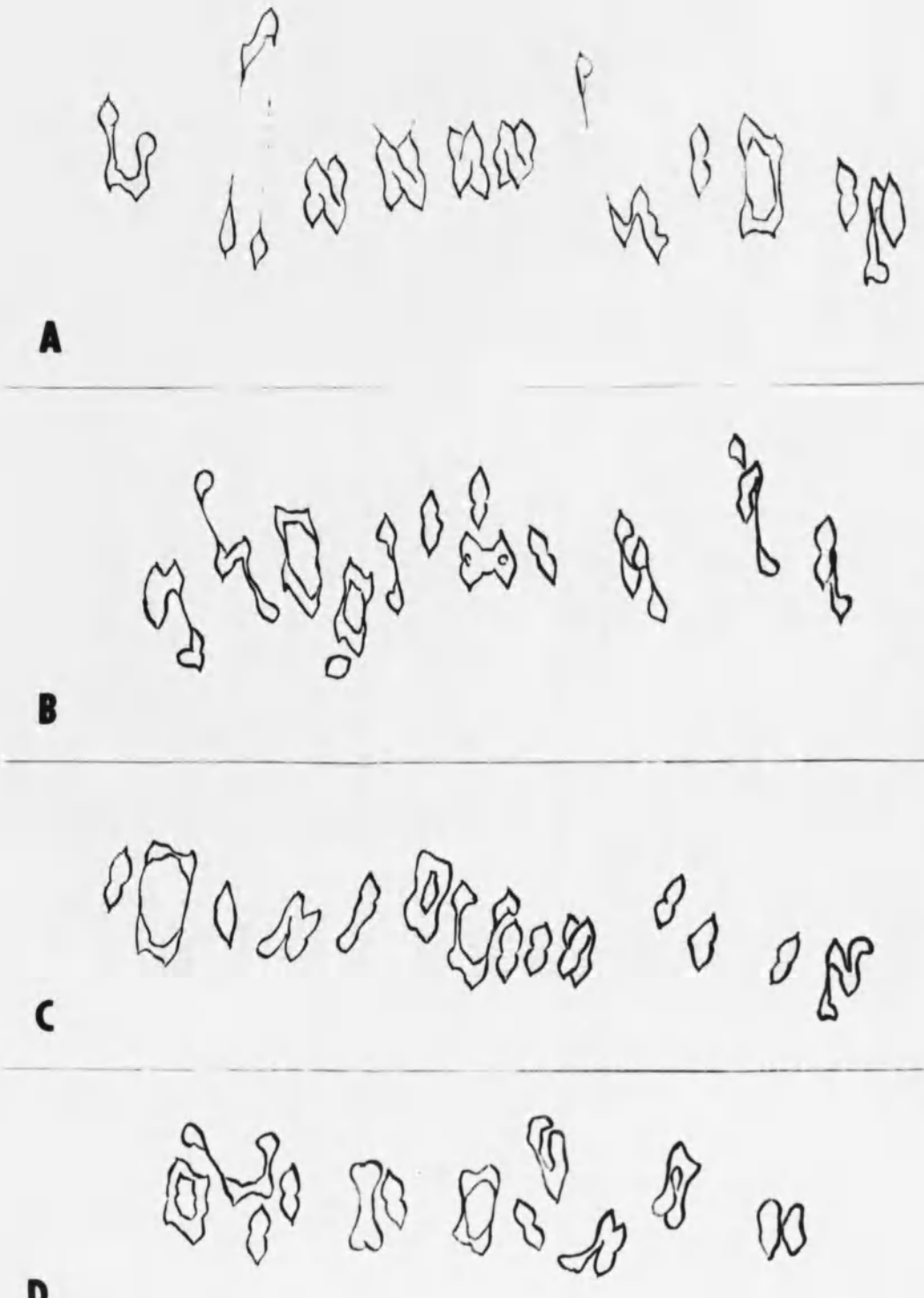

Figure 1. Camera Lucida Drawings of Metaphase I Associations in the Autotetraploid Sorghum Plants.

Figure 2.--Photomicrographs of first metaphase in the autotetraploid sorghum plants. (X97 A--1 I + 6 II + 1 III + 6 IV, B--8 II + 6 IV, C--12 II + 4 IV, D--2 I + 9 II + 5 IV)

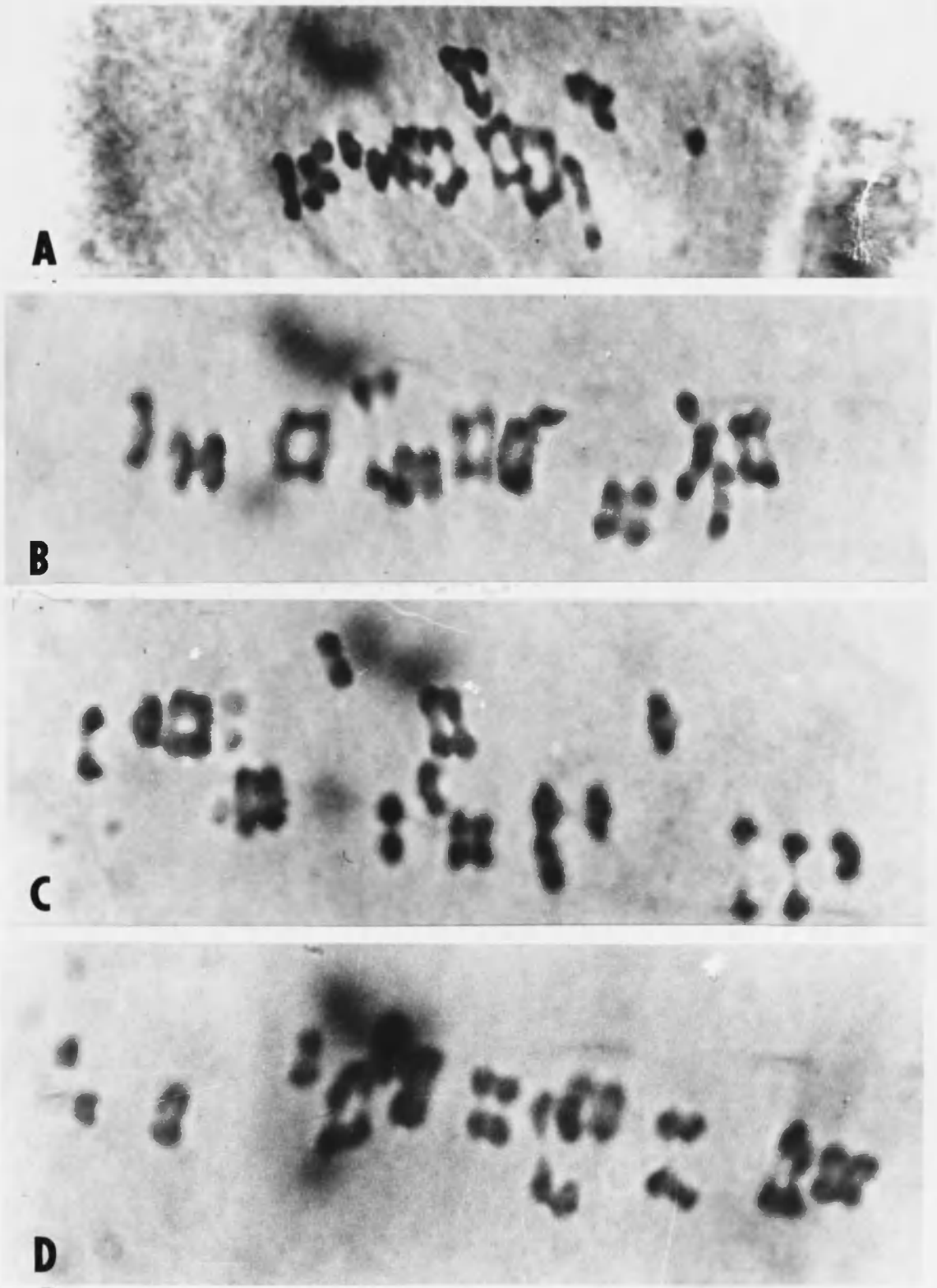

Figure 2. Photomicrographs of Metaphase I Associations in the Autotetraploid Sorghum Plants.

In autotetraploids pairing within each set of four homologous chromosomes is predominately two bivalents or a quadrivalent. It can be seen in Tables 1 and 3 that pairing in autotetraploid sorghum is almost exclusively II's and IV's. Thus based on random pairing, one would expect four homologues to form equal frequencies of bivalents and multivalents, i.e., any four homologs would be expected to pair half of the time as two bivalents and the other half of the time as a quadrivalent. Therefore, the expected distribution of multivalents in a sample of cells can be calculated with the binomial $(a + b)^{10}$, or where a = bivalent frequency = 1/2, b = the multivalent frequency = 1/2 and the power 10 is the total number of groups of homologous chromosomes.

Table 4 shows the results of the Chi square test of goodness of fit for the binomnal distribution. This test was made to determine whether the distribution of the sample sum was of a binomnal nature, which was found to be the case. The X^2 values for classes 3 and 6 multivalents per cell were the only values that were statistically significant at the 0.05 level. The remaining classes follow a normal distribution and were not statistically different from the expected values. However, total X^2 is significant, which is due to a slight but detectable shift to a higher frequency of multivalent than expected on the basis of random formation. Interpretation of statistical data

Table 4. Chi Square Test of Goodness of Fit for Binomial Distribution

Number of Multivalents Per Cell	Observed Number of Cells	Expected Number of Cells	Chi Square Values
0	0	0.2568	0.2568
1	0	2.5684	2.5684
2	9	11.5457	0.5613
3	8	30.8236	16.8999*
4	57	53.9413	0.1734
5	64	64.7243	0.0081
6	69	53.9413	4.2039*
7	39	30.8236	2.1689
8	17	11.5457	2.5767
9	0	2.5684	2.5684
10	0	0.2568	0.2568
TOTAL	263	262.9959	32.2426*

*Significant at the 0.05 level

Mean number of multivalents per cell =

$$\frac{\text{Total number multivalents}}{\text{Number of cells}} = \frac{1413}{263} = 5.37$$

also showed that the observed sample mean of 5.37 multivalents per cell was not statistically different from the expected mean of 5.0 multivalents on the basis of random pairing of the homologous chromosomes. These data show that in the autotetraploid sorghum the chromosomes pair randomly, and that there is no evidence for preferential bivalent pairing. In fact the data show that there is preferential bivalent pairing. In fact the data show that there is preferential multivalent formation.

Randomness of chromosome pairing was shown above to occur in the autotetraploid sorghums. In the present study, the mean number of multivalents varied between plants from 5.268 to 5.430 per cell which when concerted to a per cent of the total number of chromosomes in multivalents is 52.68 to 54.30 per cent. This clearly shows that random pairing is the rule in Sorghum vulgare and preferential pairing is an exception.

Figure 3 is a graphic representation of the distribution of observed and expected frequencies of multivalents per cell in the colchicine-induced autotetraploid sorghums. The graph shows that the expected and observed distribution curves are very similar.

Cytological chimeras have been reported to occur in sorghum plants following the application of colchicine to the growing coleoptile (11, 13). In the present study cytological examination of meiosis of pollen mother cells

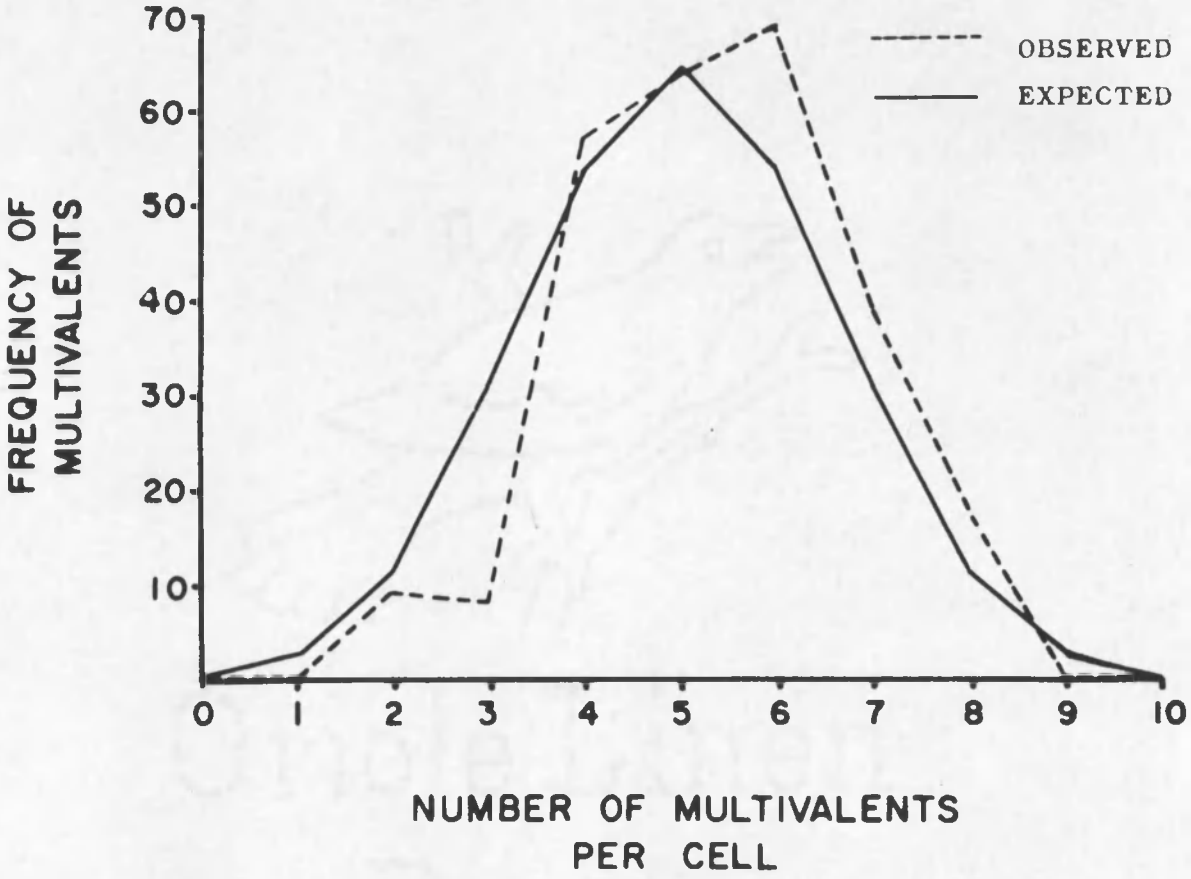

Figure 3. Distribution of the observed and expected frequencies of multivalents in autotetraploid sorghum.

from the main stalk of the E tetraploid plant showed forty chromosomes, whereas additional examinations of pollen mother cells taken later from a tiller showed the diploid chromosome number of twenty. This indicates that the plant was a chimera, which can be expected from coleoptile treatment. Twelve seeds harvested from self-pollination of tillers on three tetraploid plants B, C, and D were germinated and grown in the greenhouse to make cytological examinations on the C_1 generation. Six of the D plants were examined cytologically and all showed $2n = 20$ chromosomes. This high frequency of diploids strongly indicates that the D tetraploid plant was also a chimera.

DISCUSSION

Results presented in Tables 1 and 3 show that in autotetraploid sorghum, as in most other autotetraploid species, pairing of a group of four homologous chromosomes is almost exclusively two bivalents or a quadrivalent. In the present study, this form of pairing of the four homologous chromosomes of sorghum was shown to occur randomly, i.e., approximately one-half of the time the four homologs formed two bivalents and the other half of the time they formed a quadrivalent, with a slight but significant tendency for quadrivalent formation. This suggests that whenever one or more chromosomes are duplicated, multiple associations at Metaphase I should be present at a high frequency (at least in 50% of the cells) in any sample of cells, even in the diploid sorghum mutants.

Sanders and Franzke (29) hypothesis for the origin of diploid mutants is that homologous chromosomes are substituted for homeologous chromosomes with pairing being limited to cases where a homologue is missing. This suggests a trend toward preferential pairing as opposed to random pairing; such pairing results in plants with all bivalents and no multivalents at the diploid level which seems to be highly improbable on the bases of random pairing.

Since the results presented in the present study show that pairing of any four homologous chromosomes is predominantly two bivalents or a quadrivalent with each occurring with about an equal frequency, it is difficult to visualize from the known information on mechanism controlling synapsis how the mere substitution of one or more chromosome can limit synapsis and exchange to twos only, and completely preventing multiple associations.

Structural changes in chromosome leading to rearrangement in the order of genes are grouped into four classes: (a) deficiencies, (b) duplications, (c) inversions, and (d) translocations. The first three as a rule affect only a single chromosome whereas translocations may involve one, two, or more chromosomes.(33).

When a translocation ring or chain of four chromosomes reaches the metaphase plate, three arrangements are possible, and each has its own genetic consequences. The three arrangements are (a) adjacent-1, (b) adjacent-2, and (c) alternate. In the two adjacent orientations, adjacent chromosomes go to the same anaphase pole and the gametes formed are both deficient and duplicated for certain regions of the chromosomes, leading to inviability of gametes. The alternate arrangements differ in that alternate chromosomes go to the same anaphase pole forming two kinds of gametes, one with a normal set of chromosomes, and the other with the translocated set. Neither gamete is deficient or

duplicated; therefore gamete viability is not affected. Generally, sterility is approximately 50 per cent in reciprocal translocation heterozygotes of diploids (2).

Huang et al. (18) induced three reciprocal translocations in Sorghum vulgare variety Experimental 3. Each of these translocations when homozygous had no effect on pollen viability and seed set. However, plants that were heterozygous for any one of the reciprocal translocations were semisterile in both male and female (18, 31, 33). For each translocation approximately 50% viable pollen and 50% seed were observed, indicating that duplications and deficiencies occurred in that frequency.

Simantel and Ross (32) reported that plants heterozygous for two reciprocal translocations were completely sterile and set no seed. Endrizzi and Morgan (8), working with a reciprocal translocation in Sorghum vulgare, also observed a rather high per cent of pollen abortion (81.6%), which was almost the same frequency as the adjacent disjunction (83.8%). This shows that duplicate-deficiency chromosome structural types from reciprocal translocations in sorghum leads to gamete inviability.

All of these data point to one conclusion, namely that gametes containing duplications and deficiencies for only parts of whole chromosomes do not function in sorghum.

In Sanders' and Franzke's explanation (29) for colchicine-induced mutants in sorghum, they hypothesized

that substitution of individual chromosomes and pairs of chromosomes for their homeologues took place. Furthermore, their plants apparently had a high, if not normal, seed set.

It stands to reason that if one chromosome or one pair of chromosomes is lost and is replaced by its homeolog the resulting gametes will be deficient and duplicated for the individual chromosome or whole pairs of chromosomes, depending on the type of reductional grouping. Then, on the basis of previous information where 50% sterility was observed in plants with single reciprocal translocations and complete sterility was observed in plants with two reciprocal translocations one would expect comparable amounts of sterility to occur as result of homelogous substitution. However, fertility in the colchicine-induced mutants explained by the hypothesis seemed to be little affected. This alone cast considerable doubt on the validity of the hypothesis of not actually negating it.

Furthermore if the substitution hypothesis was correct, then one would expect diploid sorghum plants to easily tolerate the monosomic condition. However, monosomic plants have never been reported in sorghum, and sorghum has been studied cytologically by many people in several countries.

It is concluded from the present study that the hypothesis advanced by Sanders and Franzke (29) has little merit in explaining the origin of the diploid mutants in sorghum. It is concluded that the hypothesis by Harpstead *et al.* (17) in which the diploid mutants are attributed to multiple point mutations which may also involve minute structural changes is the most logical explanation.

SUMMARY

The cytological behavior, with emphasis on the frequency of multivalent formation at metaphase I, was studied in a colchcine-induced autotetraploid Sorghum vulgare, Combine Kafir 60. This study was made in order to review the literature to check the validity of the hypothesis of the origin of diploid mutants as proposed by Sanders and Franzke (29), and to cytological analyze chromosome pairing in colchicine-induced autotetraploid sorghum.

Cytological chimeras were found which can usually be expected when coleoptiles are treated with colchicine.

A mean of 5.268 to 5.430 multivalents per cell was found in the autotetraploid sorghum plants. Therefore, at least 50% of all chromosomes in autotetraploid sorghum pair as multivalents.

There was no indication of a trend toward bivalent formation in autotetraploid plants in this study. However, there was a tendency for preferential formation of multivalents.

Interpretation of statistical data clearly shows that random pairing is the rule in Sorghum vulgare and preferential pairing is an exception.

Chromosome duplication-deficiency types of gametes resulting from adjacent disjunction in one or two

heterozygous reciprocal translocations in sorghum causes 50% and 100% sterility respectively. This in itself is evidence to negate the hypothesis for the origin of colchicine-induced mutants, since fertility in the colchicine-induced mutants explained by this hypothesis seemed to be little affected.

LITERATURE CITED

1. Atkinson, G. F., C. J. Franzke, and J. G. Ross. 1957. Differential reaction of two varieties of sorghum to colchicine treatment. Jour. Hered. 48:259-264.

2. Burnham, C. R. 1956. Chromosomal Interchanges in Plants. The Bot. Rev. 22:448-450.

3. Chin, T. C. 1946. The Cytology of Polyploid Sorghum. Amer. Jour. Bot. 33:611-614.

4. Difco Manual of Dehydrated Culture Media and Reagents for Microbiological and Clinical Laboratory Procedures. 1948. Eighth Edition Difco Laboratories Inc. Detroit, Michigan. p. 151.

5. Doggett, H. 1964. Fertility improvement in auto-tetraploid sorghum. I. Cultivated Autotetraploids. Heredity 19:403-417.

6. _____. 1964. Fertility improvement in auto-tetraploid sorghum. II. Sorghum Almum Derivatives. Heredity 19:543-558.

7. Endrizzi, J. E. 1957. Cytological studies of some species and hybrids in Eu-sorghum. Bot. Gaz. 119:1-9.

8. _____, and D. T. Morgan Jr. 1955. Chromosomal interchanges and evidence for duplication in haploid Sorghum vulgare. Jour. Hered. 46:201-208.

9. Erichsen, A. W., C. J. Franzke, Me E. Sanders, and J. G. Ross. 1962. Colchicine-induced mutations in sorghum which affects qualitative characters. Jour. Hered. 53:304-308.

10. _____, and J. G. Ross. 1963. A triploid derived from a selfed haploid sorghum plant. Crop Sci. 3:99-100.

11. Franzke, C. J., and J. G. Ross. 1952. Colchicine-induced variants in sorghum. Jour. Hered. 43: 107-115.

12. _____, and _____. 1957. A linear series of mutants induced by colchicine treatment. Jour. Hered. 48:46-52.

13. _____, M. E. Sanders, and J. G. Ross. 1960. Genetic studies of diploid nontrue-breeding mutants in sorghum. Agron. Abstr. p. 47.

14. Franzke, C. J., and M. E. Sanders. 1964. Classes of true-breeding diploid mutants obtained after colchicine treatment of sorghum line E Experimental 3. Bot. Gaz. 125:170-178.

15. _____, and _____. 1965. True-breeding colchicine-induced mutants from sorghum hybrids. Amer. Jour. Bot. 52:211-221.

16. Hadley, H. H. 1953. Cytological relationship between Sorghum vulgare and Sorghum halepense. Agron. Jour. 45:139-143.

17. Harpstead, D. D., J. G. Ross, and C. J. Franzke. 1954. The nature of chromatin changes of colchicine-induced variants in sorghum. Jour. Hered. 45:254-258.

18. Huang, C. C., J. G. Ross, and H. D. Haensel. 1963. Reciprocal translocations in Sorghum vulgare. Can. Jour. Genet. Cytol. 5:227-232.

19. Kidd, H. J. 1952. Haploid and triploid sorghum. Jour. Hered. 43:204.

20. Morrison, J. W., and T. Rajhathy. 1960. Frequency of quadrivalents in autotetraploid plants. Nature 187:528-530.

21. _____, and _____. 1960. Chromosome behavior in autotetraploid cereals and grasses. Chromosoma 11:297-309.

22. Nielsen, E. L., and P. N. Drolsom. 1964. Nuclear variability in shoot primordial cells of sorghum. Crop Sci. 4:328-331.

23. Price, M. E., and W. M. Ross. 1957. Cytological studies of a triploid x diploid cross of Sorghum vulgare Pers. Agron. Jour. 49:237-240.

24. Ross, J. G., C. F. Franzke, and L. A. Schuh. 1954. Studies of colchicine induced variants in sorghum. Agron. Jour. 46:10-15.

25. _____, and C. H. Chen. 1962. Fertility differences in autotetraploid sorghum. Hereditas 48:324-331.

26. Sanders, M. E., C. J. Franzke, and J. G. Ross. 1959. Influence of environmental factors on colchicine-induced true-breeding diploid mutants in sorghum. Amer. Jour. Bot. 46:119-125.

27. _____, _____, and _____. 1962. Genetic studies of nontrue-breeding mutants in sorghum obtained after colchicine treatment. Crop Sci. 2:387-390.

28. _____, and _____. 1962. Somatic reduction of tetraploid sorghum to diploid mutants following colchicine treatment. Nature 196:696-698.

29. _____, and _____. 1964. A proposed explanation for the origin of colchicine-induced diploid mutants in sorghum. Jour. Arnold Arbor. 45:36-56.

30. Schertz, K. F. 1962. Cytology, fertility and gross morphology of induced polyploids of Sorghum vulgare. Can. Jour. Genet. Cytol. 4:179.

31. Simantel, G. M., J. G. Ross, C. C. Huang, and H. D. Haensel. 1963. Colchicine-induced somatic chromosome reduction in sorghum. II. Induction of plants homozygous for structural chromosome markers originally heterozygous in two pairs. Jour. Hered. 54:221-228.

32. _____, and _____. 1963. Colchicine-induced somatic chromosome reduction in sorghum. III. Induction of plants homozygous for structural markers in four pairs. Jour. Hered. 54:277-284.

33. Swanson, D. P. 1957. Cytology and cytogenetics. Prentice-Hall, Inc. Englewood Cliffs, New Jersey. 175-176.

34. Upcott, M. 1939. The nature of tetraploidy in Primula kewensis. Jour. Genet. 39:79-100.