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Linkage Between Chromosomal Interchanges and Colchicine Mutated Genes in Sorghum Vulgare

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LINKAGE BETWEEN CHROMOSOMAL INTERCHANGES AND COLCHICINE MUTATED GENES IN *SORGHUM VULGARE*

BY

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

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LINKAGE BETWEEN CHROMOSOMAL INTERCHANGES AND COLCHICINE MUTATED GENES IN *SORGHUM VULGARE*

Abstract

JOHN ROSS DEAKIN

Under the supervision of Professor James G. Ross

In order to investigate linkage relationships between chromosomal interchanges and a number of colchicine mutated genes, crosses were made between three homozygous interchange lines and two mutant lines. Two of the interchange lines, T165 and T231 had a common chromosome pair and the third, T396 involved the chromosome pair which carried the nucleolar organizing region. The two mutant lines, Grass III and Winner, appeared as true-breeding diploid mutants following colchicine treatment of the sorghum variety, Experimental 3.

F_2 progenies of crosses of the mutants with homozygous interchange lines were grown and certain genes, which were segregating, were tested for linkage with the interchange points. The genes studied in Interchange X Grass III progenies were those determining coleoptile color, presence of awns, purple spotting, dry or juicy midrib, plant height, number of tillers, and length of panicle branch. Genes which were investigated in Interchange X Winner progenies were those determining seed color, presence of awns, dry or juicy midrib, and number of tillers. Linkage was detected between the T231 interchange and two genes, coleoptile color (Rs,rs) and the midrib character (D,d). It was determined that these two genes probably were located on either side of the interchange point with the following recombination values:

Rs(19.1)T231(7.2)D. Linkage was also detected between the two genes with 22 per cent recombination. Evidence was obtained for linkage between a gene determining seed color and the interchange point in T396 with a crossing-over percentage of 19.0. No conclusive evidence was obtained for linkage between other genes or between any of these and the interchange points.

The interchange lines used in this study made possible the identification of five chromosome pairs. It was found that the genes for coleoptile color and the midrib character were located on a chromosome involved in the T231 interchange, but not common to the T165 interchange. A gene for seed color was located on one of the chromosomes involved in the T396 interchange. These interchanges, acting as structural markers, provide a basis from which future cytogenetic studies in sorghum can be made. Further interchange lines could be produced which would make possible the identification of all ten chromosome pairs.

The information on linkage was used to make an estimate of the number of chromosomes which carried mutated genes in the Grass III mutant. The genes for coleoptile color and the midrib were located on a specific interchange chromosome. Since the genes for awns and purple spots were not linked to interchange points, they were assumed to be on chromosomes other than those involved in the interchanges. Three genes for height were segregating and, since they are known to be independent and were not found linked to any other character in this study, they probably are located on at least two additional chromosomes. It would

appear that at least four chromosome pairs are required to carry these genes and, since this was a minimum estimate, it was not unlikely that six chromosome pairs were involved.

Since it appeared that at least four chromosomes carried mutated genes, this mutant and by inference other colchicine-induced mutants in sorghum probably have not arisen as a result of substitution of chromosomes from one genome to another (assuming that sorghum is an allotetraploid). A more reasonable explanation might be gene mutation in conjunction with somatic chromosome reduction.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Although the genetic behavior of sorghum has been studied since 1916, it has never been as extensively investigated as certain other species. Information on the cytogenetics of sorghum has been slow in accumulating, chiefly because of the difficulty in distinguishing between the chromosomes.

The use of chromosome structural markers as aids in cytogenetic studies has been suggested by Brink and Cooper (7) and Burnham (9). Chromosomal interchanges have been used successfully in some crops to determine which chromosome pair carried specific genes or groups of genes. A number of interchange lines were developed in the sorghum variety, Experimental 3, by Haensel (18) who used gamma irradiation to obtain breakage of the chromosomes. Three of these lines were later selected for use as marker stocks by Huang (19) on the basis of their meiotic behavior. These interchanges provide the possibility of determining a location of the colchicine mutated genes previously studied by Foster (15), Chen and Ross (12), and Erichsen et al. (14).

The purpose of this study was two-fold. The first objective was to test linkage relationships between the mutant genes and the break points in the interchange lines, thus determining which chromosomes carried the particular mutant genes. In addition it was desired to test for the presence of linkage between the mutant genes themselves.

The second objective was to gain information on the action of colchicine in producing true-breeding mutants. It was assumed that if chromosome substitution were responsible, the characters which were

changed would be limited to one or, at most, a small number of chromosomes. If the action of colchicine were a result of somatic reduction following gene mutation, the genes affected might be expected to be scattered at random throughout the chromosome complement. Tests for linkage between the genes and the chromosome markers should provide evidence for one of these hypotheses.

LITERATURE REVIEW

Semi-sterility

The occurrence of semi-sterility was noted by Belling (3) when a cross was made between two species of Stizolobium. In the F_1 generation, all the plants had 50 per cent of the pollen and embryo-sacs aborted, while in the F_2 half the plants showed 50 per cent abortion of the pollen and embryo-sacs and the remaining half were normal. It was assumed that this phenomenon was caused by a genetic factor K. A later investigation by Belling and Blakeslee (4) in Datura showed that non-homologous chromosomes could exchange segments. Burnham (9) reported that this discovery led Belling to the conclusion that the semi-sterility observed in Stizolobium was due to an interchange between non-homologous chromosomes.

Semi-sterility in maize was first reported by Brink (5) and was believed due to an interchange between non-homologous chromosomes. The interchange occurred naturally and was found during an investigation of the waxy character in which heterozygous plants were identified by staining the pollen with iodine.

Brink and Burnham (6) observed that crosses between semi-sterile and normal plants produced progeny which segregated in a ratio of one normal to one semi-sterile. Studies by Burnham (8), McClintock (30), and Brink and Cooper (7) indicated that the semi-sterile line was indeed the result of an interchange of terminal segments between non-homologous chromosomes. The abortion was the result of 50 per cent of the spores having received deficiencies and duplications of chromosome segments.

The functional spores consisted of two classes, one having received a normal set of chromosomes, while the other received a set bearing the interchange complement.

The use of interchanges for linkage studies was proposed by Anderson (2). He pointed out that interchanges might provide a tool for the mapping of chromosome regions which carry no known genes and that they also make possible a direct test for linkage with a specific point on the chromosome.

Burnham (8), using an interchange in maize involving chromosomes VIII and IX, was able to show that genes in both linkage groups were linked to the semi-sterility factor. By using recombination values he was able to establish the location of the genes on the chromosomes as well as their linear order.

Burnham and Cartledge (10) and Saboe and Hayes (37) studied the inheritance of smut resistance in corn using interchanges. They crossed susceptible interchange lines with a resistant normal line and a susceptible normal line, and found that the susceptibility of the F_1 progeny was about the same as that of the normal lines. Crosses were then made between the resistant normal and the interchange lines and the F_1 progeny from these crosses were backcrossed to the susceptible normal line. In the backcross progenies a 1:1 ratio of normal to semi-sterile plants was obtained and, since susceptibility entered the cross with the interchange, linkage would be indicated by a significantly higher percentage of smutted plants in the semi-sterile class. It was found that at least one factor for smut resistance was located on

chromosome II, and there was good evidence that chromosomes I, VI, and VIII also carried genes for resistance.

Nilsson (32) used the N.III interchange in Pisum as a marker to determine the relationship of various linkage groups. He was able to show that some genes were closely linked to the interchange point, and also that some previously studied linkage groups were linked together. The latter case was thought to be due to the action of the interchanges as crossing-over suppressors.

Miller (31) used a series of interchanges to study the inheritance of oil content in the maize kernel as suggested by Anderson (2). Miller's study was an attempt to determine whether oil content was conditioned by a large number of genes with small, but approximately equal effect or by a smaller number of genes having a larger and possibly unequal effect. In his analyses of backcross populations, he found that the difference between the oil content of normals and semi-steriles was usually quite small and from this he concluded that oil content was conditioned by a large number of genes distributed more or less at random among the chromosomes.

Kramer et al. (24) tested nine different interchange lines in barley for linkage with known linkage groups. The results of this study indicated that two linkage groups (III and VII), which had been previously considered independent, were in fact located on the same chromosome. They were also able to show that certain of the known linkage groups were located on specific chromosomes. Andersen (1), also working with barley interchanges, was able to establish a correspondence between linkage groups and specific chromosomes.

Chang (11) using interchanges in rice was able to detect linkage between semi-sterility and several loci which included quantitatively inherited factors as well as simply inherited genes.

Because of the difficulty of obtaining backcrosses in some species, Joachim (22) developed formulae for the expected genotypic and phenotypic frequencies in F_2 populations involving linkage between semi-sterility and a qualitative character showing a 3:1 segregation. In a study with barley, she was able to show linkage between the interchange and at least one of the characters studied.

Mather (28,29) developed a method for partitioning X^2 in linkage analysis. When two genes are being considered and all classes are viable, there are three degrees of freedom associated with the four phenotypic classes. Mather proposed that the three degrees of freedom be partitioned in the following manner: one degree of freedom for the segregation of gene A, one degree of freedom for the segregation of gene B, and the remaining degree of freedom for linkage. The linkage X^2 is obtained by subtracting the X^2 values for gene A and gene B from the total X^2, or it can be calculated directly.

Lamm (25,26) adapted this procedure for use in translocation work. In his studies with Pisum, he used semi-sterility as a marker and substituted the X^2 value for the one to one segregation of normal to semi-sterile for the X^2 value of one of the two pair of genes proposed by Mather.

Sorghum Genetics

The inheritance of plant height has been extensively investigated because of its economic importance as an agronomic trait. From the study of an F_2 population Laubscher, as reported by Quinby and Martin (35), concluded that the inheritance of height in sorghum is complex, but a more recent study by Quinby and Karper (34) showed that height was conditioned by four pairs of independent genes plus a modifying complex. The four pairs of genes exhibited partial dominance with shortness being recessive to tallness. The action of these genes inhibits internode elongation without affecting the number of nodes.

The inheritance of awns in sorghum was first studied by Vinall and Cron (48). In a cross between Dwarf Milo and Feterita they found a segregation of 188 awnless to 68 awned plants and concluded from this that the awned condition was a simple recessive. In a more comprehensive study involving a number of genotypes, Sieglinger *et al.* (39) found that there were at least three alleles which controlled the presence of awns. Awnless (A) was found to be completely dominant to strong awns (a) and tip awns (a^t), and the strong awned condition exhibited partial dominance over tip awns. This explanation was necessary to account for the presence of the four phenotypic classes which were observed.

The stalks and midribs of sorghum are either dry or juicy. The dry or juicy character of both stalks and midribs is due to the same genetic mechanism which controls the amount of juice in the plant. Most of the grain sorghums have dry midribs and stalks and most of the forage types exhibit the juicy character. Varieties with dry stalks have a pronounced white stripe in the midrib, while those with juicy

stalks have a dull green midrib. Swanson and Parker (47) found that dry (D) is dominant to juicy (d) and that F_2 progenies segregated in a 3:1 ratio. Chen and Ross (12) observed a 15:1 ratio and proposed that the character was controlled by two factors (D_1d_1,D_2d_2).

The occurrence of a gene for coleoptile color was reported by Reed (36) in 1930. He found that red was dominant to green and that the F_2 progeny of a cross between a line with a red coleoptile and a line with a green coleoptile segregated in a 3:1 ratio. He found this marker useful in measuring the amount of natural crossing. He planted seed from a line with a green coleoptile which had been allowed to cross pollinate with a line with a red coleoptile. Any F_1 plants were easily identified in the seedling stage since they exhibited a red coleoptile.

The inheritance of seed color in sorghum is complex and is not yet fully understood. This complexity is due in part to the complex nature of the seed itself. At least three of the layers, the pericarp, the mesocarp, and the nucellar layer, have an influence on color, but the color or structure of the outer layers may mask the color of the layer beneath it. Swanson (46) proposed that the following factors were responsible for seed color: a factor producing colored or colorless pericarp (R,r), a factor producing a vestigial or a thick mesocarp (S,s), and a factor for the presence or absence of a colored nucellar layer (B,b). Vinall and Cron (48), Sieglinger (38), and Conner and Karper (13) have found that red seed is simply dominant to white seed; but, in these cases, it appears that they were dealing only with the R gene.

A character which was responsible for the presence of purple spots on sorghum leaves has been studied by Chen and Ross (12). This character appeared in three similar variant plants as one of a number of characters which had been changed by colchicine treatment of the variety Experimental 3. The presence of purple spots (Ps) was found to be dominant to the normal non-spotted condition (ps) and a normal 3:1 ratio was obtained in F_2 progenies. In addition to this character, they also studied the inheritance of awns, coleoptile color, and the juiciness of the midrib. They found that awns segregated 3 awnless:1 awned, and that segregation for coleoptile color was 3 red:1 green. The data obtained for the midrib character gave a 15:1 segregation for dry and juicy indicating that two genes had been mutated. These genes were given the designation D_1d_1, D_2d_2.

Another mutant was studied by Erichsen *et al.* (14). This mutant line, which has been released for commercial production as the variety "Winner," was derived from the progeny of a true-breeding mutant obtained after colchicine treatment of Experimental 3. The authors studied the inheritance of awns, seed color, and the midrib character and obtained 3:1 ratios as reported by other workers. Variations of color intensity indicated that the inheritance of seed color was not monogenic, but the presence of any modifying factors did not disturb the ratio of 3 colored:1 white.

The linkage relationships of sorghum have not been investigated as extensively as those in some other species, but at least seven linkage groups have been reported.

The Q-B-Gs group was studied by Stephens and Quinby (43). This linkage group included gene pairs for red or black plant color (Q,q), for presence or absence of brown nucellar layer (B,b), and for normal or green-striped plants (Gs,gs). Quinby and Martin (35) reported that another gene, either Bw_1bw_1 or Bw_2bw_2, appeared to be located in this group close to the Q locus. These two genes, when present in the dominant condition, were found to control the development of brown color in the epicarp and in the dry anthers.

The D-Rs-P group was reported by Stephens and Quinby (44). This group included factors for dry or juicy midribs and stalks (D,d), red or green coleoptile color (Rs,rs), and purple or brown stem color (P,p).

Stephens and Quinby (45) also studied the Ms_2-A-V_{10} group. The genes found in this linkage group were normal or male-sterile flowers (Ms_2,ms_2), awnless or awned lemmas (A,a), and green or virescent-yellow plants (V_{10},v_{10}). A gene which produced normal or green-striped plants (Gs_2,gs_2) was added to this group by Stephens (41).

Stephens (42) reported the Y-V_{11}-G_2 linkage group. Phenotypes found in this group were red or white seed color (Y,y), green or virescent-yellow plant color (V_{11},v_{11}), and green or golden plant color (G_2,g_2). Linkage between the gene for seed color (Y,y) and the maturity gene (Ma_3,ma_3) was reported by Quinby and Karper (33).

Quinby and Karper (34) also investigated the linkage relationship of the maturity gene (Ma_1) and the dwarf gene (Dw_2). The phenotypes were reported to be late or early (Ma_1,ma_1) and tall or short (Dw_2,dw_2).

Linkage between a gene for green or yellow seedlings (Y_2,y_2) and a gene for starchy or waxy endosperm (Wx,wx) was reported by Karper *et al.* (23). Another linkage group involving the factor pair for loose or compact head (Pa_1,pa_1) and the one that controls the thickness of the mesocarp (Z,z) has been investigated (35).

The seven linkage groups which have been established in sorghum appear to be independent. However, no evidence has been presented to show conclusively that two or more of them do not appear on the same chromosome.

MATERIALS AND METHODS

Plant Material

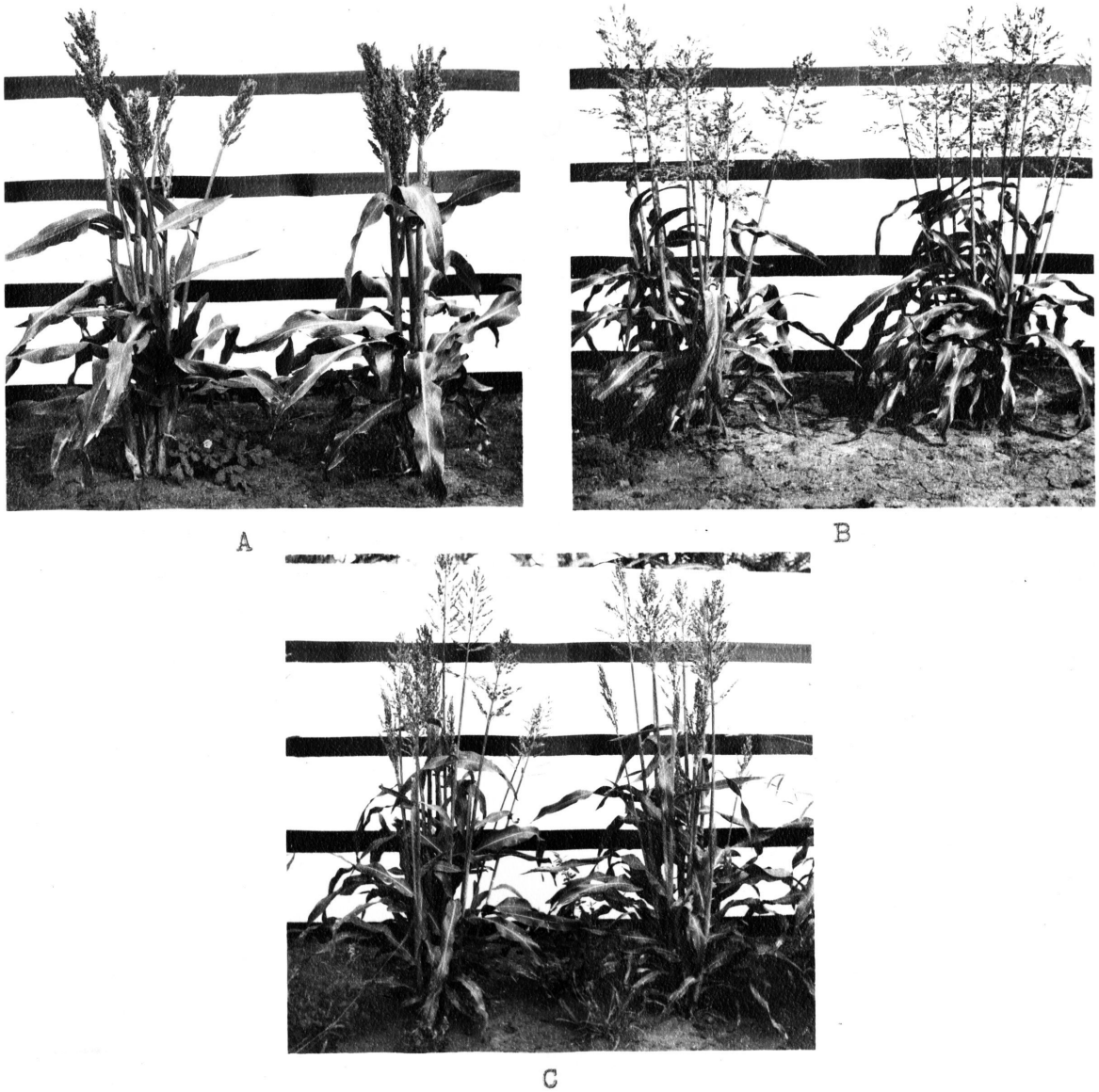
The plant materials used in this study consisted of three homozygous interchange lines obtained from the grain sorghum variety Experimental 3 which has been described by Franzke and Ross (16), two homozygous mutant lines which resulted from the treatment of Experimental 3 with colchicine, and F_2 and backcross progenies of crosses between mutant lines and interchange lines.

Haensel (18) obtained a number of plants which appeared to be heterozygous for interchanges following cobalt-60 irradiation of sorghum panicles in the preanthesis stage. Three of those plants were selected by Huang (19) on the basis of their consistent formation of rings and chains at diakinesis. These plants were then selfed and individuals homozygous for the interchange were selected and increased. The lines obtained from these plants appeared to be identical to Experimental 3 and were designated T165, T231, and T396. Typical plants from these lines are shown in Figure I, A and Figure II, A.

The mutant lines used in this study were Grass III (Figure I, B) and the variety Winner (Figure II, B). Both lines originated as immediately true-breeding mutants following colchicine treatment of Experimental 3. Grass III was studied by Chen and Ross (12) and the following genes were found to be changed: red coleoptile (RR) to green coleoptile (rr), dry midrib (DD) to juicy midrib (dd), awnless (AA) to awned (aa), and non-spotted leaf (pp) to purple-spotted leaf (PP). In addition to these simply inherited characters, Grass III had more

A B C

Figure I. Lines used in Studies of Interchange X Grass III Progenies. (A) Homozygous Interchange Plants, (B) the Grass III Mutant, and (C) Interchange X Grass III F_1 Hybrids.



A B C

Figure II. Lines used in Studies of Interchange X Winner Progenies. (A) Homozygous Interchange Plants, (B) the Winner Mutant, and (C) Interchange X Winner F_1 Hybrids.

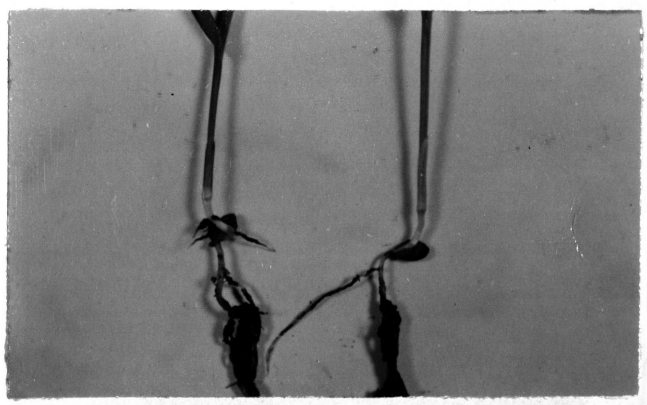
tillers, longer panicle branches, and was taller than Experimental 3. Winner was studied by Erichsen *et al.* (14) to determine the mode of inheritance of three genes which had been changed. These changes were as follows: dry midrib (DD) to juicy midrib (dd), awnless (AA) to awned (aa), and red seed color (Rs,Rs) to white seed color (rs,rs). Winner was also found to have more tillers than Experimental 3.

Methods

Each mutant line was crossed to each interchange line in the spring and summer of 1962. The F_1 plants (Figure I, C and Figure II, C) were grown and selfed to obtain F_2 seeds. The F_2 seeds from all six progenies were germinated in petri dishes, placed in plant bands, and transplanted into the field in 1963. The plants were spaced about twenty-four to thirty inches apart to reduce the effects of competition and crowding and to make observations on individual plants easier. The six F_2 progenies were also grown again in 1964 and one backcross progeny, (T165 X Grass III) X Grass III, was included.

Individual plant observations were made in both 1963 and 1964. In the Interchange X Grass III progenies, notes were taken in 1963 on the following characters: coleoptile color, dryness or juiciness of midrib, presence of awns, presence of purple spots, fertility, plant height, and number of tillers. The qualitative characters as well as the fertility differences are illustrated in Figures III and IV. The same observations were made in 1964 with the addition of notes on the length of panicle branches. Observations in the Interchange X Winner progenies consisted of the following: seed color, presence of awns,

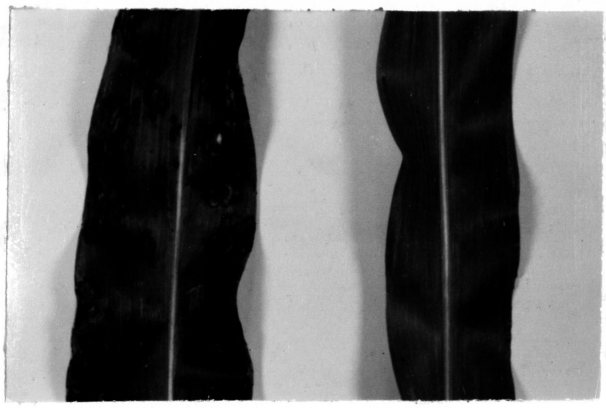
A



B

C

Figure III. Three characters segregating in Interchange X Grass III progenies. (A) left, fertile, right, semi-sterile; (B) left, red coleoptile, right, green coleoptile; (C) left, awned, right, awnless.



A

B

Figure IV. Two characters segregating in Interchange X Grass III progenies. (A) left, purple spotted, right, non-purple spotted; (B) left, dry midrib, right, juicy midrib.

dryness or juiciness of midrib, fertility, and number of tillers. These characters, except for the number of tillers, are illustrated in Figures V and VI. All observations in 1963, with the exception of coleoptile color, were made at maturity. In 1964, observations on presence of awns, presence of purple spots, and the dryness or juiciness of the midrib were made at the time of heading while notes on fertility, seed color, height, number of tillers, and length of panicle branch were made at maturity.

Notes were taken on the coleoptile color of the seedlings in the greenhouse within a week after emergence. The plants were then removed from the flats and arranged so that each progeny could be transplanted with all the plants of one color together. The segregation of red and green coleoptile was noted and the number of plants in each class recorded.

The midrib character was observed shortly after heading. This was done to avoid errors of classification since all plants tend to develop a dry midrib when the plants are mature. Purple spotting was also observed at the time of heading because spotting due to disease tends to mask the genetic character. Plants which had awns greater than approximately five millimeters in length were classified as awned, and plants with no awns or very short awns were considered awnless.

Data on fertility were recorded at maturity. Plants with more than fifty per cent seed set were considered fertile, and those with less than fifty per cent seed set were considered semi-sterile. Because of a light frost on August 11, 1964, pollen samples were taken

A

B

Figure V. Two characters segregating in Interchange X Winner progenies. (A) left, fertile, right, semi-sterile; (B) left, awned, right, awnless.

at that time from every plant which had not matured sufficiently to allow fertility notes to be taken. The pollen was stained with IKI to determine the per cent of stainable pollen. The stainability of the pollen was not considered to imply viability, but it was considered to be a reliable indicator of the presence of the interchange.

Observations on seed color were made in the Interchange X Winner progenies at maturity. Seed color was recorded as either red or white. Variations in the shade and intensity of color were observed, but no attempt was made to determine its mode of inheritance.

Height was measured to the top of the first head or to the top of the oldest head which could be found. This was necessary, since the height of the tillers of any one plant was found to be quite variable. The length of panicle branch was taken from the longest branch arising from the second node of the head on which the height measurement was made. The tiller count included all tillers which had headed at the time the count was taken.

The data were transferred to Hollerith cards in order to make the analyses faster and more accurate. A card was punched for each plant and a card sorter was used to separate the cards into the four phenotypic classes arising from the various two-factor segregations. The segregation ratios were then analysed by means of Mather's method for partitioning X^2 (28,29) as modified by Lamm (25,26). Tables devised by Joachim (22) were used for calculating linkage intensities between interchange points and simply inherited characters in F_2 progenies. Immer (21) developed tables which were used to facilitate

calculation of linkage intensities between simply inherited characters in F_2 progenies.

An electronic computer was used to obtain means and variances for the data on height, number of tillers, and length of panicle branch. Separate means were obtained for the fertile and semi-sterile segments of each F_2 population in order to test for linkage of the character with the translocation point.

EXPERIMENTAL RESULTS

Linkage Studies in Interchange X Grass III Progenies

Observations were made on Interchange X Grass III F_2 progenies which were segregating for simply inherited characters and fertility. In order to test for the presence of linkage between the genetic factors and the interchange points, a chi-square analysis was used to test the goodness to fit of a 3:3:1:1 ratio. The resulting chi-squares with three degrees of freedom were partitioned into three components, one testing the 3:1 gene segregation (X_G^2), one testing the 1:1 fertility segregation (X_S^2), and one testing for the presence of an interaction (X_I^2). The presence of a significant interaction chi-square was considered evidence for linkage of the gene and the interchange point only when the observed ratio was in agreement with the ratio which would be expected if linkage were present. It was also assumed that the presence of a significant interaction chi-square was not reliable evidence for linkage when either the gene segregation or the fertility segregation deviated significantly from the expected ratios.

Analyses of the 1963 data from the Interchange X Grass III F_2 progenies are summarized in Table 1. In all but one instance, it was found that there were significant deviations from the expected 3:3:1:1 ratios. These deviations, however, cannot be considered indicative of linkage since partitioning of the total chi-square values indicated that a major portion of the deviation in each case could be attributed to deviations from the expected ratios for gene segregation (3:1) or semi-sterility segregation (1:1).

Table 1. Tests for Linkage Between Simply Inherited Characters and Semi-sterility in 1963 Interchange X Grass III F_2 Progenies. Total $X_{(3)}^2$ is Partitioned Into Three Components: Gene Segregation (X_G^2), Semi-sterility (X_S^2), and Interaction (X_I^2).

Cross		No. in Progeny	Coleoptile Color	No. in Progeny	Midrib	Awns	Purple Spots
T165 X Gr III	$X_{(3)}^2$	225	12.64**	226	13.29**	39.67**	41.78**
	X_G^2		5.88*		4.30*	31.44**	33.19**
	X_S^2		6.76**		7.08**	7.08**	7.08**
	X_I^2		0.82		1.91	1.16	1.51
T231 X Gr III	$X_{(3)}^2$	142	9.28*	207	14.67**	30.31**	2.25
	X_G^2		2.69		9.06**	30.22**	0.04
	X_S^2		2.82		0.005	0.005	0.005
	X_I^2		3.76		5.61*	0.08	2.205
T396 X Gr III	$X_{(3)}^2$	178	8.64*	206	22.92**	65.12**	4.69
	X_G^2		0.61		18.18**	58.41**	1.09
	X_S^2		4.40*		3.28	3.28	3.28
	X_I^2		3.63		1.46	3.42	0.32

* Significant at 5% level
** Significant at 1% level

Linkage may be indicated in two instances. The significant interaction between T231 and the midrib character indicated a possible linkage but since a significant deviation from the 3:1 ratio was observed this evidence was not considered reliable. In the second instance interaction between T231 and coleoptile color approached significance (P .10), again indicating a possible linkage.

In 1964, data were collected from the F_2 progenies of the same crosses and, in addition, one backcross progeny was included. Results of the chi-square analyses are shown in Table 2. It will be noted that, in general, the segregations more nearly fit the expected ratios than in 1963. This can be attributed to more accurate classification of the phenotypes in the second year. In the F_2 progeny, linkage was indicated between the midrib character and T165, but it was not considered reliable because of the lack of fit for the 3:1 gene segregation. Furthermore, since the backcross progeny provided no evidence for linkage between T165 and the midrib character they were considered independent. Evidence for linkage between the interchange point in T231 and the genes for coleoptile color and the midrib character were much stronger as indicated by the highly significant chi-square values. Recombination values were calculated using the tables developed by Joachim (21). Recombination between T231 and the midrib character was estimated to be 7.2 ± 3.46 per cent, while a value of 19.1 ± 5.25 per cent was estimated for recombination between T231 and the gene for coleoptile color. Evidence for linkage between the genes for coleoptile color and the midrib character is shown in Table 3. The per cent recombination

Table 2. Tests for Linkage Between Simply Inherited Characters and Semi-sterility in 1964 Interchange X Grass III F_2 Progenies and One Backcross Progeny. Total $X_{(3)}^2$ is Partitioned Into Three Components: Gene Segregation (X_G^2), Semi-sterility (X_S^2), and Interaction (X_I^2).

Cross		No. in Progeny	Coleoptile Color	Midrib	Awns	Purple Spots
T165 X Gr III	$X_{(3)}^2$	149	4.25	11.23*	1.25	4.54
	X_G^2		1.18	6.77**	0.65	3.40
	X_S^2		0.33	0.33	0.33	0.33
	X_I^2		2.74	4.13*	0.27	0.81
T231 X Gr III	$X_{(3)}^2$	157	10.25*	43.24**	0.81	3.32
	X_G^2		1.12	3.23	0.17	2.90
	X_S^2		0.16	0.16	0.16	0.16
	X_I^2		8.97**	39.85**	0.48	0.26
T396 X Gr III	$X_{(3)}^2$	329	0.38	10.51*	8.76*	3.51
	X_G^2		0.05	8.39**	8.39**	1.87
	X_S^2		0.25	0.25	0.25	0.25
	X_I^2		0.08	1.87	0.12	1.39
(T165 X Gr III) X Gr III	$X_{(3)}^2$	35	2.60	0.54	1.91	
	X_G^2		0.03	0.26	0.26	
	X_S^2		0.26	0.26	0.26	
	X_I^2		2.31	0.03	1.40	

* Significant at the 5% level
** Significant at the 1% level

Table 3. Tests for Linkage Between Simply Inherited Characters in Interchange X Grass III Progenies Indicated by Partitioned X^2 Analyses.

F_2 Progenies		No. in Progeny	Midrib	No. in Progeny	Awns	No. in Progeny	Purple Spots
Coleoptile Color R,r	$X_{(3)}^2$	157	58.65**	635	4.39	635	2.57
	X_A^2		1.12		1.47		1.47
	X_B^2		3.23		2.22		1.06
	X_I^2		54.30**		0.69		0.03
Midrib D,d	$X_{(3)}^2$			157	3.42	157	8.94*
	X_A^2				3.23		3.23
	X_B^2				0.17		2.90
	X_I^2				0.02		2.81
Awns A,a	$X_{(3)}^2$					635	5.21
	X_A^2						2.22
	X_B^2						1.06
	X_I^2						1.93

Table 3 (continued)

Backcross Progeny		No. in Progeny	Midrib	No. in Progeny	Awns	No. in Progeny	Purple Spots
Coleoptile Color R,r	$X_{(3)}^2$	35	10.60*	35	0.31		
	X_A^2		0.03		0.03		
	X_B^2		0.26		0.26		
	X_I^2		10.31**		0.03		
Midrib D,d	$X_{(3)}^2$			35	0.54		
	X_A^2				0.26		
	X_B^2				0.26		
	X_I^2				0.03		

* Significant at the 5% level
** Significant at the 1% level

between these loci was estimated in three separate populations, and values ranging from 21.5 per cent to 24.5 per cent were obtained. These results agree with those obtained from the tests with the T231 interchange. No evidence was obtained for linkage between any of the other simply inherited characters.

In order to detect linkage between interchange points and genes determining quantitative characters, means were obtained for the fertile and semi-sterile classes in all three interchange lines and the differences between the means for the classes in each interchange progeny were tested for significance. The means and confidence intervals are shown in Table 4. It was assumed that if any major gene for one of the quantitative characters was located near the interchange point, this could be detected as a difference in the means. Height and tillering data obtained in 1963 were analysed. A difference in the height means was detected in T165.

Table 4. Tests for Linkage Between Semi-sterility and Quantitative Traits in 1963 Interchange X Grass III F_2 Progenies as Indicated by Means and Confidence Intervals

Cross		No. in Progeny	Mean No. of Tillers	No. in Progeny	Mean Height (inches)
T165 X Gr III	F	92	5.5 ± 0.56	92	72.2 ± 2.37
	SS	131	5.2 ± 0.54	131	66.7 ± 2.63
T231 X Gr III	F	61	5.1 ± 0.76	104	71.9 ± 2.93
	SS	81	4.6 ± 0.48	103	71.3 ± 2.81
T396 X Gr III	F	76	5.8 ± 0.64	90	77.1 ± 2.73
	SS	105	5.4 ± 0.44	118	73.9 ± 3.00

In 1964, three quantitative characters were studied: height, number of tillers, and length of panicle branch. An attempt was made to estimate the number of genes responsible for the differences in length of panicle branch and height. From the frequency distribution of panicle branch length in the Interchange X Grass III F_2 progenies (Figure VII), it appeared that the effect was the result of at least one major gene. In order to determine the number of genes segregating for height, measurements were made on Interchange lines, Grass III, and F_1 plants from these two lines. The height means were as follows: Interchange lines, 32.80 inches; Grass III, 50.15 inches; and F_1 plants, 73.83 inches. This was what might be expected if the proposal of a three gene difference between the parents were true. If the genotype of the Interchange lines is $Dw_1Dw_1dw_2dw_2dw_3dw_3$ and Grass III is $dw_1dw_1Dw_2Dw_2Dw_3Dw_3$, the F_1 would have the genotype $Dw_1dw_1Dw_2dw_2Dw_3dw_3$. Since the genes are dominant for tallness, the F_1, with a dominant gene at three loci, would be expected to be taller than the tall parent by the same amount that the tall parent exceeded the short parent. The parents were found to differ in height by 18 inches and the difference between the F_1 and the tall parent was found to be 23 inches. Since the results agreed well with the hypothesis, it appeared that three genes were segregating.

The three quantitative characters were tested for linkage with the interchange points in the same manner as in 1963. The difference in height means obtained in 1963 was not found in 1964, as shown in Table 5. Because of a better fit of the 1:1 ratio for the fertility

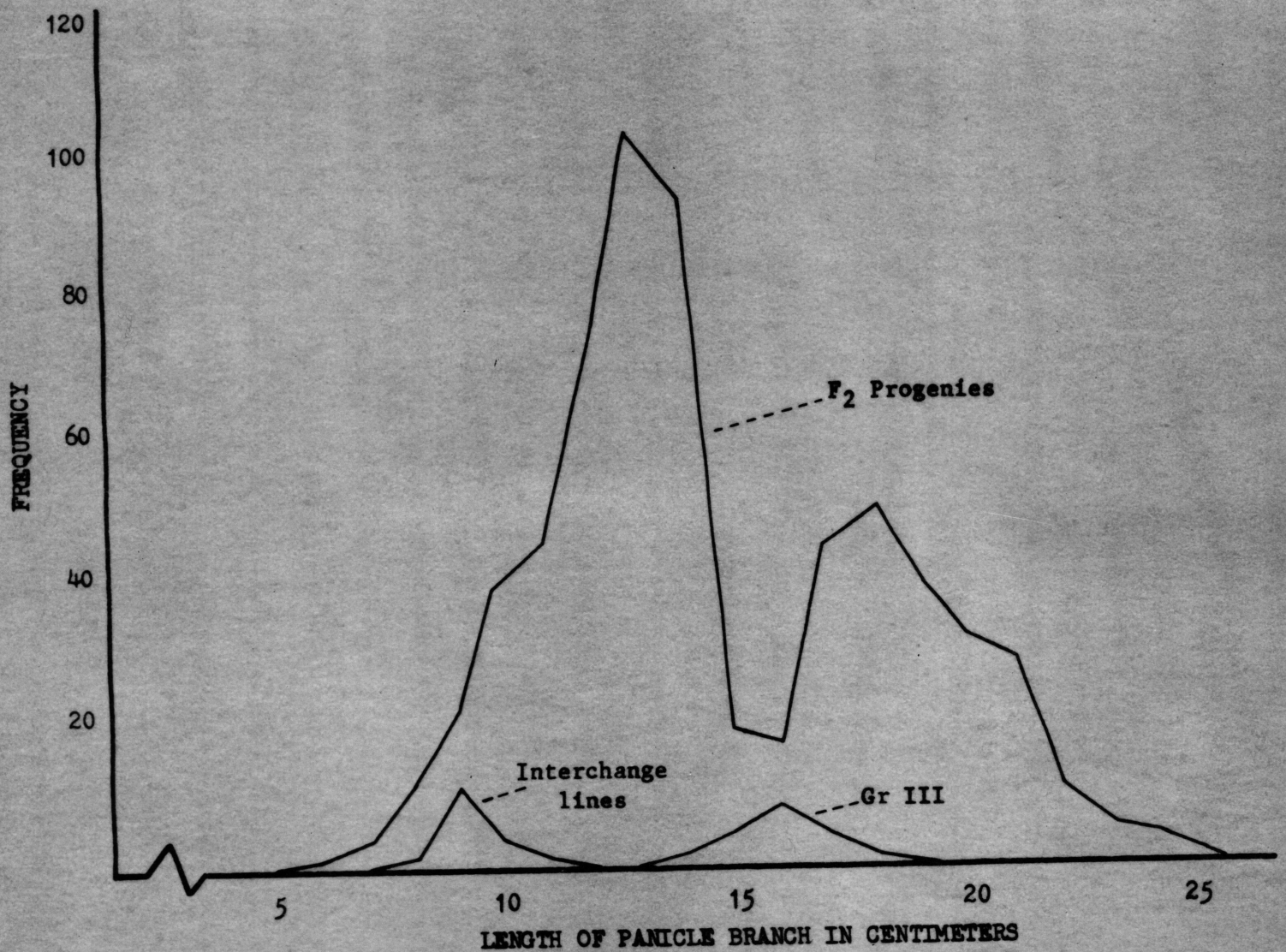


Figure VII. Distribution of panicle branch length of Interchange lines, Grass III, and the Interchange X Grass III F_2 Progenies.

segregation, the 1964 data were considered more reliable. A difference in tiller means was detected in the 1964 data, but the difference was not in the direction expected, so this was not considered evidence for linkage.

Table 5. Tests for Linkage Between Semi-sterility and Quantitative Traits in 1964 Interchange X Grass III F_2 Progenies as Indicated by Means and Confidence Intervals

Cross		No. in Progeny	Mean No. of Tillers	Mean Height (inches)	Mean Length of Panicle Branch (in.)
T165 X Gr III	F	71	6.2 ± 0.72	59.9 ± 2.98	14.5 ± 0.86
	SS	78	6.0 ± 0.56	61.0 ± 2.84	14.4 ± 0.76
T231 X Gr III	F	76	7.5 ± 0.50	61.0 ± 3.00	14.1 ± 0.86
	SS	81	5.7 ± 0.78	60.5 ± 2.90	14.1 ± 0.82
T396 X Gr III	F	160	7.3 ± 0.48	66.4 ± 1.92	15.7 ± 0.61
	SS	169	7.0 ± 0.46	65.2 ± 2.00	14.6 ± 0.55

The four qualitative characters were also tested for linkage with the quantitative characters. Means and standard deviations of the quantitative characters were obtained for each gene expression and confidence intervals were calculated. These data are given in Table 6. The means and variances were pooled for all progenies if two requirements were met. The first requirement was that the segregation fit a 3:1 ratio, and the second was that the variances were equal. If either of these requirements were not met, the samples were considered to be from different populations and were not pooled. In cases where the progenies could not be pooled, the progeny with the greatest difference between means was tested for significance. A significant difference

was found between the panicle branch means for the purple spotting character, but, since the differences observed in the other populations were much smaller, the deviation was probably due to chance rather than linkage.

Table 6. Tests for Linkage Between Simply Inherited Characters and Quantitative Traits in Interchange X Grass III Progenies as Indicated by Means and Confidence Intervals

Character	No. in Progeny	Mean Height (inches)	No. in Progeny	Mean No. of Tillers	No. in Progeny	Mean Length of Panicle Branch (in.)
Rs	463	63.46 ± 1.17	463	7.18 ± 0.27	463	14.86 ± 0.34
rs	172	63.30 ± 1.97	172	6.60 ± 0.50	172	14.48 ± 0.57
Ps	465	63.88 ± 1.18	127	6.49 ± 0.53	127	14.57 ± 0.67
ps	170	63.06 ± 1.92	30	6.90 ± 1.53	30	12.43 ± 0.95
D	108	60.85 ± 2.48	108	6.15 ± 0.54	108	14.06 ± 0.67
d	49	60.35 ± 3.98	49	7.49 ± 1.08	49	14.39 ± 1.22
A	236	60.67 ± 1.62	120	6.30 ± 0.55	116	14.27 ± 0.66
a	70	60.29 ± 3.28	37	7.40 ± 1.24	33	15.21 ± 1.07

Correlations between quantitative characters were obtained to provide a test for linkage between these characters. The following correlation coefficients were obtained: between height and number of tillers, 0.36; between height and length of panicle branch, 0.18; and between number of tillers and length of panicle branch, 0.16. Of these three values, only the correlation between height and number of tillers could be considered both significant and meaningful, but it is not large enough to be a good indication of possible linkage between these two characters.

Linkage Studies in Interchange X Winner Progenies

A chi-square analysis was performed on the Interchange X Winner progenies in the same manner as previously described. The results of the 1963 study are shown in Table 7. As in the Interchange X Grass III progenies, the expected segregation of genes and fertility was not obtained in some cases, but in the other cases accurate tests for linkage were possible. Highly significant interaction chi-squares for T231 vs. seed color and T396 vs. seed color were obtained, but the expected interaction between T231 and the midrib character was not found. In the case of T231 and the midrib character, any interaction might well have been obscured by the large deviation from a 3:1 gene segregation.

The results of the 1964 study, which are shown in Table 8, indicate that much better segregation ratios were obtained since none of the gene segregations or the fertility segregations deviated significantly from the expected ratios. The interaction chi-square for T231 vs. the midrib character was highly significant and substantiates the results obtained in the Interchange X Grass III progenies. The interaction chi-square for T396 vs. the midrib character was also significant, but examination of the data indicates that this interaction is not due to linkage since the segregation does not fit the expected linkage pattern.

A highly significant chi-square for the interaction between T396 and seed color was again observed and was considered to be evidence for linkage. In addition, a highly significant interaction

Table 7. Tests for Linkage Between Simply Inherited Characters and Semi-sterility in 1963 Interchange X Winner F_2 Progenies. Total $X_{(3)}^2$ is Partitioned Into Three Components: Gene Segregation (X_G^2), Semi-sterility (X_S^2), and Interaction (X_I^2).

Cross		No. in Progeny	Seed Color	Midrib	Awns
T165 X W	$X_{(3)}^2$	197	12.64**	13.42**	6.68
	X_G^2		7.15**	8.06**	1.42
	X_S^2		4.88*	4.88*	4.88*
	X_I^2		0.61	0.49	0.38
T231 X W	$X_{(3)}^2$	211	10.51*	28.15**	3.26
	X_G^2		0.002	23.90**	0.01
	X_S^2		2.09	2.09	2.09
	X_I^2		8.42**	2.16	1.15
T396 X W	$X_{(3)}^2$	229	25.89**	5.96	5.96
	X_G^2		1.22	2.45	2.45
	X_S^2		1.93	1.93	1.93
	X_I^2		22.74**	1.59	1.59

* Significant at the 5% level
** Significant at the 1% level

Table 8. Tests for Linkage Between Simply Inherited Characters and Semi-sterility in 1964 Interchange X Winner F_2 Progenies. Total $X_{(3)}^2$ is Partitioned Into Three Components: Gene Segregation (X_G^2), Semi-sterility (X_S^2), and Interaction (X_I^2).

Cross		No. in Progeny	Seed Color	Midrib	Awns
T165 X W	$X_{(3)}^2$	172	9.83*	0.40	0.65
	X_G^2		0.78	0.03	0.28
	X_S^2		0.09	0.09	0.09
	X_I^2		8.96**	0.28	0.28
T231 X W	$X_{(3)}^2$	193	1.17	8.11*	2.47
	X_G^2		0.50	0.62	1.45
	X_S^2		0.63	0.63	0.63
	X_I^2		0.04	6.85**	0.39
T396 X W	$X_{(3)}^2$	207	10.96*	6.27*	2.22
	X_G^2		0.002	1.01	1.01
	X_S^2		0.39	0.39	0.39
	X_I^2		10.57**	4.87*	1.55

* Significant at the 5% level
** Significant at the 1% level

chi-square was obtained for T165 vs. seed color. This discrepancy between years was possibly due to errors in classification since the seed color is often difficult to classify.

The three simply inherited genes which were segregating in the Interchange X Winner progenies were tested for independence. The results shown in Table 9 indicate that there is no linkage between any of the characters studied.

Table 9. Tests for Linkage Between Simply Inherited Characters in Interchange X Winner F_2 Progenies Indicated by Partitioned X^2 Analyses

	No. in Progeny	Midrib (D,d)		Awns (A,a)	
Seed color (R,r)	572	$X_{(3)}^2$	2.93	$X_{(3)}^2$	1.14
		X_R^2	0.76	X_R^2	0.76
		X_D^2	0.93	X_D^2	0.009
		X_I^2	1.24	X_I^2	0.38
Midrib (D,d)	572			$X_{(3)}^2$	3.04
				X_R^2	0.93
				X_D^2	0.009
				X_I^2	2.10

Data were taken on the number of tillers in the Interchange X Winner progenies in both 1963 and 1964. Table 10 shows tiller means and confidence intervals for the fertile and semi-sterile classes in the three Interchange X Winner progenies. A significant difference was

obtained for the T165 X Winner progeny in 1963, but the difference was not large. Since there was no difference in the same progeny in 1964 when a 1:1 ratio of fertile to semi-sterile was obtained, there is little evidence for an association between tillering and the interchange point.

Table 10. Tests for Linkage Between Semi-sterility and Gene Determining the Number of Tillers in Interchange X Winner Progenies as Indicated by Means and Confidence Intervals

Cross		1963		1964	
		No. in Progeny	Mean No. of Tillers	No. in Progeny	Mean No. of Tillers
T165 X W	F	70	2.88 ± 0.36	99	3.44 ± 0.34
	SS	100	3.62 ± 0.29	108	3.79 ± 0.35
T231 X W	F	49	3.39 ± 0.65	88	3.05 ± 0.36
	SS	82	3.62 ± 0.39	84	3.29 ± 0.34
T396 X W	F	28	3.87 ± 0.38	91	2.80 ± 0.23
	SS	47	4.31 ± 0.33	102	3.09 ± 0.23

The three qualitative characters were tested for linkage with the quantitative characters. Means and standard deviations were obtained for each gene expression and confidence intervals were calculated. Means and variances were pooled for all progenies where possible, and when this was not possible, means with the greatest difference were used. Since no differences were obtained at the 5 per cent level as shown in Table 11, these characters were considered independent.

Table 11. Tests for Linkage Between Simply Inherited Characters and Genes Determining the Number of Tillers in Interchange X Winner Progenies as Indicated by Means and Confidence Intervals

Character	No. in Progeny	Mean No. of Tillers
D	279	3.35 ± 0.38
d	100	3.59 ± 0.19
R	52	3.55 ± 0.28
r	155	3.83 ± 0.51
A	281	3.32 ± 0.20
a	98	3.66 ± 0.32

DISCUSSION

In comparing data obtained in 1963 and 1964, it can be seen that in 1964 much smaller deviations from the expected 3:1 and 1:1 ratios in the Interchange X Grass III progenies were obtained than in 1963. Several explanations can be proposed to account for these differences. One explanation is that notes were taken at different times in the different years. In 1963, all notes were taken at maturity; while in 1964, notes on the midrib character, awns, and purple spots were taken shortly after heading. The time of observation was changed since it was noted that the genetic differences for purple spots and the midrib character tended to become obscured by age. As the plants approached maturity difficulties in classifying the purple spotting character were encountered as a result of disease which tended to mask the genetic character. Also, increasing maturity caused the leaves to lose moisture and plants with juicy midribs were difficult to distinguish from those with dry midribs.

In the case of the awned character, the method of classification was changed. In 1963, plants which had awns of any length were considered awned, resulting in the awned class being too large. Observations on F_1 plants in 1964 revealed that those plants, which should have been awnless, often had awns from 1 to 4 mm. in length. In view of this, plants in the F_2 progenies which had awns less than 5 mm. long were considered awnless. This method of classification resulted in segregation ratios which corresponded quite well with the expected 3:1 ratio except in one case.

In 1964, the segregation of fertile:semi-sterile was closer to the expected 1:1 ratio than in 1963. The classification of fertility in 1963 was based entirely on visual estimates of floret fertility, while in 1964 the visual observations were supplemented by the examination of pollen samples. The estimates of pollen stainability as a measure of fertility used in conjunction with visual estimations of floret fertility provided a much more precise classification of the fertility of the individual plants than floret fertility alone. Another explanation for the greater precision in 1964 is that the poor floret fertility of the female parent, Experimental 3, was not taken into account in 1963. Because of the low fertility of one of the parents, individual F_2 plants might be expected to exhibit low fertility even if a normal chromosome complement were present. This factor was considered in 1964, and plants with 70-80 per cent seed set were classified as fertile.

Discrepant ratios were also obtained in the Interchange X Winner progenies in 1963, but, in 1964, all segregations fit the expected ratios. These discrepancies are probably attributable to some of the same causes as those observed in the Interchange X Grass III progenies.

It will be noted that linkage was indicated between each of the interchanges and the seed color character in the Interchange X Winner progenies. In both years, linkage was indicated between the seed color character and T396. The recombination value obtained in 1963 was 12.5 ± 3.3 per cent, while the value obtained in 1964 was 19.0 ± 4.0 per cent. Although these values were different, the difference was

small enough that they could be considered estimates of the same value.

It will be noted from the 1963 data that linkage was indicated between T231 and seed color, while in 1964 the presence of linkage between T165 and seed color was indicated. Two explanations for these discrepancies can be proposed. The first is that errors in classification might have been made, but this seems unreasonable since a good fit for both the 3:1 and 1:1 ratios was obtained in both cases. The second explanation is that error in classification occurred because of the segregation of more than one pair of genes. It has been reported by Quinby and Martin (35) that there are at least three pairs of genes which condition seed color in sorghum. Two of these factors (R,r and Y,y) segregating in a population can result in a ratio of 9 red:3 yellow:4 white. In addition to this, there is a factor (I,i) which intensifies color when present in the dominant condition. If Experimental 3 is assumed to be RRYYII and Winner is assumed to be rryyII, the result might be a 9 red:3 yellow:4 white ratio in which a clear distinction could not be made between red and yellow.

Erichsen et al. (14) noted that although a 3:1 segregation for colored seed:white seed was obtained, there were variations in the intensity of color. The same variations were noted in this study, possibly as a result of the genetic mechanism proposed above. If it is true that more than one gene is segregating, this might explain the indication of linkage between T165 and seed color or T231 and seed color.

The results obtained in 1963 and 1964 indicate that linkage was detected in three cases in the Interchange X Grass III progenies. The genes affecting coleoptile color and the midrib character were found linked to the T231 interchange and, in addition, the genes themselves were also found to be linked. If the estimate of 22 per cent recombination between the genes for the midrib character and coleoptile color obtained from the backcross progeny was used, it appeared probable that the genes were located on opposite sides of the interchange point (D — T231 — Rs). This recombination value of 22 per cent agreed well with the 21.5 per cent obtained by Stephens and Quinby as reported by Martin (26).

Two restrictions were made in the interpretation of the data in order to estimate the effects of colchicine on the genetic make-up of the Grass III mutant. (1) Any evidence for linkage was interpreted as such, in order to maximize the size of linkage groups. (2) In addition to the four genes which had monofactorial ratios, three genes were assumed to be segregating for height and at least one each for number of tillers and length of panicle branch.

The first restriction was imposed in order to minimize the number of chromosome pairs required to carry the mutated genes. In this case three linkage groups were assumed to be present. These three linkage groups are based on the following evidence: (1) there were highly significant interactions between T231 and the genes for coleoptile color and the midrib character as shown in Table 2, (2) a difference in the panicle branch means for the purple spotting character in Table 6

indicated a possibility of linkage between these characters, and (3) a significant correlation was found between height and number of tillers which could indicate linkage between one of the height genes and a tiller gene.

The second restriction was based in part on a study by Chen and Ross (12) of the qualitative characters of Grass III and on observations made in 1963 and 1964. It was also based partially on observations by Foster (15) on the colchicine mutant C15 which closely resembles Grass III. Foster studied F_3 families from the cross C15 X Experimental 3 which were segregating for height and estimated that there were three genes which had been changed. From 1964 observations of Experimental 3 interchange lines, Grass III, and F_1 progenies of these two lines, it appeared that Grass III also had three height genes which had been changed. Foster also estimated that at least one gene had been changed for tiller number. The assumption of one gene change for panicle branch length was based on observations made in 1964. The frequency distribution showed peaks at 13 cm. and 18 cm. with approximately twice as many plants at 13 cm. than at 18 cm. This indicates that there was at least one gene segregating for panicle branch length as reported by Martin (26).

On the basis of these restrictions, some conclusions may be drawn:

1. Genes for coleoptile color and the midrib character are on the chromosome pair involved in the T231 interchange which was not common to the T165 interchange (Huang et al., 20).

2. No other characters were found linked to the interchange points, so they probably were on the five chromosome pairs not involved in the interchanges.
3. Genes for awns and purple spots were not found to be linked together or with any other gene, except as shown in Table 4. Therefore, they probably were on separate chromosome pairs.
4. The gene for panicle branch length was on the same chromosome pair as the gene for purple spots.
5. The three genes segregating for height were independent according to Quinby and Karper (34). They could be assumed to be on separate chromosome pairs not occupied by previously mentioned characters since no evidence for linkage between height and any of these was noted.
6. A gene for number of tillers was located on the same chromosome pair as one of the height genes.

From these conclusions, it appeared that mutated genes were possibly located on six chromosome pairs as follows: one interchange chromosome as indicated in conclusion 1, two non-interchange chromosomes as indicated in conclusions 3 and 4, and three other non-interchange chromosomes as indicated in conclusions 5 and 6. This proposal ignored the fact that some genes may be located on the same chromosome pair, but were more than fifty crossover units apart. It should be noted, however, that the three interchanges sampled five or perhaps six of the twenty chromosome arms. Because the break points in most cases were close to the centromeres, linkage tests should be

accurate throughout much of the length of the five chromosomes involved in the interchanges. Therefore, even if it were assumed that the genes for awns and purple spots were located at opposite ends of a single chromosome pair, at least two chromosome pairs would be required to carry the four simply inherited characters.

When the qualitative characters are considered, it would appear that at least two additional chromosome pairs were involved. Quinby and Karper (34) reported that the genes for height were independent, so it would be unreasonable to assume that all three were carried on one chromosome. Therefore, since they were not found linked to any of the interchanges or any of the qualitative characters, they probably were on two additional chromosome pairs. From this it would seem that at least four chromosome pairs carry mutated genes, and, as previously indicated, six pairs would not be unreasonable.

It has been proposed by Franzke and Sanders (17) that sorghum is an allopolyploid and that the effect of colchicine in producing true-breeding mutants is the result of a substitution of chromosome pairs from one genome to another. It was also proposed that there was a gene present which permitted chromosome pairing only by pairs, instead of formation of trivalents or quadrivalents. If this were the case, the maximum number of chromosome pairs which could be affected in any one instance would be five. The results obtained in this study indicate that, in the Grass III mutant, there were mutated genes located on a minimum of two, and possibly six chromosome pairs.

A more reasonable explanation for the mutagenic effect of colchicine would be that gene mutations had occurred, followed by somatic reduction. Simantel (40) found true-breeding mutants after colchicine treatment of F_1 plants from Interchange X Normal Experimental 3 crosses. Cytological examination of the progeny of Normal Experimental 3 X Mutant and Interchange X Mutant crosses indicated that the chromosome complement of the mutants was normal. In addition, F_1 plants of Interchange X Interchange crosses were treated and mutants were obtained which had normal chromosome complements. This was considered evidence for somatic reduction. The results of this study indicated that, although chromosome substitution was not completely ruled out, the substitution which gave rise to Grass III probably involved at least eight of the ten chromosome pairs with four pair from one genome being substituted for four pair from the other genome. When this aspect was considered along with the evidence obtained for somatic reduction by Simantel (40), it appeared that gene mutation followed by somatic reduction is the more reasonable explanation for the appearance of true-breeding mutants following colchicine treatment.

SUMMARY

The objectives of this investigation were (1) to investigate linkage relationships between a number of colchicine mutated genes and interchange points in three lines, as well as the linkage relationships between the genes themselves, and (2) to gain information on the action of colchicine in producing true-breeding mutants.

Good evidence was obtained for linkage between T231 and the gene for coleoptile color, between T231 and the gene for the midrib character, and between the two genes themselves. The results indicated that the T231 interchange point was located between the two genes. The indicated order with crossover percentages was found to be D,d(7.2)T231(19.4)Rs,rs. Recombination between the genes was estimated at 22 per cent and agrees well with the individual values obtained if it is assumed that the presence of the interchange inhibits crossing-over to a certain extent.

Evidence for linkage between T396 and a gene for seed color was obtained, but this evidence is not conclusive because of discrepancies noted in the other interchange progenies. No reliable evidence was obtained for linkage between any of the interchange points and the quantitative characters, or between any genes other than those indicated above.

Results obtained in this study indicated that the mutated genes were probably located on at least four and possibly six chromosome pairs. If this were the case, chromosome substitution would seem to be an unlikely explanation for the appearance of true-breeding mutants following colchicine treatment. In this respect, data obtained in this

study appeared to substantiate the evidence obtained by Simantel (40) for gene mutation followed by somatic reduction.

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