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Genetic Similarity of Colchicine-induced Grass-type Mutants in Sorghum

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GENETIC SIMILARITY OF COLCHICINE-INDUCED GRASS-TYPE MUTANTS IN SORGHUM

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

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INTRODUCTION

The induction of true-breeding diploid mutants in certain varieties of Sorghum vulgare Pers. by application of 0.5 per cent colchicine in lanolin to the coleoptile of the seedlings has been reported by Franzke and Ross (13) and Ross et al. (23). The original assumption to explain the occurrence of this phenomenon, stating that a reduction division of the chromosomes with subsequent restoration to the diploid number occurred to give a concentration of chromatin from one of the constituents, received no support from pachytene analysis of the mutants and of the F_1 hybrids between mutants and the lines from which they arose (Harpstead et al., 18). Both cytological and genetical evidences obtained by various investigators (8, 9, 10, 11, 12, 18, 23) have indicated the occurrence of gene mutations previous to or concurrent with the reduction division.

The repeated occurrence of mutants with identical phenotypes involving whole complexes of characters raises the problem of whether identical major chromatin rearrangements or identical mutagenic effects are responsible.

This study was designed to determine whether the phenotypically similar mutants are also genotypically similar. Genetical and cytological observations were made on three phenotypically similar grass mutants which arose from the sorghum variety Experimental 3 after colchicine treatment in different years. The main part of the work was carried out at the Agronomy Farm in the summer of 1960.

LITERATURE REVIEW

The occurrence of diploid variants as the result of application of colchicine to coleoptiles of sorghum seedlings of the true-breeding grain variety Experimental 3 was first reported by Franzke and Ross in 1952 (13). From progeny tests, some of the variant plants were found to be true-breeding while others segregated for some characters. Cytological observations of meiosis revealed no irregularities in either the variants or the original variety. Reductional groupings of chromosomes, similar in appearance to those reported by Huskins (17), were observed in sorghum root tips after one hour in a 0.5 per cent aqueous colchicine solution. Since the variant plants had characteristics which were similar to those of ancestral lines, it was "proposed that such plants could originate through" reduction of "somatic chromosomes so that a concentration of chromosomes containing gene blocks originating from one of the ancestors . . . might occur in one cell. This cell, by virtue of its inherent and perhaps environmental competitive advantage, could form a new growing point and produce a plant with a genotype entirely different from that of the original zygote; in fact, homozygous diploidy" might "thus be induced."

A further experiment dealing with measurements and observations of characteristics of agronomic importance made on progenies from colchicine-treated and untreated full sibs from Experimental 1 and from Experimental 3 (Ross et al., 23) confirmed the results obtained in previous studies. It was noted that the characteristics of a dwarf variant line with cylindrical head, termed "rat tail," produced by retreatment

of a variant previously obtained from treated Experimental 1 was not known to occur in the ancestry of the original line. They then proposed that colchicine caused a somatic reduction with concurrent chromatin rearrangement or point mutations followed by restoration to the diploid number.

However, from cytological observations made at meiosis in the colchicine variants, in the original material from which the variants were derived, and in their F_1 hybrids, Harpstead *et al.* (18) indicated that neither detectable irregularity nor rearrangement of chromatin was found at the pachytene stage. They assumed the appearance of new phenotypes to be due to genic mutation or cryptic structural changes in the chromatin.

In genetical analyses of F_2 populations resulting from crosses between colchicine-induced mutants and the original variety, Foster *et al.* (8, 9, 11) noted simple Mendelian inheritance for two qualitative characters studied. No correlation was observed between these two genes or between them and the mutated quantitative characters studied, indicating that the mutagenic effect of colchicine had caused the simultaneous mutation of large number of loci on different chromosomes. From the F_2 and F_3 data of a cross between the grass-type mutant C15 and its original variety Experimental 3, Foster *et al.* (12) also made an estimate of at least 12 genes which affected the expression of six different characters that had been mutated by colchicine treatment. The characters studied and the estimated mutated genes for each of them were: days to heading, 5 or 6; plant height, 3; width of leaf, at least 1; number of tillers, at least 1; seedling-base color, 1; and awn development, 1.

Franzke and Ross (14) also found from studies of a lineal series of mutants obtained from the variety Experimental 1 by repeated colchicine treatments that these mutants were quite different from any known ancestor of the treated material. This provided further evidence that the immediately true-breeding nature of these mutants appeared to be due to gene mutation followed by somatic reduction with subsequent restoration to the diploid number.

Differential reaction to colchicine in different varieties of sorghum was observed by Atkinson et al. (1, 2). They found that varieties Experimental 3 and Norghum differed in mutation rate after colchicine treatment. Cytological examination during meiosis of 30 treated plants of each variety indicated only diploid plants in Experimental 3, but three tetraploid plants in Norghum.

Ross et al. (25) crossed reciprocally a colchicine-induced grass variant, M15, to an irradiated mutant; both of them were strong awned and originated from Experimental 3. The F_2 plants all were strong awned while segregation for some other characters was noted. In the F_2 populations of the cross between M15 and Black Amber Cane, one of the ancestors of Experimental 3, all plants had long awns and segregated for other characters. Since no evidence of sterility and no transgressive segregation for the awn condition was observed in the progeny of either of the two crosses, they concluded that both cases of mutation would appear to be at the same locus.

The modes of inheritance of the characters in sorghum related to the present studies have been reported by others as follows.

1. Seedling-base color.

Reed (22) first demonstrated that the inheritance of seedling stem color was monogenic with red stem dominant over green. However, the data obtained by Woodworth (31) from an F_2 population and F_3 progeny test of the cross between green-colored Shallu and red-colored Black Spanish broomcorn showed a 9:7 ratio of segregation for red and green indicating a digenic inheritance.

Harpstead et al. (18) observed F_1 hybrids between the green seedling-based colchicine variants 12, 15, and the red-based untreated line; the F_1 seedlings exhibited red base. When a cross was made between treated variants 12 and 15, all the F_1 seedlings were found to be green. In the cross between Experimental 3 and its colchicine-induced grass-type variant M15, Foster (9) and Foster et al. (11) calculated a segregation ratio of 3:1 for red and green. This indicated that the gene governing green seedling base had originated from that for red seedling base as a result of either spontaneous or induced mutation.

2. Spotting on leaf blade.

The so-called "spotting" that frequently occurs on the leaves of Sudan grass, Sorghum vulgare var. Sudanese (Piper) Hitchc, is not caused by a pathogen but is due to a genetic effect. Garber and Chilton (15) made a survey among 464 lines of Sudan grass which had been selfed for 2 years or more and found that 400 lines carried red spots, 59 were tan, and the remaining 5 lines were apparently segregating for red and tan. The F_1 plants from the cross of red-spotted Sudan grass with tan-spotted line were red spotted. In the F_2 generation 482 plants had red spots and 162 had tan spots on the leaf blades. They interpreted these results

as showing that color of spotting on the leaf blade was monogenic with red dominant.

3. Dry midrib.

In 1916, Hilson (cf. Quinby and Martin, 20) first reported a single factor difference between dry and juicy stalks with dry stalks dominant over juicy. Swanson and Parker (29) assigned the symbols D and d to the factors for dry and juicy stalks. Ayyanger (cf. Quinby and Martin, 20) indicated that dry-stalked sorghum had a white midrib whereas the juicy-stalked variety had a dull midrib. Casady and Anderson (5) found that the segregation for the dry vs. juicy-stalk character (D vs. d) of the F_2 progenies of four autotetraploid Sudangrass x (Johnson grass x 4n Sudangrass) crosses appeared to be intermediate between random chromosome and random chromatid segregation. The ratio obtained appeared to be somewhat closer to 35:1 than 20.8:1.

4. Awn condition.

The awn is an extension of the tip of the lemma of the fertile floret, and its development is subject to environmental influences. Vinall and Cron (30) obtained an awnless F_1 and a ratio of 3 awnless to 1 awned in a segregating F_2 population from a cross between strong-awned Dwarf Milo and tip-awned Feterita. In 1924, Ramanthan (21) reported a segregation ratio of 3:1 long to short-awned plants in sorghum. Parker (16) reported a segregation of awnless to awned plants approaching a 3:1 ratio in a cross between Kansas Orange Sorgo (awnless) and Dwarf Yellow Milo (awned). Sieglinger et al. (27) established three classes of awn conditions: strong-awned, tip-awned, and awnless. Awnless was inherited as a simple dominant to both strong-awned and tip-awned. The strong-awned

character was inherited as a simple but partial dominant to the tip-awned character. They explained the inheritance on the assumption that there are three pairs of multiple allelomorphic characters; namely, AA (awnless), aa (strong-awned), and $a^t a^t$ (tip-awned).

From the cross between colchicine mutant M15 (long-awned) and its original variety Experimental 3 (awnless), Foster (9) and Foster et al. (11) obtained an F_2 segregation fitting closely a ratio of 3 awnless to 1 awned. Colchicine had caused the mutation of the dominant gene for awnless in Experimental 3 to the recessive gene for awns in M15. This ratio was confirmed by Foster (10) based on the results obtained from F_3 progeny tests.

MATERIALS AND METHODS

Three phenotypically similar grass-type mutants derived from one variety after colchicine treatments by C. J. Franzke in 1948 and 1955, and by M. E. Sanders and C. J. Franzke in 1957, repsectively, and the original variety, Experimental 3, were involved in this study. Their morphological characters considered in this study are described as follows:

Experimental 3--Dwarf grain type with red seedling base, dry midrib, white-blotched leaf blade and awnless spikelet.

Grass I--Medium tall, forage type with green seedling base, juicy midrib, purple-spotted leaf blade, and long-awned spikelet.

Grass II--Similar to those of Grass I.

Grass III--Similar to those of Grass I.

Photographs of the original variety Experimental 3 and the three grass-type mutants--Grass I, Grass II, and Grass III--are illustrated in Figures I and II. Figure II clearly shows the morphological similarities among these three mutants.

Seeds of five categories of cross populations (namely, F_1's and F_2's of the crosses between grass-type mutants and their original variety, F_1's of intercrosses between any two of three grass-type mutants, back-crosses of grass mutants in combination with Experimental 3, and outcrosses of F_1 plants involving one grass mutant and Experimental 3 to another grass mutant) were obtained by C. J. Franzke, M. E. Sanders, A. W. Erichsen and H. D. Haensel in the spring and summer of 1958 and spring of 1959. These seeds and those of the parental materials were grown in 17-foot rows in the field at the Agronomy Farm by the author in the summer of 1960.

F_2 populations of intergrass crosses were grown in the summer of 1961. The different categories of crosses are listed below.

1. F_1 hybrids:

   Grass I x Experimental 3

   Grass II x Experimental 3

   Grass III x Experimental 3

   Experimental 3 x Grass II

   Experimental 3 x Grass III

2. F_1 intergrass crosses:

   Grass I x Grass II

   Grass I x Grass III

   Grass II x Grass I

   Grass II x Grass III

   Grass III x Grass I

   Grass III x Grass II

3. F_2 populations:

   Grass I x Experimental 3

   Grass II x Experimental 3

   Grass I x Grass II

   Grass I x Grass III

   Grass II x Grass I

   Grass II x Grass III

   Grass III x Grass I

   Grass III x Grass II

4. Backcross populations:

   Grass I x (Grass I x Experimental 3)

Grass II x (Grass II x Experimental 3)

(Grass I x Experimental 3) x Grass I

(Grass II x Experimental 3) x Experimental 3

Experimental 3 x (Grass I x Experimental 3)

Experimental 3 x (Grass II x Experimental 3)

5. Outcross populations:

(Grass I x Experimental 3) x Grass II

(Grass I x Experimental 3) x Grass III

(Grass II x Experimental 3) x Grass I

Individual plant notes were taken on (1) seedling-base color, (2) dryness of midrib, (3) spotting on leaf blade, (4) awnedness, and (5) plant height. The methods of recording these observations are described below.

1. Seedling-base color.

Whether the coleoptile was red or green was recorded at about three days after emergence of the seedlings.

2. Dry midrib.

For this character, plants were divided into two classes. Those with midribs which were entirely free of any pithy stripe or with broken stripes were classified as juicy. All others having different degrees of continuous striping were classified as dry.

3. Spotting on leaf blade.

This character is classified into two categories, i.e., purple-spotted and non-purple-spotted. Notes were taken on mature leaves after heading of the plants.

4. Awnedness.

The presence or absence of awns was recorded from the main panicle at heading stage. Plants which have an extension of the tip of the lemma 5 mm. or longer are classified as awned whereas those which are 4 mm. or less in length or have no extension of the tip of the lemma are classified as awnless.

5. Plant height.

Ten plants of each F_1 hybrid and 30 plants in F_2 populations of intergrass crosses were chosen at random and measured in inches from the ground surface to the tip of the main panicle after maximum height was reached.

From these data, chi-square values for each of the qualitative characters in different segregating F_2 populations were calculated to test the goodness-of-fit to the expected ratios. Comparisons of hybrid vigor as measured by height of F_1 plants in the intergrass crosses and in the crosses between mutants and their original variety were made.

For cytological studies, two or three young panicles were collected from tillers of healthy plants from each combination of F_1 hybrids as well as from each parent and fixed in a solution of three parts of ethyl alcohol and one part of glacial acetic acid for more than 24 hours. Slides were prepared using the propionic-carmine squash technique. The number of chiasmata per PMC were counted at diakinesis in such a way that a ring or closed bivalent contributed two counts, an open bivalent, one, and two univalents, zero.

Figure I. Experimental 3, a dwarf grain sorghum

Figure II. Three phenotypically similar grass-type mutants arose from Experimental 3 by colchicine treatments in different years (From left to right: Grass I, Grass II, and Grass III)

EXPERIMENTAL RESULTS

Genetic Analyses of Four Qualitative Characters

1. Inheritance of seedling-base color

Red seedling-base color was found in Experimental 3 and in the F_1 plants in all the crosses between three grass mutants and Experimental 3. Green color, on the other hand, was observed in the intergrass crosses and their parents. The segregations for this character in certain F_2, backcross, and outcross populations are shown in Table 1.

The segregation ratio for seedling-base color in the F_2 populations was three red to one green when Experimental 3 was crossed with either Grass I or Grass II. Chi-square tests for this ratio showed P-values of 0.95 and 0.25, respectively, showing a good fit to the ratio expected for monogenic inheritance with red dominant over green.

All seedlings showed uniformly red bases in all backcrosses in which F_1 plants between either one of two grass mutants and Experimental 3 were crossed with Experimental 3. A 1:1 segregation ratio for red and green seedling-base color was obtained in the three backcrosses in which the F_1 plants were crossed back to their respective grass parents. This confirms the results obtained from the F_2 populations and also indicates that the mutant character of the green seedling base was changed from the red as the result of the effect of colchicine.

No maternal effect is involved in this character; otherwise, it would have shown in different genetical ratios between direct and reciprocal crosses of certain combinations. Therefore, it would seem that the mode of inheritance of seedling-base color is of the simple Mendelian fashion.

Plants in all of the outcross populations showed a 1:1 ratio for red and green just as in the corresponding backcross combinations. This gives further evidence that the genes which govern seedling-base color in all three grass mutants are located at the same locus on the chromosomes.

Table 1. Genetic Analyses of F_2, Backcross, and Outcross Populations Involving Sorghum Line, Experimental 3, and Three Grass-Type Mutants to Test Segregation of Seedling-Base Color by Chi-Square Tests

Combination	No. plants observed	No. plants showing red	green	Segregation ratio	Calculated chi-square	Approx. P-value
F_2 populations:						
Grass I x Experimental 3	79	59	20	3:1	0.0043	0.95
Grass II x Experimental 3	72	60	12	3:1	1.3334	0.25
Backcrosses:						
Grass I x (Grass I x Experimental 3)	87	40	47	1:1	0.5632	0.40
(Grass I x Experimental 3) x Grass I	78	46	32	1:1	2.5138	0.15
Experimental 3 x (Grass I x Experimental 3)	11	11	0	2:0		
Grass II x (Grass II x Experimental 3)	115	60	55	1:1	0.2174	0.70
(Grass II x Experimental 3) x Experimental 3	54	54	0	2:0		
Experimental 3 x (Grass II x Experimental 3)	60	60	0	2:0		
Outcrosses:						
(Grass I x Experimental 3) x Grass II	39	19	20	1:1	0.0026	0.95
(Grass II x Experimental 3) x Grass I	27	16	11	1:1	0.9260	0.45
(Grass I x Experimental 3) x Grass III	68	38	30	1:1	0.9412	0.45

2. Inheritance of purple spots on leaf blade

Purple-spotted leaf blade, which is one of the pronounced characteristics of the grass-type mutants, was present in the F_1 hybrids of the crosses between any one of the three grass mutants and the non-purple-spotted original variety, Experimental 3, and in the F_1 hybrids of the six possible intergrass crosses.

The genetic analyses of this character in the F_2 and segregating backcross and outcross populations are presented in Table 2.

As indicated in Table 2, a chi-square test of the segregation for three purple to one non-purple spotting in the F_2 population of the cross between Grass I and Experimental 3 gives the probability of occurrence of such ratio as high as 0.95, indicating a simple Mendelian inheritance for this character with purple dominant over non-purple spotting. The low value of probability for the occurrence of the monogenic segregation ratio in the cross, Grass II x Experimental 3, might be due to certain disease or insect lesions which masked or inhibited the appearance of purple spots on leaf blades in some of the plants observed, since an identical ratio of 1:1 for purple and non-purple appeared in the backcross populations in which Experimental 3 was backcrossed to its hybrid in combination with either Grass I or Grass II. Again, the plants in the outcross populations were invariably purple-spotted as were those in backcross progenies in which the F_1 hybrids were crossed with their respective grass parents.

These results imply that the gene governing non-purple spotting in Experimental 3 has been changed to the dominant condition which controls purple spotting on the leaf blade of the grass mutants, and that

the purple-spotting genes in the grass mutants are located at the same locus.

Table 2. Genetic Analyses of F_2, Backcross, and Outcross Populations Involving Sorghum Line, Experimental 3, and Three Grass-Type Mutants to Test Segregation of Leaf Spotting by Chi-Square Tests

Combination	No. plants observed	No. plants showing purple spotting	No. plants showing non-purple spotting	Segregation ratio	Calculated chi-square	Approx. P-value
F_2 populations:						
Grass I x Experimental 3	79	60	19	3:1	0.0380	0.95
Grass II x Experimental 3	72	39	33	3:1	8.3334	0.01
Backcrosses:						
Grass I x (Grass I x Experimental 3)	87	87	0	2:0		
(Grass I x Experimental 3) x Grass I	78	78	0	2:0		
Experimental 3 x (Grass I x Experimental 3)	11	5	6	1:1	0.0910	0.75
Grass II x (Grass II x Experimental 3)	115	115	0	2:0		
(Grass II x Experimental 3) x Experimental 3	54	23	31	1:1	1.1852	0.30
Experimental 3 x (Grass II x Experimental 3)	60	32	28	1:1	0.2666	0.60
Outcrosses:						
(Grass I x Experimental 3) x Grass II	39	39	0	2:0		
(Grass II x Experimental 3) x Grass I	27	27	0	2:0		
(Grass I x Experimental 3) x Grass III	68	68	0	2:0		

3. Inheritance of awn condition

The F_1 plants of all of the crosses between strong-awned mutants and awnless Experimental 3 have awns the length of which varies from 0 to 4 mm. and are classified as awnless. The long-awned (9-11 mm.) F_1 plants appeared in the intergrass combinations. The genetic analyses of the awn development in the F_2, backcross, and outcross populations between grass mutants and the original variety are illustrated in Table 3.

A monogenic inheritance for this character was observed in the F_2 plants of the combinations of Experimental 3 with either Grass I or Grass II. The P-value obtained from the chi-square tests for the occurrence of a simple Mendelian 3:1 segregation in these two combinations are 0.35 and 0.30, respectively, indicating that the awnless character of Experimental 3 is dominant.

A 1:1 ratio of awnless to awned plants, found in each instance of the three backcross populations made of the crosses between the F_1 hybrids and their respective recessive parents, confirms the one-gene hypothesis concerning the awn condition in the mutants as well as the original variety.

Similarly, an identical segregation ratio (1:1) found in each of the outcrosses indicates that mutated genes which control the development of awns are at the same locus in all the mutants.

Table 3. Genetic Analyses of F_2, Backcross, and Outcross Populations Involving Sorghum Line, Experimental 3, and Three Grass-Type Mutants to Test Segregation of Awnedness by Chi-Square Tests

Combination	No. plants observed	No. plants awnless	showing awned	Segre-gation ratio	Calculated chi-square	Approx. P-value
F_2 populations:						
Grass I x Experimental 3	79	63	16	3:1	0.9493	0.35
Grass II x Experimental 3	72	50	22	3:1	1.1852	0.30
Backcrosses:						
Grass I x (Grass I x Experimental 3)	87	36	51	1:1	2.5862	0.15
(Grass I x Experimental 3) x Grass I	78	31	47	1:1	3.2820	0.06
Experimental 3 x (Grass I x Experimental 3)	11	11	0	2:0		
Grass II x (Grass II x Experimental 3)	115	48	67	1:1	2.0954	0.15
(Grass II x Experimental 3) x Experimental 3	54	54	0	2:0		
Experimental 3 x (Grass II x Experimental 3)	60	60	0	2:0		
Outcrosses:						
(Grass I x Experimental 3) x Grass II	39	18	21	1:1	0.2308	0.65
(Grass II x Experimental 3) x Grass I	27	12	15	1:1	0.3334	0.60
(Grass I x Experimental 3) x Grass III	68	27	41	1:1	2.8822	0.07

4. Inheritance of dry midrib in leaf blade

The three grass mutants have a juicy midrib in the leaf blade while the original variety has a dry midrib. F_1 plants of the crosses between grass mutants were distinctly juicy. All plants in F_1 progenies in crosses between mutants and the original variety exhibited dry midrib. Therefore, the dry midrib character is dominant over juicy, as reported by previous authors.

Segregation ratios for this character in various crosses (Table 4) were analyzed by using chi-square tests. P-values for a segregation ratio of 15 dry to one juicy in both F_2 populations indicate that the data fit the expected ratio well. It probably reflects a digenic inheritance for this character.

Segregations of three dry to one juicy midrib were obtained in three backcrosses when F_1 hybrids of grass-type mutants and Experimental 3 were backcrossed to their respective grass parents. P-values for such 3:1 ratios are 0.05, 0.70 and 0.80, respectively, indicating significant chi-square values. No juicy midrib plants were found in backcross populations when F_1 hybrids between grass-type mutants and Experimental 3 were crossed to Experimental 3. These data confirm the result obtained from the F_2 populations. The two recessive genes affecting this character in the mutants were derived from the dominant genes producing the dry midrib of Experimental 3 as a result of colchicine treatment.

The chi-squares for testing the goodness-of-fit for a 3:1 segregation for dry versus juicy midrib in all of the outcrosses showed P-values which indicate no significant difference from the expected ratio.

This again gives evidence that the genes which govern the dry-midrib character are located at the same loci in each mutant.

Table 4. Genetic Analyses of F_2, Backcross and Outcross Populations Involving Sorghum Line, Experimental 3, and Three Grass-Type Mutants to Test Segregation of Dry Versus Juicy Midrib by Chi-Square Tests

Combination	No. plants observed	No. plants showing dry	No. plants showing juicy	Segregation ratio	Calculated chi-square	Approx. P-value
F_2 populations:						
Grass I x Experimental 3	79	71	8	15:1	2.0218	0.20
Grass II x Experimental 3	72	66	6	15:1	0.5333	0.45
Backcrosses:						
Grass I x (Grass I x Experimental 3)	87	73	14	3:1	3.9820	0.05
(Grass I x Experimental 3) x Grass I	78	35	11	3:1	0.2246	0.70
Experimental 3 x (Grass I x Experimental 3)	11	11	0	4:0		
Grass II x (Grass II x Experimental 3)	115	85	30	3:1	0.0724	0.80
(Grass II x Experimental 3) x Experimental 3	54	54	0	4:0		
Experimental 3 x (Grass II x Experimental 3)	60	60	0	4:0		
Outcrosses:						
(Grass I x Experimental 3) x Grass II	39	27	12	3:1	0.6923	0.40
(Grass II x Experimental 3) x Grass I	27	19	8	3:1	0.3087	0.60
(Grass I x Experimental 3) x Grass III	68	56	12	3:1	1.9608	0.15

Analysis of Heterosis in F_1 Plants

The F_1 plants of the crosses between the mutants and Experimental 3 are much greater in height and panicle size than either of the parents. The average heights calculated from ten plants of each cross between the grass mutants and Experimental 3 and of each parent are listed in Table 5. These are used to test for the presence of hybrid vigor.

Table 5. Mean Heights Based on Ten Plants of Parents and F_1 Hybrids of Crosses Involving Experimental 3 and Grass Mutants

	Mean height (in.)	
	Mean	S. D.
Parents:		
Experimental 3	32.6	0.40
Grass I	50.8	1.99
Grass II	49.8	2.94
Grass III	51.2	1.56
F_1 hybrids:		
Grass I x Experimental 3	68.0	3.30
Experimental 3 x Grass II	69.6	3.02
Grass III x Experimental 3	63.7	3.95
Experimental 3 x Grass III	63.4	1.71

Table 6. Analysis of Variance for Measurements of Height of Parents and F_1 Hybrids Involving Experimental 3 and Grass Mutants

Sources of variance	D. F.	S. D.	M. S.	F-value
Among varieties & hybrids	7	10789.3875	1541.3411	223.698**
Within variety or hybrid	72	496.1000	6.8903	
Total	79	11285.4875		

Analysis of variance of plant height (Table 6) gives a highly significant F-value, indicating the existence of real differences among the materials analyzed. Further tests for the differences between the F_1 hybrids and their parents by use of Duncan's D test (28) (the calculated significant D-value is 3.65) indicate that (1) the grass mutants are much taller than Experimental 3 but no difference in plant height is shown among the mutants themselves, (2) the height of each hybrid is significantly greater than both its grass parent and Experimental 3, and (3) F_1 plants in the crosses between Experimental 3 and Grass III are shorter than those in Grass I x Experimental 3 or in Experimental 3 x Grass II. The differences in height among the F_1 hybrids of the crosses between Experimental 3 and three grass mutants are very small even though some are statistically significant between the F_1 with Grass III and the other two F_1's.

The considerable increase in vigor of the F_1 plants would appear to be a result of heterozygosity indicating differences in genotype between the parents (Figures III, IV, and V). The significant differences between F_1 groups may represent a degree of difference between genotypes of the mutants or more likely a difference that may be traceable to location in the field. The second would seem to be the case since no differences in vigor or other phenotypic characters were observed between the mutants and F_1 plants of crosses among them (Table 7).

Table 7. Mean Heights Based on Ten Plants of Grass Mutants and F_1 Hybrids Involving All Possible Combinations

	Mean height (in.)	
	Mean	S. D.
Grass I	50.8	1.99
Grass II	49.8	2.94
Grass III	51.2	1.56
Grass I x Grass II	49.5	2.76
Grass II x Grass I	49.2	2.93
Grass II x Grass III	50.3	1.77
Grass III x Grass II	51.0	3.13
Grass I x Grass III	51.2	2.34
Grass III x Grass I	50.3	2.26

Table 8. Analysis of Variance for Measurements of Height of Grass Mutants and F_1 Hybrids Involving All Possible Combinations

Sources of variance	D. F.	S. S.	M. S.	F-value
Among mutants or hybrids	8	44.2	5.52	0.91
Within mutant or hybrid	81	492.7	6.08	
Total	89	536.9		

Analysis of variance of plant height for F_1 plants of the inter-grass crosses and their parents (Table 8) gives a non-significant F-value indicating that no real difference in height exists among the hybrids of different grass combinations or between the hybrids and their respective parents. It would also mean that no heterozygosity has been brought about by crossing the grass mutants. Figure VI shows F_1 plants of the cross between Grass II and Grass I. The absence of heterosis is clearly indicated when these plants are compared to the parents shown in Figure II.

Observations on F_2 Populations of Intergrass Crosses

Like the grass mutants as well as their F_1 hybrids, the plants of F_2 populations of the six intergrass crosses also had green seedling-base, long-awned spikelets, juicy midribs, and purple-spotted leaf blades (Table 9).

Table 9. Observations on Four Genetic Characters in F_2 Populations of Intergrass Crosses

Combination	No. plants observed	Seedling base		Leaf spotting		Awn condition		Midrib	
		Red	Green	Purple	Non-purple	Awnless	Awned	Dry	Juicy
Grass I x Grass II	210	0	210	210	0	0	210	0	210
Grass II x Grass I	205	0	205	205	0	0	205	0	205
Grass II x Grass III	152	0	152	152	0	0	152	0	152
Grass III x Grass II	130	0	130	130	0	0	130	0	130
Grass I x Grass III	250	0	250	250	0	0	250	0	250
Grass III x Grass I	260	0	260	260	0	0	260	0	260

No instance of segregation for plant height in any of the F_2 intergrass crosses has been noted, giving further evidence for genetic homology of the three grass mutants studied (Figure IX, Table 10).

Table 10. Measurement of Plant Heights of F_2 Intergrass Populations and Their Parents

Combination	No. plants measured	Mean height (in.)	
		Mean	S. D.
Grass I	10	56.30	2.5841
Grass II	10	56.90	1.3703
Grass III	10	56.60	1.5776
Grass I x Grass II	30	56.63	1.4739
Grass II x Grass I	30	56.47	1.7370
Grass II x Grass III	30	56.67	1.8261
Grass III x Grass II	30	56.47	1.1064
Grass I x Grass III	30	56.77	1.5017
Grass III x Grass I	30	56.83	1.3352

Figure III. F_1 plants of the cross between Grass I and Experimental 3 showing a considerable degree of hybrid vigor and uniformity

Figure IV. F_1 plants of the cross between Experimental 3 and Grass II showing a considerable degree of hybrid vigor and uniformity

Figure V. F_1 plants of the cross between Grass III and Experimental 3 showing a considerable degree of hybrid vigor and uniformity

Figure VI. F_1 plants of the cross between Grass II and Grass I showing no hybrid vigor and indicating apparent genetic homology of both parents

Figure VII. F_2 progeny of the cross between Grass I and Experimental 3 showing segregation for many characters

Figure VIII. F_2 progeny of the cross between Grass II and Experimental 3 showing segregation for many characters

Figure IX. F_2 population of the cross between Grass I and Grass II showing no segregation. F_2 progenies of Grass I x Grass III and Grass II x Grass III likewise showed no segregation.

Cytological Studies

Ten bivalents were observed at either diakinesis or metaphase I of the meiotic cells in all F_1 hybrids as well as in their parental lines. No irregularity of division was found in either pre- or postmetaphase stages of meiosis.

The average numbers of chiasmata formed per cell at diakinesis in hybrids among the grass mutants and Experimental 3 and in the parents are presented in Table 11.

Table 11. Average Number of Chiasmata Per PMC at Diakinesis as Measured by Number of Open Bivalents in Hybrids Among Grass Mutants and Experimental 3 and in the Parents

Combination	No. of cells observed	Total no. of open bivalents	No. of chiasmata per cell	
			Mean	S. D.
Parents:				
Experimental 3	50	10	19.80	0.40
Grass I	60	13	19.78	0.43
Grass II	51	8	19.83	0.36
Grass III	55	11	19.80	0.38
F_1 hybrids:				
Grass I with Experimental 3	50	9	19.82	0.38
Grass II with Experimental 3	50	8	19.84	0.36
Grass III with Experimental 3	60	12	19.80	0.38
F_1 intergrass crosses:				
Grass I with Grass II	50	9	19.82	0.38
Grass II with Grass III	50	8	19.84	0.36
Grass I with Grass III	50	8	19.84	0.36

As indicated in Table 11, the average number of chiasmata per cell in each instance is around 19.80. Apparently, there is no significant difference in number of chiasmata among the materials studied. There

seems to be no indication of any change involving chromatin blocks, i.e., no chromatin rearrangement. Instead, the grass mutants could occur as the result of identical, simultaneous mutations at different loci after colchicine treatment of the original variety.

DISCUSSION

The information obtained from the genetic analyses of four qualitative characters (red vs. green seedling base, dry vs. juicy midrib, purple vs. non-purple spotting on leaf blade, and awnless vs. awned) studied in F_1, F_2, and backcross populations indicates that the segregation of each character obeys the Mendelian laws, and that segregation for each particular character in the populations of each specific category performed in the same fashion no matter which one of the three grass mutants was crossed to the original variety Experimental 3.

The simple Mendelian segregation for seedling-base color, for leaf spotting, and for awn condition indicates a difference of a single pair of genes for each of the three contrasting characters between the grass mutants and the original variety, whereas a dihybrid segregation for dry midrib comprises a difference of two pairs of genes for this character. Since the same segregating ratios were found in the outcrosses involving crosses of F_1 plants to a different grass mutant, as in backcrosses of the F_1 plants to the same grass parent, it would seem that identical loci were mutated in each of the grass mutants. Further support for this conclusion was obtained when no indication of genic interaction in each of the four characters was noted in the F_1 hybrids of the grass crosses. The complete uniformity of the F_2 progenies of the intergrass crosses makes the conclusion inevitable that not only the genes studied but also other mutated genes not analyzed have been mutated identically in each of the mutants.

A considerable and uniform degree of heterosis was observed in F_1 hybrids between each of the grass mutants and Experimental 3. According to Quinby and Karper (19) the heterozygous condition of the gene Ma, which controls plant response to photoperiod and influences time of floral initiation in sorghum, produced plants that were larger than homozygous genotypes of comparable growth duration. They assumed that heterosis in sorghum resulted from stimulation of cell division and considered that homozygotes are unable to make full use of the nutrients available to them whereas heterozygotes are able to make greater use of the same nutrient supply. As indicated in this experiment, five pairs of heterozygous genes are detectable in each of the F_1 hybrids between the grass mutants and Experimental 3 for the characters studied. Quantitative characters were not investigated. The increase of vigor exhibited by these hybrids may be attributed to heterozygosity. On the other hand, the lack of heterosis observed in F_1 intergrass plants, and lack of segregation in F_2 progenies for any characters including those studied, may be indicative of the homozygosity of the F_1 plants and of the identical nature of the genetic constitutions of the three grass mutants.

The mechanism of the occurrence of the genetically identical mutants after colchicine treatment of a single variety is a matter of speculation. According to Franzke and Ross (14), gene mutation prior to somatic reduction and followed by restoration to the diploid number might be responsible for the appearance of true-breeding diploid mutants. The effect of colchicine in mutagenesis has been found to be spread widely over many loci on different chromosomes, hence not restricted to one locus of one chromosome. As reported by Foster *et al.* (12), at least 12 genes had

been changed for the characters studied in F_3 data from a cross between another grass-type colchicine mutant and the original variety. Thus, the probability for occurrence of such mutants having similar genetic constitutions must be extremely low. Perhaps some chromosome aberration after colchicine treatment could have been involved in the simultaneous change of a number of characters in the mutants. Nevertheless, this assumption has received no support from pachytene analyses of colchicine mutants and their F_1 hybrids with the original line (Harpstead et al., 18) or from the examination of stages of meiosis (including chiasma counts on the mutants, the original variety, and their F_1 hybrids) made by the author. The occurrence of definite types might be expected to result from chromatin duplication as was observed by Blakeslee (4) for trisomics in Datura, but if this were so, extra chromosomes or multivalents among homologous chromosomes or parts of chromosomes at meiosis should be observed. The observation of a constant chromosome configuration of ten bivalents at diakinesis and metaphase I in both grass mutants and their F_1 hybrids does not support this hypothesis. From genetic investigations, Foster et al. (8, 9) found normal genetic behavior with no detectable linkage between mutated genes studied in F_2 populations from crosses between mutants and their original lines. Erichsen et al. (7) also indicated independent segregation of mutated genes in another mutant. These results do not agree with the hypothesis proposing rearrangement of chromosome blocks or chromatin duplication.

After examining F_2 populations of crosses between different awned lines (a colchicine-induced grass mutant, one of the parents of Experimental 3, and an irradiation-induced mutant), Ross et al. (25) reported

lack of transgressive segregation for this character indicating that the same locus was involved in each instance. Such a precise location of the awned locus in the colchicine mutant would not be expected if duplication or concentration of already existing chromatin were involved in the formation of mutant genotypes. From preliminary results which indicate that after colchicine treatment somatic chromosome numbers have been reduced from 4n to 2n (Sanders and Franzke, unpublished), and that structurally heterozygous chromosomes have become homozygous (Simantel, unpublished), it seems now possible to conclude that somatic reduction does take place as a result of treatment. Genic change occurring prior to reduction of the chromosomes and followed by restoration to the diploid number seems to be a plausible explanation for the homozygosity of the mutants.

As recognized by biologists, colchicine is a polyploidizing agent. The mechanism by which mutations are effected in sorghum seedlings which still retain the original diploid number after colchicine treatment must be considered. Since such simple organic compounds as ethylene oxide and ethylene immine have been found to be very effective as mutagenic agents (6, 24), the mutational effect of colchicine could possibly be ascribed to some simple substances produced as the result of degeneration of the metabolites in the colchicine-induced swellings within the coleoptile of treated seedlings rather than to the colchicine itself.

No case of such identical mutants resulting from gene mutations at so many identical loci has been hitherto reported. Without the weight of cytological observations against it, the obvious a priori conclusion would be that the repeated occurrence of similar phenotypes results from

chromosome changes such as the trisomics described by Blakeslee (3, 4). Unless chromatin duplication undetectable by classical cytological techniques occurs (26), the changes must be ascribed to mutagenic action on the genic level. It would seem that the identical genotypes occurring in each of the mutants might possibly have arisen from the same mutagenic effect being present in each instance within the swollen coleoptile resulting from treatment with colchicine. This suggests that directed mutations are not beyond the realm of possibility.

SUMMARY

Three phenotypically similar true-breeding diploid grass-type mutants and the grain sorghum variety, Experimental 3, from which these mutants were induced by colchicine treatment, were used to investigate the genotypic similarities of the mutants. The modes of inheritance of four different contrasting characters, namely, red vs. green seedling base, dry vs. juicy midrib, purple vs. non-purple spotting on leaf blade, and awnless vs. awned, were analyzed from the results obtained from five categories of crosses.

Heterosis was shown uniformly in F_1 plants of all crosses between Experimental 3 and the grass-type mutants indicating a marked genetic difference between the mutants and the original variety. The uniformity between F_1 plants in each cross indicates that the mutants and the original variety are homozygous. No heterosis was shown in F_1 hybrids from the six possible crosses between the grass-type mutants, and no phenotypic variability was found, indicating their genetic similarity.

Single Mendelian inheritance for seedling-base color, leaf spotting, and awn condition, and dihybrid segregation for dry midrib were the same for all three grass-type mutants in F_2, backcross, and outcross populations in combination with Experimental 3.

The identical phenotypes of the three grass-type mutants were also found in intergrass F_1 plants and in the completely uniform intergrass F_2 progenies. These results, along with the identity of backcrosses involving the grass parent and outcrosses, indicate that the genotypes were also identical and that all the mutated genes, as well as the five

studied in this investigation, were identical in each of the mutants.

No evidence of structural chromatin change was found in meiotic cells of parental materials or of F_1 plants derived from all possible crosses among the mutants and the original variety. It may be concluded, therefore, that these mutants are genotypically as well as phenotypically the same. The identical genotypes occurring in the mutants might possibly have arisen as a result of the same mutagenic effect being present in each instance within the swollen coleoptile resulting from treatment with colchicine. If this is so, the possibility of being able to direct mutation is indicated.

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