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A Study of Progenies of a Colchicine Treated F1 Hybrid of Zea mays L

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A STUDY OF PROGENIES OF A COLCHICINE TREATED F_1 HYBRID OF ZEA MAYS L.

BY

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A STUDY OF PROGENIES OF A COLCHICINE TREATED F_1 HYBRID OF ZEA MAYS L.

Abstract

Under the Supervision of Professor D. Boyd Shank

Field studies of 140 F_2 lines derived from colchicine treated plants of the maize single cross SD5 x SD7 were made to establish the possibility of duplicating the production of true-breeding homozygous variants as reported for sorghum. These 140 lines were compared with 60 nontreated F_2 lines out of the same cross. The results obtained did not indicate differences between the treated and untreated material, either as entire classes or as individual lines, for these six phenotypic measurements: plant height, ear height, stalk diameter, ear length, ear diameter and ear weight. Nor were differences in variability within individual treated progenies, or treated progenies as a class, established in comparison to the controls. No clear-cut evidence for the recovery of homozygous variants or inbred lines in one generation as a result of colchicine treatment of the single cross could be demonstrated. Some reasons for the differential performance of this single cross as compared to the sorghum variety Experimental 3 are given, and some suggestions are made with regard to the direction of further work.

A STUDY OF PROGENIES OF A COLCHICINE TREATED

F_1 HYBRID OF ZEA MAYS L.

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

Thesis Adviser

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INTRODUCTION

Since the development of hybrid corn, improvement has been through the production of new inbreds and elimination of undesirable characteristics in existing lines. This work has resulted in more vigorous inbreds, higher yields in hybrids and the maintenance of heterosis levels under stress of disease, drought, insects and other limiting factors.

In view of the desired objectives, several methods have been developed for obtaining lines. Many research workers have proposed recurrent selection systems permitting selection pressure to operate efficiently during the inbreeding process. However, this method may even increase the number of generations required to obtain homozygous lines. On the other hand, Chase (4), developed a technique for obtaining homozygous lines in one generation. This system allows the worker to sample either more sources or larger numbers in a given germ plasm.

With a better understanding of the natural and potential variation which exists in diseases, insects and viruses, and the impact which such variations may have upon the crop, there is an urgent need for a method of inducing and determining new genetic variation in plants. If the induced genetic variation were in an immediate homozygous condition both the time and variability problems would be solved. Mutation-breeding through the use of irradiation and chemicals has shown much

promise in this area.

Considerable information exists on the potential role of colchicine in effecting these desired changes. True-breeding variants in sorghum were obtained at the South Dakota Station by an adaptation of the polyploidizing use of this alkaloid. Attempts were made to extend these findings to Zea mays, but here in true-breeding variants did not appear. It was assumed that they appeared less often and/or were not evident because of the population sizes used.

The purpose of the present study was to determine if colchicine treatment of large numbers of corn seedlings would give rise to segregates that were homozygous or true-breeding, as reported for sorghum.

The hypothesis that colchicine treatment of corn seedlings would result in the induction of homozygous mutants was studied by treating F_1 single cross seedlings and using a variance study of vegetative measurements of the resulting progenies from treated versus untreated selfed plants.

REVIEW OF LITERATURE

Colchicine became a tool for the plant geneticists and plant breeders around 1937. It was discovered that colchicine would induce chromosomal doubling. Eigsti and Dustin (6) indicate that colchicine is effective as a polyploidizing agent in concentrations ranging from 1.0 to 0.01 per cent.

In 1952, Franzke and Ross used colchicine to produce true-breeding variants in sorghum (8). A great deal of intensive work at the South Dakota Station has resulted in two hypotheses to explain the effects of concentrations of 0.5 per cent colchicine on these species. They are: (1) colchicine induces homozygosity through a reductional division in somatic tissue which is followed by doubling of the haploid cell. This now homozygous cell reconstitutes a new growing point. (2) colchicine acts as a mutagenic agent.

Huskins first discussed the possibility of somatic reduction in his 1948 paper (15) and furnished preliminary information on reductional groupings as causing this variation. He challenged the accepted concept of uniform chromosome constitution of living organisms, and furnished preliminary evidence for the role of reductional groupings as causing this variation. Further work by Huskins and Cheng (16) indicated that a wide range of types of segregation were found, from reductional divisions following pairing with and without chiasmata, to segregation,

and prophase without spindle formation or pairing. They indicated haploid cells do occur but that segregation without prior pairing and restoration to the diploid number by an abortive second division was of more common occurrence. Wilson and Cheng (23) working with Trillium found evidence for segregation of homologues at greater frequency than would be due to chance alone following sodium nucleate treatment. Wilson et. al. (24) and Allen, Wilson and Powell (2), all working with colchicine and sodium nucleate induced reductional groupings, found increases for both chemicals over chance alone. Ross and Holm (21) found the frequency and size of spots of the aurea mutant in tomatoes indicating somatic segregation for homologous tissue was apparently increased following colchicine treatment at the seedling stage.

Hanson (11), in an attempt to test the somatic reduction hypothesis worked with colchicine seedling treated material of maize. The maize germ plasm used carried chromosome translocations and both recessive and dominant gene markers. He found few if any true-breeding variants. He indicated that the variants obtained could possibly be explained by contamination during pollination, seed mixture, inadequate sampling, nonheritable phenotypic colchicine effects or mutation from other known causes.

Harpstead, Ross and Franzke (13) made cytological studies of F_1 hybrids between mutant and untreated sorghum lines. These studies showed no chromosomal irregularities. Using the same genetic material

Foster (10) found the mutant characters to be independently inherited. He suggested the possibility of colchicine induced point mutations made homozygous by somatic reduction and the development of a new growing point. Atkinson, Ross, and Franzke (3) found colchicine treated true-breeding stocks of Experimental 3 and Norghum to show different frequencies of mutants. Twenty-four out of 43 treated Experimental 3, but only four out of 54 treated Norghum plants were considered to be mutants. Cytological examination at meiosis of 30 treated plants of each variety indicated only diploid plants in Experimental 3 while three out of the 30 plants of Norghum were tetraploids. They concluded a varietal difference in response to colchicine treatment for both mutation rate and propensity to polyploidy.

Dirks, Ross and Harpstead (5) treated F_1 seedlings of a Crystal x B-5128 flax cross and found true-breeding chimeral sectors for flower color, seed color and rust resistance. This recovery of parental genes in homozygous condition from a heterozygous plant was proposed as evidence that reduction division of somatic tissue with subsequent doubling is not necessarily associated with the mutagenic effect of colchicine.

Franzke and Ross (9) in 1957 reported a lineal series of mutants which were induced by colchicine treatment. They treated the true-breeding grain sorghum, Experimental 3 (cited in previous articles), and obtained a true-breeding mutant line having a taller growth habit. Retreatment of this mutant gave rise to two dwarf mutants. These also

gave pure line progenies. Retreatment of the dwarfs gave new nonsegregating lines, some of which resembled the original untreated variety. These data were interpreted as furnishing evidence of gene mutation effects by colchicine. Somatic reduction was proposed to account for homozygosity or pure line response in the generation following treatment.

In 1958 Moore (18) reported data on SD 7, an inbred line of corn developed by C. J. Franzke at the South Dakota Experiment Station, and a colchicine derivative of this inbred. This "Derivative" had been previously obtained by selection in treated SD 7. This first treatment was part of a preliminary screening of corn inbreds for colchicine response. No attempt was made to attribute this Derivative to colchicine treatment directly; however, the inbred SD 7 had been selfed for many generations with no similar variations appearing prior to colchicine treatment. Moore treated SD 7 and the colchicine derivative. Progeny rows of SD 7, SD 7 treated, Derivative and Derivative treated were compared through vegetative character measurements. Moore concluded the Derivative was more susceptible to treatment and to genetic changes following treatment than SD 7. The evidence of the latter was based on statistically analyzed data which showed populations of SD 7 differing from SD 7 treated and those of Derivative differing from Derivative treated. All measurements showed significant mean differences between original SD 7 and SD 7 treated once or SD 7 Derivative retreated. The data also indicated support of the homozygosity theory as treated progenies were

consistently more uniform than progenies of untreated plants.

The two theories that have been proposed by those working with colchicine in sorghum and corn seem to be tenable with the findings of Fahmy and Fahmy (7). Working with carcinogens and tumor inhibitors in comparison to X-radiation, they concluded that chemicals induce many changes differing in phenotype and genetic position from those induced by the physical agent. Nearly twice as many small deficiencies and less major structural rearrangements of the chromosomes were found following chemical treatment than with X-ray. This may explain the results of Harpstead et. al. (13) and Atkinson et. al. (3) where no chromosomal irregularities could be detected in their cytological studies of sorghum colchicine variants.

Heiner, Konzak, Nilan, and Legault (14) working with barley treated with Diethyl Sulfate and Gamma rays reached a conclusion similar to that of the Fahmys (op. cit.). They found a lower frequency of chromosome structural changes and higher frequency of mutations for the sulfate in comparison to Gamma rays.

Mutation-breeding dates back to Muller's experiments in Drosophila. He concluded that X-rays would induce genetic deviants similar to naturally occurring ones. Stadler soon showed that X-rays could induce high mutation rates of undesirable genes in barley and maize. Despite this beginning, numerous workers such as Nilsson-Ehle, Gustafsson, Tedin, Froier, Anderson and Olsson, all of Sweden investi-

gated the potential of mutation-breeding on many agonomic and horticultural crops. Allard (1) states that, "Two facts must be taken into account in judging the economic feasibility of searching for favorable mutants: (1) deleterious changes outnumber constructive changes by a factor of several hundred to one; and (2) most mutants are recessive. It follows, therefore, that very large second-generation populations are necessary if the desired improvements are to be obtained and detected." He also indicates most successful results in mutation-breeding will be derived only in the areas where efficient screening techniques are available for detecting genetic characteristics desired.

If colchicine induces homozygosity as well as mutations, as is indicated by the sorghum work, many of the problems encountered to date in mutation-breeding would be solved. Deleterious recessives could be recognized immediately. Lethal recessives would immediately eliminate themselves. More germ plasm sources could be sampled allowing greater probability of obtaining desired character improvements.

MATERIALS AND METHODS

Selection of Plant Material and Description

Initial work on the research for this dissertation was started in 1953. Six inbreds, (SD7, Wf9, Oh56A, SD26, SD5, SD48) and the single cross (SD5 x SD7) were treated in the seedling stage in the greenhouse with colchicine. The treated plants which survived were selfed and progeny-rowed in the field in 1954 and 1955. Selfing was used to maintain each line. In terms of reaction to colchicine treatment as measured by C-tumor formation and other colchicine responses that have been associated with genetic changes, the following rating was established: Wf9, SD5 and (SD5 x SD7), excellent; SD48, good; SD26, fair; SD7 and Oh56A, poor.

The SD7 Derivative subsequently used by Moore (18) for his investigations was selected in these studies. He found it to vary significantly from SD7 in smut resistance and in vegetative characters: length of shank, diameter of ear node, leaf width, total plant height, height to ear node, and number of internodes.

Since the single cross (SD5 x SD7) was found to be an excellent reactor it was further studied in 1954 and 1955 with the objective of improving treatment technique and greenhouse care following treatment. In this work numerous mutants varying from small phenotypic changes to gross changes were observed. It became apparent that either colchicine

was bringing about the type of responses reported by Franzke, Ross and others in sorghum, or that such changes were due to the accidents of sampling, residual variability, contamination and natural mutation. It seemed unlikely that these latter forces could be responsible for the consistently noted variants recovered in this preliminary work. The use of a single cross and treatment of the F_1 seedlings followed by testing of their selfed progenies and subjection of the data to statistical analysis, should differentiate between natural variation and the induced homozygosity hypothesis.

Once the decision had been made to study a single cross, (SD5 x SD7) was a natural choice. It had been used in preliminary studies and sufficient seed was available so that the same source of seed could be used throughout the study. The inbreds, SD 5 and SD 7 were developed by C. J. Franzke at the South Dakota Experiment Station. SD7 was developed by inbreeding the open-pollinated variety, Sundstrom 90, while SD5 came from inbreeding in (Brookings 86 x (1210 x B107) x Brookings 86). Brookings 86 is a local adaptation of the Minnesota 13 variety. The line 1210 was described as the highest yielding male out of a Close Bred Corn Breeding system carried on by A. N. Hume prior to a hybrid corn program at the South Dakota Station. B107 was a Brookings 86 selection. Seed of the single cross was obtained from the Foundation Seed Stocks Division at South Dakota State College and was Certified seed.

Treating Technique

The mechanics of the application of the colchicine to the seedlings were similar to that used by workers producing mutations in sorghum.

Seed was treated with Arasan prior to germination. Seed treatment was found necessary as the seedlings would often remain in the early germinating stage for considerable periods following colchicine application. Many seedlings were completely rotted prior to emergence from the C-tumor stage despite seed treatment.

The seeds were placed between germination blotters in petri dishes and germinated in the dark at greenhouse temperature. When the coleoptile had elongated .2-.3 of a centimeter, the pericarp was carefully removed from the embryo area of the seed. Sufficient 0.5% colchicine in lanolin was applied, by using a toothpick, to completely cover the coleorhiza, cotyledonary node, first internode and coleoptile. Primary root contact with the chemical was avoided since it had been found that this would usually kill the root and such treated seedlings would fail to resume growth following the C-tumor stage. It was found that a tight seal of the colchicine-lanolin mixture around the area treated increased seedling response. During application, the chemical mixture was warmed to keep the lanolin in a liquid state. This temperature was not high enough to injure the embryonic plant parts. The liquid state of the chemical mixture allowed for a better seal of the embryo parts covered.

Treated seeds were placed in two gallon crockery jars filled about two-thirds full of sterilized sand. The treated seed was placed so that the developing root could readily penetrate the moisture laden sand and yet the sand did not cover the embryo area of the seed (Fig. 1). The jars were covered with glass and placed on a greenhouse bench where light was available but direct rays of the sun were prevented from contacting the kernels. C-tumor development of the seedlings took place in these so-called sweat jars. The developing plants were left in these jars until the plumule emerged from the C-tumor. Then the young seedlings were transplanted to a greenhouse ground bed and allowed to develop under greenhouse conditions. Figure 1 illustrates both C-tumor sizes and the approximate stage of growth at which seedlings were transplanted from the "sweat jars" to the ground bed. Treated seedlings were rated using a scale of 1-5 as to intensity of C-tumor development and plumule growth prior to transplanting, with 1 being the largest C-tumor and the longest period before plumule growth. Figure 2 shows a comparison of the 1, 2, and 3 C-tumor ratings at the time they were made. These ratings were made in the 1956 treated material but not in 1957. A record was kept of the rating for each treated seedling and an attempt was made to use progenies from the lower numerical ratings for field studies. This was not possible in all cases because the more severe the colchicine reaction at treatment time, the greater was the mortality in the dormant C-tumor stage. Figures 3 through 7 show regrowth and phenotypic appearance of

an average plant from each rating group, 1 to 5. It was noted that the severe colchicine effect at germination time retarded the further development of the plant. Thirteen treated seedlings were rated 1 for severity of colchicine effect. Of these 13 only 2 produced an ear with sufficient selfed seed for use in the field study.

This treatment method seems to be in agreement with the conditions found to be most conducive to induction of mutants in sorghum by Sanders, Franzke and Ross (22). They found improved conditions such as agar media rather than sand, presence of major minerals in media, and presence of up to 2% sucrose increased the rate of early regrowth and survival following treatment but decreased induced mutants.

All surviving treated and transplanted plants were self pollinated and grown to maturity in the greenhouse. Untreated single cross plants were also produced under the same conditions and self pollinated.

Field Plot Design and Data Recorded

The comparison of treated progenies to untreated progenies out of the single cross was carried on for 2 years. Each year 70 treated progenies and 30 untreated progenies were compared. A completely randomized block design was used; it had 3 replications with single row plots 35 feet long. A check plot of the single cross was included every tenth plot to determine uniformity of soil over the test area and to aid in making measurement comparisons. The seed was hand planted, one kernel

every 14 inches. A purple marker corn was used for replanting where missing hills occurred to give competition. Ten plants out of the 30 possible were selected in each row for measurements. These plants were randomly selected from those with plants 14 inches on either side in the row. The selected plants were numbered 1 to 10 in each row and all measurements were recorded so that the six measurements taken were on the same plant. Three of these measurements, total plant height to tassel tip in inches, ground level to top ear node in inches, and diameter of the internode just below the top ear node in centimeters were made on the individual plants shortly after pollination had taken place. The ears from these same plants were harvested and individually bagged. After they were dried artificially at 100° F. to uniform moisture, the other three measurements, ear length in centimeters, ear diameter at mid-ear in centimeters, and ear weight in grams were made. For the two years a total of 140 treated, 60 untreated and 23 single cross progenies were measured. At 10 plants per replication, 3 replications and 6 measurements per plant this totalled to 40,140 individual measurements made over the two year period.

Statistical Analysis

Data of all measurements were placed on IBM cards. Calculations for each measurement were made for within row variances and means based on plant to plant variation. An analysis of variance of the within row variances was run to determine if colchicine treatment had induced homozygosity within lines derived from a normal segregating population. Graphs were drawn for line distribution and within line variance means.

Field Plot Conditions

The field studies were conducted on the Agronomy Farm at the South Dakota State College Experiment Station. The land selected for trials was in a two year small grain, one year corn rotation. Fertility on these plots is maintained by application of 150 pounds of 16-20-0 mixed fertilizer each year previous to small grain and 10 tons of manure previous to corn.

Figure 1. The appearance of colchicine treated seedlings just prior to transplanting from "sweat jars" to the greenhouse groundbed.

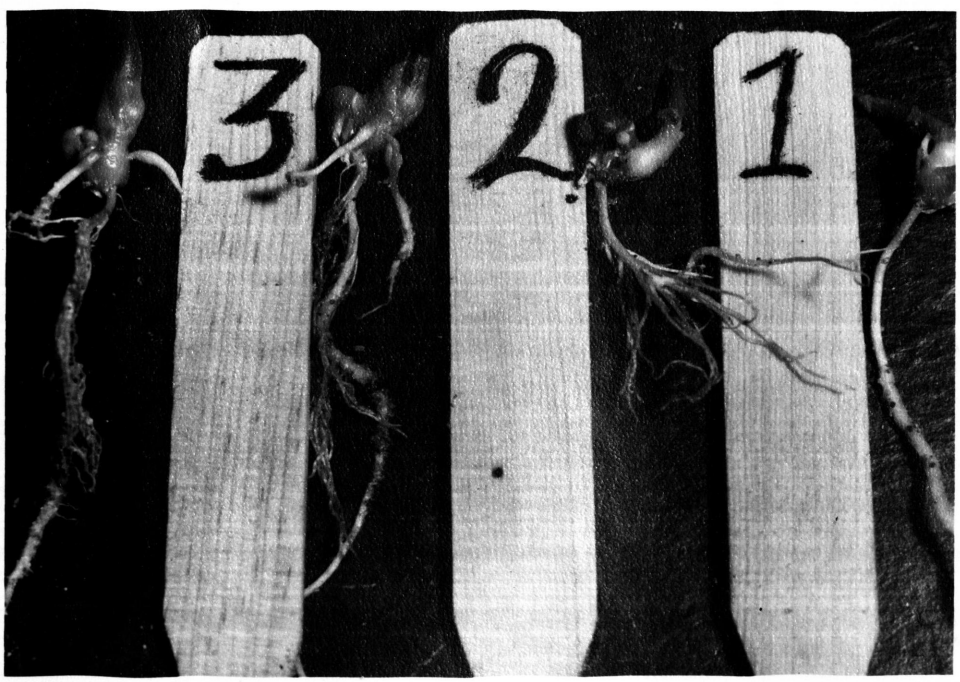


Figure 2. A comparison of C-tumor ratings 1, 2 (center 2 plants), and 3 made prior to transplanting to greenhouse groundbed.

Figure 3. A plant approximately 2-3 weeks after transplanting with a C-tumor rating of 1.

Figure 4. A plant approximately 2-3 weeks after transplanting with a C-tumor rating of 2.

Figure 5. A plant approximately 2-3 weeks after transplanting with a C-tumor rating of 3.

Figure 6. A plant approximately 2-3 weeks after transplanting with a C-tumore rating of 4.

Figure 7. A plant approximately 2-3 weeks after transplanting with a C-tumor rating of 5.

EXPERIMENTAL RESULTS

Favorable climatic conditions for corn yields prevailed during both growing seasons. Above normal rainfall for the entire growing season and warm weather in June of 1956 favored good corn development. The plot appeared uniform for germination and seasonal development. It was necessary to replant a purple marker corn in only a few isolated hills to obtain the plant competition desired for making plant measurements. In 1957 growth and development of plants appeared normal. Several treated lines gave evidence of poor germination ability but by planting in purple marker corn soon after emergence of the test lines sufficient plants per row with competition were obtained.

Table I presents the complete analysis of variance for the 1956 measurements of plant height, ear height and stalk diameter. The stalk diameter proved to be a poor measurement as it was very difficult to determine the same place in the internode for each plant. It will be noted in this table as well as in the following tables that the test areas were uniform as in only one case was any significance found for replications (or that replications were so large as to be adequate subsamples of the entire field).

The 1956 plant height measurements showed a significant difference in progenies. This difference was found to be between progeny groups and in the comparison of the single cross check versus the treat-

ed plus untreated groups. Since the treated and untreated groups were the F_2 generation from the single this difference can be explained by the normal genetic difference between generations of a hybrid.

Figures 8 and 9 present line graphs of the progeny variances and means for plant height in 1956, respectively. All curves indicate normal populations. Treated and untreated show similar curves as would be expected if no mutations or induced homozygosis had occurred as a result of treatment.

Ear height in 1956 gave a highly significant difference for progenies. This was found to be among treated and among single cross lines. The expected difference between F_1 progenies versus treated plus untreated F_2 was shown. Figures 10 and 11 show a two peaked curve for both treated progeny means and variance means with less evidence of this for untreated progenies. The mean curve of treated progenies in Figure 11 shows a slight shortening of the plant for ear height following treatment. This was not apparent in the total plant height for 1956. As was previously mentioned the plant diameter measurement was difficult to take accurately. This seems to be indicated in the analysis of this measurement in Table I and in Figures 12 and 13, where no difference was found among or between any progeny groups. The curves for means and progeny means appear normal.

The analysis of variance of variances for ear measurements in 1956 are presented in Table II. Figures 14, 15, 16, 17, 18, and 19 show

frequency distributions of means and progeny means for these data, respectively.

Inbreeding in a heterozygous corn population normally brings about a shortening of ear length. The analysis in Table II shows this same pattern as the treated and untreated F_2 groups were found to vary significantly at the 1 per cent level from the F_1. Figure 14 presents a normal curve for treated line variances while the untreated and single cross lines are more bunched. Figure 15, showing the means of the progenies, indicates as would be expected, the treated and untreated lines had consistantly shorter ears than the single cross. The curves of the treated and untreated, however, appear much alike with only a slight indication of shorter ears in the treated.

Ear diameter data in 1956 followed a similar pattern to ear length. The expected difference between F_2 and F_1 lines was found. Figure 16, however, shows variance curves for treated and untreated to be almost identical. The reason for the two peaked curves in both cases could be due to sample size or normal segregation occuring in F_2 progenies. The variance range designation for all curves was selected at random with no attempt to produce curves of predetermined shape. It will be noted in Figure 17 that the means of treated and untreated gave almost identical curves and showed again the expected smaller ear due to inbreeding from the F_1 generation. It should be noted that the large percentage of the single cross lines had nearly identical ear diameter means. This

could be expected if the plot area and other environmental conditions were fairly uniform.

Analyses of the ear weight data, Table II, for 1956 showed no difference between treated and untreated groups; however, there was a significant difference between F_1 and F_2 groups. Figure 18 indicates a bi-modal curve for the variances in treated lines. The variances of the F_1 lines were found to cover a range similar to the treated and untreated F_2. This was not found to be the case for any other measurement except ear weight in 1957. Figure 19, showing the line means for the 1956 ear weight measurement, shows the F_1 progeny means to be actually greater than the means of F_2 lines, as expected. The populations of all three groups appear normal with little difference between the treated and untreated groups.

The analysis of data from the 1957 trials are presented in Tables III and IV. For the three plant measurements the second year's data is almost identical with that of the first year. Plant height was found to vary between the single cross F_1 progenies and the F_2 progenies. There was a difference in untreated progenies at the 5 per cent level. Variance mean curves in Figure 20, however, show little difference between treated and untreated lines. The two curves for progeny means of treated and untreated lines (Figure 21) are almost identical and are less than those for the F_1 lines.

Ear height was found to vary between the single cross lines and

the treated plus untreated lines as well as among the treated lines. The variance means curve for treated lines in Figure 22 indicate one progeny falling outside a fairly normal curve. This progeny variance was based on only 12 plants over the 3 replications instead of the regular 30 plants. This may account for the 1.75 variance compared to the others falling between 2.50 and 6.00. The progeny means for the treated lines gave a normal curve while the untreated was bi-modal (Figure 23).

The plant diameter measurement in 1957 gave similar results as in 1956, however, a significant difference was found between F_1 versus treated plus untreated F_2 groups which had not been indicated in 1956. The variance means and progeny means curves were similar with no evidence of departure from normal.

The ear measurement data (Table IV) gave no difference between treated and untreated lines for any measurement in 1957. Ear length and ear diameter resulted in a significant and a highly significant difference between the F_1 and F_2 groups respectively. Curves of variance means and line means (Figures 26 and 27) for ear length showed greater variances and greater means than in 1956, however, the curves themselves were similar. Ear diameter curves (Figures 28 and 29) show the untreated lines to form a bi-modal curve of variance means while the treated lines curve is nearly normal. The progeny means show a nearly normal curve again for the treated while the untreated appears skewed to the right. Sample size may be a factor in the curves for the

untreated material. Figures 30 and 31, showing ear weight line variance means and line means respectively, indicate similar populations for treated and untreated. The F_1 progenies are shown to have nearly the same variance range as the treated and untreated, but reflect the expected greater line means. The difference in line means for the F_1 of 185 to 209 grams would indicate uniformity in plot area. This is a variation of only 24 grams, whereas the treated varied 39 grams from the least heavy progeny to the heaviest and the untreated, 44 grams.

TABLE I. Analysis of Variance of Mean Squares for Plant Measurements as Obtained in 1956. (Plant Height in Inches, Ear Height in Inches and Diameter of Stalk in Centimeters).

		PLANT HEIGHT			EAR HEIGHT			PLANT DIAMETER		
Source of Var.	DF	SS	MS	F	SS	MS	F	SS	MS	F
Total	335	942.93			300.47			1.14		
Rep.	2	4.49	2.243	.92	3.65	1.826	2.36	.01	.005	1.54
Prog.	111	354.90	3.197	1.30*	125.10	1.127	1.46**	.38	.004	1.02
Reps. x Prog.	222	583.55	2.447		171.71	.774		.75	.003	
Bet. Prog. Groups	2	19.42	9.711	3.97*	9.80	4.898	6.33*	.01	.006	1.76
T. vs U.	1	3.57	3.573	1.46	1.37	1.371	1.77	.0004	.0004	.12
S.C. vs (T + U)	1	15.85	15.848	6.47*	8.43	8.425	10.89**	.01	.012	3.53
Among T.	69	201.99	2.927	1.08	66.13	.958	1.60**	.11	.002	1.36
Among U.	29	65.44	2.257	1.07	36.45	1.257	1.29	.24	.008	.98
Among S. C.	11	68.04	6.185	1.64	12.72	1.157	5.39**	.02	.002	.69
Reps. of T.	2	3.08			3.91			.0005		
Reps. of U.	2	2.03			5.30			.02		
Reps. of S. C.	2	3.12			10.02			.01		
Reps. x T.	138	374.66	2.715		94.67	.599		.19	.001	
Reps. x U.	58	122.33	2.109		56.75	.978		.48	.008	
Reps. x S. C.	22	82.82	3.764		4.72	.215		.06	.003	
Reps. x Bet. Groups	4	3.74	.935		15.58	3.895		.02	.004	

* Significant at 5% level
** Significant at 1% level
Rep. = Replication Prog. = Progenies T. = Treated U. = Untreated S. C. = Single Cross

FIGURE 8 1956 PLANT HEIGHT

VARIANCE MEANS FOR PROGENY LINES

FOR THREE REPLICATIONS

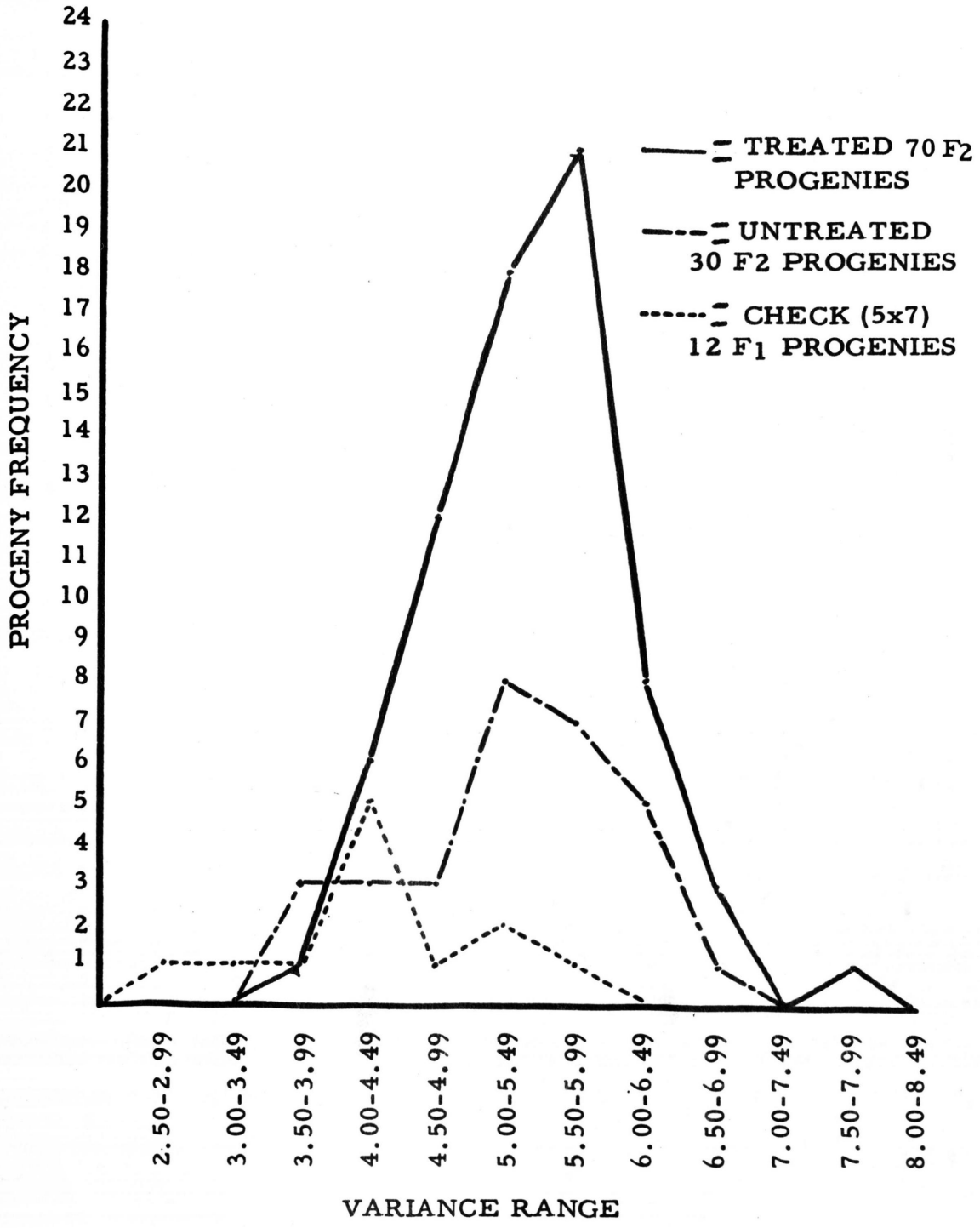


FIGURE 9 1956 PLANT HEIGHT MEANS FOR PROGENY LINES FOR THREE REPLICATIONS (IN INCHES)

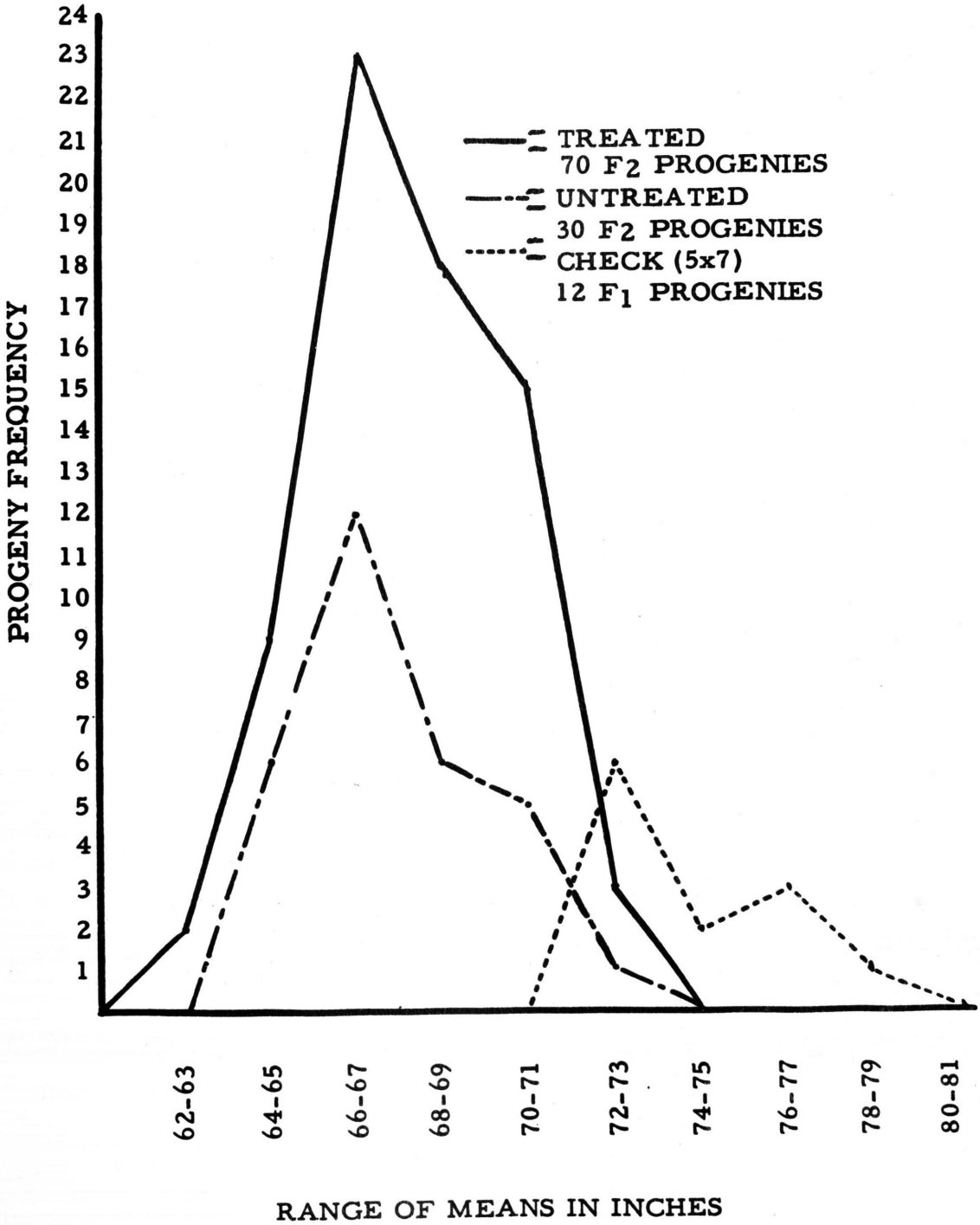


FIGURE 10 1956 EAR HEIGHT

VARIANCE MEANS FOR PROGENY LINES

FOR THREE REPLICATIONS

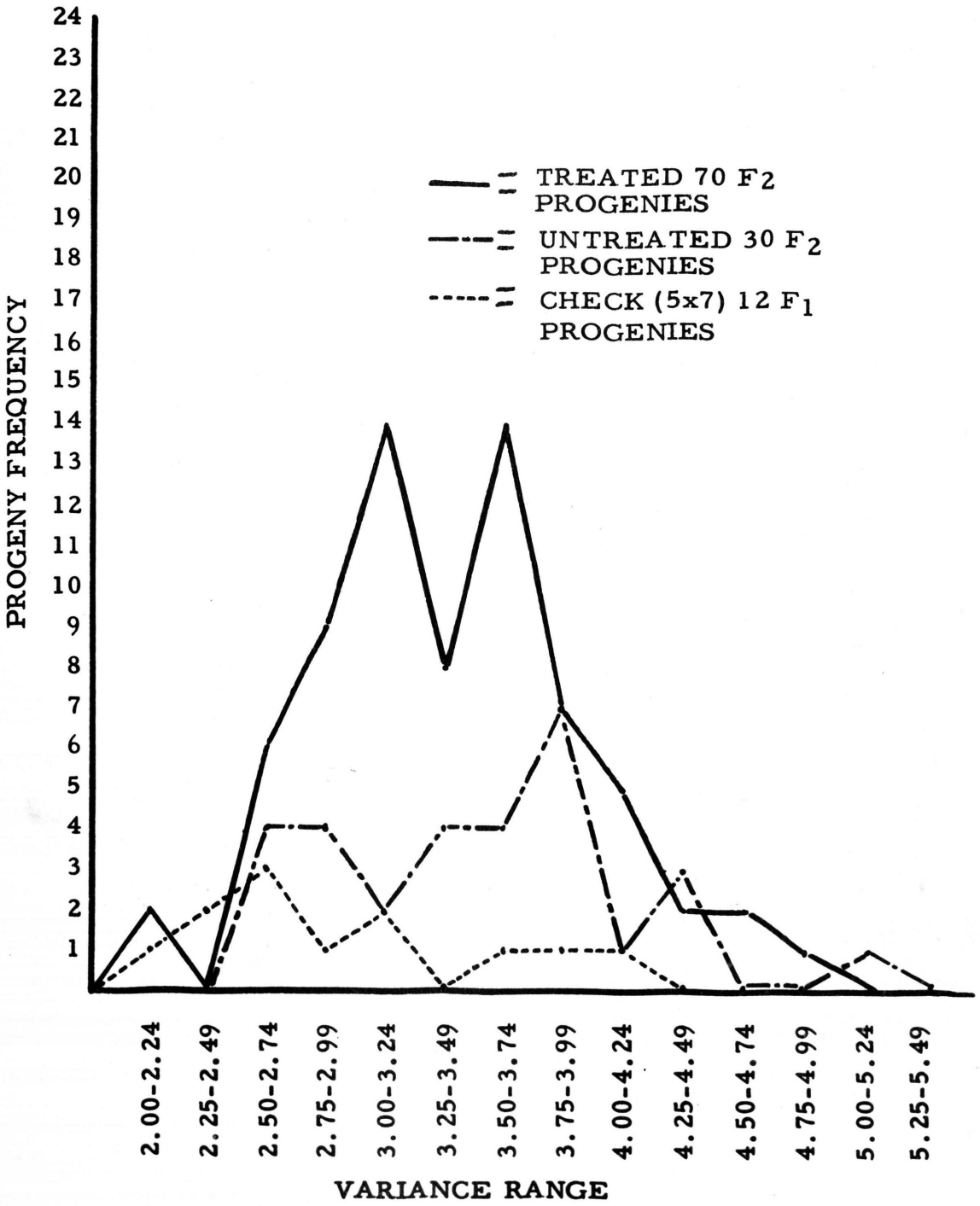


FIGURE 11 1956 EAR HEIGHT

MEANS OF PROGENY LINES FOR THREE REPLICATIONS(IN INCHES)

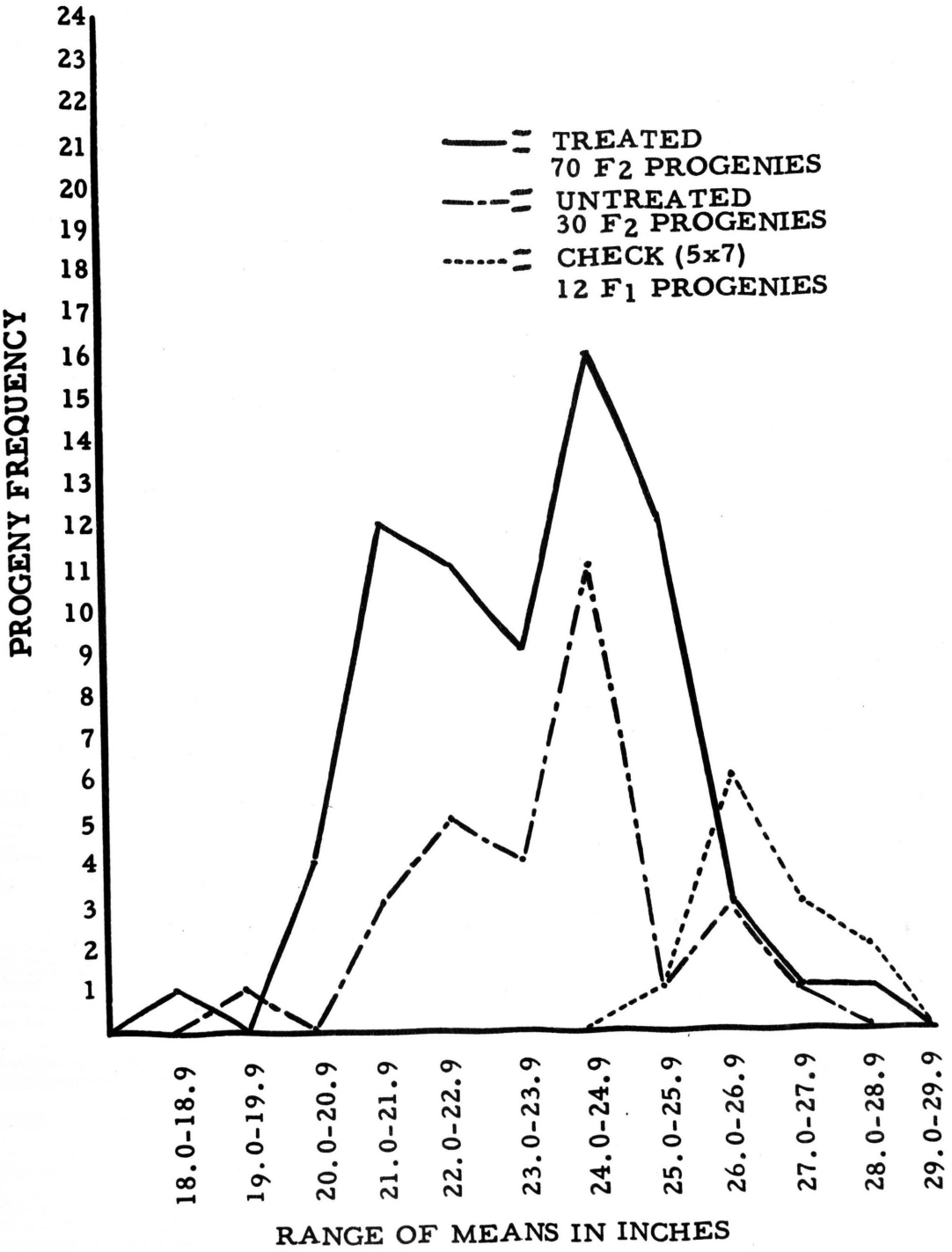


FIGURE 12 1956 PLANT DIAMETER

VARIANCE MEANS FOR PROGENY LINES

FOR THREE REPLICATIONS

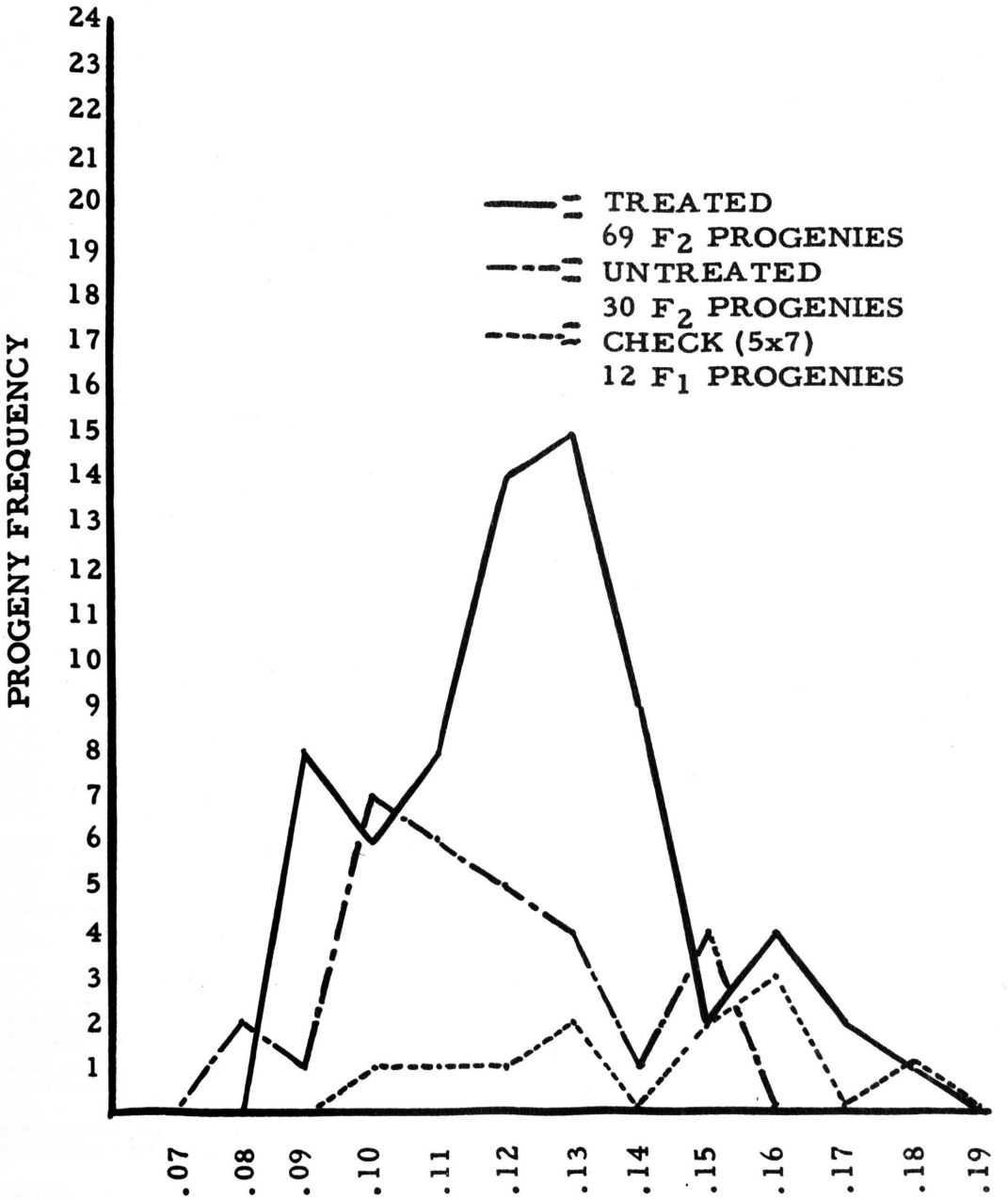


FIGURE 13 1956 PLANT DIAMETER MEANS OF PROGENY LINES FOR THREE REPLICATIONS(IN CENTIMETERS)

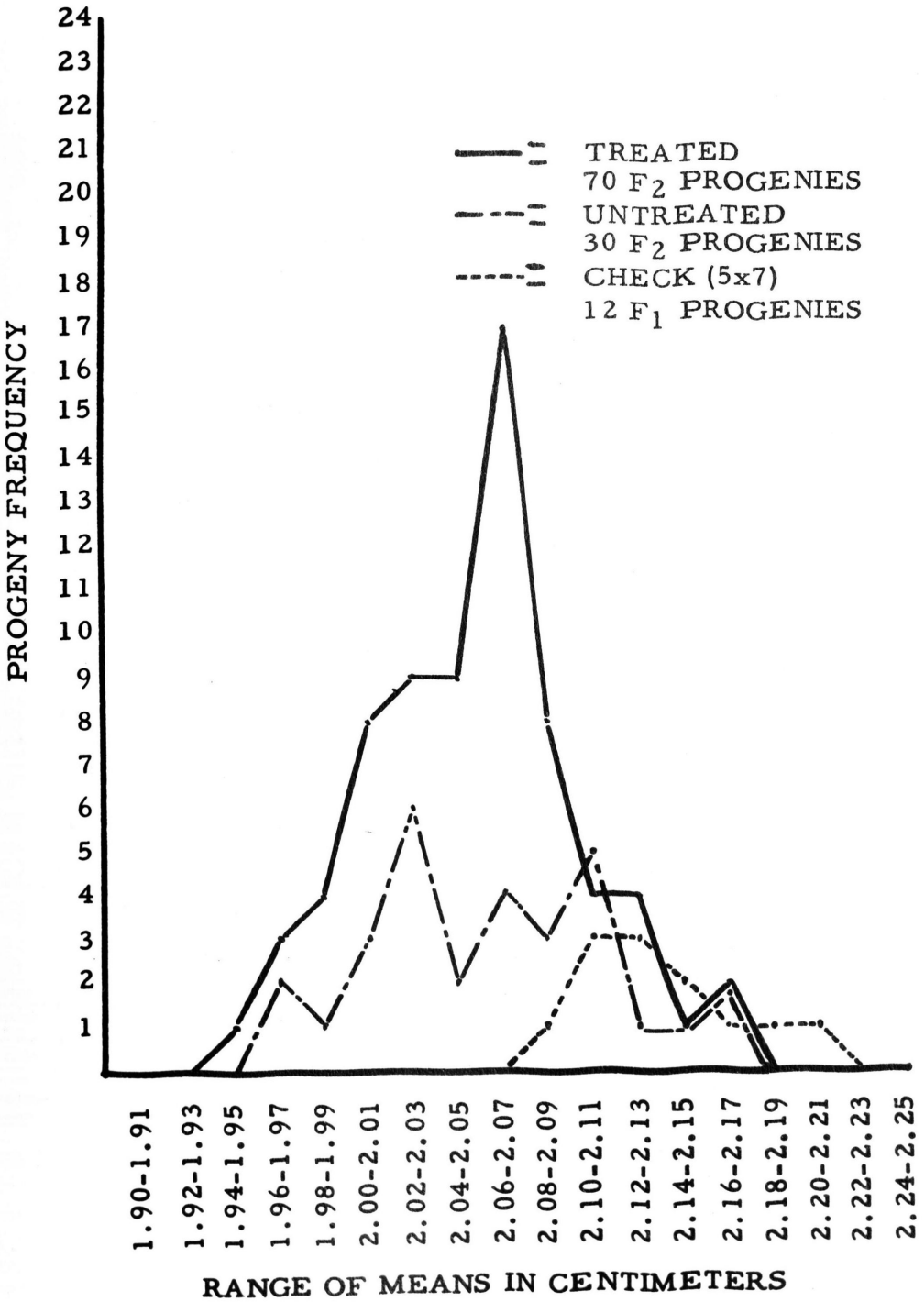


TABLE II. Analysis of Variance of Mean Squares for Ear Measurements as Obtained in 1956. (Ear Length in Centimeters, Ear Diameters in Centimeters, and Ear Weight in Grams).

		EAR LENGTH			EAR DIAMETER			EAR WEIGHT		
Source of Var.	DF	SS	MS	F	SS	MS	F	SS	MS	F
Total	331⊙	111.59			2.74			31,979.69		
Rep.	2	.82	.410	1.33	.01	.006	.78	210.91	105.455	1.10
Prog.	110	43.54	.396	1.29*	1.07	.010	1.28	10,774.71	97.952	1.02
Rep. x Prog.	219⊙	67.23	.307		1.66	.008		20,994.07	95.863	
Bet. Prog. Groups	2	7.41	3.706	12.07**	.19	.096	12.59**	609.68	304.842	3.18
T. vs U.	1	.006	.006	.02	.0002	.0002	.03	127.85	127.847	1.33
S.C. vs (T↑ U)	1	7.41	7.405	24.12**	.19	.191	25.16**	481.84	481.835	5.03*
Among T.	68	24.02	.348	1.12	.64	.010	1.22	7,362.06	108.266	1.14
Among U.	29	10.65	.367	1.01	.19	.007	1.02	1,947.48	67.155	.67
Among S. C.	11	1.47	.134	1.20	.04	.004	.43	855.49	77.772	.90
Rep. of T.	2	.87			.01			117.61		
Rep. of U.	2	.20			.03			175.48		
Rep. of S. C.	2	1.12			.002			251.95		
Rep. x T.	136	42.31	.311		1.05	.008		12,920.81	95.006	
Rep. x U.	58	21.12	.364		.38	.007		5,828.03	100.483	
Rep. x S.C.	22	2.44	.111		.18	.008		1,911.11	86.868	
Rep. x Bet. Groups	4	1.36	.340		.04	.010		334.13	83.532	

* Significant at 5% level

** Significant at 1% level

⊙ Loss of one degree of freedom for data supplied by missing plot technique

Prog. = Progenies Rep. = Replication T. = Treated U. = Untreated S. C. = Single Cross

FIGURE 14 1956 EAR LENGTH

VARIANCE MEANS FOR PROGENY LINES

FOR THREE REPLICATIONS

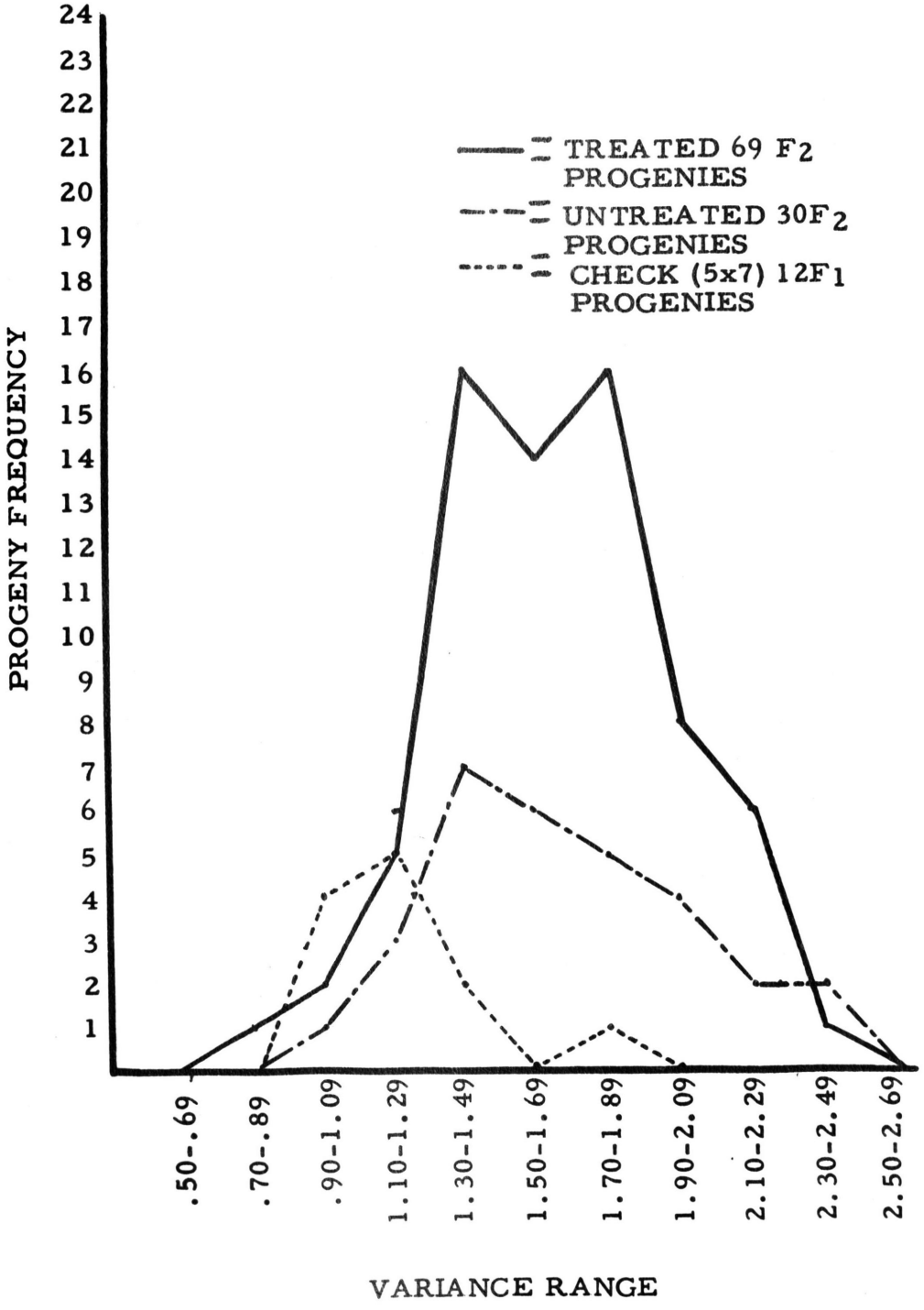


FIGURE 15 1956 EAR LENGTH

MEANS FOR PROGENY LINES FOR THREE REPLICATIONS (IN CENTIMETERS)

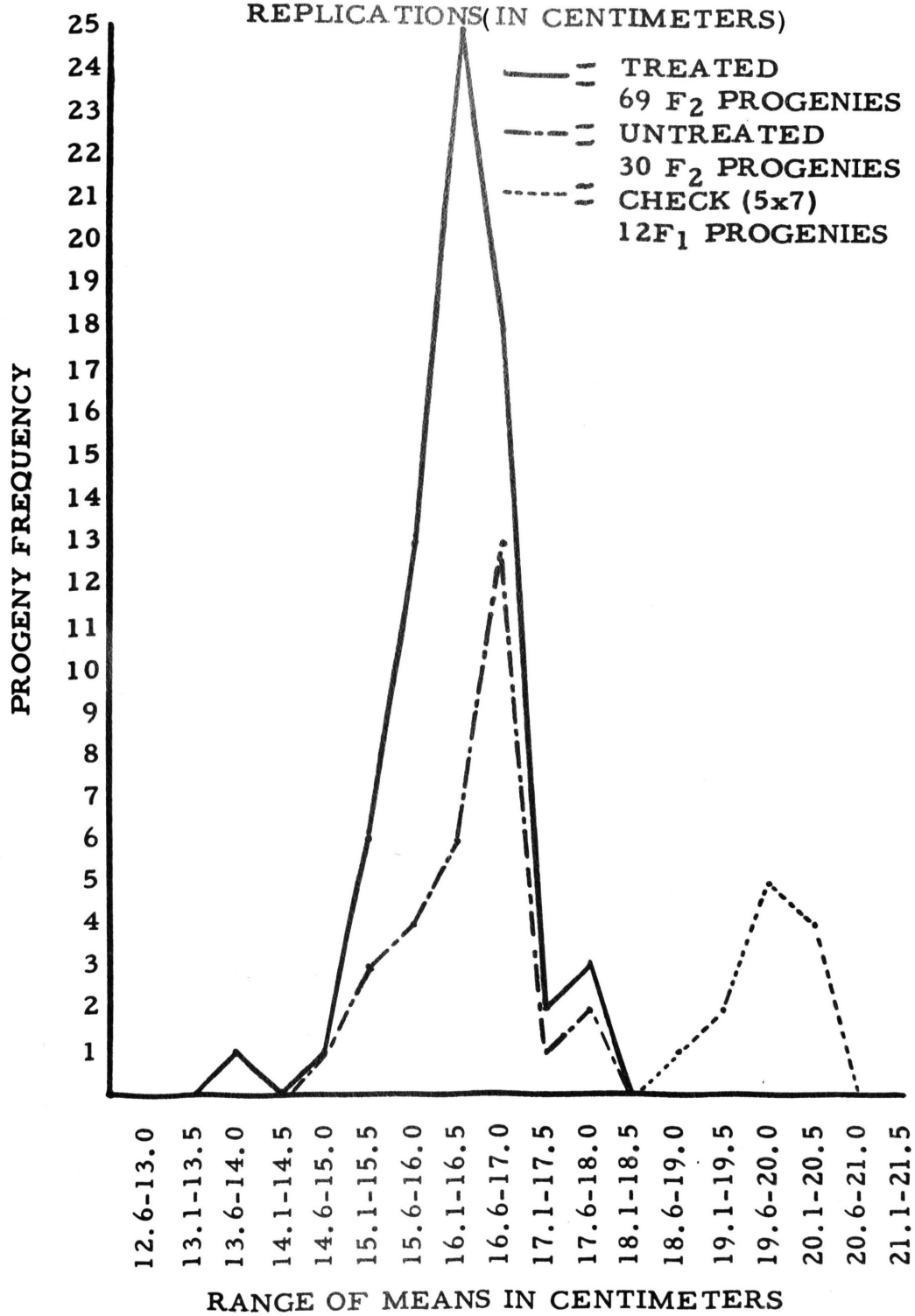


FIGURE 16 1956 EAR DIAMETER

VARIANCE MEANS FOR PROGENY LINES

FOR THREE REPLICATIONS

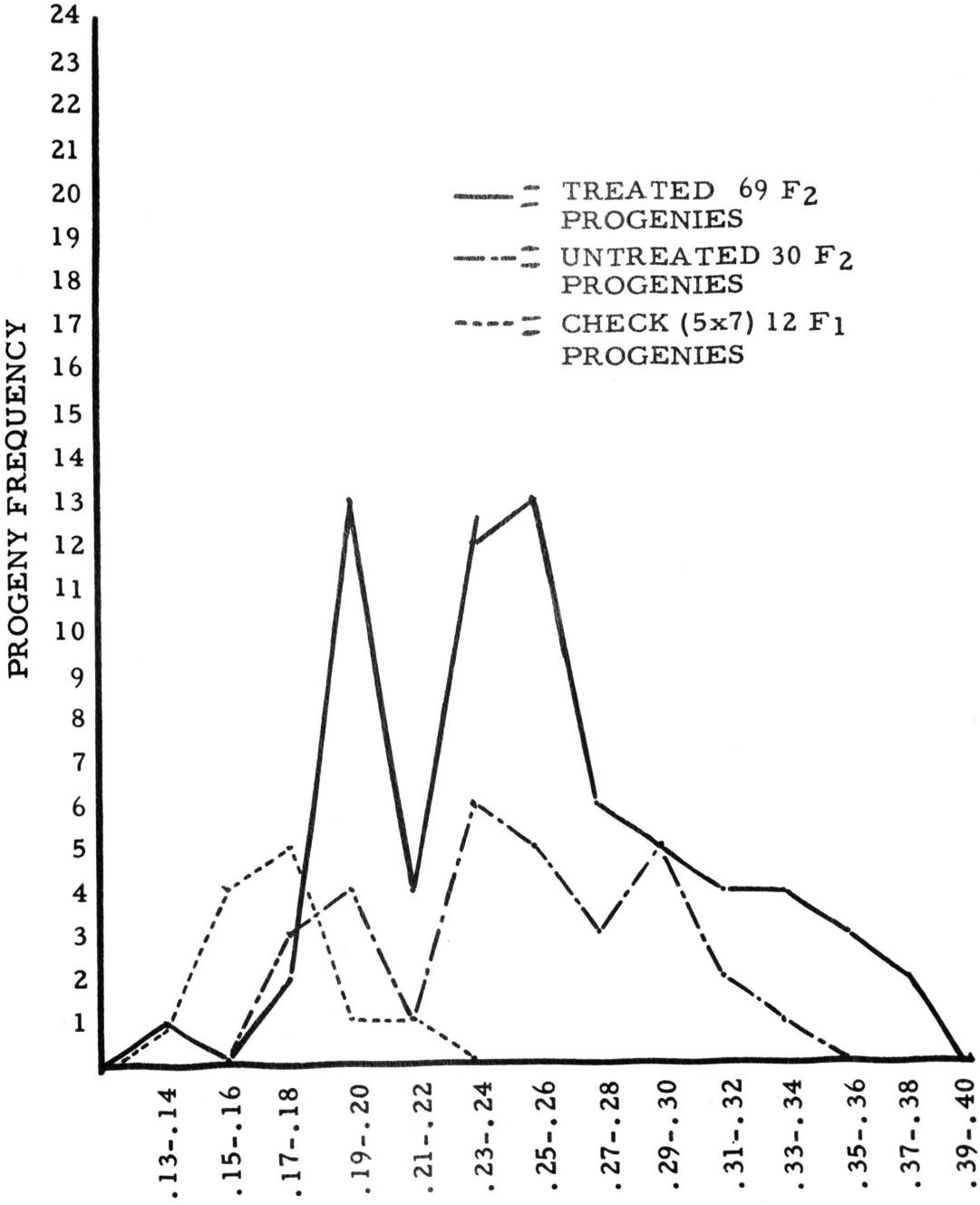


FIGURE 17 1956 EAR DIAMETER

MEANS OF PROGENY LINES FOR THREE REPLICATIONS IN (CENTIMETERS)

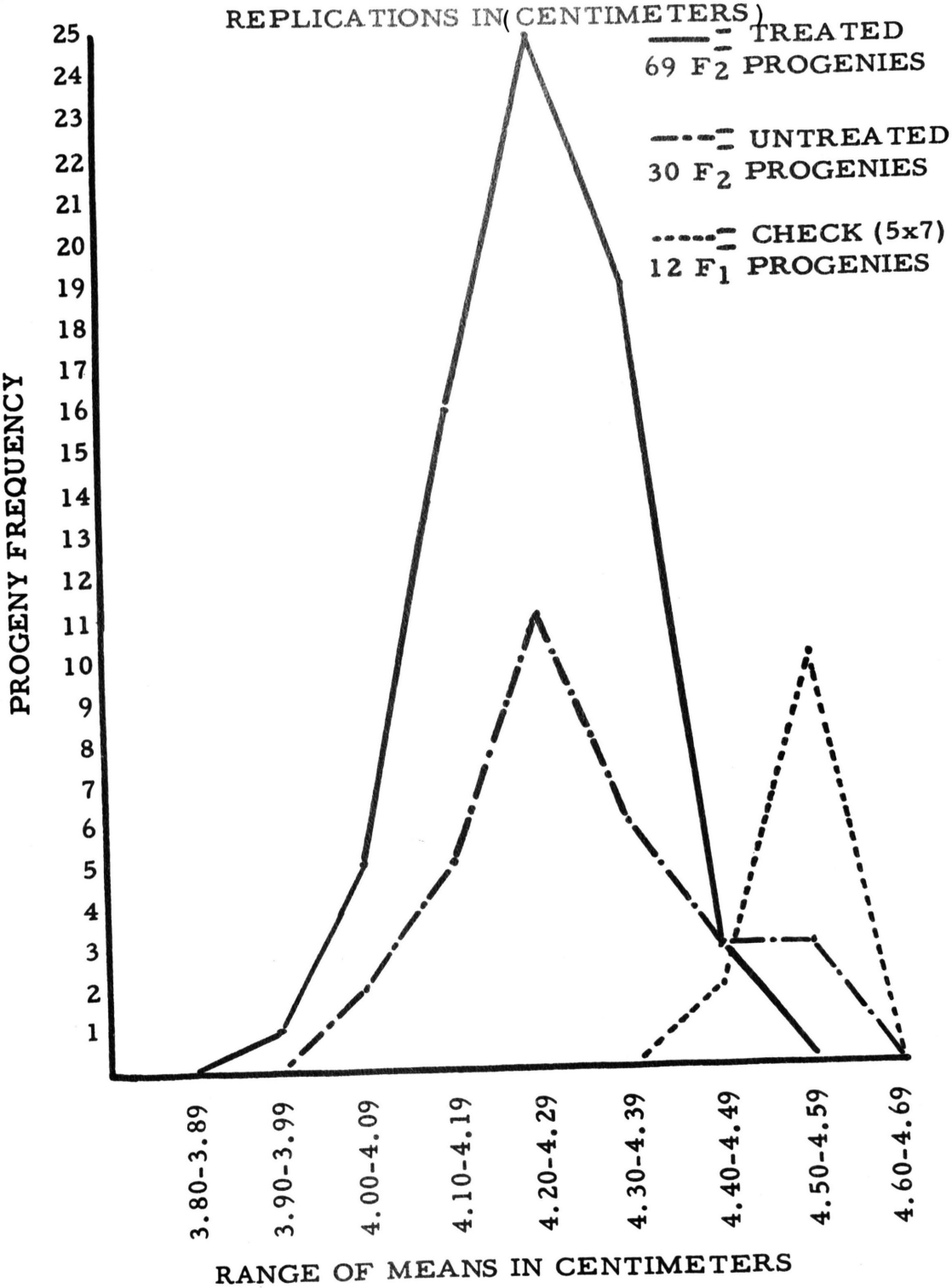


FIGURE 18 1956 EAR WEIGHT

VARIANCE MEANS FOR PROGENY LINES

FOR THREE REPLICATIONS

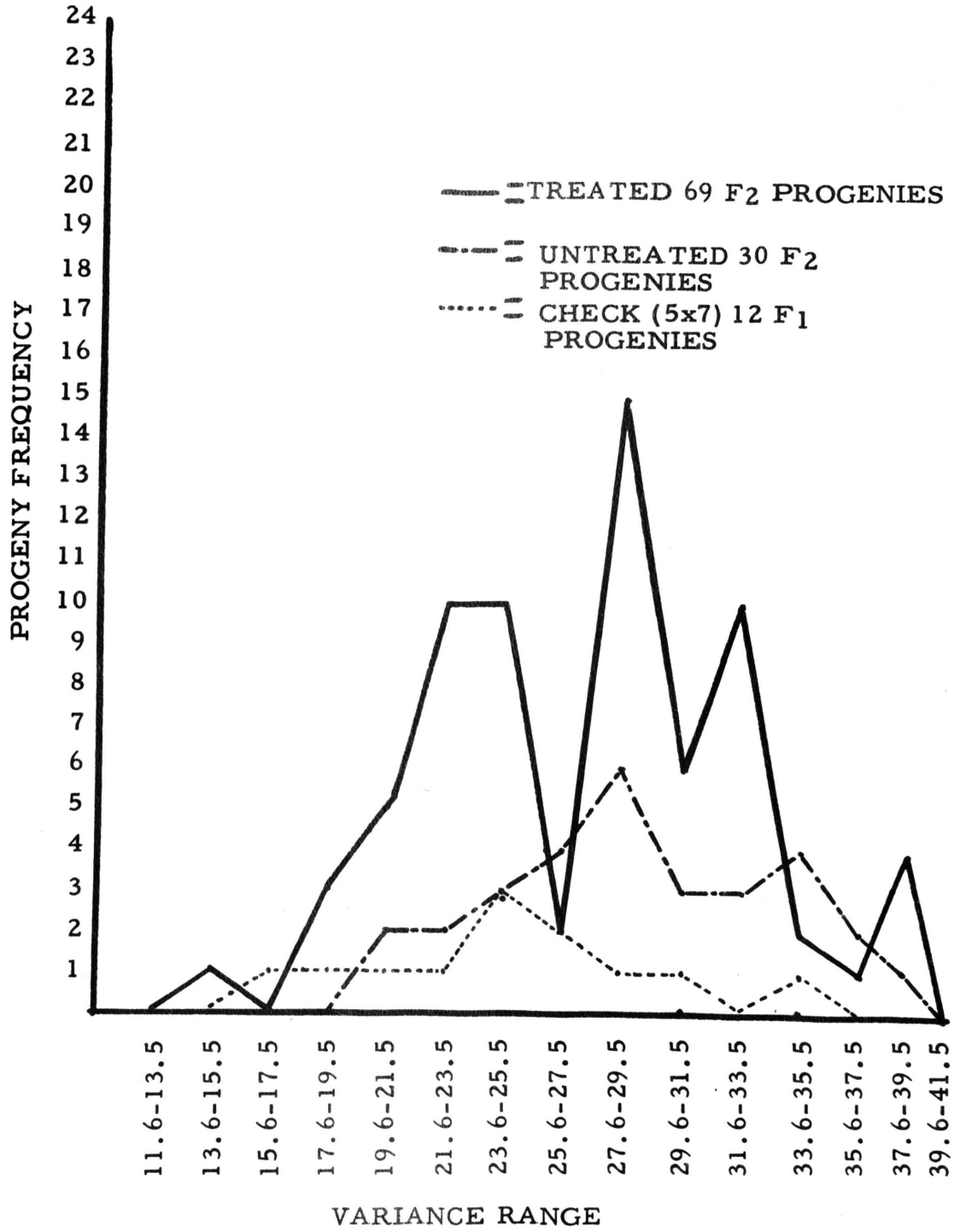


FIGURE 19 1956 EAR WEIGHT

MEANS FOR PROGENY LINES FOR THREE REPLICATIONS (IN GRAMS)

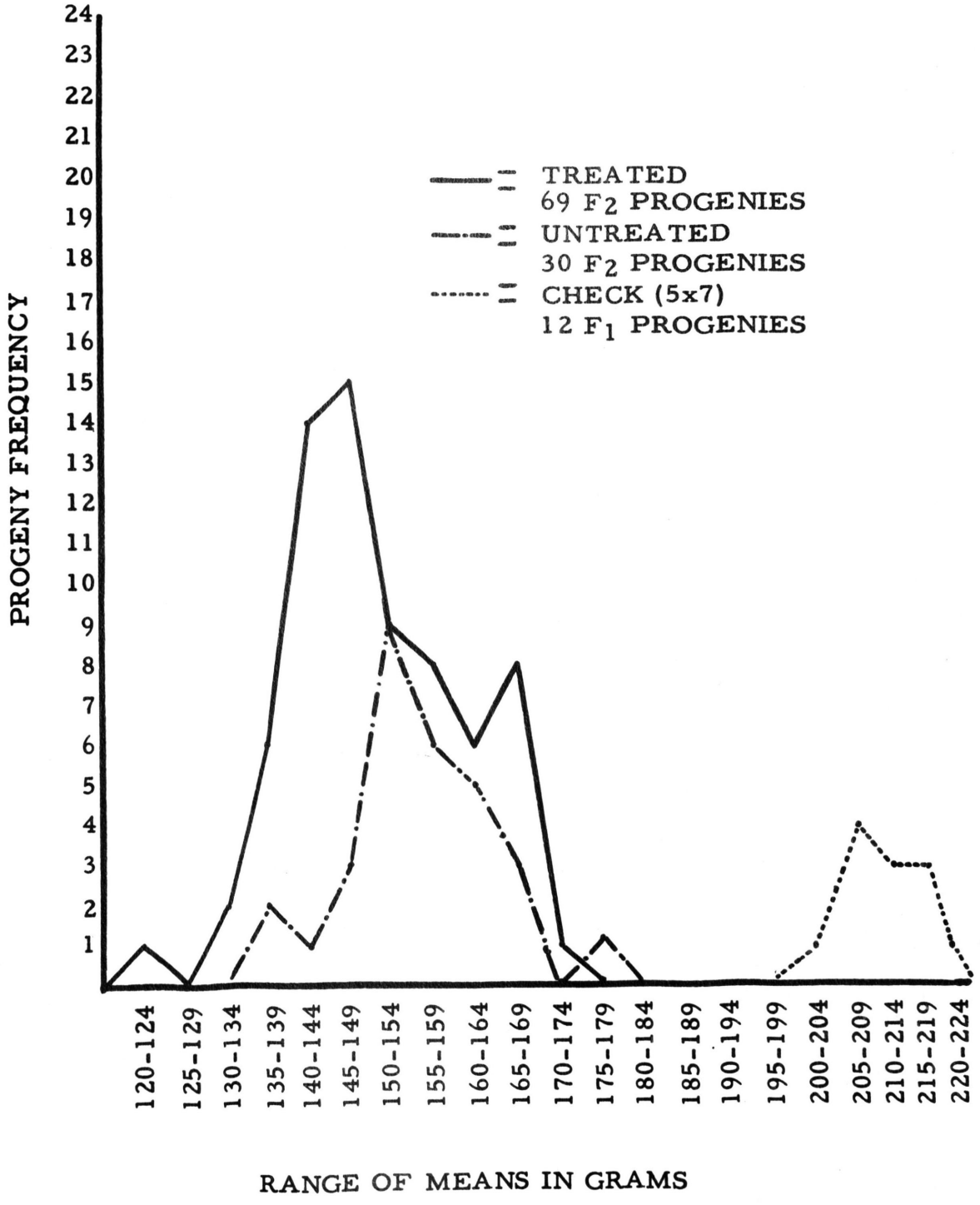


TABLE III. Analysis of Variance of Mean Squares for Plant Measurements as Obtained in 1957 (Plant Height in Inches, Ear Height in Inches and Plant Diameter in Centimeters).

		PLANT HEIGHT			EAR HEIGHT			PLANT DIAMETER		
Source of Var.	DF	SS	MS	F	SS	MS	F	SS	MS	F
Total	332	1,098.73			491.88			3.870		
Rep.	2	16.40	8.200	2.31	10.31	5.155	3.76*	0.070	.035	2.92
Prog.	110	307.86	2.799	.79	207.45	1.886	1.38*	1.169	.011	.88
Rep. x Prog.	220	782.67	3.556		274.12	1.371		2.631	.012	
Bet. Prog. Groups	2	44.40	22.200	6.24	18.30	9.150	6.67*	.056	.028	2.33
T. vs U.	1	1.00	1.000	.28	.97	.970	.71	.004	.004	.33
S. C. vs (T+U)	1	43.40	43.400	12.21**	17.33	17.330	12.64**	.052	.052	4.33*
Among T.	69	134.26	1.950	.46	137.74	1.996	1.54*	1.062	.015	.86
Among U.	29	115.00	3.970	1.74*	42.62	1.470	1.14	.042	.002	.84
Among S. C.	10	13.81	1.380	.51	8.79	.879	.78	.009	.001	1.64
Reps. of T.	2	7.47			4.79			1.070		
Reps. of U.	2	4.24			3.14			.006		
Reps. of S. C.	2	5.89			3.60			.005		
Reps. x T.	138	587.47	4.260		175.71	1.300		2.476	.018	
Reps. x U.	58	132.22	2.280		74.72	1.288		.099	.002	
Reps. x S. C.	20	53.97	2.700		22.47	1,124		.011	.0006	
Reps. x Bet. Groups	4	9.01	2.250		1.22	.305		.045	.011	

* Significant at 5% level
** Significant at 1% level
Rep. = Replications Prog. = Progenies T. = Treated U. = Untreated S. C. = Single Cross

FIGURE 20 1957 PLANT HEIGHT

VARIANCE MEANS FOR PROGENY LINES

FOR THREE REPLICATIONS

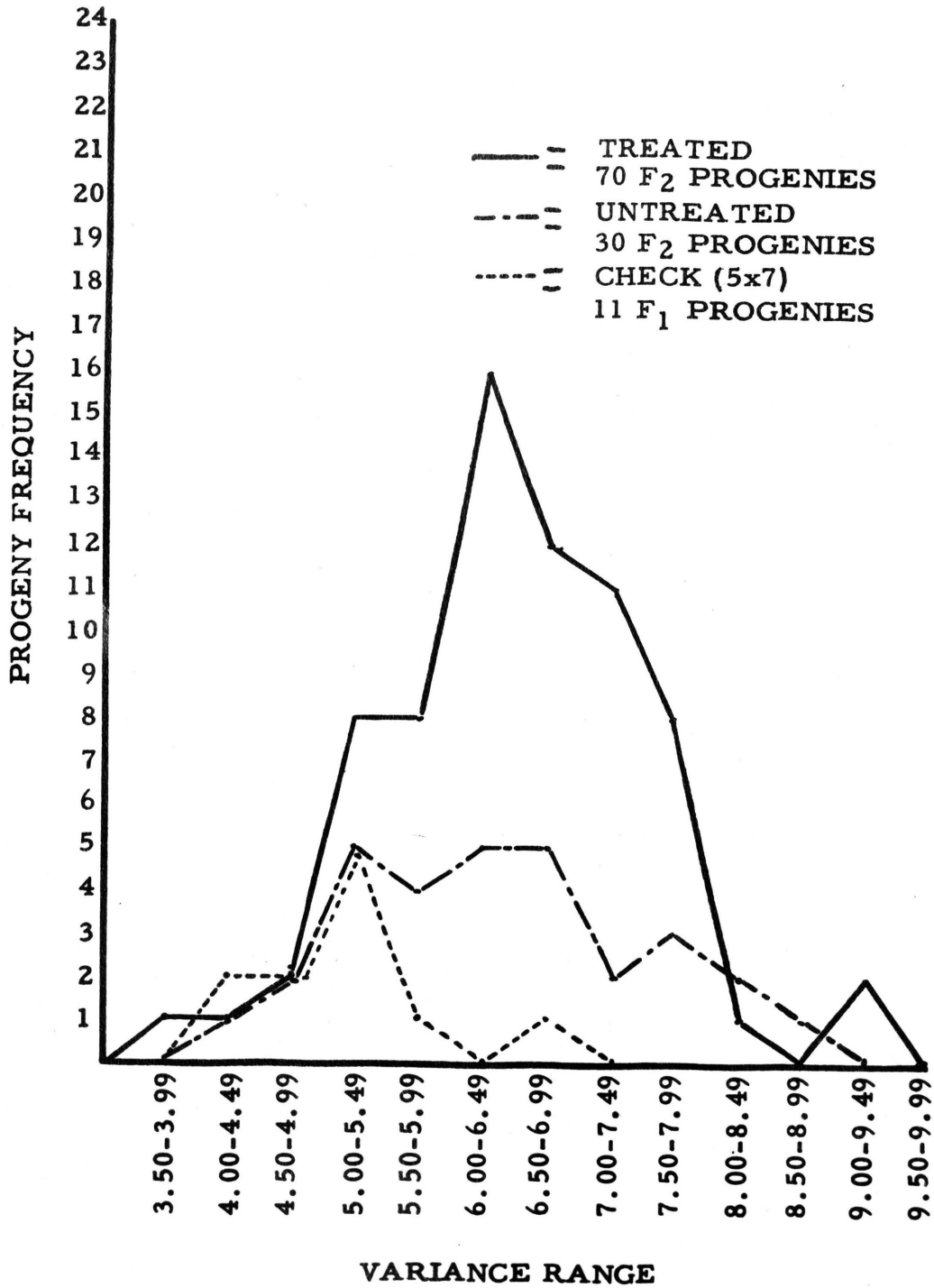


FIGURE 21 1957 PLANT HEIGHT

MEANS OF PROGENY LINES FOR THREE

REPLICATIONS(IN INCHES)

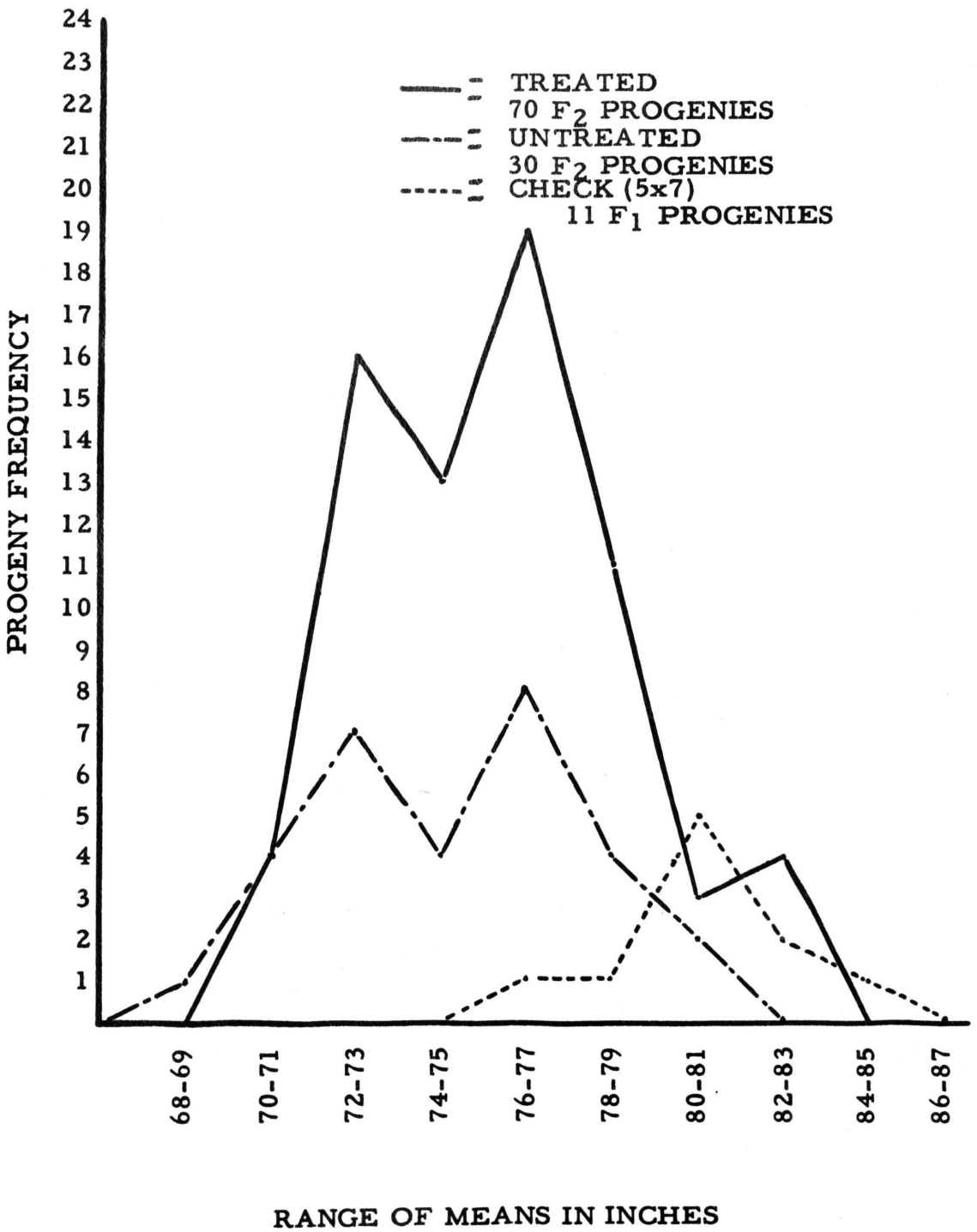


FIGURE 22 1957 EAR HEIGHT

VARIANCE MEANS FOR PROGENY LINES

FOR THREE REPLICATIONS

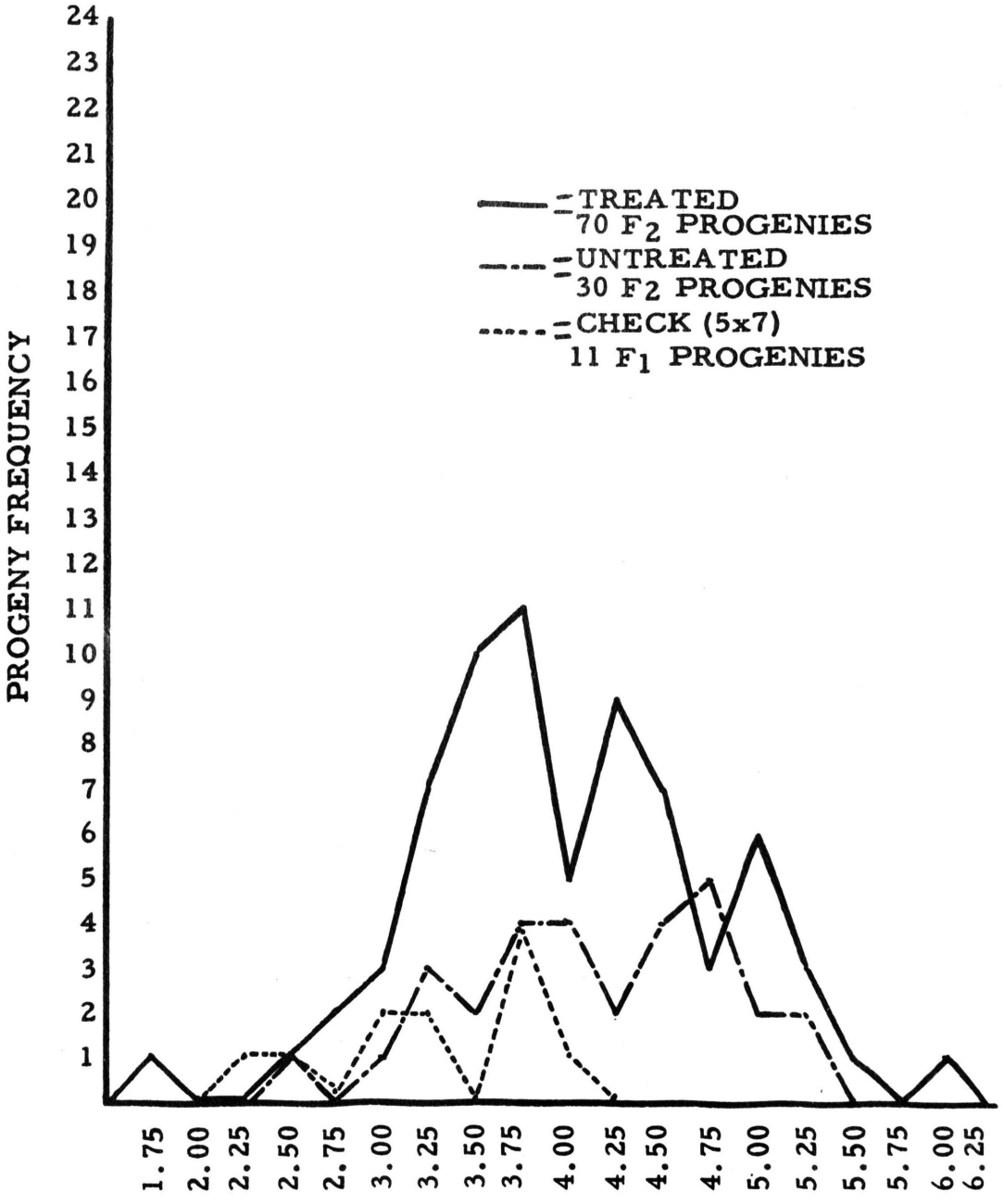


FIGURE 23 1957 EAR HEIGHT

MEANS OF PROGENY LINES FOR THREE

REPLICATIONS (IN INCHES)

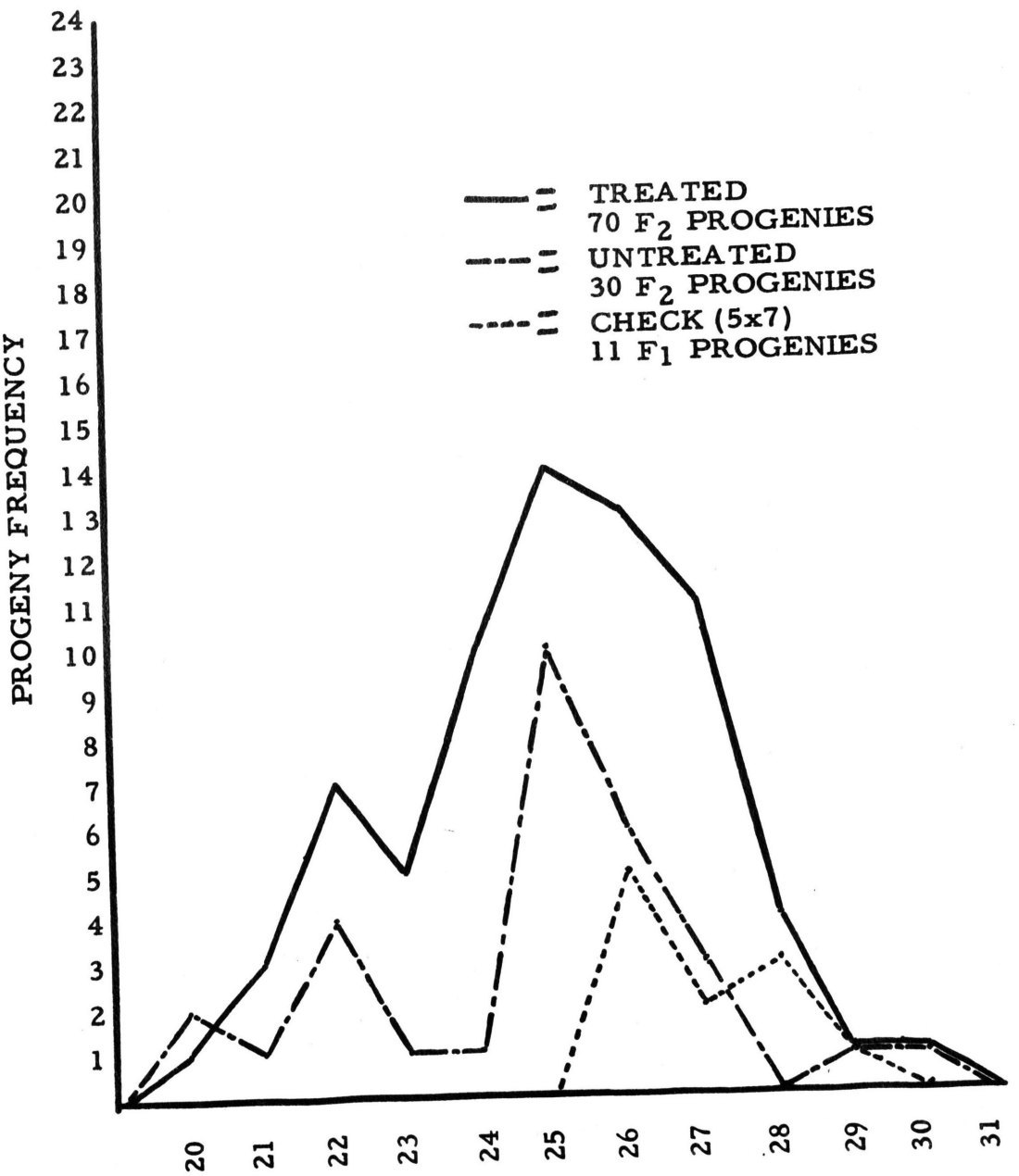


FIGURE 24 1957 PLANT DIAMETER

VARIANCE MEANS FOR PROGENY LINES

FOR THREE REPLICATIONS

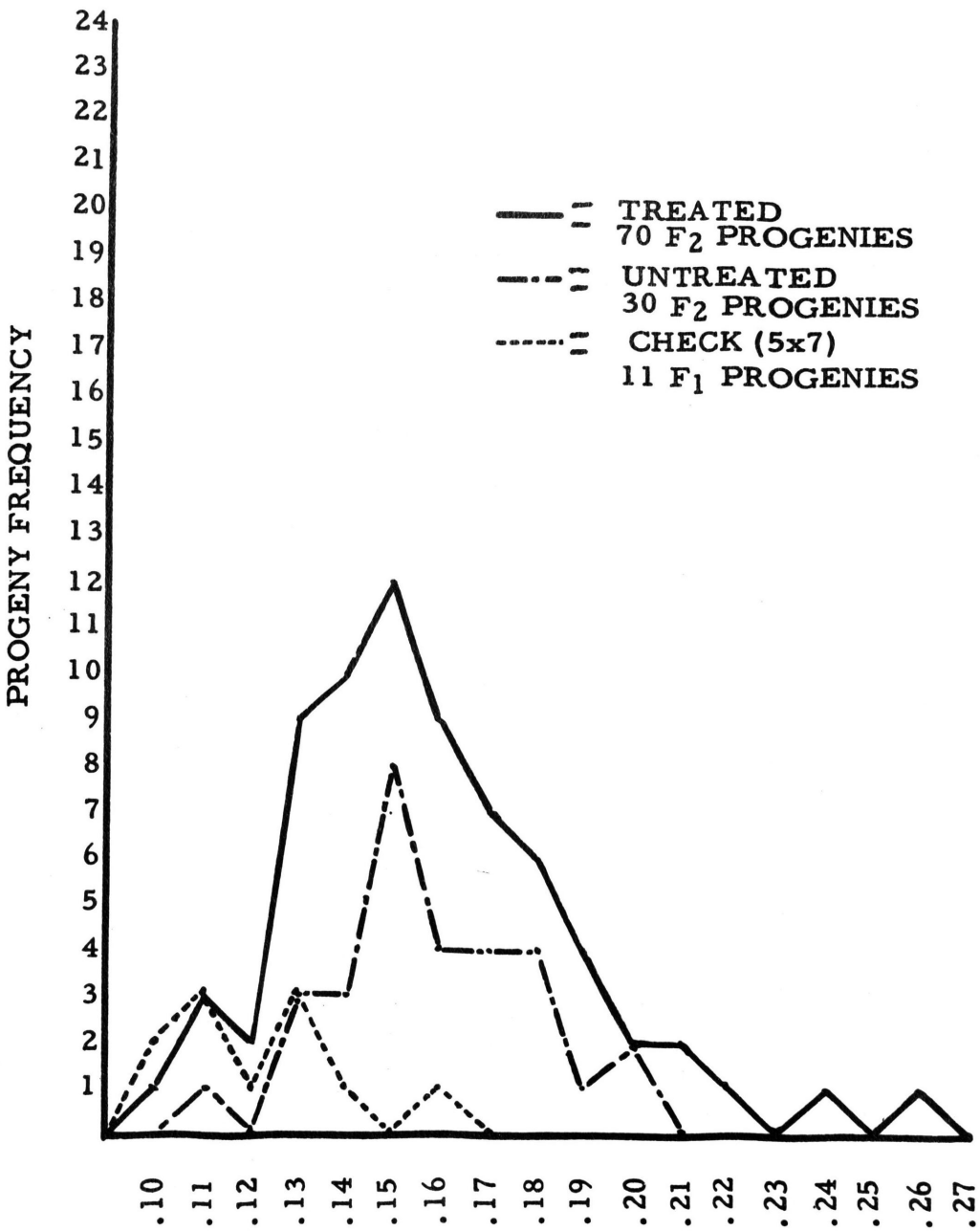


FIGURE 25 1957 PLANT DIAMETER

MEANS OF PROGENY LINES FOR THREE

REPLICATIONS(IN CENTIMETERS)

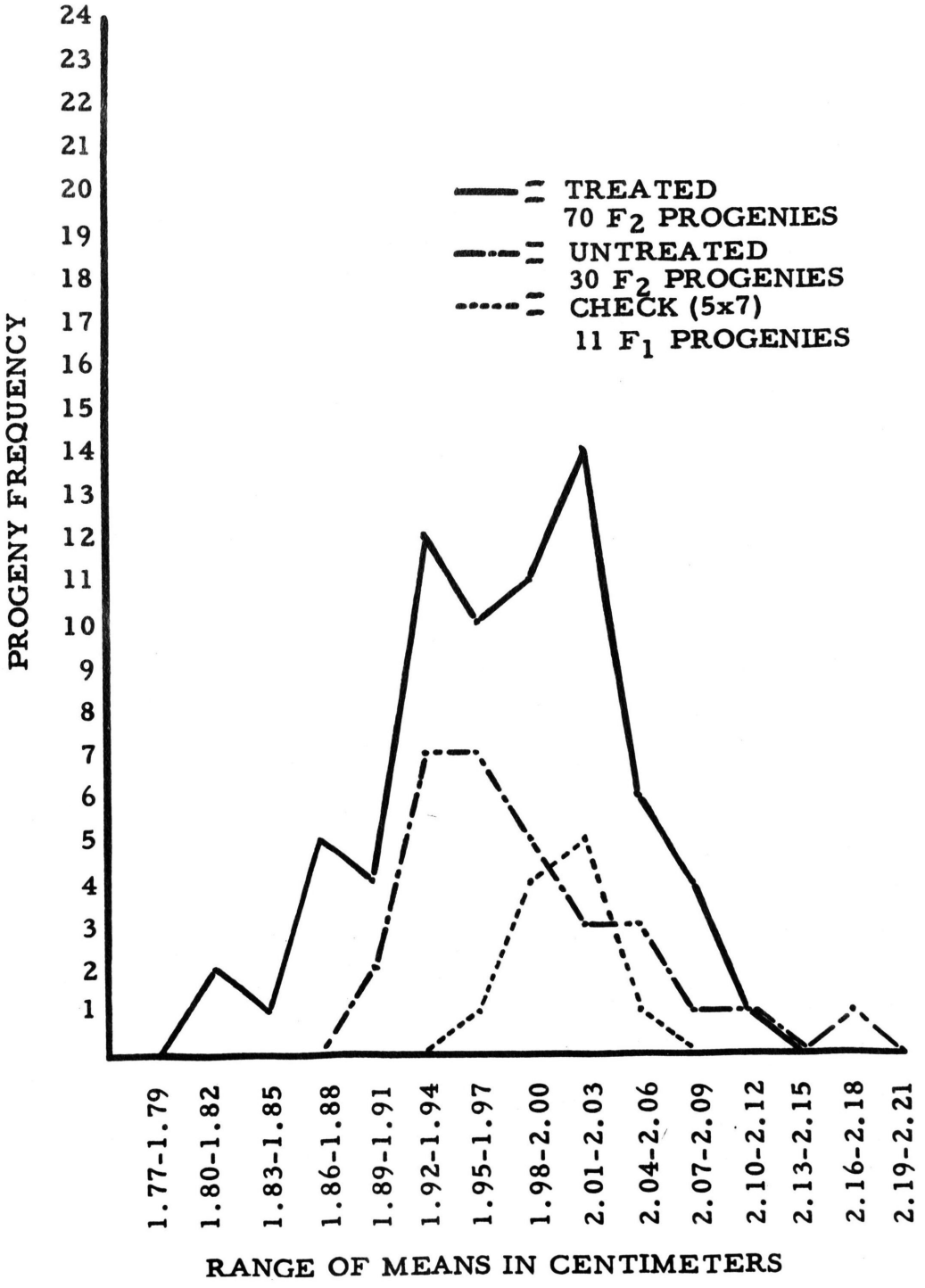


TABLE IV. Analysis of Variance of Mean Squares for Ear Measurements as Obtained in 1957. (Ear Length in Centimeters, Ear Diameter in Centimeters, and Ear Weight in Grams).

		EAR LENGTH			EAR DIAMETER			EAR WEIGHT		
Source of Var.	DF	SS	MS	F	SS	MS	F	SS	MS	F
Total	328 ⊙	214.34			3.50			29,104.09		
Rep.	2	.17	.087	.14	.03	.013	1.27	182.38	91.191	1.04
Prog.	109	80.20	.736	1.19	1.20	.011	1.05	9,885.93	90.697	1.03
Rep. x Prog.	217 ⊙	133.97	.617		2.27	.011		19,035.78	87.722	
Bet. Prog. Groups	2	4.00	1.978	3.20	.14	.072	6.82**	83.17	41.587	.47
T. vs U.	1	.08	.083	.13	.002	.002	.19	41.10	41.102	.47
S.C. vs (T + U)	1	3.87	3.873	6.27*	.14	.141	13.44**	42.07	42.071	.48
Among T.	68	49.92	.734	1.42*	.67	.010	.88	5,590.85	82.218	1.05
Among U.	29	11.28	.389	.59	.29	.010	1.10	2,914.26	100.492	.95
Among S. C.	10	15.04	1.504	1.32	.09	.009	1.01	1,297.65	129.765	1.21
Rep. of T.	2	.53			.04			117.91		
Rep. of U.	2	.83			.01			92.87		
Rep. of S. C.	2	2.10			.009			25.06		
Rep. x T.	136	69.44	.511		1.52	.011		10,678.36	78.517	
Rep. x U.	58	38.37	.662		.53	.009		6,157.07	106.156	
Rep. x S. C.	20	22.87	1.143		.18	.009		2,146.88	107.344	
Rep. Bet. Groups	4	3.29	.822		.03	.008		53.46	13.365	

** Significant at 1% level

* Significant at 5% level

⊙ Loss of one degree of freedom for data supplied by missing plot technique

Rep. = Replication Prog. = Progenies T. = Treated U. = Untreated S. C. = Single Cross

FIGURE 26 1957 EAR LENGTH

VARIANCE MEANS FOR PROGENY LINES

FOR THREE REPLICATIONS

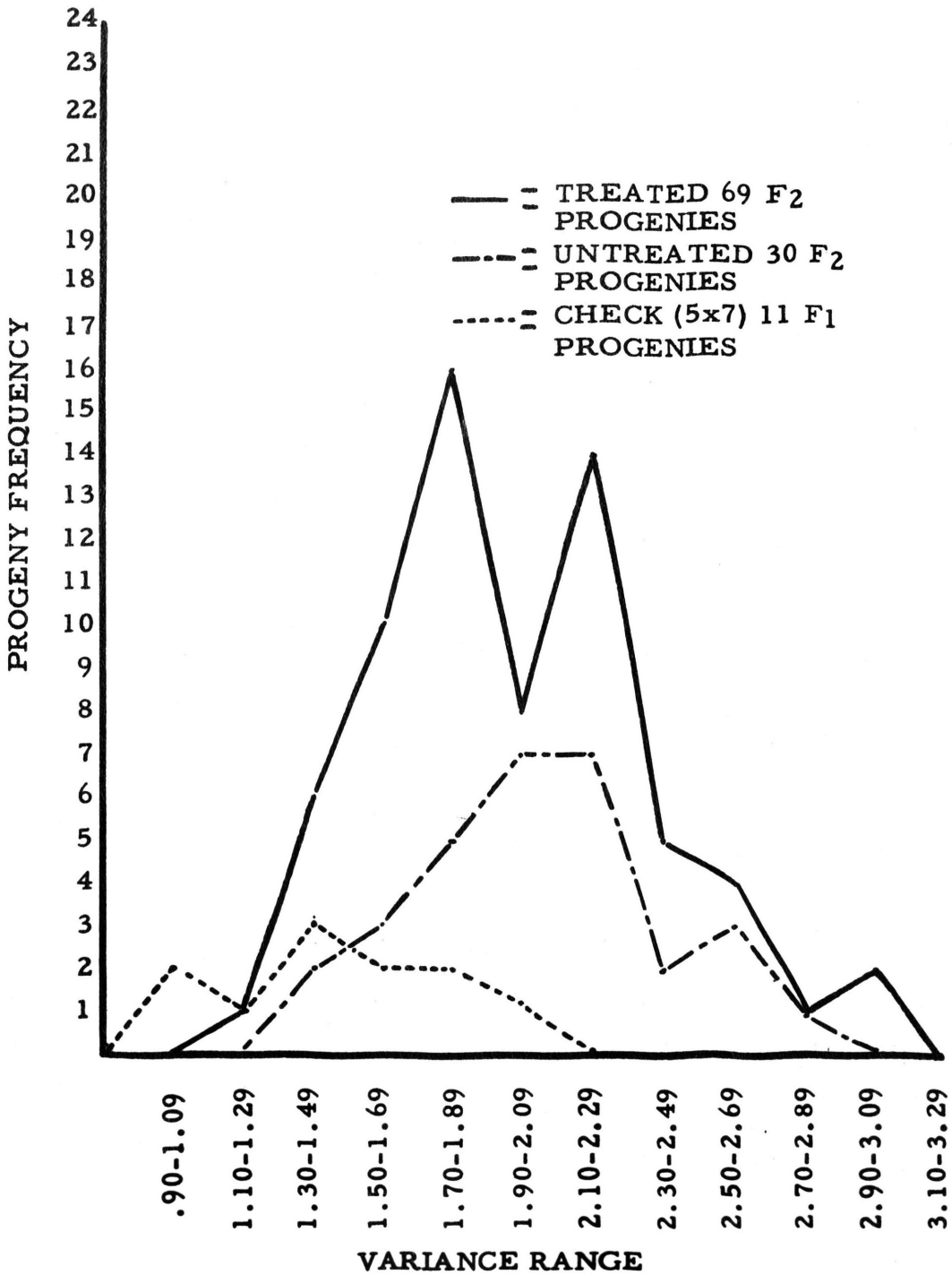


FIGURE 27

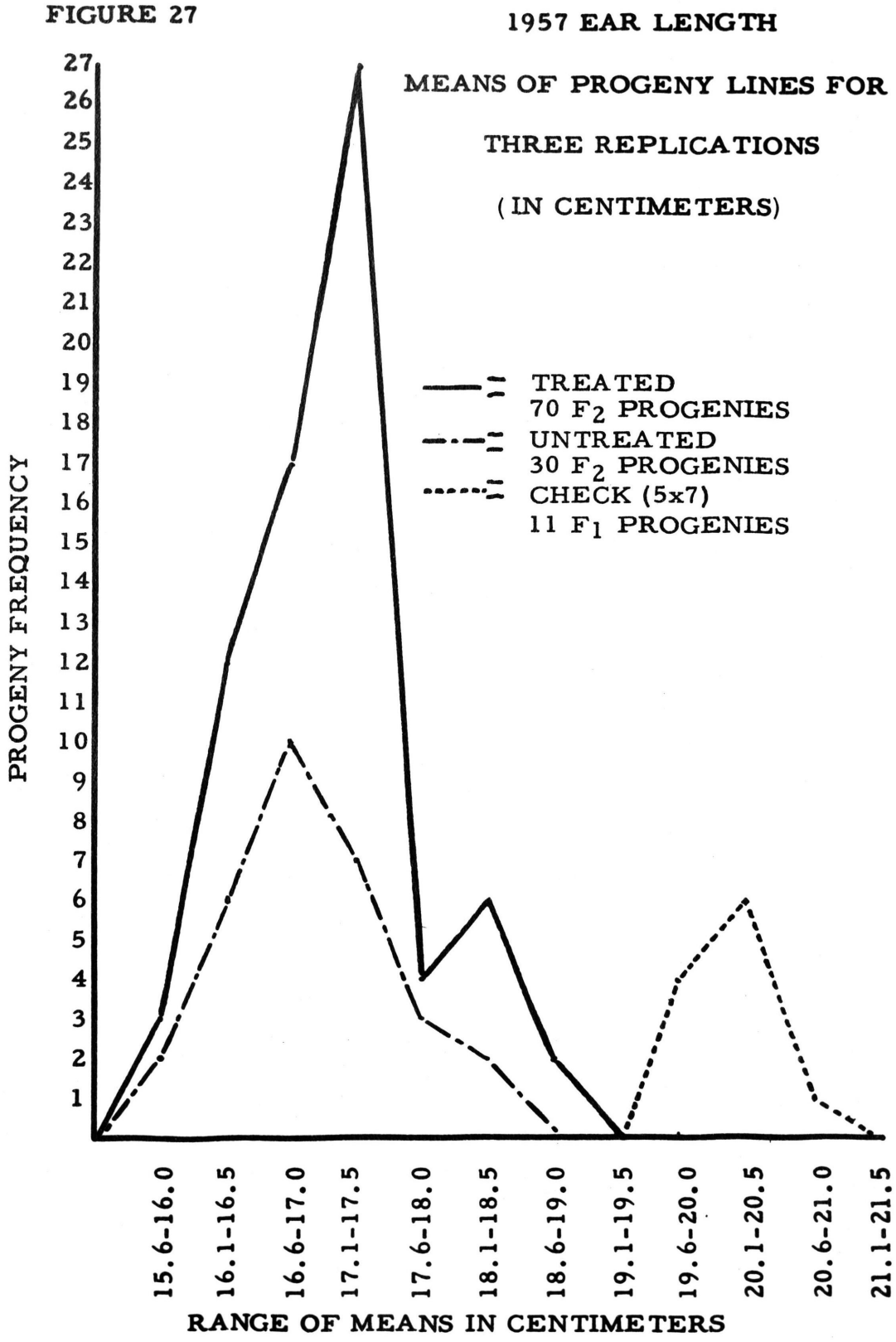

1957 EAR LENGTH
MEANS OF PROGENY LINES FOR
THREE REPLICATIONS
(IN CENTIMETERS)
TREATED
70 F2 PROGENIES
UNTREATED
30 F2 PROGENIES
CHECK (5x7)
11 F1 PROGENIES
PROGENY FREQUENCY
27
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
15.6-16.0
16.1-16.5
16.6-17.0
17.1-17.5
17.6-18.0
18.1-18.5
18.6-19.0
19.1-19.5
19.6-20.0
20.1-20.5
20.6-21.0
21.1-21.5
RANGE OF MEANS IN CENTIMETERS

FIGURE 28 1957 EAR DIAMETER

VARIANCE MEANS FOR PROGENY LINES

FOR THREE REPLICATIONS

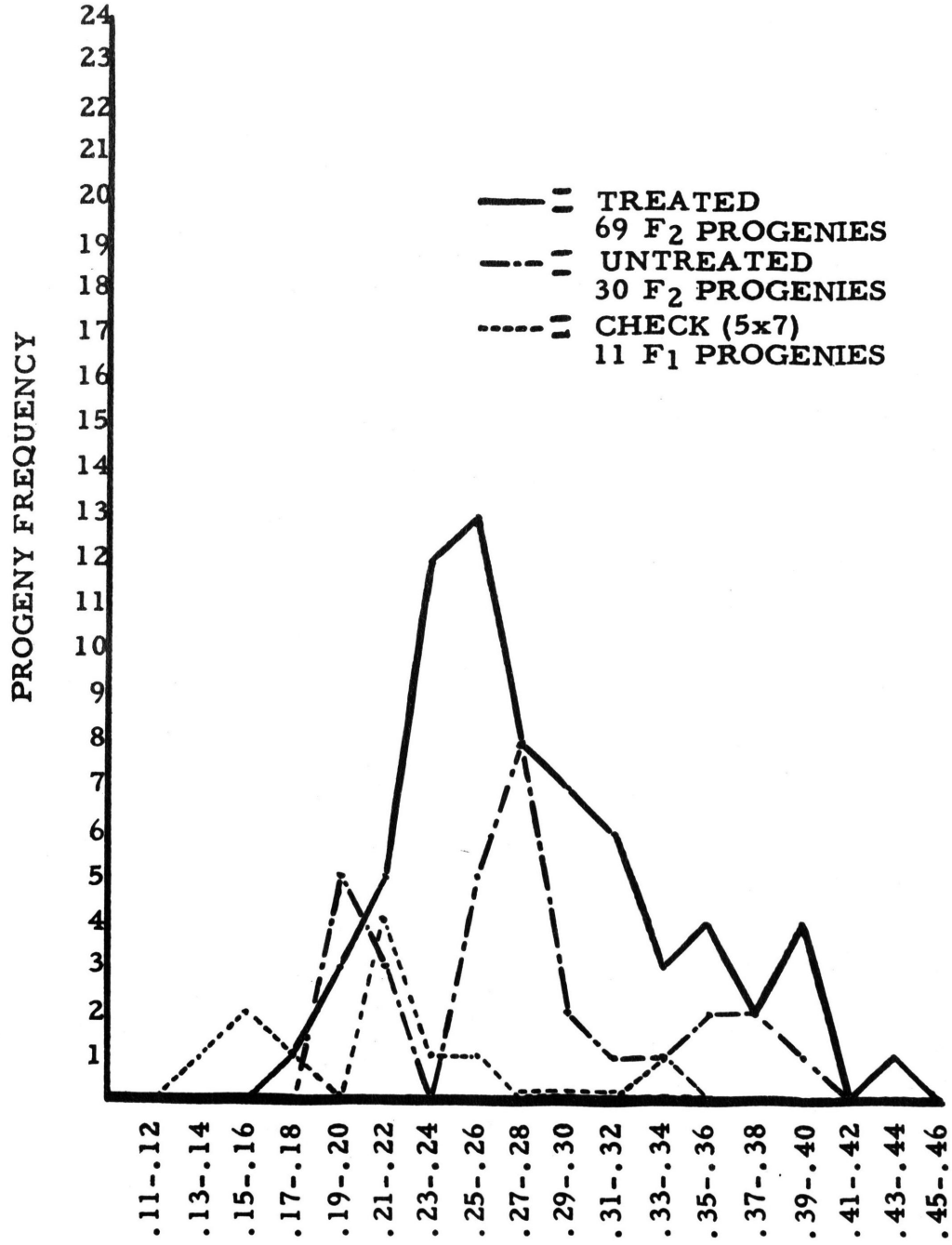


FIGURE 29 1957 EAR DIAMETER

MEANS OF PROGENY LINES FOR THREE

REPLICATIONS(IN CENTIMETERS)

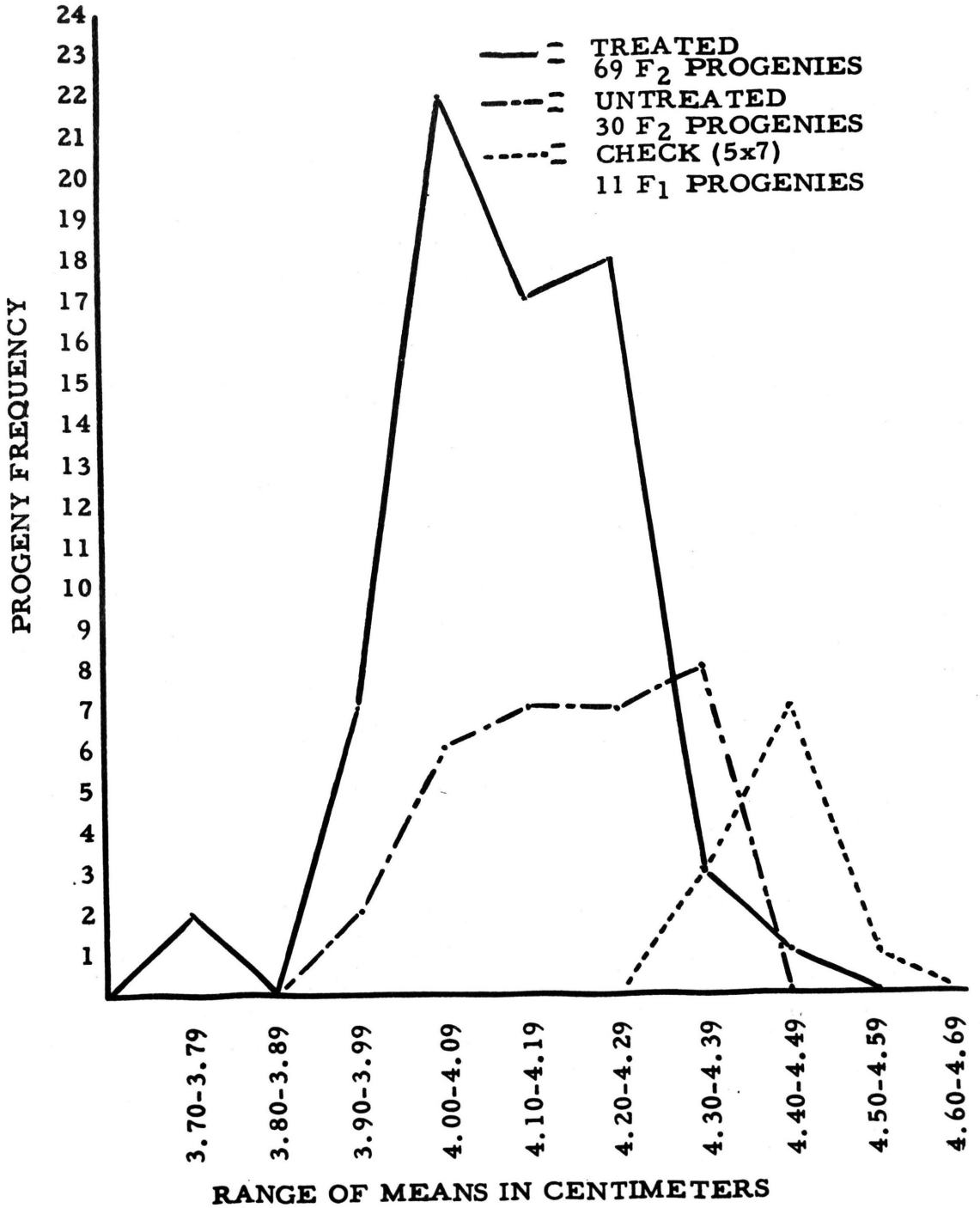


FIGURE 30 1957 EAR WEIGHT

VARIANCE MEANS FOR PROGENY LINES

FOR THREE REPLICATIONS

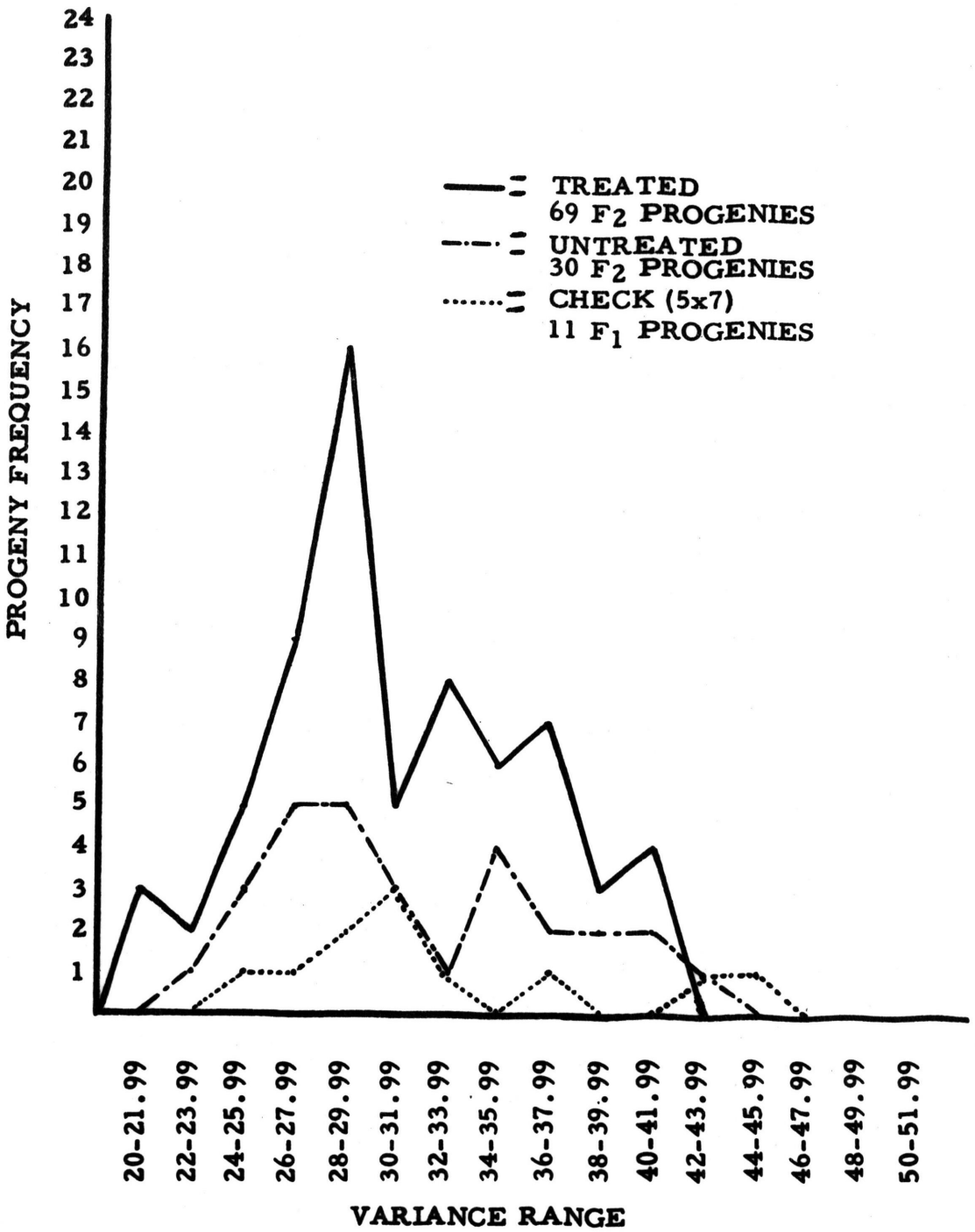
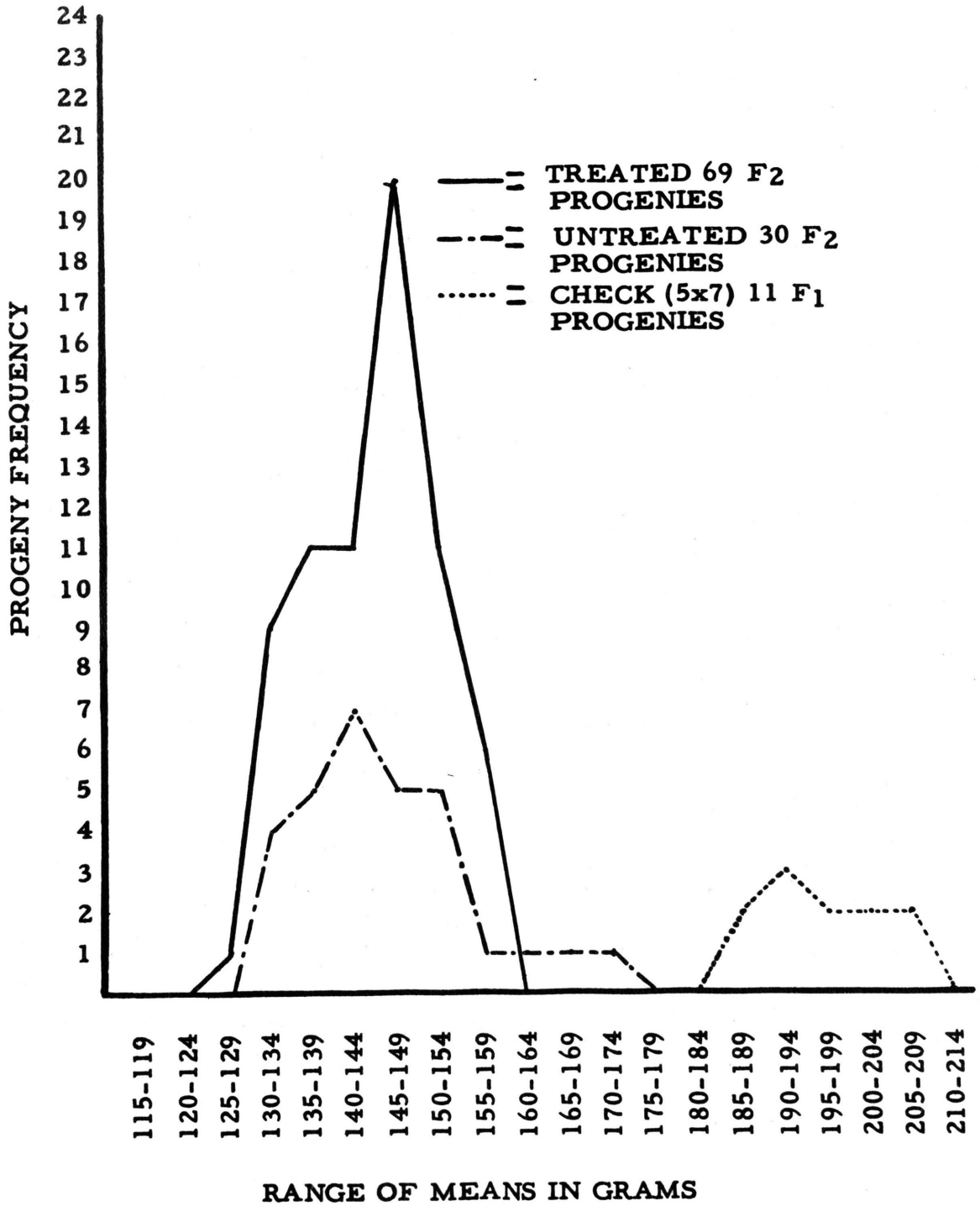


FIGURE 31 1957 EAR WEIGHT

MEANS FOR PROGENY LINES FOR THREE

REPLICATIONS(IN GRAMS)



DISCUSSION

The F_2 progenies of treated seedlings of the maize single cross, (SD5 x SD7), were compared with F_2 progenies of untreated seedlings. Comparisons were made by measuring six physical characteristics of the plant. They were: plant height, ear node height, plant diameter just below the ear node, ear length, ear diameter at mid-point and ear weight. Data obtained from these measurements were analyzed for within line variance and the variances were then taken through an analysis of variance. Progeny means and progeny variances were graphed to determine if there had been any tendency to move treated line means or the entire treated population mean away from the normal as expressed by untreated lines.

Considerable date have been reported on by Franzke, Ross, Atkinson, Harpstead, Dirks, Sanders and others on the development of true-breeding variants or mutants from colchicine treated seedlings of sorghum and flax. The data seem to leave little doubt that colchicine treatment can result in genetical changes which breed true in those generations studied following treatment. The process by which these changes take place has not been entirely clarified. Most of the workers propose the combined action of gene mutation with somatic reduction followed by development of a new growing point which then contains the mutation in a homozygous state. The evidence that somatic reduction

occurs under normal conditions and probably at a rate above that previously accepted has been surveyed in the Review of Literature.

Several workers have studied the effects of colchicine on maize. Hanson found few, if any, induced variants that could not be explained by other means. Moore's work with a single inbred line indicated a susceptibility to colchicine treatment resulting in apparent genetic changes. The results presented here were designed to furnish some conclusive evidence with regard to the effect of colchicine treatment of corn. The data give no indication of any effect at all. With regard to the specific measurements the picture indicates a uniformly negative situation.

The data obtained over a two year period for plant height did not show a difference between treated and untreated lines. In 1957 a difference was found among untreated lines. These differences could probably be explained by insufficient numbers of lines studied. These progenies which brought about the significant differences among groups could have been sampling errors or chance progenies out on the lower or upper ends of the normal population curve which would not have been striking had larger numbers of lines been used. This seems evident since the means of the variances for the groups are nearly identical.

The ear height data showed no differences between treated and untreated groups. However, difference among treated lines occurred in each year. The curves, Figures 11 and 22, of variances indicates

there are lines of treated that have less variation as well as more variation than untreated lines, however, the greater number of lines fall into nearly the same variance classes. Means for this measurement present similar curves for treated and untreated.

Plant diameter showed no differences between the F_1 and the F_2 progenies in either year. This measurement was difficult to take accurately as the stalk varies in size from the center of the internode to the node. The exact distance from the node to use as the point of measurement could not be determined. The lack of difference between F_1 plants and F_2 selfed plants cannot be explained unless it was an inaccuracy of measurement.

Ear measurement data followed much the same trend as in the plant measurements. There were no differences found between treated and untreated groups for any ear measurement in either year.

The preliminary studies made before initiating the major aspects of this study indicated a close relationship between the response induced by colchicine treatment of maize and that in sorghum. The C-tumor growth in maize follows the same pattern as in sorghum. This was studied by Hanson (11). Seedling regrowth following treatment appears to take place much the same in both species. If there is a varietal response in maize similar to that found by Franzke and Ross in sorghum it would seem entirely possible that the single cross selected for this study could be a poor reactor. This could possibly be true even though

the preliminary work had indicated that the inbred SD5 is a good reactor and the inbred SD7 is a poor reactor.

Although the hypothesis of gene mutation is fundamental to the explanation of the data reported in sorghum, it is not known how many genes and what mechanism may be involved in the production of one variant. Harlan in 1956 (12) indicated that a genotype of sorghum could be crossed with Sudan grass from which an F_2 of a wide range of material can be obtained. The selection and selfing of any plant will result in immediate homozygosity.

The measurements used here may have been poorly selected to determine genetical changes within this particular single cross. Visual observation of the plot area both years gave the impression that treated lines were more uniform than untreated. In 1956 this seemed most apparent. The measurements taken were selected in an attempt to express this visual appearance.

In 1959 Sanders, Franzke and Ross (22) indicated that controlled environmental conditions favoring growth were detrimental to recovery of colchicine changes in sorghum. No attempt was made in this study to control post-colchicine conditions. The treated seedlings were subjected to the same technique that is used by Franzke in treating sorghum. Whether greenhouse conditions of December 1955 and 1956 were more favorable for normal growth than those of other years is not known. The studies reported on sorghum would indicate that even susceptible sorg-

hum lines vary in the number of mutants obtained from treatment series to treatment series.

It was thought that the numbers used in comparing treated to untreated in this study would be sufficient to test the hypothesis. However, Prakken (19) states that treatment of 10,000 barley seeds with 12,000 r X-rays may produce the following schedule:

(1) 10,000 seeds treated (M_1-seeds) of which

(2) † 5,000 seeds germinated (M_1-plant) of which

(3) † 4,000 plants produced mature seed of which

(4) † 2,000 segregations in 4,000 M_2-families of which

(5) † 1,000 segregations for sterility (mainly structural mutations)

(6) † 450 segregations for lethal chlorophyll mutations

(7) † 50 segregations for dwarfs and (sub) lethal forms

(8) † 200 more or less normally visible and easily recognizable morphological or physiological mutations

He states that it is this last group that would be reproductive and usable. His comparison of this class for several mutagens is:

after neutron radiation † 4%

after X-ray treatment † 5%

after ethylene epoxide † 9%

after ethylene epimine †15%

Assuming Prakken's method of calculation and a 1 per cent frequency of a mutant we would with a Poisson Distribution of mutants expect that

40 per cent of samples of 100 will have no mutants. Prakken's survey indicates that chemical mutagens are more effective in causing this class of mutations than radiation. If one classes colchicine as a mutagen and it falls anywhere near the range of those above we find in application of this percentage to the present data as follows: There were a total of 140 progenies from treated seedlings compared over the two year period. This would then result in approximately only 10-15 progenies that could be expected to be mutants. With this frequency the probability of mutations occurring at loci controlling the physical measurements used would be very small and would not be detected in the analysis.

The lack of significant differences among within progeny variances in the treated F_2 lines precludes an examination of individual lines on a line by line basis, even though extremes in variance exist and fall outside the range of variances among F_1 lines.

The latter range must of necessity be held to be a measure of environmental effects. Since genetic variation among plants is very small, it will not be possible to correlate phenotypic variation to it and thus variance among F_1 progenies must be considered a mean estimate of the error or environmental variance.

The variance among normal F_2 families is generally accepted as containing a genetic component in addition to the environmental compon-

ent. While $s^2_{F1} = s^2$ * and $s^2_{F2} = s^2 + 3\, s^2_y$

The genetic term s^2_y may be subidivided further into an additive and a dominance effect so that we have $s^2_{F_2} = s^2 + \frac{3}{2} s^2_G + \frac{3}{4} s^2_H$ and at the individual line level, the variance (of the individual line) is $s^2_{F_2}$ line = $s^2_{sp} + \frac{30}{2} s^2_G + \frac{30}{4} s^2_H$. The effect of induced homozygosity in an individual F_2 line would be to reduce the within line variance to $s^2_{F_2}$ line = s^2_{sp}. Such a line present in the population would reduce the mean of the S^2's of the population of treated lines, as any extreme departure from mean expectation inevitably does.

The occurrence of an individual mutation at any specific locus will do one of the following, (assuming the usual excess of mutation to the recessive): (1) A mutant at a locus for which the F_1 is heterozygous would reduce the genetic variation within the line at that locus in the same manner as above, Aa ——— aa

$$s^2_{sp} + \frac{30}{2} s^2_G + \frac{30}{4} s^2_H \text{ ——— } s^2_{sp}$$

(2) For loci for which the F_1 is homozygous dominant,

AA ——— Aa

$$s^2_{sp} \text{ ——— } s^2_{sp} + \frac{30}{2} s^2_G + \frac{30}{4} s^2_H$$

---

*s is being used for σ as variance estimate
s^2 = sampling error at plot level
s^2_{sp} = sampling error, single plant level
s^2_y = genetic variance (all)
s^2_G = additive genetic variance
s^2_H = dominance variance
$s^2_{F_1}$ and $s^2_{F_2}$ = generation designation

Since the general trend of mutation would be in the recessive direction, the accumulation of recessives would tend to depress the line mean (and population mean) except in areas involving overdominance or overdominance effects. These are apparently limited (17) and will not be considered.

The change in variance due to mutation among treated lines will depend on the relative frequency of heterozygous loci as compared to homozygous dominant loci in the single cross, (SD5 x SD7), where loci involved would be those capable of a mutational response to the chemical. If it is assumed that the two are about equal, then variances of the variances would remain at the same level, while the means of the variances were reduced.

The validity of these techniques depends on the demonstration of significance among progeny comparisons. Since this could not be demonstrated, this procedure cannot be implemented with this material.

The ability to bring about individual genetic changes by mutations within adapted varieties without passing through the genetic melting pot of recombination with genes of unadapted varieties would be of extreme value to the plant breeder. The possibility of inducing small or localized genetic changes has been investigated by many workers in all parts of the world. X-ray, neutrons, and chemical mutagens of all types and descriptions are being used in extensive programs to extend this area of knowledge.

Colchicine as reported by the sorghum workers would be such a tool. Colchicine treatment of maize has given favorable and unfavorable results. It could be concluded from this work that there is little justification for expecting rapid progress in mutation-breeding in maize through this method. However, preliminary work and the work of others, Hanson and Moore, suggest that there should be continued study of colchicine and its effects from the genetic view. It would seem that several approaches are warranted. (1) There should be an extensive survey made of many of the existing inbred lines to ascertain if possible a source of reactive material. This should be done using considerable numbers of each inbred. (2) Single crosses should be surveyed to find those with maximum segregation in the F_2 generation following selfing. These then could be treated in the F_1 and also in the F_2 selfed generations. If this were done, it would probably be advisable to first determine what means should be used to measure changes. A thorough study of the genetical inheritance in sorghum mutants to date and the exact physical changes that have occurred may aid in this approach. (3) Since the probability of obtaining a number of mutants without using extreme numbers of treated individuals is low, it may be of some value to investigate techniques of treating and propogation other than those used here. This could be done in sorghum since the sorghum plant to date seems to be most susceptible to colchicine mutation.

These results do not disprove the possibility of colchicine as a mutagen in corn. They do, however, indicate frequencies of an order not likely to be practical unless other avenues of corn improvement are exhausted. They also suggest need for devising rapid techniques for selection of colchicine mutants in corn. In order to be practical, selection must be at least comparable to the techniques used by Chase (4) to recover haploids at frequencies of 1/4000 and better.

Multiple heterozygous genetic stocks could be used to advantage in the suggested methods of study. At the present time the available genetic marker stocks are limited in the area of their adaptation and maturity. Many of these genetic marker stocks have been developed in areas ecologically different from South Dakota. Suitable markers could be incorporated into adapted lines for any area, of course. These could then be used not only for colchicine work but in the entire mutagenic field.

SUMMARY

An attempt was made to obtain proof of the hypothesis that colchicine treatment of germinating seeds induces mutations that are true-breeding or homozygous. A study was made of the F_2 progenies following colchicine treatment in the seedling stage in maize. Preliminary investigations for technique of treatment and germ plasm to be used resulted in the selection of a single cross of two South Dakota developed corn inbred lines, SD5 and SD7. Treated F_1 seedlings were grown in the greenhouse and selfed. The F_2 seeds were planted in replicated plots, with treated and untreated progenies randomized and with the original F_1 included so that uniformity of plot area, loss by inbreeding and normalcy of frequency distributions for measured characters could be determined.

Treated seedlings were rated for C-tumor using a 1 to 5 rating. F_2 progenies were measured for plant height, ear node height, plant diameter below ear node, ear length, ear diameter, and ear weight. The resulting data were analyzed statistically for significance by determining the variance of each progeny and running an analysis of variance of variances.

The hypothesis that colchicine treatment of seedlings results in true-breeding mutants was not supported in this study. No treated progenies were found to vary either less or more than untreated progenies

for any measurement taken. Although not reported in the results, the C-tumor rating gave no evidence of significance for mutation expectation. The C-tumors rated 1 (most severe) were found to have the least chance of survival and reproduction. It could be possible that these extreme tumors involved lethal mutations; evidence in support of this is not yet available.

Among treated lines, ear height showed a significant difference in both years; ear length in 1957 only. F_1 progenies were found to vary significantly for ear height in 1956.

The use of colchicine as a breeding tool for the production of true-breeding stocks through the mutation-somatic reduction-mutant growing point sequence cannot be supported by these results. Further investigations should be carried on with other maize germ plasm since the sorghum workers have shown a distinct varietal response to colchicine in this species.

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