ESTIMATION OF PROTEIN CONTENT IN F_2 SEGREGATING POPULATIONS OF SORGHUM BY THE UDY DYE-BINDING METHOD

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CHAPTER I

INTRODUCTION

Grain sorghum is an important warm season crop in the United States particularly in the Southwest, where the climate is too hot and too dry for corn.

In the Oklahoma sorghum breeding program are several sources of high-protein containing lines from the Purdue breeding program. Crosses with some of them have been made and F_1 and F_2 segregating populations were available from the 1968 and 1969 crops for analyses.

Of the methods available for determining protein content, the dye-binding method described by Udy, offers a rapid and economical procedure. It gives results fairly comparable to the standard Kjeldahl test when operated carefully. Although it has been used on wheat, rice, and a number of grains and feed stuffs, it has not been extensively used on sorghum grain.

The purpose of this study was to evaluate the dye-binding method of determining sorghum protein and to use the method to study the segregation of protein content in F_2 populations of sorghum.

CHAPTER II

LITERATURE REVIEW

Grain sorghum (Sorghum bicolor (L) Moench) is cultivated throughout Africa and extensively in India, China, Manchuria and the United States. The estimated world production of sorghum grain for the period 1934 to 1938 averaged 24,172,105 tons. Approximately 18,069,400 tons or 75 percent of the total was used as human food, and 3,865,953 tons or 16 percent as livestock feed (8).

Comparisons have been made between low-protein and high-protein diets and their effect on health. Stefansson and coworkers as reported by Peyton (15) disproved the theory that high-protein diets cause hypertension and digestive disorders. On the other hand, there is proof that a diet too low in protein causes edema (retention of fluid in the tissue) and malnutrition. Complete proteins come from animal products and incomplete proteins which are low or lacking in one or more of the essential amino acids come from plants (15).

Heller and Sieglinger (6) noted that a complete chemical analysis of 28 varieties of Oklahoma sorghum grain from Perkins and Woodward indicated that there was some variation among varieties but environmental conditions, especially temperature and moisture, were of the most important. The protein, ash, fat, and carbohydrate analysis of the sorghums was similar to corn and other cereals.

Barham et al. (1) reported that the protein content of the grain of a group of miscellaneous sorghum varieties was higher than that of the kafir varieties. Miller and coworkers (12), from protein analyses of samples collected in 1958, indicated that hybrids were somewhat lower in protein content than old standard varieties. This could be expected since work with hybrid corn indicated that protein in general decreased and yields increased due to hybridization. Worker and Ruckman (23) reported that protein analysis of samples collected from 1961 through 1966 indicated some grain sorghum hybrids contained somewhat less protein than varieties normally grown in the Imperial Valley of California, whereas others contained more. This variability may be explained in part by the absence of hybrid vigor in certain combinations. Burleson et al. (2) indicated that in a field experiment with grain sorghum, applications of 60 and 120 pounds of nitrogen per acre gave significant increases in both content and yield of protein in the grain and forage. Zuber and coworkers (24) confirmed that the crude protein content of corn grain was significantly altered by applications of nitrogen. Also, they indicated that varietal differences existed since the hybrid, Mo. 804, had a higher crude protein content than did US 13 or Dixie 17. Increasing the plant population caused a slight decrease in crude protein content. Application of 50 pounds of nitrogen gave a significantly lower protein content in the grain than where no nitrogen was applied. The percentage crude protein in stover was increased as additional nitrogen was applied. Sumner et al. (19) reported that with 200 pounds or more of applied nitrogen it was possible to maintain a very satisfactory crude protein level in the forage of Piper sudangrass (Sorghum

sudanense, Piper) throughout the season in Yolo and Kings Counties of California.

Pond et al. (18) concluded that lysine is the first limiting amino acid and threonine is probably the second limiting amino acid in milo grain for growth. Hogan (7) found that lysine is the first limiting factor in kafirin and cystine is the second. Lysine is indispensable for the maintenance of young animals. Heller and Green (5) concluded that the analysis of Darso grain sorghum showed it to be low in histidine, and Hogan (7) proved that kafirin was deficient in cystine and lysine. Osborne and coworkers (14) noted that at least insofar as nutrition and growth is concerned, the normal synthesis of new tissue is limited by the supply of lysine. No amount of energy or protein, however abundant, induced growth of the animals in the absence of lysine. The animal organism apparently cannot synthesize lysine, which is evidently not essential for maintenance in the sense of preservation of body-weight.

Pickett (16) reported that the genetic variation in grain yield and percent lysine was caused by nearly equal amounts of additive and non-additive gene action. Pickett (17), from the world's collection of sorghum, noted that the percent oil, in addition to being an excellent indication of embryo percentage, is also an indicator of highest percent lysine and protein.

Levi and Anderson (9) found that wheat heads with high protein tended to occur on the shorter tillers of plants containing more than three tillers. The protein content of spikelets tended to decrease towards the top from about the top third of the head. The top two spikelets of each head generally had decidedly lower protein content

than the remaining spikelets.

McNeal and Davis (11) showed that the average protein of the spring wheat varieties 'Supreme', 'Rescue', and 'Lee' was 14.98 percent for main spikes and 14.73 percent for tiller spikes, and that these levels of protein were not significantly different. McNeal and Davis (12) also reported that the lateral kernels in a spikelet had a higher protein content than did the central kernels, and grain from the middle of the spike was higher in protein content than that from the top of the spike. These differences indicate that the earlier formed and maturing kernels contained the highest protein content.

Amino Acid Classification

Proteins are complex substances of high molecular weight which consist largely of nitrogen, carbon, oxygen and hydrogen. They are produced on the surface of ribosomes, spheroid particles about 0.02μ in diameter and composed largely of high molecular weight RNA. When proteins are broken down, amino acids are the product. There are approximately 22 amino acids classified into (3):

a) Simple amino acids. - glycine, alanine, valine, leucine and isoleucine.

b) Hydroxy amino acids. - serine and threonine.

c) Sulfur-containing amino acids. - cysteine, cystine and methionine.

d) Basic amino acids. - lysine and arginine.

e) Acidic amino acids. - aspartic acid and glutamic acid.

f) Amino acid amides. - asparagine and glutamine.

g) Heterocyclic amino acids. - tryptophan, histidine, proline

and hydroxyproline.

h) Aromatic amino acids. - phenylalanine and tyrosine.

Measurement of percent protein has been made by two principal methods. One is the Kjeldahl determination and the other is the dye-binding technique. Fraenkel-Conrat and Cooper (4) indicated that micro-analytical methods had been developed for the estimation of the number of acidic and basic groups of proteins. These were based on the tendency of the polar groups to bind dyes of the opposite charge, resulting in a precipitation of the protein dye complex. The proposed micromethods were applicable to both soluble and insoluble proteins. Udy (20) confirmed that acidic and basic groups of protein molecules have been measured quantitatively on fractions of wheat protein by dye-binding techniques. These fractions were essentially soluble proteins, gluten proteins, a combination of these two, and proteins nondispersible in dilute acetic acid. Udy (21) found that the wheat proteins react with the disulfuric acid dye, orange G, at pH 2.2 to form an insoluble complex. The amount of dye bound per gram of sample may be used to provide estimate of protein content. In practice, the estimate is based on the concentration of unbound dye as measured colorimetrically using a light filter (470 mμ).

Neill (13) reported that the Kjeldahl protein test is a nitrogen test. Protein is the term applied to a combination of amino acids which are united by chemical bonds. Actual determination of the protein content by the Kjeldahl procedure, is based on the total amount of nitrogen in the sample. The amount of nitrogen times a factor of 5.7 for wheat and flour or a factor of 6.25 for feed gives the amount

of crude protein. The factors are based on the percent of nitrogen in various protein molecules.

CHAPTER III

MATERIALS AND METHODS

The sorghum material selected for study was grown at the Agronomy Research Station, Perkins, Oklahoma in 1968 and 1969. The F_1 plants of selected crosses and their parents were grown in 1968, while the segregating F_2 populations were grown in 1969. All heads were bagged to ensure selfing and harvested by hand. These heads were threshed and packaged individually.

The varieties used in the study consisted of two groups, one from the Oklahoma improvement program, and the other from Purdue University where a number of high protein varieties were identified. The crosses studied were B Wheatland x P0355, B Wheatland x P2190, B Wheatland x P3237, BOK 8 x P2486, B Wheatland x P4503, BOKY 25 x P5126 and B Redlan x P5185. Three backcrosses studied were B Wheatland-P0355 x B Wheatland, B Wheatland-P3237 x B Wheatland, and B Wheatland-P3237 x BOKY 29. Protein determinations were made on grain from F_1 and parental plants grown in 1968. A total of 873 heads from parental plants and segregating F_2 and backcross population plants grown in 1969 were available for estimating protein percentage.

A 100 g sample of grain of each of the varieties B Wheatland, B Redlan, BOKY 54, and ROKY 62 was obtained for a special study of the methods of protein analysis. Each variety was analyzed by the Udy method using the following conditions: 2 particle sizes, 3 sample

weights, and 3 shaking times. For comparison, each variety was analyzed by the Kjeldahl method with the variables 2 particle sizes, 2 sample weights, and 2 digestion times.

Ninety-six grain samples of AKS 614 hybrid grain sorghum from the various treatments of a fertility trial at the Eastern Oklahoma Pasture Station, Muskogee, were also analyzed. The field design was a split plot using combinations of N, P, and K as the main plots and the source of nitrogen (ammonium nitrate and urea) as the sub-plots. The correlation between grain yields and percent protein was calculated.

Another 41 grain samples of AKS 614 hybrid grain sorghum from the various treatments of two fertility trials at the Agronomy Research Station, Perkins, were analyzed. The trial design in each investigation was a randomized complete block with nitrogen from urea and ammonium nitrate applied as a band, immediately covered and left for 72 hours prior to covering in the first experiment, and four levels of ammonium nitrate and urea immediately covered in the second.

The simple correlation between grain yield and percent protein was calculated in the following manner:

Let Y_{ijk} represent the observed value for the yield of grain sorghum obtained in the ith replication of jth treatment of kth nitrogen source. In the same manner let X_{ijk} represent the percent protein in the grain from the same plant. A new available $Z_{ijk} = X_{ijk} + Y_{ijk}$ was constructed. Analysis of variance was run on each of X, Y, and Z.

The mean square (EMS) for error (b) for the three variables were substituted in the formula

$$r = \frac{1/2[\text{EMS for Z} - (\text{EMS for X} + \text{EMS for Y})]}{\sqrt{(\text{EMS for X})(\text{EMS for Y})}}.$$

Of the methods available for determining protein content, the dye-binding method described by Udy (22) offers a very rapid and economical procedure. The dye-binding procedure for analysis of wheat (22) was modified for grain sorghum as follows:

1. Clean the sample, remove all foreign material including shrunken and diseased kernels.

2. Pulverize 5 to 10 g of the sample in the cyclone grinder, utilizing a 0.015 mm mesh screen and vacuum.

3. Blend the ground sample thoroughly and weigh out 1,000 mg of sorghum grain into a 2-ounce reaction bottle.

4. Add 40 ml of the reagent dye.

5. Shake in the Eberbach shaker for 2 hours.

6. Set the meter needle of the colorimeter exactly to zero, and then turn on the light to warm up 1 to 2 hours prior to the analysis.

7. Set the colorimeter meter to 42% transmission when the cuvette is filled with reference dye.

8. After shaking pour the sample solution into the funnel fitted with a fiber-glass filter disc and cap. Introduce the filtrate into the cuvette.

9. Read the meter needle when it has stabilized which takes approximately 40 seconds.

10. Convert the percent transmission to percent protein by Udy's (22) standard wheat conversion chart.

The equipment used in the dye-binding method was Udy's (22) protein analyzer Model S. All samples were run twice for percent protein in this study.

Protein analyses were determined by the following modified Kjeldahl method:

1. Add 1 g sample to flask.

2. Add 10 g of sodium sulfate (1 scoop) and 2 or 3 granules of selenium.

3. Add 25 ml sulfuric acid.

4. Put flask on digester for 90 minutes. Let cool for approximately 20 minutes.

5. Add 300 to 350 ml of water to each sample.

6. Add 50 ml of boric acid with methyl red and methylene blue indicator into receiver flasks and put under receiver tubes.

7. Add 75 ml of sodium hydroxide (50% solution) and 2 or 3 pieces of zinc to each sample.

8. Distill 150 to 200 ml from the Kjeldahl flasks into the receiver flasks.

9. Titrate with 0.1253 N of sulfuric acid to a grayish blue color or until no green shows when looking through receiver flask.

10. One ml of acid is equivalent to one percent protein.

CHAPTER IV

RESULTS AND DISCUSSION

Protein Percentage in F_1 and Parental Plants

The results of percent protein in F_1 and parental plants grown in 1968 are given in Table I. The inadequacy of the number of plants involved for making a definite conclusion was realized. In general the percent protein in the F_1 plants varied extensively when compared with their parents. The average percent protein of the F_1 of B Redlan x P5185 was intermediate when compared with its parents. The averages of the F_1 of B Wheatland x P4503, B Wheatland x P3237, B Wheatland x P2190, and B Wheatland x P0355 were higher than the parents available for comparison. However, the averages of the F_1 of BOKY 25 x P5126 and BOK 8 x P2486 were lower than either parent. The ranges of percent protein in the F_1 of B Redlan x P5185, B Wheatland x P4503, B Wheatland x P2190, BOKY 25 x P5126, and BOK 8 x P2486 were 9.86 to 11.81, 12.25 to 12.76, 13.48 to 15.85, 11.81 to 14.11, and 11.73 to 14.14, respectively.

The highest percent protein in the F_1 population, 16.71, was found in the cross, B Wheatland x P3237. The highest percent protein in the parents was P2197 with 17.07 percent protein.

The effect of the environment on protein percentage was evident among the F_1 plants. Although for any one particular cross the F_1 are

TABLE I

AVERAGE AND RANGE IN PERCENT PROTEIN IN F_1 AND PARENTAL PLANTS GROWN IN 1968

Parent or F_1	Number of plants	Percent protein	
		Average	Range
B Redlan x P5185	4	11.18	9.86-11.81
B Redlan	1	10.86	
P5185	1	12.28	
B Wheatland x P4503	3	12.45	12.25-12.76
B Wheatland	1	12.28	
B Wheatland x P3237	1	16.71	
P3237	1	15.83	
B Wheatland x P2190	4	14.57	13.48-15.85
B Wheatland	1	10.90	
P2190	1	13.78	
B Wheatland x P0355	1	13.84	
B Wheatland	1	12.89	
BOKY 25 x P5126	4	12.82	11.81-14.11
BOKY 25	1	16.33	
P5126	1	15.98	
BOK 8 x P2486	4	12.68	11.73-14.14
BOK 8	1	12.73	
P2486	1	14.73	
P2197	1	17.07	

theoretically identical and should have had similar analyses for protein percentage, wide ranges were obtained. However, one should note that too few plants were analyzed to be able to draw definite conclusions. The large effect of the environment should be remembered in subsequent attempts to draw conclusions from data in F_2 segregating populations.

Protein Percentage of Segregating Populations

The means and ranges of the determinations for percent protein in F_2, parental and backcross populations grown in 1969 are shown in Table II. Frequency distributions are presented in Figures 1 to 8. Figures 1, 2, 4, 5, 7, and 8 show the distribution of percent protein for F_2 and parental plants while Figures 3 and 6 include the distribution of percent protein in backcross populations. The class interval for percent protein was 0.5.

Protein Percentage in F_2 and Parental Plants

Percent protein in the F_2 population of B Redlan x P5185 given in Figure 1 and Table II ranged from 9.25 to 13.75 and showed a fairly normal distribution about the mean, 11.12, and suggested quantitative inheritance. The protein percentages in B Redlan and P5185 ranged from 11.75 to 12.25 and 10.75 to 12.75, respectively. These ranges were toward the higher end of the F_2 frequency distribution, but since they did not range as widely as the F_2 population, it might be concluded that transgressive segregation had taken place.

The distribution shown in Figure 2 of percent protein in the F_2 population of B Wheatland x P4503 approached a normal distribution.

TABLE II

AVERAGE AND RANGE IN PERCENT PROTEIN IN F_2 AND PARENTAL PLANTS GROWN IN 1969

Parent or F_2	Number of plants	Percent protein	
		Average	Range
B Redlan x P5185	51	11.12	9.25-13.75
B Redlan	3	12.23	11.75-12.25
P5185	5	11.66	10.75-12.75
B Wheatland x P4503	149	10.76	7.75-13.75
B Wheatland	5	10.59	10.25-10.75
B Wheatland x P3237	54	11.66	8.75-14.75
B Wheatland	6	10.39	9.25-11.75
P 3237	8	10.47	8.25-13.25
B Wheatland-P3237 x B Wheatland	38	10.97	8.25-13.75
B Wheatland-P3237 x BOKY 29	26	10.33	8.25-13.25
B Wheatland	4	10.58	10.25-11.25
BOKY 29	6	10.10	9.25-10.75
B Wheatland x P2190	59	12.24	7.75-15.25
B Wheatland	6	10.39	9.25-11.75
P2190	4	13.52	12.25-14.75
B Wheatland x P0355	55	12.34	9.25-16.25
B Wheatland	6	11.43	10.75-12.75
B Wheatland-P0355 x B Wheatland	17	11.97	9.75-14.75
BOKY 25 x P5126	177	12.82	8.75-18.25
BOKY 25	5	13.29	12.25-15.25
P5126	6	13.96	13.75-14.75
BOK 8 x P2486	173	12.39	9.25-17.25
BOK 8	4	11.56	10.75-12.25
P2486	8	13.64	12.25-15.25

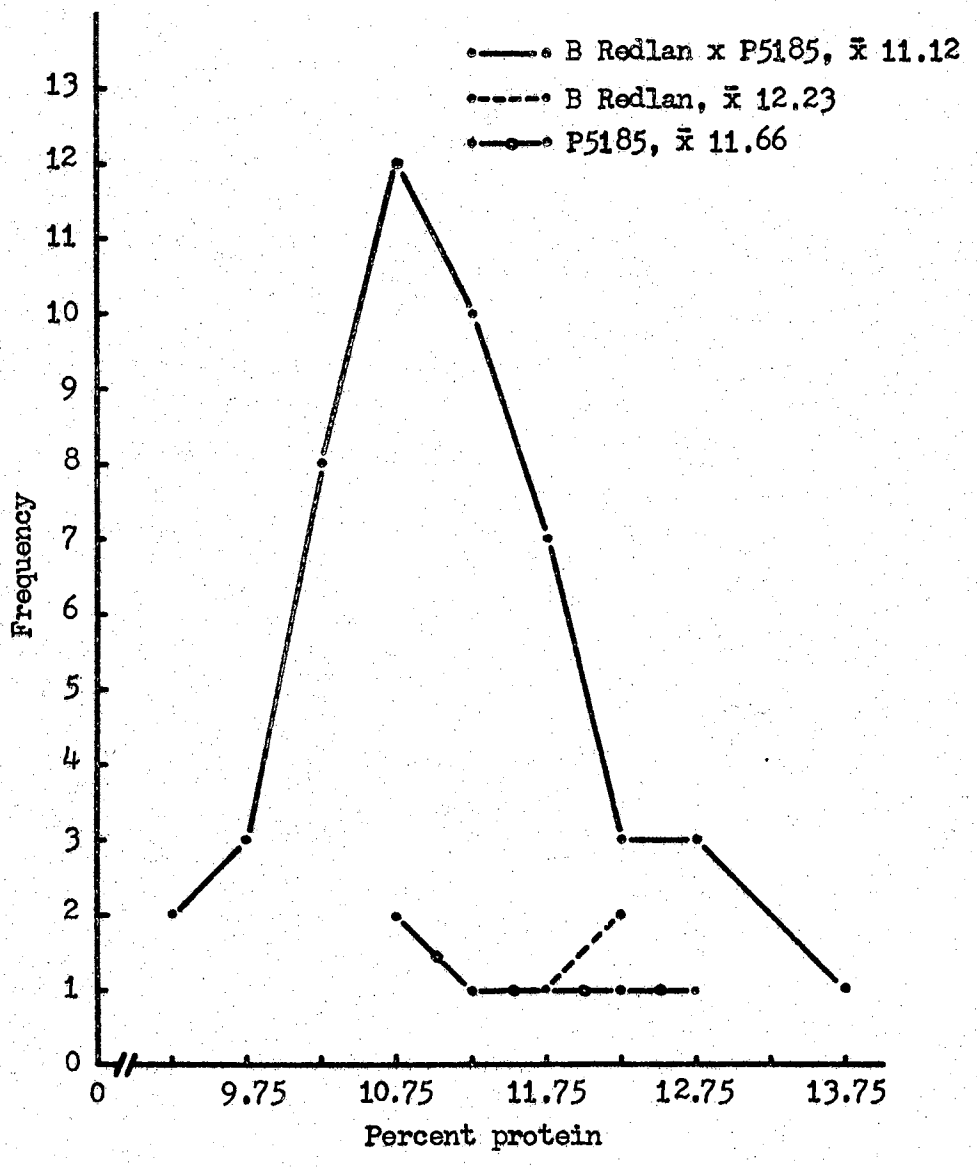

Figure 1. The Distribution of Percent Protein for the F_2 Segregating Population and Parents of the Cross, B Redlan x P5185.

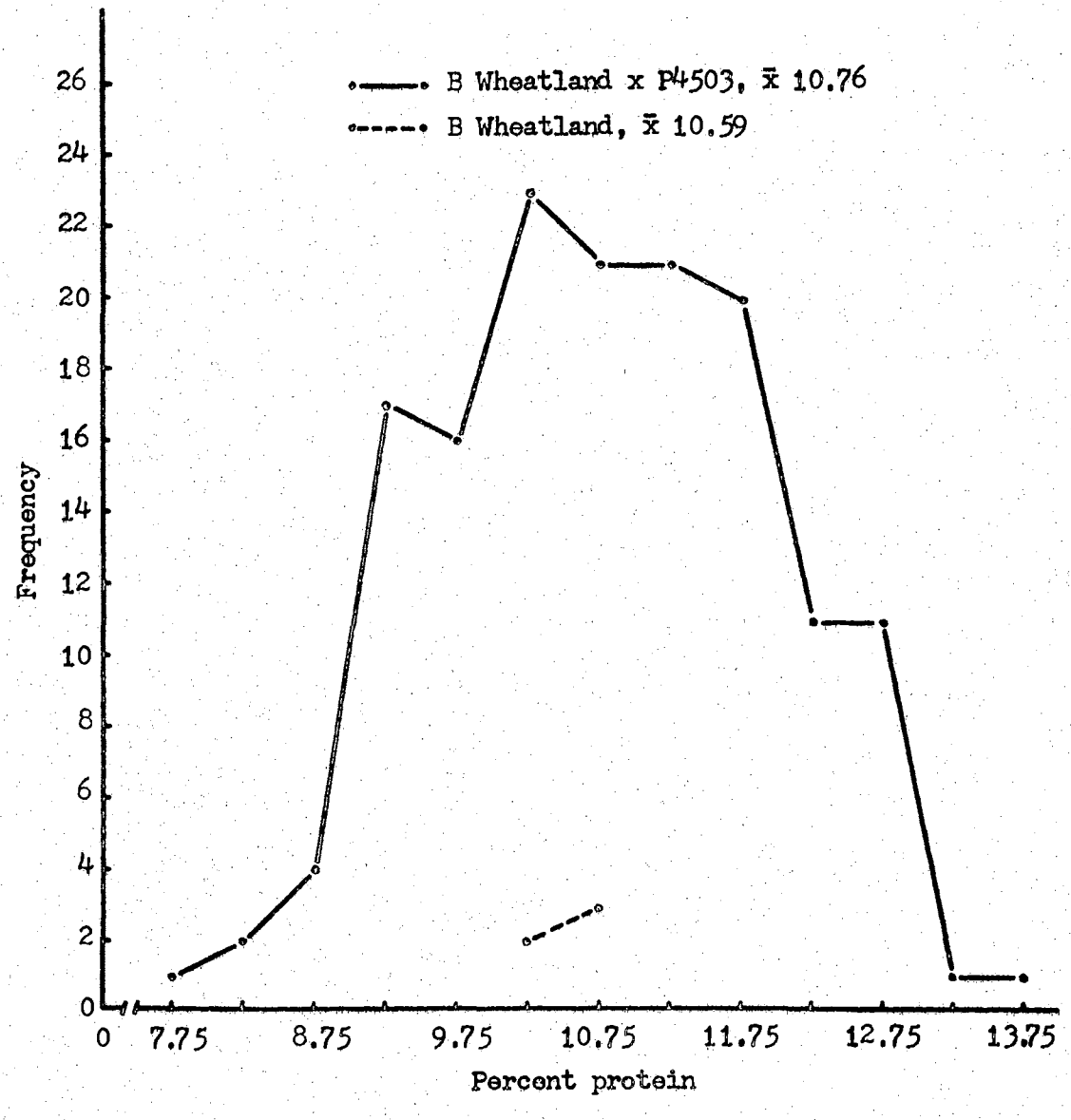

Figure 2. The Distribution of Percent Protein for the F_2 Segregating Population and Parents of the Cross, B Wheatland x P4503.

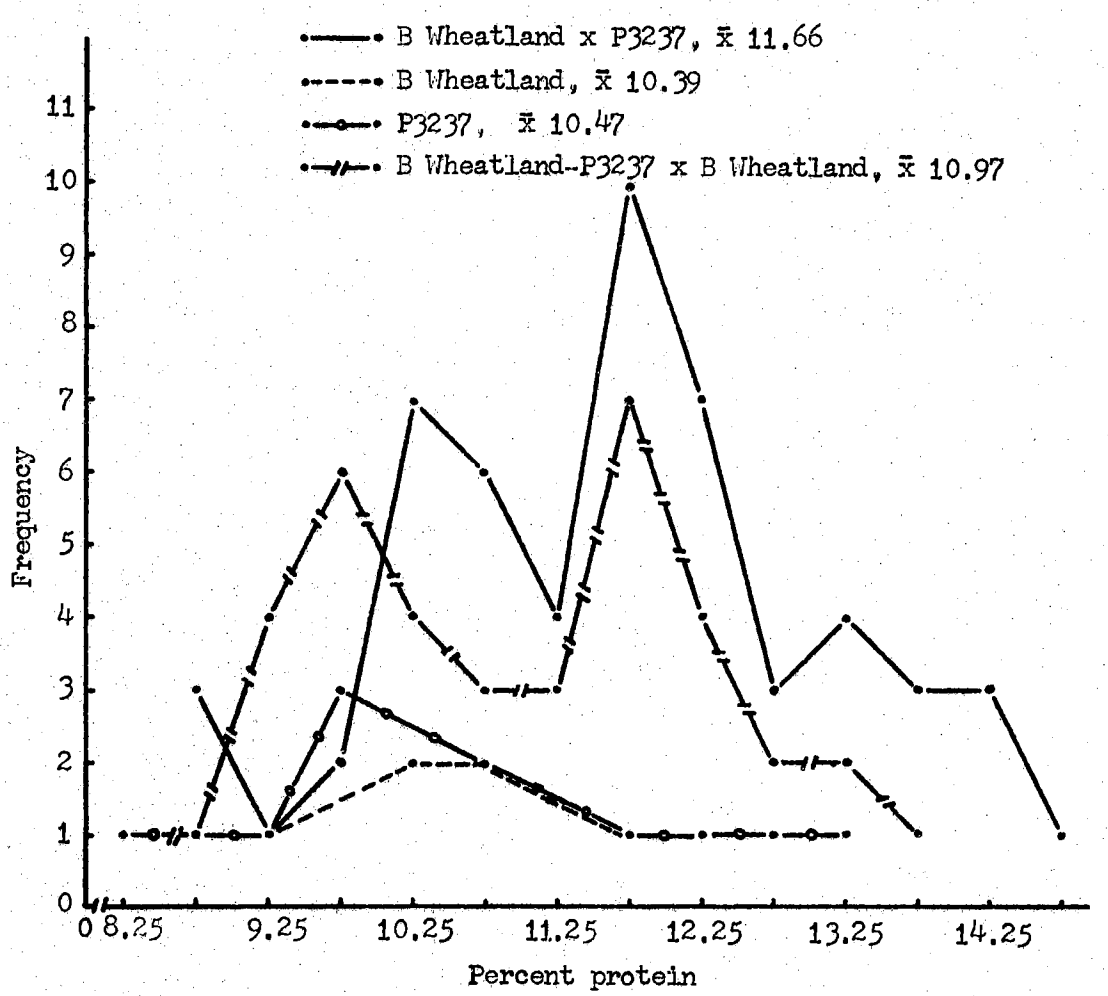

Figure 3. The Distribution of Percent Protein for the F_2 Segregating Population, Backcross, and Parents of the Cross, B Wheatland x P3237.

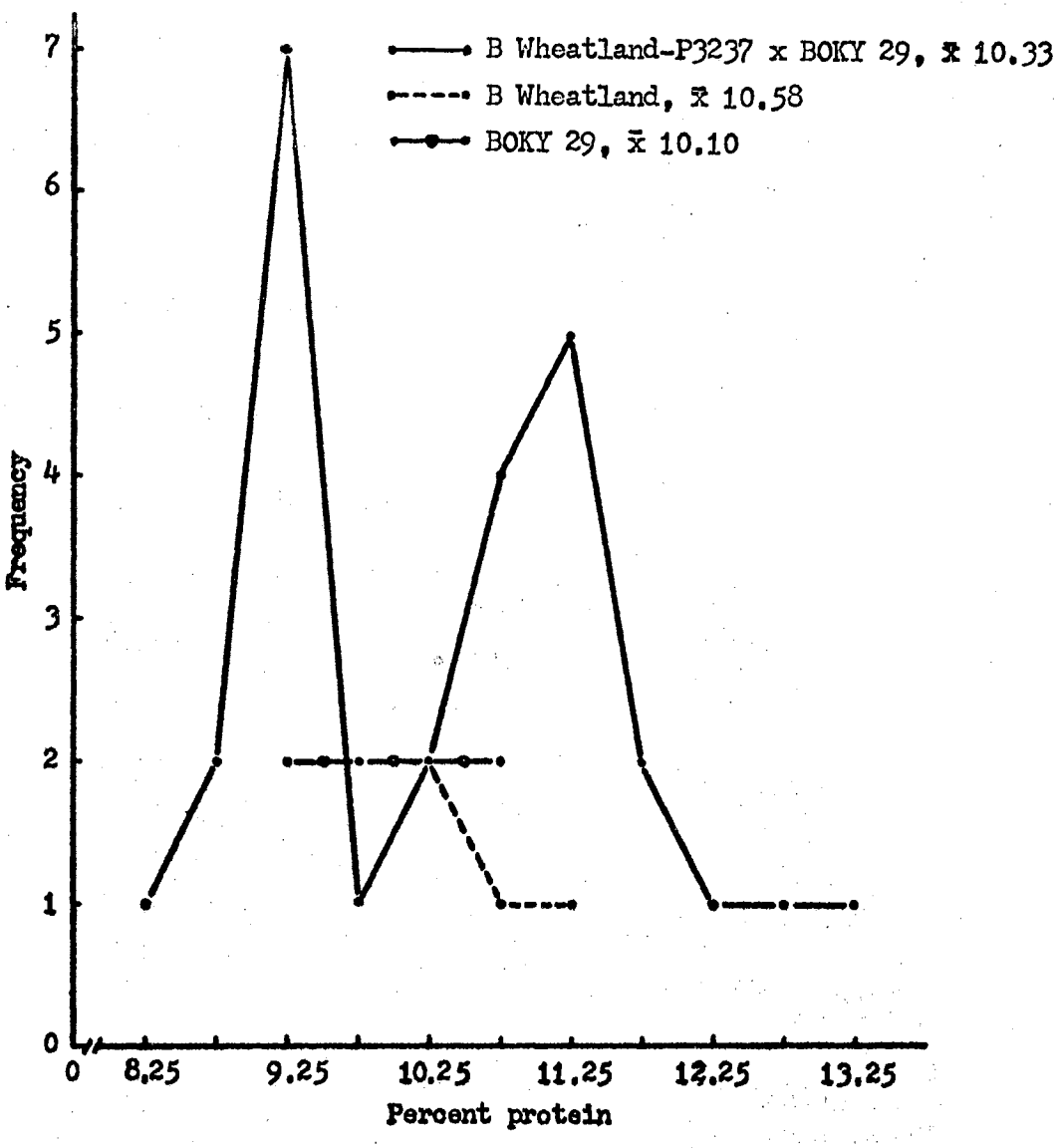

Figure 4. The Distribution of Percent Protein for the F_2 Segregating Population and Parents of the Cross, B Wheatland-P3237 x BOKY 29.

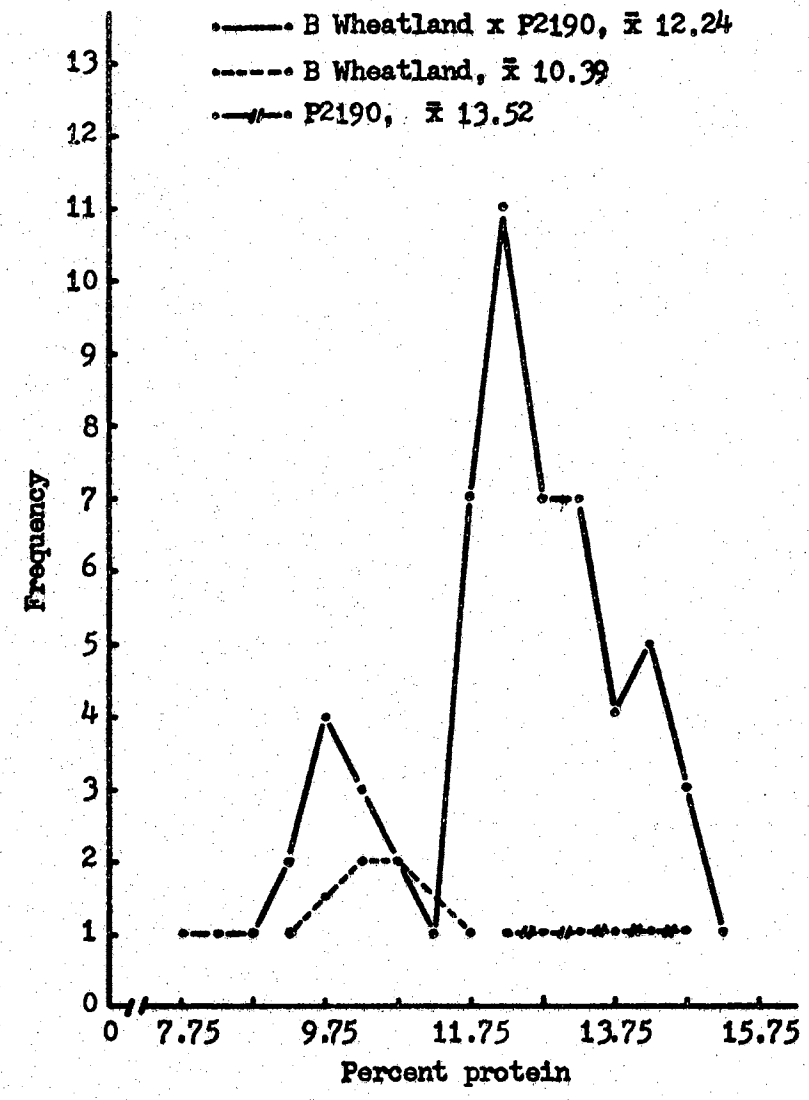

Figure 5. The Distribution of Percent Protein for the F_2 Segregating Population and Parents of the Cross, B Wheatland x P2190.

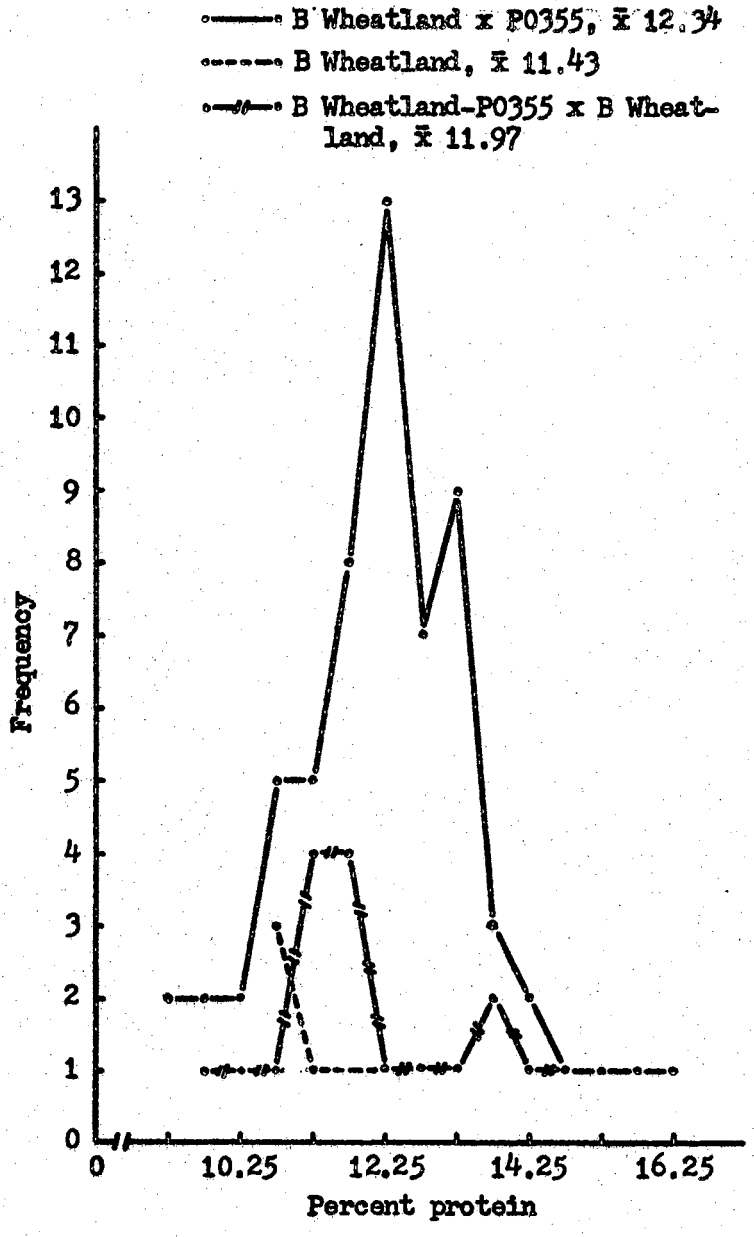

Figure 6. The Distribution of Percent Protein for the F_2 Segregating Population, Backcross, and Parents of the Cross, B Wheatland x P0355.

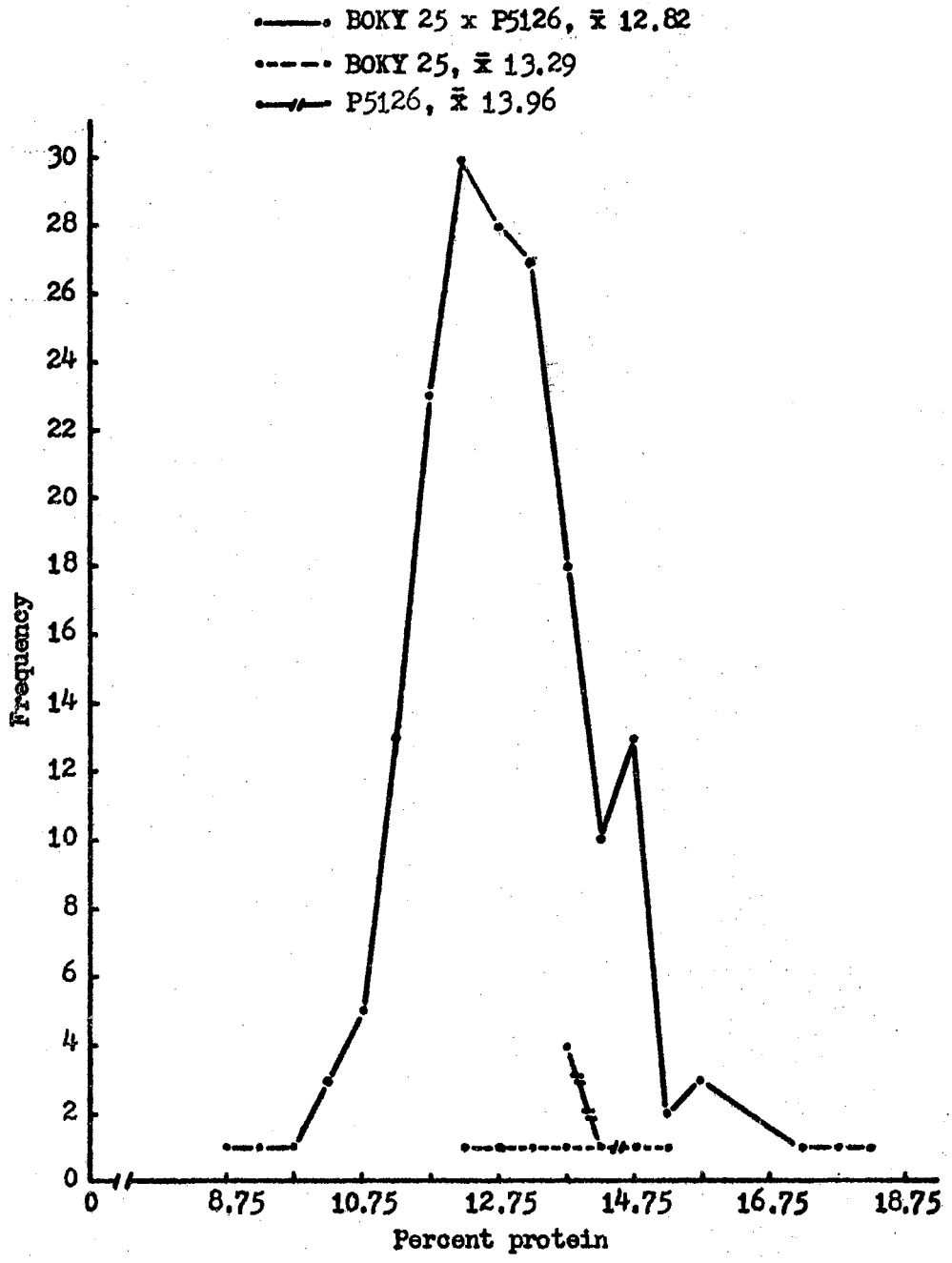

Figure 7. The Distribution of Percent Protein for the F_2 Segregating Population and Parents of the Cross, BOKY 25 x P5126.

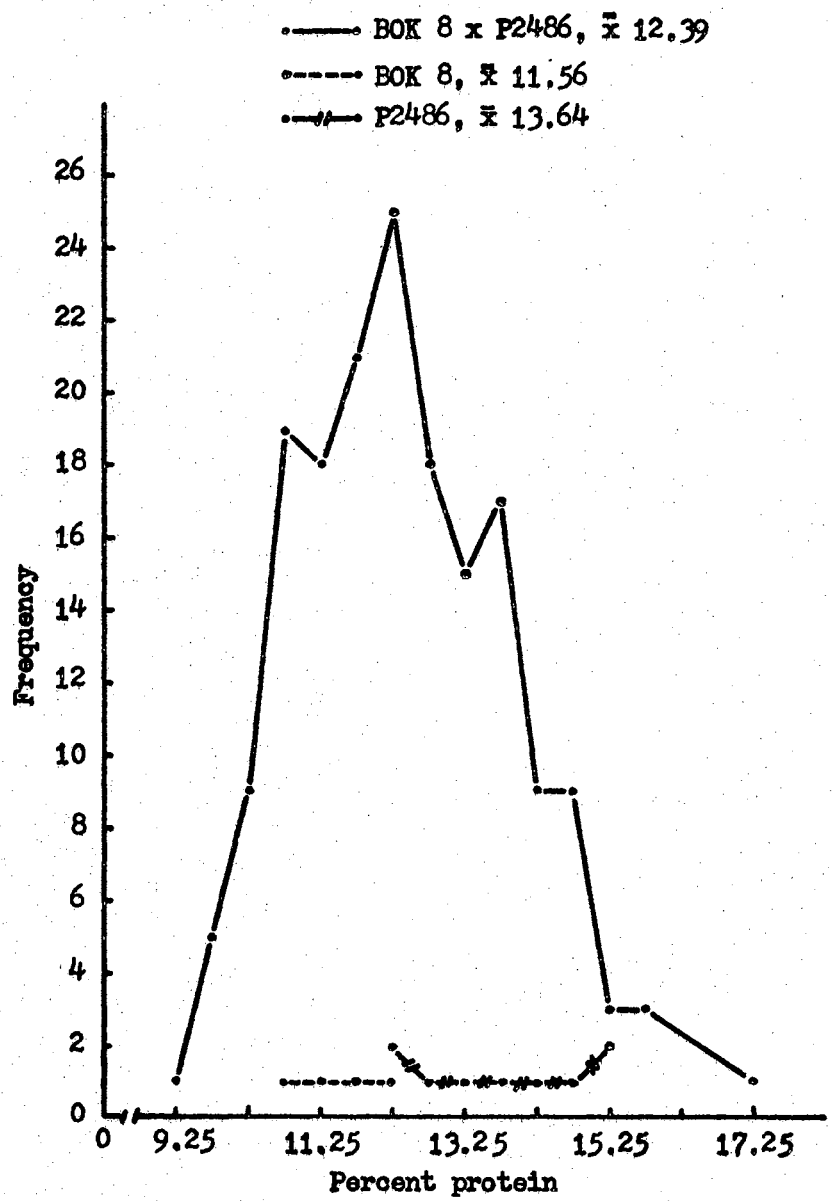

Figure 8. The Distribution of Percent Protein for the F_2 Segregating Population and Parents of the Cross, BOK 8 x P2486.

The range of percent protein for the segregating F_2 population was 7.75 to 13.75. Its mean was 10.76 percent protein. Only B Wheatland parent was available for comparison and it fell approximately in the middle of the range of protein percentages.

The frequency distribution shown in Figure 4 of percent protein in the population, B Wheatland-P3237 x BOKY 29 showed two peaks, one at 9.25 and the other at 11.25 percent protein. The ranges of percent protein in each segment of the distribution curve were 8.25 to 9.75 and 9.75 to 13.25. The bimodal nature of the frequency distribution curve suggested a backcross ratio of 1 to 1. The Chi-square test gave a value of 0.50, indicating a good fit. However, the protein analysis of the parental plants did not fall under the peaks in the curve. Instead, the percent protein in the parental plants, B Wheatland and BOKY 29 were near the low point on the curve with ranges of 10.25 to 11.25 and 9.25 to 10.75, respectively, while the percent protein of the third parent, P3237, spread over the entire range of the backcross population (See Figure 3 and Table II).

The frequency distribution of percent protein in the F_2 population of B Wheatland x P2190 in Figure 5 was bimodal in nature suggesting a one gene segregation. The distribution of percent protein in the parental plants, B Wheatland and P2190, scarcely overlapped and extended toward the peaks in the F_2 distribution. The ranges of percent protein in F_2, B Wheatland and P2190 (Table II) were 7.75 to 15.25, 9.25 to 11.75 and 12.25 to 14.75, respectively. A Chi-square test of the F_2 distribution gave a value of 0.07 indicating a high probability of a good fit to a 3:1 ratio.

The frequency distribution of percent protein in the F_2 population of BOKY 25 x P2156 in Figure 7 was a fairly normal curve suggesting quantitative inheritance. The distributions of percent protein in the parental plants, BOKY 25 and P5126, fell under the F_2 curve but toward the upper end. The ranges of percent protein in the F_2, BOKY 25 and P5126 were 8.75 to 18.25, 12.25 to 15.25 and 13.75 to 14.75, respectively.

The frequency distribution of percent protein in the F_2 population of BOK 8 x P2486 in Figure 8 was a fairly normal curve suggesting quantitative inheritance. The distributions of percent protein in BOK 8 and P2486 fell under the F_2 curve, but they did not overlap, nor were there any apparent peaks in the F_2 curve over the parental means. The range of percent protein in the F_2, BOK 8, and P2486 were 9.25 to 17.25, 10.75 to 12.25, and 12.25 to 15.25, respectively.

Protein Percentage of F_2, Backcrosses and Parental Plants

The frequency distribution patterns of percent protein in the F_2 population, B Wheatland x P3237 and in the backcross, B Wheatland-P3237 x B Wheatland are shown in Figure 3. They suggest the possibility of a bimodal curve giving a simple 3 to 1 segregation in F_2 and a 1 to 1 segregation in the backcross. One parent covered the lower range of percent protein while the other covered most of the F_2 range. The Chi-square test of the F_2 segregation gave a value of 2.99 indicating an acceptable fit to a 3 to 1 ratio. The backcross population fell in an exact ratio of 1 to 1. The ranges of percent protein of the F_2 population, backcross, B Wheatland, and P3237 (Table II) were 8.75 to 14.75, 8.25 to 13.75, 9.25 to 11.75 and 8.25 to 13.25, respectively.

The frequency distribution patterns of percent protein in the F_2 population, B Wheatland x P0355 in Figure 6 appeared to be a normal curve suggesting quantitative inheritance. The distribution of the backcross, B Wheatland-P0355 x B Wheatland in Figure 6 showed two peaks but they could not be interpreted as exhibiting a backcross ratio of 1 to 1. The ranges of percent protein of B Wheatland x P0355, B Wheatland-P0355 x B Wheatland, and B Wheatland were 9.25 to 16.25, 9.75 to 14.75, and 10.75 to 12.75, respectively.

The highest estimate of percent protein was 18.25 and it came from the F_2 population of the cross BOKY 25 x P5126.

From the populations examined, the inheritance of protein percentage would seem to be controlled by a sufficient number of genes to give fairly normal frequency distributions as determined by the data from the segregating populations shown in Table II and Figures 1, 2, 6, 7, and 8. However, Chi-square tests of the segregating populations shown in Figures 3, 4, and 5 indicated that the inheritance of protein percentage was controlled by a single gene pair. This discrepancy cannot be explained except to say that different sources of protein may be inherited differently. Data in Figures 3 and 4 are based on the same source of high protein while all the rest are based on different sources. In addition, the conclusion of single gene inheritance in certain of the populations was made from rather small numbers increasing the possibility of obtaining a misleading sample of data.

Percent Protein, Grain Yield, and Their Correlation for a Grain Sorghum Fertility Trial at the Eastern Oklahoma Pasture Station, Muskogee, 1969

The Udy dye-binding method was used to estimate percent protein of the grain sorghum. From the analysis of variance shown in Table III it can be seen that there was a highly significant difference among treatments and there was not a significant difference due to nitrogen source, replications, or any of the interactions among them. For grain yield there was a significant difference among treatments at the 0.01 level and among replications at 0.05 level. The correlation between grain yield and percent protein was calculated using the error mean squares for X, Y and Z found in Table III, 0.1259, 31,703.5265, and 31,695.5069 as follows:

$$r = \frac{1/2\left[31{,}695.5069 - (0.1259 + 31{,}703.5265)\right]}{\sqrt{(0.1259)(31{,}703.5265)}} = -0.06.$$

These data indicated a tendency for the amount of N, P and K fertilizer in various combinations to give different effects on percent protein and grain yield. The combination of N, P and K fertilizer which gave the highest percent protein, 12.65, was ammonium nitrate in the analysis 80-60-0 (See Table IV). The highest grain yield of 2,001 pounds per acre was obtained by the application of urea in the combination 80-60-80 (See Table IV).

A negative correlation between percent protein and grain yield would indicate that when yield is high, the percent protein will be low. However, this correlation was not significant.

TABLE III

ANALYSIS OF VARIANCE OF PERCENT PROTEIN, GRAIN YIELD, AND THEIR SUM FOR A GRAIN SORGHUM FERTILITY TRIAL AT THE EASTERN OKLAHOMA PASTURE STATION, MUSKOGEE, 1969

		Mean square		
Source	d.f.	Percent protein	Yield	Percent protein + yield
Corrected total	87	1.2063	96,909.9246	97,006.6272
Replication (R)	3	0.7902	105,302.4962*	105,417.0917
Treatment (T)	10	7.7409**	555,578.5568**	556,483.9030
Error (a)	30	0.6429	34,830.9629	34,802.7770
N source (N)	1	0.0180	100,305.0114	100,219.9007
T x N	10	0.1711	36,802.0114	36,823.1326
Error (b)	33	0.1259	31,703.5265	31,695.5069

Correlation = -0.06.

*significant at 0.05 level. **significant at 0.01 level.

TABLE IV

MEANS OF PERCENT PROTEIN AND GRAIN YIELD FOR A GRAIN SORGHUM FERTILITY TRIAL USING AMMONIUM NITRATE AND UREA AS NITROGEN SOURCES, AT THE EASTERN OKLAHOMA PASTURE STATION, MUSKOGEE, 1969

Treatments	Percent protein		Yield (lb/A)	
	Ammonium nitrate	Urea	Ammonium nitrate	Urea
0-60-40	9.26	9.14	1,255	1,255
40-60-40	10.97	10.86	1,535	1,431
80-60-40	11.83	12.50	1,592	1,572
120-60-40	12.07	12.53	1,888	1,763
80-0-40	12.08	12.01	1,261	1,150
80-30-40	12,23	12,32	1,403	1,567
80-90-40	11.63	11.68	1,840	1,875
80-60-0	12.65	12.41	1,276	1,198
80-60-80	11.85	11.68	1,946	2,001
120-90-0	12.61	12.63	1,615	1,296
120-90-80	12.40	12.13	1,815	1,577

Percent Protein, Grain Yield, and Their Correlation for a Grain Sorghum Fertility Trial of Ammonium Nitrate and Urea both Covered and Exposed at the Agronomy Research Station, Perkins, Oklahoma, 1969

The analysis of variance in Table V showed that for percent protein, there was a highly significant difference among replications and a significant difference between nitrogen sources using the Udy dye-binding method. There was no significant difference in grain yield. The correlation calculated for Table V between percent protein and grain yield was positive, but was not significant.

These data indicated that nitrogen sources, urea and ammonium nitrate, gave different effects on percent protein but not on grain yield. Ammonium nitrate covered gave the highest percent protein, shown in Table VI.

The positive value of the correlation between percent protein and grain yield indicated a tendency for protein and grain yield to increase or decrease together.

Percent Protein, Grain Yield and Their Correlation for a Grain Sorghum Fertility Trial Using Four Levels of Ammonium Nitrate and Urea, Covered, at the Agronomy Research Station, Perkins, Oklahoma, 1969

The analysis of variance in Table VII showed that there were no significant differences in percent protein, grain yield, nor their interactions even though different nitrogen sources and rates were used. The highest mean of percent protein, 12.36, was obtained by

TABLE V

ANALYSIS OF VARIANCE OF PERCENT PROTEIN, GRAIN YIELD, AND THEIR SUM FOR A GRAIN SORGHUM FERTILITY TRIAL USING AMMONIUM NITRATE AND UREA BOTH COVERED AND EXPOSED AT THE AGRONOMY RESEARCH STATION, PERKINS, OKLAHOMA, 1969

Source	d.f.	Mean square		
		Percent protein	Yield	Percent protein + yield
Corrected total	19	0.0733	70,204.0421	70,257.4236
Replication	4	0.2227**	85,668.5750	85,823.5487
Exposure (E)	1	0.0072	36,465.8000	36,498.3118
N source (N)	1	0.1514*	156.8000	166.6981
E x N	1	0.0562	25,205.0000	25,180.3377
Error	12	0.0240	77,447.9083	77,470.9588

Correlation = 0.24.

TABLE VI

MEANS OF PERCENT PROTEIN AND GRAIN YIELD OF GRAIN SORGHUM FROM A FERTILITY TRIAL, USING AMMONIUM NITRATE AND UREA, BOTH COVERED AND EXPOSED AT THE AGRONOMY RESEARCH STATION, PERKINS, OKLAHOMA, 1969

Source	Exposure (hours)	Mean	
		Percent protein	Yield (lb/A)
Ammonium nitrate	0	11.83	2,306
Ammonium nitrate	72	11.77	2,320
Urea	0	11.55	2,229
Urea	72	11.70	2,386

TABLE VII

ANALYSIS OF VARIANCE OF PERCENT PROTEIN, GRAIN YIELD, AND THEIR SUM FOR A GRAIN SORGHUM FERTILITY TRIAL USING FOUR LEVELS OF AMMONIUM NITRATE AND UREA COVERED, AT THE AGRONOMY RESEARCH STATION, PERKINS, OKLAHOMA, 1969

Source	d.f.	Mean square		
		Percent protein	Yield	Percent protein + yield
Corrected total	23	0.084	21,850.694	21,853.517
Replication	2	0.213	14,331.542	14,331.163
Rate of N (R)	3	0.125	41,238.819	41,165.290
N source (N)	1	0.043	4,676.042	4,704.567
R x N	3	0.021	24,988.264	24,988.134
Error	14	0.074	19,324.685	19,343.123

Correlation = 0.27.

application of 300 pounds per acre of ammonium nitrate, shown in Table VIII. The highest grain yield, 2,752 pounds per acre, was obtained where no nitrogen was applied. The correlation of percent protein and grain yield was positive, but was not significant. This indicated a tendency for percent protein and grain yield to increase or decrease together.

The correlation of percent protein and grain yield at the Agronomy Research Station, Perkins and the Eastern Oklahoma Pasture Station, Muskogee, possibly were opposite because of environmental effects. The experiment was indicated to be irrigated but water was not available and moisture was lacking at the Agronomy Research Station, Perkins, while moisture was adequate at the Eastern Oklahoma Pasture Station, Muskogee. When soil moisture is sufficient, moderate application of nitrogen fertilizer will more likely give an increase in grain yield than in percent protein. When soil moisture is insufficient, nitrogen fertilizer will be less likely to give an increase in grain yield, but the protein percent may be increased.

Comparison of Percent Protein in Sorghum Grain of B Wheatland, B Redlan, BOKY 54 and ROKY 62, Using the Udy Dye-binding and Kjeldahl Methods

The analysis of variance for the Udy dye-binding study in Table IX shows that there were highly significant differences in percent protein among varieties, variety x shaking time, particle sizes, particle size x shaking time, particle size x variety, and sample weight. Significant differences (at the 0.05 level) were found among shaking time, sample weight x shaking time, sample weight x variety and sample weight x

TABLE VIII

MEANS OF PERCENT PROTEIN AND GRAIN YIELD OF GRAIN SORGHUM FROM A FERTILITY TRIAL, USING AMMONIUM NITRATE AND UREA AS NITROGEN SOURCES AT 0, 150, 300, AND 450 AND 0, 110, 220 AND 330 POUNDS PER ACRE, RESPECTIVELY, AT THE AGRONOMY RESEARCH STATION, PERKINS, OKLAHOMA, 1969

Source	Mean	
	Percent protein	Yield (lb/A)
No nitrogen	11.90	2,752
150 lb/A of ammonium nitrate	12.10	2,604
300 lb/A of ammonium nitrate	12.36	2,626
450 lb/A of ammonium nitrate	12.15	2,664
110 lb/A of urea	12.12	2,635
220 lb/A of urea	12.13	2,702
330 lb/A of urea	12.03	2,470

particle size.

These differences indicated that a higher percent protein was obtained from certain varieties, from the smaller particle size, as well as from a longer shaking time, and higher sample weight. It was also indicated that the varieties did not respond uniformly to different shaking times, nor to different particle sizes. In addition the two particle sizes reacted differently to varying lengths of shaking time.

The analysis of variance of percent protein obtained by the Kjeldahl method in Table X showed a highly significant difference among varieties, sample weight, and variety x sample weight. The analysis

TABLE IX

ANALYSIS OF VARIANCE OF PERCENT PROTEIN IN SORGHUM GRAIN, B WHEATLAND, B REDLAN, BOKY 54 AND ROKY 62, USING THE UDY DYE-BINDING METHOD

Source	d.f.	MS
Trial (T)	1	6.167
Shaking time (S)	2	14.246*
Error (a)	2	0.441
Variety (V)	3	81.271**
V x S	6	0.268**
Error (b)	9	0.013
Particle size (P)	1	120.707**
P x S	2	1.631**
P x V	3	2.203**
P x V x S	6	0.060
Error (c)	12	0.045
Sample weight (W)	2	33.695**
W x S	4	0.042*
W x V	6	0.051*
W x P	2	0.057*
W x V x S	12	0.039
W x P x S	4	0.034
W x P x V	6	0.013
W x P x V x S	12	0.009
Error (d)	48	0.016

*significant at 0.05 level. **significant at 0.01 level.

TABLE X

ANALYSIS OF VARIANCE OF PERCENT PROTEIN IN SORGHUM GRAIN, B WHEATLAND, B REDLAN, BOKY 54 AND ROKY 62, USING THE KJELDAHL METHOD

Source	d.f.	MS
Replication (R)	1	0.0032
Variety (V)	3	53.2695**
Error (a)	3	0.0232
Particle size (P)	1	0.0329
V x P	3	0.0539
Error (b)	4	0.0118
Digestion time (D)	1	0.0375
D x V	3	0.0111
D x P	1	0.0032
D x V x P	3	0.0015
Error (c)	8	0.0317
Sample weight (W)	1	17.2744**
W x V	3	0.1088**
W x P	1	0.0172
W x D	1	0.0113
W x V x P	3	0.0214
W x D x V	3	0.0130
W x D x P	1	0.0010
W x D x V x P	3	0.0050
Error (d)	16	0.0086

*significant at 0.05 level. **significant at 0.01 level.

indicated that different varieties and different sample weights gave different protein percentages. Also, the varieties did not respond uniformly to an increase in sample weight. There were no significant differences in particle size, digestion time, or their interaction in this method, while there were significant differences for those variables when the Udy dye-binding method was used.

It is apparent from the analysis of variance and the means shown in Table XI that some of the factors have a greater influence on the results obtained from the dye-binding method than from the Kjeldahl method. An increase in shaking time from 1 to 2 to 3 hours continued to give higher readings with the dye-binding method, while 90 minutes of digestion time with the Kjeldahl method was adequate. The smaller particle size gave a noticeably higher reading with the dye-binding method, but did not influence the Kjeldahl results. Both methods gave a linear relationship between sample size and percent protein. Both methods revealed differences due to variety, but the Kjeldahl method gave a higher percent protein than the Udy method for three of the four varieties, when the analysis was averaged over all determinations. This average included results from the 1 hour shaking time and from the 0.024 mm particle size tests which were shown to underestimate the protein percentage. The fourth variety, the only one with white seed, gave a higher protein percentage by the Udy dye-binding method. The pigment in the colored varieties may have reduced light transmission in the Udy test, thereby giving lower percent protein readings. In the lower part of Table XI more critical comparisons of the Udy and Kjeldahl methods were made. When the manufacturers recommendations for the dye-binding method were used (as described in footnote 3) and the

TABLE XI

MEANS OF PERCENT PROTEIN OF INDIVIDUAL VARIABLES AVERAGED OVER ALL OTHER VARIABLES, AND FOR CERTAIN COMBINATIONS OF VARIABLES SELECTED TO GIVE CRITICAL COMPARISONS OF THE UDY DYE-BINDING AND THE KJELDAHL METHODS

Source	Mean of percent protein	
	Udy	Kjeldahl
B Wheatland	8.63	9.33
B Redlan	10.36	11.29
BOKY 54	11.50	12.08
ROKY 62	8.26	8.07
Particle size 0.015 mm	10.78	10.21
Particle size 0.024 mm	8.78	10.17
Sample weight 0.95 g	8.87	9.67
Sample weight 1.00 g	9.71	-
Sample weight 1.05 g	10.53	10.71
Shaking time 1 hour	9.11	-
Shaking time or digestion time 2 hours[1]	9.80	10.12[2]
Shaking time or digestion time 3 hours	10.19	10.22
B Wheatland	9.56[3]	9.27[4]
B Redlan	11.69[3]	11.17[4]
BOKY 54	12.49[3]	12.04[4]
ROKY 62	9.14[3]	8.14[4]
B Wheatland	9.48[5]	9.33[6]
B Redlan	11.70[5]	11.29[6]
BOKY 54	12.39[5]	12.08[6]
ROKY 62	9.05[5]	8.07[6]

[1] Shaking time in case of Udy and digestion time for Kjeldahl analysis; [2] Digestion time was 90 minutes; [3] Recommended procedure, 0.015 mm particle size, 1 g sample, and 2 hours shaking time, average of 2 determinations; [4] Recommended procedure, 0.015 mm particle size, average of 0.95 and 1.00 g sample, and 90 minutes digestion, average of 4 determinations; [5] Average of 6 determinations including 0.015 mm particle size, 0.95, 1.00 and 1.05 g sample weights, and 2 hours shaking time; [6] Average of all Kjeldahl determinations (16) regardless of factor.

results were compared with the most common Kjeldahl procedure (as described in footnote 4) protein percentages were slightly higher by the dye-binding method. The additional comparisons of the methods in Table XI included more analyses, and the results were very similar. Statistical tests for differences were not made because the experiment was not designed to make such tests.

Since these latter comparisons were considered to be more appropriate and critical than those of the varieties averaged over all factors (upper part of Table XI) it was concluded that the Udy dye-binding method of determining protein percentage gave results fairly comparable to the standard Kjeldahl method when used according to the manufacturers recommendations.

CHAPTER V

SUMMARY AND CONCLUSION

A study was conducted to determine the inheritance of protein content in segregating populations of grain sorghum grown in 1968 and 1969 using the Udy dye-binding method. One gram each of the ground samples (0.015 mm), 40 ml of reagent dye, and 2 hours of shaking time were used in this method. A total of 33 analyses from F_1 and parental plants and 873 analyses from F_2 segregating populations and parental plants were completed.

The highest percent protein from F_1 and parental plants was 16.71 from B Wheatland x P3237 and 17.07 from P2197, respectively. The ranges of percent protein in F_1 population and parental plants were 9.86 to 16.71 and 10.86 to 17.07, respectively.

The highest percent protein in F_2 population (18.25) and parental plants (15.25) was found in BOKY 25 x P5126 and BOKY 25, respectively. The ranges of percent protein in F_2 population and parental plants were 7.75 to 18.25 and 8.25 to 15.25, respectively. Fairly normal frequency distributions about the mean in the F_2 populations were found in B Redlan x P5185 (Figure 1), B Wheatland x P4503 (Figure 2), B Wheatland x P0355 (Figure 6), BOKY 25 x P5126 (Figure 7), and BOK 8 x P2486 (Figure 8). The bimodal curve suggesting a backcross ratio of 1 to 1 was found in B Wheatland-P3237 x BOKY 29 (Figure 4), and B Wheatland-P3237 x B Wheatland (Figure 3). The bimodal curve in the F_2

distribution patterns of B Wheatland x P3237 (Figure 3) and B Wheatland x P2190 (Figure 5) suggested a single segregating gene pair with a ratio of 3 to 1.

Apparently the inheritance of some sources of protein is controlled by numerous genes while others are controlled by only a single gene pair.

The 96 samples of grain sorghum taken from the fertility trial with various combinations of N, P and K fertilizer, using urea and ammonium nitrate as sources of nitrogen, at the Eastern Oklahoma Pasture Station, Muskogee, 1969, were analyzed for percent protein, using the Udy dye-binding method. The resulting data and the corresponding grain yields were analyzed statistically and the correlation between them was determined.

There were highly significant differences in percent protein and grain yield from the various combinations of N, P and K fertilizer. A significant difference in replications was found in grain yield but not in percent protein. The estimated correlation of percent protein and grain yield was -0.06, indicating no relationship between the two variables.

The highest mean for percent protein, 12.65, was obtained by using ammonium nitrate as the nitrogen source from the analysis 80-60-0. The highest mean for grain yield, 2,001 pounds per acre, was obtained by using urea as a nitrogen source from the analysis 80-60-80.

The 20 samples of grain sorghum taken from the fertility trial of covered and exposed urea and ammonium nitrate, at the Agronomy Research Station, Perkins, Oklahoma, 1969, were analyzed for percent protein using the Udy dye-binding method. The resulting data and the grain

yields were analyzed statistically and the correlation between them was calculated.

There was a highly significant and a significant difference for percent protein for replications and nitrogen sources, respectively. The correlation of percent protein and grain yield was 0.24, indicating a positive but nonsignificant relationship between the two variables.

The highest mean for percent protein, 11.83, was obtained by using ammonium nitrate covered. The highest mean for grain yield, 2,386 pounds per acre, was obtained by using urea exposed 72 hours.

The 21 samples of grain sorghum taken from the fertility trial of 0, 110, 220, and 330 and 0, 150, 300, and 450 pounds per acre of urea and ammonium nitrate, respectively, at the Agronomy Research Station, Perkins, Oklahoma, 1969 were analyzed for percent protein, using the Udy dye-binding method. These data and the grain yields were analyzed statistically and the correlation between them was determined.

There was no significant difference for percent protein or grain yield in rates of nitrogen, nitrogen sources, or their interactions. The correlation of percent protein and grain yield was 0.27, indicating a positive but nonsignificant relationship between the two variables.

It was concluded that moderate application of fertilizer with adequate moisture such as the trial at Muskogee often results in increased yield without a corresponding increase in protein percentage. The tests at Perkins, however, had inadequate moisture which results in a tendency for grain yield and protein percentage to increase together.

A comparison of the Udy dye-binding and the Kjeldahl methods of estimating protein percentage was conducted using B Wheatland, B Redlan,

BOKY 54 and ROKY 62. Three shaking times (1, 2 and 3 hours), two particle sizes (0.024 and 0.015 mm), and three sample weights (0.95, 1.00 and 1.05 g) were used in the estimation of percent protein using the Udy dye-binding method. With the Kjeldahl method two digestion times (90 and 180 minutes), two particle sizes (0.024 and 0.015 mm), and two sample weights (0.95 and 1.05 g) were used. The results were analyzed statistically.

In the Udy dye-binding method, there were highly significant differences for percent protein due to varieties, particle sizes, sample weights, variety x shaking time, particle size x shaking time and particle size x variety. Also, there were significant differences due to shaking time, sample weight x shaking time, sample weight x variety, and sample weight x particle size.

With the Kjeldahl method, there were highly significant differences due to varieties, sample weights, and the interaction of variety and sample weight.

The data indicated that the estimation of protein percentage using the Udy dye-binding method was affected by more factors than the Kjeldahl method. The Udy dye-binding method, however, when used according to the manufacturers recommendations gave results fairly comparable to the Kjeldahl method. Results indicated the possibility of interference of grain pigments with the test dye in the Udy analysis.

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