

MUTATION INDUCTION FOR PROTEIN
IN WHEAT

BY

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CHAPTER I

INTRODUCTION

Approximately one and a half billion people or 35 percent of the world's population rely on wheat as their principal food. Wheat exhibits wide adaptation and excellent productivity in many different environments of the world. It is very desirable as a human food grain, especially at lower economic levels of the population in less developed countries. It is also the main source of protein in these less developed countries. Wheat like other cereal grains is generally low in grain protein content and inadequate for human nutritional needs. Consequently, better nutrition is possible with the improvement of the grain protein percentage of the plant.

Grain protein may be increased by the application of nitrogen fertilizer, by conventional breeding programs, or by induced mutation for higher protein. Since the first two methods have shown limited success, we initiated mutation induction research in an attempt to obtain high protein variants for use in wheat breeding.

The first efforts at inducing mutations to improve plants were not successful because of the lack of understanding of the biological process involved in mutation, and the procedure fell into disfavor. In contrast, in recent years the very rapid increase in the numbers of released varieties and improved characters resulting from induced

mutations shows that induced mutations are now being used successfully in plant breeding programs.

At the present time, the measurement of grain protein percentage is almost all done by two well known methods: the Kjeldahl which is used as a standard procedure, and the Udy dye-binding method which requires shorter time and less expense.

The objectives of this study are to:

- (1) Determine if mutations were produced for grain protein.
- (2) Determine which lines or cultivars among those tested have the highest grain protein percent.
- (3) Determine which lines or cultivars among those tested have the highest grain protein per unit area.
- (4) Determine the relation between the Kjeldahl and the Udy dye-binding methods for measuring grain protein in wheat.

CHAPTER II

LITERATURE REVIEW

Triticum aestivum L. is the second largest United States grain crop and the most widely cultivated of all cereals in the world (19). It is the principal food in most areas of the world. Because of the lack of animal proteins in the human diet of much of the world, the amount of protein in wheat grain is important. Attention has been given to improvement of grain protein in wheat as well as its milling and baking quality.

In the mid 1950's, the first concentrated attempt to improve grain protein in hard red winter wheat was recorded at the Nebraska Agronomy Research Station using the soft wheat variety "Atlas" as a genetic source of high grain protein (16). Various breeding methods have been used in attempts to improve wheat protein. One of these is mutation induction. Although this method offers many possibilities, it also offers difficulties.

Mutation induction began in 1927 when an American geneticist, Muller, announced his discovery that mutation frequency was increased following irradiation of the fruit fly Drosophila melanogaster with X-rays (33). Stadler (33) showed a similar increase in mutation frequency in barley following seed irradiation at about the same time. It was not until about 1950 that a renewed interest in mutation induction was shown. This interest followed the important research on

tion was shown. This interest followed the important research on nuclear energy as mutagenic agents (27). In recent years, mutation induction has received renewed attention due to the success achieved. At least eleven wheat varieties utilizing induced mutations have so far been released (39).

Until fairly recently, X-rays and gamma rays were the principal mutagens employed in mutation induction, but today there is no problem in getting genetic variability by chemical mutagens as well as by radiation. Prakken (27) stated that the most effective chemicals are several derivatives of sulfonic acid, notably ethylmethanesulfonate (EMS). From investigations concerning the effect of mutagens, investigators concluded that ethylmethanesulfonate (EMS) is a very effective agent when compared with other chemicals and with X-rays because it is capable of inducing considerably higher mutation frequencies (34).

Siddiqui and Arain (31) stated that allohexaploidy of bread wheat presents many opportunities for the induction of mutations for useful agronomic traits. In an experiment on wheat, M_6 mutants derived from gamma rays, ethylmethanesulfonate, and combined mutagenic treatment were tested for yield performance. They found that most of the mutant strains were low yielding; however, some were high yielding compared with their respective controls. The result of a study by Khan (17) on improvement of quality and yield of wheat by mutation induction indicated that protein content of wheat can be increased without any reduction in yield.

Favorable results are not always easy to achieve by mutagenic treatment since the desired mutant trait may be governed by a gene

located on the same chromosome as deleterious genes which give rise to undesirable characteristics (33).

Composition of Wheat Protein

Knowledge of the composition of the kernel particularly the different protein fractions seems to be necessary for improving the nutritional value of the wheat. Bland (6) reported that in a well-filled wheat kernel composition of the kernel would be 3% germ, 15% bran, and the remainder, endosperm. The bran layers contain about 19% of the total grain protein, the germ--8%, and the endosperm--70-75% (26).

Osborne (23) classified wheat proteins on the basis of solubility. He concluded that wheat proteins comprise five fractions: the albumin, which is soluble in water and comprises 2.5% of the total protein; the globulin, which is soluble in dilute salt solution and comprises 5% of the total protein; protease which comprises 2.5% of the total protein; the prolamins (gliadin), which is soluble in 70% alcohol; and the glutelin (glutenin), which is soluble in dilute acids and alkalis. The gliadin and glutenin which comprise about 90% of total protein make up the water-insoluble gluten which makes the wheat protein unique and is an important factor in the bread baking quality.

Pence and Elder (24) characterized the albumins as having a tryptophan content higher than that of other wheat proteins and as having a low amide-nitrogen content. The globulin is characterized by both low tryptophan and low amide-nitrogen and relatively high arginine content. The gliadin has a high amide-nitrogen content compared to albumins and globulins.

Vogel et al. (37) in an experiment on USDA world wheat collection

reported that "Atlas 66" was high in grain protein content because of its high endosperm protein content. On the other hand, they indicated that the bran protein contains nearly twice as much lysine as the endosperm protein. They suggested that it should be possible to improve both the protein and lysine content of the endosperm of wheat by breeding.

Relationship Between Udy and Kjeldahl

Udy (36) found that the Udy and Kjeldahl procedures agreed with each other on wheat and wheat flour. Banasik and Gilles (4) also found a positive relationship between the Udy and Kjeldahl methods. They stated that if 100 samples were determined by the Udy Analyzer, 95 of them would be analyzed correctly when compared to the Kjeldahl method.

The results of a study by Greenaway (11) showed that the Udy dye-binding method does differ from the Kjeldahl method for all classes of wheat except hard red spring. The major differences between the dye-binding and the Kjeldahl procedures occurred in wheats of low protein content. The greatest difference noted between the Kjeldahl and dye-binding procedures was 1.6%. Greenaway also noted that protein contents read from the Udy conversion chart may be significantly high at protein levels above 18%. On the other hand, Banasik and Gilles (4) reported that the Udy protein analyzer consistently gave low values in the high protein range when compared with the Kjeldahl method.

Dalaroche and Fowler (8) noted that the Udy-dye test for protein was strongly influenced by daily variation in testing conditions. Differences in dye concentration, particle size of samples, and reaction time with dye are believed to be the causes of this variation.

They also stated that differences between Kjeldahl replicates may be the result of the loss of nitrogen due to fluctuations in digestive temperatures. It is noteworthy that reporting averages of data from replacement of repeat determinations not only reduced error estimates but also more clearly demonstrated systematic errors within the laboratory.

Methods of Protein Determination

In grain marketing at the present time, there is an urgent need for a method of determining wheat protein content which is rapid, easy to perform, and which produces results not significantly different from results by the Kjeldahl method for different classes of wheat (11). The determination of protein content has been made by two well known methods, the Kjeldahl method, which is accepted as the standard procedure, and the dye-binding method, which is faster and less expensive.

McDonald (20) described the Kjeldahl test as a nitrogen test. He stated that amino acids are the building blocks of protein, and they contain carbon, hydrogen, oxygen, sulfur, and nitrogen. Actual determination of the protein content is based on the total amount of nitrogen in the sample. The nitrogen found times a factor of 5.7 for wheat and 6.25 for other grains gives the content of crude protein. Neil (22) also indicated that these factors are based on the percent of nitrogen in the various protein molecules. He stated that this protein test includes the soluble proteins, amino acids, gluten, and all other organic material containing nitrogen and is reported as total protein.

Williams (38) noted that the precision of the Kjeldahl test for measuring wheat protein content is of the order of plus or minus 0.15% of the true result. With a single analysis, there is a possibility that, occasionally, there will be a wide variation from the actual value (20). This method is accepted as the standard procedure but is rather expensive.

The Udy dye-binding method was developed by Udy (36). He found that wheat proteins react with disulfonic 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 an accurate estimate of protein content. In practice, the estimate is based on the concentration of unbound dye, as measured colorimetrically using a light filter (470 mu).

Relationship Between Yield and Its Components

Austenson and Walton (3) stated that the relationship between grain yield and its components can be expressed in the multiple equation: $\text{yield} = C_0 + C_1X_1 + C_2X_2 + C_3X_3$, where C_0, C_1, C_2, C_3 are constants and X_1 is the number of spikes per plant or per unit area, X_2 the number of seed per spike, and X_3 kernel weight. They reported that spike number was the most important component of yield. Hsu and Walton (12) in their study with spring wheat also observed that spike number per plant was the most important component in determining yield per plant. A study by McNeal and Davis (21) also confirmed that yield increases are the result of increasing one of these components. On the other hand, Johnson, Schmidt and Mekasha (15) working on four winter wheat varieties reported that the highest yielding variety produced more

kernels per spike, but its kernel weight and spike number were less than the others.

Sidwell et al. (32) reported that tiller number had a high positive phenotypic correlation and an intermediate genetic correlation with grain yield. Also, they observed a negative association between kernel weight and tiller number and between kernel weight and kernels per spike. The result of a study by Hsu and Walton (12) indicated that spike number and kernels per spike were positively associated with total yield per plant, while kernel weight was not. They also observed a negative correlation between tiller number and 1000-kernel weight and a positive correlation between spike number and number of seeds per spike.

Knott and Talukdar (18) noted that weight per seed was positively correlated with yield and negatively correlated with number of kernels per plot. However, the increase in seed weight had a greater effect on yield than the reduction in seed number. Thirty varieties of wheat were studied by Jaimini, Goyal and Tikka (13), with grain yield and its components being determined. According to this experiment, grain yield was positively and significantly correlated with test weight and number of spikes per plant. The data suggested that number of spikes per plant was primarily responsible for the high yield.

Although kernel weight certainly accounts for a portion of grain yield, Sethi and Singh (29) found a negative genotypic correlation for grain yield with grain weight. They also indicated a positive genotypic correlation for grain yield and plant height, but the path-coefficient analysis revealed that its direct contribution is negative.

On the other hand, Pepe and Heiner (25) reported that plant height did not influence grain yield or protein percentage.

In wheat high grain yield is usually associated with low grain protein. McNeal and Davis (21), Stuber et al. (35), Busch et al. (7), Gill and Brar (10), and Bhatia (5) have reported a negative correlation between grain yield and percent protein. Fonesca and Patterson (9) stated that the tendency for high yielding lines to have a lower grain protein percentage appears to result from a limited or diluted source for protein production. Stuber et al. (35) found that the lateral kernels in a spikelet had a higher protein content than the central kernels, and grain from the middle of the spike was higher in protein content than that from the top of the spike, averaging .60% more in the lower portion. This is in agreement with McNeal and Davis (21) and Ali et al. (1). This suggests that the supply of nitrogen needed for protein production may become limited before the additional kernels produced by high yielding varieties develop.

A significant positive relationship was reported in a hybrid population by Shebeski (30). Johnson et al. (14) also indicated a positive correlation for some winter wheats.

Ries and Euerson (28), working on protein content and kernel size relationships, suggested that the source of wheat seed may affect the crop growth and yield. Within a genotype, seed that has a higher protein content or is larger will sometimes produce higher yield. Stuber et al. (35) suggested that, since grain protein is expressed as a percentage, the protein expression is a ratio of protein to non-protein constituents. Therefore, factors which affect either components will affect the magnitude of the percentage value.

Bhatia (5) reported that grain protein per unit area was positively correlated with grain yield, grain weight, grain number, and grain protein percent. He suggested that the grain protein yield per unit area provides the best criterion for making early generation selection in breeding programs aimed at improving protein productivity per unit area.

CHAPTER III

MATERIALS AND METHODS

This study consisted of thirty-seven lines and three checks (one parent line and two high protein varieties). It was carried out in the 1976-1977 season at the North Central Agronomy Research Station, Lahoma, Oklahoma. The soil was a Pond Creek silt loam. A brief description of the lines is presented below.

The seed of a pure line of wheat (5*KAW//DS28A/PNC) was treated in the fall of 1972-1973 with 0.5% ethylmethanesulfonate solution (EMS), (KH_2PO_4) buffer solution at pH 7, for 24 hours with aeration. The temperature during treatment was 20°C. After treatment, the seed was washed with distilled water and dried to remove excess surface moisture. Treated seed, along with checks, was sown in the field in rows three meters long and thirty centimeters apart as the M_1 generation.

Individual spikes from individual M_1 plants were harvested and threshed and planted as M_2 . The M_2 generation was grown as a single-spike progenies in 1973-1974. In the M_2 generation there were 3,040 rows, each row from one spike selected from M_1 plants. Every M_2 plant was harvested separately. Because of the lack of seed, protein analysis was not conducted on every M_2 plant. However, from every row, which contained approximately 20 M_2 plants, a sample of seed was taken (a few seeds from each plant of that row) and composited. These 20 M_2 plants traced to one M_1 spike. Protein was conducted on the composite

sample from each M_2 plant row. Ten to 20 plants from each high protein row were selected and planted for the next generation.

The M_3 generation was grown as plant rows from putative mutants of the M_2 generation in 1974-1975. Each row traced back to an individual M_2 plant which tested high in protein, so this represented only part of the M_2 population. In this generation, individual rows were harvested and protein was run on a row basis. Ten high protein lines out of each 100 plant rows were selected and planted for the next generation.

The M_4 generation was grown as progeny rows in 1975-1976. Each row traced back to one M_2 plant and one M_3 plant progeny. In this generation individual rows were harvested and protein was run on bulk seed from the row.

Those lines which were high in protein both in the M_3 and M_4 generations were selected for the M_5 generation and were grown in a yield nursery in 1976-1977. Through these procedures, 37 lines were selected and their characters were studied in the M_5 generation. Selection for protein was made in the M_2 , M_3 , and M_4 generations by the Udy dye-binding method.

Design and Field Layout

The experimental design was a randomized complete block design with four blocks, each block containing 40 entries. The plots were three meters long and sixty centimeters wide. The plots were seeded at the rate of 7.5 grams per row.

Characters Investigated

The following characters were observed and measured on all plots:

(a) grain yield, (b) tiller number, (c) plant height, (d) kernels per spike, (e) 1000-kernel weight, and (f) protein content.

Grain Yield

This trait was calculated by harvesting 1.44 square meters from each plot. The yield of the grain was recorded as grams per row.

Tiller Number

This trait was determined by counting the number of spike-bearing tillers in 0.09 square meter of row and recorded as number of tillers per 0.09 square meter. Thus, the number of tillers per 0.09 square meter is equivalent to the number of spikes per 0.09 square meter.

Plant Height

This was measured before harvesting, and corresponded to the distance in centimeters from the soil surface to the tips of spikes, excluding the awn.

Kernels per Spike

The average number of kernels per spike was calculated from randomly selected spikes in 0.09 square meter of row.

1000-Kernel Weight

This was calculated based on the number of kernels taken from randomly selected spikes in 0.09 square meter of row.

Protein Content

Protein content was determined for all material used in this study by both the Udy dye-binding method and the Macro Kjeldahl. Each sample was ground to a particle size of 0.015 mm using a Udy cyclone hammermill equipped with a vacuum collecting device. The ground samples were blended and 1000 mg subsamples were weighed out for protein determination by both the Udy and Kjeldahl methods.

The dye-binding method used in this study was the procedure used by cereal chemists (2) as follows:

1. Dispense 800 mg of well-blended sample into sample bottle and add 40 ml of the standard reagent dye.
2. Place on Eberbach shaker and agitate for 30 minutes. The shaker would hold 44 samples at once and the samples were prepared and placed on the shaker at one minute intervals which permitted reaction of a large number of samples while maintaining the optimum reaction time.
3. Adjust meter needle to zero, then turn on color analyzer to allow maximum time for stabilization.
4. Adjust temperature to 25 ± 1 c by water bath.
5. Fill cuvette with reference dye and adjust meter needle to 12% protein, reading as indicated in Udy wheat conversion table for the particular color analyzer.
6. After shaking, pour the sample solution into the funnel fitted with a fiberglass filter disc and cap. Introduce the filtrate into the cuvette and read percentage of transmission when needle reading is constant.

7. Convert meter reading into percent protein using Udy wheat conversion table for the particular color analyzer.

All samples were run twice for percent protein in this study.

The Kjeldahl method used in this study was the Boric acid modification and was as follows:

1. Add 1 gm ground sample into digestion flask.
2. Add polyethylene packet of catalyst and 2 granules of selenium.
3. Add 25 ml sulfuric acid.
4. Put flasks on to digest for 45 minutes; remove and cool for approximately 20 minutes, but don't allow to crystallize.
5. Add 250-300 ml water to each sample.
6. Add 50 ml of boric acid-methyl red-methylene blue indicator into receiver flasks and put them under receiver tubes.
7. Add 75 ml of sodium Hydroxide (50%) and 2 or 3 pieces of Zinc to each sample.
8. Boil until all ammonia has distilled (at least 150 ml of distillate).
9. Titrate distillate to neutrality, with standard 0.1253 N sulfuric acid, using buret (with automatic zero).
10. Read ml of acid used directly from buret and report as percent protein.

Statistical Analysis

The statistical analyses of variance for the data collected were made by the Statistical Analysis System at the Oklahoma State University Computer Center. Analyses of variance were performed for each character.

LSD was used to compare the means of the lines for each character. To evaluate the possible relationship between two different protein determination methods (Udy and Kjeldahl), a regression coefficient was used.

CHAPTER IV

RESULTS AND DISCUSSION

Grain Yield

The results of the analyses of variance for grain yield and its components are presented in Table I. This table indicates that there were significant differences due to varieties and lines for grain yield at the 0.01 level of probability. The average grain yield of thirty-seven lines and three checks are compared in Table II. Grain yield expressed in grams per row is the average of four blocks. The three highest yielding entries were Lancota, Plainsman V, and 5*KAW//DS28A/PNC. Two of the high yielding entries (Lancota and 5*KAW//DS28A/PNC) tended to be low in protein percentage (Tables VII and VIII). In contrast, Plainsman V, which ranked second in grain yield, had the highest protein percentage. This shows that a wheat line with high grain yield may produce either low or high protein grain. Negative correlations between grain yield and protein were recorded by McNeal and Davis (21). Whereas, positive correlations were recorded by Johnson et al. (14). A comparison between thirty-seven putative mutant lines and their parent (5*KAW//DS28A/PNC) shows that the parent has a higher grain yield than the lines.

TABLE I
 MEAN SQUARES FOR GRAIN YIELD, PROTEIN PERCENTAGE, KERNEL WEIGHT,
 NUMBER OF SEED PER SPIKE, NUMBER OF TILLERS, AND HEIGHT
 FOR FORTY WHEAT VARIETIES AND LINES

Source of Variation	d.f.	Grain Yield	Protein	1000 Kernel Weight	Kernel Per Spike	Number of Tillers	Height
Rep.	3	6691.78	1.27	5.52	15.84	82.69	208.11
Var.	39	8556.87**	2.79**	19.32**	15.84**	93.10*	167.46
Rep. x Var.	117	759.33	0.36	1.75	5.72	59.88	112.27
Corrected Totals	159	2783.86	0.97	6.13	8.39	68.46	127.62
F Value		11.26	7.68	11.01	2.76	1.55	1.44
OSL		0.0001	0.0001	0.0001	0.0001	0.0370	0.0530

*Significant at the 0.05 Level of probability.

**Significant at the 0.01 Level of probability.

TABLE II
 AVERAGE GRAIN YIELD OF FORTY WHEAT VARIETIES AND LINES
 GROWN AT LAHOMA, OKLAHOMA IN 1977

Rank	Variety	Entry Number	Grain Yield (g/Row)
1	LANCOTA	1	373
2	PLAINSMAN V	2	353
3	5*KAW//DS28A/PNC	40	331
4	LA7626621	16	277
5	LA7627414	24	275
6	LA7626305	9	271
7	LA7626333	26	260
8	LA7627313	8	259
9	LA7627274	23	256
10	LA7626450	32	254
11	LA7627378	38	254
12	LA7627472	39	249
13	LA7626301	36	247
14	LA7626535	18	246
15	LA7627230	7	237
16	LA7627043	30	230
17	LA7627229	25	229
18	LA7627554	19	227
19	LA7627418	4	225
20	LA7626451	31	224
21	LA7627407	20	224
22	LA7626459	3	223
23	LA7626793	37	222
24	LA7626458	33	221
25	LA7626313	29	221
26	LA7627355	5	219
27	LA7627310	21	218
28	LA7627273	22	218
29	LA7627312	27	216
30	LA7627231	13	213
31	LA7626691	35	198
32	LA7627558	28	197
33	LA7627390	12	191
34	LA7627610	17	189
35	LA7627357	15	186
36	LA7626419	11	178
37	LA7626310	6	174
38	LA7627064	34	171
39	LA7626476	14	167
40	LA7626993	10	156

LSD .05 = 39

TABLE III

NUMBER OF TILLERS PER 0.09 SQUARE METER FOR FORTY WHEAT
VARIETIES AND LINES GROWN AT
LAHOMA, OKLAHOMA IN 1977.

Rank	Variety	Entry Number	Number of Tillers
1	LA7626418	4	50.0
2	LA7626313	29	49.0
3	LA7627378	38	49.0
4	PLAINSMAN V	2	47.7
5	LA7627230	7	47.2
6	LA7626451	31	47.0
7	LA7626621	16	45.5
8	LA7627274	23	44.7
9	LANCOTA	1	44.2
10	LA7627472	39	43.5
11	5*KAW//DS28A/PNC	40	43.2
12	LA7627554	19	43.2
13	LA7626691	35	41.2
14	LA7627313	8	41.0
15	LA7627558	28	40.7
16	LA7627610	17	40.7
17	LA7627414	24	40.2
18	LA7627355	5	40.0
19	LA7626793	37	39.7
20	LA7627043	30	39.7
21	LA7627229	25	39.0
22	LA7626301	36	38.7
23	LA7626476	14	38.7
24	LA7626419	11	38.5
25	LA7626535	18	38.2
26	LA7627064	34	38.2
27	LA7626333	26	37.5
28	LA7626993	10	37.5
29	LA7626450	32	37.2
30	LA7627312	27	37.2
31	LA7626310	6	37.0
32	LA7626305	9	36.7
33	LA7627273	22	36.7
34	LA7626459	3	36.2
35	LA7627231	13	35.2
36	LA7627310	21	33.7
37	LA7627357	15	33.5
38	LA7627390	12	32.5
39	LA7627407	20	32.0
40	LA7626458	33	31.7

LSD .05 = 10.8

Number of Tillers

The entry effect on the number of tillers was significant at the 0.05 level of probability (Table I). The average number of tillers per 0.09 square meter for thirty-seven lines and three checks are presented in Table III. LA7626418 had the highest number of tillers which was not significantly different from the next nineteen varieties and lines. All seven lines in the low ranking group (Entries 33, 20, 12, 15, 21, 13, and 3) were also low in grain yield, expressing the contribution of tiller number to grain yield. Austenson and Walton (3) and Hsu and Walton (12) reported that tiller number is positively associated with grain yield. However, high tillering ability alone does not ensure high grain yield. LA7626418 ranked first for number of tillers; however, it ranked nineteenth in grain yield. LA7626305 ranked thirty-second for number of tillers; however, it ranked sixth in grain yield.

Height

The entry effect on height was not significant at 0.05 level of probability (Table I). The average height in centimeters for thirty-seven lines and three checks are compared in Table IV. The tallest entry was LA7627472 which was not significantly different from the next thirty-five entries. Plant height did not influence grain yield nor protein content. The average height for all entries was 85.5 centimeters.

Number of Kernels Per Spike

A highly significant difference was found among varieties and lines for number of seeds per spike (Table I). The average number of kernels

TABLE IV
 HEIGHT OF FORTY WHEAT VARIETIES AND LINES
 GROWN AT LAHOMA, OKLAHOMA IN 1977.

Rank	Variety	Entry Number	Average Height (cm)
1	LA7627472	39	96
2	LA7626301	36	95
3	LA7627313	8	94
4	LA7626993	10	93
5	LA7627043	30	93
6	LA7627357	15	93
7	LA7626535	13	93
8	LA7627355	5	93
9	LA7626333	26	93
10	LA7626476	14	93
11	LA7626231	13	92
12	LA7626378	38	92
13	LA7626691	35	92
14	LA7627414	24	92
15	LA7626418	4	91
16	LA7627229	25	91
17	LA7626621	16	91
18	LA7626793	37	91
19	LA7626450	32	90
20	LANCOTA	1	90
21	LA7627554	19	90
22	LA7626313	29	90
23	LA7627312	27	90
24	LA7626305	9	89
25	LA7626458	33	89
26	LA7626459	3	89
27	LA7626451	31	88
28	LA7627230	7	88
29	LA7626419	11	88
30	LA7627273	22	88
31	LA7627310	21	87
32	LA7627407	20	87
33	LA7627274	23	85
34	LA7627558	28	85
35	LA7627064	34	85
36	LA7627390	12	84
37	LA7627610	17	78
38	PLAINSMAN V	2	72
39	5*KAW//DS28A/PNC	40	70
40	LA7626310	6	67

LSD .05 = 15

TABLE V

NUMBER OF KERNELS PER SPIKE FOR FORTY WHEAT VARIETIES AND LINES
GROWN AT LAHOMA, OKLAHOMA IN 1977

Rank	Variety	Entry Number	Number of Kernels per Spike
1	LANCOTA	1	24.8
2	LA7626333	26	22.9
3	LA7627312	27	21.9
4	LA7626793	37	21.0
5	LA7626301	36	21.0
6	LA7627310	21	20.7
7	LA7626450	32	20.7
8	LA7627274	23	20.7
9	LA7626691	35	20.3
10	LA7627390	12	20.3
11	LA7627229	25	20.3
12	LA7627273	22	20.2
13	LA7627472	39	20.2
14	LA7627407	20	20.0
15	LA7627313	8	19.8
16	LA7627357	15	19.7
17	LA7627231	13	19.5
18	LA7626459	3	19.3
19	LA7627378	38	19.2
20	LA7626993	10	19.2
21	5*KAW//DS28A/PNC	40	19.1
22	LA7626458	33	19.0
23	LA7627043	30	18.9
24	LA7627355	5	18.7
25	LA7627414	24	18.5
26	LA7627064	34	18.3
27	LA7626621	16	18.1
28	LA7626310	6	18.1
29	LA7626418	4	18.0
30	PLAINSMAN V	2	17.7
31	LA7626305	9	17.7
32	LA7627230	7	17.0
33	LA7626535	18	16.9
34	LA7627554	19	16.7
35	LA7626451	31	16.7
36	LA7627610	17	16.6
37	LA7626419	11	16.6
38	LA7627558	28	16.2
39	LA7626313	29	16.0
40	LA7626476	14	15.1

LSD 0.5 = 3.4

per spike for thirty-seven lines and checks are presented in Table V. Lancota had the highest number of kernels per spike, which was not significantly different from the next two entries (LA7626333 and LA7627312). Entries with high numbers of kernels per spike tended to produce more grain yield (Tables II and V).

Kernel Weight

The entry effect on kernel weight was significant at the 0.01 level of probability (Table I). The average kernel weights for all entries are compared in Table VI. 5*KAW//DS28A/PNC had the highest kernel weight and LA7626993 had the lowest. Knott and Talukdar (18) reported a positive correlation for grain yield and kernel weight.

Protein Content

Protein percentage showed a mean square of 2.79 which was significant at 0.01 level of probability (Table I). The protein content was determined for all thirty-seven lines and three checks by both the Udy dye-binding and the Kjeldahl methods. The average percent protein for all varieties and lines are presented in Tables VII and VIII. Plainsman V, LA7626476, and LA7627558 were in the highest protein percentage group by both the Udy and Kjeldahl procedures. 5*KAW//DS28A/PNC had the lowest protein percentage. The first determination of protein by the Udy dye-binding method was discarded because it did not show any agreement with the Kjeldahl method, which is used in this study as standard, nor with the second Udy determination.

Figure 1 shows the comparison of percent protein by the Udy

TABLE VI

KERNEL WEIGHT FOR FORTY WHEAT VARIETIES AND LINES
GROWN AT LAHOMA, OKLAHOMA IN 1977

Rank	Variety	Entry Number	Kernel Weight g/1000
1	5*KAW//DS28A/PNC	40	34.6
2	LA7626621	16	33.3
3	LA7626485	33	32.4
4	LA7626313	29	31.7
5	LA7627357	15	31.6
6	LA7626301	36	31.6
7	LA7626535	18	31.4
8	LA7626305	9	31.3
9	LA7626459	3	31.2
10	PLAINSMAN V	2	31.0
11	LA7627310	21	30.9
12	LA7626793	37	30.5
13	LANCOTA	1	30.4
14	LA7627355	5	30.3
15	LA7627231	13	30.2
16	LA7626450	32	30.0
17	LA7627313	8	30.0
18	LA7627043	30	29.7
19	LA7627414	24	29.6
20	LA7627273	22	29.3
21	LA7627312	27	29.3
22	LA7626476	14	28.6
23	LA7627407	20	28.4
24	LA7627274	23	28.3
25	LA7627390	12	28.2
26	LA7627610	17	28.2
27	LA7627558	28	28.0
28	LA7627472	39	27.9
29	LA7626310	6	27.8
20	LA7626451	31	27.7
31	LA7627229	25	27.4
32	LA7627230	7	27.3
33	LA7627378	38	27.1
34	LA7627554	19	26.9
35	LA7626333	26	26.8
36	LA7626419	11	26.8
37	LA7627064	34	26.7
38	LA7626418	4	26.7
39	LA7626691	35	26.1
40	LA7626993	10	24.0

LSD .05 = 1.9

TABLE VII

PERCENT GRAIN PROTEIN FROM FORTY WHEAT VARIETIES AND LINES
GROWN AT LAHOMA, OKLAHOMA IN 1977 (UDY METHOD)

Rank	Variety	Entry Number	Protein Percent
1	LA7627558	28	19.0
2	LA7627355	5	18.5
3	LA7626993	10	18.4
4	LA7627610	17	18.2
5	LA7626476	14	17.9
6	PLAINSMAN V	2	17.9
7	LA7627230	7	17.7
8	LA7627390	12	17.7
9	LA7626310	6	17.6
10	LA7627378	38	17.4
11	LA7626451	31	17.1
12	LA7626535	18	16.8
13	LA7627313	8	16.8
14	LA7627357	15	16.8
15	LA7626450	32	16.7
16	LA7627472	39	16.7
17	LA7627273	22	16.7
18	LA7626419	11	16.6
19	LA7627554	19	16.6
20	LA7627407	20	16.6
21	LA7626459	3	16.5
22	LA7627231	13	16.4
23	LA7626458	33	16.4
24	LA7627229	25	16.3
25	LA7626418	4	16.3
26	LA7626793	37	16.3
27	LA7627064	34	16.1
28	LA7627043	30	15.7
29	LA7627274	23	15.7
20	LA7626621	16	15.7
31	LA7626691	35	15.6
32	LA7627312	27	15.6
33	LA7627414	24	15.5
34	LA7626333	26	15.4
35	LA7626305	9	14.9
36	LA7626313	29	14.9
37	LA7627310	21	14.5
38	LA7626301	36	14.3
39	LANCOTA	1	14.1
40	5*KAW//DS28A/PNC	40	13.8

LSD .05 = 1.1

TABLE VIII

PERCENT GRAIN PROTEIN FROM FORTY WHEAT VARIETIES AND LINES
GROWN AT LAHOMA, OKLAHOMA IN 1977 (KJELDAHL METHOD)

Rank	Variety	Entry Number	Protein Percent
1	PLAINSMAN V	2	18.9
2	LA7626476	14	18.5
3	LA7627558	28	18.2
4	LA7627610	17	18.0
5	LA7627472	39	17.7
6	LA7626451	31	17.6
7	LA7627378	38	17.6
8	LA7626419	11	17.6
9	LA7626310	6	17.4
10	LA7627390	12	17.4
11	LA7627230	7	17.4
12	LA7627355	5	17.4
13	LA7626535	18	17.2
14	LA7627313	8	17.2
15	LA7627064	34	17.1
16	LA7626450	32	17.1
17	LA7626459	3	17.0
18	LA7626458	33	17.0
19	LA7627554	19	17.0
20	LA7627407	20	16.8
21	LA7626793	37	16.8
22	LA7626418	4	16.8
23	LA7627231	13	16.8
24	LA7627312	27	16.7
25	LA7627229	25	16.6
26	LA7627414	24	16.6
27	LA7627357	15	16.5
28	LA7627043	30	16.5
29	LA7626621	16	16.5
30	LA7626993	10	16.5
31	LA7626305	9	16.4
32	LA7627273	22	16.3
33	LA7626333	26	16.1
34	LA7627310	21	16.1
35	LA7626313	29	15.9
36	LA7626691	35	15.9
37	LA7627274	23	15.6
38	LANCOTA	1	15.4
39	LA7626301	36	15.3
40	5*KAW//DS28A/PNC	40	15.2

LSD .05 = 0.8

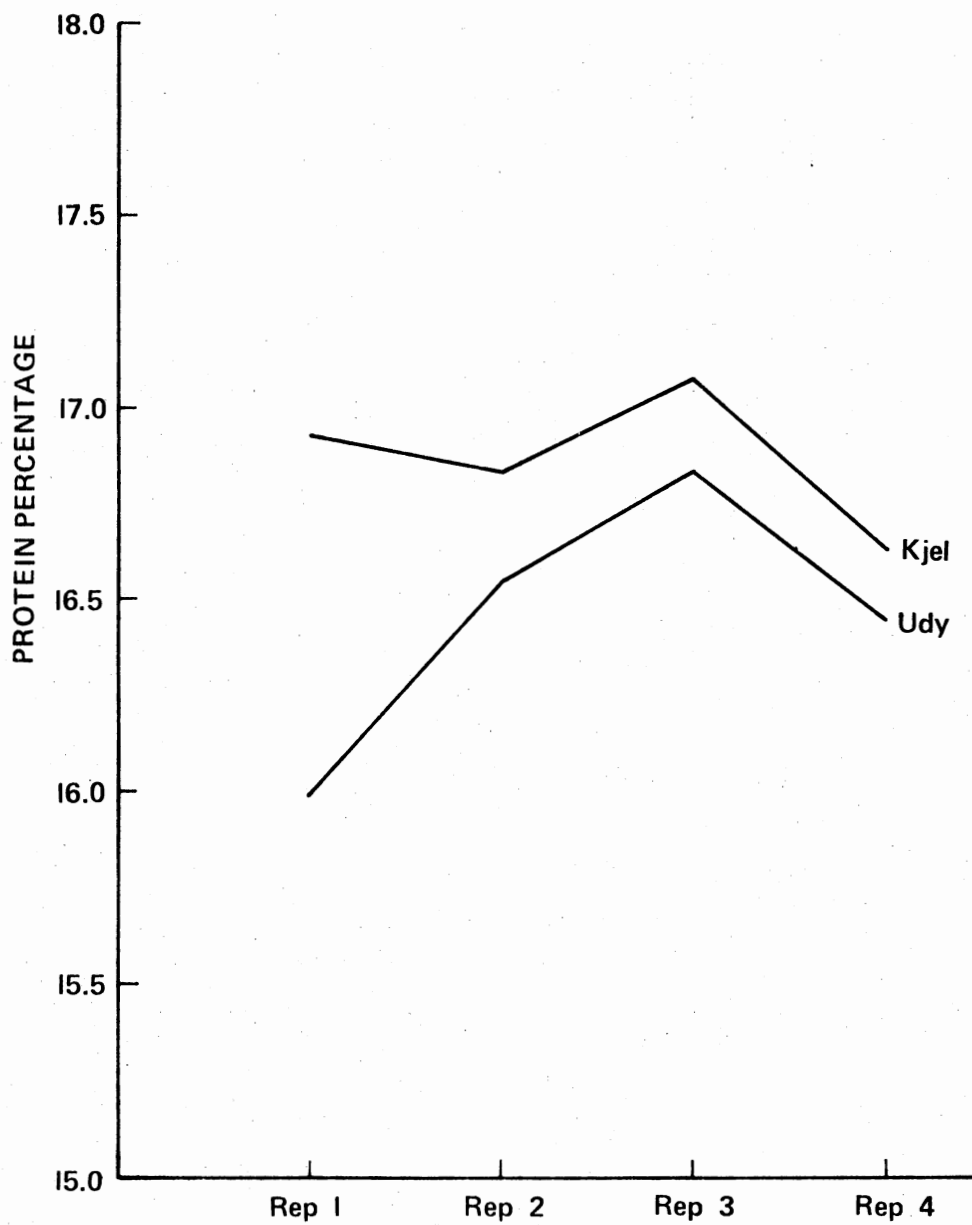


Figure 1. Comparison of Percent Protein by Udy and Kjeldahl Methods on Four Replications

TABLE IX
SOURCES OF ERROR IN UDY METHOD FOR PROTEIN

-
1. Sampling.
 2. Moisture determination.
 3. Moisture loss in grinding.
 4. Uniformity and fineness of grinding.
 5. Mixing of sample prior to weighing.
 6. Calibration and operation of balance, including weighing process.
 7. Calibration and operating of dye dispenser.
 8. Preparation of reagent dye, and inconsistencies between batches of dye concentrate.
 9. Storage and transport of reagent dye.
 10. Shaking time.
 11. Warm-up time of colorimeter.
 12. Standing time between shaking and filtration.
 13. Preparation of filter.
 14. Spillage of dye in colorimeter.
 15. Dirty cuvet.
 16. Type and grade of grain being analyzed.
 17. Recording and documentation.
 18. General carelessness.
-

TABLE X
SOURCES OF ERROR IN THE KJELDAHL METHOD FOR PROTEIN

-
1. Sampling.
 2. Moisture determination.
 3. Moisture loss during grinding.
 4. Uniformity and fineness of grinding.
 5. Storage of sample prior to analysis.
 6. Mixing of sample prior to weighing.
 7. Calibration and operation of balance, including weighing process.
 8. Wet Kjeldahl flasks.
 9. Purity of chemicals (i.e., reagent blanks).
 10. Purity of distilled water, which affects end-points in titration.
 11. Digestion procedure.
 12. Voltage fluctuation, which affects digestion temperature.
 13. Low dilution volume.
 14. Inadequate mixing of standard solutions.
 15. Wrong acid or alkali normality.
 16. Inadequate storage of standard solutions, particularly standard alkali.
 17. Inaccurate preparation of receiving acid solution.
 18. Inaccurate dispensing of receiving acid solution.
 19. Loose stoppers in distillation.
 20. Dirty burets.
 21. Incorrect zeroing of burets.
 22. Color blindness.
 23. Incorrect reading of burets due to parallax.
 24. Recording and documentation.
 25. General carelessness and excessive speed of execution.
 26. Introduction and training of new staff.
 27. Introduction of new equipment.
-

determination and the Kjeldahl methods on all replications. The Udy and Kjeldahl methods were well correlated in Replicates 2, 3, and 4, but the correlation was poor in Replicate 1. There are many factors that can affect protein results. Williams (38) and McDonald (20) classified the sources of error in the testing procedure for protein content. They are presented in Tables IX and X. McDonald (20) concluded that even with the best technicians and equipment, a few results can still vary from the true or correct value. The average protein content by the Udy and the Kjeldahl methods indicated that the Udy and Kjeldahl methods gave almost the same results as far as the ranking is concerned.

To find a good relationship between Udy protein and Kjeldahl protein, regression coefficients were fitted using Kjeldahl protein as the dependent variable. The regression equation for the data was as follows: $\text{Kjeldahl protein} = 18.83 - 0.64U + 0.03U^2$ where U is the Udy protein. By developing the quadratic equation, a curve was obtained which is shown in Figure 2. The lowest and highest protein percentage found in this study by the Udy method was 13.8% and 19%. This Figure indicated that the Udy dye-binding and the Kjeldahl methods do agree with each other for samples below 19% and over 13.8% in protein content.

Both Udy and Kjeldahl showed that 5*KAW//DS28A/PNC was the lowest in protein content in comparison with thirty-seven lines which were derived from it. Putative mutant lines which showed an increase over the parent in protein content were isolated.

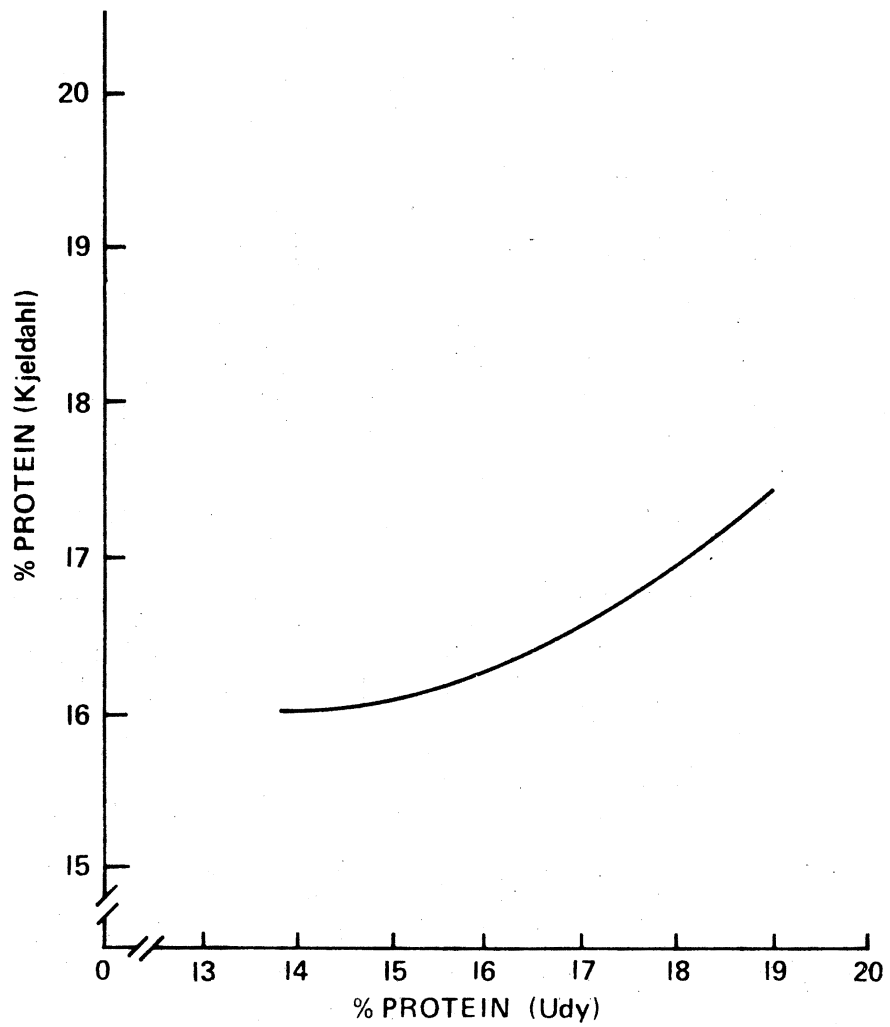


Figure 2. Relation Between Kjeldahl and Udy Methods by Linear Regression

Protein Per Unit Area

Protein per unit area was calculated on the basis of grain protein percent and grain yield. It was calculated as:

$$\text{Protein Yield} = \text{Grain Yield} \times \text{Protein Percent.}$$

The average protein per unit area was recorded as grams per row and compared in Tables XI and XII. Plainsman V had the highest protein per unit area and was significantly different from the next variety (Lancota). Lancota, which was second in protein per unit area, was significantly higher than 5*KAW//DS28A/PNC. 5*KAW//DS28A/PNC ranking third in protein per unit area was not significantly different from the next twelve lines (Table XI). As far as protein per unit area is concerned, both the Udy and Kjeldahl methods gave almost the same results. Table XIII shows the ten highest and ten lowest ranking entries in protein per unit area, using protein percentage which was determined by the two different methods.

5*KAW//DS28A/PNC, the parent of the putative mutant lines produced higher protein per unit area in comparison with the lines. In contrast, the protein percent of the thirty-seven wheat lines ranged from 18.5% to 15.3%, while in the parent it was only 15.2%. On the other hand, grain yield for the mutant lines was consistently lower than the parent causing a lower protein per unit area for the lines.

The yield, protein per unit area, and protein percent of the mutant lines and parent are presented in Table XIV. This shows that there is a possibility that mutations were produced for protein in these lines. However, mutations may have been produced which lowered grain yield and resulted in lower protein per unit area. Sigurbjornsson (33) reported

TABLE XI

PROTEIN PER UNIT AREA FOR FORTY WHEAT VARIETIES AND LINES
GROWN AT LAHOMA, OKLAHOMA IN 1977 USING THE UDY METHOD

Rank	Variety	Entry Number	Protein Per Unit Area
			(g/row)
1	PLAINSMAN V	2	63.2
2	LANCOTA	1	52.6
3	5*KAW//DS28A/PNC	40	45.7
4	LA7627378	38	44.2
5	LA7627313	8	43.5
6	LA7626621	16	43.5
7	LA7627414	24	42.6
8	LA7626450	32	42.4
9	LA7627230	7	41.9
10	LA7627472	39	41.6
11	LA7626535	18	41.3
12	LA7627355	5	40.5
13	LA7626305	9	40.4
14	LA7627274	23	40.2
15	LA7626333	26	40.0
16	LA7626451	31	38.3
17	LA7627554	19	37.7
18	LA7627558	28	37.4
19	LA7627229	25	37.3
20	LA7627407	20	37.2
21	LA7626459	3	36.8
22	LA7626418	4	36.7
23	LA7627273	22	36.4
24	LA7626458	33	36.2
25	LA7626793	37	36.2
26	LA7627043	30	36.1
27	LA7626301	36	35.3
28	LA7627231	13	34.9
29	LA7627610	17	34.4
30	LA7627390	12	33.8
31	LA7627312	27	33.7
32	LA7626313	29	32.9
33	LA7627310	21	31.6
34	LA7627357	15	31.2
35	LA7626691	35	30.9
36	LA7626310	6	30.6
37	LA7626476	14	29.9
38	LA7626419	11	29.5
39	LA7626993	10	28.7
40	LA7627064	34	27.6

LSD .05 = 6.9

TABLE XII

PROTEIN PER UNIT AREA FOR FORTY WHEAT VARIETIES AND LINES GROWN
AT LAHOMA, OKLAHOMA IN 1977 USING THE KJELDAHL METHOD

Rank	Variety	Entry Number	Protein Per Unit Area g/row
1	PLAINSMAN V	2	66.7
2	LANCOTA	1	57.4
3	5*KAW//DS28A/PNC	40	50.3
4	LA7626621	16	45.7
5	LA7627414	24	45.7
6	LA7627378	38	44.7
7	LA7627313	8	44.5
8	LA7626305	9	44.4
9	LA7627472	39	44.1
10	LA7626450	32	43.4
11	LA7626535	18	42.3
12	LA7626333	26	41.9
13	LA7627230	7	41.2
14	LA7627274	23	39.9
15	LA7626451	31	39.4
16	LA7627554	19	38.6
17	LA7627355	5	38.1
18	LA7627229	25	38.0
19	LA7627043	30	38.0
20	LA7626459	3	37.9
21	LA7626418	4	37.8
22	LA7626301	36	37.8
23	LA7627407	20	37.6
24	LA7626458	33	37.6
25	LA7626793	37	37.3
26	LA7627312	27	36.1
27	LA7627558	28	35.9
28	LA7627231	13	35.8
29	LA7627273	22	35.5
30	LA7626313	29	35.1
31	LA7627310	21	35.1
32	LA7627610	17	34.0
33	LA7627390	12	33.2
34	LA7626691	35	31.5
35	LA7626419	11	31.3
36	LA7626476	14	30.9
37	LA7627357	15	30.8
38	LA7626310	6	30.3
39	LA7627064	34	29.2
40	LA7626993	10	25.7

LSD .05 = 7.0

TABLE XIII

COMPARISON OF PROTEIN PER UNIT AREA (G/ROW) FOR TOP
TEN AND LOW TEN VARIETIES AND LINES BY USING
KJELDAHL AND UDY

Rank	Variety	<u>KJEL Protein</u> g/row units	Rank	Variety	<u>Udy Protein</u> g/row units
1	PLAINSMAN V	66.7	1	PLAINSMAN V	63.2
2	LANCOTA	54.4	2	LANCOTA	52.6
3	5*KAW//DS28A/PNC	50.3	3	5*KAW//DS28A/PNC	45.7
4	LA7626621	45.7	6	LA7626621	43.5
5	LA7627414	45.7	7	LA7627414	42.6
6	LA7627378	44.7	4	LA7627378	44.2
7	LA7627313	44.5	5	LA7627313	43.5
8	LA7626305	44.4	12	LA7626305	40.4
9	LA7627472	44.1	10	LA7627472	41.6
10	LA7626450	43.4	8	LA7626450	42.4
30	LA7626313	35.1	32	LA7626313	32.9
31	LA7627310	35.1	33	LA7627310	31.6
32	LA7627610	34.0	29	LA7627610	34.4
33	LA7627390	33.2	30	LA7627390	33.8
34	LA7626691	31.5	35	LA7626691	30.9
35	LA7626419	31.3	38	LA7626419	29.5
36	LA7626476	30.9	37	LA7626476	29.9
37	LA7627357	30.7	34	LA7627357	31.2
38	LA7626310	30.3	36	LA7626310	30.6
39	LA7627064	29.2	40	LA7627064	27.5
40	LA7626993	25.7	39	LA7626993	28.7

TABLE XIV

COMPARISON BETWEEN PARENT AND LINES FOR GRAIN YIELD
 PROTEIN PER UNIT AREA AND PROTEIN PERCENTAGE

Variety	<u>Grain Yield</u> g/row units	<u>Protein</u> <u>Per Unit Area</u> g/row	<u>Protein</u> <u>Percent</u>
5*KAW//DS28A/PNC	331	50.3	15.2
LA7626621	277	45.7	16.5
LA7627414	275	45.7	16.6
LA7626305	271	44.4	16.4
LA7626333	260	41.9	16.1
LA7627313	259	44.5	17.2
LA7627274	256	39.9	15.6
LA7626450	254	43.4	17.1
LA7627378	254	44.7	17.6
LA7627472	249	44.1	17.7
LA7626301	247	37.8	15.3
LA7626535	246	42.3	17.2
LA7627230	237	41.2	17.4
LA7627043	230	38.0	16.5
LA7627229	229	38.0	16.6
LA7627554	227	38.6	17.0
LA7626418	225	37.8	16.8
LA7626451	224	39.4	17.6
LA7627407	224	37.6	16.8
LA7626459	223	37.9	17.0
LA7626793	222	37.3	16.8
LA7626458	221	37.6	17.0
LA7626313	221	35.1	15.9
LA7627355	219	38.1	17.4
LA7627310	218	35.1	16.1
LA7627273	218	35.5	16.3
LA7627312	216	36.1	16.7
LA7627231	213	35.8	16.8
LA7626691	198	31.5	15.9
LA7627558	197	35.9	18.2
LA7627390	191	33.2	17.4
LA7627610	189	34.0	18.0
LA7627357	186	30.8	16.5
LA7626419	178	31.3	17.6
LA7626310	174	30.3	17.4
LA7627064	171	29.2	17.1
LA7626476	167	30.9	18.5
LA7626993	156	25.7	16.5

that other characteristics can be affected in a given mutant variety because the mutant gene which was selected governs characteristics of the plant other than the one sought, or the mutant variety may represent more than one mutation.

CHAPTER V

SUMMARY AND CONCLUSIONS

This study was conducted with a replicated wheat yield nursery grown at the North Central Agronomy Research Station, Lahoma, Oklahoma. The nursery consisted of thirty-seven putative mutant lines and three checks (one parent line and two high protein cultivars). The objectives of this study were to: (1) determine if mutations were produced for protein, (2) determine which lines or cultivars among those tested have the highest protein percent, (3) determine which lines or cultivars among those tested have the highest protein per unit area, (4) determine the relation between the Kjeldahl method and the Udy dye-binding method for measuring protein in wheat. The experiment was carried out during the 1976-1977 growing season. It contained four replications. The plots were three meters long and 0.60 meters wide. The characters investigated were grain yield, tiller number, plant height, kernels per spike, 1000-kernel weight, and protein content. Analyses of variance were calculated for all the traits.

Analyses of variance indicated that there were significant differences due to varieties and lines for all characters investigated except height. Grain yield, protein percentage, kernels weight, and kernels per spike were significantly different at the 0.01 level and tiller number was significant at the 0.05 level of probability. The least significant difference was used for the comparison of the averages.

The three high yielding varieties were Lancota, Plainsman V, and 5*KAW//DS28A/PNC. The variety Lancota and the line 5*KAW//DS28A/PNC tended to be low in protein percentage. Plainsman V, which ranked second in grain yield, had the highest protein percentage. This shows that a variety with high grain yield can also produce high protein grain. A comparison between thirty-seven putative mutant lines and their parent (5*KAW//DS28A/PNC) shows that the parent has a higher grain yield than the lines. The entry effect on the number of tillers was significant at the 0.05 level of probability. LA7626418 had the highest number of tillers which was not significantly different from the next nineteen varieties and lines. All seven lines in the low ranking group (Entries 33, 20, 12, 15, 21, 13, and 3) were also low in grain yield, expressing the contribution of tiller number to grain yield. LA7626305 ranked thirty-second for number of tillers; however, it ranked fifth in grain yield. The tallest entry was LA7627472 which was not significantly different from the next thirty-five entries. Plant height did not influence grain yield nor protein content. Lancota had the highest number of seeds per spike, which was not significantly different from the next two entries. The entries with high numbers of seeds per spike tended to produce more grain yield. 5*KAW//DS28A/PNC had the highest kernel weight and LA7626993 had the lowest. The entries 1, 2, 40, 16, 24, and 9, which were high in grain yield were, respectively, high in kernel weight.

Plainsman V had the highest protein percentage and 5*KAW//DS28A/PNC had the lowest. 5*KAW//DS28A/PNC was the lowest in protein content in comparison with thirty-seven lines which were derived from it. This

shows that putative mutant lines with an increase over the parent in protein content were isolated.

Plainsman V had the highest protein per unit area. 5*KAW//DS28A/PNC, the parent of the putative mutant lines, produced higher protein per unit area in comparison with the lines.

Udy and Kjeldahl, by using regression coefficients, agreed with each other for wheat samples below 19% and over 13.8% protein content.

In conclusion, the performance and protein content of the mutant lines and parent shows that there is a possibility that mutations were produced for protein in these lines. However, mutations may have been produced which lowered grain yield and resulted in lower protein per unit area. Plainsman V had the highest protein percentage which was not significantly different from the next two entries (LA7626476 and LA7627558) by Kjeldahl. It also had the highest protein per unit area.

The Udy dye-binding method is faster, less expensive, and easier than the Kjeldahl method and is suitable to use as a preliminary screening method in a protein improvement breeding program. In this study, it does agree with the Kjeldahl method on wheat samples below 19% and over 13.8% in protein content.

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