GENETIC AND ENVIRONMENTAL FACTORS INFLUENCING PROTEIN CONTENT IN WHEAT

by

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Major Professor

To my wife Delphine

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INTRODUCTION

Increasing yield capacity has been and still is the primary objective task of wheat breeding. However grain protein is an important characterisitc of wheat because of its relation to baking quality and nutrition. The increase of grain protein quantity and quality would hopefully increase the net production of protein and help alleviate the world protein deficit. Grain protein content can be increased through breeding; but breeding for high protein content appears to be difficult because of the interaction with the environment. Use of nitrogen fertilizer can also increase protein content. Mutations have been induced to develop new genetic sources of higher protein content.

To obtain information on the feasibility of increasing protein content of wheat under experimental conditions, the effect of environment, nitrogen fertilization, and genetic factors on grain protein of wheat were studied.

LITERATURE REVIEW

As early as 1908, Snyder found that an increase of nitrogen in the soil gave higher protein content in the wheat kernel, but Leclerc and Leavitt in 1910 pointed out that climate had a much greater influence of protein content of wheat than soil fertility. This was confirmed by Shaw (1913) who claimed that the available nitrogen in the soil had comparatively little if any influence upon the protein content of wheat, and the climate was found to be the most important factor. He also concluded that it was impossible to increase protein of wheat by increasing the available nitrogen of the soil. Davidson and Leclerc (1917) studied the effect of sodium nitrate applied at different stages of growth on the yield, composition and quality of wheat. They concluded that the presence of sodium nitrate in the soil at the time of heading gave a better quality of grain with reference to color and protein content; the vegetative growth was however, not in the least affected. The idea of unimportance of the soil effect was criticized by Neidig and Snyder (1922) who pointed out that both climate and available nitrogen played an important role on the quality and yield of wheat. Swanson (1924) making extensive studies on the effect of different systems of cropping, tillage and fertilization on wheat yield and quality, concluded that the concentration of available nitrogen in the soil solution and the amount of that solution were the two most important factors determining yield and protein content of wheat. He also found varietal differences and pointed out that

moisture is the other most common factor. The importance of moisture was confirmed by Neidig and Snyder (1924) who reported that under field conditions, a high moisture content properly distributed during the growing period, on the average might produce a high yield of wheat with a low protein content. At the same time they reported that a highly fertile soil under the same conditions produced a high yield of wheat and might also show an increase in protein content if sufficient available nitrogen was present during the entire life cycle of the plant. Gericke (1920), through single applications of 100 lbs of nitrogen per acre to spring wheat at different times during the growing period, found a significant relationship between the stage of development of the plant and when the nitrogen fertilizer can be most effectively utilized by the plant in the production of high protein wheat. In his experiments, the highest protein content was obtained by application of nitrogen 72 and 112 days after planting. Mangels (1925) claimed that different previous crops influenced the protein content of wheat. Murphy (1930) confirmed the importance of soil nitrogen supply on wheat protein content. Malloch and Newton (1934), studying the effect of climate on yield and protein content of wheat, found a significant inverse relation between those two traits. Bayfield (1936) confirmed the effect of climate as the prime factor affecting wheat quality and also was able to show that applications of nitrogen at the time of heading had a much greater effect on wheat protein than earlier applications, but had no effect on yield. Waldron, Harris, Estoa, and Sibbitt (1940) studied the effect of seasonal conditions upon the quantity and the quality of protein in eight varieties of wheat grown for four years at four locations and found that the mean protein con-

tent of the wheats varied decidedly from year to year but relatively little from one locality to another. Finney et al. (1957), by foliar spraying Pawnee wheat a varying number of times with concentrations of urea nitrogen at different periods before and after flowering, found significant increases in protein content when spraying occurred at or shortly before flowering time, but the increases were much greater when more than one spraying was made. Experiments conducted by Peterson (1952) on Utah soils that had received no nitrogen showed an increase in yield, protein content, or both when nitrogen fertilizer was applied to winter wheat. It was also reported that test weight was seldom increased by fertilization. Hobbs (1953) confirmed the increase of protein content of winter wheat by spring application of nitrogen fertilizers and reported that there was an increase in yield, especially on the plots that were adequately supplied with phosphate. Coic (1953) in France confirmed the fact that there was a significant relation between the stage of development of wheat and an efficient use of nitrogen fertilizer. Other French workers namely Carles, Soubles and Gadet (1955) stated that the nitrogen supply of the wheat plant was critical at two stages: the first at tillering time, to have enough straw and well developed spikes and the second at the translocation time so that the protein content in the grains might be high. Jagdish (1956) made a comparative study on nitrogen utilization in two high protein and two low protein wheat varieties, found that the difference in protein content between the two kinds of varieties occurred only during the period of kernel formation, and stated that "the differences in protein content of high protein varieties and low protein varieties seemed to be associated with the difference in the rate of protein

formation in the developing kernels." Russell, Smith and Pittman (1956) obtained results that showed high rates of nitrogen fertilizer caused significant increases in protein over the unfertilized plants, but in the same year Rennie (1956) could not find any effect of nitrogen fertilization on protein content of wheat, rather this trait was affected more by soil type and climate. Wahhab and Hussain (1957) in West Pakistan, confirmed the effect of nitrogen fertilization and reported that the late application of nitrogen fertilizer increased the protein content of wheat grain. Fernandez and Laird (1959) in Mexico obtained an increase of protein content of wheat by application of large amount of nitrogen. Fajer (1961) reported significant varietal, nitrogen fertilization, seasonal, interaction year-variety, year-fertilizer effects on the protein content of wheat. He also showed the influence of time of nitrogen application every year, but he did not get any significant effect of the interaction variety by fertilization suggesting that all the varieties had similar response to nitrogen fertilization.

Many workers tried to increase the protein content of crops through induced mutations. Haq et al. (1966) irradiated seeds of eight varieties of rice with gamma rays, found that five of the eight mutants which resulted had higher protein content than their parents in M_4 generation. In 1968, working with 545 mutant lines selected in the M_3 generation after gamma radiation of a rice variety, Tanaga and Tagagi in Japan found that in the M_6 and M_7 generation, some lines were higher in protein percent by 1% or more than the original parents, although some morphological anomalies were observed on the mutant lines. By using EI (ethyleneimine), EMS (ethyl methane sulfonate) and irradiation

to induce mutation on a wheat variety, Dumanovic et al. in Yugoslavia reported in 1969 that some of the treated plants had higher grain protein content than untreated plants and also the EMS treated plants gave the highest protein content.

MATERIALS AND METHODS

Experiment I

In 1972 and 1973, 12 selections and 1 check were seeded at Manhattan and Hutchinson with the recommended fertilizer which was an equivalent of 30 lbs of nitrogen and 40 lbs of P_2O_5 per acre, to study the effect of late nitrogen application and also effect of genotype on protein content of wheat. The 12 genotypes selected were developed to be genetically higher in grain protein than the control (Heyne et al.) and were pure line selections made in the F_6 generation or later. The 12 selections were:

7 selections from the cross Kaw/Atlas 66

- KS 710177
- KS 710156
- KS 71067
- KS 71077
- KS 71090
- KS 71915
- KS 71949

5 selections from the cross Kaw/Atlas 50

- KS 681396
- KS 681402
- KS 681459
- KS 71516
- KS 71714

The check was Kaw CI 12872. Compared to bread wheat, Atlas 50 and Atlas 66 has short mixing time, inferior mixing tolerance, low flour yield, below average test weight, and non-adaptation to Kansas environmental conditions. Kaw had a medium-long mixing time, good mixing tolerance, high test weight, an average flour yield (Lofgren et al.).

A split plot design with four replications and two levels of nitrogen (0 lb/a and 100 lbs/a applied at blooming time) was used at each location. The following measurements were made:

1. Protein percent was obtained using the Kjeldhal method (nitrogen x 5.7). The protein was determined for each replication in 1972, but in 1973 it was determined on the bulk of the four replications.
2. Yield: weight of dry grain expressed as grams per plot.
3. Height: distance (in inches) from ground level to top of the head.

Experiment II

Two selections (KS 71077 from Kaw/Atlas 66 and KS 681402 from Kaw/Atlas 50) and Kaw as check were seeded at ten locations in 1973 and 1974 to study the effect of environment on grain protein in wheat. A randomized block design was used with four replications in each location. Data on yield, test weight, and protein percent were collected. In 1973, one reading was made on each replication. In 1974, one reading was made on the bulk of equal amount from each replication.

Experiment III

Seeds from individual plants of four cultivars Kaw, Parker, Shawnee and Concho*2 Triumph were treated with a 0.40M solution of

EMS (Ethyl Methane Sulfonate). The treated seeds were space-planted and grown to maturity in 1969. The two hundred most vigorous plants were selected from each cultivar and twenty seeds of each plant were spaced planted in 1970 using a randomized block design with two replications, each replication having ten plants. Only good plants were saved to be planted in 1971 in three replications; only two replications were harvested. In 1973, lines selected on the basis of M_1 and M_2 were seeded at Manhattan and Hutchinson in a single eight foot row: eighty eight lines plus some checks were planted. In 1974, essentially all the 1973 lines were reseeded as a single eight-foot row at Manhattan and Hutchinson. In 1975 all the 1974 lines were seeded at the same locations in four-row plots but only the central two rows of each plot were harvested. The following data were collected:

1970: only one reading on protein content based on only one replication

1971: one reading on protein percent obtained from average of two replications

1973: one reading by bulking equal amounts of seeds from Manhattan and Hutchinson

1974: one reading exactly like in 1973

1975: data on height, test weight, yield and protein were collected on each plot at each location.

RESULTS

Experiment I

The data on protein percentage of Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 are given in Tables 1 and 2 and the analysis of variance in Table 3. The analysis of variance showed a highly significant difference among the genotypes. There was a large variation within the Kaw/Atlas 50 and Kaw/Atlas 66 genotypes but no significant variation among the checks. The variance of the checks, which was an estimate of the environmental effect at each location, was 0.047 at Manhattan and 0.037 at Hutchinson, whereas the variance of the 13 genotypes put together was 0.196 at Manhattan and 0.357 at Hutchinson. Therefore with the two locations combined, the variance due to environment represented about 17% of the variation among the genotypes. Also Table 1 showed that on the average, Kaw/Atlas 50 and Kaw/Atlas 66 lines had higher grain protein content than the checks at both locations and both years.

Table 1 showed that in 1972, Kaw/Atlas 50 and Kaw/Atlas 66 lines had higher protein content at Hutchinson than Manhattan whereas Kaw (checks) had more protein at Manhattan than Hutchinson. But in 1973 all the lines had more protein in the grains from Manhattan than from Hutchinson. The analysis of variance (Table 3) confirmed the significant location and location by genotype interaction effect on the protein content.

Table 1. Protein percentage of Kaw, Kaw/Atlas 50, Kaw/Atlas 66 seeded at Manhattan and Hutchinson in 1972 and 1973.

Genotypes	Selection No.	Manhattan 1972	Manhattan 1973	Hutchinson 1972	Hutchinson 1973
Kaw/Atlas 50	KS681396	15.2	13.4	16.2	11.4
"	KS681402	15.4	14.2	15.7	12.9
"	KS681459	14.8	13.6	15.6	13.1
"	KS71516	14.4	12.8	15.2	12.4
"	KS71714	14.9	13.3	15.4	12.7
Kaw[a]	CI12871	14.5	12.3	14.0	11.9
Kaw/Atlas 66	KS710177	15.8	13.7	16.0	13.1
"	KS710156	15.0	13.2	16.3	13.1
"	KS71067	16.6	13.9	16.0	12.8
"	KS71077	14.8	13.6	16.5	13.4
"	KS71090	13.8	12.9	15.3	12.9
"	KS71915	14.1	12.8	15.9	12.9
"	KS71949	15.9	14.2	16.2	13.8

[a]Average of 6 entries

Table 2. Increase of protein percentage in Kaw/Atlas 50[a] and Kaw/Atlas 66[b] genotypes as compared with Kaw at Manhattan and Hutchinson for 1972 and 1973.

Genotypes	Locations	Year	Average	Range
Kaw/Atlas 50	Manhattan	1972	0.5	0.3-1.0
Kaw/Atlas 50	Manhattan	1973	1.2	0.5-1.9
Kaw/Atlas 50	Hutchinson	1972	1.6	1.2-2.1
Kaw/Atlas 50	Hutchinson	1973	0.9	0.5-1.2
Kaw/Atlas 66	Manhattan	1972	1.1	0.3-2.1
Kaw/Atlas 66	Manhattan	1973	1.2	0.5-1.9
Kaw/Atlas 66	Hutchinson	1972	2.0	1.2-2.5
Kaw/Atlas 66	Hutchinson	1973	1.2	0.9-1.9

[a]Average of 5 entries.

[b]Average of 7 entries.

Table 3. Analysis of variance for protein content in Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 at Manhattan and Hutchinson for 1972.

Source	Df	Mean Square	F ratio
Location (L)	1	5.50	19.64**
Replication (R)	6	0.28	0.88
Nitrogen (N)	1	29.64	92.63**
L x N	1	0.96	6.75
R x N	6	0.32	2.29
Genotype (G)	17	8.57	60.41**
L x G	17	2.78	19.58**
N x G	17	0.16	1.09
L x N x G	17	0.13	0.90
Error	204	0.14	

**Significant at 1% level.

The year effect was analyzed by taking the 1973 data (for bulks of four replications) as one replication; thus in the analysis of variance, five replications were considered at each location: replications 1, 2, 3, 4 for 1972 and replication 5 for 1973. By comparing replication 5 against replications 1, 2, 3, 4 at each location, a good estimate of year effect could be obtained; Table 4 showed a highly significant year effect at each location. Also Table 2 gave a good indication of year by location interaction effect on the protein content.

The late application of 100 pounds of nitrogen per acre caused a significant increase of grain protein over the unfertilized plots used as checks in 1972 and 1973; however there was no significant difference in the response to the late nitrogen application as indicated by the nonsignificant fertilizer by genotype interaction at both locations (Tables 5, 6); for example they all gave on the average the same amount of increase in protein content compared with the unfertilized plots. However the increase in grain protein due to nitrogen application was higher in 1973 than in 1972 (Table 7); the average increase was 0.70% in 1972 and 3.30% in 1973. The statistical analysis for year by fertilizer interaction was done by comparing the difference between the two levels of fertilizer in replication 5 against the difference between the two levels of fertilizer in replications 1, 2, 3, 4 (standing for 1972) at each location, in such a way that any significant difference between replication 5 and replications 1, 2, 3, 4 would be a good indication of year by fertilizer interaction; Table 4 indicated that the interaction was significant.

Yields, in grams per plot are given in Table 8 and percent of Kaw, in Table 9. The analysis of variance calculations are given in

Table 4. Analysis of variance on the year effect on protein content using 1973 as Replication 5 and 1972 as Replications 1, 2, 3 and 4.

Source	Df	Mean Square	F ratio
Replication/Loc.	8	7.01	0.50
R_5 vs R_1 R_2 R_3 R_4 at L_1	1	6.64	18.97**
R_5 vs R_1 R_2 R_3 R_4 at L_2	1	47.37	135.34**
Remainder	6	0.35	
Replication x Nitrogen	8	14.00	73.68**
R_5 vs R_1 R_2 R_3 R_4 at L_1	1	49.24	140.69**
R_5 vs R_1 R_2 R_3 R_4 at L_2	1	60.66	173.31**
Remainder	6	0.35	

**Significant at 1% level.

Table 5. Analysis of variance of protein percentage in Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 at Manhattan for 1972.

Source	Degree of freedom	Mean Square	F ratio
Replication	3	0.37	0.37
Nitrogen	1	20.63	40.46**
R x N	3	0.51	
Genotype	17	3.77	23.75*
N x G	17	0.14	0.89
Error	102	0.16	

*Significant at 5% level.

**Significant at 1% level.

LSD 0.05 for fertilizer 0.1314

LSD 0.05 for genotype 0.3942

Table 6. Analysis of variance of protein percentage in Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 at Hutchinson for 1972.

Source	Degree of freedom	Mean Square	F ratio
Replication	3	0.18	1.29
Nitrogen	1	9.98	71.29**
R x N	3	0.14	
Genotype	17	7.58	60.64**
N x G	17	0.14	1.12
Error	102	0.12	

**Significant at 1% level.

LSD 0.05 for fertilizer 0.1167

LSD 0.05 for genotype 0.3502

Table 7. Increase of protein percentage due to nitrogen in Kaw, Kaw/Atlas 50[a] and Kaw/Atlas 66[b] compared with the unfertilized plots at Manhattan and Hutchinson for 1972 and 1973.

Genotypes	Locations	Year	Average	Range
Kaw/Atlas 50	Manhattan	1972	0.7	0.4-1.1
Kaw/Atlas 50	Manhattan	1973	3.1	2.6-3.5
Kaw/Atlas 50	Hutchinson	1972	0.6	0.3-0.9
Kaw/Atlas 50	Hutchinson	1973	3.3	2.6-4.7
Kaw/Atlas 66	Manhattan	1972	0.8	0.6-1.4
Kaw/Atlas 66	Manhattan	1973	3.4	2.6-3.8
Kaw/Atlas 66	Hutchinson	1972	0.6	0.4-0.9
Kaw/Atlas 66	Hutchinson	1973	3.5	2.3-5.0
Kaw[c]	Manhattan	1972	0.7	0.4-1.1
Kaw	Manhattan	1973	3.5	3.3-3.7
Kaw	Hutchinson	1972	0.6	0.2-0.9
Kaw	Hutchinson	1973	3.4	3.0-3.7

[a]Average of 5 entries

[b]Average of 7 entries

[c]Average of 6 entries

Table 8. Yield, in grams per plot, of Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 genotypes seeded at Manhattan and Hutchinson in 1972 and 1973.

Genotypes	Selection No.	Manhattan 1972	Manhattan 1973	Hutchinson 1972	Hutchinson 1973
Kaw/Atlas 50	KS681396	466	346	435	404
"	KS681402	421	279	466	477
"	KS681459	470	392	451	538
"	KS71516	472	353	419	417
"	KS71714	534	304	427	458
Kaw*	CI12875	463	328	441	471
Kaw/Atlas 66	KS71915	554	360	474	543
"	KS71949	472	358	416	489
"	KS71067	432	389	375	463
"	KS71077	404	303	356	517
"	KS71090	482	358	401	436
"	KS710156	414	313	374	503
"	KS710177	490	370	436	506

*Average of 6 entries

Table 9. Yield, in percent of Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 at Manhattan and Hutchinson in 1972 and 1973.

Genotypes	Selection No.	Manhattan 1972	Manhattan 1973	Hutchinson 1972	Hutchinson 1973
Kaw/Atlas 50	KS681396	100	105	99	86
"	KS681402	91	86	106	101
"	KS681459	102	120	102	114
"	KS71516	102	108	95	89
"	KS71714	115	93	97	97
Kaw[a]	CI12871	100	100	100	100
Kaw/Atlas 66	KS71915	120	110	107	115
"	KS71949	102	109	94	104
"	KS71067	93	119	85	98
"	KS71077	87	92	81	110
"	KS71090	104	109	91	93
"	KS710156	89	95	85	107
"	KS710177	106	113	99	107

[a]Average of 6 entries

Table 10 and 11. A highly significant genotype effect on yield in 1972 and 1973 was indicated. At both locations and both years, some of the Kaw/Atlas 50 and Kaw/Atlas 66 lines yielded more than the checks; for example selection KS 71915 and Kaw/Atlas 66 was consistently higher than the checks. Also selection KS 710177 which was among the highest protein content lines in the Kaw/Atlas 66 series seemed to yield more than the checks although it showed a slight decrease at Hutchinson in 1972.

Significant effect of location on yield is shown in Table 8. In 1972, the selections yielded more at Manhattan than Hutchinson on the average. But in 1973, materially higher yields were obtained at Hutchinson than at Manhattan (Table 8). At the same time, there seemed to be, in general, a better performance of the genotypes in 1972 than in 1973.

The analysis of variance indicated that there was no significant effect of late nitrogen application on yield (Tables 10 and 11).

Significant difference in height among the genotypes was indicated in Tables 11, 12, 13. The Kaw checks were more uniform (details of data not given), whereas a wide variation was observed in the Kaw/Atlas 50 and Kaw/Atlas 66 lines probably because of their heterogeneity. On the average the Kaw/Atlas 50 lines were the tallest and the Kaw/Atlas 66 the shortest; however in both years KS 710156 of Kaw/Atlas 66 was the tallest.

The analysis of variance (Tables 13 and 14) showed a significant location effect. On the average plants were taller at Manhattan than at Hutchinson both years. Also the plants seemed to be taller in 1973 than in 1972.

Table 10. Analysis of variance for yield of Kaw, Kaw/Atlas 50, and Kaw/Atlas 66 at Manhattan and Hutchinson in 1972.

Source	Degree of freedom	Mean Square	F ratio
Location	1	117854.56	42.25**
Replication	6	2789.56	
Nitrogen	1	369.02	0.17
L x N	1	1343.43	0.45
R x N	6	2192.62	
Genotype	17	15034.04	4.98**
L x G	17	5173.57	1.71*
N x G	17	2220.97	0.74
L x N x G	17	3772.42	1.25
Error	204	3018.29	

*Significant at the 5% level.

**Significant at the 1% level.

Table 11. Analysis of variance for yield of Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 at Manhattan and Hutchinson in 1973.

Source	Degree of freedom	Mean Square	F ratio
Location	1	1367031.00	50.55**
Replication	6	27043.09	
Nitrogen	1	8756.14	0.32
L x N	1	1300.57	0.36
R x N	6	27600.51	
Genotype	17	12011.97	3.34**
L x G	17	10127.65	2.81**
N x G	17	3473.26	0.96
L x N x G	17	1510.06	0.42
Error	204	3601.17	

**Significant at 1% level.

Table 12. Height in percent of Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 at Manhattan and Hutchinson in 1972 and 1973.

Genotypes	Selection No.	Manhattan 1972	Manhattan 1973	Hutchinson 1972	Hutchinson 1973
Kaw/Atlas 50	KS681396	102	104	113	107
"	KS681402	92	98	113	109
"	KS681459	96	98	97	100
"	KS71516	102	104	108	111
"	KS71714	98	102	113	107
Kaw[a]	CI12871	100	100	100	100
Kaw/Atlas 66	KS71915	98	98	92	98
"	KS71949	98	100	100	100
"	KS71067	94	100	97	98
"	KS71077	98	100	105	100
"	KS71090	90	94	90	98
"	KS710156	102	108	113	111
"	KS710177	92	96	90	95

[a]Average of 6 entries

Table 13. Analysis of variance for height of Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 at Manhattan and Hutchinson in 1972.

Source	Degree of freedom	Mean Square	F ratio
Location	1	4983.36	200.46**
Replication	6	24.86	
Nitrogen	1	11.68	14.97*
L x N	1	5.01	2.03
R x N	6	0.78	0.32
Genotype	17	69.08	27.96**
L x G	17	28.13	11.39**
N x G	17	0.87	0.35
L x N x G	17	1.41	0.57
Error	204	2.47	

*Significant at the 5% level.

**Significant at the 1% level.

Table 14. Analysis of variance for height of Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 at Manhattan and Hutchinson in 1973.

Source	Degree of freedom	Mean Square	F ratio
Location	1	813380096.00	18.27**
Replication	6	44516336.00	
Nitrogen	1	3124374.00	0.56
L x N	1	55538.28	0.02
R x N	6	5553242.00	
Genotype	17	43891248.00	17.43**
L x G	17	7241821.00	2.88**
N x G	17	2713229.00	1.08
L x N x G	17	1217312.00	0.48
Error	204	2517610.00	

**Significant at 1% level.

Significant effect of late application of nitrogen on height was observed in 1972 (Table 13), but no significant effect was found in 1973 (Table 14).

No statistical analysis was conducted for test weight because the replications were bulked in both years and test weight taken on the bulked material. However, Table 15 did indicate that no important effect of late nitrogen application occurred. Also there seemed to be a large difference among the genotypes; for example, Kaw generally was heavier than Kaw/Atlas 50 and Kaw/Atlas 66 in both years. Location and location by year interaction is also observed; for example in 1972 the grains were heavier at Manhattan than at Hutchinson whereas in 1973, the grains were heavier at Hutchinson than at Manhattan.

Correlations were determined first by grouping the genotypes into 3 series: Kaw, Kaw/Atlas 50, and Kaw/Atlas 66. Then the analysis was conducted for each series at different levels of fertilizer and at each location. Table 16 showed that non-significant r values were obtained between protein and yield for each group. However by considering each group at different nitrogen levels at different locations, different levels of significance of the r values were found (Table 17); the general trend was that the r values between yield and protein tended to be less negative when 100 pounds of nitrogen was applied at blooming time.

Also different significance levels of the correlation coefficient r were obtained between protein and height in Table 17.

Experiment II

A significant genotype effect was indicated in Table 17, Kaw/Atlas

Table 15. Test weight, in kilograms per hectoliter, of Kaw, Kaw/Atlas 50, and Kaw/Atlas 66 at Manhattan and Hutchinson in 1972 and 1973.

Genotype	Selection No.	Manhattan				Hutchinson			
		1972		1973		1972		1973	
		N_1[a]	N_2[b]	N_1	N_2	N_1	N_2	N_1	N_2
Kaw/Atlas 50	KS681396	79.7	80.2	79.1	79.6	76.6	76.8	80.2	80.8
"	KS681402	79.1	80.8	78.8	79.1	78.3	77.9	82.4	82.0
"	KS681459	81.3	81.5	81.0	80.5	80.0	79.9	82.7	81.9
"	KS71516	82.4	81.9	80.6	80.8	80.1	80.2	83.2	82.4
"	KS71714	81.3	81.4	80.9	80.4	78.4	78.0	82.2	81.7
Kaw[a]	CI12871	83.1	82.9	81.5	81.4	81.8	81.8	83.9	83.7
Kaw/Atlas 66	KS71915	83.3	82.9	81.5	81.3	80.0	79.6	83.7	83.2
"	KS71949	82.3	82.3	81.5	80.8	80.4	80.4	83.9	82.9
"	KS71067	82.8	82.6	81.1	80.1	79.3	79.5	83.7	82.8
"	KS71077	78.0	78.2	78.7	79.9	73.0	74.2	81.5	81.3
"	KS71090	82.3	81.3	80.5	80.2	79.1	78.8	83.7	83.2
"	KS710156	79.2	80.0	78.9	79.6	74.3	74.7	81.3	80.8
"	KS710177	82.6	81.9	79.9	80.0	79.5	79.3	83.7	83.2

[a]No nitrogen applied at blooming time.

[b]100 pounds of nitrogen per acre applied at blooming time.

Table 16. Pooled correlation coefficients between protein percent and yield, and protein and height for each group of genotypes.

Genotypes	Protein vs Yield	Protein vs Height
Kaw[a]	-0.19	-0.31*
Kaw/Atlas 50[b]	-0.15	+0.18
Kaw/Atlas 66[c]	+0.19	+0.04

*Significant at 5% level.

[a]6 entries

[b]5 entries

[c]7 entries

Table 17. Correlation coefficients between protein and yield, and protein and height for each group of genotypes at each location in 1972.

Genotypes	Locations		Protein vs Yield	Protein vs Height
Kaw	Manhattan	N_1	-0.42*	-0.45*
		N_2	-0.04	-0.25
	Hutchinson	N_1	-0.39	-0.40
		N_2	-0.02	-0.38
Kaw/Atlas 50	Manhattan	N_1	-0.57**	-0.32
		N_2	0.0421	-0.03
	Hutchinson	N_1	0.27	0.11
		N_2	0.30	0.57**
Kaw/Atlas 66	Manhattan	N_1	-0.36	-0.00
		N_2	-0.00	-0.37
	Hutchinson	N_1	-0.26	0.45
		N_2	-0.02	0.38

*Significant at 5% level.

**Significant at 1% level.

50 and Kaw/Atlas 66 having more protein in the grains than Kaw (check) at almost all the 10 locations (Table 18).

A significant difference among the 10 locations studied was observed (Table 19) on the effect of location and year on protein content. Also a significant year by location interaction was obtained by using the same analysis method for bulk data as in experiment I and confirmed the observations in Table 18; for example in 1973 the wheat at Hays contained more protein in the grain than any other location, whereas Colby and Dodge City had the lowest protein content grain that same year. In 1974 Dodge City produced grains with more protein content than any other location, whereas Parsons had the lowest protein content grain.

Tables 20 and 21 showed that there was a large difference among the genotypes and also among the locations for yield. The most interesting result is the consistently higher yield of the Kaw/Atlas 66 over the check at almost all locations except Colby and Tribune in both years. However, no significant difference among the 3 genotypes was observed in the analysis of variance (Table 21).

Experiment III

Four genotypes Kaw, Parker, KS 644 and Shawnee were treated with EMS. The M_1, M_2, M_3 generations were screened for protein content, those with the highest amount of protein were retained. Table 22 showed that some of the EMS treated lines contained consistently more protein compared with the parent materials every year (about 1% or more). However at the same time the mutants were lower in yield (only one years data) than the present materials. Also there was a general

Table 18. Protein content of Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 seeded at ten locations in 1973 and 1974.

Locations	Selections					
	Kaw CI12871		Kaw/Atlas 50 KS681402		Kaw/Atlas 66 KS71077	
	1973	1974	1973	1974	1973	1974
Hays	15.5	12.8	17.2	14.0	15.9	15.4
Belleville	14.9	10.4	15.0	12.7	16.6	13.0
Manhattan	13.6	10.6	14.3	12.8	14.4	12.5
Parsons	12.5	9.7	13.0	11.1	14.4	12.0
Newton	12.8	-	13.0	-	13.8	-
Powhattan	12.3	9.5	12.2	11.8	13.5	11.7
Tribune	11.8	12.6	11.6	14.0	12.7	14.4
Hutchinson	11.1	12.2	12.0	14.5	12.1	14.4
Dodge City	8.8	13.2	8.9	14.9	9.6	14.4
Colby	8.3	11.2	9.2	11.8	9.3	12.8
Average	12.2	11.4	12.6	13.1	13.2	13.4

Table 19. Analysis of variance for protein content in Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 at ten locations for 1973.

Source	Degree of freedom	Mean Square	F ratio
Location	9	67.77	50.57**
Replication	30	1.34	4.12**
Genotype	2	11.29	34.67**
L x G	18	0.84	2.58**
Error	60	0.33	

**Significant at 1% level.

Table 20. Yield in percent of Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 seeded at ten locations in 1973 and 1974.

Locations	Selections			
	Kaw/Atlas 50		Kaw/Atlas 66	
	1973	1974	1973	1974
Hays	83	100	103	111
Belleville	145	83	95	122
Manhattan	94	90	110	106
Parsons	97	139	138	135
Newton	63	-	105	-
Powhattan	146	97	112	121
Tribune	90	88	97	93
Hutchinson	103	121	111	145
Dodge City	115	84	100	100
Colby	89	95	84	95
Average (10 loc.)	100	99	106	112

Table 21. Analysis of variance for yield of Kaw, Kaw/Atlas 50 and Kaw/Atlas 66 at ten locations for 1973.

Source	df	Mean Square	F ratio
Location	9	758679.31	4.66**
Replication/Loc.	30	162877.19	1.22
Genotype	2	171498.94	1.29
L x G	18	375592.88	2.82
Error	60	133285.00	

**Significant at 1%.

Table 22. Protein content, yield, test weight and height of some EMS treated lines grown at Manhattan and Hutchinson in 1971-1975.

Parent	Line	Protein percent				Yield[a] (g)	Test weight[a] (Kg/hl)	Height[a] (cm)
		1971	1973	1974	1975			
KS644	1[c]	14.7	15.5	11.1	12.9	460	81.8	109
	2	16.2	16.6	12.0	14.2	414	81.8	99
Kaw	1[c]	13.2	13.8	10.8	12.5	468	82.6	112
	2	14.2	14.9	11.9	13.2	389	81.5	109
	3	14.9	15.3	12.4	13.0	409	81.5	110
	4	17.0	16.2	13.1	14.2	375	80.9	108
	5	16.0	15.4	12.3	13.3	396	81.7	113
Parker	1[c]	12.3	13.8	11.4	13.1	516	81.5	104
	2	15.2	15.5	14.2	15.1	310	76.9	94
	3	14.2	15.2	13.3	14.1	361	80.2	101
	4	13.6	15.0	13.3	13.7	420	80.0	94
	5	15.8	16.0	12.7	14.2	471	80.1	106
Shawnee	1[c]	12.9	12.9	11.5	12.9	518	79.9	106
	2	14.2	13.6	13.1	14.2	353	76.9	106
	3	14.8	14.5	12.4	14.0	413	76.9	101
	4	14.6	13.8	13.5	14.2	382	78.9	96
	5	15.0	13.9	13.1	14.0	345	75.2	94
	6	15.0	13.8	14.2	14.7	256	74.4	98
	7	15.7	14.0	13.2	14.3	328	71.6	93

[a]1975 data only

[c]Control (untreated)

KS644 = Concho/* 2 Triumph

decrease in test weight of the "high protein" mutants, except the KS 644 mutants.

The correlation analysis (Table 23) showed a highly significant negative correlation between protein and yield except in KS 644, and a highly significant negative correlation between protein and test weight except in KS 644 and Parker series; also highly significant negative r values between protein and height were obtained for all four genotypes. A highly signiticant positive correlation between yield and test weight was obtained, except in the KS 644 series, and also a highly significant positive correlation was found between yield and height, except for Shawnee.

Table 23. Correlation coefficients among protein percent, yield, test weight (T.W.), and height for the EMS lines for 1975.

	Kaw	KS644	Parker	Shawnee
Protein vs yield	-0.61**	-0.17	-0.57**	-0.64**
Protein vs T.W.	-0.57**	-0.10	-0.26	-0.44**
Protein vs height	-0.80**	-0.28*	-0.72**	-0.36**
Yield vs T.W.	0.63**	0.11	0.72**	0.69**
Yield vs height	0.57**	0.44**	0.45**	0.30
T.W. vs height	0.62**	-0.07	0.23	-0.005

*Significant at 5% level.

**Significant at 1% level.

DISCUSSION

The results indicate that the genetic control of grain protein in wheat is stronger than environmental effects and can be expected under a range of environmental conditions. The increase of protein content by breeding can be achieved without necessarily any decrease in yield. Furthermore, the results suggest that some of the high protein lines also may yield more than the checks under different environments.

The strong influence of year and location on grain protein in wheat is in agreement with the results obtained by previous workers. No satisfactory explanation for the differences among the locations and for the significant year by location interaction could be made by correlating the protein percentage with the temperature and rainfall alone, suggesting that other factors, such as soil properties, probably are involved. Bayfield (1936) found that protein content in wheat increased as soil became heavier in texture and also as the relative fertility of the soil increased.

An increase in grain protein in wheat by late nitrogen application can be obtained without any major change in vegetative growth and yield. The nitrogen applied at or near blooming time was utilized in kernel development. This is in agreement with the results obtained by Bayfield (1936). No selection for response to nitrogen fertilizer is possible since all the lines gave the same responses. The difference in protein increase due to fertilizer among the two years probably are attributable to timely rains in 1973 and the greater uptake of nitrogen

by the wheat plant. Fajer (1961) also found a significant year by nitrogen fertilizer interaction.

Protein increase also was obtained by induced mutation, but the increase seemed, in most cases, to be associated with lighter grains or shorter culms. The decrease in carbohydrate accumulation in the grains due to the mutation can explain in part the low yield of the mutants in 1975. However, that some lines do not show any significant correlation between protein and test weight and also protein and yield, indicate that there might not be any linkage between gene(s) which affect those traits and those controlling protein content; therefore the change in yield, test weight, and height can be attributed to different and independent groups of genes that mutated simultaneously after the application of the EMS. However, that is only an assumption and only the use of the mutants in crosses could verify whether there are mutant lines that have genes for higher protein content.

There seemed to be a genotype by environment interaction effect on the relationship between protein and yield, suggesting that with certain genotypes under certain environments, the negative correlation between protein and yield can be broken. Also there is a different relationship between protein and height under different environments; for example under certain conditions high grain protein might be incompatible with reduced plant height (McNeal et al. 1952), while under other conditions there might be some association of shortness with high grain protein (Johnson et al. 1973). That relationship depends also upon the genotype. It seems therefore that the relationship of grain protein with other agronomic traits in wheat should be studied for each genotype grown under different environments.

CONCLUSIONS

I. If enough nitrogen is applied to wheat during the flowering stage, the protein level of the wheat grain can be increased without any major change in yield, test weight and culm length.

II. Protein increase can also be obtained by genetical means: breeding and induced mutation.

III. There seems to be a genotype by environment interaction effect on the relationship between protein percentage, yield, test weight and height of wheat, suggesting that under certain conditions selection for high protein content can be made without affecting yield.

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GENETIC AND ENVIRONMENTAL FACTORS INFLUENCING PROTEIN CONTENT IN WHEAT

by

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Three individual experiments were conducted to study the effect of genetic and environmental factors on the grain protein content of wheat. Experiment I was conducted at Manhattan and Hutchinson in 1972 and 1973 to study the effect of applying nitrogen (100 pounds per acre) at blooming time, and the effect of genotype on grain protein content of wheat. Thirteen genotypes were used: five from the cross Kaw/Atlas 50, seven from the cross Kaw/Atlas 66 and Kaw used as a control. The Kaw/Atlas 50 and Kaw/Atlas 66 genotypes were previously found to be genetically higher in grain protein than Kaw. Experiment II was conducted at ten locations in 1973 and 1974 in order to test two of the twelve high protein content selections (KS681402 from Kaw/Atlas 50 and KS71077 from Kaw/Atlas 66) not only as far as the protein content of the grain was concerned, but also to test other agronomic traits under a wide range of environments. Experiment III was conducted to determine whether new genotypes for higher protein content could be obtained by mutation. Ethyl Methyl Sulfonate (EMS) (0.40M) was used to treat seeds of four cultivars (Concho/*2Triumph, Kaw, Parker, Shawnee). Protein content was determined on M_2 plants and M_3 - M_5 generation lines. Data on yield, test weight and height were taken on the M_5 lines.

The results indicated that Kaw/Atlas 50 and Kaw/Atlas 66 genotypes contained consistently more protein than the control without necessarily any reduction in yield. There was a significant increase in grain protein by late application of 100 pounds of nitrogen over untreated plots. The increase due to nitrogen application was higher in 1973 than in 1972, probably because the timely rains facilitated more nitrogen uptake in 1973. Significant and consistent grain protein increase (1% or more) was obtained by induced mutation although the 1975 data indicated that the EMS treated

lines may yield less than the parent lines.

The significant effect of location, year, and year by location interaction confirmed the strong influence of environment on grain protein content in wheat.

Correlation data indicated that the relationship among grain protein and yield, test weight, and height can be influenced by both the genotype and environment.