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EFFECT OF PROTEIN CONTENT IN WINTER WHEATS ON LEVELS OF ESSENTIAL AMINO ACIDS

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The importance of wheat as a human food in the world is equal to that of rice. Countless millions of people rely upon wheat for their daily sustenance. The protein of wheat possesses unique physical properties that permit the use of leavening agents to produce palatable and highly desirable bread foods.

Wheat, like all of the other major cereal food crops (rice, corn, sorghum), lacks the amino acid balance necessary for maximum utilization of its protein for tissue synthesis and body growth. It is most deficient in the amino acids lysine, methionine, and threonine probably in that order.

Improvement of wheat nutritionally can be accomplished in different ways. The first and obvious approach is to increase its protein content. If the increase is not associated with a less desirable ratio or balance of the essential amino acids, the higher protein content would have the effect of providing more of these essential amino acids per unit quantity of wheat consumed. A second approach would be to improve the ratio of essential amino acids in the wheat protein. For example, more lysine in wheat protein would permit the protein to be more fully utilized to satisfy nutritional requirements. Lysine and other limiting essential amino acids can be added directly to the processed wheat. This approach encounters serious difficulties of implementation in countries where there is greatest reliance on wheat as the main food staple.

Quality of protein in wheat from a nutritional viewpoint has received little serious attention until recent years. The pressure of rapidly increasing world population and widespread food shortages in many countries has provided the impetus for much accelerated research into the nutritional quality of wheat and other cereals. The recent discovery by Nelson and Mertz of Purdue University of a gene in corn that significantly increases the lysine content of corn protein has stimulated the search for genes with similar effect in the other cereal crops.

The Agricultural Research Service and the Nebraska Agricultural Experiment Station have engaged in cooperative research on wheat protein since 1955. Initially, the research centered around the establishment of the effectiveness of genes for higher protein in wheat and the transfer of these genes into winter bread wheats. Subsequent research has been concerned with physiological aspects of the high protein phenomenon in wheat and the study of amino acid composition of high protein lines.

We are currently engaged in the systematic screening of some 17,000 wheats in the World Wheat Collection for significant and useful differences in protein and lysine content.

Quantity of protein. When Atlas 66 was used as a source of high protein in crosses with the hard winter wheat Comanche, the F_2 population gave a near-normal frequency distribution (Figure 1). The parental varieties were widely different in protein content of their grain and the F_2 mean was intermediate to the parents.

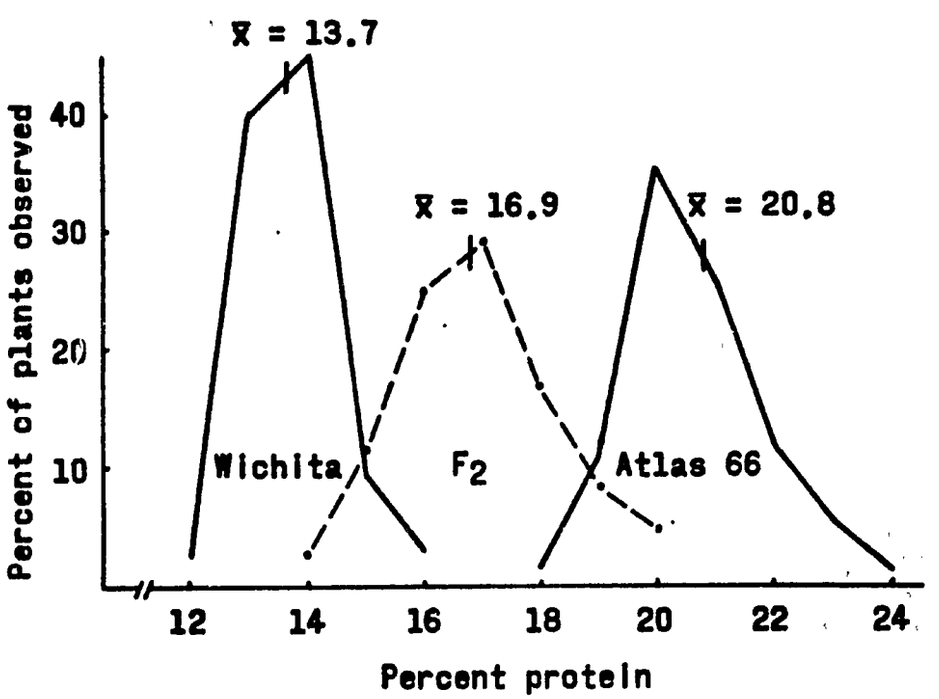

Figure 1. Grain protein frequency distribution of plants of Wichita and Atlas 66 and their F_2 generation grown at Lincoln, Nebraska, in 1957.

Atlas 66 possesses adult resistance to leaf rust. Its gene for resistance is linked with a gene for high protein. Resistant segregates averaged 17.0 percent protein whereas susceptible segregates averaged only 13.5 percent protein (Figure 2). There also appears to be some linkage of high protein with soft grain texture and low adult resistance or tolerance to stem rust.

A number of advanced selections from crosses of Atlas 66 with Wichita and Comanche were evaluated in 1965 (Table 1). Atlas 66 was 4 percent higher in protein than Comanche and Wichita. Progeny lines ranged from 15.3 to 18.7 percent protein with a mean of 16.9 percent.

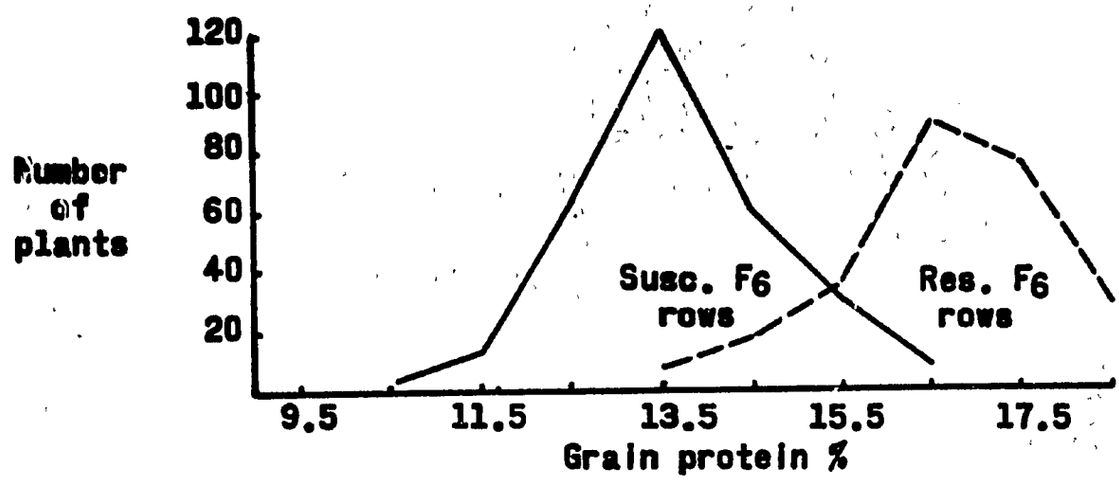

Figure 2. Grain protein frequency distribution of F_8 rows of Atlas 66 X Wichita homozygous for reaction of leaf rust.

Table 1.--Grain protein content of parent varieties and high protein progeny lines grown at Lincoln, Nebraska, in 1965

Item	Protein, %
Atlas 66	18.3
Wichita	14.0
Comanche	14.3
Progeny lines (range)	15.3-18.7
Progeny lines (mean)	16.9

in 1966 Comanche was higher in protein than in 1965, but it was 3.3 percent lower than Atlas 66 (Table 2). High protein progeny lines ranged from 16.2 to 18.3 percent protein in their grain with a mean of 17.4 percent.

Table 2.--Grain protein content of parent varieties and high protein progeny lines grown at Lincoln, Nebraska, in 1966

Item	Protein, %
Atlas 66	18.3
Wichita	14.1
Comanche	15.0
Progeny lines (range)	16.2-18.3
Progeny lines (mean)	17.4

The percentage increase in protein among the high protein lines over the Comanche and Wichita parent varieties in 1965 and 1966 is shown in Table 3. As much as 30.7 percent increase (Atlas 66 X Comanche) was recorded in 1965 and 26.5 percent (Atlas 66 X Wichita) in 1966.

Table 3.--Percentage increase in the protein content of high protein progeny lines over their low protein parent

Progeny lines	1965	1966
Atlas 66 X Comanche lines	6.7-30.7	7.6-21.7
Atlas 66 X Wichita lines	12.1-19.9	22.5-26.5

High protein wheat would have little practical value if it was less productive than other wheat. Our results have indicated that higher protein levels can be achieved by breeding without sacrificing yield. Note in Table 4 that the high protein lines 60306 and 60305 were intermediate in yield to the varieties Scout and Warrior and their grain possessed as much as 3 percent more protein.

Table 4.--Average heading date, grain yield, and grain protein content of Scout, Warrior, and the high protein selections 60305 and 60306 at Lincoln, Nebraska, 1962-1964

Variety	Date headed, May	Grain yield, bu./acre	Protein content,[1] %
Scout	19	41	13.6
60306	19	38	16.1
60305	22	36	16.5
Warrior	22	31	13.4

[1] Calculated at 14 percent moisture.

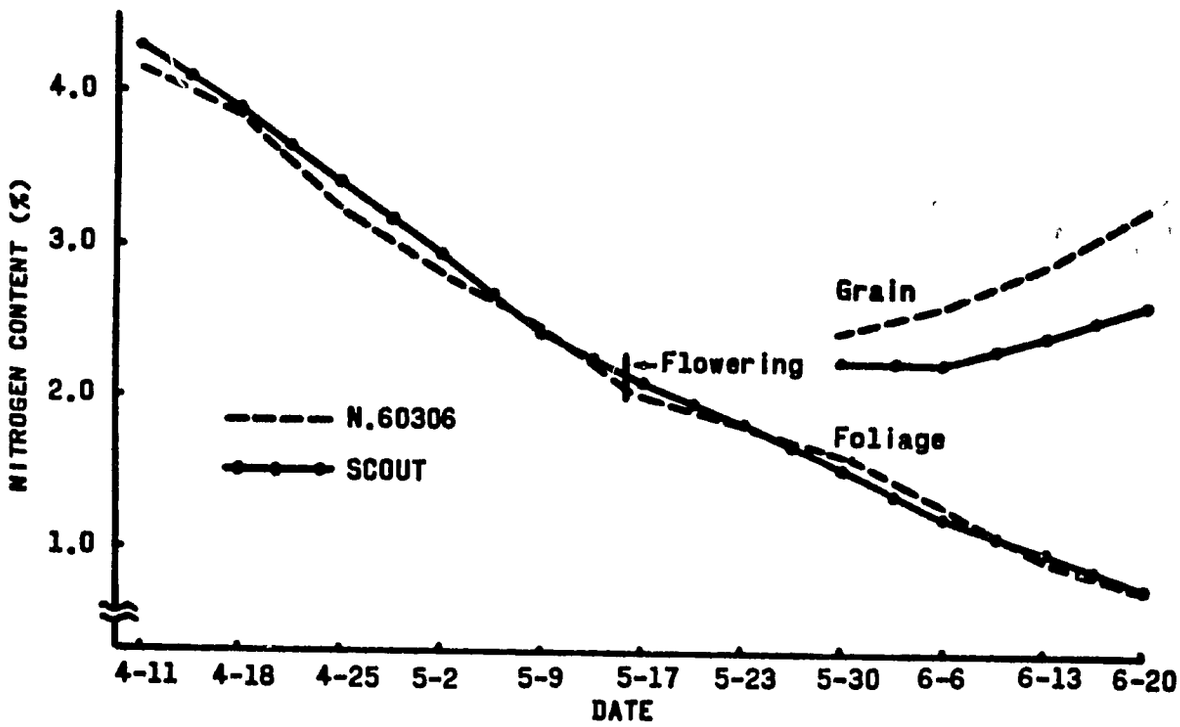

Figure 3. Nitrogen content of the foliage and grain of two winter wheat varieties at Lincoln, Nebraska, 1963.

Physiologic basis for high protein. In order to learn something of the physiologic basis for the high protein phenomenon in wheat, the nitrogen content of the plants and grain of high and low protein varieties was assayed at weekly intervals. As soon after anthesis as the developing grain could be separated from other plant parts, it was analyzed separately.

Nitrogen relations in the high protein selection 60306 are compared with the variety Scout in Figure 3. At no time during the spring growing season were there significant differences in the plant nitrogen content of the two varieties. Yet, from the early stages of kernel development, the grain of 60206 had a higher nitrogen content than the grain of Scout. Nitrogen increased in the grain of both varieties as kernel development progressed, but the rate of increase was more rapid in 60306.

Warrior characteristically produces moderately low protein grain. Yet, when compared with high protein selection 60305, the plants of Warrior contained significantly more nitrogen than 60305 at all sampling dates throughout the spring (Figure 4). Selection 60305, with a much lower plant nitrogen content, produced grain with significantly higher nitrogen content at all stages of kernel development.

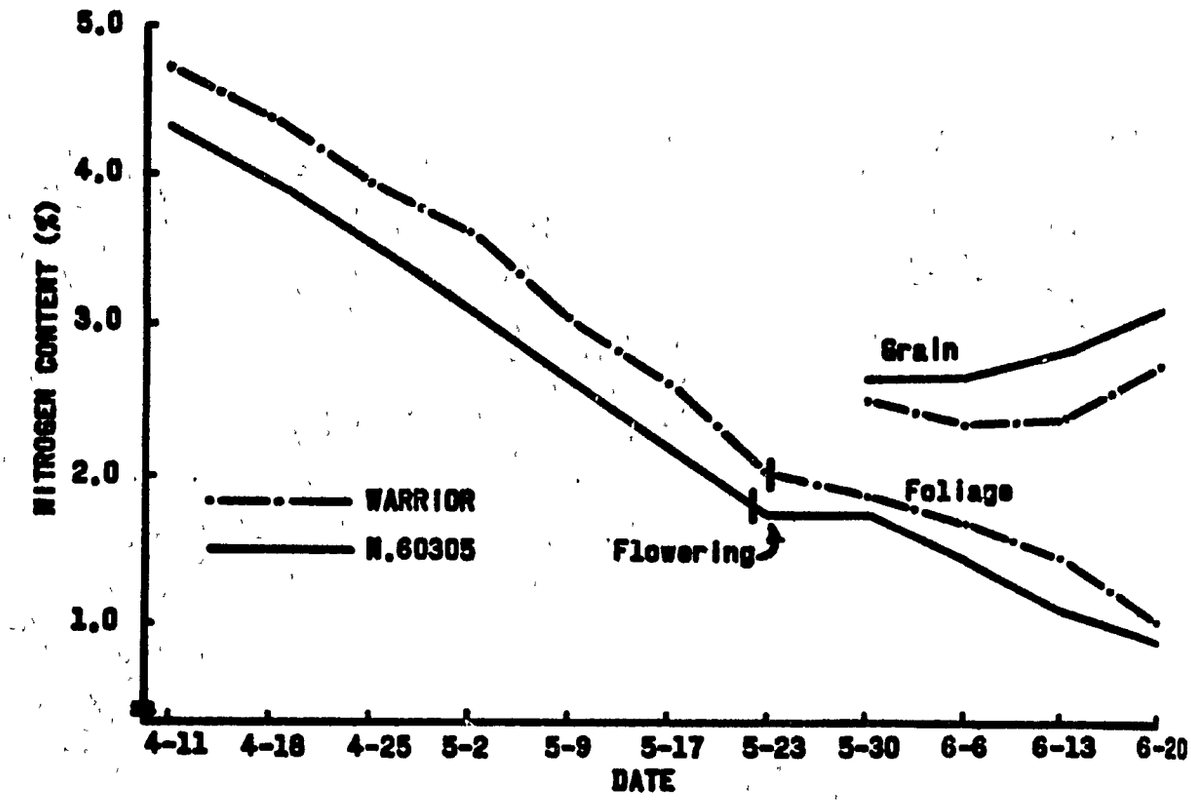

Figure 4. Nitrogen content of the foliage and grain of two winter wheat varieties at Lincoln, Nebraska, 1963.

Our data suggest that the high protein genetic trait in wheat is not associated with differential nitrogen uptake or nitrogen accumulation in the plant. Varieties may differ in their plant nitrogen content but such differences appear to be unrelated to inherent grain protein differences. More complete and efficient translocation of nitrogen from the plant to its grain seems to be the physiologic basis of high grain protein.

Nutritional quality of the protein. The balance of essential amino acids determines the nutritional value of protein since the protein is utilized in relation to the level of its first limiting essential amino acid. If high protein wheats possessed a less favorable balance of the essential amino acids, their nutritional value would be decreased. Lysine, methionine, and threonine are particularly critical since they are the most deficient in wheat. The levels of these amino acids in selected high protein lines of Atlas 66 X Comanche are compared with the parent varieties in Table 5. Atlas 66 and Comanche are similar in lysine and threonine but Atlas 66 is substantially the lowest in methionine. All of the experimental lines are comparable to the parent varieties in lysine. Line 2507 is considerably higher in lysine than either parent. Most lines are as high in methionine as Comanche. Three lines are lower in threonine than either parent variety. Line 2509 exhibits the best balance of all three amino acids.

Table 5.--Lysine, methionine, and threonine levels in selected high protein lines of Atlas 66 X Comanche

Variety	Protein, % DWB[1]	Lysine, % protein	Methionine, % protein	Threonine, % protein
Comanche	15.0	3.23	1.67	3.54
Atlas 66	18.0	3.33	1.11	3.35
Atlas 66 X Comanche 2507	17.7	3.72	1.74	2.62
Atlas 66 X Comanche 2509	18.3	3.45	1.83	3.32
Atlas 66 X Comanche 2504	17.9	3.38	1.14	3.69
Atlas 66 X Comanche 2510	16.5	3.37	1.67	3.22
Atlas 66 X Comanche 2499	18.2	3.29	1.68	3.10
Atlas 66 X Comanche 2500	18.3	3.20	1.65	3.16

1/Percent of total dry weight.

Among 17 high-protein Atlas 66 X Comanche and Atlas 66 X Wichita lines studied, the range in lysine (expressed as percent of protein) was from 6.5 less to 13.0 percent more than the low-protein parents Comanche and Wichita (Table 6.) For methionine the range was from -31.7 to +13.8 percent and for threonine it was from -26.0 to +4.2 percent. Lysine content among 17 lines averaged 5.0 percent more than the low protein parent but methionine averaged 0.8 percent less and threonine 9.1 percent less than the low protein parent.

Table 6.--Percent increase in amino acid content (g./100 g. protein) of 17 high-protein lines over their low-protein parent

Amino acid	Range	$\bar{x}$
Lysine	-6.5 to 13.0	+5.0
Methionine	-31.7 to 13.8	-0.8
Threonine	-26.0 to 4.2	-9.1

To adequately assess the contribution of higher protein to the nutritional value of wheat, it is necessary to measure amino acid levels on a grain weight basis. Values shown in Table 7 were calculated on a grain weight basis. The 17 high-protein lines averaged 22.3 percent more lysine, 15.7 percent more methionine, and 7.7 percent more threonine than contained in the low-protein hard wheat parents.

Table 7.--Percent increase in amino acid content per unit weight of grain among 17 high-protein lines over their low-protein parent

Amino acid	Range	x
Lysine	8.0 to 34.7	22.3
Methionine	-21.0 to 33.1	15.7
Threonine	-3.9 to 24.2	7.7

All essential amino acids except tryptophan were analyzed in 17 high-protein lines. The amount of each amino acid in relation to Comanche is shown for Selection 2509 in Table 8. Note that Selection 2509 contained 22 percent more protein than Comanche. It was equal to or better than Comanche in all essential amino acids except threonine (9 percent less) and phenylalinine (2 percent less).

Table 8.--Protein and essential amino acid (g./100 g. protein) in Atlas 66 X Comanche Selection 2509

Percent higher or lower than Comanche in:	
Total protein	+22
Lysine	+7
Methionine	+10
Threonine	-9
Histidine	+10
Valine	+2
Isoleucine	0
Leucine	+2
Phenylalinine	-2
Tryptophan	Not analyzed

Similar information for Selection 2507 is shown in Table 9. Although it is only 14 percent higher in protein than Comanche, Selection 2507 exhibits considerably poorer amino acid balance than Selection 2509. It has 26 percent less threonine than Comanche and 5 percent less phenylalinine. The apparent deficiency of threonine is particularly important since it is the third most limiting essential amino acid in wheat. The lysine content of 2507 was 15 percent higher than Comanche.

It seems clear from our data that high protein in wheat need not be associated with an altered, less favorable amino acid balance. In our high protein Atlas 66-derived lines there has been no nutritional penalty in lysine and methionine and only a small one in threonine. Our data suggest the possibility of effective selection for improved amino acid balance in our high protein materials.

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Table 9.—Protein and essential amino acid (g./100 g. protein) in Atlas 66 X Comanche Selection 2507

Percent higher or lower than Comanche in:	
Total protein	+14
Lysine	+15
Methionine	+4
Threonine	-26
Histidine	+6
Valine	+3
Isoleucine	+3
Leucine	0
Phenylalinine	-5
Tryptophan	Not analyzed

The Nebraska Experiment Station in cooperation with the Agricultural Research Service is systematically analyzing all of the common wheats in the World Collection maintained by the U.S. Department of Agriculture to identify new sources of high protein and high lysine. The research is being supported by the Agency for International Development, U.S. State Department. More than 2,000 wheats from the Collection have been analyzed to date.

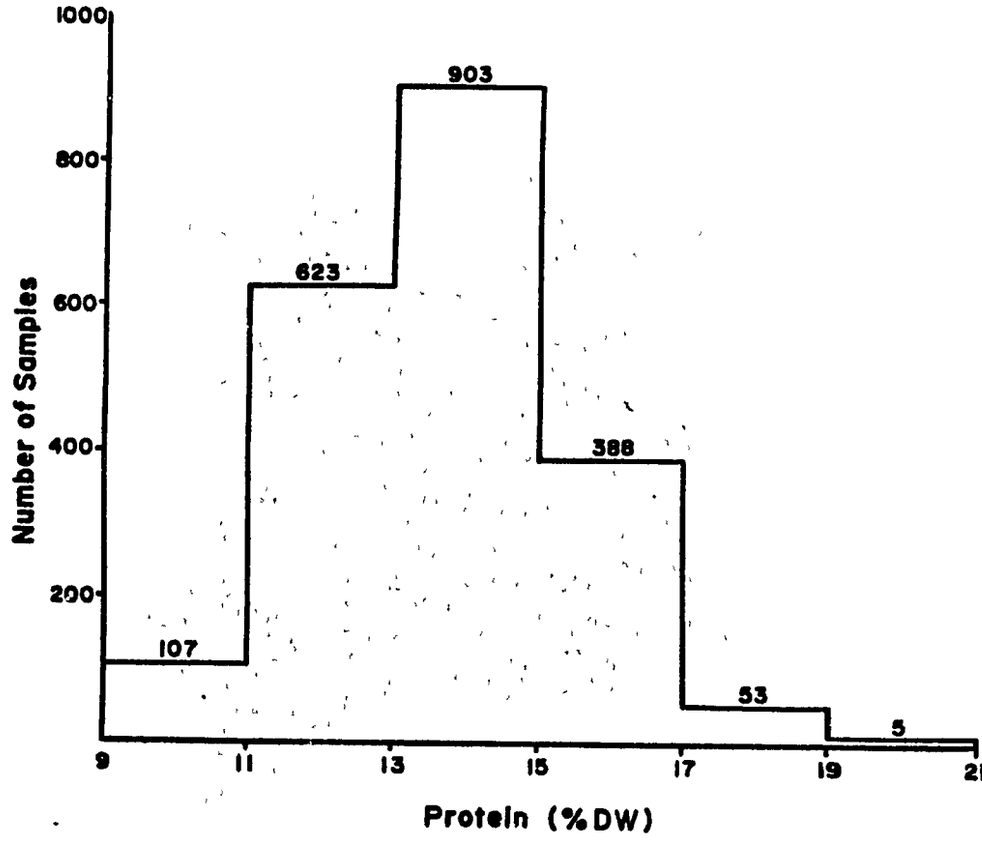

Figure 5. Protein (percent of dry weight) frequency distribution for 2079 wheats from the World Collection.

The protein frequency distribution for 2,079 wheats, is shown in Figure 5. The largest number of samples fell in the 13-to 15-percent protein class. Only 58 had a protein content of 17 percent or more while 107 were below 11 percent in protein.

The frequency distribution for lysine expressed as percent of protein appears in Figure 6. More than one-half of the samples possessed lysine in the range of 2.9 to 3.2 percent. Forty-one samples exceeded 3.5 percent and only six were less than 2.3 percent in lysine.

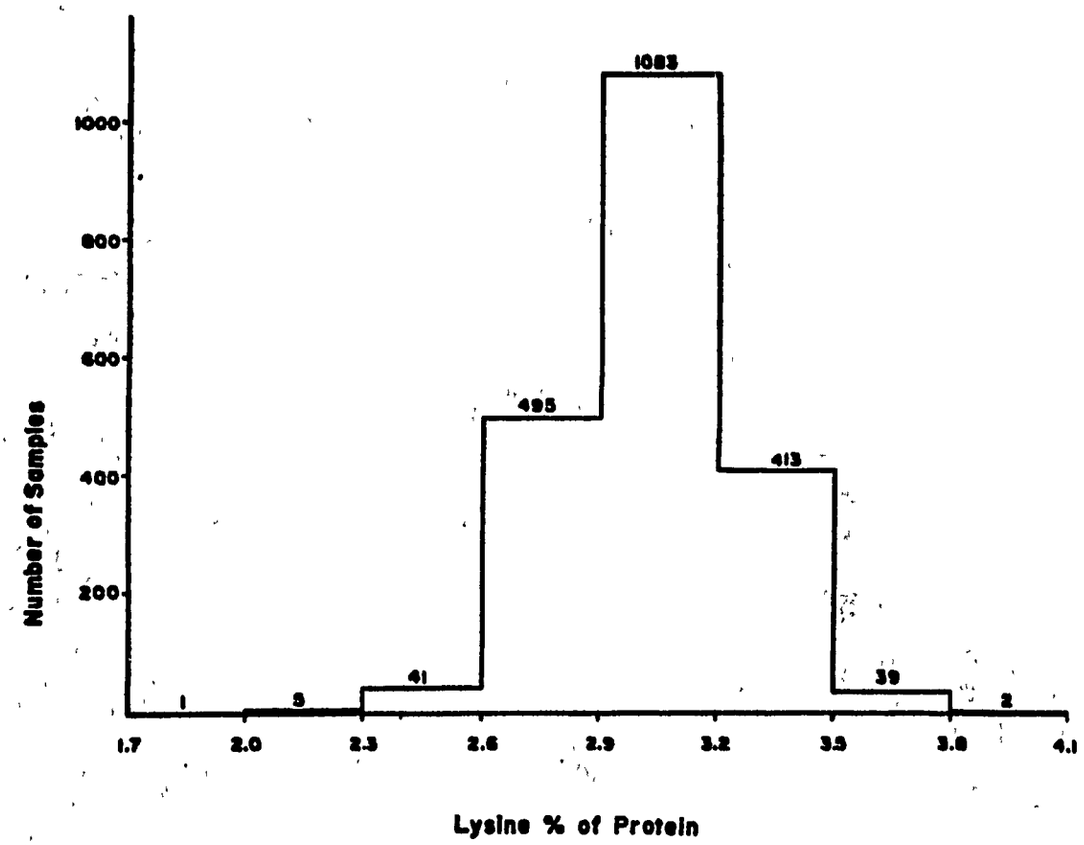

Figure 6. Lysine (percent of protein) frequency distribution for 2,079 wheats from the World Collection.

The effect of protein level on mean lysine content expressed as percent of total dry weight is shown graphically in Figure 7. The shaded area indicates the range of lysine variation encountered at each protein level. Note the very uniform and substantial increase in lysine with protein. This would be expected since lysine is a constituent of protein and thus would be affected by changes in protein.

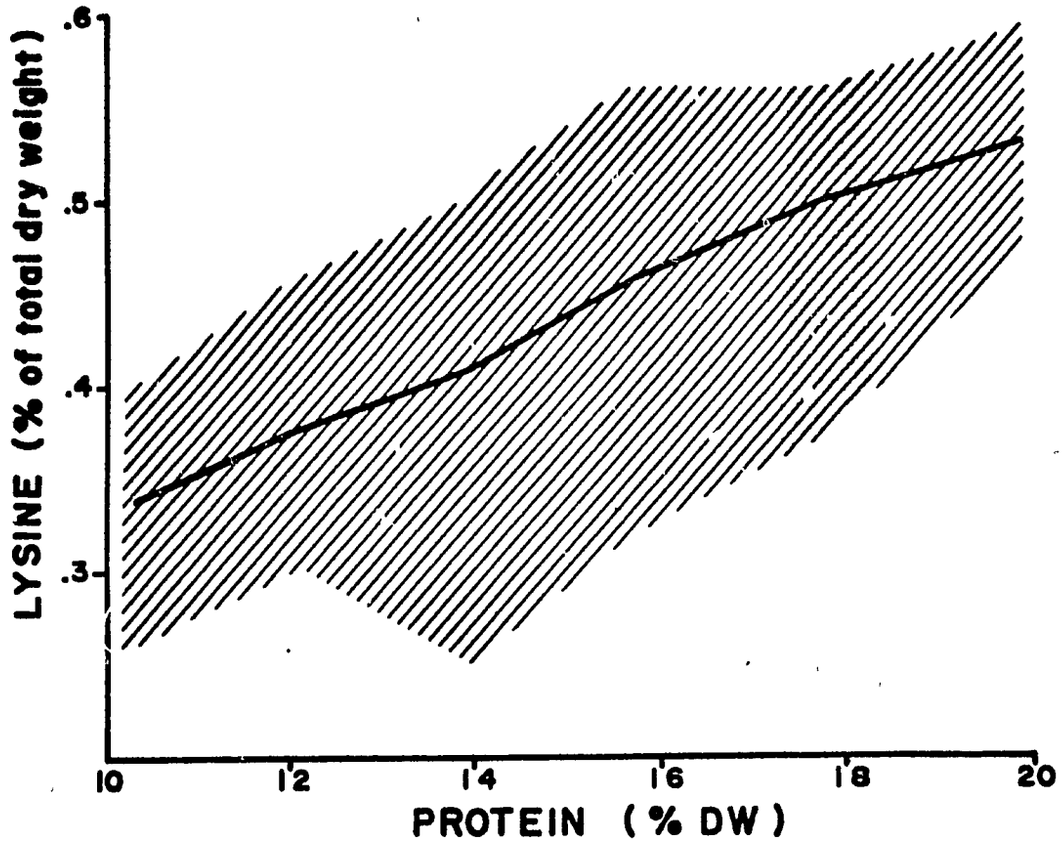

Figure 7. Relationship of lysine (percent of total dry weight) to protein among 2,079 wheats from the World Collection.

When lysine is expressed as percent of protein, its relationship with protein over a range of protein values is a negative one (Figure 8). However, the effect of protein level on lysine is relatively modest. Average lysine content decreased from 3.31 percent at 10.3 percent protein to 2.71 percent at 19.9 percent protein among 2,079 wheats analyzed. The decrease in lysine appears to be somewhat largest in the 10 to 14 percent protein range.

The correlation of protein with lysine (expressed as percent of dry weight) over the entire range of protein encountered among 2,079 wheats was +0.81 (Table 10). However, the correlation of lysine with protein within 2 percent protein increments is relatively modest--ranging from +0.46 in the 13.0 to 14.9 percent protein level to only +0.03 at the 17.0 to 18.9 percent protein level. This would indicate that lysine variation within restricted ranges of protein is largely random.

A low negative correlation of -0.48 was obtained when lysine as a percent of protein was correlated with protein (Table 11). The correlations were even lower when correlations were based on samples within restricted protein ranges.

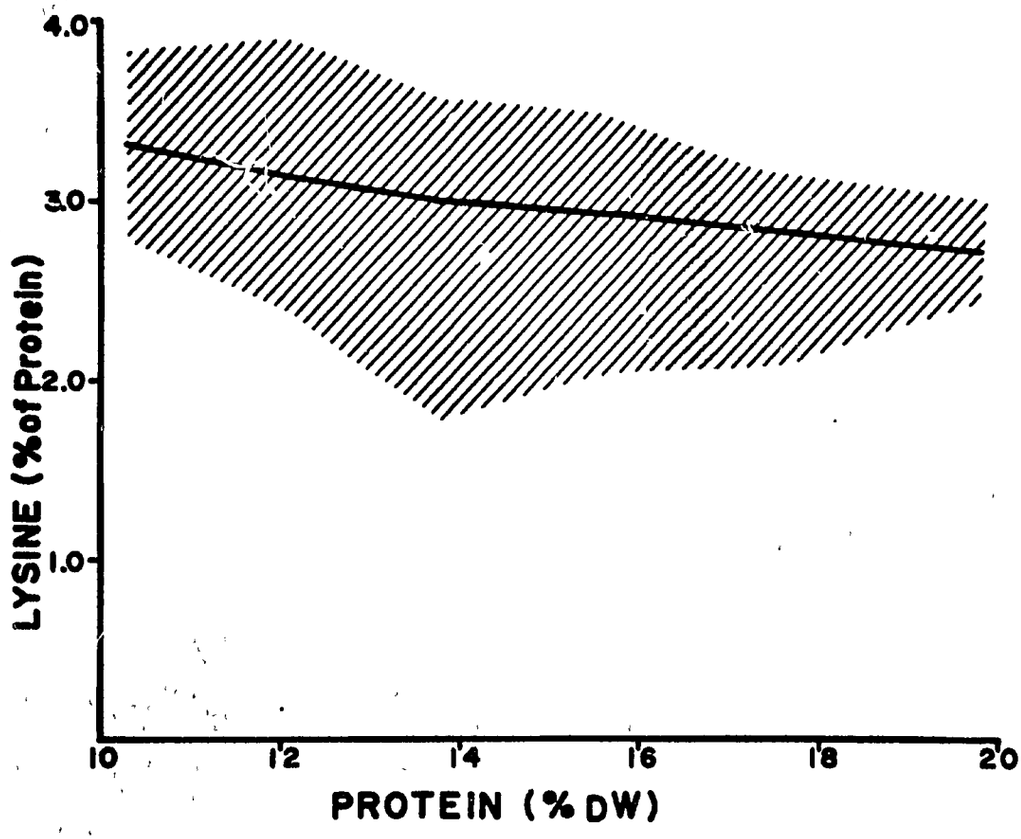

Figure 8. Relationship of lysine (percent of protein) to protein among 2,079 wheats from the World Collection.

Table 10.—Correlation of protein and lysine expressed as percent of dry weight of grain

Protein range, %	n	r
9.0 to 21.0	2,079	+0.81
9.0 to 10.9	107	+0.40
11.0 to 12.9	623	+0.34
13.0 to 14.9	903	+0.46
15.0 to 16.9	388	+0.34
17.0 to 18.9	53	+0.03

Table 11.—Correlation of protein and lysine expressed as percent of protein

Protein range, %	n	r
9.0 to 21.0	2,079	-0.48
9.0 to 10.9	107	-0.29
11.0 to 12.9	623	-0.33
13.0 to 14.9	903	-0.11
15.0 to 16.9	388	-0.14
17.0 to 18.9	53	-0.34

Our data indicate that level of lysine is influenced by protein level but that protein can account for only a portion of the lysine variation we have encountered. Thus, it would seem that selection for high lysine in wheat may be feasible.

The protein and lysine contents of selected wheats from the World Collection are shown in Table 12. The mean protein content of 2,079 wheats from the World Collection was 13.6 percent. Lysine expressed as percent of protein averaged 3.04 percent. The lowest lysine encountered was 1.77 percent in a 14.3 percent protein wheat from Russia. Seven of the wheats included in Table 12 appear to have promise as possible sources of high lysine. None is abnormally low in protein and two are higher in protein than the average of the 2,079 wheats analyzed.

Table 12.--Protein and lysine content of selected wheats from the World Collection

Sample No.	Origin	Protein,[1/] %	Lysine	
			Dry wt., %	Protein, %
1180	USA	11.5	0.44	3.87
22	Arabia	12.1	0.44	3.66
15	USA	12.5	0.46	3.65
667	China	12.5	0.45	3.58
610	Sweden	13.3	0.47	3.57
20	Spain	15.1	0.52	3.44
1335	USA	14.1	0.47	3.36
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$\bar{x}$ (2079 samples)		13.6	0.40	3.04
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229		14.3	0.25	1.77

1/ Dry weight basis.

Whether these wheats have promise for breeding higher lysine will depend upon whether their high lysine values are real or only apparent. The effect of environment on lysine level is not known. A selected number of spring wheats from the World Collection were grown at four locations in the United States in 1967. A comparable number of winter wheats were seeded at three locations this fall. These will be analyzed to ascertain the magnitude of the environmental effect on level of lysine in wheat.

We are optimistic that wheats with superior levels of lysine will be found. Improved lysine level combined with high protein would constitute a major step in the improvement of the nutritional value of wheat.