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HIGH PROTEIN--LYSINE RICH WHEATS

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At the Fifth National Conference on Wheat Utilization held at Fargo, N. Dak. in 1967, we reported on 12 years of cooperative USDA and Nebraska effort to improve protein content in wheat. We also summarized our work efforts of 1 year on a project supported in part by the Agency for International Development, U.S. Department of State, to screen the USDA World Wheat Collection to identify possible genetic sources of higher lysine and protein containing wheats. At that time we reported on screening data from 2,000 samples. The present report will include data on an additional 10,500 common wheat samples. Screening has been completed but the data have not been calculated for the first 1,000 samples in the durum collection. The remaining 2,500 durum samples will be finished in a few months, and our screening effort on the World Wheat Collection will be completed.

Genes for High Protein Wheat

Twelve years ago the variety Atlas 66 was the only known available genetic source for higher protein content in wheat. Since then the variety Aniversario (P.I. 168714, C.I. 12578) was identified as a high protein genetic source. J. Craddock of the U.S. Department of Agriculture sent seed to Nebraska in 1962. It was increased and used in the breeding program in 1963 greenhouse crosses. The Nebraska fertility-restoring experimental line NB 542437, recently identified in our project, is an additional genetic source to improve protein content.

These three sources for high protein have been crossed in greenhouse experiments in combination with each other to determine if additive effects are possible.

Other wheats with high protein contents have been identified in our screening efforts on the World Wheat Collection. The protein frequency distribution for the first 7,000 samples is shown in figure 1. Protein values ranged from 6.9 to 22.0 percent. Selected data for high protein samples are shown in table 1.

Greenhouse crosses have been made between the highest protein materials and the three known genetic sources for high protein. A new genetic source for high protein would be identified if a cross shows an increase in protein content over the parental material.

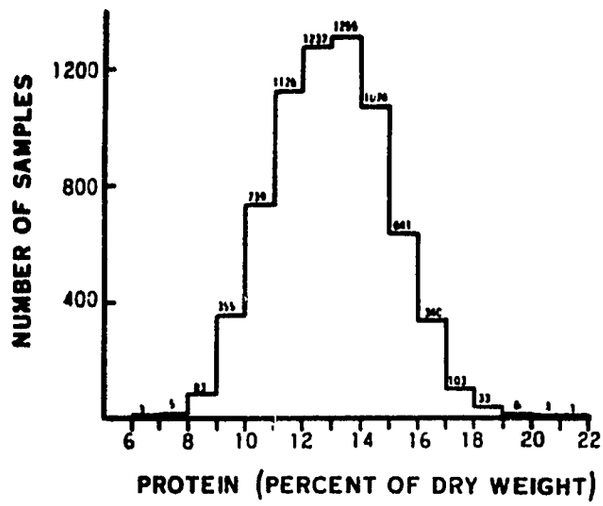

Figure 1.--Frequency distribution for protein content of 7,000 samples from the World Wheat Collection

Table 1.-- Selected high protein samples from World Wheat Collection

Sample No.	P.I. No. 1/	Dry Weight Basis Protein	Dry Weight Basis Lysine	Lysine/ Protein	Lysine/protein adjusted 2/
		Percent	Percent	Percent	Percent
10382	225252	21.4	0.58	2.74	3.17
9855	202800	20.9	0.62	3.00	3.40
9883	204008	20.7	0.61	2.98	3.37
10378	225248	20.7	0.60	2.90	3.29
10375	225244	19.7	0.57	2.93	3.26
9678	192812	19.5	0.60	3.08	3.41
9643	192750	19.4	0.58	2.99	3.31
9855	203985	18.9	0.55	2.93	3.23
9637	192738	18.6	0.57	3.09	3.37
9741	196903	18.4	0.56	3.09	3.36
7739	181329	6.9	0.29	4.26	3.89

1/ P.I. - Plant Introduction number.

2/ Values adjusted to 13.5 percent protein.

Strong influence of environment on the level of grain protein does not permit fixing protein content at a specific level. Except under conditions of excessively high soil nitrogen availability, high yields are usually associated with depressed protein content in the grain.

Genes that increase protein content must function in an array of environments and soil fertility levels. Data from wheat performance trials in Nebraska have been reported, which provided evidence of the effectiveness of genes from Atlas 66 to increase protein content in productive varieties. Also, we demonstrated in 1968 that Atlas 66 x Comanche Selection 65307 (a hard red winter wheat selection) maintained its protein superiority over the Nebraska variety, Lancer, in a range of soil fertility levels (fig. 2). In 1969, we extended the level of soil nitrogen and

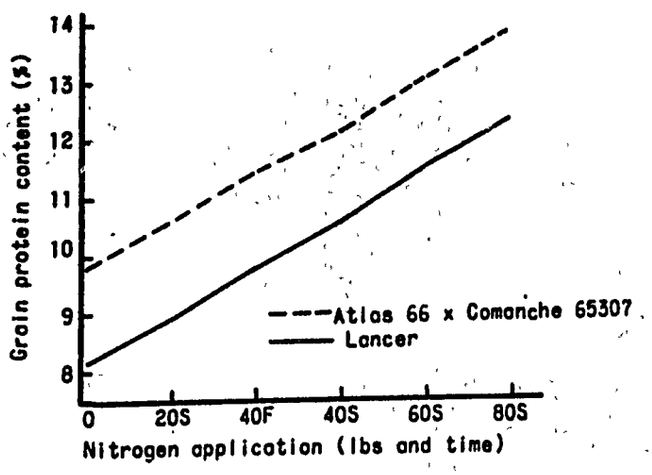

Figure 2.--Response of protein content to increasing nitrogen applications for two varieties of wheat. S=spring; F=fall.

again found superiority at all nitrogen levels. Averages for three growing locations in 1969 are listed in figure 3.

We have several higher protein hard red winter wheats, derived from Atlas 66, in advanced quality testing. The decision to release a line as a new wheat variety may be made soon.

The recent establishment of an International Winter Wheat Performance Nursery in which high protein wheats are being evaluated will provide additional information on the stability and effectiveness of all known high protein genes.

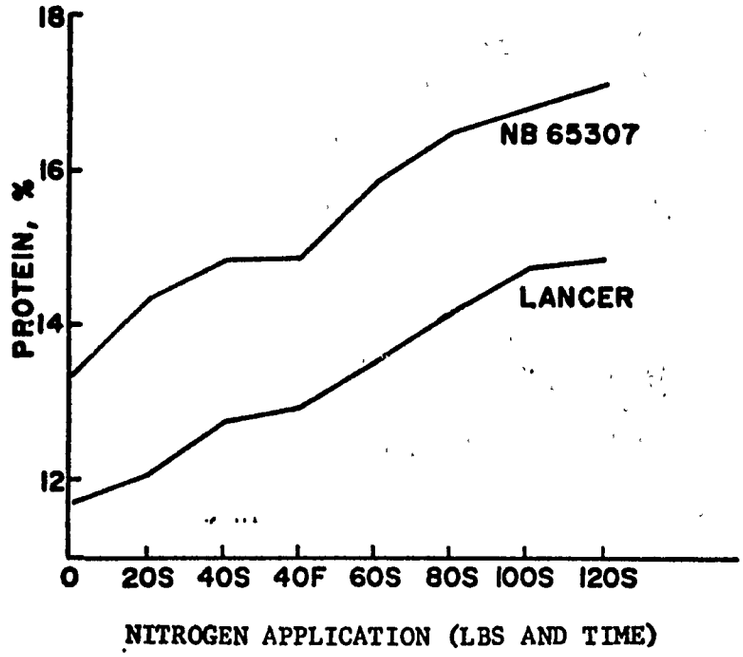

Figure 3.--Averages of results at three locations, showing responses of protein content to nitrogen applications. S=spring; F=fall.

The Search for High Lysine in Wheat

A gene in wheat with major effect on lysine, such as that of opaque-2 in maize, has not been identified. We have analyzed more than 12,500 wheats from the World Collection to date. Lysine values for the first 7,000 samples ranged from 1.7 to 4.15 percent of the protein (fig. 4). The data for the remaining 5,500 also fell within these same general limits.

Wide variations in protein were encountered. The degree to which the measured differences in lysine are genetic has not been established. Lysine data from greenhouse crosses made in the spring of 1969 with high lysine identified materials from the World Collection show some promise. The strong influence of environment on protein level is already well established. Our data suggest that there is also a strong environmental influence on the amino acid composition of the protein.

Lysine level in wheat tends to be negatively correlated with protein. We have recorded highest lysine values for wheats with lowest protein content. The negative regression of lysine (percent of protein) on protein among 7,000 wheats from the World Collection is shown in figure 5. The shaded area indicates the

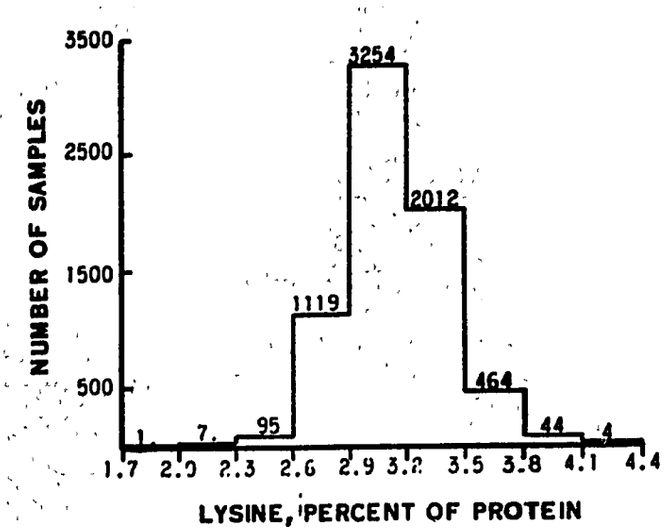

Figure 4.--Frequency distribution for lysine content (as percent of protein) of 7,000 samples from the World Wheat Collection

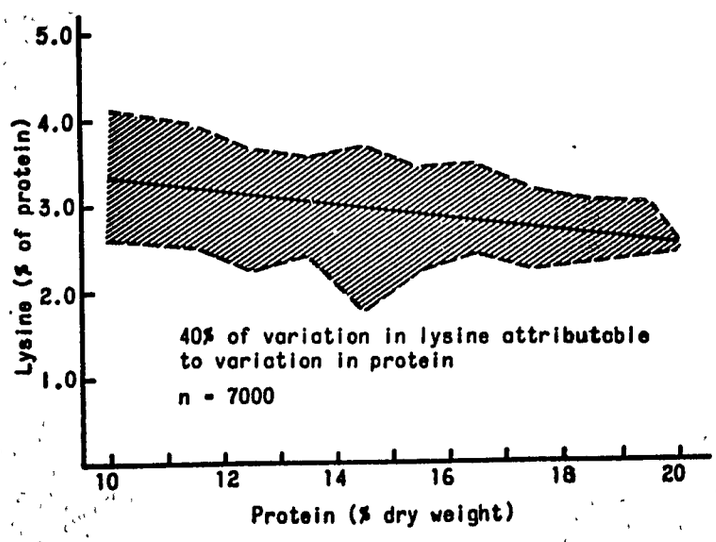

Figure 5.--Relationship of lysine content (as percent of protein) to protein content of 7,000 wheats

range of dispersal of actual values about the regression line. Note that 40 percent of the variation in lysine could be attributed to variation in protein. We now utilize the regression coefficient to adjust lysine values to a common protein level. This provides a means of making lysine comparisons among wheat differing

in protein content. The lysine frequency distribution of 7,000 wheats after adjustment of lysine values to 13.5 percent protein is shown in figure 6. The number of wheats with adjusted lysine values higher than 3.5 percent is only 125 compared with 512 wheats with unadjusted values above 3.5 percent.

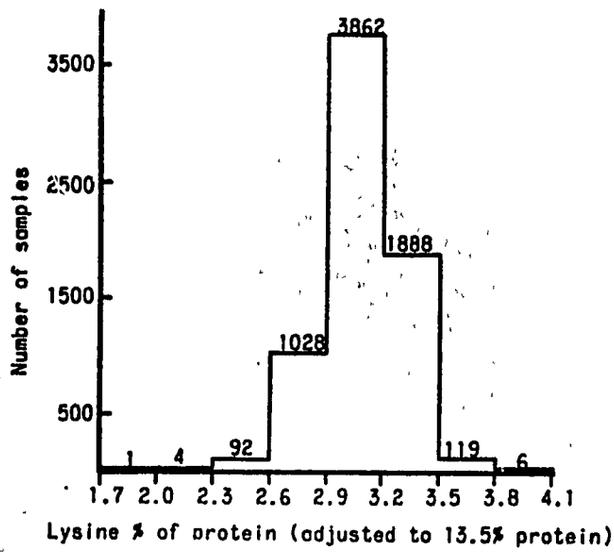

Figure 6.--Frequency distribution for lysine content (as percent of protein) calculated to a 13.5-percent protein basis of 7,000 wheats from the World Wheat Collection

Protein and lysine values of selected high lysine wheats among the first 7,000 analyzed appear in table 2. The wheats are from seven different countries and possess both spring and winter habits of growth. The protein levels of the wheats are low compared to the mean protein level of the 7,000 wheats analyzed. Therefore, adjustment of lysine values to 13.5 percent protein reduced the highest value from 4.15 to 3.91 percent.

Pedigrees of some of the high lysine varieties can be identified. They are shown for eight varieties in table 3. Some possibly significant relationships emerge. C.I. 13252 has the pedigree Norin x Brevor, Sel. 14. C.I. 13447 and C.I. 13449 are derivatives of Norin 10 x Brevor, Sel. 14. Further examination of pedigrees reveals that the old Australian varieties Florence, Federation, and Hard Federation appear frequently. Marquis and Crimean also are in evidence. We believe that some of these wheats may be contributing genes for lysine.

Table 2.--Protein and lysine content of selected high lysine wheats from the World Collection

C.I. or P.I. No. 1/	Origin	Growth habit	Protein content	Lysine (percent of protein) Unadj.	Adj. 2/
			Percent	Percent	Percent
13449	U.S.A.	Winter	9.2	4.15	3.91
142509	Iran	"	9.2	4.15	3.91
11721	Canada	Spring	10.2	4.08	3.89
11696	U.S.A.	Winter	11.1	3.97	3.83
117421	Turkey	Spring	7.7	4.12	3.80
135073	Afghanistan	"	7.5	4.09	3.76
121815	India	"	8.1	4.04	3.74
112344	England	Winter	9.1	3.95	3.70
13447	U.S.A.	"	10.3	3.87	3.69
109595	Turkey	Spring	9.1	3.91	3.66
$\bar{x}$ (7,000 wheats)		--	12.9	3.12	3.08
5483	USSR	Spring	14.3	1.77	1.81

1/ C.I. - Cereal Investigation No.
P.I. - Plant Introduction No.

2/ Values adjusted to 13.5 percent protein.

Comparison of Essential Amino Acids from Selected High Protein and High Lysine Wheats Identified During Screening of the USDA World Wheat Collection

Seed increases for the USDA World Wheat Collection are made sequentially at Mesa, Ariz. Many samples, therefore, are not adapted to the one environment. Also, the samples represent a number of different growing seasons. However, we were interested in analyzing high protein and high lysine samples for their amino acid content.

A comparison of the essential amino acids, for selected wheats, is shown in table 4. The high lysine selections were superior in lysine, methionine, valine, and tryptophan.

We have noted a correlation of lysine and tryptophan for these 24 high lysine samples and are continuing to evaluate this association.

Table 3.--Parental relationships among high lysine wheats

C.I. No.	Lysine content	Pedigree[1/]
13449	3.91	Norin 10-Brevor, Sel. 14 x Sister to Brevor
11721	3.89	Pentad x Marquis
11696	3.83	Ridit x Utah Kanred
13447	3.61	Norin 10-Brevor, Sel. 14 x Rio-Rex
11723	3.58	Hope x Ridit
13058	3.51	Marfed x Merit-28
12052	3.50	Comet-1110 x H44-Ceres
13253	3.50	Norin 10 x Brevor, Sel. 14

1/ Brevor (Florence, Federation)
Kanred (Crimean)
Hope (Marquis, Hard Federation)
Ridit (Turkey, Florence)
Rio-Rex (Crimean, Hard Federation)
H44 and Hope (Marquis)

Table 4.--Comparison of essential amino acids from selected high protein and high lysine wheats

Amino acid	High protein[1/]	High lysine[2/]
	percent	percent
Lys	2.89	3.58
Ile	3.68	3.69
Leu	7.12	7.37
Met	1.49	1.67
Phe	5.20	4.88
Thr	2.97	3.37
Val	4.48	4.78
Trp	1.12	1.53

1/ Average 24 samples.

2/ Average 55 samples.

Non-endosperm proteins are richest in lysine (over 4 percent) whereas endosperm proteins are relatively poor in lysine (approximately 2 percent). Differences in kernel morphology could alter the ratio of endosperm to non-endosperm proteins, thereby affecting the overall lysine content of the kernel. Kernel size of four varieties as established by passing the grain over appropriately sized sieves had no effect on either protein content or lysine level (table 5). Kernel configuration determined by kernel plumpness and crease characteristics strongly affected the protein

content of kernels but had little effect on lysine expressed as percent of protein (table 6).

Table 5.--Average effect of kernel size on the protein and lysine content of the grain of four wheat varieties

Kernel size	On sieve	Protein	Lysine/ dry wt.	Lysine/ protein
		Percent	Percent	Percent
Large	# 8	12.7	0.38	3.1
Medium	# 7	12.2	.38	3.2
Small	# 6	12.5	.38	3.1

Table 6.--Average effect of kernel size and configuration on the protein and lysine content of the grain of four wheat varieties

Kernel					
Size	Configuration	Crease	Protein	Lysine/ dry wt.	Lysine/protein
			Percent	Percent	Percent
Large	Plump	Closed	12.0	0.37	3.15
"	"	Open	12.6	.39	3.13
"	Wrinkled	--	14.3	.43	3.02
Small	Plump	Closed	11.8	.38	3.31
"	"	Open	12.9	.40	3.24
"	Wrinkled	--	15.4	.37	3.04

Contribution of increased protein content to the nutritional value of cereals must be recognized. When wheat with 17.2 percent protein is compared with high lysine wheat at 8.3 percent protein, 100 grams of the high protein grain provide more of all essential amino acids than does an equivalent quantity of the high lysine wheat (table 7).

Food and Agriculture Organization (FAO) standards for amino acids and nitrogen intake of 6.65 grams per day for an adult human weighing 70 kilograms were utilized to compute the weight of grain needed to provide minimal amounts of each essential amino acid. Grain weights for different types of wheat are contained in table 8. Only 355 grams of wheat with 17.2 percent protein would be required to provide man with his minimal amino acid requirements compared to 548 grams ordinary wheat with 11.1 percent protein and 649 grams for high lysine wheat at 8.3 percent protein.

The problem to maximize the protein nutrition of wheat is obvious. One must identify a genetic source for lysine and com-

bine it with the best genetic source of high protein. In addition, production practices must be followed to insure adequate protein levels.

Table 7.--Comparative essential amino acid composition of normal, high lysine and high protein wheat

Amino acid	Normal 11.1 percent protein		High lysine 8.3 percent protein		High protein 17.2 percent protein	
	Percent of protein	Grams per 100 g. grain	Percent of protein	Grams per 100 g. grain	Percent of protein	Grams per 100 g. grain
Lysine	3.0	0.33	3.6	0.29	2.9	0.49
Isoleucine	3.5	.39	3.7	.31	3.7	.64
Leucine	7.0	.78	7.3	.61	7.1	1.22
Methionine	1.5	.17	1.7	.14	1.6	.28
Phenyl-alanine	4.9	.54	4.9	.41	5.2	.89
Threonine	3.0	.33	3.4	.28	3.0	.51
Valine	4.3	.48	4.7	.39	4.5	.77
Tryptophan	1.3	.14	1.5	.12	1.1	.19

Table 8.--Weight of wheat[1/] needed to provide an adult human weighing 70 kg. with his minimal essential amino acid requirement

Amino acid	Normal 11.1 percent protein	High protein 17.2 percent protein	High lysine 8.3 percent protein
	Gram	Gram	Gram
Lysine	524	355	600
Isoleucine	448	271	561
Leucine	259	165	330
Methionine	548	325	649
Phenylalanine	216	131	285
Threonine	351	229	418
Valine	365	226	446
Tryptophan	404	306	485

1/ Dry-weight basis.

International Winter Wheat Performance Nursery

High protein selections from the screening project have been crossed with known genetic sources for high protein to identify still other genetic factors for increasing protein content in wheat. In addition, wheats with highest lysine levels have been crossed with known high protein materials.

An International Winter Wheat Performance Nursery was established in 1968 in cooperation with Agricultural Research Service of the U.S. Department of Agriculture; the Centro Internacional de Mejoramiento de Maiz y Trijo (CIMMYT-Mexico); and the Food and Agriculture Organization of the United Nations. It was grown at 23 sites in 16 countries in 1969 (fig. 7). Seed

Figure 7.--Locations of International Winter Wheat Performance Nurseries (23 sites; 16 countries)

harvested at each site is returned to Nebraska for protein and lysine analyses. Certain selected samples will be analyzed for total amino acid constituents.

This international nursery will be valuable to determine the range of environmental effects and to identify genetic sources that are most stable in diverse environments.