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GENETIC STUDIES OF WHEAT PROTEINS

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~~This presentation is concerned with wheat protein research at the University of Nebraska and the outlook for enhancement of nutritional value in wheat by genetic means.~~

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CHAPTER 9

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Genetic Studies of Wheat Proteins

INTRODUCTION

The average protein content of wheat usually exceeds 12%. This is higher than the protein content of rice, maize, or sorghum, the other widely utilized food cereals. Production environment exerts a strong influence on protein level of wheat. Protein values as high as 18% and as low as 7% are relatively common in the United States.

The cereal grains lack the balance of essential amino acids required for maximum utilization of their protein when they are the main or sole source of daily nutrition. Lysine is in shortest supply in the cereal proteins; thus, it largely fixes the degree of protein utilization.

Increases in the quantity of protein in the grain of wheat and/or improvement in the ratio of essential amino acids in the protein would enhance significantly its nutritional value. The discovery in 1963 (Mertz *et al.* 1964) of the effect of the *opaque-2* gene in maize on the lysine and tryptophan content of its seed protein pointed to the opportunity for significant genetic improvement of protein quality in the cereal species. A gene in barley with effect on lysine similar to that of *opaque-2* was subsequently reported (Hagberg and Karlsson 1969).

This presentation will be concerned with wheat protein research at the University of Nebraska and the outlook for enhancement of nutritional value in wheat by genetic means.

THE NEBRASKA-ARS RESEARCH PROGRAM

Our efforts to improve the nutritional value of wheat by breeding date back to 1954 when Atlas 66, a soft winter wheat from North Carolina was introduced into our wheat breeding program. Atlas 66, selected from a cross Redhart/No11/2/Frondoso, already had been determined to be higher in grain protein than other soft wheats. Our early research was supported in part by funds from the Nebraska Wheat Commission. It encompassed investigation of the heritability of grain protein, magnitude and stability of the genetic effect, the relationship of grain protein to grain yield, and physiological aspects of the high protein phenomenon.

The Nebraska-ARS protein research was broadened in 1966 to include investigation of the amino acid composition of wheat protein. Funds from the Agency for International Development, U.S. Dept. of State, were utilized to screen the

World Collection of Wheats for protein and lysine differences and to enlarge our breeding program for wheat with improved nutritional value. An International Winter Wheat Performance Nursery has been established in 26 countries to identify superior winter wheat genotypes and measure the impact of environment on nutritional quality.

RESEARCH RESULTS

Protein Content

Grain protein content of wheat is an heritable trait. We have computed estimates of heritability as high as 0.83 (Stuber *et al.* 1962). The high protein trait is readily transferable among common wheats. Its inheritance is relatively simple. Our data suggest the operation of two major genes from Atlas 66, CI 12561, our main genetic source of high protein to date. One of the genes is linked genetically with a gene that conditions adult plant leaf rust resistance (Johnson *et al.* 1963).

We have achieved protein increases from the Atlas 66 genetic source that range from 20 to 25% (Johnson *et al.* 1968A, 1969). Thus, varieties with 15% protein are possible in production situations in which ordinary varieties produce only 12%.

Our studies of nitrogen relations in high and low protein winter wheats show that high grain protein results from more efficient and complete translocation of nitrogen from the wheat plant to its grain (Johnson, *et al.* 1967, 1968A). We have been unable to demonstrate differential nitrogen uptake as a basis for high grain protein in Atlas-derived lines. The nitrate reductase enzyme transport system has been implicated by Illinois researchers (Croy and Hageman 1970).

Protein Stability

Grain protein content cannot be fixed at a specified high level by breeding. Production environment, particularly soil fertility, has strong influence on protein content as well as on grain yield. However, our data do show that high protein varieties can be expected to be superior in protein level to ordinary varieties in an array of environments and soil fertility situations.

Atlas 66 and a hard winter wheat experimental variety, NB 67730, with genes for high protein from Atlas 66 were consistently higher in protein content than other varieties tested in an International Winter Wheat Performance Nursery (IWWPN) in 1969. Atlas 66 averaged 17.4% and NB 67730 16.4% protein over 16 international test sites (Table 9.1). No ther variety shown in Table 9.1 averaged higher than 14.6%.

The high protein experimental variety NB 65307 was compared with the popular Lancer variety at sites in western Nebraska in 1968. Nitrogen fertilizer ranging from 20 to 80 lb per A was applied. NB 65307 maintained approximately 2% protein advantage over Lancer in all fertility levels (Johnson *et al.* 1970).

TABLE 9.1

AVERAGE GRAIN YIELD AND PROTEIN CONTENT OF 12 VARIETIES OF WINTER WHEAT GROWN IN AN INTERNATIONAL WINTER WHEAT PERFORMANCE NURSERY AT 16 SITES IN 1969

Variety	Grain Yield (Bu/A)	Protein Content (%)
Atlas 66	50	17.4
Atl 66/Cmn, NB 67730	53	16.4
Triumph 64	54	14.6
Gage	56	14.4
Parker	60	14.3
Sturdy	62	14.2
Scout 66	57	14.0
Lancer	56	13.9
Shawnee	56	13.9
Winalta	48	13.7
Bezostaia	68	13.3
Gaines	47	12.8

High Protein and High Grain Yield

The high protein trait was transferred to many experimental lines of hard winter wheat. Lines selected from the first cycle of breeding were not sufficiently productive for consideration as commercial varieties. Their yields frequently equalled the parent varieties but were generally lower than the commercially-grown Nebraska varieties. Additionally, there were other agronomic as well as milling and baking deficiencies that were unacceptable in U.S. hard winter wheats.

The performance of selections from the second cycle of breeding are highly encouraging. Our data indicate that there can be simultaneous advances in both grain yield and grain protein content (Table 9.2). Atlas 66/Comanche//Lancer, NB 701132, produced an average yield of 59.4 bu per A at 3 test sites in 1970 compared to 51.8 bu for the widely grown Scout variety. The grain protein content of NB 701132 was 14.4% compared to only 11.8% for Scout. This is a 23% advance in protein content combined with 13% yield increase.

Seed of 12 experimental varieties from the second breeding cycle is being increased in 1971 in Nebraska and Arizona. Some may have good potential as commercial varieties.

Effect of High Protein on Lysine

Does the high protein trait in wheat alter the ratio of essential amino acids? The Nebraska-ARS research provides useful information. Amino acid profiles for several high protein Atlas 66-derived lines from the first breeding cycle were

TABLE 9.2

GRAIN YIELD AND PROTEIN CONTENT OF SECOND CYCLE ATLAS 66-DERIVED LINES GROWN AT THREE SITES IN NEBRASKA IN 1970

Variety	CI or Sel. No.	Grain Yield (Bu/A)	Grain Protein Content (%)	Grain Protein Content: Advantage over Scout 66	Increase in Protein over Scout 66 (%)
Atl 66/Cmn//Lancer	NB 701132	59.4	14.4	2.3	23
Atl 66/Cmn//Lancer	NB 701134	55.2	14.3	2.2	22
Atl 66/Cmn//Lancer (Wrr/2/Atl 66/Cmn) F_1/3/Lancer	NB 701137	53.9	14.1	2.0	20
	NB 701154	53.4	14.5	2.4	24
Lancer//Atl 66/Cmn	NB 70654	50.7	14.4	2.3	23
Scout 66	13996	51.8	11.8	–	–

determined in our laboratory (Johnson *et al.* 1968B; Mattern *et al.* 1968). Several were comparable to their low protein parent in lysine, methionine, and threonine expressed as percentage of protein. Others showed some depression in these amino acids. Most important, in every line there was an increase in the amount of the three amino acids per unit weight of grain. Other things equal, such lines would be more nutritional than lower protein varieties.

A limited number of high protein lines from the second breeding cycle were analyzed for amino acid content. These lines had been selected only for productivity, desirable agronomic traits, and high grain protein. There were no prior analyses for amino acid profiles of their protein. The amino content of 3 of the lines grown at 2 Nebraska sites is compared with that of a check variety, NB 66403, in Table 9.3. At Mead, Nebraska, all of the high protein lines had lower lysine content of their protein than the check variety. Lysine per grain weight was higher than the check in two of the lines but lower in the third, NB 68570. The lysine content of protein in NB 68513 and NB 701137 grown at Sidney, Nebraska was no different than the check variety. As at Lincoln, NB 68570 was lower. All of the lines produced substantially more lysine per grain weight than did the check. There was some variation in methionine and threonine at both sites.

Lysine Variability

Fifteen thousand hexaploid and tetraploid wheats in the World Collection maintained by the Agricultural Research Service, USDA, have been screened for protein and lysine in the Nebraska laboratory. Complete amino acid profiles have been done on a limited number. Mean protein was approximately 13.0% with a range from 7.0 to 22.0%. The range in lysine was from 2.2 to 4.2% with a mean of approximately 3.0%.

TABLE 9.3

EFFECT OF HIGH PROTEIN ON THE AMINO ACID CONTENT OF THREE ATLAS 66-DERIVED WHEATS GROWN AT TWO SITES IN NEBRASKA IN 1970

Variety	Selection Number	Protein Content (%)	Lysine Protein (%)	Lysine Dry Wt. (%)	Methionine Protein (%)	Methionine Dry Wt. (%)	Threonine Protein (%)	Threonine Dry Wt. (%)
			Mead, Nebraska					
Standard	NB 66403	13.6	2.9	.40	1.5	.20	3.2	.43
High Protein Sel.	NB 68513	17.5	2.5	.43	1.4	.24	3.0	.53
High Protein Sel.	NB 701137	17.0	2.6	.44	1.2	.20	3.0	.51
High Protein Sel.	NB 68570	17.1	2.3	.39	1.4	.24	3.1	.53
			Sidney, Nebraska					
Standard	NB 66403	14.0	2.7	.38	1.3	.18	2.9	.40
High Protein Sel.	NB 68513	16.2	2.7	.43	1.4	.23	2.9	.47
High Protein Sel.	NB 701137	15.5	2.7	.42	1.5	.23	3.0	.47
High Protein Sel.	NB 68570	17.3	2.5	.43	1.4	.23	2.9	.49

Lysine expressed as percentage of protein is negatively correlated with protein. The correlation coefficient for 7000 wheats was −0.63. Lysine per protein decreased from 3.2 to 2.7% as protein increased from 10 to 20%. Lysine expressed as percentage of grain weight is positively correlated with protein. The coefficient for 7000 wheats was +0.83. Lysine per dry grain weight increased from 0.33 to 0.55% as protein increased from 10 to 20%. The +0.83 value is particularly significant in assessment of nutritional impact, because it indicates that high protein wheats can be expected to provide more lysine per unit weight of grain than lower protein wheats; this despite the tendency for high protein to be associated with depressed lysine per protein.

Selected high protein wheats are compared with a group of high lysine wheats from the World Collection in Table 9.4. The high protein group had only 2.75% lysine per protein but provided substantially more lysine, methionine and threonine per unit weight of grain than did the high lysine group or ordinary winter wheat. In contrast, the low protein content of the high lysine group resulted in no increase of the three amino acids per weight of grain.

The high protein experimental variety NB 67730 and three other varieties grown in the 1969 International Winter Wheat Performance Nursery are compared in Table 9.5. Although its lysine per protein content was lower, NB 67730 provided more lysine per grain weight than the other three varieties.

Reevaluation of high lysine wheats from the World Collection following their propagation in an array of environments has shown that most of the variation initially measured was environmentally induced. The genetic component of variation appears to be only approximately 0.5%. This is shown in Table 9.6 in

TABLE 9.4

AVERAGE LYSINE, METHIONINE, AND THREONINE CONTENT OF SELECTED HIGH-PROTEIN AND HIGH-LYSINE WHEATS FROM THE WORLD COLLECTION COMPARED WITH AVERAGE VALUES FOR WINTER WHEAT

Measurement	High Protein Samples (N = 46)	High Lysine Samples (N = 72)	Avg. for Winter Wheat[1]
Protein (%)	18.9	9.6	12.3
Lysine (% of protein)	2.75	3.40	2.76
Lysine (gm/100 gm wheat)	0.52	0.33	0.34
Methionine (% of protein)	1.32	1.52	1.54
Methionine (gm/100 gm wheat)	0.25	0.15	0.19
Threonine (% of protein)	2.94	3.32	2.85
Threonine (gm/100 gm wheat)	0.56	0.32	0.35

[1] Amino acid content of foods, USDA Publication.

TABLE 9.5

AVERAGE PROTEIN AND LYSINE PRODUCTION OF FOUR VARIETIES GROWN IN THE INTERNATIONAL WINTER WHEAT PERFORMANCE NURSERY IN 1969

Variety	Protein Content (%)	Lysine	
		Protein (%)	Gm/100Gm Grain
Atl 66/Cmn, NB 67730	16.4	2.75	0.45
Triumph	14.7	2.88	0.42
Scout 66	14.1	2.88	0.41
Bezostaia	13.4	2.90	0.39

which Aniversario, a high protein wheat, and Pearl, a high lysine wheat, are compared with Nap Hal which we believe to be high in both protein and lysine. The difference in lysine per protein between Nap Hal and Aniversario at a comparable level of protein is about 0.5%. The impact of combined high protein and high lysine can be noted for Nap Hal. It produced 0.60 gm of lysine, 0.33 gm of methionine, and 0.67 gm of threonine per 100 gm of wheat. The amounts of these amino acids were much lower for Aniversario and Pearl.

The large effect of environment on lysine content suggests the possibility that changes in kernel morphology induced by environment could be involved. Eight

TABLE 9.6

LYSINE, METHIONINE, AND THREONINE CONTENTS OF SPRING WHEAT VARIETIES GROWN IN THE GREENHOUSE AT LINCOLN, NEBRASKA IN 1969

Measurement	Aniversario (High Protein)	Pearl (High Lysine)	Nap Hal (High Protein and High Lysine)
Protein (%)	20.7	19.4	21.3
Lysine (% of protein)	2.36	2.83	2.81
Lysine (gm/100 gm wheat)	0.48	0.54	0.60
Methionine (% of protein)	1.32	1.91	1.57
Methionine (gm/100 gm wheat)	0.27	0.37	0.33
Threonine (% of protein)	2.88	3.11	3.18
Threonine (gm/100 gm wheat)	0.59	0.60	0.67

seed morphology traits were correlated with protein and lysine in 280 wheats from the World Collection that exhibited wide differences in protein and lysine content. Protein level and lysine per grain weight were most strongly affected by degree of kernel shriveling. R-values were 0.54 and 0.55, respectively. Degree of kernel shriveling did not affect lysine per protein. Other seed traits showed little or no correlation with protein and lysine content of the seed.

Genetic Sources of High Protein and High Lysine

We are utilizing various wheat varieties as genetic sources of high protein and high lysine. Our classification should be considered tentative—particularly for high lysine—until there is additional confirmative data. The most promising are listed:

High Protein		High Lysine	
Atlas 66	CI 12561	Nap Hal	PI 176217
Nap Hal	PI 176217	Pearl	CI 3285
Hybrid English	CI 6225	Hybrid English	CI 6225
Fertility Restorer	NB 542437	April Bearded	CI 7337
Aniversario	CI 12578	Fultz/Hungarian	CI 11849
Atlas-derived lines		Fultz Sel/Hungarian//	
Aniversario-derived lines		Minturki/Fultz Sel	CI 12756

Detailed protein and lysine analyses of hybrid populations involving the above wheats are in progress. Nap Hal is of particular interest because it has consistently produced grain in greenhouse studies with both above-normal protein and lysine. Hybrid English has produced grain with higher-than-normal protein and lysine but of less magnitude than Nap Hal.

DISCUSSION AND OUTLOOK

Estimated amino acid requirments for man were published by FAO in 1957.

These were compared with availability of the amino acids in corn, rice, soybeans and wheat (VanEtten *et al.* 1967). We have computed for wheat the deviation of essential amino acids from requirements of man from these data (Table 9.7).

TABLE 9.7

DEVIATION OF ESSENTIAL AMINO ACIDS IN WHEAT PROTEINS FROM REQUIREMENTS OF MAN, FAO-1957

Amino Acid	Deviation from Requirement (%)
Lysine	−55.0
Methionine[1]	−12.5
Isoleucine	−16.7
Leucine	+30.4
Tyrosine	+ 9.7
Phenylalanine	+40.4
Threonine	− 3.4
Valine	+ 4.5

[1] Adjusted provisional amino acid pattern (WHO 1965).

Clearly, lysine is the critical essential amino acid in wheat. It would appear that a substantial increase in lysine could take place before any other amino acid would emerge as the limiting amino acid in wheat protein utilization.

The literature usually lists lysine, methionine, and threonine as the amino acids in shortest supply in wheat protein. The calculations would indicate that isoleucine may be more critical in wheat than either methionine or threonine.

Data have been reported (Benton *et al.* 1956) indicating that leucine in excess of requirements can interfere with utilization of isoleucine and valine. If so, reduction of leucine in wheat protein could possibly achieve a beneficial effect on its utilization.

A gene for lysine in wheat with the impact of *opaque-2* in maize has not been identified. Lysine differences that appear to be genetic were detected in our search of the World Wheat Collection. Their magnitude is relatively small compared to the total lysine variation encountered. However, the 0.5% genetic component suggested by our data represents a 17% potential advance in lysine content by breeding.

Segregating populations from crosses in which the potentially best genetic sources of higher lysine were combined with high protein varieties are being propagated. Protein and lysine analyses of selections from such populations

should provide valuable information on high lysine-high protein breeding expectations.

Common wheat is a hexaploid plant whereas durum wheat has a tetraploid genetic constitution. The presence of more than one genome in common and durum wheat may have contributed to our failure to turn up large genetic differences in lysine content. A gene in one genome with large effect on lysine could be masked by genes in the other genomes. It may be significant that maize and barley in which genes with large effect on lysine have been identified, are both diploid species.

The negative relationship between level of protein and lysine per protein adds to the problem of making simultaneous advances in both. Fortunately, depression in lysine per protein associated with increased protein content is of insufficient magnitude to offset the increase in lysine per unit weight of grain due to higher protein content. Thus, the net effect of higher protein on amount of lysine per weight of grain is positive.

Our data point to increased protein content as the key to nutritional improvement of wheat. Maximum enhancement of nutritional value should come from combining genes for high protein with genes for higher lysine. Variations of lysine content among our high protein selections indicate that selection for high protein must be accompanied by lysine analyses to achieve the highest lysine per protein. Wheats with 25% more protein have been achieved. Lysine increases of 15 – 17% also may be possible. Combination of the two would represent a very significant nutritional step in wheat.

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