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# GENETIC ADVANCES IN WHEAT PROTEIN QUANTITY AND COMPOSITION[1]

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The protein content of wheat grain averages approximately 12%. The nutritional quality of wheat protein is limited by its short supply of the essential amino acid lysine. Less than adequate level of lysine is a deficiency of all cereal grains. The Agricultural Research Service, U. S. Department of Agriculture, and the Nebraska Agricultural Experiment Station have conducted cooperative research on wheat protein since 1955. The research initially was aimed at genetic improvement of protein content. Since 1966 it has encompassed investigation of amino acid composition and its modifications to improve the biological value of wheat.

## PROTEIN CONTENT

The soft winter wheat variety Atlas 66 has been our principal genetic source of high protein. Genes for high protein in Atlas 66 are believed to come from the South American variety Frondoso. Protein increases in the range of 2 to 3% have been accomplished in selections from Atlas 66 x hard winter wheat crosses (JOHNSON *et al.*, 1972a).

We encountered some difficulty in combining the high-protein trait from Atlas 66 with acceptable bread-wheat baking characteristics in high-protein selections from the first breeding cycle. This appears to have been overcome in advanced second-cycle selections currently undergoing performance evaluation in Nebraska and regional trials.

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## Environmental Effects

The strong influence of environment on protein content does not permit fixed levels of protein as a breeding goal. In this respect, protein content is little different from yield. Varieties with high yield potential do not always make high yields. Varieties with high grain-protein potential do not always produce grain with high protein content. We are forced, therefore, to rely in our breeding programs on relative levels of protein in comparably produced material.

Phenotypic expression of high protein derived from Atlas 66 is good. In a 3-year experiment involving different levels of nitrogen-fertilizer application on low-fertility soils, the high-protein experimental variety CI14016 maintained a 2% protein advantage over Lancer at all levels of fertilizer application (JOHNSON *et al.*, 1973). Both varieties responded strongly to increments of fertilizer with higher protein content of their grain. The grain-protein advantage of CI14016 persisted throughout the range of treatments, including those in which it was more productive than Lancer.

Atlas 66 and high-protein selections from the Nebraska program have been evaluated in the International Winter Wheat Performance Nursery since 1969. The nursery is currently grown at 52 sites in 33 countries. Atlas 66 and its derivatives consistently produced grain with higher protein content than other varieties evaluated in the nursery (STROIKE *et al.*, 1971, 1972). Their protein content was higher than less productive as well as more productive varieties in the nursery. Their protein advantage was equally pronounced at sites where the general level of protein was low or high (Table 1).

Correlation of yield and protein for individual varieties over international nursery sites ranged from -0.43 to zero (Table 2). This suggests that all varieties may not exhibit similar yield-protein relationships. A more meaningful correlation involves the relationship of yield and protein at individual nursery sites. When protein was correlated with yield for a group of varieties not known to be genetically different in protein potential at each of several nursery sites, r values ranged from -0.61 to +0.65 (Table 3). A relatively small part of protein variation could be accounted for by differences in grain yield, even at sites for which the largest negative correlations were computed.

Table 1. Phenotypic expression of the high grain protein trait at sites of the International Winter Wheat Performance Nursery in 1970 at which the mean protein level among varieties was high (Stillwater, Oklahoma), intermediate (Martonvasar, Hungary), and low (Cambridge, England)

| Variety | Grain protein content (%) in: Oklahoma | Hungary | England |
|---|---|---|---|
| Nursery $\bar{x}$ | 17.8 | 15.8 | 12.5 |
| Bezostaya 1 | 16.5 | 14.3 | 12.3 |
| Lancer | 16.2 | 14.6 | 12.1 |
| Yorkstar | 16.0 | 13.7 | 11.2 |
| Gaines | 16.5 | 14.1 | 10.6 |
| Atl 66/Cmn (NE67730) | 20.9 | 18.4 | 14.2 |
| Purdue 28-2-1[1] | 20.8 | 20.3 | 13.7 |
| Atlas 66 | 20.6 | 19.4 | 13.5 |

[1]High protein line derived from Frondoso

The Atlas 66 genes for high protein have been ineffective in promoting protein in spring wheats in the northern USA plains states higher than that of currently grown varieties (R. C. FROBERG, pers. comm.). Reasons for this are obscure. U. S. hard spring wheats

produce grain with higher protein content than hard winter wheats. Some have attributed this to lower productivity of the spring wheats and possible higher soil fertility in the spring wheat region. It is also possible that the same genes for high protein that are carried by Atlas 66 already are present in several of the currently grown spring varieties.

Table 2. Correlation coefficients for yield, protein, and lysine computed for nine varieties grown in the International Winter Wheat Performance Nursery in 1969 and 1970

| Variety | Yield vs protein | Protein vs lysine |
|---|---|---|
| Berostaya 1 | -0.11 | -0.73** |
| Scout 66 | 0.02 | -0.82** |
| Triumph 64 | -0.01 | -0.74** |
| Winalta | -0.03 | -0.76** |
| Heine VII | -0.43** | -0.12 |
| Bankuti 1201 | -0.18* | -0.74** |
| Atlas 66 | -0.20* | -0.61** |
| Atl66/Cmn (NE67730) | 0.05 | -0.75** |
| Purdue 28-2-1 | -0.06 | -0.79** |

*Significant at the 5% level
**Significant at the 1% level

## Protein Variability-World Wheat Collection

All common and durum wheats in the World Collection maintained by the Agricultural Research Service, USDA, except recent accessions, have been analyzed for protein and lysine content in our laboratory. They total 12,613 common and 3,400 durum wheats. Protein frequency distribution for the common wheats is shown in Figure 1. Most values were in the 10 to 16% range, although total protein variation extended from 6 to 22%. Protein variation among the durums was similar to that of the common wheats.

We believe that most of the variation in protein among the World Collection wheats is of the non-genetic kind. The seed analyzed was produced at Mesa, Arizona, but not all in the same year. Differing maturities and degrees of adaptation to the Arizona production environment resulted in variability of seed size and plumpness. The latter has been demonstrated to strongly affect protein content (Table 4).

Table 3. Yield and protein correlations for a group of 24 winter wheat varieties[1] at selected International Winter Wheat Performance Nursery sites in 1969 and 1970

| Site | Year | Mean Yield | Mean Protein | r | $r^2$ |
|---|---|---|---|---|---|
| | | q/ha | % | | |
| Stillwater | 1970 | 26 | 17.3 | -0.61** | 0.37 |
| Svalof | 1969 | 57 | 16.1 | -0.57** | 0.32 |
| Kabul | 1969 | 55 | 13.2 | -0.56** | 0.32 |
| Kabul | 1970 | 63 | 16.6 | -0.49** | 0.24 |
| Wageningen | 1969 | 42 | 13.1 | -0.45** | 0.20 |
| Novi Sad | 1969 | 48 | 14.7 | -0.42** | 0.18 |
| Cambridge | 1970 | 33 | 12.4 | -0.35** | 0.12 |
| Ankara | 1970 | 36 | 13.8 | -0.25* | 0.06 |
| Svalof | 1970 | 51 | 15.6 | -0.24* | 0.06 |
| Novi Sad | 1970 | 42 | 15.9 | -0.19 | 0.04 |
| Fundulea | 1970 | 36 | 14.6 | -0.09 | 0.01 |
| Rieti | 1969 | 48 | 12.4 | +0.24* | 0.06 |
| Eskisehir | 1969 | 45 | 12.8 | +0.65** | 0.42 |

[1]None of varieties known to possess genes for high protein.

*Significant at 5% level of probability.
**Significant at 1% level of probability.

We have utilized seed production from several sites to identify varieties genetically different in grain-protein potential. Potentially useful genetic sources of high protein in wheat in addition to the Atlas wheats have been identified. These include:

| | |
|---|---|
| Aniversario | CI12578 |
| Nap Hal | PI176217 |
| Nebraska Fertility Restorer | NE542437 |
| April Bearded | CI7337 |
| Hybrid English | CI6225 |
| Hume[2] x Nb[4]-Agrus-Tc[7] | SD69103 |

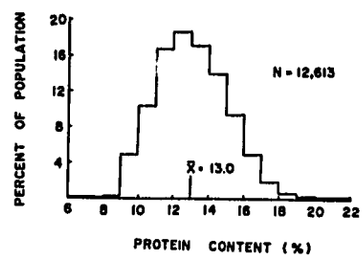


Figure 1. Frequency distribution for grain protein content of 12,613 common wheats in the USDA World Wheat Collection

Nap Hal is of particular interest because, in addition to high protein, it also has higher-than-normal lysine content.

## Inheritance of High Protein

$F_2$ progeny bulks from Nap Hal/Atlas 66 were grown in the $F_3$ generation in 1971 and in the $F_4$ generation in 1972 at Yuma, Arizona. Frequency distributions for protein are shown in Figure 2. Protein content of the parent varieties exceeded 20% in both years. Mean protein of the $F_3$ and $F_4$ populations approximated the mid-parent value. Forty-four percent of the $F_3$ population exceeded the protein range of the parents. Thirteen % of the $F_4$ population produced protein values outside the parental range. We attribute the apparent transgressive segregation for protein content to different genes in Nap Hal and Atlas 66.

Table 4. Average effect of kernel size and configuration on the protein and lysine content of the grain of four winter wheat varieties

| Size | Configuration | Crease | Protein content | Lysine per unit protein |
|---|---|---|---|---|
| | | | % | % |
| Large | Plump | Closed | 12.0 | 3.15 |
| " | " | Open | 12.6 | 3.13 |
| " | Wrinkled | --- | 14.3 | 3.02 |
| Small | Plump | Closed | 11.8 | 3.31 |
| " | " | Open | 12.9 | 3.24 |
| " | Wrinkled | --- | 15.4 | 3.04 |

Correlation of protein $F_2$ progeny bulks in the $F_3$ and $F_4$ generations was only 0.34 but highly significant statistically. The $F_3$-$F_4$ correlation for protein among 56 $F_2$ progeny bulks in which there was selection for seed plumpness was 0.43. This suggests that different

degrees of seed shrivelling among the progeny rows contributed to the relatively low $F_3$-$F_4$ correlation of protein content in unselected $F_2$ progeny rows.

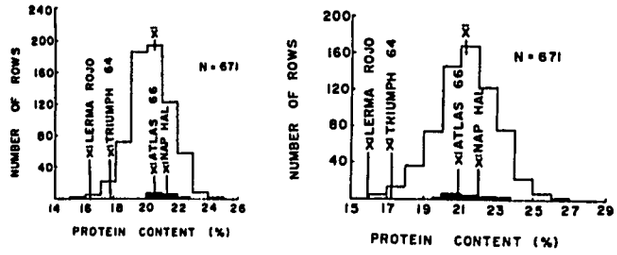


Figure 2. Frequency distribution for grain protein of $F_2$ progeny bulk rows of Nap Hal/Atlas 66 grown in the $F_3$ (left) and $F_4$ (right) generations at Yuma, Arizona, in 1972.

$F_2$ progeny bulks from Nap Hal/13449 were analyzed for protein content in the $F_3$ generation grown in 1972 at Yuma, Arizona. The frequency distribution for protein among 437 rows appears in Figure 3. In this population in which only one parent variety was high in protein, only one progeny row had a protein value outside the parent range. A population mean of 16.7% protein compared to 15.5% for CI13449 and 20.2% for Nap Hal, suggests partial dominance of low protein. Protein frequency distributions similar to that of Nap Hal/CI13449 occurred in populations derived from Nap Hal/Lancer and Nap Hal/Yang Kwang (JOHNSON *et al.*, 1972b).

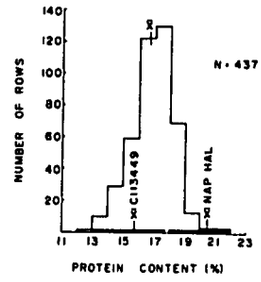


Figure 3. Frequency distribution for grain content of $F_2$ progeny bulk rows of Nap Hal/CI13449 grown in the $F_3$ generation at Yuma, Arizona in 1972

## Effect of Plant Height on Protein Content

Development of varieties with high resistance to lodging is an important objective in most wheat-breeding programs. It is necessitated by high-yielding varieties, heavy use of nitrogen fertilizer, and irrigation of wheat in some important production areas. Improved lodging resistance has been accomplished by shorter straw combined with increased straw strength.

Higher protein content also has become an important wheat-breeding objective in many programs. Little is known concerning the effect of reduced plant height on protein content or amino acid composition of the grain. McNEAL *et al.* (1971) suggested that high grain protein may be incompatible with reduced plant height.

CI13449 is a semi-dwarf variety. It produces grain with normal protein content. Nap Hal is moderately tall and produces grain with high protein content. If plant height influences protein content, tall $F_2$ progeny rows from Nap Hal/CI13449 would be expected to produce grain with higher protein content than short rows. Mean plant height of 430 rows was correlated with protein content of the grain. Several rows in which there was wide segregation for height were eliminated from the computation.

Plant height ranged from 60 to 119 centimeters. The r value for plant height versus protein content was -0.24. This relatively small but statistically significant negative correlation indicates some association of shortness with high grain protein under the conditions of this experiment.

## Protein Quality

The nutritional quality of wheat protein is limited by its short supply of the essential amino acid lysine. Nearly one-half of wheat protein is comprised of alcohol-soluble prolamin, which is low in lysine content. Genetic improvement of lysine content, similar to that provided by the opaque-2 gene in maize, would involve reduction of the prolamin fraction.

## Lysine Variation

Little was known concerning amino acid variation in wheat protein at the time of initiation of the ARS-Nebraska research on nutritional quality. Lysine analyses among the common and durum wheats in the World Collection were undertaken in 1966. They now are completed except for recent accessions to the Collection. The frequency distribution for lysine among the common wheats is shown in Figure 4. Ly ine values ranged from 2.2 to 4. % with a mean of 3.2% (lysine expressed as percent of protein). The majority of values were in the 2.8 to 3.6% range.

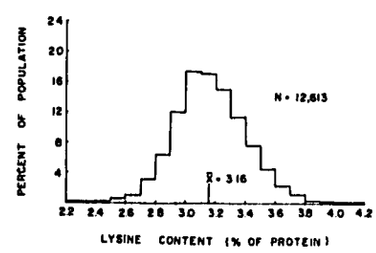


Figure 4. Frequency distribtuion for lysine per unit protein of 12,613 wheats in the USDA World Wheat Collection

## Effect of Protein Level on Lysine

Lysine expressed as percent of protein is negatively correlated with protein (Figure 5, left). The relationship is strongly curvilinear. Protein differences at the lower levels of protein exert a marked effect on lysine--an effect which decreases as protein becomes higher and disappears altogether above 16%.

Lysine expressed as percent dry grain weight is positively correlated with protein content (Figure 5, right). The relationship is mildly curvilinear and is most pronounced at the high protein levels. The contribution of protein to lysine per unit weight of grain more than compensates for the tendency for high protein to be associated

with depressed lysine content of the protein. This also has been observed in rice, where protein differences above the 10% level have little or no effect on lysine content of the protein (JULIANO, 1972).

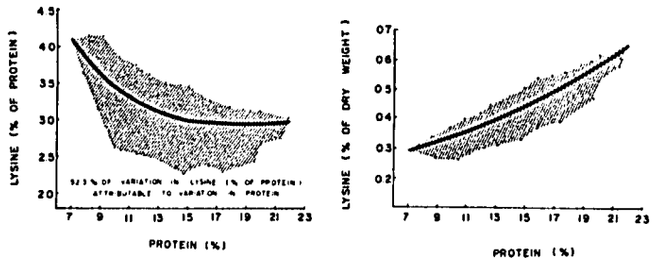


Figure 5. Relationship of grain protein content to lysine per unit protein (left) and lysine per unit weight of dry grain (right) among 12,613 wheats in the USDA World Wheat Collection

## Adjustment of Lysine

Initially, we adjusted lysine values to a common protein level using linear regression of lysine on protein. The adjustment is necessary to permit lysine comparisons among individuals that differ in protein content. The relationship evident in Figure 5 (left) shows that lysine adjustments based on linear regression are inappropriate, since there would be over-correction in some portions of the protein distribution and under-correction in others. We now routinely utilize the curvilinear relationships shown in Figure 5 (left) to compute a prediction equation for lysine adjustments to a common level of protein.

The frequency distribution for lysine per unit protein adjusted to 13% protein is shown for 12,613 common wheats in the World Collection in Figure 6. Note that, with adjustment, the frequency of wheats with high lysine values is sharply reduced from that for unadjusted values shown in Figure 4. World Collection wheats with the highest adjusted values are listed in Table 5.

An acceptable adjustment of lysine values to minimize or reduce the effect of protein level is especially important in analysis of populations in which there is genetic segregation for protein and lysine. Nap Hal/CI13449 and Nap Hal/Atlas 66 are such populations. In each, segregation for protein produced wide differences in protein level among $F_2$ progeny bulk rows.

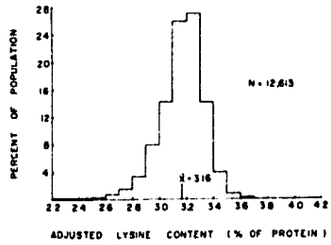


Figure 6. Frequency distribution for adjusted lysine per unit protein among 12,613 wheats in the USDA World Wheat Collection

The prediction equation for adjustment of lysine values is based upon protein-lysine relationships among wheats in the World Collection in which protein values ranged from 8 to 21%. Protein values above 16% had little effect on lysine. Lysine adjustments, therefore, would be most useful for protein variation in the 8 to 16% range.

Nap Hal/Atlas 66 produced $F_2$ progeny bulk rows that ranged in protein content from 16 to 26%. The correlation of protein with lysine per unit protein among 671 rows was -0.38 in the $F_3$ generation

Table 5. Protein and lysine values for common wheats in the World Collection with highest and lowest adjusted lysine values

| P.I. or C.I. number | Rank | Protein content | Lysine per unit wt. of grain | Lysine per unit protein | Adj. lysine per unit protein[1] | Deviation from predicted[2] |
|---|---|---|---|---|---|---|
| | | % | % | % | % | % |
| 13449 | 1 | 9.2 | .38 | 4.2 | 3.7 | .55 |
| 9364 | 2 | 11.5 | .43 | 3.8 | 3.7 | .49 |
| 271075 | 3 | 15.1 | .52 | 3.5 | 3.6 | .47 |
| 10517 | 4 | 14.5 | .50 | 3.5 | 3.6 | .47 |
| 94460 | 5 | 12.4 | .45 | 3.6 | 3.6 | .46 |
| 117628 | 6 | 15.8 | .54 | 3.4 | 3.6 | .45 |
| 94504 | 7 | 12.6 | .45 | 3.6 | 3.6 | .45 |
| 13447 | 8 | 10.3 | .39 | 3.9 | 3.6 | .45 |
| 12189 | 9 | 14.9 | .51 | 3.5 | 3.6 | .44 |
| 13058 | 10 | 14.2 | .49 | 3.5 | 3.6 | .44 |
| 2092 | 11 | 15.1 | .52 | 3.4 | 3.6 | .44 |
| | . | | | | | |
| | . | | | | | |
| | . | | | | | |
| | . | | | | | |
| | . | | | | | |
| 135050 | 12610 | 9.4 | .25 | 2.8 | 2.4 | -.81 |
| 10013 | 12611 | 10.4 | .26 | 2.6 | 2.4 | -.81 |
| 166475 | 12612 | 9.8 | .26 | 2.7 | 2.3 | -.83 |
| 9050 | 12613 | 9.9 | .25 | 2.6 | 2.3 | -.88 |

[1]Values adjusted to 13% protein
[2]Based on prediction from curvilinear regression equation

grown in 1971 and -0.29 in the $F_4$ generation grown in 1972. Adjustment of lysine values according to our prediction equation did not lower these values. This was anticipated because of the high level of protein, which, for many progeny rows, exceeded the range on which the prediction equation was based. A single lysine adjustment of 0.2% was applied to all progeny rows to reflect anticipated lysine values at the 13% protein level and permit comparisons of lysine across different populations.

Protein values among 437 $F_2$ progeny bulk rows derived from Nap Hal/CI13449 ranged from 12 to 21%. The correlation of protein with lysine per unit protein was -0.42. Adjustment of lysine values according to our prediction equation reduced the r value to -0.30. While it helped to reduce the effect of protein difference on lysine, the adjustment did not entirely eliminate it.

## Inheritance of Lysine

CI13449, the wheat with the highest adjusted lysine value in the World Collection, was crossed with Nap Hal. The lysine frequency

distribution for $F_2$ progeny bulks grown at Yuma, Arizona, in the $F_3$ generation appears in Figure 7. The mean lysine level of the Nap Hal parent variety was 3.23%, and, for CI13449, 3.35%. Eleven percent of the $F_2$ progeny bulks had adjusted lysine levels outside the parent range. Twenty-two rows exceeded 3.5%. These results suggest transgressive segregation for lysine in the cross and opportunity to select lines with higher lysine than either parent variety.

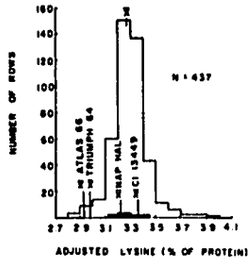


Figure 7. Frequency distribution for adjusted lysine per unit protein of $F_2$ progeny bulk rows of Nap Hal/CI13449 grown in the $F_3$ generation at Yuma, Arizona, in 1972

Lysine distributions for $F_2$ progeny bulks from Nap Hal/Atlas 66 grown in the $F_3$ and $F_4$ generations at Yuma, Arizona, are plotted in Figure 8. Since all protein values in both years exceeded 16%, lysine probably was not being differentially affected by protein differences. Therefore a standard adjustment at 0.2% was made to bring the lysine values to the 13% level of protein.

Atlas 66 is not known to be higher than normal in lysine. Nap Hal consistently produces grain that is 0.2 to 0.4% higher in lysine than Atlas 66 and other varieties with which it has been compared. The lysine frequency distributions shown in Figure 8 indicate segregation for lysine. The population mean in

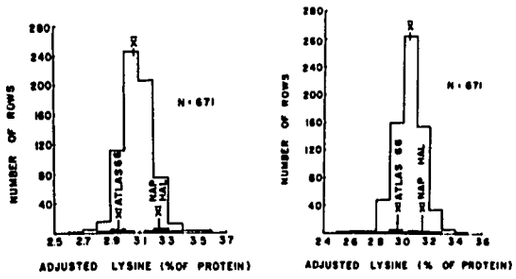


Figure 8. Frequency distribution for adjusted lysine per unit protein of $F_2$ progeny bulk rows of Nap Hal/Atlas 66 grown in the $F_3$ (left) and $F_4$ (right) generations at Yuma, Arizona in 1972.

each year approximated the mid-parent value. There was recovery of parental levels of lysine but little evidence of transgressive segregation as in the cross of Nap Hal/CI13449. More than 98% of the population in each year was within the parental range of lysine variation.

## Bioassay of Nutritional Quality

A mouse bioassay laboratory was established in 1971 to study high-protein and high-lysine wheats from our program. We will utilize it to establish the degree to which in-vitro laboratory analyses reflect true biological value.

Atlas 66-derived high-protein lines were bioassayed in 1972. As-yet-unexplained large variations in weight gain among animals receiving a common ration occurred during 28-day feeding trials. Test results were grouped according to the protein content of wheat samples being studied (Table 6). High-protein groups of samples produced higher mean weight gains and better feed-efficiency ratios than the lower protein groups.

Table 6. Mean mouse weight gains and feed efficiency ratios (FER) from wheat samples differing in protein content fed to weanling mice for a 28-day period (5 mice per sample)

| No. of samples | Protein range | Mean protein | Mean weight gain | Mean FER[1] |
|---|---|---|---|---|
| | % | % | g | |
| 14 | 17.0 - 18.6 | 17.5 | 15.1 | 8.6 |
| 16 | 15.1 - 16.8 | 16.1 | 12.9 | 9.4 |
| 14 | 13.5 - 14.8 | 14.3 | 13.5 | 9.3 |
| 5 | 10.8 - 12.9 | 11.7 | 10.5 | 11.1 |

[1]Feed consumption ÷ weight gain

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