AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523

**BIBLIOGRAPHIC INPUT SHEET**

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1. SUBJECT CLASSIFICATION

A. PRIMARY: Agriculture

B. SECONDARY: Cereal crops

2. TITLE AND SUBTITLE

Nebraska Research on Wheat Protein Improvement

3. AUTHOR(S)

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| 4. DOCUMENT DATE | 5. NUMBER OF PAGES | 6. ARC NUMBER |
|---|---|---|
| October 1974 | 10p. | ARC |

7. REFERENCE ORGANIZATION NAME AND ADDRESS

University of Nebraska
Dept. of Agronomy
Lincoln, Nebraska 68503

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*

9. ABSTRACT

Genetic factors and the production environment affect the protein content and amino acid composition of wheat grain. Fixed levels of protein in wheat cannot be achieved by breeding alone, but genetic potential for high protein can be manipulated genetically.

Genes for high protein in Atlas 66 were successfully transferred to a productive, disease-resistant experimental hard winter variety with excellent milling and baking characteristics. The variety produces grain with 1 to 2.5 percentage points more protein than ordinary varieties under Nebraska conditions. It is undergoing seed multiplication for probable release to growers in 1975.

One of the genes for high protein in Atlas 66 is closely associated with a gene for leaf rust resistance. Some association of the high protein trait from Atlas 66 with acid soil tolerance has been detected by researchers in the Netherlands.

Genes for high protein different from those in Atlas 66 have been identified and, when combined with the Atlas 66 genes, produce protein levels higher than that of Atlas 66. Most of the high-protein effect resides in the kernel endosperm and is retained in white milled flour.

10. CONTROL NUMBER

PN-AAB-263

11. PRICE OF DOCUMENT

12. DESCRIPTORS

Genetic factors, Production environment, Amino acid composition, Breeding, Diseas resistance, Milling, Baking

13. PROJECT NUMBER
931-17-130-471

14. CONTRACT NUMBER
AID/CSD-1208

15. TYPE OF DOCUMENT
Research paper

AID 590-1 (4-74)

# NEBRASKA RESEARCH ON WHEAT PROTEIN IMPROVEMENT[1]

by

V. A. Johnson[2]

Wheat protein research at the University of Nebraska dates to 1954 when it was established that the soft winter varieties 'Atlas 50' and 'Atlas 66' had genetic potential for significantly higher grain protein content than ordinary U. S. wheat varieties. Both varieties are derived from the Brazilian soft spring variety 'Frondoso' which was utilized for its resistance to leaf and stem rust. It is now well established that Frondoso also contributed the genes for high grain protein in Atlas 50 and Atlas 66.

Two considerations prompted our wheat protein research. The Hard Red Winter Wheat region of the United States, of which Nebraska is a part, has experienced during the last two decades significant depression of the protein content of the wheat produced -- especially in the High Plains portion in which little nitrogen fertilizer was used. The importance of wheat as a major provider of protein as well as calories was the second consideration.

Our research was supported initially by funds from the Nebraska Division of Wheat Utilization and Development. Since 1966, the Agency for International Development, U. S. Department of State, has provided major financial support. In the beginning, the research was concerned

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with increasing the protein content of wheat. It was broadened in 1966 to include modification of the amino acid composition of the wheat protein.

Success of the research required answers to numerous questions relating to protein. Our findings as they relate to these questions are discussed.

Is the high protein trait from Frondoso readily transferred to other wheat varieties?

Yes, it is. Our information suggests that a low number of major genes is involved -- perhaps as few as two. We base this on ease of recovery of parental levels of protein in segregating populations from crosses involving Atlas 66. We have computed heritability estimates for protein content as high as 0.8. Additive gene action appears to be important.

Have linkages of genes for high protein with undesirable traits been found?

None have been established to date. Some associations of protein genes from Atlas 66 with desirable traits have been found.

One gene for high protein in Atlas 66 is closely associated with a simply inherited dominant gene for leaf rust resistance. Atlas 50 and Atlas 66 were selected for resistance to leaf rust transmitted from the Frondoso parent variety. Both also have genes for high protein. High protein lines selected in Nebraska from Atlas 66/Comanche and Atlas 66/Wichita also carried the Atlas 66 leaf rust resistance.

Our data indicate the presence in Atlas 66 of at least one additional gene for high protein not associated with leaf rust resistance.

Atlas 66 possesses tolerance to high soil acidity derived from Frondoso. Mesdag and associates at Wageningen, The Netherlands, studied the association of high protein and soil acidity tolerance in $F_3$ and $F_4$ populations from Atlas 66 crossed with U. S. hard winter wheats. Since the high protein of Atlas 66 is controlled by a relatively small number of major genes and its soil acidity tolerance is due to more than one dominant gene, they interpreted a correlation coefficient of 0.39 between the two traits to mean that only parts of both complexes are genetically linked.

Atlas 66 produces grain with poor dough-mixing and bread-making properties. High protein $F_2$-derived families from Atlas 66/Comanche exhibited widely different dough-mixing properties indicating that the high protein trait was not linked with unacceptable mixing properties. Subsequent studies have shown this to be true. NE701132, a high protein hard red winter wheat selected from Atlas 66/Comanche//Lancer, has entirely acceptable bread wheat milling and baking properties.

## Is the high protein trait from Atlas 66 compatible with high yield?

High yields of wheat may be accompanied by depressed protein content of the grain. This has been interpreted by some as evidence that yield and protein are incompatible and that one can be achieved only at the expense of the other. Our data do not support this.

When yield and protein for each of nine varieties grown in the International Winter Wheat Performance Nursery (IWWPN) were correlated over 45 sites, yield level provided no predictive

value for protein content of the grain produced. Statistically significant negative and positive correlations were computed for a group of 24 varieties not known to possess genes for high protein at each of 13 international test sites. Only a small part of protein variation could be accounted for by differences in grain yield, even at sites where the correlations were the largest.

High protein lines selected from first breeding cycle crosses of Atlas 66 with U. S. hard red winter wheats at the Nebraska Agricultural Experiment Station were less productive than the better adapted hard winter varieties. However, we have been successful in combining high grain protein with excellent productivity and other acceptable agronomic traits in a second-cycle selection from the cross Atlas 66/Comanche//Lancer that is currently undergoing seed multiplication for probable commercial release in 1975. The new variety has excellent milling and baking quality.

## How stable is the high protein trait from Atlas 66?

Since environment strongly influences the protein content of wheat, fixed protein levels are no more assured by breeding alone than is high grain yield. Our experimental data indicate that varieties carrying the Atlas 66 genes will produce grain with higher protein content than comparably productive varieties grown in the same environment.

We compared the responses to nitrogen fertilizer of the high protein line CI14016 and Lancer grown on low fertility soils in

Nebraska during a 3-year period. Yield responses were minimal under the low rainfall test conditions, but there were strong protein responses in both varieties. The average protein content of each variety was increased by more than three percentage points with application of 120 pounds per acre of nitrogen. Most important, CI14016 maintained an approximate 2 percentage points protein advantage over Lancer throughout the entire range of nitrogen applications.

Atlas 66 and its winter derivatives have consistently produced grain with significantly higher protein content than other varieties tested in the IWWPN since 1969. This has been so even when they were compared with less productive varieties in the nursery.

How much genetic variability for protein content is there among the world's wheats?

We have systematically analyzed over 13,000 common wheats and 3,500 durums for protein and lysine since 1966. Total variation for protein ranged from 7 to 22 percent. Our data suggest the genetic component of this total variation to be no more than five percentage points of protein. Several genetically useful high protein varieties have been identified and are being used in our breeding program. One of these, 'Nap Hal', is of particular interest because it also produced grain with above-normal lysine content.

Do increases in the protein content of wheat affect the amino acid composition of its protein?

Increases in protein content up to 15 percent are associated with decreased lysine per unit protein. Further protein increases above 15% produce little, if any, changes in lysine per unit of protein.

Lysine is expressed on a grain weight basis, increases with protein content. Since lysine is the limiting essential amino acid in wheat, high protein wheat would be expected to be more nutritious than lower protein wheat because it contains more of this amino acid. Wheat with 17.2 protein provides more of each essential amino acid than does an equal amount of 10.0% protein opaque-2 maize.

Can the level of protein in wheat be further increased by breeding?

Strong evidence of transgressive segregation for high protein was obtained in the $F_3$-$F_5$ generations from a cross of the high protein varieties Nap Hal and Atlas 66. $F_2$ progeny bulks that were 2-4 percentage points higher in protein than the parent varieties were identified. $F_5$ selections from these also were significantly higher in protein content than the parent varieties.

We conclude from these data that Nap Hal and Atlas 66 have different genes for high grain protein content which together produce protein levels exceeding that of either variety. The development of wheat varieties with genetic potential for 5 percentage points higher protein than ordinary varieties may be possible.

**How much genetic variability for lysine is there in common and durum wheat?**

Variability for lysine per unit protein among 12,600 common wheats in the World Collection ranged from 2.2 to 4.0 percent. Most of this variation was subsequently shown to be environmentally induced and, therefore, non-fixable. A portion of the variation on the high side of the distribution was demonstrated to be an artifact of protein variation. The genetic component of total lysine variation appears to be no more than 0.5 percentage point. This is less than one-half the increase in lysine needed to bring it into reasonable balance with other essential amino acids in wheat protein. Lysine variability in World Collection durum wheats is similar to that in the common wheats.

We have obtained experimental evidence of transgressive segregation for high lysine in the $F_3$-$F_5$ generations from the cross Nap Hal/CI13449. CI13449 had the highest adjusted lysine value among common wheats in the World Collection. Selections from this cross are higher in lysine than ordinary wheats by 0.4 to 0.8 percentage point.

**Where in the wheat kernel do the high protein and high lysine effects reside?**

The high protein of Atlas 66 resides entirely in the starchy endosperm portion of the kernel. In the case of Nap Hal, the high protein effect appears in both starchy endosperm and non-starchy endosperm fractions. The very high protein selections from Nap Hal/Atlas 66 all have very high protein levels in the

starchy endosperm. This means that the effect will be maintained in the white milled flour.

The elevated lysine of Nap Hal appears to reside mainly in the non-endosperm fraction. In contrast, the high lysine effect in CI13449 is entirely in the starchy endosperm fraction of the kernel.

Does high protein in wheat result in higher nutritive value?

Limited data from feeding trials of weanling mice indicate that it does. High protein combined with high lysine, as seems to be the case in triticale, produces higher nutritive value than high protein alone.

Have high protein-high lysine materials from the Nebraska project been made available to breeders in other countries?

In 1974 we assembled 280 experimental lines that have genetic potential for high protein and/or high lysine. These were distributed to over 20 sites in the northern hemisphere and they will be sent to three sites in Latin America for local evaluation. The group of wheats include strong winter, weak winter, intermediate, and spring habit types. The wheats range in height from tall to semi-dwarf. We are requesting that a seed sample from each harvested row at each site be returned to us for protein and lysine analyses.

## SUMMARY

### NEBRASKA RESEARCH ON WHEAT PROTEIN IMPROVEMENT

V. A. Johnson

Genetic factors and the production environment affect the protein content and amino acid composition of wheat grain. Fixed levels of protein in wheat cannot be achieved by breeding alone, but genetic potential for high protein can be manipulated genetically.

The Agricultural Research Service, U. S. Department of Agriculture in cooperation with the Nebraska Agricultural Experiment Station and, with funding from the Agency for International Development, U. S. Department of State, is engaged in research on the nutritional improvement of wheat. The establishment of an International Winter Wheat Performance Nursery network in 1969 is a part of this research activity.

Genes for high protein in Atlas 66 were successfully transferred to a productive, disease-resistant experimental hard winter variety with excellent milling and baking characteristics. The variety produces grain with 1 to 2.5 percentage points more protein than ordinary varieties under Nebraska conditions. It is undergoing seed multiplication for probable release to growers in 1975.

One of the genes for high protein in Atlas 66 is closely associated with a gene for leaf rust resistance. Some association of the high protein trait from Atlas 66 with acid soil tolerance has been detected by researchers in the Netherlands.

Genes for high protein different from those in Atlas 66 have been identified and, when combined with the Atlas 66 genes, produce protein levels higher than that of Atlas 66. Most of the high-protein effect

resides in the kernel endosperm and is retained in white milled flour.

Modest but useable genetic variation for lysine has been found among 12,600 common wheats in the World Collection maintained by the Agricultural Research Service, USDA. Lines have been selected from a cross of two high lysine varieties that exhibit lysine levels higher than that of either parent variety.

Protein content of wheat is strongly affected by soil nitrogen availability. At most fertility levels, genes from Atlas 66 promote protein content 2 to 3 percentage points higher than the protein content of ordinary varieties.

Increases in protein content up to 15% protein are associated with decreased lysine per unit protein. Further increases in protein content above 15% do not affect lysine level. Higher protein in wheat increases the amount of lysine per unit weight of grain and promotes higher weight gains in weanling mice.

Experimental lines of wheat with unusual combinations of high protein and/or high lysine were made available to breeders in other countries in 1974.