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Johnson,V.A.; Schmidt,J.W.; Mattern,P.J.

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Cereal Breeding for Better Protein Impact[1]

V. A. JOHNSON, J. W. SCHMIDT, AND P. J. MATTERN[2]

The problem of nutrition in the world is related primarily to a shortage of protein. Protein efficiency on a nutritional scale is determined by the content and balance of those amino acids that are essential for tissue synthesis and body growth. Animal proteins are nutritionally complete proteins, whereas many plant proteins are deficient in one or more essential amino acids. The major cereal food crops of the world, wheat, rice, corn and sorghum, are deficient in lysine. Each is deficient in other essential amino acids as well.

Adequate protein balance from plant sources can be achieved by selection of plant foods that complement one another in essential amino acids. For example, soybeans are rich in lysine and can complement the lysine-deficient cereals to provide a nutritionally improved diet. Unfortunately, the likelihood of achieving protein balance through selection of plant foods is remote in those countries in which dietary deficiencies are the most acute. Economic limitations and lack of understanding of nutritional requirements rule out food selection as a realistic approach to improved diets.

Improved protein balance could also be accomplished by the addition of lysine or other essential amino acids directly to the processed cereals. This approach is receiving serious consideration by some agencies (2). The probable shortcoming of the approach again relates to poor understanding of nutrition among people and the difficulty of implementation in those areas of the world that are in the greatest need.

Better nutrition for the millions of people who rely upon the cereals as their principal food may well depend upon the improvement of the inherent nutritional quality of the cereals. Recent research developments in the cereals hold out the hope for early attainment of this goal.

The plant breeder relies upon genetic variability for the improvement of plants through breeding. He has successfully utilized such variability to accomplish spectacular improvements in the productiveness of wheat, corn, sorghum and the other cereals. The cereal breeder who seeks to improve nutritional quality must likewise assess the magnitude of genetic variability and find ways to utilize it if his efforts are to be successful. There has been a notable lack of information on the existing genetic variation in quantity and nutritional quality of the protein in cereal crops. This lack of information has been due, in part, to the lack of rapid measurement techniques. For example, assessment of genetic variability in amino acid content of protein has had to await the development of an amino acid analyzer that would permit efficient and rapid analyses.

The cereal breeder who seeks to improve nutritional quality must be concerned with both the quantity and quality of the protein. Increases in quantity of protein in a cereal species are of limited value if achieved at the expense of a less favorable balance of essen-

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[2] Research Agronomist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and Professor of Agronomy, University of Nebraska; Professor of Agronomy; and Associate Professor of Agronomy, University of Nebraska, respectively.

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tial amino acids. Less than full value is realized from improved amino acid balance, if it is associated with decreased quantity of protein. Similarly, yield itself cannot be ignored in breeding efforts to improve nutritional quality of cereals, because the amount of protein available for food purposes is determined largely by the quantity of grain produced. Also, the acceptance of nutritionally improved types and varieties by farmers will be determined primarily on the basis of productivity.

Quantity and nutritional quality of protein in the cereals have received little attention by breeders until recent years. It has been known for a long time that the crude protein content of corn could be doubled by selection. However, since the increased protein was largely in the form of the nutritionally poor zein, there was little breeding effort to increase the protein content of commercial lines and hybrids. In wheat, environmental effects on protein quantity were large compared to genetic differences. Consequently, breeding for high protein in wheat was considered by breeders to be an unfruitful line of endeavor. Efforts to improve the nutritional quality of cereal proteins by breeding have had to await improved technology for protein constituent analyses.

Protein Research in Corn

The Opaque-2 and Floury-2 Effects

The discovery by Nelson and Mertz in 1964 that the gene opaque-2 significantly increases the lysine and tryptophan content of corn has had a significant impact on corn breeding (10). The opaque-2 gene causes a floury condition of the endosperm. A second endosperm gene, floury-2, also increases the level of lysine in the corn protein (14). The two genes are indistinguishable phenotypically. It is ironical that the opaque-2 gene was identified more than 30 years ago but its effect on lysine content remained unknown until 1964. Since ordinary corn is deficient in both lysine and tryptophan, there is likelihood that increases in these two amino acids produce a proportional increase in the overall nutritive value of corn protein. The impact of high lysine corn will be the greatest in the tropical belt around the world where corn is the major source of food for 200 million people (18).

The opaque-2 corn stocks have been distributed widely. The o_2 gene is being incorporated into locally adapted lines and varieties by corn breeders in many countries. The International Maize and Wheat Improvement Center in Mexico is analyzing its collection of Latin American corn to determine the extent to which the o_2 and fl_2 genes are present, and to ascertain whether there are additional genes which affect quality. The o_2 gene is being transferred to five outstanding lowland racial complexes of maize identified by the Center (18). Wellhausen has suggested that the effect of the o_2 and fl_2 genes on protein quality may explain, in part, why the Latin American Indians preferred and maintained the floury types of corn.

Opaque-2 kernels are usually lighter in weight than normal kernels on the same ear. However, in certain genetic backgrounds o_2 kernels are equal in mass to their normal siblings (1). Thus, it would seem that the kernal weight deficiency associated with the o_2 gene probably can be overcome.

Nelson studied the o_2 fl_2 double mutants in corn (13). The double mutant kernels were translucent, rather than opaque, and were nearly normal in appearance. They were only slightly lower in lysine than o_2 o_2 and about the same for the other amino acids. Nelson has suggested that the o_2 fl_2 double mutants may be the key to the economic production of high quality protein in corn in the United States. Dense endosperm corns possessing large amounts of corneous starch and high quality protein should be possible via the double mutant approach.

Purdue research also has demonstrated that o_2 lines can be constituted that have high levels of high quality protein. This is shown in Table I. From the cross of Illinois High Protein (IHP) with opaque-2, there was recovery of a line possessing the high lysine of the o_2 parent combined with the high protein of the IHP parent. The lysine level of the recovery line was equal to that of the o_2 parent and its protein content was more than doubled over the o_2 parent.

It also has been shown that the floury-2 gene exerts its effect in a high protein background. This may be seen in Table II.

Floury kernel segregates which would be composed equally of the floury gene in two and in three doses, combine moderately high lysine with moderately high protein.

Many corn breeders believe that yield is reduced by selection for high protein. This is based mainly on data published in 1952 (19). Evidence obtained by Nelson that the high lysine and high protein traits can be combined in corn provides strong incentive for a thorough re-examination of the yield-protein level question Improved nutrition for 200 million people may be at stake.

Nutritional Studies

The projected improvement in the nutritive value of high lysine opaque-2 corn has been demonstrated in nutrition trials. Rat feeding data indicate that opaque-2 corn has a protein efficiency ratio 62 to 110% greater than ordinary corn and, when fed at a 15% protein level, is equal to milk protein supplemented with cystine (11, 12). Differences of similar magnitude in favor of opaque-2 corn over ordinary corn have been recorded in swine feeding experiments (15). Human nutrition studies have indicated the nutritional value of opaque-2 corn to be 90% of skim milk for children (3). In adults, the regression of nitrogen retention on the quality of the corn consumed was linear which indicates the contribution of additional lysine to the nutritive value of the corn (4).

Protein Research in Sorghum

Sorghum exhibits a wide range in the protein content of its seed. Protein levels ranging from 8 to 18% were reported in 1964 (5). There has been little study of the amino acid composition of sorghum protein, nor has there been serious breeding effort to modify the protein content and composition of sorghum, until recently.

Purdue University, in 1965, initiated a comprehensive investigation of the inheritance and improvement of protein quality and content in sorghum The research is supported by funds from the Agency for International Development, United States State Department The project involves a search for new sources of high protein in the world collection of sorghums and amino acid analyses of promising materials in the collection. Particular attention is being given to the amino acids lysine, threonine, tryptophan, methionine, and isoleucine. Lines with the highest levels of these essential limiting amino acids will be combined with high protein lines as the first step in the development of productive varieties and hybrids with superior nutritional quality.

Variation in crude protein ranging from 7.4 to 25.9% was found among the first 400 sorghum samples analyzed from the world collection (R. C. Pickett, pers. comm.). Ly-

TABLE I

PROTEIN AND LYSINE CONTENT OF A RECOVERY LINE FROM OPAQUE-2 CROSSED WITH ILLINOIS HIGH PROTEIN (DATA FROM NELSON, HIGH-LYSINE CORN CONFERENCE)

Endosperm constituent	Illinois High Protein	O_2 recovery line from ((IHP × o_2) × IHP) ⊗	opaque-2
Protein (%)	20.4	17.4	7.9
Lysine (mg/g protein)	1.3	3.6	3.6

TABLE II

PROTEIN AND LYSINE CONTENT OF TRANSLUCENT AND FLOURY RECOVERY LINES FROM FLOURY-2 CROSSED WITH ILLINOIS HIGH PROTEIN (DATA FROM NELSON, HIGH LYSINE CORN CONFERENCE)

Endosperm constituent	Illinois High Protein	(IHP × fl_2) ⊗		floury-2
		Translucent	Floury	
Protein (%)	20.4	17.8	17.0	13.6
Lysine (mg/g protein)	1.3	1.5	2.7	3.3

sine values ranged from 0.54 to 2.84 g per 100 g protein in the group. Subsequent analyses of additional samples from the collection have revealed lysine contents as high as 3.80 g per 100 g protein. Several relatively good combinations of high protein and high lysine have been found. Substantial variations in threonine, methionine, and leucine levels were detected. Lines with the highest yields and protein and lysine contents are being subjected to complete amino acid analyses.

The early results from the Purdue sorghum protein research are encouraging. Evaluation of new genetic combinations under different levels of soil fertility will be made in 1967. Promising lines and combinations are being increased for nutritional studies.

Protein Research in Wheat

The first good evidence of the existence of large inherent differences in the protein content of wheat was obtained by Middleton et al. (9) who reported in 1954 that 'Atlas 66', a leaf-rust-resistant soft winter wheat variety, synthesized significantly more protein in its grain than other soft winter varieties. In comparisons with hard winter wheats in Oklahoma and Texas, 'Atlas 66' produced more protein in its grain but was significantly lower in yield than the hard winter varieties in most tests.

Cooperative research to further study 'Atlas 66' and utilize it in breeding for higher protein in bread wheats was initiated at the University of Nebraska in 1956 (6, 7, 8, 16, 17). Our protein research program now encompasses the analyses of amino acid composition in our high protein wheat selections and the systematic screening of 16,000 wheats in the world collection to identify heritable protein and lysine differences. Our discussion of protein breeding in wheat will be based mainly on the research results from the ARS-Nebraska program.

Genetic Studies of High Protein

'Atlas 66' was initially crossed with 'Comanche' and 'Wichita', two commercially grown hard red winter varieties. Selections from these crosses have been utilized in our genetic and physiologic studies of the high protein phenomenon in wheat.

The grain protein content of individual F_2 and parent plants was assayed (Fig. 1).

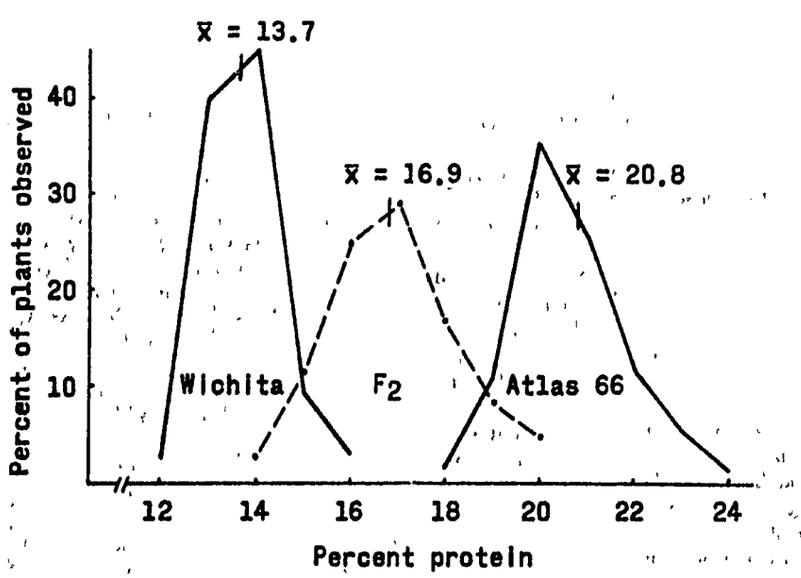

Fig. 1. Grain protein frequency distribution of plants of 'Wichita' and 'Atlas 66' and their F_2 generation grown at Lincoln, Nebraska, in 1957.

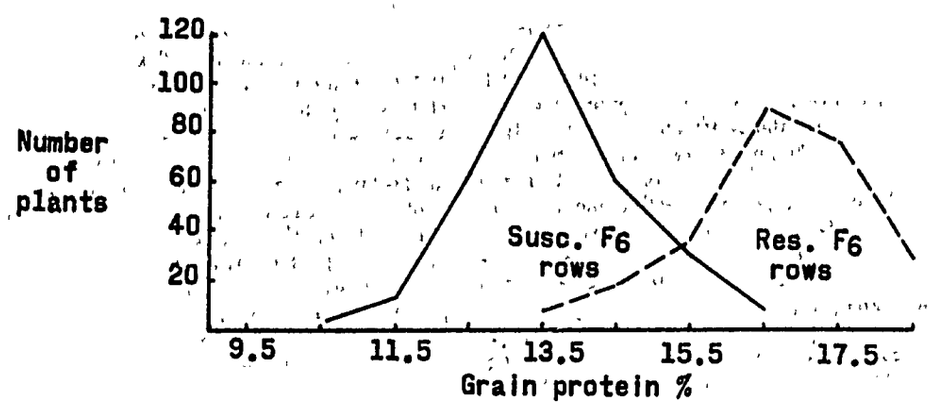

Fig. 2. Grain protein frequency distribution of F_6 rows of 'Atlas 66' × 'Wichita' homozygous for reaction to leaf rust.

The mean protein content of 'Atlas 66' was nearly 21% but 'Wichita' averaged less than 14%. The F_2 segregates ranged from 14 to 20% with a mean of 16.9%. The F_2 distribution approached normality.

'Atlas 66 'is resistant in the adult stage to the physiologic races of leaf rust (*Puccinia recondita*) prevalent in central United States. 'Wichita' is susceptible to these races. Leaf-rust susceptible F_6 lines produced grain that averaged only 13.5% protein (Fig. 2). Leaf-rust-resistant lines average nearly 17.0% protein This suggests a close genetic linkage between high protein and leaf rust resistance. Similar results have been obtained by Caldwell and associates at Purdue University. In no instance have we recovered high protein lines from 'Atlas 66' crosses that are susceptible to leaf rust. Some susceptible lines with grain of intermediate protein content have been identified, indicating the operation of more than one major gene for high protein. However, the ease with which we recovered high protein lines would suggest that the number of major genes for high protein in 'Atlas 66' is not large.

Grain yield and grain protein content in wheat tend to be inversely related. Unproductive high protein lines would be questionable as genetic sources for high protein. The yields and protein contents of two 'Atlas 66' × 'Comanche' lines and the varieties 'Scout' and 'Warrior' were compared during a three-year period. Results are summarized in Table III. The lines were more productive on the average than 'Warrior' but somewhat less productive than 'Scout.' Their grain was nearly 3% higher in protein than either variety These and other data convince us that significant increases in the protein content of wheat without sacrificing yield can be achieved through breeding. We have recovered the high prot trait in productive lines that exhibit an array of bread wheat milling and baking properties (8).

Physiologic Basis of High Protein in Wheat

Establishment of the existence of genes for high protein from 'Atlas 66' raised the question of the physiologic basis of high

TABLE III

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	Grain yield	Protein content*
	May	bu /a.	%
Scout	19	41	13.6
60306	19	38	16.1
60305	22	36	16.5
Warrior	22	31	13.4

*Calculated at 14% moisture.

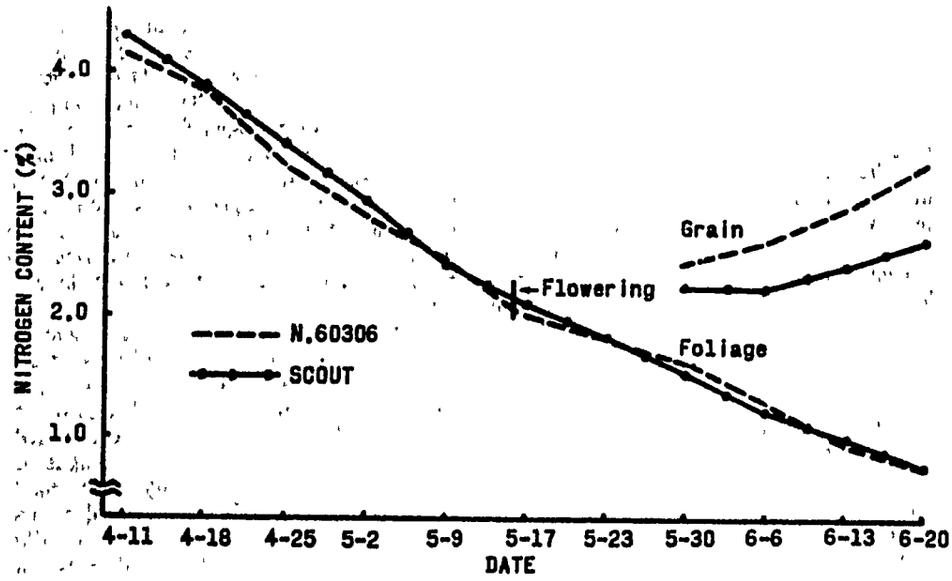

Fig. 3. Nitrogen content of the foliage and grain of two winter wheat varieties at Lincoln, Nebraska, 1963.

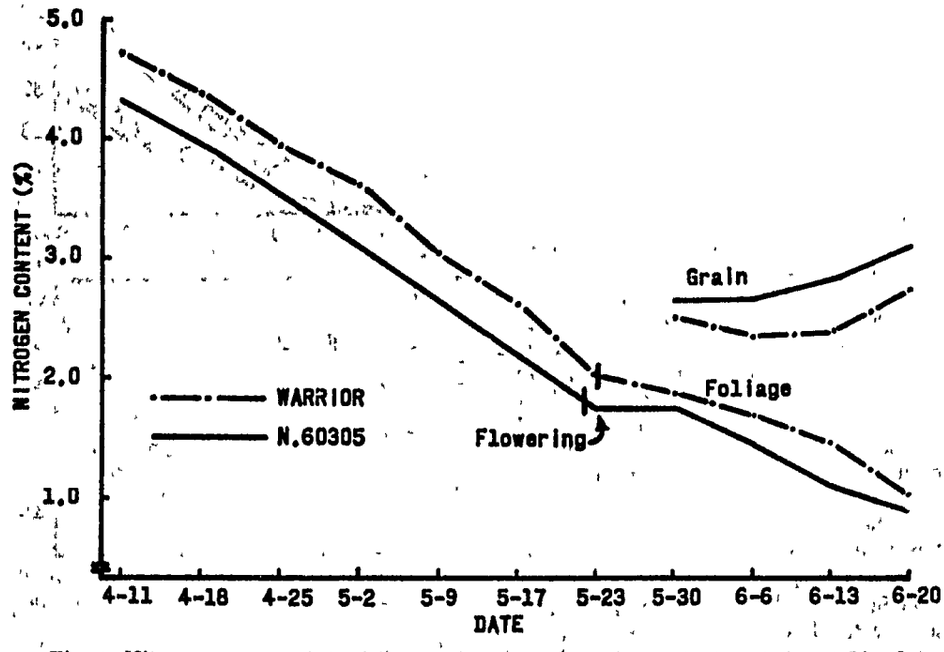

Fig. 4. Nitrogen content of the foliage and grain of two winter wheat varieties at Lincoln, Nebraska, 1963.

protein in wheat. Was the high-protein trait associated with greater nitrogen uptake and accumulation in the wheat plant; or was it the result of more efficient and complete translocation of nitrogen from the plant to its grain? The usefulness of the trait would be determined, in part at least, by answers to these questions. If differential nitrogen uptake was involved, high availability of soil nitrogen probably would be required for phenotypic expression of the protein genes. Much wheat in the world is produced on relatively infertile, low-nitrogen soils. If, on the other hand, differential translocation of nitrogen in the plant was the basis for high-protein grain, then the trait could be expected to express itself phenotypically in an array of production situations.

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' and the the variety 'Scout' are shown in Fig. 3. At no time during the spring growing season were there significant differences in the plant nitrogen contents of the two varieties. Yet, from the early stages of kernel development, the grain of '60306' 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.'

Nitrogen relations in '60305' and 'Warrior' are compared in Fig. 4. 'Warrior' characteristically produces moderately low protein grain. It was surprising to discover that the plants of 'Warrior' contain significantly more nitrogen than the high protein 'Selection 60305' and other varieties with which it was compared. 'Selection 60305' with a much lower plant nitrogen content produced grain which was significantly higher in nitrogen throughout the entire grain maturation period. Four additional years of experimentation with these anl other varieties have produced similar results each year.

We conclude from our data that the high protein genetic trait in wheat is not associated with differential nitrogen uptake or nitrogen accumulation in the plant. We have good evidence that varieties may differ in their plant nitrogen content but such differences appear to be unrelated to inherent grain protein differences. The evidence strongly points to more efficient and complete translocation of nitrogen from the plant to the grain as the physiologic basis of high grain protein. Varieties possessing the 'Atlas 66' genes could be expected to exhibit superior levels of grain protein over a range of fertility situations. This is supported by several years of data from the Nebraska stations.

Grain weight and grain nitrogen content in relation to total plant weight and total plant nitrogen of 'Selection 60305' and 'Warrior' are shown in Fig. 5. The grain of 'Selection 60305' and 'Warrior' constituted approximately 30% of the total weight of the plant at maturity. Only 56% of the total plant nitrogen of 'Warrior' resided in its grain at maturity, whereas in '60305' slightly more than 60% of the total nitrogen was in

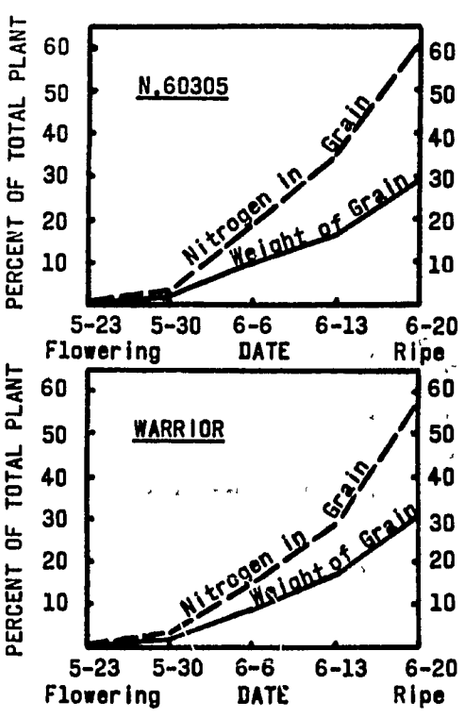

Fig. 5. Grain weight and grain nitrogen content in relation to the total plant weight and total plant nitrogen during grain maturation.

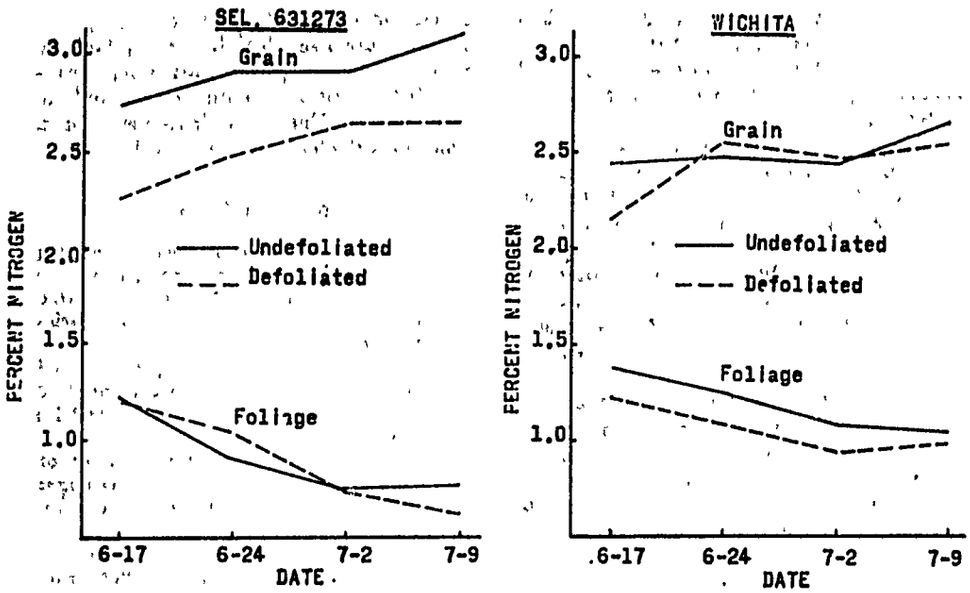

Fig. 6. Nitrogen content of the foliage and grain of defoliated and undefoliated plots of high protein 'Selection 631273' and 'Wichita.'

its grain. This is further evidence for differential nitrogen translocation as the basis for high grain protein.

The leaves of the wheat plant constitute an important repository for nitrogen from which it may move, probably in the form of amino acids, to the wheat kernels during grain maturation. Diseases, insects, or other conditions that would prematurely defoliate the wheat plant could adversely affect the level of protein in the grain. To test this, we artificially defoliated varieties at heading time. The effect of defoliation on the high protein 'Selection 631273' and 'Wichita' is shown in Fig. 6. Defoliation produced a sharp reduction in the grain nitrogen content of '631273', whereas the effect on the lower protein Wichita was negligible. Defoliation of '631273' reduced its grain nitrogen level to approximately that of 'Wichita.' The importance of foliage retention for the phenotypic expression of high protein in wheat is indicated by these data.

Nutritional Quality of the Protein

The nutritional value of protein is determined by the balance of its constituent essential amino acids. Protein will be utilized in relation to the level of its first limiting essential amino acid. If high protein wheats contained a less favorable balance of these essential amino acids, their value would be reduced considerably. The levels of lysine, methionine, and threonine would be particularly critical since these are the first limiting essential amino acids in wheat. The most promising high protein lines from the ARS-Nebraska program were subjected to amino acid analyses. Results of the lysine, methionine, and threonine assays appear in Table IV.

'Atlas 66' and 'Comanche' are similar in lysine and threonine but 'Atlas 66' is substantially lower 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.

It seems clear from our data that high protein in wheat need not be associated with

an altered, less favorable amino acid balance. On the contrary, the data would indicate that selection for improved amino acid balance might be effective in high protein materials.

The Nebraska Experiment Station is engaged in the systematic analyses of all the common wheats in the World Collection maintained by the United States Department of Agriculture, in an effort to identify genetic sources of high lysine and new sources of high protein. The research is being supported by the Agency for International Development of the United States Department of State.

Wheats in the World Collection found to have high lysine will be grown in several different environments to assess the stability of level of lysine and its relationship to protein level. Those which exhibit consistently high lysine properties will be used in crosses with high protein wheat to achieve nutritionally superior varieties.

Efforts to improve the nutritional quality of the cereals are not limited to corn, sorghum, and wheat. The International Rice Research Institute in the Philippines is engaged in nutritional studies of rice. The world rice collection is being surveyed for differences in protein and lysine content. A promising source of high protein in oats was recently discovered.

Research on nutritional quality has added a new dimension to cereal improvement. Rapidly accumulating evidence indicates that the biological scientist can approach this new area of research with reasonable expectation of success. The need is urgent. The tools are available. Nature apparently has provided the genetic variability necessary for nutritional improvement of the cereal species. These are ingredients of a tremendous challenge for the cereal breeder.

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TABLE IV

LYSINE, METHIONINE, AND THREONINE LEVELS IN SELECTED PROTEIN LINES OF 'ATLAS 66' × 'COMANCHE'

Variety	Protein % DWB*	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
Atl 66 × Cmn 2507	17.7	3.72	1.74	2.62
Atl 66 × Cmn 2509	18.3	3.45	1.83	3.32
Atl 66 × Cmn 2504	17.9	3.38	1.14	3.69
Atl 66 × Cmn 2510	16.5	3.37	1.67	3.22
Atl 66 × Cmn 2499	18.2	3.29	1.68	3.10
Atl 66 × Cmn 2500	18.3	3.20	1.65	3.16

*Percentage of total dry weight.

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