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WHEAT PROTEIN IMPROVEMENT

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## Wheat protein improvement

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

Protein increases in wheat as large as 25 percent have been achieved by breeding. Atlas 66 has been the main genetic source of high protein in the ARS-Nebraska program. At least two genes condition protein level in Atlas 66. One of them is linked with a gene for leaf-rust resistance. Although the level of protein in wheat is variable due to environment, the protein advantage of lines derived from Atlas 66 over other similarly grown wheats persists in a wide array of environments. High protein in wheat is compatible with high yield, desirable agronomic traits, and satisfactory processing quality. New sources of high protein have been identified. High protein wheats provide more lysine and other essential amino acids per unit weight of grain than do ordinary wheats. At low protein levels, lysine per unit protein is negatively correlated with protein but no significant correlation exists at high protein levels. Lysine differences ranged from 2 to 4 percentage points among 15,000 common and durum wheats in the USDA world collection. The genetic component of lysine variation appears to be only 0.5 percentage points. High lysine in wheat protein is mainly compensated for by lower levels of the non-essential amino acids, glutamic acid and proline.

### INTRODUCTION

A short supply of amino acid lysine is the principal nutritional limitation of wheat protein.

Cooperative work between the Agricultural Research Service of the U.S. Department of Agriculture and the Nebraska Agricultural Experiment Station to improve the nutritional value of wheat began in 1954 when Atlas 66, a soft winter wheat from North Carolina, was introduced into our breeding program. Middleton, Bode, and Bayles (1954) showed that Atlas 66, selected from the cross Redhart x No11/2 x Frondoso, had more protein in its grain than other soft wheats. In our early research we investigated the heritability of grain protein, the magnitude and stability of the genetic effect, the relationship of grain protein to yield and processing characteristics, and the physiology of high protein content in wheat. Funds from the Nebraska Division of Wheat Development Marketing and Utilization and the Northern Utilization Research and Development Division of USDA aided this early research.

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The ARS-Nebraska protein research was broadened in 1966 to include investigation of the amino acid composition of wheat protein. Supported by funds from the U.S. Agency for International Development, we screened the USDA world collection of wheats for protein and lysine differences and expanded our breeding program for wheat with improved protein. We established an International Winter Wheat Performance Nursery (fig. 1) in 1969 to identify superior winter wheat genotypes and to measure the impact of environment on nutritional quality.

The difference between nutritional quality and milling and baking quality should be clearly understood. The latter refers primarily to the suitability of wheat varieties for a highly mechanized wheat food processing industry. It has little to do with nutritional value.

The nutritional improvement of wheat protein may not be entirely compatible with accepted standards of bread wheat processing quality of the western world. High-grade white flour is composed largely of kernel endosperm. Endosperm protein, however, is relatively poor in lysine (2%) compared with the non-endosperm proteins (over 4%). Since the non-endosperm proteins are mostly eliminated from wheat flour during milling, increasing their lysine content would not change the lysine content of wheat flour much.

In countries where the whole wheat grain is used for food, the site of the lysine-rich protein in the wheat kernel would be of little consequence. Increases in the quantity of any of the proteins or increases in their lysine content would significantly raise the nutritional value of the wheat.

#### GENETIC VARIATION IN PROTEIN

We have identified substantial genetic differences in the grain protein content of wheat. The source of high protein most extensively used in our program has been Atlas 66. We have been able to raise the level of grain protein by as much as one-fourth in selections from crosses of Atlas 66 with hard winter wheat varieties (Johnson et al., 1963). The high protein trait is conditioned by more than one

Table 1. Average grain yield and protein content of Lancer, a normal wheat variety, NE65307, and a high protein wheat variety, grown with various nitrogen fertility levels in western Nebraska (USA) in 1969 and 1970.

Nitrogen applied (kg/ha)	Yield (t/ha)			Protein* (%)		
	Lancer	NE65307	Difference	Lancer	NE65307	Difference
0	2.57	2.55	-0.02	10.8	12.6	1.8
22	2.96	2.71	-0.25	11.2	13.3	2.1
44	3.11	2.92	-0.19	11.8	14.0	2.2
66	3.12	2.97	-0.15	12.6	14.9	2.3
88	3.09	3.01	-0.08	13.2	15.4	2.2
110	3.05	3.02	-0.03	13.6	15.8	2.2
132	2.99	3.08	0.09	14.1	16.2	2.1

\*Nitrogen x 5.7. Measured at 14% moisture.

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1. Fourth International Winter Wheat Performance Nursery -- 44 sites in 27 countries.

gene. The ease of recovery of high protein segregates from crosses involving Atlas 66 indicates that the number of genes is not large.

A major gene for high protein in Atlas 66 is closely linked with a gene for leaf rust resistance in adult plants. We have recovered no high protein lines that are susceptible to leaf rust. Our recovery of lines with intermediate protein that are either resistant or susceptible to leaf rust provides evidence of the existence of a second gene for high protein that is not linked with resistance to leaf rust.

### **Heritability of protein**

Environment has a large influence on grain protein. The heritability of protein level is not as high as the heritability of other economic traits in wheat. We have computed estimates of heritability ranging from 0.3 to 0.8 depending on the method of determination (Stuber, Johnson, and Schmidt, 1962).

### **Stability of protein**

Grain protein level cannot be genetically fixed in wheat any more than grain yield can be fixed. The environment in which the wheat is grown is the major influence on both traits. But the genetic potential for high protein and high yield can be built into varieties. We have determined that high protein selections from our program will produce grain with more protein than ordinary wheats grown in the same environment. Our data indicate that the protein genes from Atlas 66 do not affect nitrogen uptake by roots. Rather, they promote more efficient and complete translocation of nitrogen from the plant to its grain (Johnson, Mattern, and Schmidt, 1967).

Evidence of the relative stability of the high protein trait is provided by 2 years of data from Nebraska tests in which a high protein line, NE65307, was compared with variety Lancer with a wide range of nitrogen fertilization rates (Table 1). NE65307 maintained a consistent protein advantage of 2 percentage

**Table 2. Average grain yield, protein, and lysine content of nine wheat varieties grown in an international Winter Wheat Performance Nursery in 1969 and 1970.**

Variety	Yield (t/ha)	Protein <sup>a</sup> (%)	Lysine content	
			(% of protein)	(g/100 g grain)
Atlas 66	3.08	17.9	2.7	0.48
Purdue 28-2-1	3.00	17.5	2.8	0.49
NE67730	3.15	16.9	2.8	0.47
Triumph 64	3.28	15.2	2.8	0.43
Scout 66	3.66	14.5	2.9	0.42
Winalta	3.02	14.1	2.9	0.41
Bezostaia	4.34	13.8	2.9	0.40
Gaines	2.87	13.3	3.0	0.40
Yorkstar	3.34	12.8	3.1	0.40
LSD (5%)	6.4	1.1	0.3	—

<sup>a</sup>Dry wt basis.

points over Lancer at increasingly high levels of protein induced by the nitrogen fertilization.

Further evidence for the relative stability of the high protein trait in diverse environments comes from International Winter Wheat Performance Nurseries grown in 1969 and 1970 (Table 2). Atlas 66, Purdue 28-2-1, and NE67730, all of which possess genes for high protein in common, maintained an average protein advantage of 1.7 to 4.1 percentage points over other varieties with comparable grain yields. The high protein trait was expressed equally well at low yielding and high yielding nursery sites.

## COMPATIBILITY OF HIGH PROTEIN WITH OTHER TRAITS

### High protein and bread quality

Milling wheat into flour reduces the protein content of the flour below that of the whole grain. The protein reduction ranges from 0.5 to 1.5 percentage points or roughly 10 percent. High protein wheats derived from Atlas 66 exhibit the same magnitude of reduction in protein from milling as do lower protein varieties (Johnson et al., 1963). Thus the high protein trait involves an increase of protein in the endosperm portion of the wheat kernel that permits the protein advantage to persist after milling.

High protein selections from our first cycle of breeding did not possess adequate processing quality. Most lacked the dough development and baking properties required of American bread wheats. The selections, however, had substantial variation for individual quality traits which suggested that problems of combining high protein with satisfactory processing quality would not be insurmountable. This has been borne out in selections from the second and third breeding cycles (Johnson, Mattern, and Schmidt, 1971).

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### High protein and high grain yield

High protein selections from the first breeding cycle lacked the productivity of popular commercial varieties in Nebraska. In addition, they were too tall, lacked straw strength, and had insufficient resistance to stem rust (Johnson, Mattern, and Schmidt, 1970). One of the selections, NE67730, tested in the International Winter Wheat Performance Nursery was substantially less productive than Bezostaja and several other varieties in the nursery (Stroike et al., 1971). Twenty-six of the first-cycle lines were released as germ plasm by the Nebraska Agricultural Experiment Station and ARS in 1970. The pattern of yield response of one of the lines, NE67730, to nitrogen fertilizer was different than that of Lancer in Nebraska fertilizer trials (Table 1). NE67730 was less productive than Lancer at low levels of fertilizer, but was equal to Lancer above 110 kg/ha N.

Second-cycle, high protein selections currently under evaluation show much more promise. One selection, NE701132, made an average yield of 3.96 t/ha at three sites in Nebraska in 1970 compared to only 3.45 t/ha for Scout 66. Its protein advantage over Scout 66 was 2.3 percentage points or 23 percent. NE701132 and other productive second-cycle, high protein lines combine satisfactory processing quality with moderately short stature and combined resistance to leaf and stem rust (Johnson et al., 1971).

### HIGH GRAIN PROTEIN AND AMINO ACID COMPOSITION

Analyses of the USDA world collection of common and durum wheats showed a negative correlation between lysine, expressed as a percentage of protein, and protein. The coefficient for 7,000 common wheats was  $-0.63$ . The ratio of gluten to water-soluble proteins and salt-soluble proteins in the kernel endosperm may be involved. The water-soluble and salt-soluble proteins are high in lysine (over 4%), but the gluten proteins are very low (less than 2%). The ratio of water-soluble protein to gluten-protein varies. Low protein wheats usually have a higher percentage of water-soluble protein. This probably explains the tendency of low protein wheats to have a higher percentage of lysine in their protein.

When lysine is expressed as a percentage of dry grain weight, its correlation with protein is strongly positive. The coefficient for 7,000 common wheats was  $+0.83$ . Obviously, the tendency for protein to be negatively correlated with lysine per unit protein is not sufficient in wheat to overcome the expected increase in lysine per unit weight of grain associated with an increase of protein. This is especially important because it suggests that high protein wheats should provide more lysine per unit weight of grain than wheats with lower protein.

We have used linear regression to adjust lysine values to a common protein level. This technique permits lysine comparisons among wheats that differ in protein content. It is necessary in breeding for higher lysine because it largely overcomes the direct effect of protein on level of lysine. Lysine values, unadjusted for protein variations, could be misleading from a genetic standpoint.

Amino acid profiles were compiled for several of our first-cycle, high protein lines (Mattern et al., 1968). Some lines possessed levels of lysine, methionine,

and threonine, expressed as a percentage of protein, that were equal to those of the low protein parent. Other lines did not equal the low protein parent. When lysine was expressed in terms of dry grain weight, however, all lines had higher values than their low protein parent in these essential amino acids.

The amino acid profiles of some high protein lines from the second breeding cycle also have been analyzed. As with the first-cycle lines, the lines varied in their level of lysine per unit protein. Most were superior to a standard variety in the amount of lysine synthesized per unit weight of grain.

### GENETIC SOURCES OF HIGH PROTEIN

Twenty-six high protein lines derived from Atlas 66 were released as germ plasm in 1970. Twelve second-cycle, high protein lines were increased in Nebraska and Arizona in 1971. They will be extensively tested in Nebraska and in regional trials. The best lines will be nominated for testing in the International Winter Wheat Performance Nursery. A portion of the 1971 increase seed has been sent to Turkey, Iran, and Afghanistan for agronomic evaluation. The lines combine high protein with outstanding productivity, good agronomic and processing-quality traits, and field resistance to leaf rust and stem rust under Nebraska conditions. Atlas 50, a sister selection of Atlas 66, was used in Kansas and has led to some promising high protein experimental lines.

Other wheats that appear to possess genes for high protein have been identified. One of these, Aniversario, comes from South America and may have a gene or genes for protein in common with Atlas 66 and Atlas 50. A Nebraska male-fertility restorer line, NE542437, also is a potential new genetic source of high protein. It has consistently produced grain that is higher in protein than normal varieties. In addition, it transmits the full effect to its  $F_1$  hybrids. Since the line and all of its hybrids tested to date possessed male-sterile cytoplasm, high

Table 3. Protein and lysine data for four wheat varieties grown for 18 station-years at sites in the United States from 1967 to 1970.

Variety	Protein <sup>a</sup> (%)		Lysine content <sup>b</sup> (%)		Adjusted lysine content (%)	
	Mean	Range	Mean	Range	Mean	Range
<i>Initial sample — world collection</i>						
Justin	17.0	—	2.9	—	3.1	—
CI5484	14.5	—	3.1	—	3.2	—
CI7337	14.1	—	3.3	—	3.3	—
CI5005	14.0	—	3.5	—	3.5	—
<i>Nursery data — (18 station-years)</i>						
Justin	18.2	16.2 to 23.0	2.8	2.6 to 3.0	3.1	2.9 to 3.3
CI5484	17.4	11.1 to 25.1	3.0	2.7 to 3.9	3.2	3.0 to 3.8
CI7337	19.1	14.3 to 26.4	2.9	2.7 to 3.5	3.2	2.9 to 3.7
CI5005	15.9	10.9 to 23.1	3.0	2.8 to 3.7	3.2	2.9 to 3.6

<sup>a</sup>At 14% moisture. <sup>b</sup>Based on g lysine/17.5 g N.

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protein may be associated with the cytoplasm rather than with nuclear genes. Progenies from reciprocal crosses involving NE542437 in combination with lines derived from Atlas 66 and Aniversario are being studied.

Two wheats from the USDA world collection, C16225 and P1176217, also show promise as new genetic sources of high protein. They hold special interest because they may also be higher in lysine than other varieties. In greenhouse plantings, P1176217 has consistently produced grain with above-normal protein and lysine. We have made numerous hybrid combinations of different high protein wheats to determine genetic relationships and to assess the opportunity of achieving new high levels of protein in wheat.

### LYSINE STUDIES

Fifteen thousand common and durum wheats from the world collection thus far analyzed for lysine were grown at Mesa, Arizona in large blocks over a 2-year period. Differential environmental effects should have been minimal. We obtained a range in lysine values from 2 to 4 percent of the protein with a mean of 3 percent. Forty percent of the lysine variation was attributable to variation in protein among the first 7,000 wheats analyzed. Adjustment of lysine values to 13.5 percent protein removed many wheats from the high-lysine class. Six of the 7,000 wheats had adjusted values higher than 3.8 percent and 125 were higher than 3.5 percent (Johnson et al., 1970).

#### **Environmental effect**

Some of the wheats with high initial lysine values were regrown at different sites in the United States from 1967 to 1970. Few of the high lysine values were maintained at all sites in all years. Table 3 shows three of the varieties that were tested, and the standard variety Justin. Statistical analyses of data were possible from 12 to 18 test sites. Mean differences for adjusted lysine were small. The adjusted lysine value for C15484 was significantly higher than Justin in five tests and no different from Justin in seven tests. C17337 was significantly higher than Justin in adjusted lysine in four tests and no different from Justin in eight tests. C15005, which had the highest initial adjusted value, was higher than Justin in only one test and was significantly lower than Justin in one test.

Within-year combined analyses revealed that C15484 and C17337 were significantly higher in adjusted lysine than Justin in 1968. Not one of the three experimental varieties was different from Justin in 1969, but all were significantly higher than Justin in 1970. It is apparent that environment exerts a strong effect on the lysine level of wheat. This effect complicates the identification and use of genetic sources of high lysine. We are starting research to determine the extent that changes in the ratio of component proteins of the wheat kernel are associated with the environmental effect.

#### **Genetic effect**

In wheat, no gene for lysine with the effect of the maize opaque-2 gene has been identified among common and durum wheats. The genetic component of lysine variation among wheats that we have studied appears to be no larger than

**Table 4. Protein-amino acid relationships among 90 wheat samples with low protein and high lysine.**

Amino acid	Mean <sup>a</sup> (%)	Range <sup>a</sup> (%)	Correlation <sup>b</sup>	
			Protein with amino acids	Lysine with other amino acids
Protein	9.6	6.5 to 16.5	---	---
<i>Essential amino acids</i>				
Lysine	3.4	2.6 to 3.8	-0.57	---
Isoleucine	3.6	2.9 to 4.0	ns	ns
Methionine	1.5	1.1 to 1.8	ns	ns
Threonine	3.3	2.8 to 3.7	-0.50	0.44
Valine	4.7	3.8 to 5.5	-0.35	0.49
Tyrosine	2.7	2.0 to 3.2	ns	ns
Tryptophan	1.3	0.1 to 1.8	ns	ns
Leucine	7.1	5.9 to 8.1	-0.41	ns
Phenylalanine	4.5	3.8 to 5.2	0.36	ns
<i>Non-essential amino acids</i>				
Histidine	2.4	2.2 to 2.8	ns	0.50
Arginine	5.0	3.9 to 5.7	ns	0.37
Aspartic acid	6.3	5.0 to 7.3	ns	0.59
Serine	5.2	4.3 to 5.8	ns	ns
Glutamic acid	30.3	24.3 to 35.3	0.38	-0.75
Proline	9.4	7.4 to 11.2	0.50	-0.55
Glycine	4.5	3.8 to 4.9	-0.34	0.55
Alanine	4.2	3.4 to 4.5	-0.58	0.76
Cystine	0.8	0.5 to 1.4	ns	ns

<sup>a</sup>Based on g/17.5 g N for amino acids. <sup>b</sup>Based on 72 samples only; ns = non-significant.

0.5 percent of the protein. PI176217, a variety with high protein and high lysine, was compared with Aniversario, a variety with high protein, in greenhouse tests. Their protein contents were similar but PI176217 consistently showed an advantage of 0.5 percentage point over Aniversario. The genetic component of 0.5 percentage point suggested by our data represents a potential 17-percent advance in the lysine level of wheat. More extensive analyses of other wheats from the world collection could reveal lysine differences larger than 0.5 percentage point.

It may be significant that maize and barley, in which genes with a large effect on lysine have been identified, are both diploid species. In contrast, common wheat is hexaploid and durum wheat is tetraploid. The presence of more than one genome in these wheat species may have contributed to our failure to identify large differences in lysine content. It is possible that a gene in one genome with a large effect on lysine could be masked by genes in the other genomes (Johnson et al., 1971).

#### Amino acid relationships

Four amino acids in wheat protein are in short supply according to FAO determinations of human requirements (World Health Organization, 1965).

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The lysine in normal wheat protein provides less than one-half of man's requirement and is the most critical of the essential amino acids. Isoleucine, methionine, and threonine are also deficient. Phenylalanine and leucine are strongly in excess of requirements.

Little information has been published about the interrelationships of amino acids in wheat. A change in the amount of one amino acid must compensate for a change or changes in other amino acids. We have been particularly concerned with the effect of changes in levels of protein as well as changes in lysine on the levels of other amino acids in wheat protein. Our screening of the world wheat collection afforded an opportunity to obtain such information. Complete amino acid profiles were determined for a group of 90 samples with low protein and high lysine and a group of 47 samples with high protein and low lysine.

We found that protein level was negatively correlated with lysine, threonine, valine, and leucine among the wheats with low protein and high lysine (Table 4). No significant relationship of protein with isoleucine, methionine, tyrosine, or tryptophan could be detected. Lysine was positively correlated with threonine and valine among the essential amino acids. It was not negatively correlated with any of the essential amino acids. The data suggest that selection for high lysine may not be associated with adverse downward shifts in levels of other essential amino acids. The negative correlation of lysine with protein coincides with data from the world collection at large. The negative correlation of lysine with glutamic acid and proline indicates that compensation for high lysine is largely provided by reductions in these two non-essential amino acids.

Correlations for the wheats with high protein and low lysine were notably different from those computed for the wheats with low protein and high lysine (Table 5). Only isoleucine and alanine were positively correlated with protein. Lysine, in contrast to its negative relationship with protein among the low protein wheats, showed no significant relationship with protein when the protein level was high. It can be speculated that wheats genetically high in protein will not be as low in lysine per unit of protein as the general regression of lysine on protein among ordinary wheats would indicate.

Lysine level among the high protein group of wheats was unrelated to levels of other essential amino acids except threonine and valine which were positively correlated with lysine. Among the non-essential amino acids, only glutamic acid was negatively correlated with lysine. These data lend support to our contention that improved levels of protein and lysine can be achieved in wheat without adverse effects upon the levels of other essential amino acids.

### Sources of above-normal lysine

Screening of the world collection of common and durum wheats for lysine differences is essentially complete except for recent accessions to the collection. Based upon additional study we have tentatively identified the following as potentially usable genetic sources of improved lysine level: P1176217, C13285, C15484, C16225, C17337, C111849, and C112756. The lysine content of the protein of these wheats is rarely more than 0.5 percentage point higher than that of ordinary wheats. The strong effect of environment may make the advan-

**Table 5. Protein-amino acid relationships among 47 wheat samples with high protein and low lysine.**

Amino acid	Mean <sup>a</sup> (%)	Range <sup>a</sup> (%)	Correlation <sup>b</sup>	
			Protein with amino acids	Lysine with other amino acids
Protein	19.0	17.5 to 22.5	—	—
<i>Essential amino acids</i>				
Lysine	2.8	2.5 to 3.1	ns	—
Isoleucine	3.6	3.2 to 3.9	0.50	ns
Methionine	1.3	0.9 to 1.5	ns	ns
Threonine	3.0	2.8 to 3.1	ns	0.52
Valine	4.5	4.1 to 4.8	ns	0.40
Tyrosine	2.5	2.1 to 2.8	ns	ns
Tryptophan	1.1	0.7 to 1.5	ns	ns
Leucine	7.0	6.5 to 7.5	ns	ns
Phenylalanine	4.9	4.4 to 5.2	ns	ns
<i>Non-essential amino acids</i>				
Histidine	2.4	2.2 to 2.7	ns	0.50
Arginine	4.8	3.8 to 5.4	ns	0.72
Aspartic acid	5.5	4.8 to 6.0	ns	0.50
Serine	5.1	4.6 to 5.4	ns	ns
Glutamic acid	33.4	29.8 to 36.3	ns	-0.70
Proline	10.6	9.4 to 12.0	ns	ns
Glycine	4.1	3.8 to 4.3	ns	ns
Alanine	3.6	3.3 to 3.8	0.52	0.44
Cystine	1.1	0.1 to 1.4	ns	ns

<sup>a</sup>Based on g/17.5 g N for amino acids. <sup>b</sup>Based on 46 samples only; ns = non-significant.

tage disappear in some production situations. Because they also exhibit above-normal protein, PI176217 and C16225 are being used extensively in our breeding program.

### OUTLOOK

The quantity of protein in the grain of wheat can be modified genetically. Although we have increased protein content by as much as 25 percent by breeding, we believe further increases are possible as additional sources of high protein are found.

Varieties possessing genes for high protein maintain their protein advantage relative to other varieties in a wide spectrum of environments. Thus, it would seem that such genes can be effectively used in most winter wheat areas of the world to increase the protein content of wheat.

What is the true contribution of higher protein in wheat to improved nutritional value? At the present time we have only *in vitro* laboratory tests to guide us. They indicate that an increase, on a dry grain weight basis, in lysine and other essential amino acids that are in shortest supply is associated with higher protein content. Theoretically, such wheats should be more nutritious because they provide more of these amino acids.

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Our wheat research group in cooperation with University of Nebraska nutritionists recently began feeding tests with mice to better measure the biological value of our high protein varieties. Present nutritional guidelines seem inadequate. Feeding trials with small animals will provide highly useful additional information but our trials must eventually be supplemented with human nutritional tests.

Genetic modification of the amino acid content of wheat protein apparently presents a more difficult problem. Lysine differences that we can identify as genetic are small compared with the total variability of lysine. Incorporation of lysine increases as small as 0.5 percentage point into agronomically acceptable varieties will be difficult.

Moreover, the analytical techniques needed to measure lysine can be effectively done by a few laboratories in the world. The ion-exchange chromatographic system for amino acid determinations is the most reliable method for accurate determinations. The dye-binding technique developed in Sweden offers an apparently adequate alternative method for lysine screening of large numbers of samples generated by breeding programs. Loss of accuracy appears minimal.

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### **Discussion: Wheat protein improvement**

S. K. SINHA: Are there lines with high protein content as well as stability in protein level?

V. A. Johnson: As I pointed out, it is not possible to fix the protein content of wheat or any other crop at a pre-determined level by breeding. Environment exerts a major influence. But we can fix a potential for high protein in wheat, which we have done. Such potential is expressed as an advantage in protein content of high protein varieties over other varieties grown in the same environment, whatever the general level of protein content might be.

B. O. JULIANO: Has the lack of association between foliage and grain nitrogen in wheat been recently verified using your more advanced lines, and leaf blades instead of foliage? We recently found a high level of leaf nitrogen in rices that have a high yield of protein in the brown rice.

V. A. Johnson: No. We have not yet repeated this experiment with our more advanced high protein lines. We plan to do so.

P. R. JENNINGS: Would you speculate why wheat has more protein than rice?

V. A. Johnson: I suppose it is because wheat is grown under lower temperatures and on drier land than rice.

L. T. EVANS: Perhaps the essential difference between wheat and flooded rice in this connection is the extent and duration of their root growth. Roots are a major source of amino acids, and dryland conditions often lead to increased root growth. In support of this postulate, we find that the wild diploid wheat, *T. boeoticum*, which can have up to 38 percent protein in its grain, invests far more in root growth than do modern wheats. Similarly, upland rice may have a more extensive root system than lowland rice.

K. KAWANO: Generally speaking, rice yields more than wheat. Do you think that the generally higher protein content of wheat is responsible for this yield difference?

V. A. Johnson: No, I think not. Wheat and rice are different species that have been developed under quite contrasting ecological situations throughout the world. Even in those production environments in which the yields of wheat and rice are the same, wheat has a sizeable protein advantage over rice. One can only speculate on the reasons for this.

T. H. JOHNSTON: Is there information available on what happens to protein content in the extremely high yielding wheat varieties grown under irrigation and at very high levels of nitrogen fertilization?

V. A. Johnson: We have no information on this except the response of winter wheat varieties in the International Winter Wheat Performance Nursery. In Kabul, Afghanistan, under high fertilization and high productivity of the crop in 1969, the protein content of the varieties remained relatively high.

H. I. OKA: How much is the heritability value of protein and lysine in selection?

V. A. Johnson: We have computed heritability values for protein content in wheat ranging from 0.3 to 0.8 depending on the method of computation. I question the usefulness of such computations even though we have made them. Our research on lysine content has not progressed to the point where heritability estimations were possible.

H. L. CARNAHAN: Were the data showing an association between rust reaction and protein content obtained from seed produced on plants that were not affected by rust?

V. A. Johnson: No. However, phenotypic expression of the high protein trait derived from Atlas 66 lines has been obtained on many occasions in which leaf rust was not present. In other words, although there is linkage between a gene for high protein and one for leaf rust resistance in Atlas 66, expression of the high protein trait is unrelated to the presence of leaf rust.

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