### Improvement of Wheat Protein by Traditional Breeding and Genetic Techniques<sup>1</sup>

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#### **ABSTRACT**

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Genetic variation for grain protein concentration amounting to five percentage units has been demonstrated in wheat and related species. Atlas 66 and Nap Hal possess major genes which regulate amount of protein. Amount of grain protein tends to be negatively correlated with yield, but correlation coefficients seldom exceed 0.6, indicating that much of the variation in protein is independent of yield and that simultaneous breeding

advances in yield and protein are possible. This was demonstrated by the successful transfer of the Atlas 66 genes for high-protein content to the productive hard red winter wheat cultivar Lancota. Genetic variation for lysine, the first limiting essential amino acid in wheat protein, is too limited in wheat germ plasm to be successfully utilized by breeders.

The grain of wheat commonly contains protein that ranges in concentration from 8 to 15%. Among U. S. market classes, durum wheat and hard red spring wheat can be expected to be highest in protein, whereas the soft white winter and soft red winter classes generally are lowest in protein.

Wheat protein exhibits an imbalance of essential amino acids that prevents maximum utilization of the protein by monogastric animals. Lysine is present in only one-half the concentration needed for balance with the other essential amino acids. It is the critical amino acid in wheat protein because, among the essential amino acids, it is in shortest supply.

Nutritional improvement of wheat grain can be accomplished by modifying its protein. The protein concentration can be increased or the ratio of essential amino acids in the protein can be modified to better achieve the balance required by monogastric animals for maximum utilization of the protein.

The USDA Agricultural Research Service (ARS), in cooperation with the University of Nebraska, has conducted research on genetic modification of wheat protein by traditional means since 1954. Key findings from this research effort are summarized.

The protein content of wheat grain is subject to wide variation. Although protein content is under genetic control, it is strongly affected by production environment as well. Because grain protein expressed as percent of total dry weight reflects the ratio of protein

to nonproteinaceous components of the grain, changes in either component will affect the magnitude of the percentage value.

Breeding improvement of wheat protein requires the existence of genetic variation for protein in wheat or related species, and this component of variation must be separable from nongenetic effects. To be useful, protein advances must be achievable without negative effects on other important traits such as yield.

#### PROTEIN CONCENTRATION

The ARS-Nebraska group systematically analyzed most of the common wheats in the ARS World Wheat Collection to ascertain the magnitude of variation in protein and lysine (Vogel et al 1973). Total variation in grain protein concentration among 12,600 wheats was more than 15%, ranging from 7 to 22%. Subsequent analyses of selected accessions and genetic studies identified genetic variation in concentration of grain protein amounting to 5%. Although substantial, this genetic variation is not as large as nongenetic variation frequently associated with production environment. This has made genetic manipulation of protein concentration difficult.

Genes affecting the protein content of wheat grain appear to be widespread in Triticum aestivum L. germplasm. The genes, for the most part, produce small effects and are difficult to manipulate and follow in genetic studies. Large genetic differences in grain protein concentration in common wheat were reported initially by Middleton et al (1954). The soft wheat cultivars Atlas 66 and Atlas 50, developed in North Carolina, produced grain significantly higher in protein than other soft wheat cultivars, and both were competitive in grain yield. Both cultivars were selected from the cross of the South American cultivar Frondoso with Redhart-Noll. Frondoso is believed to have contributed the genes for high protein to the Atlas cultivars. Frontana and Frontiera, South American cultivars related to Frondoso, probably carry the same genes as Frondoso for elevated protein. Field trials conducted in the southern part of the U.S. hard red winter wheat region revealed that the Atlas wheats were equal in yield but significantly higher in

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grain protein content than the hard wheat cultivars Comanche and Wichita (Haunold et al 1962).

Genes for protein with similarly large effects also were discovered in the common wheat introduction Nap Hal. Nap Hal was reported as a potential genetic source of high protein in common wheat initially by Watson et al (1966). Atlas 66 and Nap Hal were used extensively in the Nebraska-based protein research. In each, the high-protein effect is in a genetic background in which there are numerous undesirable agronomic and quality traits. These have been difficult to eliminate in high-protein progeny from crosses involving Atlas 66 and Nap Hal and suggest some degree of genetic linkage of protein genes with those conditioning the agronomic and quality traits.

#### GENETIC STUDIES AND BREEDING ADVANCES

Genes coding for endosperm storage proteins occurs at nine complex loci on six different wheat chromosomes (Payne et al 1984). Differences in protein quality for breadmaking have been associated with allelic variation in genes coding for high and low molecular weight glutenins located on chromosomes 1A, 1B, and 1D. Chromosomes 6A, 6B, and 6D carry protein genes coding for  $\alpha$ - and  $\beta$ -gliadins with alleles that confer only minor differences in protein quality. Morris et al (1978) identified major and minor regulatory genes for protein concentration in the cultivar Atlas 66. The genes were located on chromosomes 5D and 5A, respectively.

Nebraska research established that the protein genes in Atlas 66 and Nap Hal are different (Johnson et al 1973, 1975).  $F_3$  progeny from the cross Atlas  $66 \times$  Nap Hal exhibited strong transgressive segregation for protein on the basis of whole-kernel analyses. Identification of lines from the cross that consistently produced grain with higher protein concentration than either parent provided corroborative evidence of different genes controlling protein in these two cultivars. The parent cultivars have been used extensively in second and third cycle crosses.

Further evidence that the Atlas 66 and Nap Hal protein genes are different came from protein analysis of kernel fractions (Johnson et

TABLE I
Site of the High-Protein Effect in the Grain of Nap Hal and Atlas 66

Cultivar or Line	Protein Content (%)			
	Whole Grain	Endosperm Fraction	Nonendosperm Fraction	
Nap Hal	19.6	18.9	24.5	
Atlas 66	19.4	19.3	19.8	
CI 13449	15.5	14.5	19.6	
Centurk	15.4	15.0	19.6	

al 1975, Vogel et al 1978). Data are shown in Table I. Both of the high-protein wheats produced grain with high-protein endosperm, whereas only Nap Hal expressed the high-protein effect in the nonendosperm fraction. Genetic analysis of protein concentration of the endosperm fraction of progeny lines from the Atlas  $66 \times \text{Nap}$  Hal cross revealed transgressive segregation for protein (Vogel et al 1978).

Inheritance studies have shown that both additive and nonadditive gene action may influence grain protein concentrations. Kraljevic-Balalic et al (1982) found a preponderance of nonadditive gene action in crosses of five divergent cultivars with presence of partial dominance affecting the inheritance of protein content. Atlas 66 was found to possess mainly recessive genes for protein concentrations. Halloran (1975) indicated a generally additive nature of genetic control of protein content with evidence of dominance. The study suggested that the genetic control of protein content, whether by dominant or recessive genes, may change depending on the influence of environment on segregating populations.

High-protein lines were extracted from a complex cross involving parental cultivars from five different countries (Table II; Johnson et al 1979). Each parent was a potential donor of genes for high protein based on earlier analyses. The lines were higher in protein than the high-protein check variety Lancota and much higher than the Centurk check at Lincoln, NE, and Yuma, AZ. Additionally, all had equal or higher yields, shorter straw, and produced larger seed than the check cultivars.

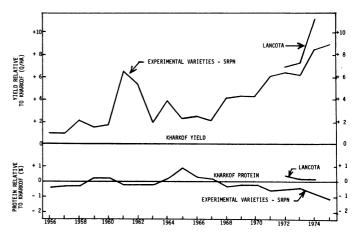


Fig. 1. Grain yield and protein trends among hard red winter wheats evaluated in the Southern Regional Performance Nursery since 1956.

TABLE II
Promising High-Protein Experimental Winter Wheats Grown at Lincoln, NE, in 1975 and at Yuma, AZ, in 1976

1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2				
Plot No.	Plant Height (cm)	100-Kernel Weight (g)	Grain Yield (q/ha)	Grain Protein Content (%)
$\overline{x}$ of 18	102	•••	35.9	15.6
x̄ of 18	97		37.5	17.5
12 201	0.1			
		•••		19.8
	84	•••	41.1	19.3
12,297	81		42.0	19.1
12,327	86	***	41.0	18.7
				10.7
$\bar{x}$ of 14	•••	3.6	54.0	11.6
$\bar{x}$ of 14		3.7	45.0	12.9
343	•••	4.5	51.0	14.3
444	•••			14.1
457	•••			14.3
	•••			14.3
	No. $\frac{\overline{x}}{\overline{x}}$ of 18 $\overline{x}$ of 18 $\overline{x}$ of 18 12,291 12,293 12,297 12,327 $\overline{x}$ of 14 $\overline{x}$ of 14 343	Plot No.         Height (cm)           \$\overline{x}\$ of 18         102           \$\overline{x}\$ of 18         97           12,291         81           12,293         84           12,297         81           12,327         86           \$\overline{x}\$ of 14            \$\overline{x}\$ of 14            343            444            457	Plot No.         Plant Height (cm)         100-Kernel Weight (g)           x of 18 x of 18         102           x of 18 y of 18         97           12,291 81            12,293 84            12,297 81            12,327 86            x of 14            x of 14	Plot No.         Plant Height (cm)         100-Kernel Weight (g)         Grain Yield (q/ha) $\overline{x}$ of 18         102          35.9 $\overline{x}$ of 18         97          37.5           12,291         81          41.0           12,293         84          41.1           12,297         81          42.0           12,327         86          41.0 $\overline{x}$ of 14          3.6         54.0 $\overline{x}$ of 14          3.7         45.0           343          4.5         51.0           444          4.6         51.0           457          4.3         50.0

The ARS-Nebraska group successfully transferred the Atlas 66 genes into a high-quality, agronomically competitive hard red winter wheat (Johnson et al 1968, Johnson 1977). The Lancota cultivar released in 1975 produces grain consistently higher in protein concentration by 0.5-1.5% than other cultivars of a decade ago. Yield and protein trends among experimental cultivars evaluated in the hard red winter wheat Southern Regional Performance Nursery are shown in Figure 1. The pronounced advances in yield of experimental cultivars since 1965 have been accompanied by a depression in protein of more than 1%. Lancota, however, over a three-year period of testing in the nursery, was high yielding and produced grain equal to or higher in protein than the check cultivar Kharkof. Despite its outstanding performance in regional trials, Lancota did not achieve wide-scale grower acceptance. There was little economic incentive for producers to grow high-protein cultivars. Additionally, the straw of Lancota was too tall, and its lack of winter hardiness made production in Nebraska more precarious than other available cultivars.

Protein genes from Atlas 66 function through their effect on N metabolism in the plant. The high-protein Lancota cultivar was compared with Lancer in a comprehensive two-year study in Nebraska. Data from the study are summarized in Table III. Lancota produced higher yields than the Lancer cultivar by an average 4.5 q/ha, and its grain was 1.7% higher in protein. Its seed was substantially larger than that of Lancer, and its ratio of straw to grain was better than Lancer. Grain protein production that was higher, together with lower residual straw protein, indicated more efficient and complete N translocation in Lancota. The activity of NO<sub>3</sub> reductase, believed to be the rate-limiting enzyme in wheat N metabolism, was highest in Lancota. Lower residual N in the soil under Lancota plots after harvest indicated that differential N uptake also was involved in the higher grain protein of Lancota.

Protein genes have been moved successfully from wheat relatives to common wheat. Plainsman V, developed by Seed Research Associates in Kansas, apparently carries a gene(s) from Aegilops that consistently boosts grain protein concentration by as much as 2 to 3% (Finney 1978). Although it is relatively nonproductive in most central plains environments, Plainsman V yielded well in some Kansas trials in which its grain protein advantage over other cultivars was maintained. More recently, collections of the wild wheat Triticum dicoccoides in Israel were discovered to produce seed with exceedingly high protein concentration. A large research effort is in progress to move the T. dicoccoides gene(s) to cultivated wheat (Kushnir and Halloran 1982).

## RELATIONSHIP OF PROTEIN GENES WITH OTHER TRAITS IN WHEAT

Clearly, the protein content of wheat grain can be increased by conventional breeding. There are constraints, however, some of which are difficult to overcome or circumvent. The first of these, and the one about which most wheat breeders are concerned, is the negative correlation between grain yield and grain protein

TABLE III
Performance of Lancota (High Protein) and Lancer in Replicated Trials
at Clay Center, NE, in 1973 and 1974

Measurement	Lancer	Lancota	Lancota Advantage (%)
Grain yield (q/ha)	27.9	32.4	16
1,000-kernel weight (g)	27.9	31.9	14
Straw:grain ratio	2.35	2.16	$-9^{\mathrm{b}}$
Grain protein (%)	14.6	16.3	12
Grain protein production (q/ha)	4.1	5.3	29
Straw protein production (q/ha)	3.3	2.7	$-26^{\rm b}$
Total protein production (q/ha)	7.4	8.0	8
NO <sub>3</sub> -Reductase activity (μ moles/gfw/hr)	7.45	8.17	10
Residual soil NO <sub>3</sub> (g/m <sup>2</sup> )	7.8	6.9	$-13^{b}$

<sup>&</sup>lt;sup>a</sup>Data from Wilhelmi 1976.

concentration. In many, but not all, production situations, the r-value is statistically significant.

In the International Winter Wheat Performance Nursery (IWWPN) supervised by the ARS-Nebraska group, significant negative correlations between yield and protein occurred in approximately two-thirds of the trials over a two-year period (Table IV). There was no correlation in the remaining one-third of the trials. More importantly, only one trial in each year produced an  $r^2$  value higher than 0.50, as compared with 10 trials in 1976 and 22 in 1977 in which  $r^2$  was less than 0.25. Because  $r^2$  indicates the portion of protein variation that can be accounted for by variation in yield, an r-value of 0.6 would allow only one-third of the protein variation to be explained by yield changes. The international trial data indicate that the largest part of protein variation is independent of yield and that simultaneous breeding progress in improving both traits is possible. Data from Nebraska and Arizona field tests have provided strong additional evidence that the negative correlation of protein with yield, when it occurs, seldom exceeds -0.60.

The relationship of yield and protein among 64 high-protein winter lines grown in a replicated trial at Yuma, AZ, is shown in Figure 2. The productive semidwarf cultivar TAM 105 served as the yield standard in the trial. Although all of the lines were higher in protein than the check, only 13 exceeded the check in yield. Despite the negative correlation of protein with yield, the latter group maintained a protein advantage of 1-2% over the check.

Some investigators believe that the frequent negative correlations between grain yield and grain protein reflect a bioenergetic constraint. Bhatia and Rabson (1976) performed calculations based on biochemical pathways and energy requirements of reactions associated with assimilation of C and N in microorganisms. On the assumption that biochemical pathways in plants do not differ from those in microorganisms, their calculations showed that increased inputs of C assimilates and N are necessary for higher protein concentrations in high-yielding cereal grains. They concluded that increased protein in wheat grain can be expected to be associated with depression of grain yield. This bioenergetic constraint, if valid for wheat, probably would be most pronounced in the more favorable production environments in which genetic potential for yield would be most fully expressed. The constraint, if real, does not suggest that yield and protein cannot be advanced simultaneously, but rather, that the yield of a productive high-protein cultivar might be even higher if the protein were lower.

A close linkage of one gene for protein concentration with a gene for leaf rust resistance exists in Atlas 66 (Johnson et al 1968). The generally tall straw and poor seed quality of progeny from the initial crosses involving Atlas 66 and Nap Hal indicated the possibility of genetic linkages that would impede the transfer of protein genes to agronomically acceptable wheats. The identification of high-protein selections that also possessed

TABLE IV

Correlation of Yield and Protein in the International Winter Wheat

Performance Nursery in 1976 and 1977

	Sites	
	Number	%
1976 (47 sites)		
Significant negative correlation	29	62
No correlation	18	38
r <sup>2</sup> Value higher than 0.50	1	2
r <sup>2</sup> Value between 0.33 and 0.50	7	15
$r^2$ Value between 0.25 and 0.32	11	23
$r^2$ Value less than 0.25	10	21
1977 (56 sites)		
Significant negative correlation	40	71
No correlation	16	29
$r^2$ Value higher than 0.50	1	2
$r^2$ Value between 0.33 and 0.50	8	14
$r^2$ Value between 0.25 and 0.32	9	16
r <sup>2</sup> Value less than 0.25	22	39

<sup>&</sup>lt;sup>b</sup>Negative value is favorable.

moderately short straw and large seed shown in Table II diminished this concern. Subsequently, we have identified numerous large-seeded semidwarf lines that also carry high protein genes. Many of these lines have been distributed internationally from an observation nursery for high-protein wheats managed by the ARS-Nebraska wheat group.

A close positive association exists between grain protein and several trace minerals (Table 5; Peterson et al 1983). Simple r-values between protein and five mineral elements in flour were highly significant in a study of IWWPN entries grown at selected nursery sites. Even higher genetic correlations were determined for P, Fe, Mg, and Zn with protein in the flour. The positive association of protein concentration with most trace minerals is valuable from a nutritional point of view.

#### LYSINE CONCENTRATION

Because wheat protein contains only one-half the amount of lysine needed for good balance with other essential amino acids, any increase in lysine would significantly improve the nutritional quality of the protein. Analysis of accessions in the ARS Wheat Collection revealed substantial variability for lysine, which subsequent research demonstrated to be largely nongenetic. The genetic component of total lysine variation in common wheat

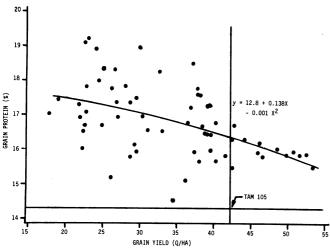


Fig. 2. Performance of high-protein experimental wheats from the ARS-Nebraska program in a replicated High Protein-High Lysine Nursery grown at Yuma, AZ, in 1983.

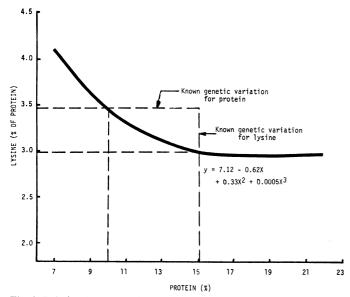


Fig. 3. Relation between lysine and protein levels in wheat grain.

protein is no more than 0.5%, far less than the 2.25–2.50% needed to achieve nutritional balance of lysine with other essential amino acids.

The lysine content of wheat protein is not fixed. As grain protein level is increased, the lysine concentration in the protein decreases. The negative protein-lysine relationship is curvilinear and most pronounced in the 10-15% range of protein (Vogel et al 1973). Protein increases in this range are due mainly to increases in the lysine-poor gliadin fraction. Wheat protein increases in the 10 to 15% range, then, result in protein of poorer nutritional quality because of less lysine.

Despite the negative relationship between protein and lysine per unit protein, increased protein concentration in wheat grain results in more lysine per weight of grain (Vogel et al 1973). Research in Nebraska has provided good evidence that higher protein grain not only provides more lysine but more of all essential amino acids. Nutritional studies involving small animals as well as humans as subjects indicate that increased grain protein concentration improves the nutritional value of wheat (Johnson et al 1975, MacLean et al 1976).

The outlook for modifying the lysine concentration of wheat protein (utilizing known genetic variation for lysine in common wheat germplasm) by conventional breeding is not favorable. Lysine analyses are time-consuming and expensive, and few laboratories are adequately equipped to perform large numbers of such analyses. Lysine variation that is independent of protein concentration in such donor wheats as Nap Hal and Cl 13449 is only in the 0.3-0.4% range. This barely exceeds the resolution capabilities for lysine determined by ion-exchange chromatography.

Known genetic variation for lysine, if fully utilized, appears to be sufficient to compensate the lysine depression normally associated with increased protein concentration in wheat grain (Figure 3). Nebraska researchers have identified some high-protein lines in which lysine per unit protein is equal to that of lines with much lower protein (Johnson et al 1975). Using these lines in further

TABLE V
Simple and Genetic Correlations of Grain Protein Content
with Mineral Levels in Flour and Bran

Mineral Element	Flour		Bran	
	Simple	Genetica	Simple	Genetica
Calcium	0.55** <sup>b</sup>	0.21	0.76**	-0.25
Phosphorus	0.37**	0.88	0.03	0.48
Iron	0.59**	0.94	0.69** -	0.46
Magnesium	0.35**	0.89	0.22**	0.13
Zinc	0.73**	0.95	0.44**	0.55

<sup>&</sup>lt;sup>a</sup>There is no test of significance for genetic correlations.

b\*\*Significant at the 0.01 probability level.

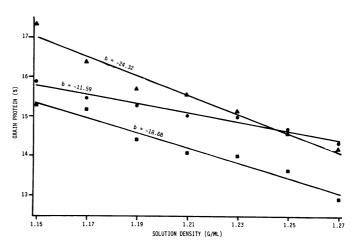


Fig. 4. Relationship of solution density to the protein content of seed separated by the flotation technique in three bulk hybrid wheat populations.

TABLE VI Protein Content of High- and Low-Protein Seed Fractions in Six Bulk Hybrids and Lancota Resulting from Separation by Solvent Flotationa

	Protein Content (%)		
Population	High-Protein Fraction	Low-Protein Fraction	
Experimental hybrid 1	19.0	16.1	
2	18.1	16.3	
3	17.2	15.2	
4	18.6	13.8	
5	17.4	14.3	
6	18.1	15.0	
Lancota (check)	14.3	12.5	

<sup>&</sup>lt;sup>a</sup> Based on method of A. Garzon Trula, Ministry of Agriculture, Madrid, Spain.

crosses to improve productivity and agronomic worth would require large numbers of lysine analyses to avoid losing the lysine genes in the progeny.

#### **CONSTRAINTS**

Many breeders do not have access to protein analysis equipment needed for breeding improvement of wheat. Neither do they have adequate funds to pay for large numbers of protein analyses required to breed effectively for higher protein grain. The ARS-Nebraska group is currently evaluating a simple, inexpensive, nondestructive technique developed by A. Garzon Trula, Madrid, Spain, for separation of wheat kernels on the basis of protein content. Preliminary results indicate that the selection technique may be effective in increasing the frequency of genes for high grain protein in early generation segregating bulk hybrid populations (Peterson et al 1985).

The technique is based on the differential water absorption capacities of protein and starch and their relative densities when hydrated. Dry protein has a specific density of approximately 1.4, whereas that of dry starch approaches 1.6. A. Garzon Trula found that protein will absorb appoximately five times more water than starch and that the differential density of the components is significantly increased when hydrated. High- and low-protein seed then can be effectively separated on the basis of kernel density.

Wheat seeds are screened to a relatively uniform size to reduce the large amount of nongenetic variation in protein content of individual kernels. Seed then is soaked in water for 7 to 10 days at 1°C to imbibe water without germinating. A solution with a density of approximately 1.25 g/ml will allow separation of the higher protein, less dense seed from the lower protein, more dense seed. If the seed fractions are dried after separation, the seed can be stored and will germinate normally upon planting. A mixture of carbon tetrachloride and hexane was proposed by Garzon Trula as a density medium for separations. The toxicity of CCl4, however, requires that special precaution be taken to avoid contact during use. The ARS-Nebraska team has determined that a mixture of NaCl and sucrose in water can be substituted sucessfully for CCl<sub>4</sub>hexane as a safe, effective solution for density separations.

The relationship of solution density to the protein content of seed separated by this technique is shown for three bulk populations in Figure 4. Seed with increasing protein content will be separated as the density of the solution is decreased. Substantial differences in the protein contents of high- and low-protein fractions following density separation of six bulk hybrid populations are shown in Table VI. When high-protein seed separated by the technique is planted, the increase in grain protein content of the derived bulk populations may be significant compared to unselected materials (Table VII). The increase in protein shown is small relative to the protein differential immediately following density separation and probably reflects the substantial amount of nongenetic variation influencing the protein content of individual kernels.

TABLE VII Protein Gains in Winter Wheat Bulk Hybrid Populations Derived from Seed Separation by Solvent Flotation in the Generation Following Selection

		Grain Protein (%)		Protein
Population No.	Generation of Separation	Unselected Bulk	Selected Bulk	Increase (%)
Grown at Ver	rnon, TX (1984)			
90307	$F_3$	19.6	20.7	1.1
90412	$F_4$	17.7	18.5	0.8
90205	$F_2$	20.1	20.8	0.7
90302	$F_3$	18.2	18.9	0.7
90310	$F_3$	18.5	19.2	0.7
90427	$F_4$	18.6	19.3	0.7
Grown at Lir	ncoln, NE (1984)			
9238	$F_3$	18.3	19.2	0.9
9250	$F_3$	17.2	17.9	0.7
9263	$F_3$	17.8	18.4	0.6
9227	$F_3$	18.1	18.6	0.5

#### **OUTLOOK**

There is sufficient genetic variation for grain protein concentration in wheat and related species to effectively breed higher protein wheats. This cannot be said for lysine, for which known genetic variation is limited. Effective modification of lysine in wheat may require the use of new nontraditional techniques. Preliminary results from research in progress at Kansas State University show promise for lysine selection using tissue culture (R. G. Sears, personal communication).

The frequent negative correlation between grain yield and grain protein content is the most troublesome constraint confronting wheat breeders. Reducing yield to achieve higher grain protein cannot be justified. Fortunately, the negative correlation is not so high as to prevent simultaneous advances for both yield and protein, but it does make them more difficult. Higher protein wheats can and will be developed; such wheats will be utilized commercially when there are economic incentives for the production of wheat with higher grain protein content.

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# Gliadin Electrophoretic Variations in Foundation Arkan Wheat Grown at 16 Kansas Agricultural Experiment Stations in 1983<sup>1</sup>

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#### ABSTRACT

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Gliadin electrophoretic patterns of composite samples of foundation Arkan wheat grain grown at 16 Kansas branch experiment stations and experimental fields in 1983 were compared with patterns of two other hard red winter wheats, Newton and TAM 105, grown at the same locations, and with patterns of Arkan's parents, Sage and Arthur. Gliadin polyacrylamide gel electrophoretic patterns were also determined for single hard- and soft-appearing kernels. Electrophoregrams of the single seeds at each location were identical, or nearly so, to bulk Arkan patterns at the

Key words: Kernel characteristics, Kernel hardness, PAGE, Wheat class

respective locations, and to typical Arkan patterns previously established. Therefore, differences in electrophoretic patterns are not the result of varietal mixtures, but more likely of biotypes. Extra bands found in some Arkan samples were usually present in one of its parents. Gliadin patterns of bulk extracts of Newton and TAM 105 from some locations also exhibited patterns different from normal. The number of locations where electrophoretic differences were found was highest for Arkan and lowest for TAM 105.

Classification of wheat by the United States Department of Agriculture, Federal Grain Inspection Service (FGIS) allows commercial enterprises to buy and sell wheat based on intended end uses; for example, hard wheats are used for pan breads, soft wheats for pastries, and durum wheats for pastas. The classification scheme has been under constant scrutiny and revision since its inception in 1916. Recently, additional pressure has been put on this classification system with the use of new exotic germ plasms and interclass breeding in varietal development, which may improve disease resistance, yield, and protein content. However, such improvements may also introduce problems, the major one being variability of kernel characteristics for a cultivar. Because FGIS identifies and determines purity of wheat cultivars by kernel characteristics (USDA-FGIS 1977) and sample homogeneity,

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respectively, new methods must now be developed to aid in identifying and classifying wheat cultivars, as long as classification standards remain unchanged.

Arkan is a recently introduced wheat cultivar that resulted from crossing a hard red winter (HRW) wheat, Sage, with a soft red winter (SRW) wheat, Arthur. Arkan exhibits milling and baking properties typical of HRW wheat, but exhibits nonhomogeneous kernel characteristics. As a result, Arkan foundation-quality seed has been graded by FGIS as a mixture of SRW and HRW wheats.

Two instrumental methods (polyacrylamide gel electrophoresis [PAGE] and high-performance liquid chromatography [HPLC]) have been reported that can identify wheat cultivars by analyzing their gliadin proteins (Bushuk and Zillman 1978, Lookhart et al 1982, Wrigley et al 1982, Bietz 1983). This paper describes variations in electrophoretic patterns of bulk Arkan and of the soft- and hard-appearing kernels of Arkan. It also compares their patterns with those of its parents, Arthur and Sage, and with two other HRW wheats, Newton and TAM 105.

#### MATERIALS AND METHODS

#### Chemicals and Reagents

Acrylamide, N, N'-methylenebisacrylamide, ascorbic acid, Coomassie Brilliant Blue R-250, methyl green, trichloroacetic acid