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"NEW APPROACHES TO BREEDING FOR IMPROVED PLANT PROTEIN"

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BREEDING FOR HIGH PROTEIN CONTENT AND QUALITY IN WHEAT*

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Abstract

BREEDING FOR HIGH PROTEIN CONTENT AND QUALITY IN WHEAT. Research on wheat protein conducted co-operatively by the Agricultural Research Service, USDA, and the University of Nebraska since 1954 is reviewed. The assumption is made that wheat, because of unique and highly desirable protein traits, will continue as a foremost food crop in the world.

Enhanced value of wheat as a human food encompasses the distinctly different but related factors of grain yield, grain protein quantity, and grain protein quality. Co-operative ARS-University of Nebraska research has demonstrated the feasibility of breeding wheat with 15 to 25% higher protein content. The protein increases are not associated with depressed productiveness or with a less desirable balance of essential amino acids. The large effect of environment on protein content presents problems in identification of genetic effects. The world Wheat Collection maintained by the US Department of Agriculture is being searched for additional genetic sources of high protein. The Collection also is being analysed for genetic differences in lysine, methionine, and threonine since the protein of existing wheat varieties is deficient is these three essential amino acids.

Lysine content of 4100 varieties in the Collection thus far analysed ranged from 1.77 to 4.15% with a mean of 3.00%. The value of 4.15 approaches the level believed necessary for optimum balance of lysine with other essential amino acids. Lysine and protein show a modest negative relationship. Only 18% of the variation in lysine/protein was attributable to variation in protein content in the wheats analysed. The effect of environment on level of lysine is being studied. Research results to date provide the basis for optimism that the lysine content of wheat protein is amenable to significant improvement by breeding procedures.

A newly established International Winter Wheat Performance Nursery to identify winter varieties with superior general adaptation characteristics is discussed. The potential usefulness of the nursery for assessment of the stability of the high protein and high lysine traits is considered.

It is my intention to examine the opportunity for significant improvement in the quantity and nutritional quality of the protein in wheat by conventional breeding techniques. The Agricultural Research Service, USDA, co-operating with the University of Nebraska, has conducted research on the quantity and quality of wheat protein since 1954. Much of what is presented here has been published or will be published soon. This presenta-

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* Joint contribution of the Crops Research Division, Agricultural Research Service, US Department of Agriculture and the Agronomy Department, University of Nebraska, Lincoln, Nebraska. The research was supported partially by funds from the Agency for International Development, US Department of State, Washington, D.C., and the Nebraska Wheat Development Division.

tion is essentially a review of our protein research findings and an assessment of their significance toward protein improvement in wheat.

The dependence of more than one billion people on wheat as their basic food is ample evidence of the importance of this cereal. The broad adaptation of wheat to many climates and environments attests to its uniqueness among the cereal grains. Equally as unique, the protein of wheat possesses properties that permit the preparation of an array of highly palatable and desirable leavened baked products. It seems reasonable, then, that wheat will continue its role as a major food crop of the world. Improvement of its protein would have significant and far-reaching nutritional consequence.

Enhanced value of wheat as a human food encompasses three distinctly different but related factors. These are the yield of grain, its protein content, and the nutritional value of the protein. Continued improvement in the productivity of wheat at the expense of protein content and quality has dubious net value from a nutritional point of view. Equally questionable would be the improvement of protein quantity and quality at the expense of productivity. Increases in protein content accompanied by undesirable shifts in the ratio of its essential amino acids would have questionable value as would improvement of amino acid balance associated with depressed protein content. The co-operative ARS-University of Nebraska research effort in wheat has encompassed all of these factors of total nutritional improvement.

Can the protein content of wheat be significantly increased by breeding? The accumulated evidence from 14 years of research at Lincoln, Nebraska indicates that it can. It has not been easy to demonstrate this because of the strong influence of environment on protein content. A single wheat genotype can produce grain varying from as low as 8% protein to as high as 18% depending on the environment in which it is grown. Genetic differences are frequently difficult to separate from those that are environmental in nature. Yield differences may strongly influence the protein content of the grain. But, this relationship does not always hold, depending on soil fertility, water availability, and other factors of environment. We have been unable, therefore, to utilize simple correlations or linear regressions for meaningful adjustment of protein values to a common productivity level. We have compromised by restricting critical protein comparisons in our breeding program to lines of comparable productiveness. While this is not a precise technique, it has permitted significant genetic advances in grain protein level.

Our protein breeding program traces to the identification of a soft winter wheat variety Atlas 66 as possessing the capacity to lay down more protein in its grain than other soft wheat varieties (Table I). The high protein trait of Atlas 66 is believed to have been transmitted from the South American variety Frondoso. The high protein trait from Frondoso is closely associated with adult resistance to leaf rust. Whether close genetic linkage, pleiotropism, or a physiological linkage is the basis for this relationship has not been fully established. The weight of evidence favours a genetic linkage.

Protein increases in the range of 15 to 25% have been achieved without reductions in productivity using Atlas 66 as a parent (Table II). This magnitude of protein increase has been demonstrated in several different environments in the United States. It has also been demonstrated at various levels of grain yield and grain protein content. Phenotypic ex-

Table I. Grain protein content of parent varieties and high protein progeny lines grown at Lincoln, Nebraska in 1965.

Population	% Protein
Atlas 66	18.3
Wichita	14.0
Comanche	14.3
Progeny lines (range)	15.3 - 18.7
Progeny lines (mean)	16.9

Table II. Average grain yield and protein content of high protein lines and standard varieties at 2 locations in Nebraska in 1965.

Variety	C.I. or Sel. No.	Grain Yield	Protein Content of Grain
		Bu/A	%
Atlas 66 x Comanche	631068	40.2	18.5
Atlas 66 x Comanche	631423	45.3	17.5
Atlas 66 x Comanche	631417	37.6	17.5
Atlas 66 x Wichita	631168	42.9	17.1
Atlas 66 x Comanche	631250	40.1	17.0
Lancer	13547	40.9	14.8
Comanche	11673	29.6	14.4
Wichita	11952	28.2	14.0

pression of the high protein trait is most difficult to demonstrate in high yielding environments in which soil nitrogen availability is limited. The complete range of environments in which expression of the high protein trait from Atlas 66 will occur has not been defined. An International Winter Wheat Performance Nursery being initiated by the Nebraska Agricultural Experiment Station in co-operation with the Agricultural Research Service, USDA, and the Agency for International Development, US State Department should help to establish this. There is also informal co-operation with the International Maize and Wheat Improvement Center, Mexico, D.F., Mexico.

The high protein from Atlas 66 is inherited as an incompletely dominant trait (Fig. 1). It is believed that more than a single gene is involved. The selection of both intermediate and high protein lines resistant to leaf rust provides evidence for the presence of a gene or genes in addition to the one linked with leaf rust resistance (Fig. 2). On the other hand, the ease with which high protein lines were selected from crosses of Atlas 66 with the hard winter wheat varieties Comanche and Wichita suggests the operation of a relatively small number of genes. Chromosomes from Atlas 66 are being individually substituted into a low protein variety by the monosomic technique to identify the chromosomal location of the genes for high protein.

What of the possibility of further enhancement of protein level in wheat by breeding? Little is known of the genetic variability of the trait among the wheats of the world. It seems reasonable to believe that major genes in addition to those from Frondoso may exist among the 17000 common wheats in the World Collection. They will not be easily identified because of the large effect of environment on protein level. However,

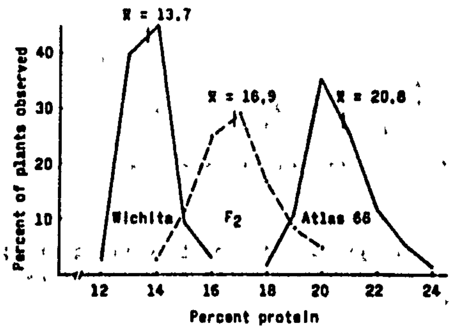

FIG. 1. Grain protein frequency distribution of plants of Wichita and Atlas 66 and their F_2 generation grown at Lincoln, Nebraska in 1957 (from JOHNSON et al., Econ. Bot. 22 (1968) 16-25).

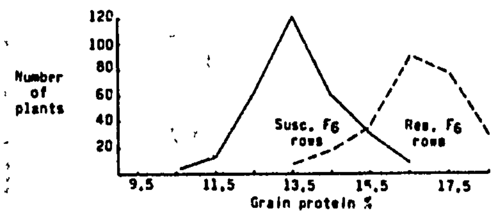

FIG. 2. Grain protein frequency distribution of F_6 rows of Atlas 66 x Wichita homozygous for reaction to leaf rust (from JOHNSON et al., Econ. Bot. 22 (1968) 16-25).

a start has been made. The University of Nebraska in co-operation with the Agricultural Research Service is systematically screening the World Collection for protein as well as for amino acid differences. The work is partially supported by funds from the Agency for International Development, US Department of State. Wheats with suspected high protein properties on the basis of the initial screening will be tested in an array of environments to establish the nature of the protein differences. Crosses of new genetically high protein wheats with Atlas 66 or Frondoso will determine whether the genes involved are different from those in Frondoso. We believe that identification of new genes can lead to increasingly higher protein plateaus in wheat by conventional breeding techniques.

Our study of the physiological aspects of the high protein phenomenon in wheat has led to some interesting and useful findings. The question of whether the high grain protein trait is associated with greater nitrogen uptake from the soil has practical significance. If differential nitrogen uptake were involved, then an adequate level of soil nitrogen availability could be a prerequisite for phenotypic expression of the high protein trait.

In no instance have we been able to demonstrate that nitrogen uptake by our high protein lines was greater than the uptake of lower protein varieties. Analyses of the foliage of varieties at weekly intervals during the spring and summer growing period revealed no greater concentration of foliage nitrogen in the high protein lines than in lower protein varieties at any time prior to anthesis (Fig. 3). In fact, the study revealed that Warrior, a low grain protein Nebraska variety, has the ability to concentrate significantly more nitrogen in its foliage than all other varieties analysed (Fig. 4). Warrior plants took up more nitrogen but obviously translocated less to their grain than other varieties.

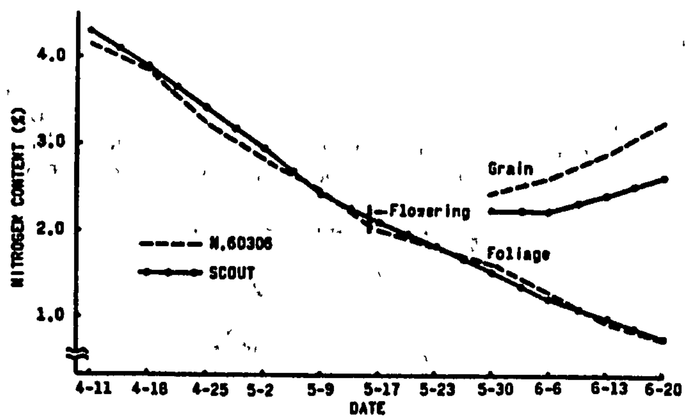

FIG. 3. Nitrogen content of the plants and grain of Atlas 66 x Comanche Selection 60306 and Scout during the spring growing season at Lincoln, Nebraska, 1963 (from JOHNSON et al., Econ. Bot. 22 (1968) 16-25).

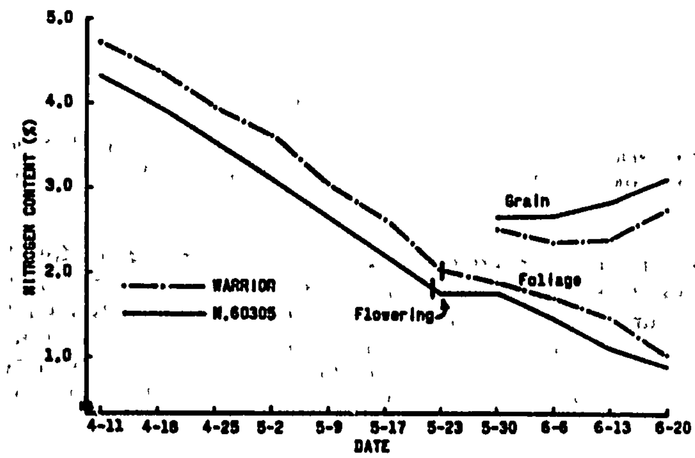

FIG. 4. Nitrogen content of the plants and grain of Atlas 66 X Comanche Selection 60305 and Warrior during the spring growing season at Lincoln, Nebraska, 1963 (from JOHNSON et al., Econ. Bot. 22 (1968) 16-25).

The concentration of nitrogen in the grain increased as grain development progressed — even during the late stages of kernel maturation. This is contrary to a commonly held notion that nitrogen is laid down early and that the latter stages of kernel development mainly involve starch deposition and, therefore, constitute a protein dilution process.

The grain of high protein lines contained higher concentrations of nitrogen than low protein varieties from the early stages of kernel development. Furthermore, the concentration of nitrogen in the grain of the high protein lines increased more rapidly during kernel maturation than in the lower protein varieties (Figs 3 and 4). All our data consistently point to more efficient and complete translocation of nitrogen from foliage to the grain as the physiologic basis of the higher protein in the grain of the Atlas 66-derived lines. We have been able to demonstrate that more of the total nitrogen in the above-ground portion of Atlas 66 derivatives resides in the grain at maturity than is the case with lower protein varieties (Fig. 5). Also, it has been possible to demonstrate the importance of leaf retention for expression of the high protein trait. Artificial defoliation of leaves after anthesis produced a larger decrease in the grain protein content of high protein lines than in low protein ones (Fig. 6).

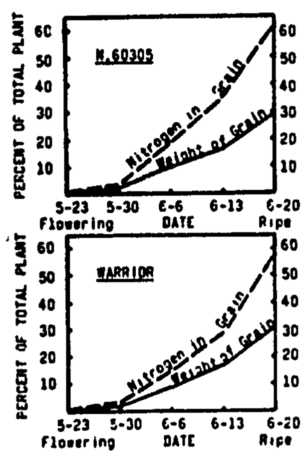

FIG. 5. Grain weight and grain nitrogen content in relation to total plant weight and total plant nitrogen during grain maturation (from JOHNSON et al . Econ. Bot 22 (1968) 16-25).

Since nitrogen uptake and nitrogen translocation from foliage to grain appear to be separate physiologic processes and varieties differ for each, it has occurred to us that enhanced levels of grain protein could accrue from combining them. For example, association of the high nitrogen uptake capacity of Warrior with the high capacity for nitrogen translocation of Atlas 66 might lead to a higher and more stable grain protein level than is possessed by either variety.

Recently we have turned our attention to the question of what influence high grain protein has on amino acid balance, hence the nutritional quality of the grain. Analyses of the essential amino acid balance of several high protein Atlas 66-derived lines indicate that high protein need not be associated with decreased lysine or methionine, two of the three most limiting amino acids in wheat (Table III). In fact, some of the high protein lines possessed higher amounts of these amino acids than other varieties (Tables IV and V). In this study, only threonine among the first three most limiting amino acids, failed to have comparable levels in high and low protein lines. However, it is particularly significant that there was an increase in the amount of each amino acid (lysine, threonine, and methionine) per unit of grain weight in the high protein lines over that of the low protein varieties with which they were compared (Tables VI and VII).

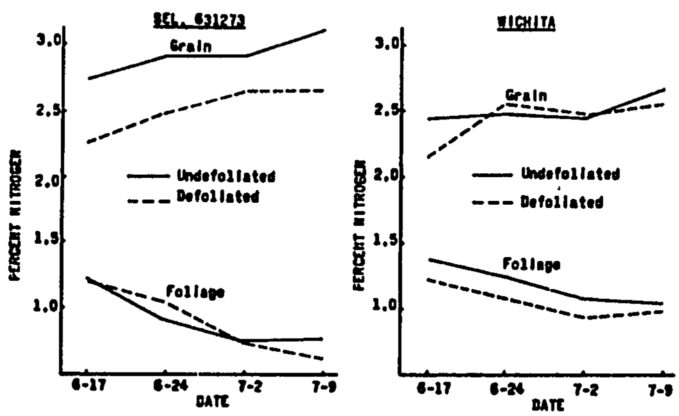

FIG. 6. Nitrogen content of the foliage and grain of defoliated and undefoliated plots of high protein selection 631273 and Wichita (from JOHNSON et al., Econ. Bot. 22 (1968) 16-25).

Table III. Lysine, methionine, and threonine levels in selected high protein lines of Atlas 66 x Comanche.

Variety	Protein % DWB1/	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 x Cmn 2507	17.7	3.72	1.74	2.62
" " 2509	18.3	3.45	1.83	3.32
" " 2504	17.9	3.38	1.14	3.69
" " 2510	16.5	3.37	1.67	3.22
" " 2499	18.2	3.29	1.68	3.10
" " 2500	18.3	3.20	1.65	3.16

1/ Percent of total dry weight.

Our analyses of 4100 common wheats from the World Collection for lysine content show a range of 1.77 to 4.15% (Table VIII). The highest lysine value obtained thus far approaches the level believed to be necessary for the nutritional balance of lysine with the other essential amino acids. If the high lysine values prove to be real from a genetic standpoint,

Table IV. Protein and essential amino acid balance in Atlas 66 x Comanche Selection 2507.

Percent higher or lower than Comanche in:	
Total protein	+14
Lysine	+15
Methionine	+ 4
Threonine	-26
Histidine	+ 6
Valine	+ 3
Isoleucine	+ 3
Leucine	0
Phenylalanine	- 5
Tryptophan	not analyzed

Table V. Protein and essential amino acid balance in Atlas 66 x Comanche Selection 2509.

Percent higher or lower than Comanche in:	
Total protein	+22
Lysine	+ 7
Methionine	+10
Threonine	- 9
Histidine	+10
Valine	+ 2
Isoleucine	0
Leucine	+ 2
Phenylalanine	- 2
Tryptophan	not analyzed

Table VI. Percent increase in amino acid content (g/100 g protein) of 17 high protein lines over their low protein parent.

Amino Acid	Range	$\bar{x}$
Lysine	- 6.5 to 13.0	+5.0
Methionine	-31.7 to 13.8	-0.8
Threonine	-26.0 to 4.2	-9.1

Table VII. Percent increase in amino acid content (g/100 g dry weight of grain) among 17 high protein lines over their low protein parent.

Amino Acid	Range	$\bar{x}$
Lysine	8.0 to 34.0	22.3
Methionine	-21.0 to 33.1	15.7
Threonine	- 3.9 to 24.2	7.7

Table VIII. Grain protein and lysine content of selected wheats from the World Collection maintained by the U. S. Department of Agriculture.

C.I. No.	Origin	Growth Habit	Protein Content1/	Lysine (% of Protein) Unadjusted	Adjusted2/
13449	USA	Winter	9.2	4.2	3.9
11721	Canada	Spring	10.2	4.1	3.9
11696	USA	Winter	11.1	4.0	3.8
13447	USA	Winter	10.3	3.9	3.7
9364	Finland	Winter	11.4	3.8	3.6
9390	China	Winter	10.0	3.8	3.6
12369	USA	Winter	16.2	3.5	3.6
1970	USA	Winter	12.5	3.7	3.6
11723	USA	Spring	10.1	3.8	3.6
11743	USA	Winter	10.4	3.8	3.6
⋮					
5483	USSR	Spring	14.3	1.8	1.8

1/ Dry weight basis.
2/ Values adjusted to 13.5% protein.

Table IX. Lysine content (% of protein) among 40 spring wheats from the World Collection grown at 3 locations in the United States.

	Minot, No. Dak.	Bozeman, Mont.	Aberdeen, Idaho Non-fert.	Aberdeen, Idaho Fertilized	4-Test Average Unadj.	4-Test Average Adjusted1/
Highest	3.33	3.41	3.88	3.79	3.43	3.50
Lowest	2.67	2.65	2.24	2.93	2.92	2.95
Justin (ck)	2.74	2.96	3.01	2.95	2.92	3.10
$\bar{x}$	2.93	3.09	3.31	3.26	3.15	3.15

1/ Lysine values adjusted to 16% protein.

then there will be opportunity for further enhancement of nutritional quality by developing high protein lines with increased lysine percentage through breeding procedures.

Limited information from three locations in the United States indicates that environment affects level of lysine (Table IX). Significant genotype-environment interactions for lysine content were obtained among 40 spring wheat varieties tested. One variety, C.I. 7337, had a significantly higher lysine/protein content than 37 other varieties at the three locations (Table X).

Linear regression analysis of 4100 wheats from the World Collection indicates that protein and lysine/protein are negatively related (Fig. 7). The relationship is modest, however. Only 18% of the variation in lysine content could be attributed to variation in protein content. In contrast,

Table X. Average yield, protein content, and lysine content of selected spring wheat varieties from the World Collection grown in 4 trials at 3 locations in the United States.

Entry No.	Source	Grain Yield Grams/Plot	Protein Content % (DWB)	Lysine Content (Adjusted)	
				% of Protein	% of Dry Wt.
31	England	496	17.9	3.50	0.56
4	USA	98	16.7	3.35	0.52
25	USSR	361	14.9	3.30	0.51
38	China	456	17.0	3.27	0.52
6	Sweden	561	16.8	3.26	0.51
27	Uruguay	444	16.5	3.25	0.51
Justin (ck)	USA	598	17.8	3.10	0.48
14	USSR	574	16.0	2.96	0.47
$\bar{x}$	40 entries	469	16.0	3.15	0.49

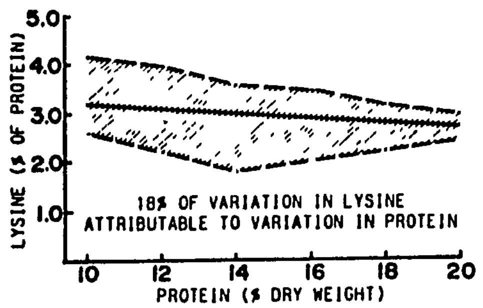

FIG. 7. Regression of lysine/protein on protein computed from the analysis of 4100 wheats from the World Collection (shaded area indicates the dispersal of actual lysine values about the regression line).

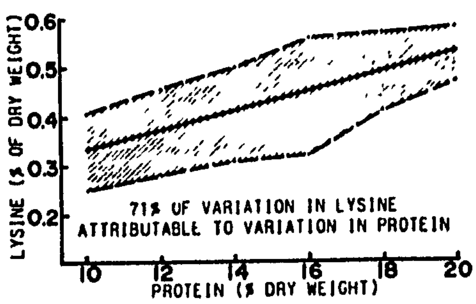

FIG. 8. Regression of lysine/dry weight on protein computed from the analysis of 4100 wheats from the World Collection (shaded area indicates the dispersal of actual lysine values about the regression line).

lysine is positively correlated with dry weight of grain. More than 70% of the variation in lysine/dry weight could be attributed to variation in protein content of the grain (Fig. 8). The adjustment of lysine values to a common protein level using the regression coefficient may be valid and potentially useful for identification of genetically high lysine varieties and the screening of populations segregating for lysine level (Tables VIII-X).

It would appear that there is sufficient genetic variation in lysine level among the wheats of the world to provide a basis for lysine modification by breeding. Numerous crosses of suspected high lysine lines with commercially acceptable varieties and with high protein lines already have been made at Lincoln. Among the spring wheats, we are utilizing the recently developed high yielding Mexican dwarf wheats as recipient varieties for the high lysine trait. Our crosses involving winter wheats are presently restricted to crosses of suspected high lysine winters with the high protein Atlas 66-derived winter lines. There is the exciting possibility of extracting from these latter crosses selections in which high protein has been combined with improved level of lysine.

There is little available information on the relative breadth of adaptation of winter wheat varieties. Such information is needed in order that the transfer of genes for high protein and high lysine be accomplished most efficiently. The newly established International Winter Wheat Performance Nursery program should permit early identification of varieties with the best potential for use as recipient genotypes for high lysine and high protein genes. Rapid assessment of the stability of the high protein and high lysine traits in widely different environments also is made possible by the nursery.

The major obstacle to the improvement of the nutritional quality of wheat protein by breeding is rapid and efficient characterization of large segregating populations for essential amino acid levels. Sophisticated and expensive laboratory apparatus is required for this. Accurate reliable data require competent technicians and a good preventative maintenance program. Relatively few wheat breeding programs have access to such laboratory facilities.

An amino acid analyser is being utilized in the Nebraska-ARS program for routine lysine analyses. We are trying to modify an auto analyser to accommodate rapid routine lysine analyses by the lysine decarboxylase method. The method shows promise but requires rigid preventative maintenance to achieve acceptable accuracy.

Improvements in protein content and amino acid balance of wheat can best be achieved in large well-equipped breeding centres. Wheat varieties best suited for the various developing countries would be utilized in breeding programs as recipient genotypes Initial identification of lines possessing the desired protein and amino acid traits would be made at these centres. Subsequent routine agronomic and quality evaluations could be conducted in the various countries. This probably is the most realistic procedure in view of the complicated and expensive laboratory procedures necessary for protein and amino acid analyses.

The success of wheat improvement by breeding is well established. Spectacular advances in grain yields have been achieved through breeding and the application of sound cropping practices. Inherent characteristics of the wheat grain have been modified to meet demanding requirements of mechanized milling and baking procedures. The feasibility of breeding wheat with higher protein content has been demonstrated. An imbalance of certain amino acids in its protein that are essential for maximum nutritional value is the principal existing shortcoming of wheat for food purposes. Chances are good that this too can be effectively modified by the application of sound breeding procedures.

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