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IMPROVEMENT OF THE NUTRITIONAL QUALITY OF WHEAT THROUGH INCREASED PROTEIN CONTENT AND IMPROVED AMINO ACID BALANCE

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Nebraska University

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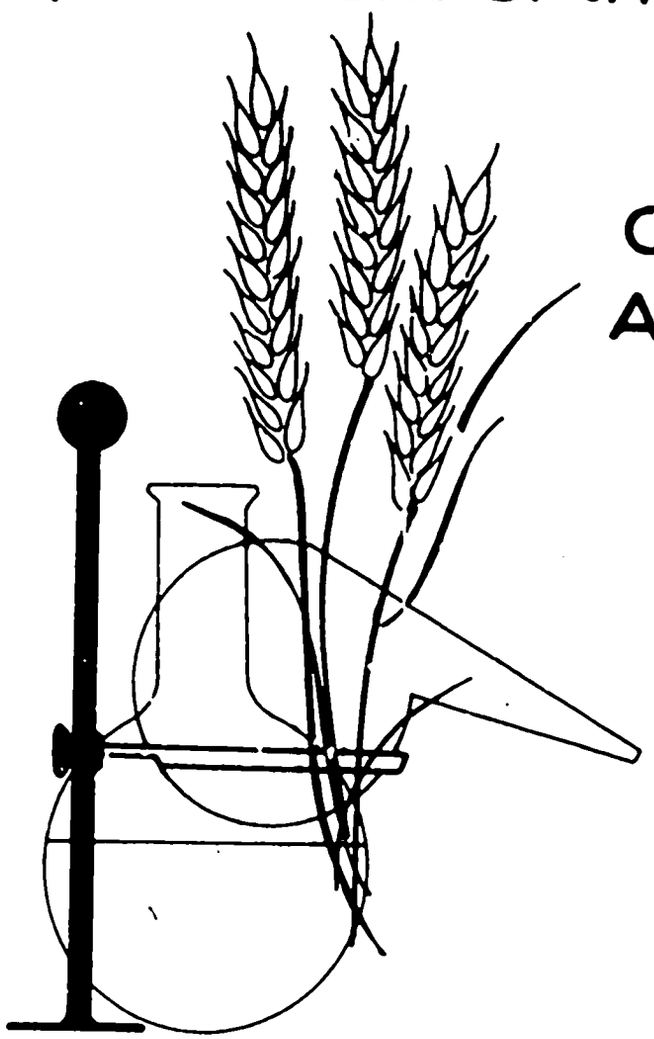
Improvement of the Nutritional Quality of Wheat through Increased Protein Content & Improved Amino Acid Balance



Summary Report of Research Findings

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JULY 1, 1966 - DECEMBER 31, 1972

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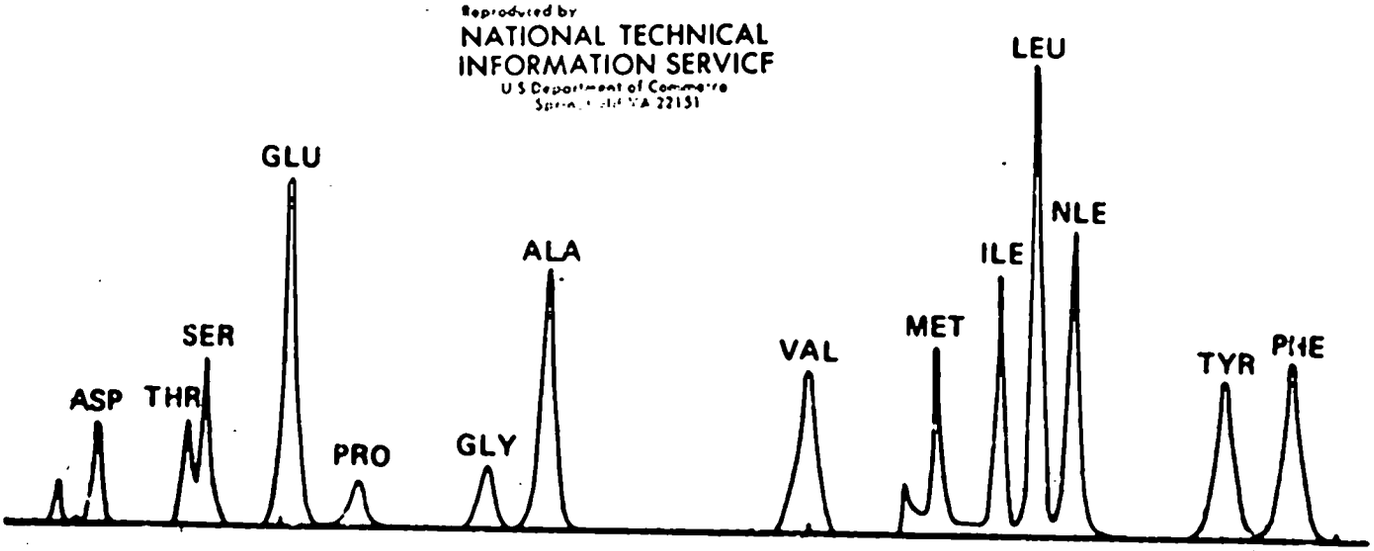

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This summary report describes the research findings on improving the nutritional quality of wheat by increasing its protein content and improving its amino acid balance from July 1, 1966 to Dec. 31, 1972.

Useful new analytical procedures have been developed and are described on sample grinding, sample blending, moisture equlibrium of samples, sample weighing, protein determinations, lysine determinations, complete amino acid determinations and in vitro nutritional assay.

Research results are reported on the world wheat collection, protein and nutritional value, kernel morphology, kernel anatomy, effect of environment on protein and lysine content, soil fertility and protein content, physiological processes associated with high grain protein, genetic studies of high protein and high lysine, breeding for improved nutritional value and the International Winter Wheat Performance Nursery.

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SUMMARY REPORT

OF

RESEARCH FINDINGS

FROM

IMPROVEMENT OF THE NUTRITIONAL QUALITY OF WHEAT THROUGH INCREASED PROTEIN CONTENT AND IMPROVED AMINO ACID BALANCE

July 1, 1966 - December 31, 1972

Prepared by

V. A. Johnson and P. J. Mattern

Contract AID/csd-1208

Agency for International Development
Department of State
Washington, D. C.

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SUMMARY OF RESEARCH PROGRESS

Research progress since 1966 under Contract AID/csd-1208 is reported. Under the contract the nutritional quality of wheat was to be improved by increasing its protein content and improving its amino acid balance. The possibility of higher protein in wheat by breeding already was established from prior research at the University of Nebraska and elsewhere but little was known concerning useful variation in amino acid composition among the wheats of the world.

Much progress has been made. Useful new analytical procedures were developed. New genetic sources of high protein were identified and utilized in breeding. Useful variation in lysine was uncovered and is being combined with high protein. Effect of production environment on protein and lysine was determined. Physiological, morphological and anatomical factors associated with protein and lysine variation were identified. Seed with potential for much improved nutritional value was distributed to several countries.

Specific research findings are listed.

Analytical Procedures

Special procedures and equipment were developed for grinding and blending small ground samples.

A humidity cabinet was devised to equilibrate ground samples to a constant moisture level. This eliminated individual moisture determinations on thousands of samples. With a known moisture content, samples for analysis were weighed directly to a dry weight basis. The need for conversion of data was thereby removed, time was saved, and possible errors during calculation reduced.

Protein was determined by macro or micro Kjeldahl procedures depending on sample size. The dye-binding procedure was employed as a fast screening method for some materials.

Several lysine screening methods were evaluated. Ion-exchange chromatography has been the most reliable method. Lysine decarboxylase and dye-binding procedures also were used.

Complete amino acid values were determined with standard ion-exchange chromatography with an automatic amino acid analyzer.

An existing in-vitro method was improved for biological evaluation of wheat protein. Ion-exchange sephadex was substituted for the original picric acid technique of concentrating the amino acids coming from the pepsin-pancreatin digest. Some wheat samples contain naturally occurring enzymes which produce ornithine during the incubation step of the digestion procedure. Ornithine is evaluated as lysine with the usual ion-exchange chromatography procedure.

Our modified procedure gives satisfactory separation of lysine and ornithine so that a more accurate Pepsin-Pancreatin Digest Residue (PPDR) Index can be achieved. A tedious calculation for the Index was computerized. Indices for wheats tested ranged from 45 to 57.

Analysis of World Wheat Collection

Sixteen thousand wheats have been analyzed to date. Large variations in protein and lysine content were found.

The genetic portion of total protein variation is at least 5 percentage points. For lysine the identifiable genetic component is 0.5 percentage points when lysine is expressed as percent of protein.

Lysine per unit protein exhibits a negative curvilinear relationship with protein. Lysine per unit grain weight is strongly positively correlated with protein.

Protein and Nutritional Value

Amino acid profiles for low and high protein wheats are different. In low protein wheats lysine per unit protein is negatively correlated with protein. It is not correlated with protein in high protein wheats.

Positive correlations of lysine with threonine and leucine indicate that genetic increases in lysine would not be associated with significant decreases in these other essential amino acids.

Chemical analyses of Nebraska high protein wheat lines (17.2%) indicate that they would provide more lysine, isoleucine, methioinine, and threonine per unit weight of grain than would opaque-2 maize containing 10.0% protein.

High protein varieties provide more of each essential amino acid than high lysine-low protein varieties from the World Collection.

Preliminary data from mouse feeding tests indicate that high protein varieties tend to produce higher weight gains and feed efficiency ratios than varieties with ordinary protein.

Kernel Morphology

Kernel size does not exert a significant influence on protein or lysine content of the kernel. Degree of kernel plumpness has a large influence on protein content but not on lysine per unit protein.

Attention to degree of kernel shriveling is important for accurate protein and lysine per unit grain weight comparisons among wheats.

Kernel Anatomy

Chemical and physical separations of kernel fractions are under way to identify those fractions associated with protein and amino acid variations in wheat. Until these are known the full contribution of protein and lysine differences to nutritional value cannot be accurately assessed.

More efficient methods of detecting and measuring protein and lysine variations should result.

Effect of Environment on Protein and Lysine

Production environment exerts a large influence on protein and lysine levels of wheat which occasionally may mask genetic differences.

Wheat varieties have been identified which produce significantly more protein and lysine than other varieties over many environments.

Soil Fertility and Protein Content

High protein genes from Atlas 66 effectively promote higher grain protein in a wide international array of environments.

Atlas-derived varieties produce grain with higher protein content than ordinary varieties under low as well as high soil fertility.

The data suggest that wheat with high grain protein (14-17%) can be achieved from genetically high protein varieties grown under high soil fertility.

The International Winter Wheat Performance Nursery (IWWPN)

Winter wheat varieties differ widely in productivity and general adaptation as measured by their performance in the IWWPN.

The superior performance of the Russian variety Bezostaia 1 in the IWWPN has led to its recent use on increasingly large acreages in Turkey, Iran, and Afghanistan. Its higher yield contributes to more adequate wheat production in these countries.

Three varieties known to possess genes for high grain protein consistently produced grain with higher protein content than other varieties at nursery sites in 23 countries.

Grain yield and protein content were not correlated when measured for individual varieties over nursery sites. Only a small portion of total protein variation could be accounted for by variations in grain yield.

International Winter Wheat Conference

The conference was held in Ankara, Turkey in 1972. It was organized by the University of Nebraska under Contract AID/csd-1208. Ninety seven wheat researchers from 24 countries participated. The Conference Proceedings have been distributed as Nebraska Miscellaneous Publication 28.

Physiological Basis for High Protein

High grain protein results from more complete nitrogen translocation from the plant to its grain. Differential N-uptake has not been demonstrated.

The nitrate reductase enzyme system has been implicated by University of Illinois research on high protein in wheat. Research is in progress under this contract which will more fully explore the role of nitrogen metabolism in the high protein phenomenon.

Genetic Studies

A number of different genetic sources of high protein in wheat have been identified.

The Indian variety Nap Hal (P.I. 176217) possesses high lysine in combination with high protein. Its genes for high protein are different from those in Atlas 66. New levels of high protein have been identified among the progeny of Atlas 66 x Nap Hal. The Nap Hal level of lysine appears to have been recovered in some of these.

The inheritance of high protein and high lysine of Nap Hal is under investigation.

Breeding for Improved Nutritional Value

The high protein trait of Atlas 66 has been transferred to attractive potentially useful lines for dryland production areas which are being evaluated in Turkey, Iran, and Afghanistan.

Twenty six high protein lines have been released publically as elite wheat germplasm.

Selections from Atlas 66 x Nap Hal with combined very high protein and high lysine will be subjected to mouse feeding trials to ascertain their true nutritional value.

Additional genetic sources of high protein and high lysine uncovered in the World Collection are being utilized for breeding purposes.

Publications

Forty seven publications of research findings from this project have been issued since 1966. Project personnel have presented invitational papers or participated in 45 regional, national and international conferences and workshops.

Seed and Reprint Requests

There have been 100 requests from 24 different countries for seed of varieties identified by the project. Most have been for small amounts for breeding and testing purposes. Foreign requests for reprints of project publications total 327.

The large number of requests for seed and research information reflect a high level of interest throughout the world in the work of the project.

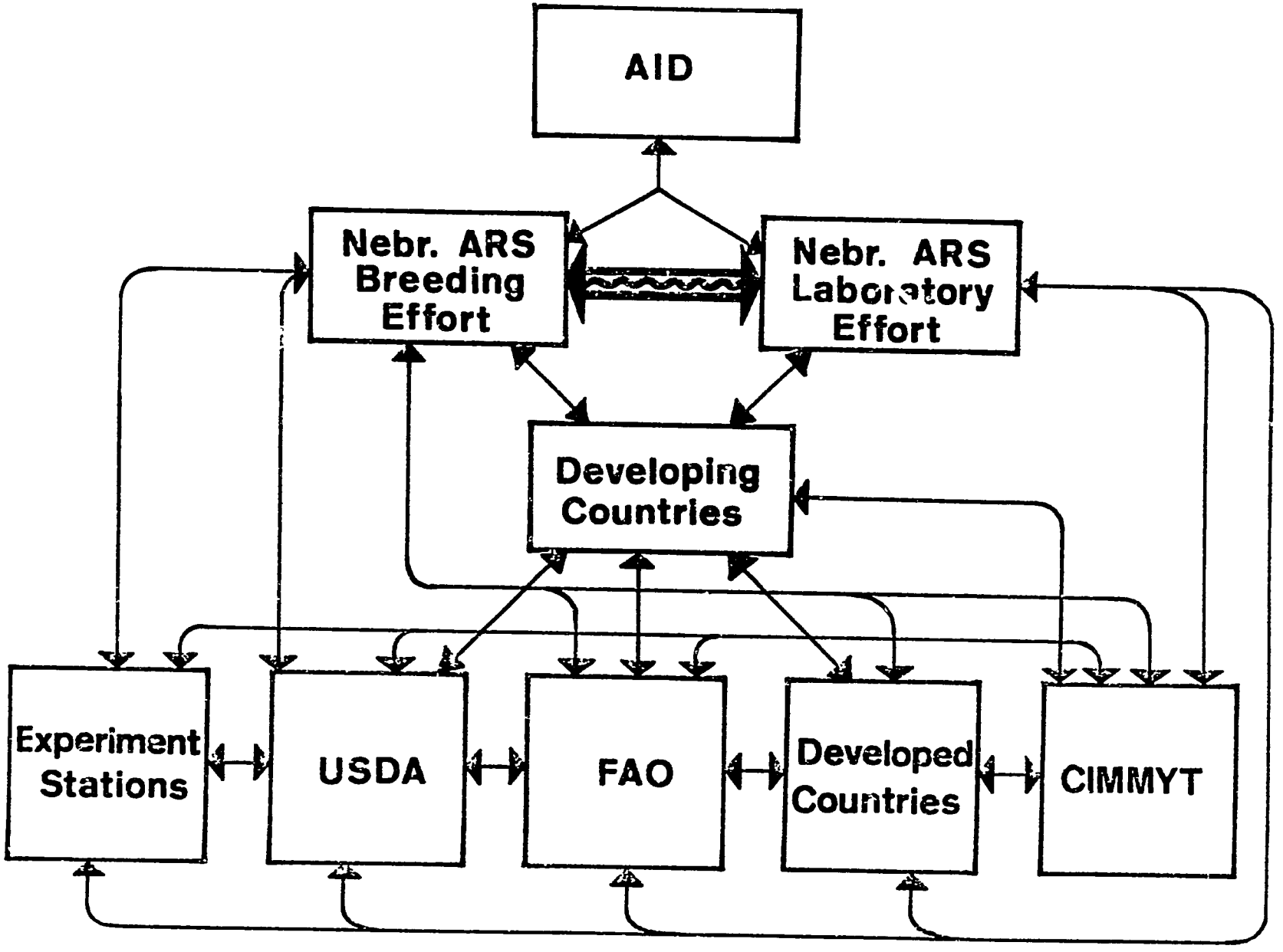
COOPERATION

The wheat nutritional improvement project at the University of Nebraska is cooperative between the University, the Agricultural Research Service, USDA, and Agency for International Development, U. S. Department of State. Financial support for the work is derived jointly from these three agencies.

There has been active but informal cooperation with the International Maize and Wheat Improvement Center, Mexico, D. F., who aided in establishing initial contacts with scientists in countries who participate in growing the IWWPN and provided guidance in details of nursery organization and management. Personnel of the Plant Production and Protection Division, Food and Agriculture Organization of the United Nations, have cooperated in arranging for movement of seed and nursery inspections at Near East sites.

There has been excellent cooperation from scientists at various Agricultural Experiment Stations in the United States and many other countries at which the IWWPN and other project materials were grown. These people provide the day-to-day supervision of planting, note-taking, harvest and processing of the nurseries. A flow chart of information and germplasm movements which follows, outlines the cooperation of the Nebraska project with other agencies and countries.

The assistance of Department of Agronomy secretaries Mrs. Katie Meierhenry and Miss Chris Kant in the typing and assembly of this report is gratefully acknowledged.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IMPROVEMENT OF THE NUTRITIONAL QUALITY OF WHEAT THROUGH INCREASED PROTEIN CONTENT AND IMPROVED AMINO ACID BALANCE

INTRODUCTION

The Nebraska Agricultural Experiment Station in 1966 was awarded a contract by the Agency for International Development, U. S. Department of State, for research on the nutritional improvement of wheat. The research was concerned specifically with modification of the quantity and quality of wheat protein. Genes for high grain protein in wheat had already been identified and research data on the inheritance and physiological mechanisms associated with the high protein phenomenon had been published by ARS-Nebraska investigators.

Discovery of the impact of the opaque-2 and floury genes on amino acid composition and nutritional value of maize protein provided impetus for similar investigations of other cereal species, all of which are notably low in lysine. Since an estimated one billion people rely on wheat as their main food staple, any improvement of its nutritional value could have enormous impact. Research findings from the first five years of the Nebraska-AID contract on nutritional improvement of wheat are summarized.

Six semi-annual progress reports covering the period July 1, 1966 through June 30, 1969 have been previously prepared and submitted. Preliminary reports of results of the 1st, 2nd, and 3rd International Winter Wheat Performance Nurseries established in 1968 also have been distributed. Significant findings emanating from the research also are largely covered in several publications of project personnel.

RESEARCH OBJECTIVES

The initial research was conducted under six broad objectives as follows:

1. Systematically analyze the wheats in the World Collection maintained by the Agricultural Research Service, U. S. Department of Agriculture, for differences in quantity of protein and lysine content of the protein.
2. Construct amino acid profiles for selected wheats from the World Collection identified for high lysine.
3. Propagate high lysine, high protein, and other nutritionally promising wheats in different geographical areas of the United States to measure the effect of environment on lysine and protein levels.
4. Hybridize different genetic sources of high protein and high lysine as they are identified to achieve new high levels of these kernel constituents.
5. Incorporate genes for high protein and high lysine into agronomically acceptable wheats for developing countries.
6. Distribute research information and nutritionally useful wheat germplasm to developing countries and other research agencies for use in genetic and breeding research.

ANALYTICAL PROCEDURES

Initial screening of the World Wheat Collection for protein and lysine was made on 10-gram samples of seed produced from irrigated increase plantings at Mesa, Arizona. Not all of the seed was produced in the same year.

Sample Grinding

Initially we used a Udy Cyclone Sample Mill[1] for sample grinding which was a modification of the Weber Brothers Laboratory Pulverizing Mill. This small hammer mill was used with a 0.024-inch screen and a vacuum-operated cyclone for sample pickup. The grinder was cleaned between samples during processing of 5-10-gram samples.

The laboratory currently utilizes a newly-designed Udy Cyclone Sample Mill in which the grinding principle has been entirely changed. In the new mill, impact fins of a propellor force grain against an abrasive surface which grinds the wheat quickly without an additional vacuum source. Mill cleanout between samples of 5 grams or larger size has proven to be unnecessary.

Cereals containing relatively high levels of lipid may adhere to the sides of the cyclone grinder. A removable plate permits rapid efficient cleaning of the grinder. We operate the mill with a .040-inch screen when grinding wheat samples for normal dye-binding protein analysis..

Sample Blending

We have found that brushing samples through two stacked sieves provides adequate blending of the ground material. Where the blended sample does not occupy all of the container space further blending is accomplished by tumbling the container.

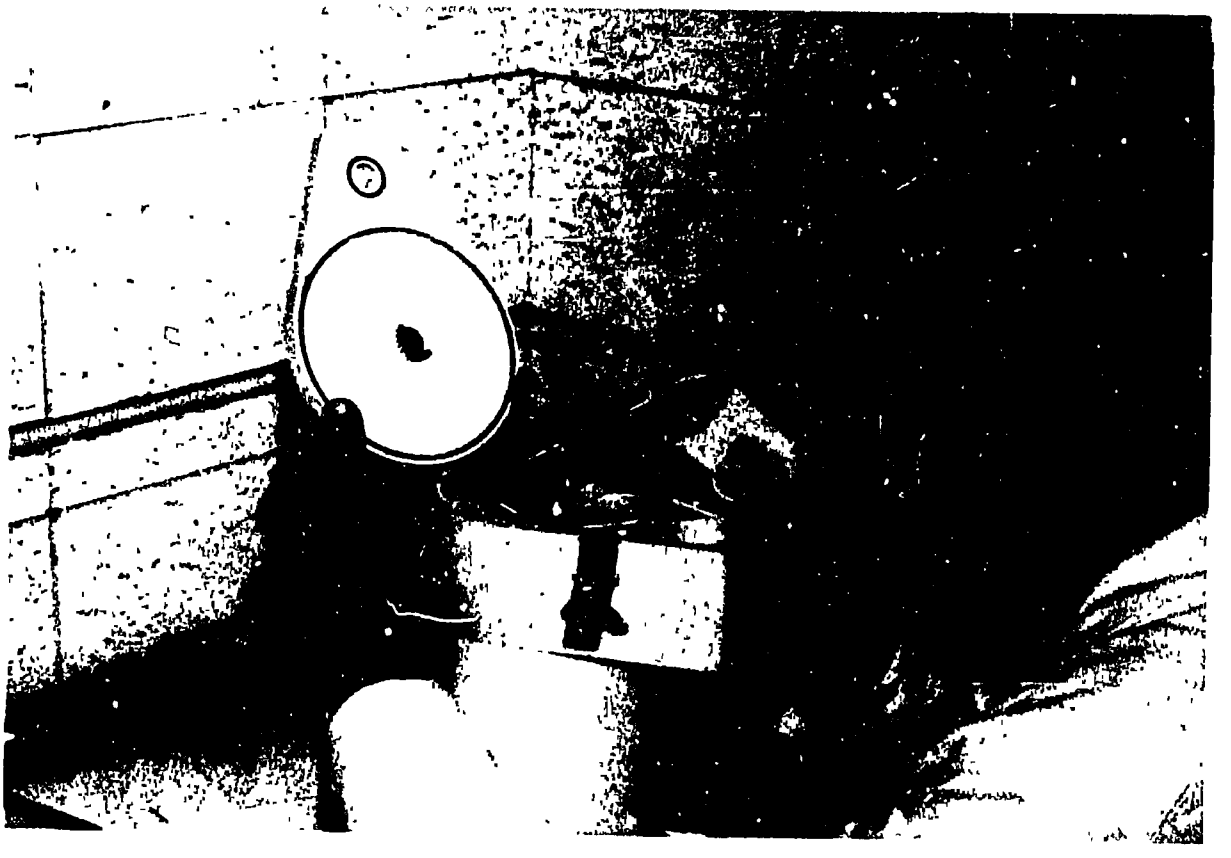
[1]Mention of firm names or trade products does not imply that they are recommended by the U. S. Department of Agriculture and Agency for International Development, U. S. Department of State, over other firms or similar products not mentioned.

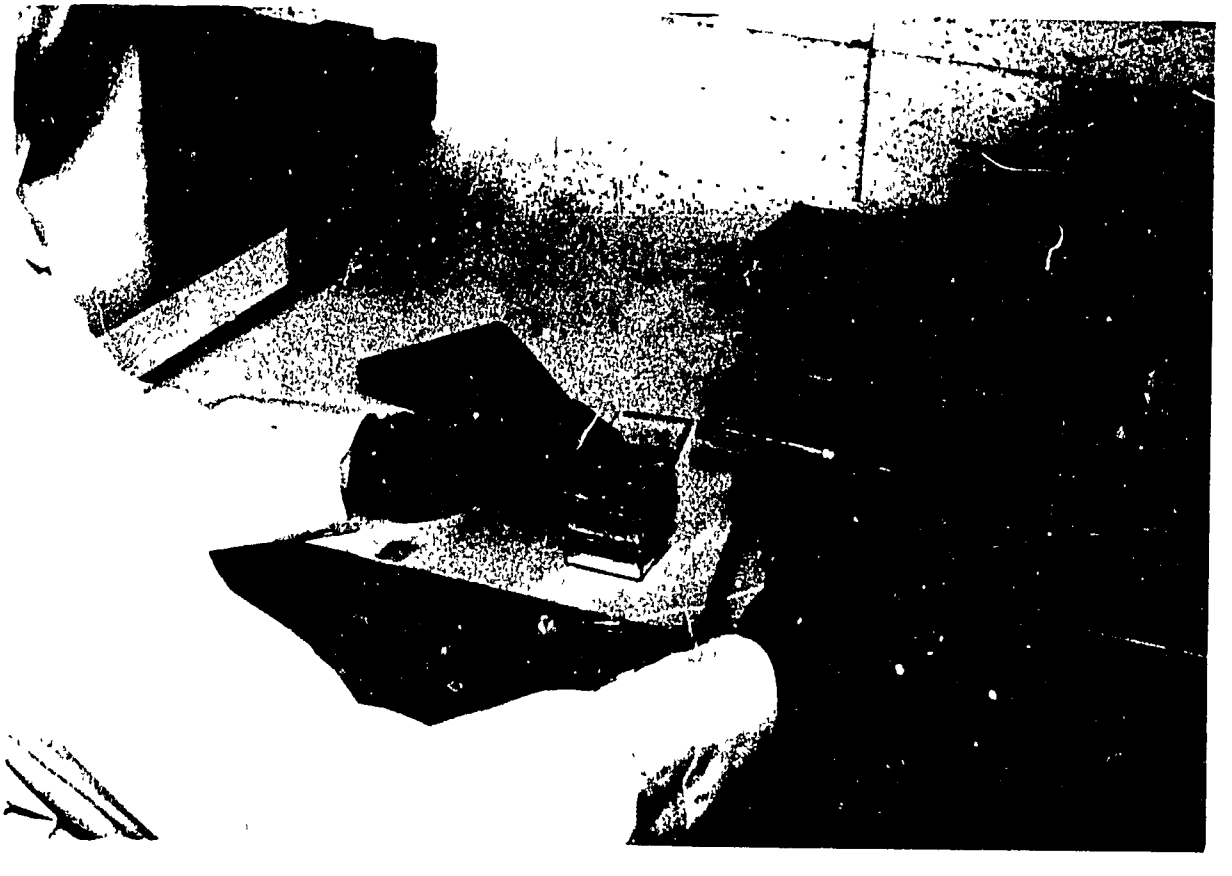
Moisture Equilibration of Samples

The moisture content of cereal samples must be known to compute analytical data to a constant moisture value. Such determinations waste sample material and are too slow for a laboratory operation involving large numbers of samples.

We utilize a baking fermentation cabinet (Figure 1) from the National Manufacturing Company, Lincoln, Nebraska, as a moisture equilibrating cabinet. Temperature control provided in the cabinet is not used since the room in which the cabinet is operated has controlled temperature. Air circulation in the cabinet is provided by a squirrel cage fan. Humidity control is achieved by a Walton Montclair Model WF-225 humidifier unit installed below the cabinet and connected by one-fourth inch I.D. pressure rubber tubing with a rubber stopper reducing unit. This arrangement was necessary to prevent over-shooting of humidity in the cabinet during the cycling of the humidity controller unit. We find that ground wheat samples can be maintained at a moisture content of 11.7-11.9% with a hygrometer controller setting of 46% relative humidity and room temperature of 21-22° centigrade.

Constant moisture content of cereal samples is achieved in 3 to 5 days for ground material and 21 days for whole grain. Each cereal comes to its own characteristic moisture level in the constant condition of the cabinet. These are shown below. Differing protein levels and prior drying treatments influence final moisture levels slightly.





Improved Udy cyclone sample mill for grinding cereal samples (above).
Stacked sieve arrangement for blending ground cereal samples (below).

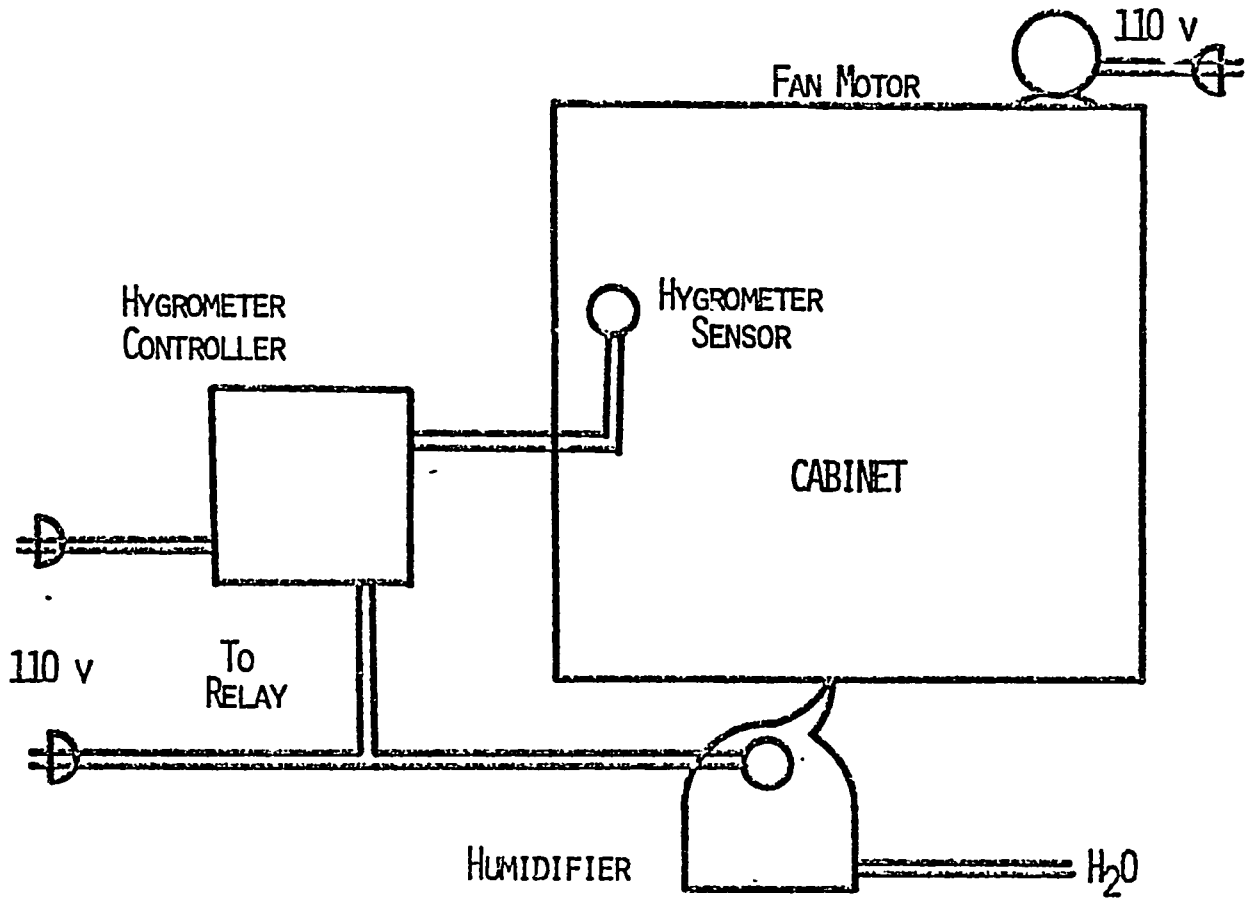


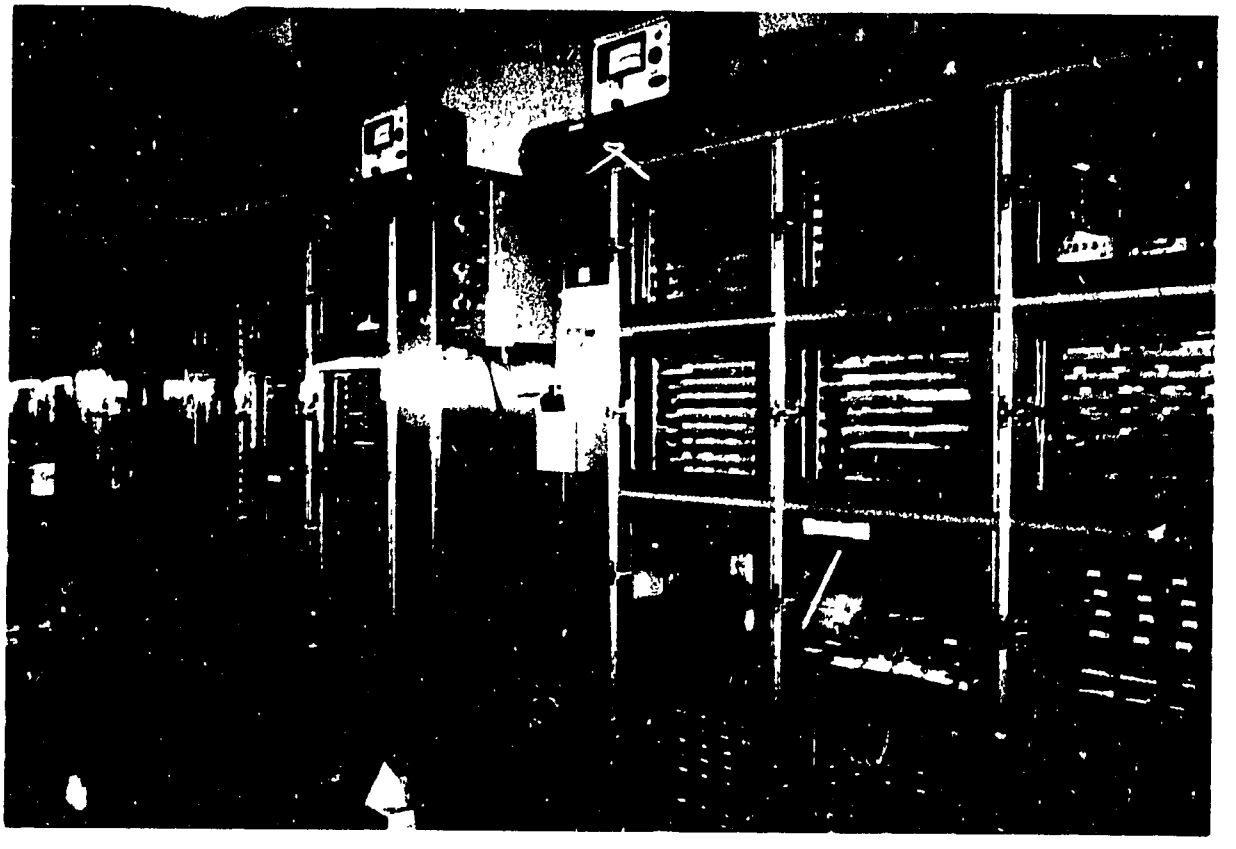
Figure 1. Moisture equilibrating cabinet used in the Nebraska Wheat Quality Laboratory.

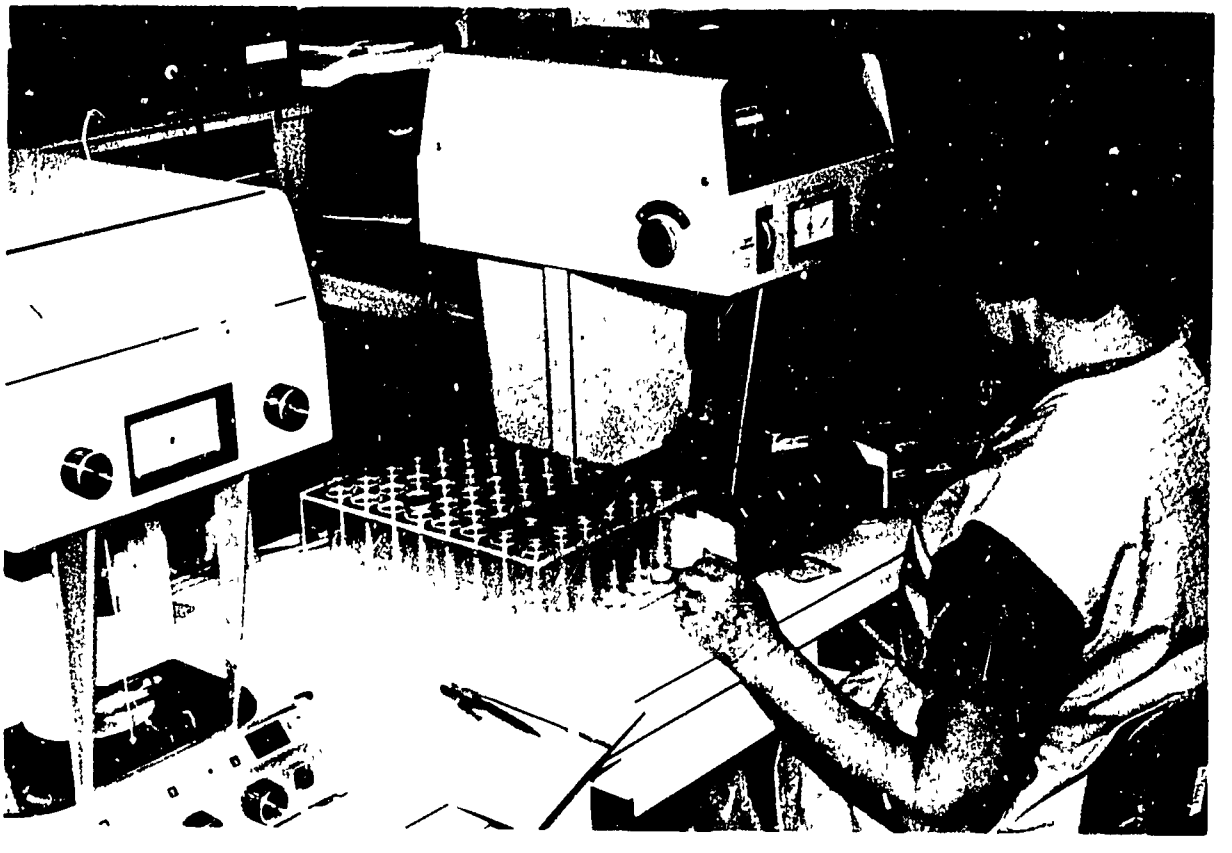
Cereal	Protein Content (%)	Moisture Level (%)[1]
Hard red winter wheat	17.0	11.95
Soft red winter wheat	17.5	11.65
Triticale	18.2	11.30
High lysine maize	12.3	11.30
Barley	--	11.40

[1]Cabinet at 46% relative humidity and 21°C temperature

The cabinet has 12 small doors, each with 9 or 10 shelf units. There are 9 drawers on each shelf. Each drawer has a capacity of 45 polystyrene sample boxes 2" x 2" x 3/4". Capacity per door is 405 samples and per cabinet 4000 samples. Adhesive teflon placed on the runner edges of the shelf minimizes friction and avoids powdered metal falling into samples.

Several control samples are weighed each morning for moisture determination. Since moisture for each kind of cereal is then known, samples can be weighed to a constant moisture content. We use prepared tables for appropriate dry sample weights at various moisture levels. This technique makes unnecessary conversions of data upon completion of an analysis.





Humidity cabinets for equilibrating ground cereal samples to constant moisture before weighing (above). Weighing operation (below).

Sample Weighing

Samples from the moisture equilibrating cabinet are weighed directly to the desired moisture basis using the following conversion table of moistures and weights:

Moisture Correction to Weigh to Dry Weight Basis

% Moisture	For 1.0 g. Dry Weight weigh:	For 0.5 g. Dry Weight weigh:
11.50	1.1299	0.5650
11.52	1.1302	0.5651
11.54	1.1305	0.5653
11.56	1.1307	0.5654
11.58	1.1310	0.5655
11.60	1.1312	0.5656

As can be determined from the table, 1.1299 g. of a sample with 11.50 moisture would be weighed to obtain a 1 g. dry weight sample. If a 1 g. sample containing 15% protein and 11.50% moisture had been weighed on a "as is" basis as is customary, conversion of analytical data to dry weight basis would utilize the following formula:

$$\frac{100 - \text{"as is" data}}{100 - \text{desired \% moisture}} = \frac{\text{\% constituent "as is"}}{x\ (\text{\% constituent at desired moisture})}$$

Computation

$$\frac{100 - 11.50}{100 - 0} = \frac{15}{x} \quad (X = 16.95\% \text{ protein at } 0\% \text{ moisture})$$

Advantages of our procedure involving the use of a moisture equilibrating cabinet are:

1. Only 2 moisture determinations per cabinet per day are necessary.
2. Pre-adjusted sample weights eliminate time-consuming moisture conversions of analytical data.
3. Samples are held to permit rechecks where necessary.

Protein Determinations

By Kjeldahl --

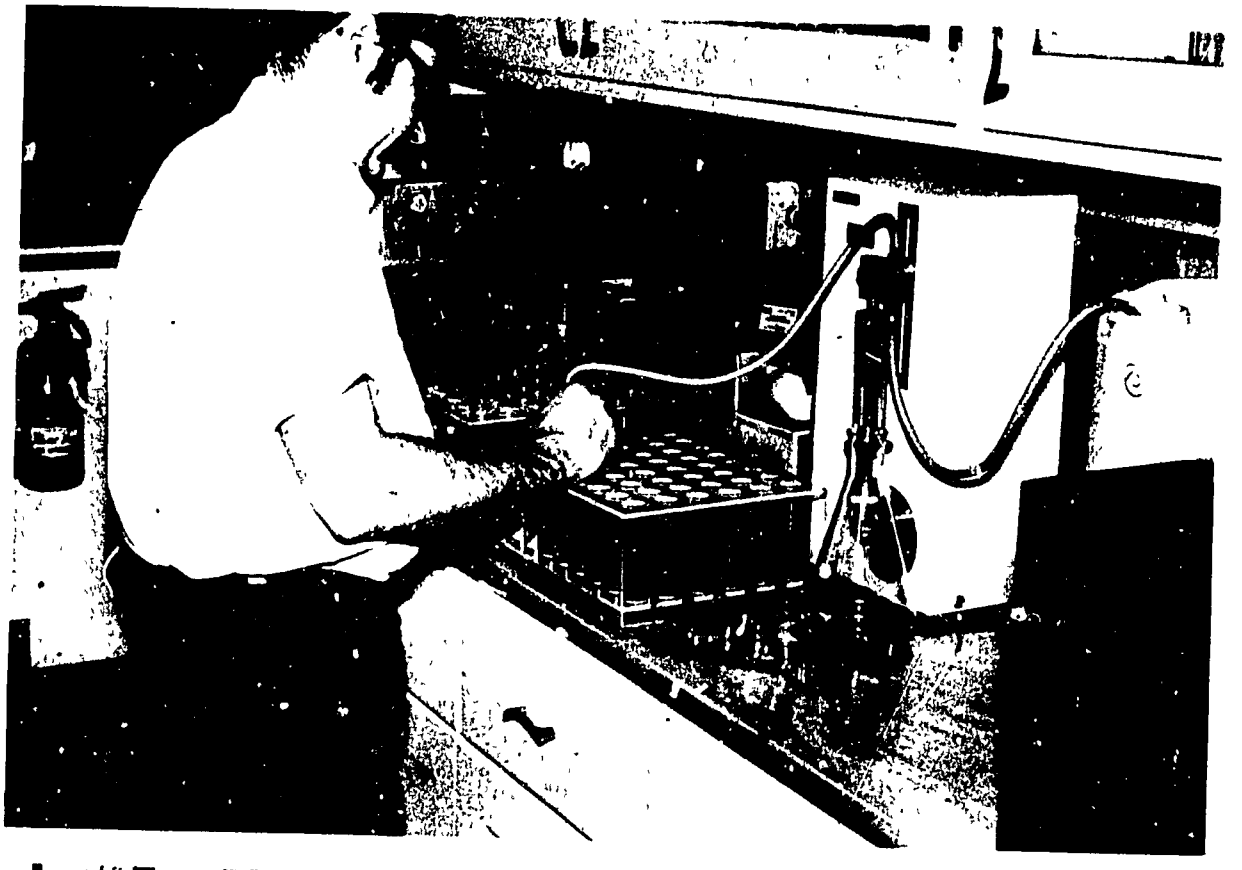
If sample size is sufficient we prefer the method for protein analyses. Although the method has been in use for more than 80 years it is not necessarily an "easy" procedure. It requires special laboratory installation and competent technicians for accurate data. We also utilize the Kjeldahl method for samples to be analyzed for complete amino acid composition.

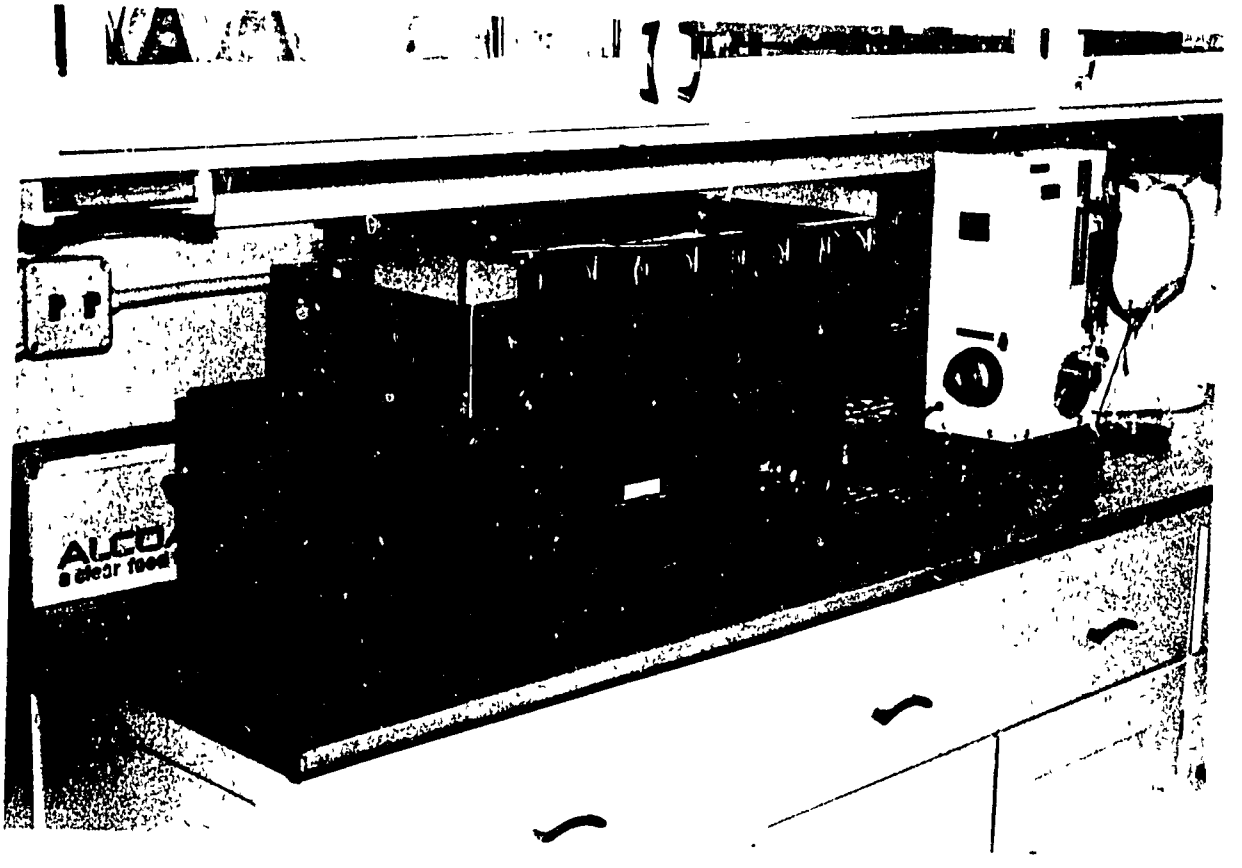
We utilize the micro-Kjeldahl technique for small samples. Digestions for the micro-Kjeldahl now are simplified by the use of a 40-unit digestor produced by Tector ab, Kronborgsgatan, Sweden. Samples are digested in 25 x 250 mm tubes. A special exhaust system is needed to adequately eliminate sulfuric acid fumes. Hengar tubes used as closures for digestion tubes reduce the fume problem. We employ the alkaline-phenol color development detection system recommended by the Technicon Corporation.

By Dye-Binding --

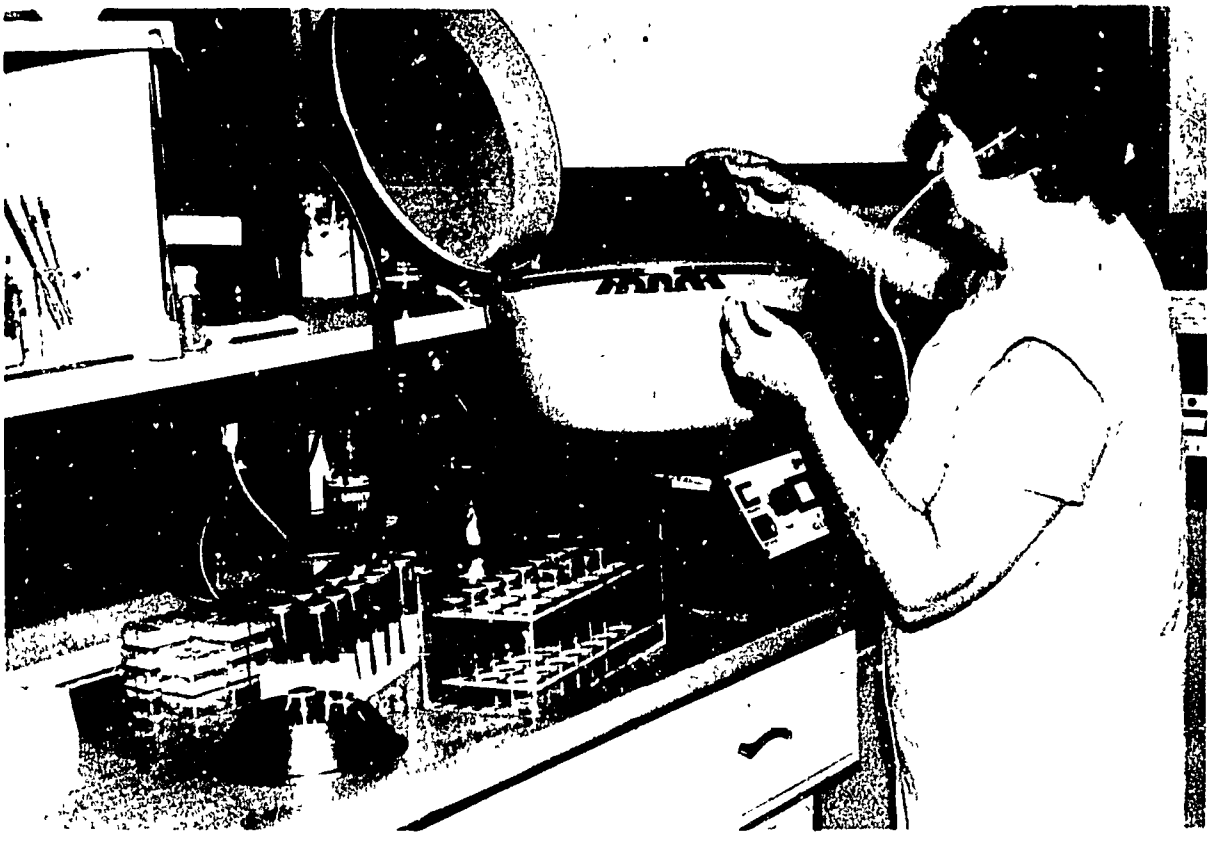
When this system is employed in our laboratory we use the azo sulfonic dye acid orange 12 (C.I.15970). The acid-buffered dye solution is mixed with the ground cereal sample and the single anionic dye bonds with the basic imidazol, quanidine, and amino groups of the protein. These groups originate from the basic amino acids histidine, arginine, and lysine or from the free amino groups at the end of the protein chains.

We employ a modification of the Udy method. One-half gram ground samples are weighed into 50 ml polycarbonate centrifuge tubes. Twenty-five ml of dye (1.3 mg/ml) are added with a mechanically driven syringe (Brewer Automatic Pipetting Machine). Tubes are capped and shaken to wet the contents before being placed in special 24-sample shaker racks on a Eberbach reciprocating shaker. The tubes are agitated in a horizontal position at 146 excursions per minute for one hour. Tubes are then centrifuged for 15 minutes at 5000 rpm





Dye solution is added to samples automatically in the dye-binding procedure for determining protein or lysine (above). Samples are reacted with the dye solution for one hour on shaker (below).



Samples being evaluated by dye-binding procedure are centrifuged (above) before reading in a colorimeter (below).

in a model GLC-1 Sorvall Clinical Centrifuge. The supernatant dye solution is read in a Udy colorimeter. Appropriate standarized conversion charts are used to convert percent light transmission to percent protein. A 0.2-gram sample with 10 ml of dye solution will provide an accurate dye binding value if the sampling is adequate.

For best results with the dye-binding procedure the following steps should be followed:

1. Grind all samples on the same mill with as uniform conditions as possible.
2. Evaluate a known standard wheat sample daily in order to check accuracy of weighing, solutions, etc.
3. Check calibration of the colorimeter with known dye solutions if the standard wheat sample is in error.
4. Use a centrifuge to remove sample from the dye solution to avoid problems associated with filtering systems.

Lysine Determinations

By Ion-Exchange Chromatography --

We consider this the most reliable method for determination of lysine and other amino acids. Automated amino acid analyzers are very expensive and require highly trained competent technicians for productive operation. Laboratories with minimal financial support and technical personnel should consider other available methods for lysine determinations.

Screening of the World Wheat Collection for lysine differences in our laboratory was done mainly with a Beckman Automatic Amino Acid Analyzer modified with four short (5 cm) columns packed with PA35 resin. Samples were acid hydrolyzed prior to analysis. Our procedure was programmed so that only the lysine peak was recorded and integrated with a Beckman Integrator Model 125. With this procedure a competent operator can analyze 50 prepared samples per day.

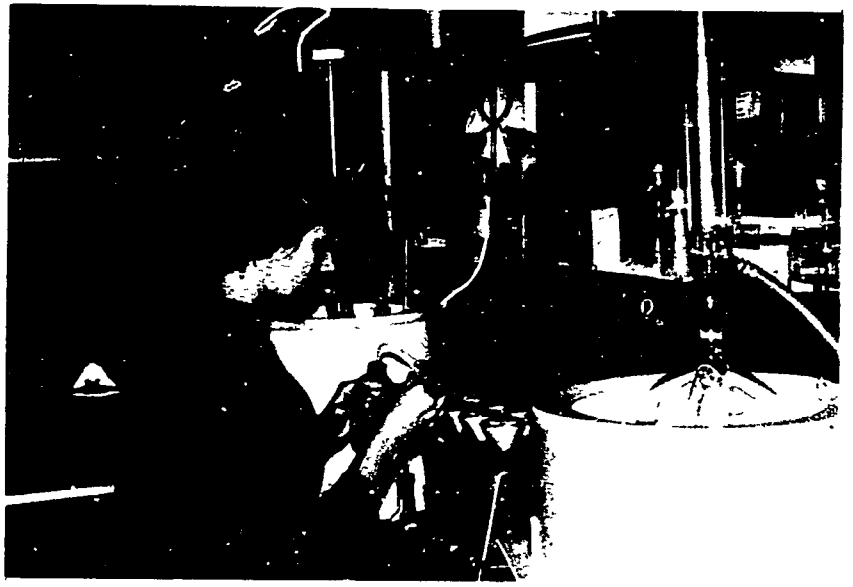
By Dye-Binding --

Mossberg (Sweden) demonstrated that dye-binding capacity (DBC) of barley, wheat, oats, rye, triticale, and maize is more highly correlated with basic amino acid content (r = 0.940) than with nitrogen and crude protein content (r = 0.767). Lysine was correlated with DBC (r = 0.819). Munck (Sweden) incorporated into the DBC procedure the weighing of samples to a constant amount of protein.

We utilize a modification of the Mossberg-Munck procedure. Crude protein content of a wheat sample is determined by Kjeldahl. A ground sample containing 65 mg of protein is shaken for one hour with 25 ml solution containing 1.3 mg/ml of dye (Udy Analyzer Company commercial dye) in a 50-ml polycarbonate centrifuge tube. The mixture is centrifuged in accordance with the Udy Method procedure. "Apparent" lysine is determined by ion exchange chromatography. Our DBC data have correlated well with standard lysine values (r = 0.78).

Complete Amino Acid Determinations

Special precautions are necessary to remove oxygen from the hydrolyzate during acid hydrolysis of the samples. We employ a 2-column system on the Beckman analyzer with 5 cm of PA35 resin in the short column for the basic amino acids and 56 cm of A6 Bio-Rod resin for the long column for determination of the neutral and acidic amino acids. One of three amino acid analyzers in our laboratory is equipped with a Bio Cal Automatic sample applicator (Model A20) and an Infotronics Integrator (Model CRS-110A). The automatic applicator has a 19-sample capacity. Approximately 4 hours are required per sample permitting 76 hours of continuous operation before reloading is necessary.



Sealing hydrolysis tubes for lysine screening (upper left). Filtering and evaporating acid hydrolysis solutions (lower left). Technicians monitoring automatic amino acid analyzers (above). Analyzer on right has been modified with 4 short columns for lysine screening.

Tryptophan --

Since tryptophan is destroyed during the normal 6NHCl acid hydrolysis procedure for the complete amino acid profile, it is determined from a separate basic hydrolyzate by a modified ion exchange method. The method utilizes an 8 cm column of PA35 resin which is equilibrated with a 3.28 pH sodium citrate buffer and developed with a 5.28 pH sodium citrate buffer.

In Vitro Nutritional Assay

We have utilized an *in vitro* assay for wheat protein to compute a Pepsin-Pancreatin Digest Index in which hog pepsin and pancreatin is used. The method was published by Akeson and Stakman in 1964. It makes use of the amino acid analyzer to determine the essential amino acids released by the proteolytic enzyme system.

The computation uses acid hydrolysis data for whole egg and the unknown wheat sample and essential amino acid data from proteolytic enzyme-treated whole egg and the wheat sample. From these four sets of data the PPDR index is determined from a prepared computer program. Whole egg serves as the reference.

We have improved the method with the use of DEAE-Sephadex to recover the essential amino acids following the pepsin-pancreatin digest. Lysine is frequently difficult to determine on the amino acid analyzer because of formation of ornithine during the assay. To effectively separate lysine from ornithine, we have used a 20-cm column of PA35 resin equilibrated with a 3.28 pH sodium citrate buffer and eluted with 5.28 pH sodium citrate buffer.

This *in vitro* test requires much sophisticated laboratory apparatus. The computations are time-consuming and tedious without the use of a computer. A small animal test laboratory would appear to offer several distinct advantages over this laboratory procedure.

Small Animal Laboratory

A small animal laboratory was established in 1971 to do *in vivo* tests of high protein wheats using the mouse as the test animal. The laboratory is cooperative between the Departments of Food and Nutrition and Agronomy of the University of Nebraska. The laboratory has a capacity of 350 samples per year. A 28-day test involving 5 animals is utilized for each sample. Data from *in vitro* tests are being compared with mouse feeding values to determine how well these data compare with FER and PER values from mouse feeding.

FIELD AND GREENHOUSE PROCEDURES

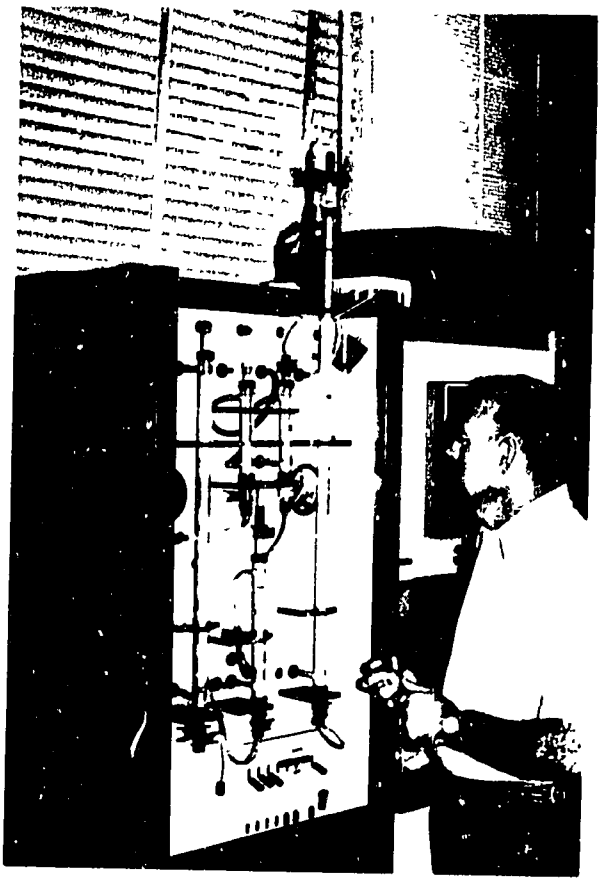
Hybridization and Propagation

Hybridization of nutritionally promising lines and propagation of F_1 hybrids is done in greenhouses on the University of Nebraska campus. Propagation and selection of segregating populations are conducted at Lincoln and Mead, Nebraska and at Yuma, Arizona.

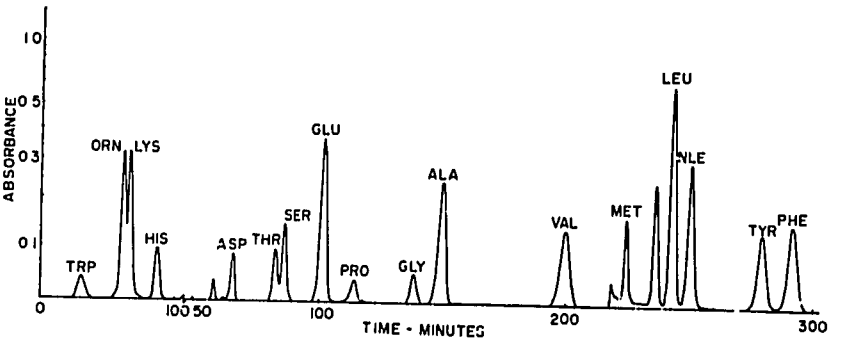
International Evaluation

An international winter wheat performance nursery was organized and grown initially in 1969 at 23 sites in 16 countries. By 1972 the number of sites had increased to 44 in 27 countries (Figure 2). The nursery is comprised of the best and most representative varieties from the winter wheat-producing countries of the world grown in 6-row plots replicated 4 times at each site.

Cooperators return seed samples from harvested plots and agronomic performance data to project personnel at the University of Nebraska for analysis and summarization. Preliminary mimeographed reports are issued to cooperators upon receipt of data from all reporting sites with full nursery data published at a later date as a Nebraska Research Bulletin.



Amino acid analyzer modified with longer ion-exchange columns for improved amino acid resolution from in-vitro protein digestion studies.



Chromatogram from the modified amino acid analyzer showing the amino acids released during an in-vitro, pepsin-pancreatin protein digestion.

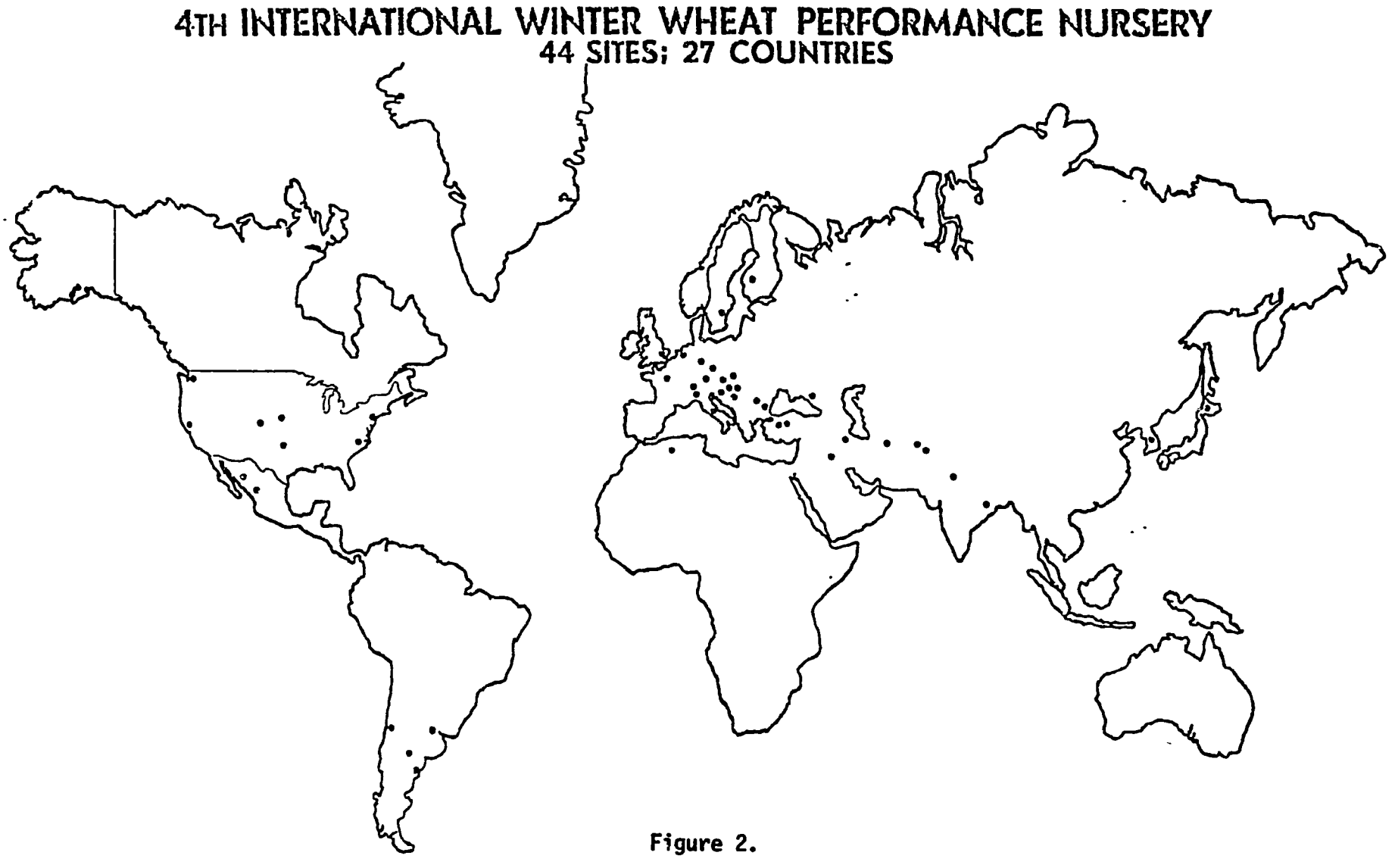

4TH INTERNATIONAL WINTER WHEAT PERFORMANCE NURSERY
44 SITES; 27 COUNTRIES

Figure 2.

United States quarantine regulations require that plant materials from foreign countries be propagated in quarantine isolation for one year. The Agricultural Experiment Station at Yuma, Arizona was selected for this activity as well as the propagation of winter-tender populations and populations segregating for growth habit generated by the Nebraska research.

Environmental Nurseries

Varieties from the World Collection that exhibited unusual levels of seed protein and/or lysine based on the initial analysis, were propagated at selected sites in the United States to provide seed for re-evaluation of protein and lysine. The nursery sites were:

Spring wheat		Winter wheat	
Minot, No. Dakota	(1967, 68, 69, 70)	College Station, Texas	(1968)
Bozeman, Montana	(1967, 68, 69, 70)	Denton, Texas	(1969, 70)
Aberdeen, Idaho	(1967, 68, 69, 70)	Pendleton, Oregon	(1968)
Lincoln, Nebraska	(1968, 69)	Mesa, Arizona	(1968)
Davis, California	(1969, 70)	Yuma, Arizona	(1969, 70)
		Lincoln, Nebraska Greenhouse	(1968, 69, 70)
		Davis, California	(1969, 70)
		Aberdeen, Idaho	(1970)

Spring wheats in the trials numbered 40 in 1967, 25 in 1968, 32 in 1969, and 32 in 1970. For winter wheats the number was 60 in 1968, 44 in 1969, and 75 in 1970.

DATA PROCESSING

All laboratory analytical data and field agronomic data are transferred to computer punchcards for machine-processing and analyses. Computer print-outs of protein and lysine data for wheats in the World Collection are transmitted to J. C. Craddock, Beltsville, Maryland for incorporation into the permanent records of the World Collection wheats. Complete print-outs also are available upon request to any agency or researcher who desires them. Linear regression is used to adjust lysine values to a common protein level to permit lysine comparisons among wheats that differ widely in the protein content of their grain.

Computer usage also provides for permanent file copies of data and permits rapid additional statistical computations when they are needed. An example of computer print-out information from protein and lysine screening of World Collection samples and lines from hybrid populations appears below:

Lab. no.	Sample no.	Protein	Integrator value	Lysine per sample	Lysine per unit protein	Adjusted lysine per unit protein
1	101	17.6	1161	0.48	2.73	2.96
2	102	15.1	1058	0.43	2.90	2.99
3	103	21.1	1399	0.58	2.75	3.16
4	104	19.3	1258	0.52	2.70	3.02
5	105	18.9	1222	0.50	2.68	2.97

Example of a print-out for complete amino acid determinations appears in the tabulation that follows. Columns 1, 2, and 3 are imput data for the computation.

AMINO ACID ANAYLSIS

LABORATORY SAMPLE A71-10442 BG ANALYZER NO. 3 CHROMATOGRAMS 3-42-72-S-6
TYPE 1 1=ACID 2=ENZYME AA=AMINO ACID MFB=MOISTURE FREE BASIS 3-41-72-L-6
CALCULATION BASED ON DATA B 302

VOL. ON SHORT COLUMN 1 ML
VOL. ON LONG COLUMN 1 ML
SAMPLE WEIGHT .1 GRAM
MOISTURE 0/0 0.00

PROTEIN 0/0 MFB. FROM KJ. OR UDY 21.00
SUM OF SIXTEEN AA PER SAMPLE 22.76
PROTEIN 0/0 MFB. CALC. AS SUM(7) 19.77
PRO RECOVERY 0/0. SUM(7)/KJ OR UDY X 100 94.16
N 0/0 MFB. FROM KJ. OR UDY 3.684
N 0/0 MFB. FROM COLUMN AS SUM(8) 3.668
N RECOVERY 0/0. KJ OR UDY N/COLUMN N X 100 99.57
N TO PROTEIN FACTOR. SUM(7)/KJ OR UDY N 5.36

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
LYSINE	3141.	50.0	0.9905	3.87	3.81	0.566	0.496	0.1005	2.51	2.49	0.154	2.47	2.70	2.10
HISTIDINE	2250.	50.0	0.9905	3.20	3.20	0.497	0.438	0.1345	2.21	2.18	0.135	2.16	2.36	1.80
AMMONIA	27603.	50.0	0.9905	57.34	0.00	0.000	0.000	0.0033	0.00	0.00	0.000	0.00	0.00	0.00
ARGININE	4216.	50.0	0.9905	5.74	5.74	1.001	0.897	0.3220	4.53	4.40	0.272	4.36	4.77	3.24
ASPARTIC ACID	2953.	100.0	1.0306	8.74	8.74	1.165	1.006	0.1225	5.09	5.12	0.316	5.07	5.55	4.94
THREONINE	1937.	100.0	1.0306	5.76	5.76	0.687	0.582	0.0807	2.94	3.02	0.186	2.99	3.27	3.25
SERINE	3557.	100.0	1.0306	11.06	11.06	1.163	0.963	0.1549	4.87	5.11	0.316	5.06	5.54	6.24
GLUTAMIC ACID	18495.	100.0	1.0306	53.75	53.75	7.910	6.940	0.7531	35.10	34.75	2.147	34.44	37.66	30.37
PROLINE	1811.	100.0	1.0306	22.09	22.09	2.544	2.145	0.3095	10.85	11.18	0.690	11.08	12.11	12.48
GLYCINE	4335.	100.0	1.0306	12.65	12.65	0.950	0.722	0.1773	3.65	4.17	0.258	4.14	4.52	7.15
ALANINE	3105.	100.0	1.0306	8.79	8.79	0.784	0.625	0.1232	3.16	3.44	0.213	3.41	3.73	4.97
HALF CYSTINE	281.	100.0	1.0306	1.67	1.67	0.203	0.172	0.0234	0.87	0.89	0.055	0.88	0.97	0.94
VALINE	2928.	100.0	1.0306	9.00	9.00	1.054	0.892	0.1261	4.51	4.63	0.286	4.59	5.02	5.08
METHIONINE	766.	100.0	1.0306	2.03	2.03	0.304	0.267	0.0285	1.35	1.34	0.083	1.33	1.45	1.15
ISOLEUCINE	2294.	100.0	1.0306	6.56	6.56	0.861	0.742	0.0919	3.75	3.78	0.234	3.75	4.10	3.70
LEUCINE	4405.	100.0	1.0306	12.12	12.12	1.590	1.371	0.1698	6.93	6.99	0.432	6.92	7.57	6.84
TYROSINE	1120.	100.0	1.0306	3.20	3.20	0.581	0.522	0.0449	2.64	2.55	0.158	2.53	2.77	1.81
PHENYLALANINE	2361.	100.0	1.0306	6.68	6.68	1.105	0.984	0.0936	4.97	4.85	0.300	4.81	5.26	3.77
TRYPTOPHAN	0.	0.0	0.0000	0.00	0.00	0.000	0.000	0.0000	0.00	0.00	0.000	0.00	0.00	0.00
COLUMN SUMS				234.34	176.99	22.963	19.773	3.6685	100.00	100.89	6.232	100.00	109.34	100.00
AMMONIA					57.34	0.976	0.976	0.0000	4.93	4.29	0.266	4.25	4.65	32.39

(1) INTEGRATOR AREA
(2) TOTAL SAMPLE VOLUME
(3) INTERNAL STANDARD CORRECTION FACTOR
(4) UMOLES AA/0.1 GM SAMPLE OR MILLIMOLES AA/100 GMS OF SAMPLE, AS IS BASIS
(5) UMOLES AA/0.1 GM SAMPLE OR MILLIMOLES AA/100 GMS OF SAMPLE, MFB
(6) GMS OF HYDROLYZED AA PER 100 GMS OF SAMPLE, MFB
(7) GMS OF ANHYDRO AA PER 100 GMS OF SAMPLE, MFB
(8) GMS N FROM EACH AA IN 100 GMS OF SAMPLE, MFB
(9) GMS ANHYDRO AA PER 100 GMS PROTEIN, MFB, PROTEIN CALC. FROM COLUMN 7
(10) GMS HYDROLYZED AA PER 100 GMS OF SIXTEEN AA, MFB
(11) GMS HYDROLYZED AA PER GM OF KJ OR UDY N, MFB
(12) GMS HYDROLYZED AA PER 100 GMS OF HYDROLYZED AA, MFB
(13) GMS HYDROLYZED AA PER 100 GMS OF KJ. OR UDY PROTEIN, MFB
(14) MOLES AA PER 100 MOLES OF AA, MFB

RESEARCH RESULTS

The World Wheat Collection

Common wheats totaling 12,681 from the World Collection have been analyzed for protein and lysine. Several hundred additional accessions to the Collection were received from the Agricultural Research Service which will also be analyzed routinely as the laboratory schedule permits. Protein level ranged from 6 to 23 percent with a mean of 12.9% (Figure 3).

Relationship of Protein and Lysine Among Common Wheats --

The range in lysine per unit protein was 2.2 to 4.2 percent with a mean of 3.2 percent (Figure 4). There is a well-established negative relationship between protein content and lysine per unit protein. The linear regression of lysine on protein was computed. Forty percent of the variation of lysine per unit protein could be attributed to variation in protein (Figure 5). When lysine is expressed as percent of dry grain weight it is positively correlated with protein and 75 percent of its variation is accounted for by variation in protein (Figure 5).

The negative linear regression of lysine per unit protein on protein was utilized to adjust lysine values of 12,581 wheats in the World Collection to a common protein level. The frequency distribution for adjusted lysine values appears in Figure 6. There are fewer wheats with high adjusted lysine values than with high unadjusted value (compare Figures 4 and 6). This reflects the effect of protein level on the lysine content of the protein.

Lysine expressed as percent of protein was plotted against protein for 12,613 World Collection wheats. A pronounced curvilinear relationship is evident (Figure 7). In the 8 to 14 percent protein range a strong negative relationship between protein and lysine per unit protein can be seen which

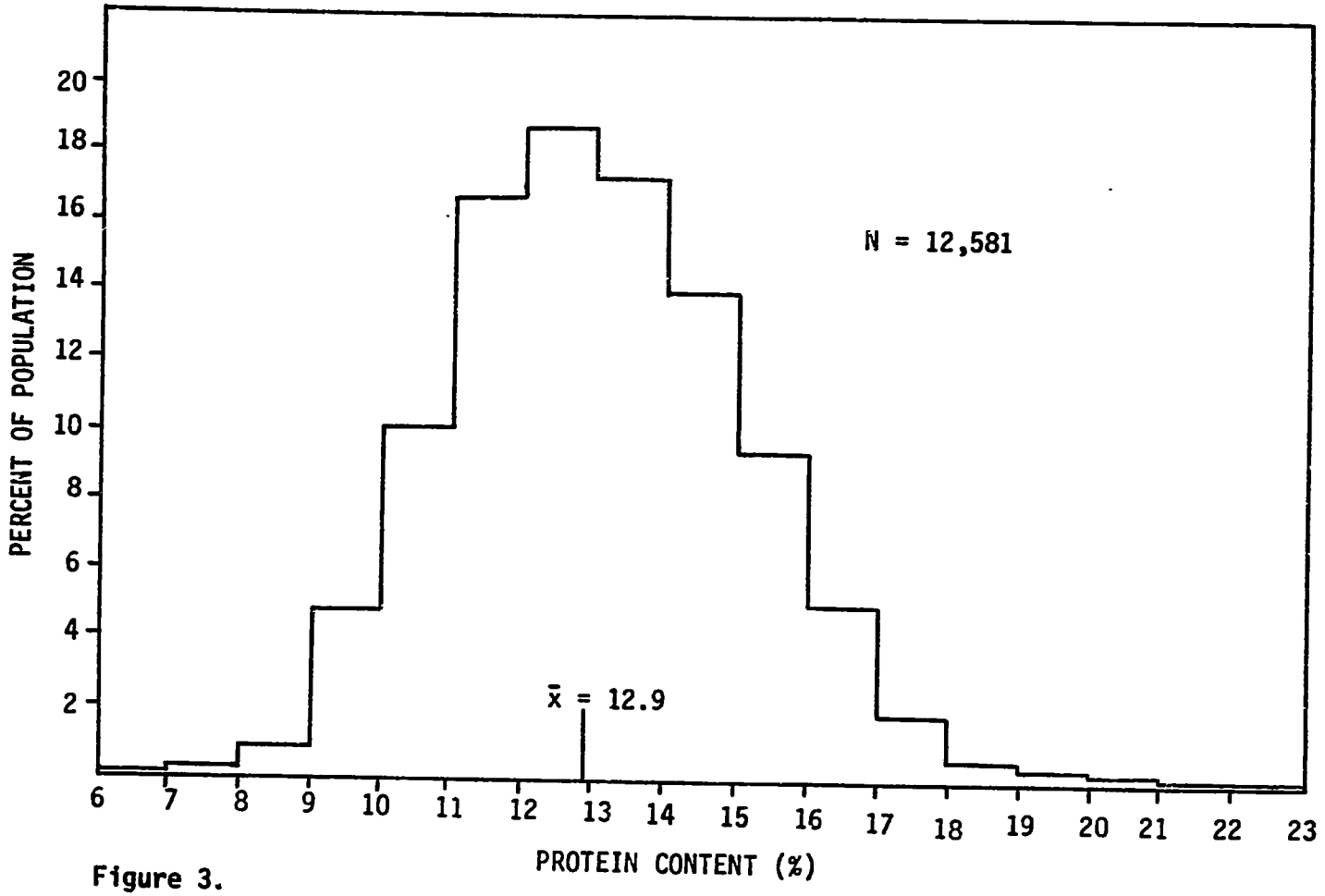


Figure 3.
Frequency distribution for grain protein content among 12,581 wheats in the USDA World Collection.

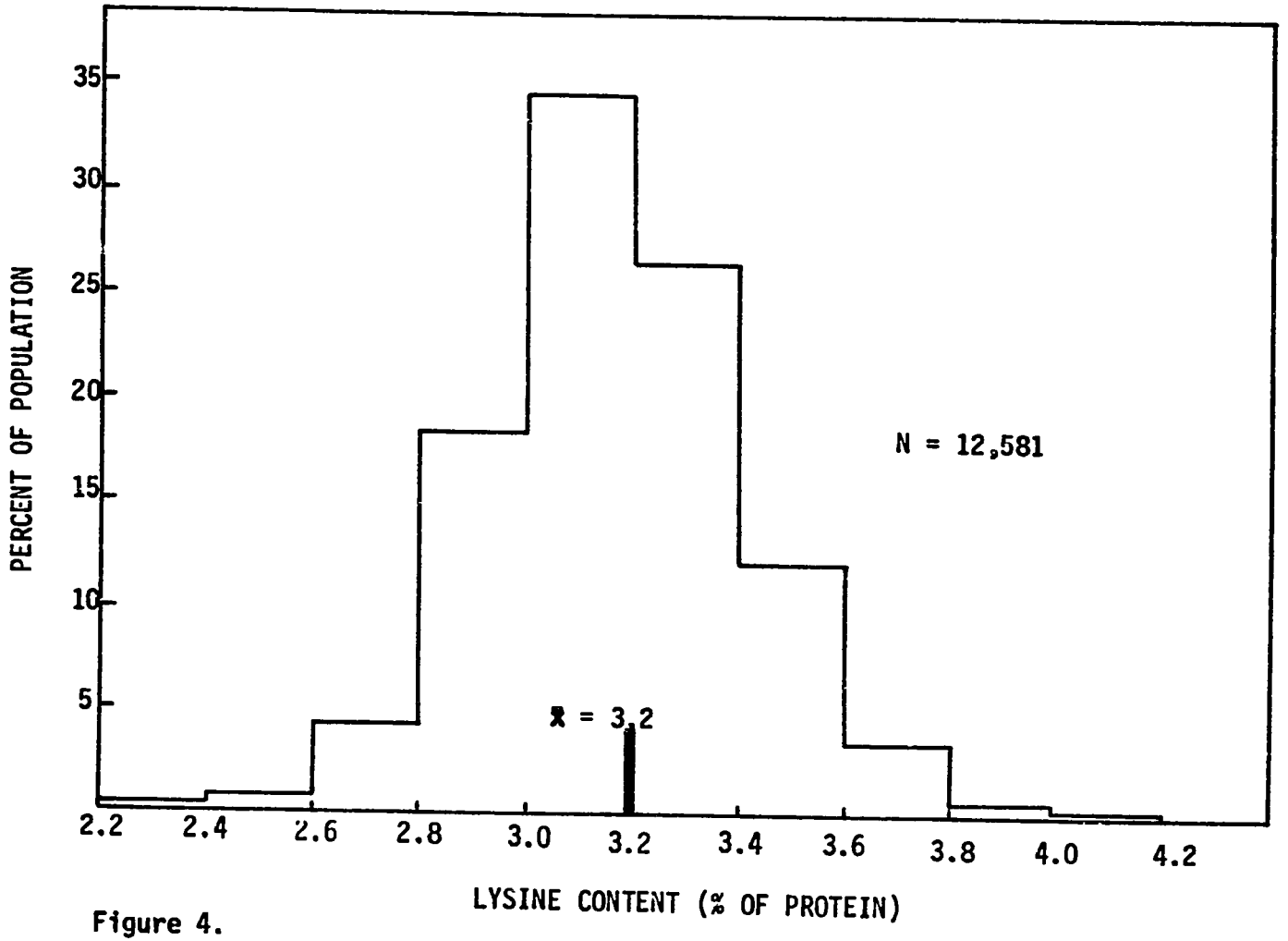
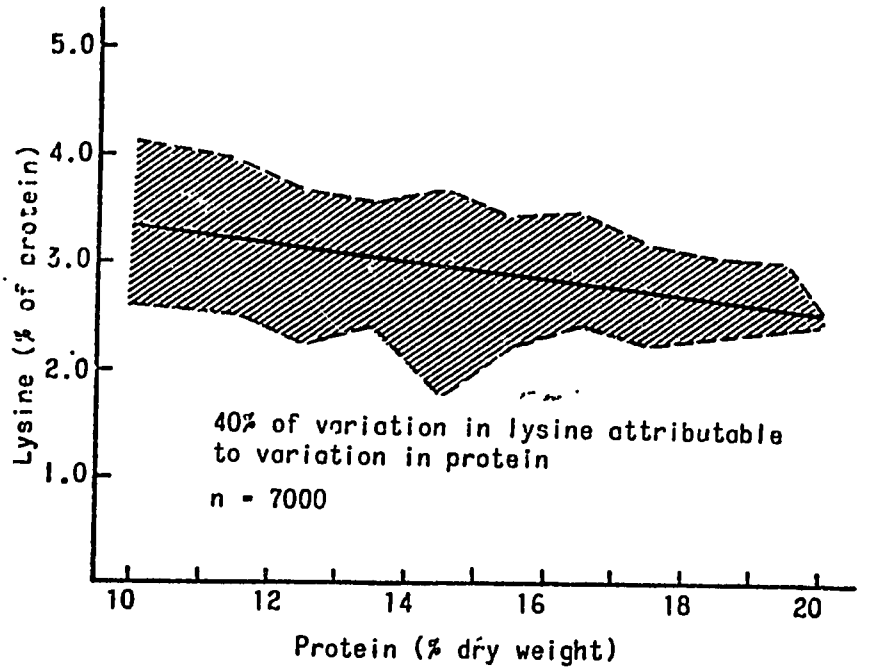


Figure 4.
Frequency distribution for lysine expressed as percent of grain protein among 12,581 wheats in the USDA World Collection.



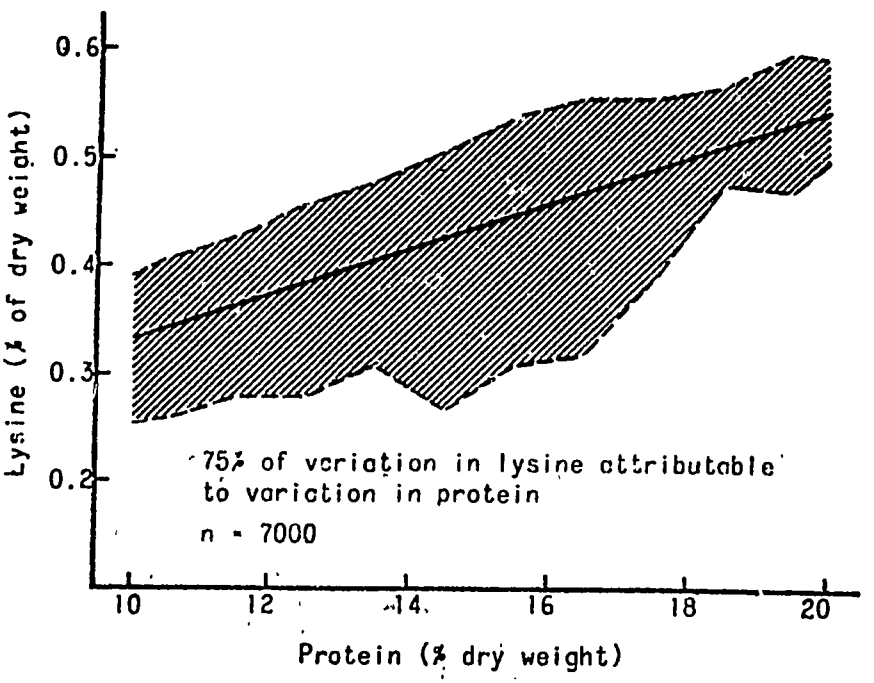


Figure 5. Regression of lysine per unit protein on protein (top) and regression of lysine per unit dry grain weight on protein (bottom) based on analyses of 7,000 wheats from the World Collection. Shaded area indicates the range of dispersal of actual values about the regression line.

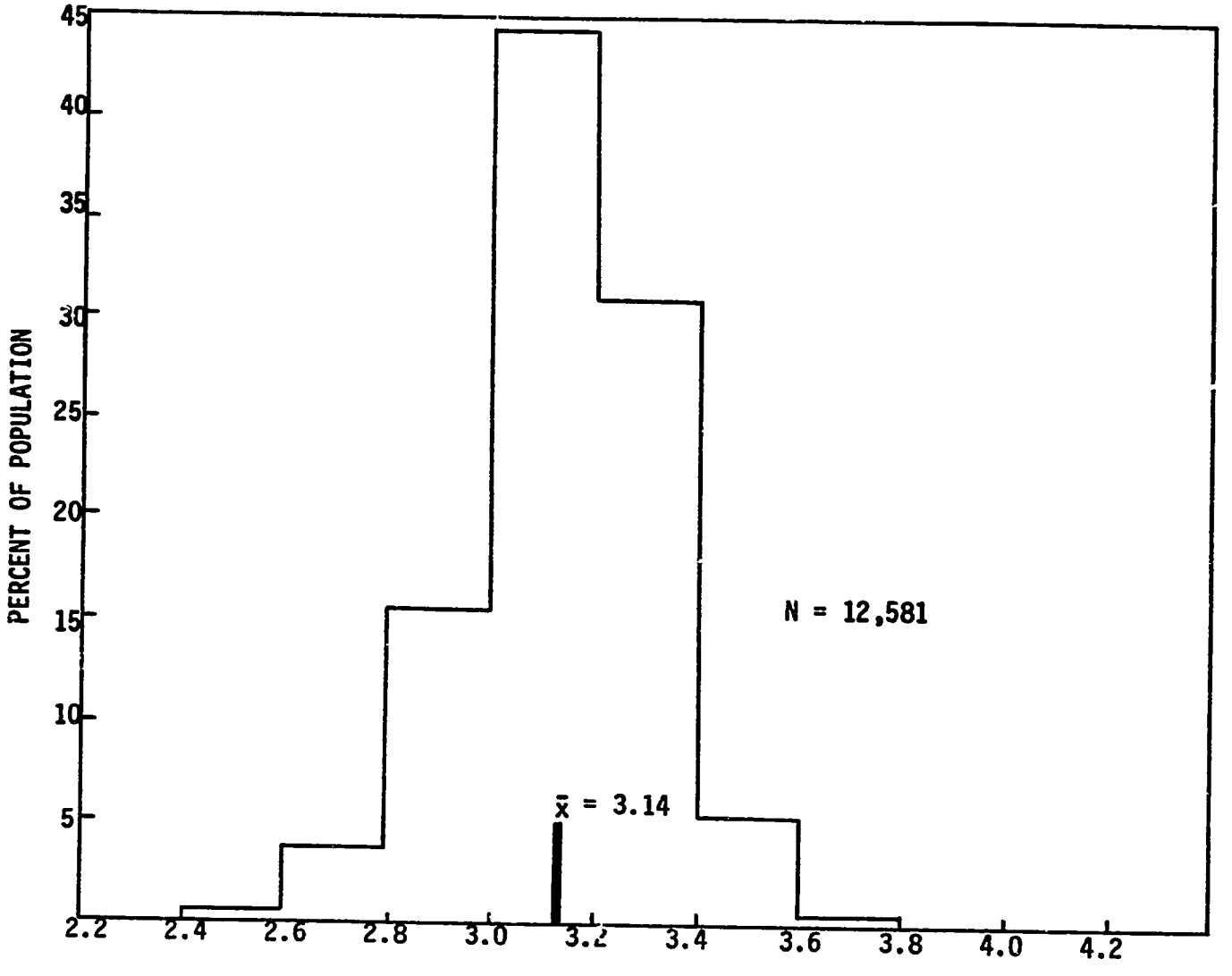


Figure 6. Frequency distribution for lysine adjusted to a common protein level among 12,581 wheats in the USDA World Collection.

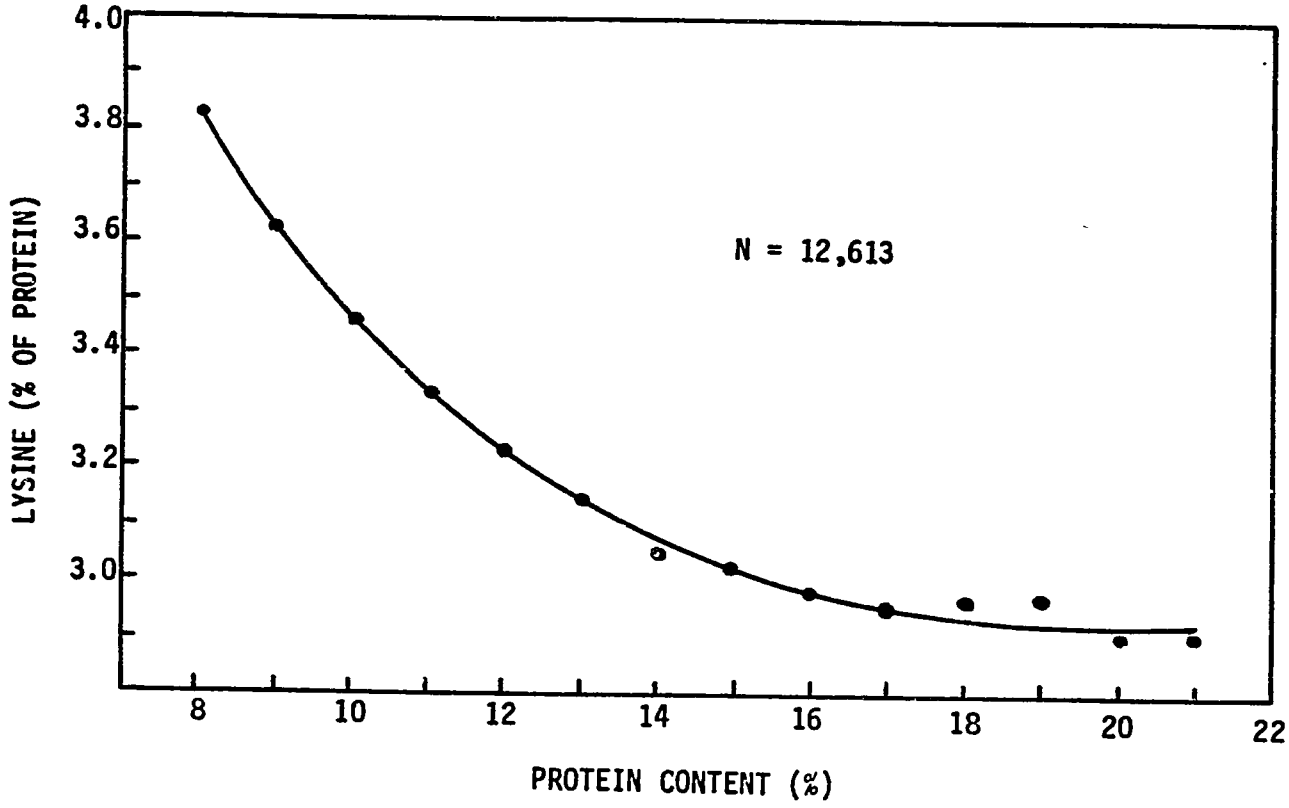


Figure 7. The relationship of grain protein level and lysine per unit protein among 12,613 wheats in the USDA World Wheat Collection.

becomes less pronounced as protein increases, and virtually disappears above 16 percent protein. This clearly indicates that the validity of a correction of lysine values based on the linear regression of lysine on protein is suspect. A computer program for adjustment of lysine values based upon the curvilinear relationship shown in Figure 7 is being prepared. Lysine per protein values of all wheats in the World Collection will be adjusted according to the new program.

When lysine per unit dry grain weight is plotted against protein for wheats in the World Collection a strong positive linear relationship emerges (Figure 8). As protein level increases from 8 to 21 percent lysine per unit dry grain weight also increases from 0.30 to 0.60 percent.

Common Wheats with Highest Protein and Lysine Values --

These are shown for wheats in the World Collection in Tables 1-3. The strong influence of environment on protein level does not permit heavy reliance upon the values for high protein appearing in Table 1. The genetic component of protein variation may be relatively small in relation to total variation. Only extensive grow-outs of these wheats with high values at several locations over a period of years can firmly establish their genetic uniqueness for high protein.

Several wheats with high unadjusted values for lysine per unit of protein appearing in Table 2 do not appear in Table 3 in which the wheats with highest adjusted lysine values are listed. In such wheats high lysine was associated with very low protein content. When the lysine values of these wheats were adjusted to a higher common protein level (13.5%) they were no longer among the highest in adjusted lysine because of the negative relationship between protein and lysine per unit protein.

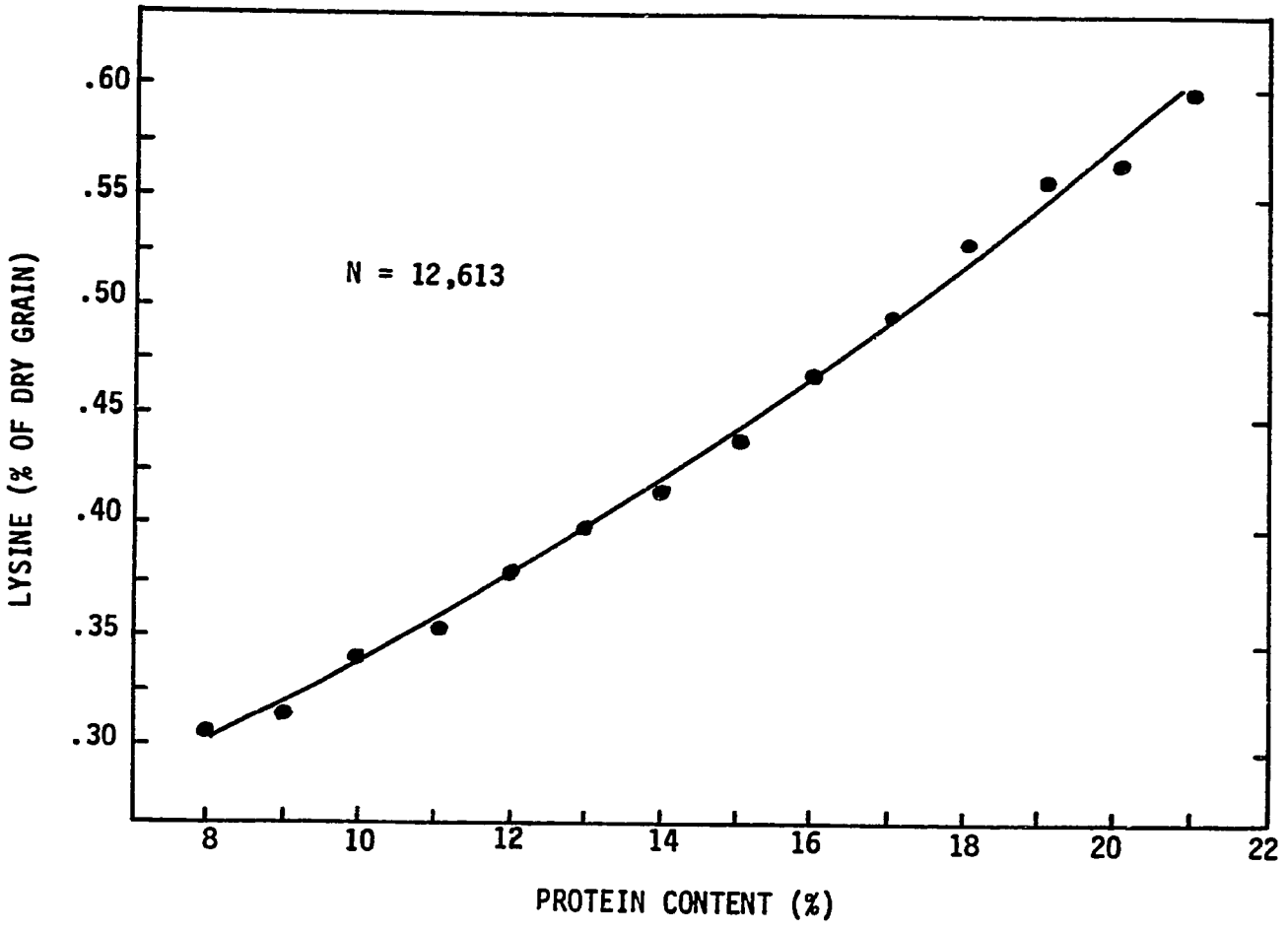


Figure 8. The relationship of grain protein level and lysine per unit weight of grain among 12,613 wheats in the USDA World Wheat Collection.

Table 1. Protein and lysine values for common wheats among 12,613 analyzed from the World Collection exhibiting the highest protein values.

C. I. or P. I. No.	% Protein 1/	% Lysine 1/	Lysine/ Protein	Adjusted Lysine/Protein 2/
185700P	22.0	.66	3.04	3.50
225252P	21.4	.58	2.74	3.17
6654	21.1	.59	2.83	3.24
272423P	21.0	.63	3.01	3.42
185343P	21.0	.60	2.86	3.27
202800P	20.9	.62	3.00	3.40
298577P	20.9	.59	2.86	3.26
204008P	20.7	.61	2.98	3.37
225248P	20.7	.60	2.90	3.29
174680P	20.6	.58	2.81	3.20
191796P	20.3	.58	2.87	3.24
185349P	20.2	.59	2.94	3.30
174701P	20.0	.55	2.79	3.14
174684P	20.0	.62	3.14	3.50
3275	20.0	.53	2.68	3.03
178005P	19.9	.56	2.84	3.19
286000P	19.8	.60	3.05	3.39
166726P	19.8	.60	3.03	3.37
184220P	19.8	.57	2.92	3.26
168794P	19.8	.56	2.84	3.18
225244P	19.7	.57	2.93	3.26
185233P	19.6	.56	2.89	3.22
185388P	19.6	.59	3.01	3.35
272422P	19.6	.53	2.73	3.06
3384	19.6	.47	2.42	2.75
192812P	19.5	.60	3.08	3.41
174702P	19.5	.59	3.04	3.37
6225	19.5	.58	2.99	3.31
13793	19.4	.58	2.99	3.31
192750P	19.4	.58	2.99	3.31
184523P	19.3	.60	3.14	3.46
298584P	19.3	.54	2.82	3.14
272426P	19.2	.56	2.94	3.25
272427P	19.2	.56	2.95	3.26
298583P	19.2	.56	2.92	3.23
198587P	19.2	.58	3.05	3.36
191160P	19.1	.58	3.07	3.37
272420P	19.1	.57	3.00	3.30
3300	19.1	.50	2.61	2.91
176223P	19.0	.57	3.00	3.30
184176P	19.0	.56	2.97	3.27
298580P	19.0	.56	2.96	3.26
285965P	18.9	.59	3.13	3.42
286004P	18.9	.58	3.09	3.38
166841P	18.9	.57	3.03	3.33

1/ Dry weight basis.
2/ Adjusted to 13.5% protein.

Table 2. Protein and lysine values for common wheats in the World Collection with the highest lysine per unit protein values.

C. I. or P. I. No.	% Protein1/	% Lysine1/	Lysine/ Protein	Adjusted Lysine/Protein2/
181329P	6.9	.29	4.26	3.89
184250P	8.1	.33	4.17	3.88
13449	9.2	.38	4.15	3.91
117421P	7.7	.31	4.12	3.80
135073P	7.5	.30	4.09	3.76
166757P	8.5	.34	4.05	3.77
121815P	8.1	.32	4.04	3.74
222670P	8.0	.32	4.03	3.73
166951P	8.3	.33	3.99	3.70
173438P	9.0	.35	3.97	3.72
268449P	9.0	.35	3.97	3.72
166946P	8.6	.34	3.97	3.70
225221P	8.3	.33	3.97	3.69
112344P	9.1	.36	3.95	3.70
234860P	9.7	.38	3.94	3.73
166759P	7.6	.29	3.94	3.62
191043P	9.5	.37	3.93	3.71
167455P	8.4	.32	3.92	3.64
166859P	8.3	.32	3.91	3.62
222671P	9.3	.36	3.90	3.67
184194P	9.4	.36	3.90	3.67
166901P	9.2	.35	3.90	3.67
225243P	8.3	.32	3.90	3.62
222674P	8.7	.33	3.89	3.63
157600P	8.4	.32	3.89	3.61
137740P	8.4	.32	3.88	3.60
13447	10.3	.39	3.87	3.69
166624P	9.3	.36	3.87	3.64
166916P	9.4	.36	3.87	3.64
167697P	9.0	.34	3.87	3.62
94540P	8.8	.34	3.87	3.61
121814P	8.7	.33	3.87	3.61
225223P	8.5	.32	3.87	3.59
166921P	8.3	.32	3.87	3.58
220358P	9.1	.35	3.86	3.61
220350P	9.0	.34	3.86	3.61
225232P	8.5	.32	3.85	3.57
135061P	8.4	.32	3.85	3.57
135070P	8.4	.32	3.85	3.57
119317P	7.7	.29	3.85	3.53
181325P	9.4	.36	3.84	3.62
137737P	9.2	.35	3.84	3.60
166674P	8.4	.32	3.84	3.56
167681P	9.0	.34	3.83	3.59
117018P	9.0	.34	3.83	3.58

1/ Dry weight basis.
2/ Adjusted to 13.5% protein.

Table 3. Protein and lysine values for common wheats in the World Collection with the highest adjusted lysine values.

C. I. or P. I. No.	% Protein[1/]	Lysine[1/]	Lysine/Protein	Adjusted Lysine/Protein[2/]
13449	9.2	.38	4.15	3.91
181329P	6.9	.29	4.26	3.89
184250P	8.1	.33	4.17	3.88
117421P	7.7	.31	4.12	3.80
166757P	8.5	.34	4.05	3.77
135073P	7.5	.30	4.09	3.76
121815P	8.1	.32	4.04	3.74
222670P	8.0	.32	4.03	3.73
234860P	9.7	.38	3.94	3.73
268449P	9.0	.35	3.97	3.72
173438P	9.0	.35	3.97	3.72
191043P	9.5	.37	3.93	3.71
112344P	9.1	.36	3.95	3.70
166946P	8.6	.34	3.97	3.70
166951P	8.3	.33	3.99	3.70
225221P	8.3	.33	3.97	3.69
13447	10.3	.39	3.87	3.69
166901P	9.2	.35	3.90	3.67
184194P	9.4	.36	3.90	3.67
222671P	9.3	.36	3.90	3.67
9364	11.5	.43	3.76	3.64
166624P	9.3	.36	3.87	3.64
166916P	9.4	.36	3.87	3.64
167455P	8.4	.32	3.92	3.64
222674P	8.7	.33	3.89	3.63
166759P	7.6	.29	3.94	3.62
166859P	8.3	.32	3.91	3.62
225243P	8.3	.32	3.90	3.62
167697P	9.0	.34	3.87	3.62
181325P	9.4	.36	3.84	3.62
94540P	8.8	.34	3.87	3.61
121814P	8.7	.33	3.87	3.61
157600P	8.4	.32	3.89	3.61
220358P	9.1	.35	3.86	3.61
220350P	9.0	.34	3.86	3.61
94442P	9.6	.36	3.82	3.60
137737P	9.2	.35	3.84	3.60
137740P	8.4	.32	3.88	3.60
178649P	11.0	.41	3.74	3.60
225223P	8.5	.32	3.87	3.59
167681P	9.0	.34	3.83	3.59
167699P	9.6	.36	3.81	3.59
192393P	10.2	.38	3.77	3.59
11723	10.1	.38	3.77	3.58
11743	10.4	.39	3.76	3.58

1/ Dry weight basis.
2/ Adjusted to 13.5% protein.

Durum Wheats --

Durum wheats in the World Collection totaling 3400 have now been analyzed for protein and lysine differences. Those with the highest protein and lysine values are listed in Tables 4-6. In general the distribution of protein and lysine among the durums was similar to the much larger common wheat group. Several of the durum varieties in the World Collection exhibit excellent high lysine combined with normal protein level.

Since analysis of the durum wheat segment of the World Collection followed the common wheat analyses, we have not yet fully summarized and evaluated the screening data. The true relationship between protein and lysine will be established and utilized, as in the common wheats, to adjust lysine values of the durums.

Significance of World Collection Data --

Large variations in protein and lysine exist among 16,000 common and durum wheats in the World Wheat Collection analyzed to date in the Nebraska laboratory.

Since the grain of these wheats was produced at the same U. S. site (Mesa, Arizona) it seems reasonable to assume that a significant portion of the total protein and lysine variation detected is genetic in nature.

Lysine, the essential amino acid in shortest supply in wheat protein, when expressed as percent of protein, exhibits a curvilinear relationship with protein. At low protein levels the relationship is strongly negative; at intermediate protein levels it is weakly negative; and at high protein levels it largely disappears.

Lysine expressed as percent of dry grain weight is positively correlated with protein level. This provides strong evidence that the nutritional value of wheat would be enhanced by increasing its protein content.

Table 4. Protein and lysine values for durum wheats in the World Collection with the highest protein content.

C. I. or P. I. No.	% Protein[1/]	% Lysine[1/]	Lysine/ Protein	Adjusted Lysine/Protein[2/]
177950P	21.3	.55	2.59	3.02
185721P	21.1	.62	2.95	3.36
185726P	20.9	.62	3.01	3.41
177949P	20.7	.56	2.72	3.11
184541P	20.6	.60	2.91	3.30
166728P	20.1	.54	2.72	3.08
185736P	20.1	.58	2.91	3.27
185729P	20.0	.52	2.61	2.96
192485P	20.0	.51	2.59	2.94
184527P	19.9	.59	3.01	3.36
185610P	19.9	.58	2.96	3.31
185728P	19.9	.58	2.91	3.26
185740P	19.9	.57	2.87	3.22
134936P	19.8	.52	2.65	3.00
184174P	19.8	.54	2.76	3.10
184540P	19.8	.59	3.00	3.35
191528P	19.8	.60	3.04	3.38
204011P	19.8	.56	2.87	3.22
295036P	19.8	.51	2.61	2.96
134935P	19.7	.54	2.74	3.08
184535P	19.7	.57	2.93	3.26
185196P	19.7	.55	2.82	3.16
185731P	19.7	.54	2.76	3.10
185749P	19.7	.56	2.86	3.19
185765P	19.7	.56	2.86	3.20
191089P	19.7	.60	3.09	3.43
165117P	19.6	.54	2.80	3.13
165154P	19.6	.56	2.87	3.21
166817P	19.6	.56	2.88	3.21
177947P	19.6	.50	2.57	2.91
185724P	19.6	.56	2.89	3.22
185738P	19.6	.56	2.87	3.20
191608P	19.6	.52	2.69	3.02
192733P	19.6	.56	2.89	3.22
192736P	19.6	.54	2.75	3.08
204038P	19.6	.58	3.00	3.33
295042P	19.6	.52	2.70	3.03
166816P	19.5	.54	2.81	3.14
184534P	19.5	.55	2.82	3.15
185764P	19.5	.56	2.89	3.22
166931P	19.4	.54	2.79	3.11
185301P	19.4	.52	2.71	3.03
185727P	19.4	.54	2.79	3.11
185760P	19.4	.53	2.75	3.07
204013P	19.4	.54	2.78	3.11

1/ Dry weight basis.
2/ Adjusted to 13.5% protein.

Table 5. Protein and lysine values for durum wheats in the World Collection with the highest lysine per unit protein values.

C. I. or P. I. No.	% Protein 1/	% Lysine 1/	Lysine/ Protein	Adjusted Lysine/Protein 2/
7528	7.7	.33	4.29	3.97
192086P	8.0	.33	4.15	3.85
220704P	7.7	.31	4.14	3.82
192129P	9.5	.37	3.99	3.77
266978P	8.0	.31	3.95	3.65
192618P	8.8	.34	3.92	3.66
268456P	8.7	.33	3.89	3.63
4921	7.3	.28	3.89	3.55
6880	8.2	.31	3.88	3.59
265005P	9.1	.35	3.87	3.62
192119P	9.2	.35	3.86	3.62
4690	7.7	.29	3.85	3.53
195089P	9.9	.38	3.84	3.64
137744P	8.1	.31	3.84	3.54
220129P	8.7	.33	3.83	3.56
3068	8.8	.33	3.82	3.56
7451	8.5	.32	3.82	3.54
262675P	8.8	.33	3.81	3.55
7502	8.2	.31	3.81	3.52
7150	8.9	.33	3.80	3.54
7453	8.4	.31	3.80	3.52
297838P	10.5	.39	3.79	3.62
192619P	10.2	.38	3.79	3.61
7408	8.9	.33	3.79	3.54
7477	8.3	.31	3.79	3.51
6649	8.1	.30	3.79	3.50
8066	9.1	.34	3.78	3.54
243792P	8.7	.32	3.78	3.51
10024	8.5	.32	3.78	3.51
265016P	8.5	.32	3.78	3.51
193867P	12.1	.45	3.77	3.69
7189	8.8	.33	3.77	3.51
94699P	8.8	.33	3.77	3.51
7466	8.4	.31	3.77	3.49
192081P	9.5	.35	3.76	3.54
7986	9.4	.35	3.76	3.54
7681	9.2	.34	3.76	3.53
7507	9.1	.34	3.76	3.52
262674P	9.1	.34	3.76	3.52
7293	9.0	.33	3.76	3.51
7402	9.0	.33	3.76	3.51
7668	9.0	.33	3.76	3.51
7439	8.8	.33	3.76	3.50
7446	8.7	.32	3.76	3.49
7983	9.6	.36	3.75	3.53

1/ Dry weight basis.

2/ Adjusted to 13.5% protein.

Table 6. Protein and lysine values for durum wheats in the World Collection with the highest adjusted lysine values.

C. I. or P. I. No.	% Protein1/	% Lysine1/	Lysine/ Protein	Adjusted Lysine/Protein2/
7528	7.7	.33	4.29	3.97
192086P	8.0	.33	4.15	3.85
220704P	7.7	.31	4.14	3.82
192129P	9.5	.37	3.99	3.77
193867P	12.1	.45	3.77	3.69
192618P	8.8	.34	3.92	3.66
266978P	8.0	.31	3.95	3.65
195089P	9.9	.38	3.84	3.64
268456P	8.7	.33	3.89	3.63
265005P	9.1	.35	3.87	3.62
192119P	9.2	.35	3.86	3.62
297838P	10.5	.39	3.79	3.62
192619P	10.2	.38	3.79	3.61
6880	8.2	.31	3.88	3.59
192120P	10.8	.40	3.74	3.59
196907P	10.5	.39	3.74	3.58
220129P	8.7	.33	3.83	3.56
3068	8.8	.33	3.82	3.56
4921	7.3	.28	3.89	3.55
262675P	8.8	.33	3.81	3.55
137744P	8.1	.31	3.84	3.54
7451	8.5	.32	3.82	3.54
7150	8.9	.33	3.80	3.54
7408	8.9	.33	3.79	3.54
8066	9.1	.34	3.78	3.54
192081P	9.5	.35	3.76	3.54
7986	9.4	.35	3.76	3.54
7391	10.0	.37	3.74	3.54
3142	9.9	.37	3.74	3.54
192107P	10.5	.38	3.71	3.54
7517	10.4	.38	3.71	3.54
8635	11.4	.41	3.65	3.54
191127P	17.9	.59	3.30	3.54
4690	7.7	.29	3.85	3.53
7681	9.2	.34	3.76	3.53
7983	9.6	.36	3.75	3.53
234871P	9.5	.35	3.75	3.53
7499	9.4	.35	3.75	3.53
192103P	9.9	.37	3.73	3.53
8123	10.3	.38	3.70	3.53
7502	8.2	.31	3.81	3.52
7453	8.4	.31	3.80	3.52
7507	9.1	.34	3.76	3.52

1/ Dry weight basis.
2/ Adjusted to 13.5% protein.

Protein and Nutritional Value

The amino acids in wheat protein are not present in the proportions required to satisfy the nutritional needs of man. Our computations of the deviations of the essential amino acids from human requirements as determined by FAO in 1957 are summarized in Table 7. Clearly, lysine is the critical amino acid and is present in wheat protein in less than one-half of the amount needed for nutritional balance. According to these calculations Isoleucine, Methionine, and threonine (in that order) are the other essential amino acids in short supply in wheat protein. Phenylalanine and leucine are present in large excesses of human adult requirements.

Amino Acid Profiles --

Amino acid profiles were determined for a group of 114 low protein and a group of 50 high protein wheat varieties from the World Wheat Collection. Designation as high or low protein was based on analyses of seed samples produced at Mesa, Arizona and does not necessarily indicate genetic differences for grain protein potential. Correlations of level of protein with individual amino acids for the two groups are shown in Tables 8 and 9.

Lysine was substantially higher in the low protein than in the high protein group. This is consistent with protein-lysine relationships determined from analyses of the entire World Wheat Collection. However, it will be noted that there was a moderately-large negative correlation between protein and lysine (-0.60) among wheats in the low protein group, but a non-significant protein-lysine correlation for the high protein group. It must be concluded that high protein wheats do not exhibit the same lysine depression that is associated with protein variation among wheats that are lower in protein.

Table 7. Deviation of essential amino acids in wheat protein from the requirements of man, FAO-1957.

Amino Acid	Percent deviation from man's requirement
Lysine	-55.0
Isoleucine	-16.7
Methionine[1/]	-12.5
Threonine	- 3.4
Valine	+ 4.5
Tyrosine	+ 9.7
Leucine	+30.4
Phenylalanine	+40.4
Tryptophan	--

1/ All data are reported on a dry weight basis.

Table 8. Protein-amino acid relationships among 114 low protein samples from the World Wheat Collection.

Measurement[1/]	Mean	Range	Correlation[2/]
Protein (5)	9.5	6.9 - 13.8	---
Total Amino Acids[3/]	9.4	6.9 - 12.7	
Essential Amino Acids (% of total amino acids)			
Lysine	3.4	3.0 - 4.0	-0.60
Isoleucine	3.7	3.3 - 4.0	ns [4/]
Methionine	1.6	1.1 - 1.9	-0.22
Threonine	3.4	3.0 - 3.6	-0.49
Valine	4.9	4.1 - 5.6	ns
Tyrosine	2.6	1.9 - 3.4	ns
Leucine	7.2	7.0 - 7.5	-0.37
Phenylalanine	4.6	4.1 - 4.9	ns
Non-Essential Amino Acids (% of total amino acids)			
Histidine	2.4	2.2 - 2.7	-0.37
Arginine	5.1	3.9 - 6.0	-0.25
Aspartic Acid	6.3	5.5 - 7.7	-0.37
Serine	5.3	4.7 - 5.7	ns
Glutamic Acid	30.8	27.8 - 32.8	+0.48
Proline	9.7	8.8 - 11.0	+0.48
Glycine	4.6	4.1 - 5.1	-0.40
Alanine	4.2	3.8 - 4.7	-0.56

1/ All data are reported on a dry weight basis.
2/ Correlation of total amino acids with each amino acid expressed as a % of total amino acids.
3/ Total amino acids means grams of the sixteen amino acids hydrolyzed per 100 grams of sample.
4/ Non-significant at the 5% level.

Table 9. Protein-amino acid relationships among 50 high protein samples from the World Wheat Collection.

Measurement[1]	Mean	Range	Correlation[2]
Protein (%)	18.8	17.6 - 22.0	---
Total Amino Acids[3]	18.8	16.2 - 21.4	---
Essential Amino Acids (% of total amino acids)			
Lysine	2.8	2.3 - 3.2	ns [4]
Isoleucine	3.6	3.4 - 3.9	+0.49
Methionine	1.3	0.9 - 1.6	+0.36
Threonine	3.0	2.8 - 3.2	-0.32
Valine	4.5	4.4 - 4.8	ns
Tyrosine	2.5	2.2 - 2.9	ns
Leucine	7.0	6.7 - 7.3	ns
Phenylalanine	4.9	4.6 - 5.2	ns
Non-Essential Amino Acids (% of total amino acids)			
Histidine	2.4	2.2 - 2.6	ns
Arginine	4.8	3.9 - 5.6	ns
Aspartic Acid	5.6	4.9 - 6.2	ns
Serine	5.1	4.8 - 5.4	-0.37
Glutamic Acid	33.8	31.9 - 37.0	ns
Proline	10.7	9.9 - 11.7	+0.38
Glycine	4.1	3.7 - 4.3	-0.31
Alanine	3.6	3.4 - 3.9	-0.53

1/ All data are reported on a dry weight basis.
2/ Correlation of total amino acids with each amino acid expressed as a % of total amino acids.
3/ Total amino acids means grams of the sixteen amino acids hydrolyzed per 100 grams of sample
4/ Non-significant at the 5% level.

Methionine, threonine, and leucine among the essential amino acids also exhibited significant negative correlations with protein level (total amino acids) among the low protein wheats whereas only threonine was negatively correlated with protein in the high protein group. Proline was positively correlated with protein level in both the high and low protein groups.

All possible correlations of 16 amino acids with each other among the high protein and low protein groups were computed and appear in tables 10 and 11. In both the low and high protein groups lysine was positively correlated with threonine, leucine, and tyrosine but negatively correlated with valine among the essential amino acids.

Wheat versus Maize --

Ordinary wheat with 11.1% protein provides more lysine, isoleucine, methionine, and threonine per unit weight of dry grain than does ordinary corn with 8.8% protein content (Table 12). When high protein wheat with 17.2% protein content is compared with opaque-2 maize with 10.0% protein it continues to provide more of each of these four essential amino acids per unit weight of grain (Table 13). Since these comparisons are based on *in vitro* laboratory analyses, they may not measure how well high protein wheat would compare with O_2 maize in true biological value based on animal feeding trials.

High Protein versus High Lysine Wheats --

Thirty-nine wheats from the World Collection with highest lysine values were compared with 16 wheats from the Collection with the highest protein content to determine which group provided the largest quantities of the essential amino acids per unit dry weight of the grain. The comparisons are summarized in Table 14. The high protein group (17.2%) provided more of every essential amino acid than the high lysine group (8.3% protein).

Table 10. All possible correlations of 16 amino acids with each other based on the 114 high lysine-low protein samples from the World Wheat Collection Each amino acid was expressed as a % of total amino acids for calculation of the correlations.

	Histidine	Arginine	Aspartic Acid	Threonine	Serine	Glutamic Acid	Proline	Glycine	Alanine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine
Lysine	+.60	+.45	+.64	+.57	-.37	+.73	-.61	+.54	+.74	-.19	ns	ns	+.26	+.21	ns
Histidine		+.56	ns	+.26	-.28	-.43	-.53	+.43	+.31	-.23	ns	ns	+.24	+.20	ns
Arginine			ns	ns	ns	-.66	-.46	+.46	+.26	ns	+.19	-.22	ns	+.52	ns
Aspartic Acid				+.47	-.33	-.59	-.40	+.32	+.60	-.21	ns	-.20	ns	ns	ns
Threonine					-.25	-.46	-.34	+.36	+.56	-.45	+.25	-.22	ns	+.22	ns
Serine						ns	ns	ns	ns	+.32	ns	-.24	ns	-.21	-.26
Glutamic Acid							+.40	-.63	-.64	ns	ns	ns	ns	-.35	ns
Proline								-.48	-.47	ns	-.24	ns	-.40	-.39	ns
Glycine									+.59	ns	ns	ns	ns	ns	-.25
Alanine										ns	ns	ns	+.20	ns	-.25
Valine											-.30	+.39	ns	-.36	-.22
Methionine												ns	ns	+.46	ns
Isoleucine													+.44	-.24	ns
Leucine														ns	ns
Tyrosine															ns

Table 11. All possible correlations of 16 amino acids with each other based on the 50 high protein samples from the World Wheat Collection. Each amino acid was expressed as a % of total amino acids for calculation of the correlations.

	Histidine	Arginine	Aspartic Acid	Threonine	Serine	Glutamic Acid	Proline	Glycine	Alanine	Valine	Methionine	Isoleucine	Leucine	Tyrosine	Phenylalanine
Lysine	+.62	+.72	+.58	+.60	ns	-.82	-.43	ns	+.53	+.43	ns	+.35	+.32	ns	+.31
Histidine		+.59	ns	ns	-.31	-.61	ns	ns	ns	+.32	+.38	+.36	+.36	ns	+.40
Arginine			+.45	+.38	-.32	-.70	-.32	ns	ns	ns	ns	ns	ns	ns	ns
Aspartic Acid				+.72	ns	-.62	-.44	ns	+.67	ns	ns	ns	ns	ns	ns
Threonine					+.32	-.68	-.55	+.43	+.79	+.41	ns	ns	ns	ns	ns
Serine						ns	-.30	+.43	+.30	ns	ns	-.42	ns	ns	ns
Glutamic Acid							+.30	-.28	-.55	-.42	-.32	-.35	-.44	-.33	-.45
Proline								-.44	-.60	-.44	ns	ns	-.36	-.31	ns
Glycine									+.54	ns	ns	ns	+.32	ns	ns
Alanine										+.48	ns	ns	+.31	ns	ns
Valine											ns	+.40	+.46	ns	ns
Methionine												+.37	ns	ns	ns
Isoleucine													+.33	ns	+.51
Leucine														+.36	+.29
Tyrosine															ns

Table 12. Comparative essential amino acid composition of normal wheat and maize.

Amino acid	Wheat (11.1% protein)*		Maize (8.8% protein)*	
	% of protein	g/100 g grain	% of protein	g/100 g grain
Lysine	3.0	0.33	3.0	0.26
Isoleucine	3.5	0.39	4.2	0.37
Leucine	7.0	0.78	14.6	1.28
Methionine	1.4	0.17	1.3	0.11
Phenylalanine	4.9	0.54	5.8	0.51
Threonine	3.0	0.33	4.1	0.36
Valine	4.3	0.48	5.7	0.50
Tryptophan	1.3	0.14	0.7	0.06

*14% moisture basis

Table 13. Comparative essential amino acid composition of high protein wheat and opaque-2 maize.

Amino acid	High protein wheat (17.2% protein)*		Opaque-2 maize (10.0% protein)*	
	% of protein	g/100 g grain	% of protein	g/100 g grain
Lysine	2.9	0.49	4.8	0.48
Isoleucine	3.7	0.64	3.4	0.34
Leucine	7.1	1.22	9.1	0.91
Methionine	1.6	0.28	2.1	0.21
Phenylalanine	5.2	0.87	4.5	0.45
Threonine	3.0	0.51	4.0	0.40
Valine	4.5	0.77	5.1	0.51
Tryptophan	1.1	0.19	1.3	0.13

* 14% moisture basis

Table 14. Comparative essential amino acid composition of high protein and high lysine wheats.

Amino acid	High protein wheats (17.2% protein)*		High lysine wheats (8.3% protein)*	
	% of protein	g/100 g grain	% of protein	g/100 g grain
Lysine	2.9	0.49	3.6	0.29
Isoleucine	3.7	0.64	3.7	0.31
Leucine	7.1	1.22	7.3	0.61
Methionine	1.6	0.28	1.7	0.14
Phenylalanine	5.2	0.89	4.9	0.41
Threonine	3.0	0.51	3.4	0.28
Valine	4.5	0.77	4.7	0.39
Tryptophan	1.1	0.19	1.5	0.12

* 14% moisture basis

Lysine, Methionine, and Threonine in High Protein Nebraska Lines --

Lysine, methionine, and threonine determinations were made on a group of high protein experimental lines of winter wheat developed at Nebraska. The contents of these amino acids in the grain of 9 high lines are compared with the parent varieties in Table 15. Five lines were equal to Comanche in lysine, one was equal to Comanche in methionine, and five were equal or higher than Comanche in threoning.

Amino acid values are expressed as percentage increases per unit weight of grain over the Comanche parent variety in Table 16. This permits better assessment of the potential contribution to nutritional value of the high protein trait in these wheats. Percentage increases in the range of 15 to 33% for lysine, 6 to 19% for methionine, and 9 to 32% for threonine were computed for the high protein wheats.

Mouse Feeding Trials --

A small animal laboratory was established in the Department of Foods and Nutrition in 1972 to assess the true nutritional value of wheats demonstrated to possess high protein and/or high lysine in *in vitro* laboratory analyses. Description of mouse feeding trials conducted from September 1971 to November 1972 is contained in Table 17.

Results from selected mouse feeding trials appear in Tables 18-21. Numerous additional trials have been completed for which data analyses and summarization are as yet unfinished. FER (feed efficiency ratio) as used in Tables 18, 20, and 21 = feed consumption ÷ weight gain. Thus, a low FER value indicates better gain per unit of feed consumed than a high value. FER values for Triumph 64 winter wheat were nearly comparable to casein and higher than a commercial wheat variety (Table 18).

Table 15. Lysine, methionine, and threonine content of Atlas 66-derived high protein lines and parent cultivars.

Name or pedigree	Laboratory number	% protein content*	Amino acid content (g/16 g N)*		
			Lysine	Methionine	Threonine
Atlas 66	65-1825	15.8	2.6	1.4	2.6
Comanche	1556	12.3	2.7	1.5	3.5
Atlas 66 x Cmn	1557	15.4	2.7	1.4	3.4
"	1558	14.3	2.7	1.4	3.6
"	1559	15.6	2.7	1.4	3.5
"	1560	16.1	2.5	1.3	3.3
"	1561	14.8	2.6	1.4	3.6
"	1562	15.8	2.5	1.3	3.3
"	1563	16.4	2.3	1.3	3.5
"	1564	14.5	2.7	1.5	3.3
"	1565	14.6	2.7	1.4	3.5

* 14% moisture basis

Table 16. Percentage increase in lysine, methionine, and threonine per unit weight of grain over the low protein parent variety Comanche among the Atlas 66-derived lines.

Pedigree	Laboratory number	% increase per unit weight of grain over low protein parent variety Comanche[1]		
		Lysine	Methionine	Threonine
Atlas 66 x Cmn	65-1557	25	19	20
"	1558	15	6	17
"	1559	33	17	24
"	1560	20	18	22
"	1561	15	12	22
"	1562	19	9	22
"	1563	15	14	32
"	1564	18	16	9
"	1565	18	11	19

[1] Calculated values rounded to nearest whole percent

Table 17. Description of mouse feeding trials conducted between September, 1971 and November, 1972. Each trial was 28 days in length and each sample was fed to 5 mice. A 10% casein diet was fed in each trial.

Trial Number	Description or Purpose
1	Preliminary study to establish procedure
2	Preliminary study to establish procedure
3	Fed 23 samples and 2 controls on an equal amount of grain basis
4	Fed 22 samples and 2 controls on an equal amount of grain basis
5	Fed 22 samples and 2 controls on an equal amount of grain basis
6	Samples fed for mouse-human comparison
7	Fed 24 samples and 2 controls on an equal amount of grain basis
8	Fed 25 samples and 2 controls on an equal amount of grain basis
9	Fed 23 samples and 3 controls on an equal amount of protein basis
10	Cooperative study with CIMMYT and Purdue

Table 18. Mean weight gain, feed consumption and FER for 3 control samples fed in 5 trials. The means are based on the values obtained from 5 mice except for casein in trial 4, Triumph 64 in trial 5 and the commercial sample in trials 4 and 8 which are based on values obtained from 4 mice.

Sample	Weight Gain (g)				
	Trial Number				
	3	4	5	7	8
Casein	11.2	14.1	19.4	13.6	23.8
Triumph 64	13.6	12.0	16.1	16.0	12.4
Commercial	9.9	9.0	9.2	10.7	10.0
	Feed Consumption (g)				
Casein	104	115			
Triumph 64	115	107			
Commercial	106	113			
	FER[1/]				
Casein	9.6	8.2			
Triumph 64	9.3	9.0			
Commercial	11.0	12.8			

1/ Feed consumption ÷ weight gain

Table 19. The comparison of weight gain data on 9 samples and 3 controls that were fed in trials 3 and 7.

Sample	Location	Protein[1/] (%)	Mice/mean : Weight gain (g) Trial Number 3	7	3	7
NE701137	N Platte	17.5	5	5	17.6	13.0
NE68513	C Center	13.9	5	4	15.2	14.9
"	N Platte	17.9	5	5	13.6	18.1
Triumph 64	Yuma	15.7	5	5	13.6	16.0
NE701152	N Platte	16.6	4	5	13.1	17.6
"	C Center	16.2	5	5	13.0	14.6
"	Yuma	17.1	3	5	12.1	14.0
NE701137	"	17.2	5	5	11.9	13.9
Casein			5	5	11.2	13.6
NE701139	Yuma	14.2	4	5	10.9	11.6
NE68513	Red Willow	12.0	3	5	9.9	11.8
Commercial		10.8	5	5	9.9	10.7
Trial Means		15.4			13.1	14.0

1/ Kj. nitrogen x 5.7, 0% moisture basis.

Table 20. Data obtained from mouse feeding trial number 3.

Sample	Location	Protein 1/	Mean	FER 2/	Weight Gain	Feed Consumption
		%			(g)	(g)
NE701132	N Platte	17.4	4	7.0	18.3	123
NE701134	"	17.2	5	7.1	16.3	114
NE68510	"	17.0	5	7.1	17.6	124
NE701137	"	17.5	5	7.2	17.6	124
NE68570	"	17.8	5	7.6	16.3	123
NE68513	C Center	13.9	5	7.6	15.2	116
NE701132	"	14.5	5	7.7	15.1	116
NE68570	"	14.6	5	8.5	13.5	112
NE68513	N Platte	17.9	5	8.6	13.6	104
NE701136	C Center	14.4	5	9.3	13.2	121
Triumph 64	Yuma	15.7	5	9.3	13.6	115
Casein			5	9.6	11.2	104
NE701152	C Center	16.2	5	9.7	13.0	119
NE701136	N Platte	16.4	5	9.8	11.6	112
NE68510	C Center	12.9	5	9.8	11.3	108
NE701152	Yuma	17.1	3	9.9	12.0	117
NE701139	N Platte	16.5	5	10.1	11.2	109
NE701134	C Center	15.1	5	10.2	11.2	103
NE701152	N Platte	16.6	4	10.2	13.1	118
NE701139	C Center	13.8	5	10.4	12.5	124
Triumph 64	Mead	15.8	5	10.5	10.4	106
NE701137	Yuma	17.2	5	10.9	11.9	114
NE68513	Red Willow	12.0	3	11.0	9.9	107
Commercial		10.8	5	11.0	9.9	106
NE701139	Yuma	14.2	4	11.6	10.9	122
NE701137	C Center	14.6	5	12.5	10.1	120
Trial Means		15.5		9.4	13.1	115
σ 3/				2.2	3.2	13
CV				23.4	24.6	11.5
F 4/				2.31	2.82	1.33
LSD 5/				2.9	4.2	

1/ Kj. nitrogen x 5.7, 0% moisture basis.
2/ Feed consumption ÷ weight gain.
3/ Obtained from residual mean square of ANOV.
4/ A significant F at the 5% level would need to be greater than 1.6.
5/ Approximate LSD.

Table 21. Data obtained from mouse feeding trial number 4.

Sample	Location	Protein 1/ %	Mice/ Mean	FER 2/	Weight Gain (g)	Feed Consumption (g)
NE70654	C Center	14.4	4	7.5	17.8	133
NE701154	"	14.5	3	8.1	14.6	111
NE701124	Yuma	17.9	4	8.1	15.3	123
NE701154	"	16.5	5	8.2	15.4	124
Casein			4	8.2	14.1	115
Triticale	N Platte	17.1	5	8.4	16.6	128
NE701124	C Center	14.2	5	8.4	15.2	126
NE69461	N Platte	16.7	5	8.6	13.4	113
Scout 66 3/	Mead	15.6	5	8.6	15.5	130
NE70654	Yuma	16.1	4	8.6	14.3	120
"	N Platte	17.2	5	8.8	16.1	139
NE701124	"	18.0	5	8.8	13.6	114
Scoutland	Mead	14.8	5	8.9	14.5	118
Gage	"	16.8	5	8.9	14.6	120
Triumph 64	Yuma	15.7	5	9.0	12.0	107
Ben Hur	"	14.0	5	9.1	13.0	113
NE701154	N Platte	17.0	4	9.3	13.1	118
Lancer	Yuma	16.0	4	9.3	11.2	98
Scout 66 3/	Mead	15.8	5	9.5	13.2	120
Ben Hur	"	16.2	5	9.8	13.1	123
NE69461	C Center	13.5	4	10.3	11.8	116
Scout 66	Yuma	14.8	4	10.5	11.2	116
Scoutland	Red Willow	12.2	5	10.9	11.1	120
4/	Mead	18.6	4	11.5	12.6	122
Commercial		10.8	4	12.8	9.0	113
Trial Means		15.6		9.2	13.7	119
σ 5/				1.8	3.9	19
CV				19.7	28.3	16.2
F 6/				1.82	1.14	0.88
LSD 7/				2.4		

1/ Kj. nitrogen x 5.7, 0% moisture basis.
2/ Feed consumption ÷ weight gain.
3/ Grown in different areas of the Mead Field Laboratory.
4/ Composite of high protein lines.
5/ Obtained from residual mean square of ANOV.
6/ A significant F at the 5% level would need to be greater than 1.6.
7/ Approximate LSD.

High protein varieties tended to produce better weight gains and better FER values than low protein varieties although there were some exceptions (Tables 20 and 21). Some changes in the conduct of the mouse feeding trials will be undertaken in an effort to reduce unexplained inter-animal variations that have reduced the effectiveness and reliability of the trials to date.

Nutritional Significance of Protein Data --

Lysine is in shortest supply and is the critical amino acid in wheat protein.

Amino acid profiles for low and high protein wheats from the World Collection showed negative correlation of lysine with protein in the low protein group but no correlation in the high protein group, thus confirming protein-lysine relationships established from analyses of 12,613 wheats in the World Collection.

Lysine was determined to be positively correlated with the other essential amino acids threonine, leucine, and tyrosine. Thus, genetic increases in lysine would not be expected to be associated with undesirable decreases in these other three essential amino acids.

Laboratory analyses of high protein Nebraska wheat lines (17.2%) indicated that they would provide more lysine, isoleucine, methionine, and threonine per unit weight of grain than would opaque-2 maize containing 10.0% protein.

High protein wheat varieties were determined to provide more of each essential amino acid per unit weight of grain than high lysine-low protein wheats.

Percentage increases of as much as 33% for lysine, 19% for methionine, and 32% for threonine were computed for Nebraska-developed high protein varieties over their low protein parent.

Data from mouse feeding tests indicated that high protein varieties tend to produce better weight gains and feed efficiency ratios than varieties with ordinary protein.

Kernel Morphology

Effect on Protein and Lysine Content -

Protein is not uniformly distributed throughout the wheat kernel. The embryo or germ and bran contain higher levels of protein than does the endosperm. Kernel size and morphology affects the ratio of kernel parts to one another. These kernel traits could, therefore, affect the content of protein in the kernel. Further, not all kernel proteins contain amino acids in the same ratios. For example, the non-endosperm proteins are richest in lysine (over 4%) whereas endosperm proteins are relatively poor in lysine (approximately 2%).

The grain of four hard red winter wheat varieties was graded according to size and protein-lysine determinations were made on each size grade. The results appear in table 22. Kernel size alone had no significant effect on either protein or lysine content. When large and small kernels were further subdivided on the basis of kernel plumpness and crease characteristics (open versus closed) a substantial effect on protein and smaller effect on lysine content was measured (table 23). Plump kernels in both size categories contained much less protein than shriveled kernels and plump kernels with closed creases contained less protein than plump kernels with open creases. Lysine trend was inverse to protein trend although differences were not large. Since lysine is negatively correlated with protein, it is likely that the effect of kernel size and morphology on lysine is a secondary one associated with changes in protein content.

Wheat samples from the World Collection totaling 280 were measured and otherwise classified for morphological traits of the kernels. The various classes then were subjected to protein and lysine analyses.

Table 22. Average effect of kernel size on the protein and lysine contents of the grain of four winter wheat varieties.

Kernel size	Protein content	Lysine per unit protein
	%	%
Large (on sieve #8)	12.7	3.1
Medium (on sieve #7)	12.2	3.2
Small (on sieve #6)	12.5	3.1

Table 23. Average effect of kernel size and configuration on the protein and lysine contents of the grain of four winter wheat varieties.

Size	Configuration	Crease	Protein content	Lysine per unit protein
			%	%
Large	Plump	Closed	12.0	3.15
"	"	Open	12.6	3.13
"	Wrinkled	--	14.3	3.02
Small	Plump	Closed	11.8	3.31
"	"	Open	12.9	3.24
"	Wrinkled	--	15.4	3.04

Means and standard deviations appear in table 24. Seed morphology measurements were correlated with protein and lysine values (table 25). R-values were low for all comparisons except seed plumpness with protein and lysine/dry weight; and protein with lysine/dry weight and lysine/protein. Adjustment of lysine/protein to a common protein level reduced the R-value to 0.06.

Significance of Data --

Kernel size does not appear to exert a significant influence on protein or lysine content of the kernel.

The largest effect is that of degree of plumpness on protein content and lysine per unit kernel weight. In contrast, kernel plumpness exerted no effect on lysine per unit protein.

The high correlation between protein content and lysine per unit weight of grain was anticipated since lysine is a constituent of protein. The negative correlation between protein and lysine per unit protein is consistent with the negative correlation obtained from analysis of the entire World Collection.

Attention to degree of kernel shriveling appears warranted for accurate protein and lysine comparisons between wheats.

Table 24. Mean values and standard deviations for kernel measurements from 280 wheats from the World Collection.

Traits	Measurement	Mean	Standard deviation
Kernel plumpness	1-5 scale: 1 = shriveled; 5 = plump	1.94	0.487
" crease	1-5 scale: 1 = open; 5 = closed	1.64	0.489
" surface	1-5 scale: 1 = wrinkled; 5 = smooth	1.56	0.444
" weight	grams: $\bar{x}$ of 20 kernels	0.65	0.115
" length	millimeters: $\bar{x}$ of 10 kernels	5.92	0.455
" width	millimeters: $\bar{x}$ of 10 kernels	2.90	0.206
Germ length	millimeters: $\bar{x}$ of 10 kernels	1.83	0.168
" width	millimeters: $\bar{x}$ of 10 kernels	1.32	0.157
Protein content	percent	13.99	2.158
Lysine/dry grain wt.	percent	0.39	0.061
Lysine/protein	percent	2.82	0.296
Adj. lysine/protein	percent: adjusted to 13.5% protein	2.84	0.279

Table 25. Correlation of four kernel morphological traits with percent protein and lysine content among 280 wheats selected from the World Collection.

	Percent protein	Lysine/ dry wt.	Lysine/ % protein	Adjusted lysine
Seed plumpness	-0.54	-0.55	+0.01	-0.22
Kernel crease	-0.06	-0.16	-0.15	-0.19
Kernel surface	0.00	-0.11	-0.19	-0.20
Percent protein	--	0.83	-0.34	0.06

d.f. = 278

$r_{.05}$ = .119

$r_{.01}$ = .155

Kernel Anatomy

Effect on Protein and Lysine Content -

Kernel fractionation is currently being studied by two graduate students working on Ph.D. degrees.

Richard Ulmer is comparing biochemical properties of wheats differing in protein content. His analyses of chemically separated kernel protein fractions should identify the fractions associated with differences in protein amount and amino acid composition. It is anticipated that the study will be completed in 1973.

The Ph.D. thesis research of Kenneth Vogel is aimed at elucidation of qualitative and quantitative factors of wheat kernels that affect their lysine content. The research involves examination of both morphological and anatomical traits of kernels. Physical separations of bran, germ, and starchy endosperm are being made on the grain of World Collection wheats exhibiting lysine content ranging from 2.25 to 4.26 percent. The variability of protein and lysine content of each kernel component will be established and appropriate correlations made. The study will be completed in 1974.

Significance of Studies -

Until the sites of differences in protein amount and/or amino acid composition of wheat grain are identified the contribution of these differences to nutritional value of wheat products cannot be accurately assessed.

Research information from the studies may lead to more efficient methods of detecting and measuring protein and lysine variations in wheat varieties.

Effect of Environment on Protein and Lysine Content

Substantial differences in protein and lysine content of the grain were detected among 13,000 wheats in the World Collection. Protein ranged from 8 to 21% and lysine from less than 2 to over 4%. The grain samples evaluated had been produced under irrigation over a 2-year period at Mesa, Arizona where the influence of variable environment presumably would be minimal. Unusual values (high or low) for protein and lysine were routinely rechecked to reduce analytical and mathematical errors in data which are reported.

The basis for differences in protein or lysine is impossible to identify until suspect varieties are grown in an array of environments. Only then can the stability of protein and lysine differences (genetic or environmental) be identified. From the outset, selected varieties with high lysine values, as they were identified, were re-grown at different sites in the United States. It became quickly apparent that most of the varieties with high lysine values based on analysis of the Arizona-grown sample were not genetically different for high lysine potential. Further, even among varieties established to be genetically superior in lysine, the superiority was of limited magnitude and of questionable value for immediate use as parents in high lysine breeding.

Spring Wheats -

A few varieties from the 1967-1970 tests have shown some promise. Among the spring wheats tested during the 1967-1970 period, the best are shown in table 26. P.I.7337 and 5484 are of the most interest -- both being six percent better in lysine content than the check variety Justin.

Table 26. 1967-1970 average values for percent protein, adjusted lysine and average yield for 5 varieties in the spring wheat environmental nursery.

	Percent protein	Percent of Jt.	Adjusted lysine	Percent of Jt.	Average yield (gm)	Percent of Jt.
PI 5005	15.3	86	3.13	103	337	96
PI 5484	16.7	94	3.22	106	247	71
PI 6127	16.4	93	3.11	102	425	121
PI 7337	18.6	105	3.23	106	346	99
Justin (Jt.)	17.7	100	3.05	100	350	100

P.I.7337 has the best potential because, in addition to its lysine, it showed consistent superiority to other varieties in protein content and was equal to Justin in grain yield (table 27). Although relatively high in adjusted lysine, P.I.5484 averaged one percentage point lower in protein than Justin and was substantially lower yielding.

Statistical significance levels for protein and lysine among spring wheat varieties tested from 1968 to 1970 are indicated for individual locations and years in table 28. Statistically significant differences in protein and lysine were detected between varieties at all stations except Bozeman, Montana in 1968 where lysine and adjusted lysine differences were non-significant. When test sites within individual years were combined, significant protein differences between varieties in 1968 could not be measured (table 29).

Winter Wheats -

Winter wheats from the World Collection tested over environments in the USA in 1969 and 1970 gave protein and lysine results similar to the spring wheats. Those with the highest combined protein-adjusted lysine values are listed in table 30. Of these Atlas 66 emerged with the highest average protein content. C.I.13449 had the highest average lysine and was 22% better than the check variety Triumph 64 in protein content. Statistically significant differences occurred at all winter wheat test sites except for adjusted lysine at Denton, Texas in 1969 and Davis, California in 1970 (table 31).

Current Evaluations -

Completion of protein and lysine analyses for the entire World Collection except the most recent accessions now permits more comprehensive assessment of findings and selection criteria for varieties to be

Table 27. 1967-1970 ranks for percent protein, adjusted lysine, and average yield for 5 wheats in the spring wheat environmental nursery.

	% Protein				Adjusted lysine				Average yield (gm)			
	1967	1968	1969	1970	1967	1968	1969	1970	1967	1968	1969	1970
PI 5005	40	23	19	28	4	17	10	13	27	6	16	9
PI 5484	32	15	7	10	2	1	4	5	35	25	31	23
PI 6127	35	22	2	15	12	2	14	15	1	14	1	1
PI 7337	1	4	1	1	1	4	3	3	13	16	10	6
Justin	2	1	4	4	35	7	19	15	2	22	11	19
Total nursery entries	40	25	32	32	40	25	32	32	40	25	32	32

Table 28. Statistical significance among entries in the spring wheat environmental nursery for individual locations and years.

	1968			1969			1970		
	% Prot.	Lys/ prot.	Adj. lys.	% Prot.	Lys/ prot.	Adj. lys.	% Prot.	Lys/ prot.	Adj. lys.
Bozeman MT	**	ns	ns	**	**	**	**	**	**
Minot ND	**	**	**	**	**	**	**	**	**
Aberdeen ID (fert.)	**	**	*	a/	a/	a/	**	**	**
Aberdeen ID (non-fert.)	*	*	*	a/	a/	a/	**	**	**
Mead NE	--	--	--	--	--	--	**	**	**
Davis CA	--	--	--	--	--	--	**	**	**

a/ Only one replication of the nursery grown

Table 29. Statistical significance among entries in the spring wheat environmental nursery with locations combined for individual years.

	1968	1969	1970
Percent protein	ns	**	**
G x E	**	a/	**
Lys/protein	**	**	**
G x E	**	a/	**
Adjusted lysine	*	ns	**
G x E	*	a/	**

a/ Location means were treated as replications in the combined analysis.

Table 30. Wheats from the 1969 and 1970 winter wheat environmental nursery which appear to have higher than normal percent protein and lysine content.

Entry	Percent protein	Percent of Triumph 64	Adjusted lysine	Percent of Triumph 64
Atlas 66	19.4	139	3.11	102
PI 135067	18.6	133	3.12	103
PI 135070	18.6	133	3.16	104
PI 166913	18.3	131	3.15	104
PI 94526	18.2	130	3.15	104
PI 173438	18.2	130	3.12	103
CI 13449	17.1	122	3.21	106
PI 11680	14.8	106	3.17	104
PI 117018	14.8	106	3.17	104
Triumph 64 (ck)	14.0	100	3.04	100

Table 31. Statistical significance among entries in the winter wheat environmental nursery for individual locations and years.

	1969			1970		
	% Protein	Lysine/ protein	Adjusted lysine	% Protein	Lysine/ protein	Adjusted lysine
Davis CA	**	**	**	**	*	ns
Denton TX	**	**	ns	**	**	**
Aberdeen ID	-	-	-	**	**	**

Note: In 1968 one replication was grown at each location

tested over environments. There is some indication that the lysine levels initially selected as cutoff points for environmental testing of varieties in 1967-1970 may have been too high and resulted in a high number of varieties that had abnormally high values and were not genetically superior to other varieties. One such example is Nap Hal which appears to be our best source of combined high protein and high lysine. Nap Hal was not among the spring wheat varieties tested in the 1967-70 environmental nurseries because its lysine value, although moderately high, was below the selection cutoff point for varieties to be included.

Several hundred common wheats from the 12,613 in the World Collection evaluated to date were grown at Yuma, Arizona in 1972 and will be grown again in 1973. They were chosen according to different selection criteria in an effort to establish which criterion most accurately identifies the varieties with genetically unusual protein and lysine properties. Among the criteria were protein content, lysine per unit protein, lysine per unit dry weight, and adjusted lysine per unit protein. Selections were not limited to the varieties with highest values but were taken from various portions of the protein and lysine spectrums.

Significance of Findings -

Production environment exerts a large influence on protein and lysine values of wheat. The environmental effect is probably larger than the genetic effect and tends to mark genetic differences.

Environmental nurseries of a few wheats from the World Collection tested during the 1967-1970 period have identified some varieties with significantly more lysine in their protein over environments than other varieties. The lysine advantage of the varieties was small.

Soil Fertility and Protein Content

It is no more possible to fix protein content at a pre-designated level by genetic means than it is possible to fix yield of grain genetically. Both traits, protein content and yield, are functions of genetic potential and production environment. The ARS-Nebraska program has led to development of winter wheats with significantly increased potential for high grain protein than ordinary wheats. Also, the research has pointed to differential and more complete nitrogen translocation from the wheat plant to its grain as the physiological key to the high protein trait. Differential nitrogen uptake from the soil has not been demonstrated to be a factor.

The significance of such findings is the expectation that there would be phenotypic expression of the high protein trait in an array of production environments and soil fertility levels. Varieties carrying the Atlas genes for high protein can be expected to be superior in protein content to other varieties whether soil nitrogen availability was high or low.

Three-year Study in Nebraska -

A three-year study was conducted at 10 sites in Nebraska in cooperation with Outstate Testing to determine the yield and protein responses of a high protein (C.I.14016) and ordinary variety (Lancer) to different levels of soil fertility. The full results of the study have been prepared for separate publication but are briefly summarized in this report.

The varieties responded significantly to nitrogen application in protein at all sites and in yield at six of 10 sites (table 32). The

Table 32. Yield and protein responses of Lancer and CI14016 to nitrogen fertilizer at 10 experimental sites in Nebraska during a 3-year period, 1968-1970.

Site number	Availability of soil nitrogen	Availability of precipitation	Grain yield response	Grain protein response
1	low	normal	**	**
2	low	normal	*	**
3	medium	normal	**	**
4	very high	low	ns	**
5[1]	medium	normal	ns	**
6	medium	low	ns	**
7	low	normal	*	**
8	medium	normal	**	**
9	very low	normal	**	**
10	high	high	ns	*

[1] Late spring frost caused partial spike sterility
* Significant at .05 level of probability
**Significant at .01 level of probability

yield of Lancer peaked at only 40 pounds of nitrogen whereas the highest yield of the high protein variety C.I.14016 was recorded for 120 pounds of applied nitrogen (table 33). Lancer was more productive than C.I.14016 at the intermediate rates of nitrogen but less productive at the highest rate.

The 1.9 percentage points protein advantage of C.I.14016 persisted whether it was less, equal to, or more productive than Lancer (table 34). This clearly demonstrates that the protein differences of these two varieties is genetically based and not merely an artifact of differences in grain yield.

The mean yield responses of both varieties were non-linear (figure 9). The second degree polynomial provided a good fit of the data. R^2 values for the regression of nitrogen on grain yield were .88 and .96 for Lancer and C.I.14016, respectively.

In contrast to yield, the protein responses of Lancer and C.I.14016 to N-fertilizer, within the limits of the fertilizer rates applied, were clearly linear (figure 10). C.I.14016 maintained its approximate 2 percentage points protein advantage over Lancer throughout all rates of nitrogen from 0 to 120 pounds per acre. R^2 values based on linear regression were very high (.99 and .98) for both varieties.

C.I.14016 also produced more protein per acre than Lancer (figure 11). This was so even at the lower rates of nitrogen application in which C.I.14016 was less productive than Lancer. The data indicate a protein production potential for C.I.14016 that had not yet been reached with application of 120 pounds of nitrogen.

Table 33. Average grain yield and protein content of a normal and a high protein wheat variety on different nitrogen fertility levels at test sites in Nebraska in 1969 and 1970.

Nitrogen applied	Grain yield			Protein content		
	Lancer	CI 14016	Difference	Lancer	CI 14016	Difference
lb/a	bu/a	bu/a	bu/a	%	%	%
0	38.5	38.3	-0.2	10.8	12.5	+1.7
20	44.4	40.7	-3.7	11.2	13.3	+2.1
40	46.7	43.8	-2.9	11.8	14.0	+2.2
60	46.2	45.5	-0.7	12.6	14.9	+2.3
80	46.3	45.1	-1.2	13.2	15.4	+2.2
100	45.7	45.3	-0.4	13.6	15.8	+2.2
120	44.9	46.2	+1.3	14.0	16.3	+2.3
$L.S.D._{.05}$	1.7	1.7	--	0.3	0.3	--

Table 34. Effect of relative grain yield on protein content of Lancer and C.I. 14016.

N-fertilizer treatment lb/a	Mean protein advantage of CI 14016 over Lancer when CI 14016 yielded: less than Lancer Percentage points difference	equal to Lancer Percentage points difference	more than Lancer Percentage points difference
0	1.8 (7) 1/	----	1.5 (3)
20	1.9 (8)	----	2.1 (2)
40	1.9 (8)	----	1.9 (2)
60	2.1 (7)	----	1.7 (3)
80	1.9 (5)	1.7 (3)	2.3 (2)
100	2.4 (2)	2.4 (2)	1.9 (2)
120	2.0 (2)	2.1 (1)	2.3 (3)
$\bar{x}$	1.9 (39)	2.0 (6)	1.9 (17)

1/ Number of comparisons comprising the mean protein value.

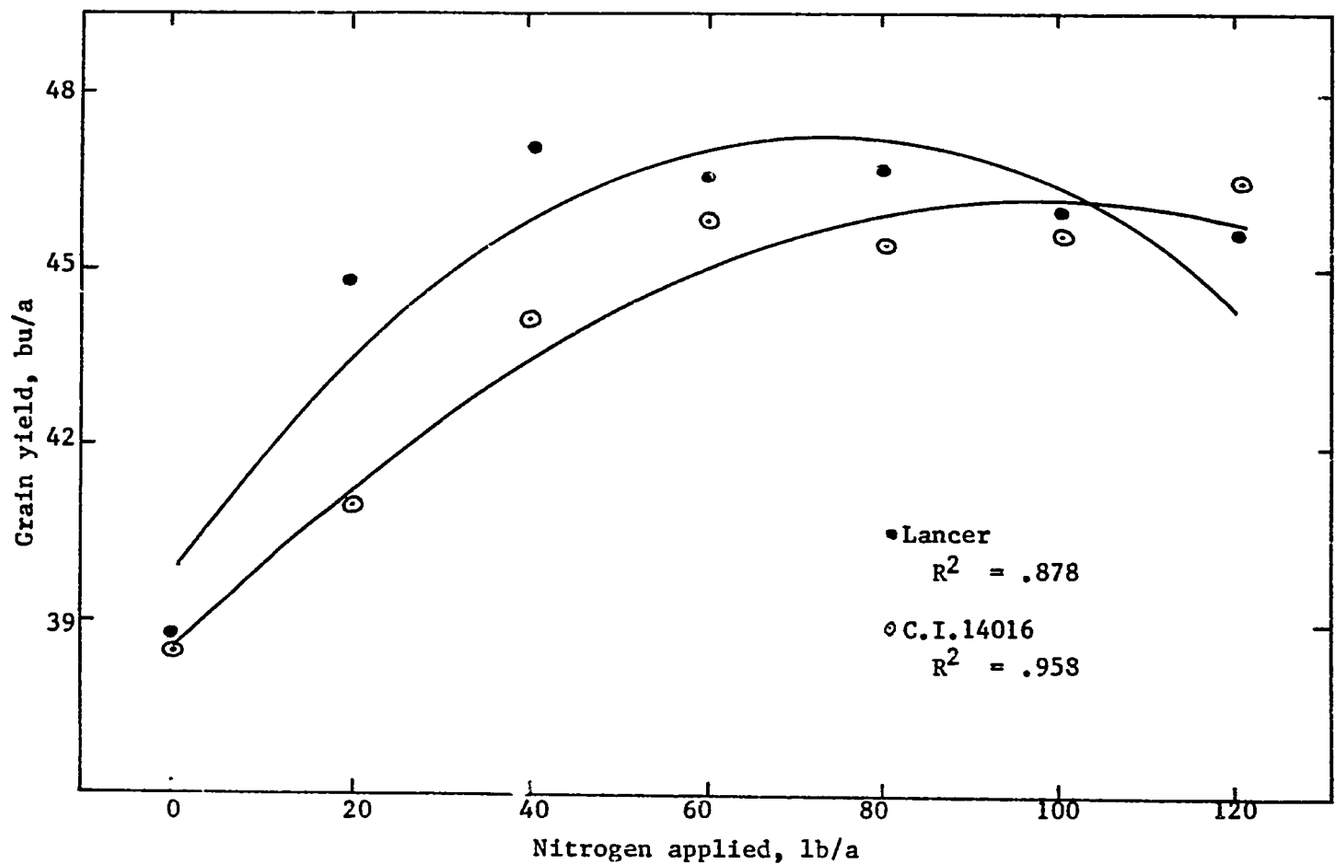


Figure 9. Mean yield response of two winter wheat varieties to nitrogen fertilizer in 6 experiments in Nebraska, 1969-1970.

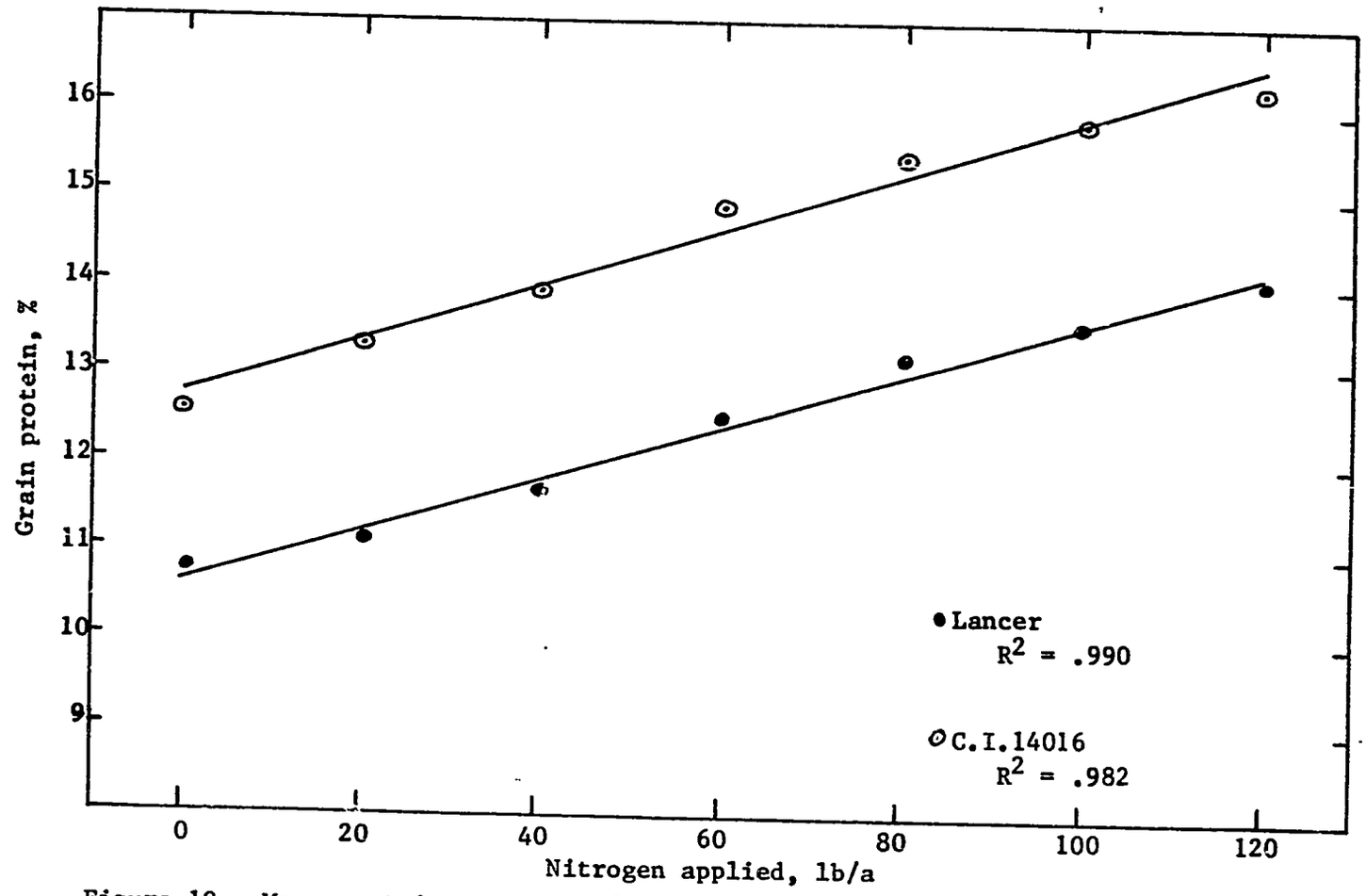


Figure 10. Mean protein response of two winter wheat varieties to nitrogen fertilizer in 6 experiments in Nebraska, 1969-1970.

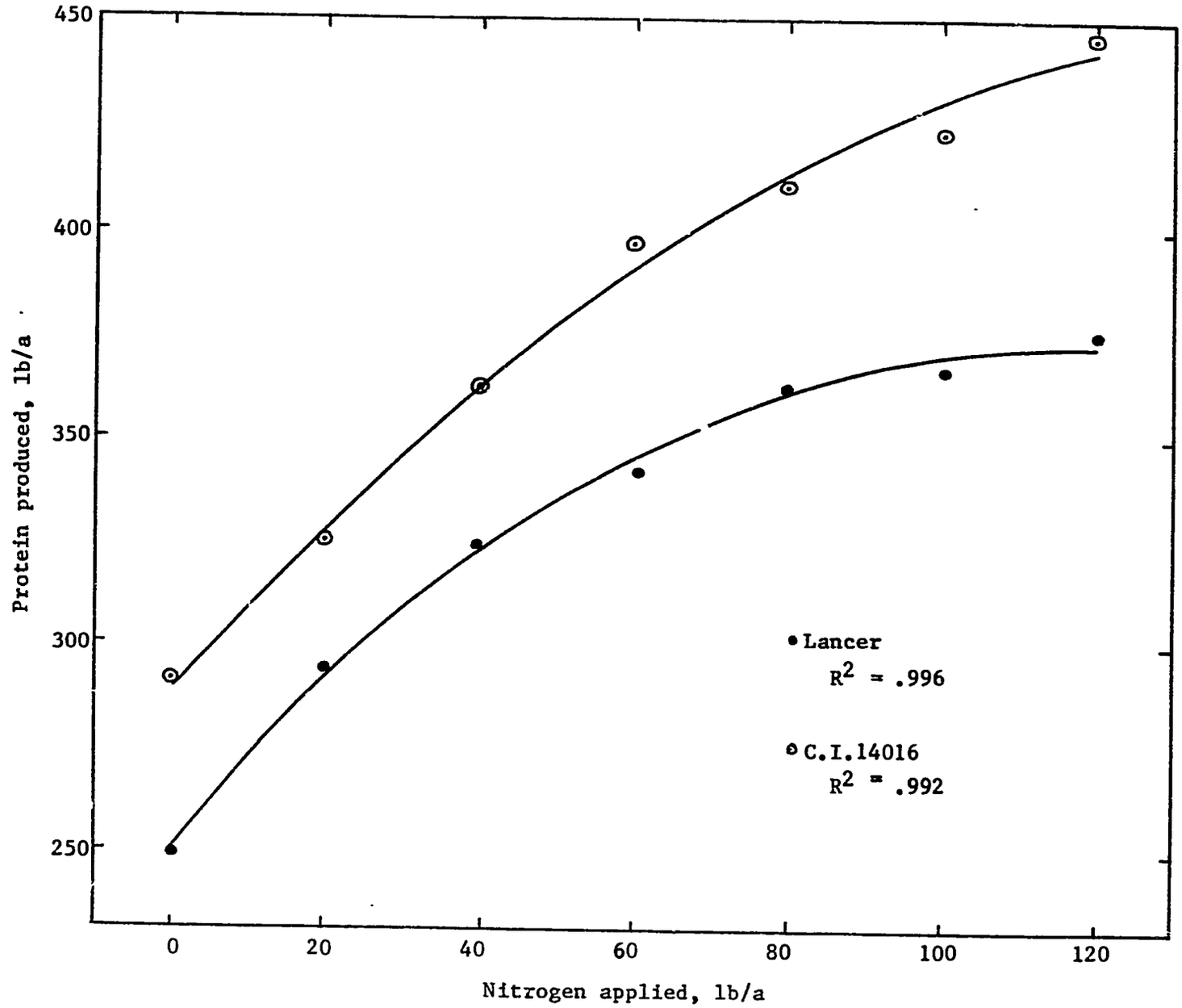


Figure 11. Mean protein production of two winter wheat varieties under different levels of nitrogen fertilizer in 6 experiments in Nebraska, 1969-1970.

Significance of the Data -

We conclude from this study that high protein genes from Atlas 66 can effectively promote higher grain protein content of wheat in a wide array of soil fertility situations.

Atlas-66 derived high protein varieties can be expected to produce grain with higher protein content than ordinary varieties under low as well as high soil fertility. Their protein advantage can be expected to be in the range of 2 to 3 percentage points.

Production of wheat with high grain protein (14 to 17%) can be achieved from genetically high protein varieties grown under high fertility situations.

The International Winter Wheat Performance Nursery

This nursery has been effectively utilized to test the stability of the Atlas-derived high protein trait in diverse production environments throughout the world as well as identify winter varieties with superior general adaptation for use in breeding programs.

Phenotypic Expression of High Protein -

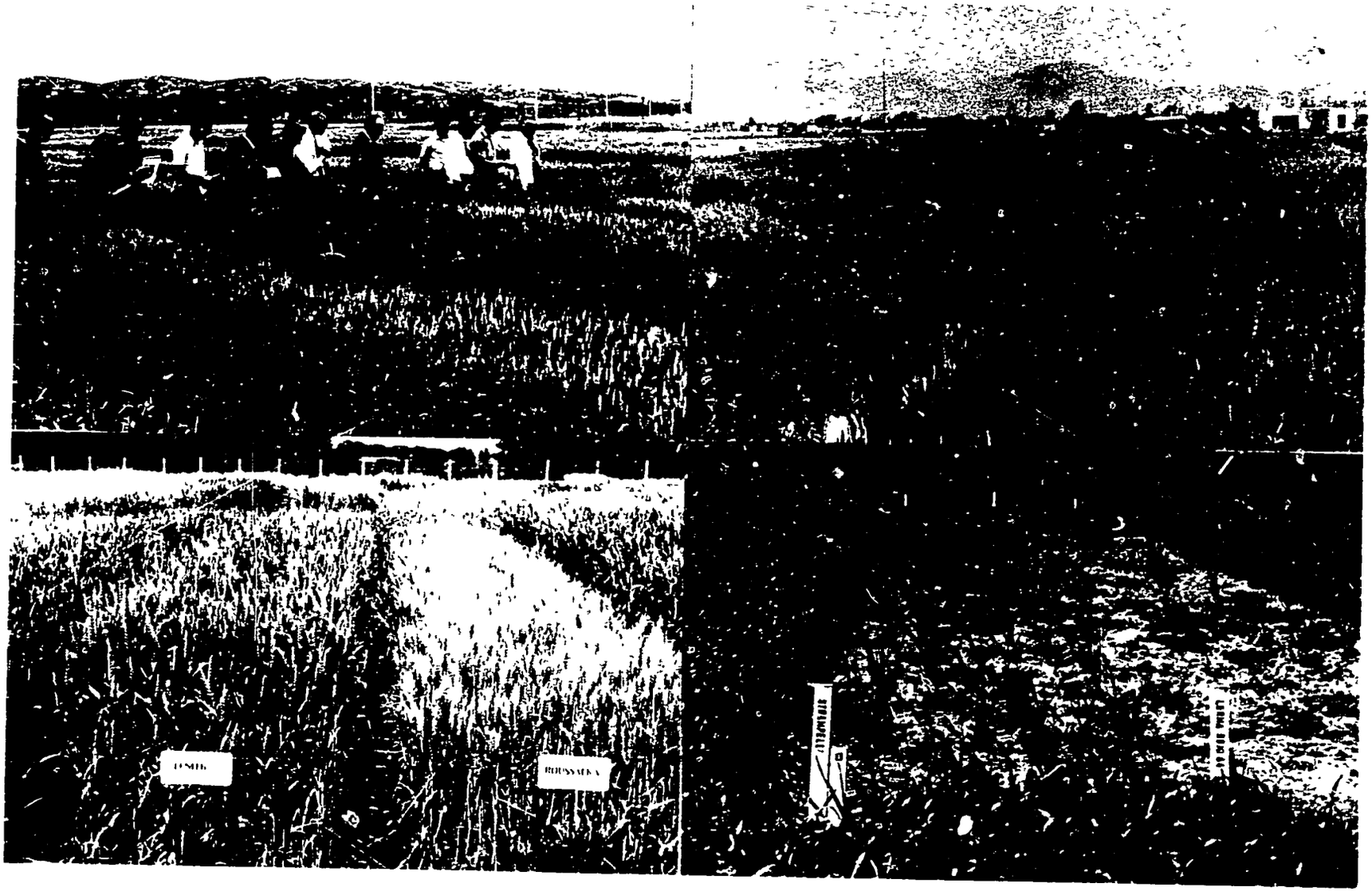
Grain yield, protein content, and lysine content of varieties tested in the nursery in 1969 and 1970 are summarized in table 35. Three varieties in the nursery possessed known genes for high grain protein content. They were Atlas 66, NB67730, and Purdue 28-2-1. The latter variety is derived from the South American variety Frondoso as is Atlas 66. NB67730 is a selection from Atlas 66 crossed with Comanche.

None of the high protein varieties were as productive on the average as were several other varieties in the nursery such as Bezostaia, Blueboy, and San Pastore. However, they did yield as well as or more than Winalta, Cappell Desprez, Gaines, Felix and Odin. The highest grain protein content in each year was produced by Atlas 66, Purdue 28-2-1, and NB67730. As an average for two years, these varieties ranged from 16.9 to 17.9 percent protein content and were from 2.1 to 3.1 percentage points higher in protein content than the nursery mean. They were significantly higher in protein content than all other varieties except Cappell Desprez.

The protein contents of Atlas 66, NB67730, and five other varieties, are compared at Kabul, Afghanistan, where grain yields were very high and at Kermanshah, Iran where the yields were low in 1969 (table 36). Mean protein level of the nursery was only one percentage point lower at Kabul than Kermanshah although mean grain yield at Kabul exceeded that at Kermanshah by three times. At both sites there was excellent phenotypic expression of the high protein trait in NB67730 and Atlas 66.

Wheat growers examine the International Winter Wheat Performance Nursery grown at Lincoln, Nebraska.

Foreign visitors to the wheat research project at the University of Nebraska in 1970 included N. Saulescu and N. Eustatiu (Romania) and T. Y. Chung and B. H. Hong (Korea).



International Winter Wheat Performance Nurseries at Eskisehir, Turkey (upper left), Kabul, Afghanistan (upper right), Krasnodar, USSR (lower left), and Malé Ripnany, Czechoslovakia (lower right) where heavy winterkilling of Strampelli and Lerma Rojo occurred.

Table 35. Grain yield, protein content, and lysine content for varieties grown in the International Winter Wheat Performance Nursery in 1969 and 1970.

Variety	Avg. grain yield				Avg. protein content				Avg. lysine content			
	1969	1970	2-year avg.	Rank	1969	1970	2-year avg.	Rank	1969	1970	2-year avg.	Rank
	q/ha	q/ha	q/ha		%	%	%		%	%	%	
Number of sites --	16	33	--	--	18	23	--	--	18	23	--	--
Winter Varieties												
Bezostaia	45.2	39.6	42.4	1	13.4	14.2	13.8	25	2.9	2.9	2.9	8
Blueboy	43.5	29.3	36.4	4	13.6	14.6	14.1	21	3.0	2.9	3.0	3
San Pastore	41.1	26.6	33.9	14	13.6	14.5	14.1	21	2.9	2.9	2.9	8
Sturdy	40.5	32.2	36.4	6	14.3	15.2	14.8	11	2.8	2.8	2.8	21
Timwin	39.9	35.5	37.7	2	14.1	15.2	14.7	15	3.0	2.9	3.0	3
Parker	39.8	32.9	36.4	5	14.4	15.1	14.8	11	2.9	2.8	2.9	8
Fertodi 293	39.3	32.5	35.9	8	14.8	15.3	15.1	8	2.8	2.8	2.8	21
Benhur	38.5	29.8	34.2	12	14.4	15.7	15.1	8	2.8	2.8	2.8	21
Scout 66	38.4	34.7	36.6	3	14.1	14.9	14.5	18	2.9	2.8	2.9	8
Yung Kwang	38.1	30.3	34.2	13	14.2	15.4	14.8	11	2.9	2.8	2.9	8
Arthur	38.1	34.1	36.1	7	14.1	15.2	14.7	15	2.9	2.9	2.9	8
Gage	37.1	32.1	34.6	10	14.4	15.2	14.8	11	2.9	2.8	2.9	8
Stadler	36.9	31.9	34.4	11	13.0	14.3	13.7	26	3.1	2.9	3.0	3
Heine VII	36.8	31.0	33.9	15	14.8	14.5	14.7	15	2.8	3.0	2.9	8
Lancer	36.6	32.7	34.7	9	14.0	14.4	14.2	20	2.9	2.9	2.9	8
Shawnee	36.5	30.3	33.4	17	13.7	14.5	14.1	21	2.9	2.9	2.9	8
Riley 67	36.3	31.5	33.9	16	14.1	14.9	14.5	18	3.0	2.9	3.0	3
Yorkstar	35.8	30.9	33.4	18	12.4	13.1	12.8	28	3.1	3.1	3.1	1
Bankuti 1201	35.6	28.4	32.0	20	15.1	15.8	15.5	5	2.8	2.8	2.8	21
Triumph 64	35.6	29.9	32.8	19	14.7	15.7	15.2	7	2.9	2.8	2.9	8
NB67730	34.8	28.2	31.5	21	16.4	17.3	16.9	3	2.8	2.7	2.8	21
Atlas 66	33.4	28.1	30.8	22	17.5	18.3	17.9	1	2.7	2.7	2.7	28
Purdue 28-2-1	32.9	27.1	30.0	24	16.6	18.4	17.5	2	2.8	2.7	2.8	21

Table 35. (Continued)

Variety	Avg. grain yield 1969	Avg. grain yield 1970	Avg. grain yield 2-year avg.	Rank	Avg. protein content 1969	Avg. protein content 1970	Avg. protein content 2-year avg.	Rank	Avg. lysine content 1969	Avg. lysine content 1970	Avg. lysine content 2-year avg.	Rank
	q/ha	q/ha	q/ha		%	%	%		%	%	%	
Winalta	32.2	28.1	30.2	23	13.8	14.4	14.1	21	2.9	2.8	2.9	8
Cappell Desprez	32.0	26.0	29.0	25	16.0	15.9	16.0	4	2.8	2.8	2.8	21
Gaines	30.7	26.7	28.7	26	13.0	13.7	13.4	27	3.1	3.0	3.1	1
Felix	29.6	24.4	27.0	27	14.9	15.3	15.1	8	2.9	2.9	2.9	8
Odin	26.7	22.5	24.6	28	15.4	15.6	15.5	5	3.0	2.9	3.0	3
Spring Varieties												
Lerma Rojo 64	35.4	29.3	32.4		14.5				2.8			
INIA 66	24.5	26.4	25.5		14.5				2.8			
Mean (Winter varieties)	36.2	30.2	33.2		14.4	15.2	14.8		2.9	2.8	2.9	
L.S.D. .05	6.46	6.50	6.26		0.92	1.27	1.19		0.12	0.13	0.12	

Table 36. Grain yield and protein content of seven varieties at high-yielding and a low yielding nursery sites of the International Winter Wheat Performance Nursery grown in 1969. (taken from data presented by V. A. Johnson at 3rd FAO/Rockefeller Foundation Wheat Seminar, April 29-May 13, 1970, Ankara, Turkey)

Variety	Kabul, Afghanistan		Kermanshah, Iran	
	Yield	Protein	Yield	Protein
	q/ha	%	q/ha	%
Nursery mean - -	52	13.8	16	14.8
Yorkstar	66	10.4	13	13.5
Heine VII	61	15.9	18	15.4
Bankuti 1201	60	14.1	16	14.7
NB67730	53	17.1	14	15.9
Riley 67	50	14.5	13	14.7
Gaines	46	10.8	11	14.0
Atlas 66	44	16.5	13	18.5

The protein contents of seven varieties at IWWPN sites at which the general level of protein was high, intermediate, or low in 1970 are compared in table 37. Grain yields were similar at each of the three sites. The protein potential of Atlas 66, NB67730, and Purdue 28-2-1 was expressed well at each of the sites -- particularly at Stillwater and Martonvasar where all were several percentage points higher in protein than the nursery mean.

Lysine Variation -

There was little difference in the mean lysine content of varieties in the nursery. Over two years, mean lysine values for varieties ranged from 2.7 to 3.1 percent. Interestingly, the three high protein varieties had lysine contents as high on the average as numerous other varieties with much lower levels of grain protein. The depression in lysine associated with high protein appears minimal at the general levels of protein attained in the nursery.

Lysine variation among varieties at individual nursery sites was larger (table 38). The range for NB67730 was from 2.3 to 3.1 percent compared to 2.4 to 3.9 percent for Sturdy. Sizeable variations in levels of other amino acids at individual sites likewise were recorded.

Correlations -

Varietal correlation coefficients for yield vs. protein, protein vs. lysine, and protein vs. adjusted lysine for nine varieties grown in the 1969 and 1970 International nurseries are reported in table 39. Protein content showed little or no correlation with grain yield over nursery environments for nine varieties. The highest coefficient was -0.43 for the German variety Heine VII. Thus, 18 percent of the protein variation of Heine VII is accountable through variation in grain yield. Less than 4 percent of protein variation of other varieties was related to grain yield differences.

Table 37. Phenotypic expression of the high protein trait at sites of the International Winter Wheat Performance Nursery in 1970 at which the general protein level among varieties was high (Stillwater, Oklahoma), intermediate (Martonvasar, Hungary), and low (Cambridge, England).

Variety	Grain protein content (%) at:		
	Stillwater Oklahoma	Martonvasar Hungary	Cambridge England
$\bar{x}$ protein content of (%) nursery	17.8	15.8	12.5
Bezostaia	16.5	14.3	12.3
Lancer	16.2	14.6	12.1
Yorkstar	16.0	13.7	11.2
Gaines	16.5	14.1	10.6
NB67730	20.9	18.4	14.2
Atlas 66	20.6	19.4	13.5
Purdue 28-2-1	20.8	20.3	13.7
Nursery $\bar{x}$ yield (q/ha)	25.4	32.7	31.7

Table 38. Protein and amino acid data for four varieties grown at 15 locations (4 reps per location, 60 samples per variety) in the First IWWPN. The protein data are expressed as a % of the sample and the amino acid data are expressed as shown in the footnotes.

Measurement	Variety								
	NB67730[1]		Triumph 64[2]		Bezostaya[2]		Sturdy		
	Mean	Range	Mean	Range	Mean	Range	Mean[2]	Mean[3]	Range[3]
Protein	16.0	11.4-21.9	14.7	11.3-18.6	13.2	9.8-15.2	14.0	14.6	10.7-18.3
Lysine	2.7	2.3-3.1	2.7	2.5-3.1	2.8	2.6-3.2	2.8	2.7	2.4-3.9
Histidine	2.3	1.9-2.8	2.3	2.0-2.5	2.4	2.1-2.7	2.2	2.1	2.0-2.5
Arginine	4.4	3.6-5.3	4.6	4.2-5.1	4.6	4.2-5.1	4.5	4.3	3.9-5.7
Aspartic Acid	5.6	4.7-6.5	5.5	4.8-6.3	5.6	5.1-6.4	5.8	5.5	5.0-7.1
Threonine	3.2	2.8-3.5	3.2	2.9-3.5	3.2	3.0-3.5	3.1	3.0	2.7-3.7
Serine	5.5	4.7-6.2	5.4	4.9-6.3	5.6	4.7-6.1	5.4	5.1	4.0-5.6
Glutamic Acid	36.8	28.2-47.8	35.3	30.1-42.5	35.7	31.1-46.6	36.1	34.6	26.8-37.3
Proline	11.6	9.3-14.2	11.4	9.8-13.3	11.6	9.8-13.8	11.4	11.0	8.6-12.1
Glycine	4.3	3.6-4.8	4.4	4.0-4.8	4.4	4.0-5.1	4.6	4.4	4.1-5.1
Alanine	3.7	3.1-4.2	3.6	3.2-4.0	3.8	3.5-4.1	3.8	3.6	3.3-4.8
Valine	4.7	3.6-5.2	4.9	4.3-5.5	5.1	4.6-5.6	5.0	4.8	4.4-5.5
Methionine	1.4	1.2-1.8	1.5	1.2-1.8	1.4	1.1-1.7	1.4	1.3	1.0-1.6
Isoleucine	3.6	2.7-4.4	3.8	3.4-4.3	4.0	3.6-4.3	3.9	3.8	3.5-4.0
Leucine	7.3	6.0-8.4	7.3	6.8-7.9	7.5	6.8-8.1	7.4	7.1	6.8-7.6
Tyrosine	2.7	1.8-3.1	2.6	2.1-3.1	2.4	1.8-2.9	2.0	1.9	1.5-2.6
Phenylalanine	5.0	4.0-5.9	5.1	4.7-5.7	5.1	4.5-5.6	5.3	5.1	4.6-5.3

[1]Protein determined by the Udy dye binding procedure except for two samples out of the range which were determined by the Kjeldahl procedure; amino acid expressed as a % of the protein.
[2]Protein determined by the Udy dye binding procedure and amino acids expressed as a % of Udy protein.
[3]Protein determined by the Kjeldahl procedure and amino acids expressed as a % of Kjeldahl protein.

Table 39. Correlation coefficients for yield, protein and lysine computed for nine varieties grown in the International Winter Wheat Performance Nursery in 1969 and 1970.

Variety	Yield vs. protein	Protein vs. lysine	Protein vs. adj. lysine
Bezostaia	-0.11	-0.73	-0.23
Scout 66	0.02	-0.82	-0.29
Triumph 64	-0.01	-0.74	-0.04
Winalta	-0.03	-0.76	-0.19
Heine VII	-0.43	-0.12	0.02
Bankuti 1201	-0.18	-0.74	0.11
Atlas 66	-0.20	-0.61	0.33
NB67730	0.05	-0.75	0.11
Purdue 28-2-1	-0.06	-0.79	0.08

Large and significant negative correlations of protein with lysine per unit protein were recorded for all nine varieties except Heine VII which had a very low relationship of lysine with protein. A standard correction factor based on the regression of lysine on protein for wheats in the World Collection was utilized to adjust lysine values to a common protein. Correlation of protein with the adjusted lysine values indicates an under-correction for the lower protein varieties like Bezostaia but over-correction for the high protein varieties - particularly Atlas 66. We believe this results from a curvilinear relationship of lysine with protein over the entire protein range. The strong negative relationship of lysine with protein in the lower protein range tends to disappear as protein becomes higher. Application of a single adjustment factor, therefore, would under-correct lysine values in the low protein range but over-correct in the high range of protein.

The most useful correlation involves yield-protein relationships at individual test sites. The yields of a group of 24 varieties from the first and second IWWPNs, not known to be genetically different in grain protein potential, were correlated with protein at each of 13 individual nursery sites (table 40).

Protein was negatively correlated with yield at 9 sites; the highest value was -0.61 at Stillwater, Oklahoma in 1970. Yield and protein showed no correlation at 2 sites and were positively correlated at 2 sites. The highest positive correlation was +0.65 at Eskesehir, Turkey in 1969.

R^2 values indicate the amount of protein variation accounted for by variation in grain yield at each site. At those sites at which protein was negatively correlated with yield, the accountable portion of the protein variation ranged from 37% at Stillwater, Oklahoma in 1970 to only 6% at Svalof, Sweden and Ankara, Turkey in 1970. At the two sites where protein was positively

Table 40. Yield and protein correlations for a group of 24 winter wheat varieties[1/] at selected IWWPN sites in 1969 and 1970.

Site and Year		Mean Yield	Mean Protein content	r	$r^{2/}$
		q/ha	%		
Stillwater	1970	26.0	17.3	-0.61	0.37
Svalof	1969	56.7	16.1	-0.57	0.32
Kabul	1969	54.8	13.2	-0.56	0.32
Kabul	1970	62.8	16.6	-0.49	0.24
Wageningen	1969	42.3	13.1	-0.45	0.20
Novi Sad	1969	48.2	14.7	-0.42	0.18
Cambridge	1970	32.7	12.4	-0.35	0.12
Ankara	1970	35.7	13.8	-0.25	0.06
Svalof	1970	50.5	15.6	-0.24	0.06
Novi Sad	1970	42.3	15.9	ns[2/]	--
Fundulea	1970	35.6	14.6	ns	--
Rieti	1969	48.3	12.4	+0.24	0.06
Eskisehir	1969	44.6	12.8	+0.65	0.42

1/ None of varieties known to possess genes for high protein (Atlas 66, Purdue 28-2-1, NB67730, Cappelle Desprez, Lerma Rojo, and INIA excluded from computation).

2/ Non-significant at 5% level of probability.

correlated with yield the accountable protein variation was from 42% at Eskisehir, Turkey to only 6% at Rieti, Italy.

J. E. Stroike who manages the IWWPN, did an in-depth analysis of nursery data from 1969 and 1970 as a Ph.D. thesis. The findings have been published as Nebraska Research Bulletin 251. Three performance stability parameters were computed as a measure of general adaptation of each variety, mean yield, regression coefficient, and deviation from regression. Each parameter provides a measurement of a different aspect of variety performance. We believe that all are needed to describe a variety's performance potential.

Significance of IWWPN Data -

Winter wheat varieties differ strongly in general adaptation as measured by their yield performance in the IWWPN.

The Russian variety Bezostaia was found to be highly productive and to possess broad international adaptation. It performs well relative to other winter wheat varieties in widely different production situations.

There was excellent phenotypic expression of high protein in three U. S. varieties known to possess high protein genes which were tested in the IWWPN in 1969 and 1970. The genes were equally effective in promoting high protein at high as well as low yield nursery sites.

High protein varieties in the IWWPN had lysine contents as high as numerous other varieties with much lower protein levels. The depression of lysine associated with high protein was minimal at the generally high protein levels of the nurseries.

Yield and protein showed little or no correlation when measured for individual varieties over nursery sites. Protein and lysine per unit protein were strongly negatively correlated.

P. P. Lukyanenko (right), developer of Bezostaia 1, discusses his variety with V. A. Johnson (left) at Krasnodar, USSR in 1972. S. Rajki (Martonvasar, Hungary) examines a field of Bezostaia 1 near Istanbul, Turkey in lower photo during a tour in 1972.

Adjustment of lysine by linear regression reduced the magnitude of the negative correlation between protein and lysine.

When yield and protein of a group of varieties not known to be genetically different in protein potential were correlated at individual nursery sites, significant negative as well as positive correlations occurred.

A relatively small portion of total protein variation among varieties could be accounted for by variations of grain yield.

The First International Winter Wheat Conference

The conference was organized by personnel of the Nebraska Agricultural Experiment Station-Agricultural Research Service wheat research team with funding from AID via Contract No. AID/csd-1208. The conference site was Ankara, Turkey. It was officially hosted by the Turkish Ministry of Agriculture.

Ninety-seven wheat researchers from 24 countries met in Ankara June 5-9, 1972. Conference sessions which lasted until noon of June 8, were followed by a 2-day tour of wheat research sites in Ankara, Eskisehir, Adapazari, and Yesilkoy.

The International Winter Wheat Performance Nursery provided impetus for the conference. A survey of nursery cooperators made in 1971 indicated much interest in an early conference to assess the significance of nursery findings and to develop procedures to accelerate the agronomic and nutritional improvement of winter wheat.

The Proceedings of the Conference have been printed and distributed as Nebraska Miscellaneous Publication 29 (344 pages). Conference participants unanimously recommended that a second winter wheat conference be held in 1975 or 1976.

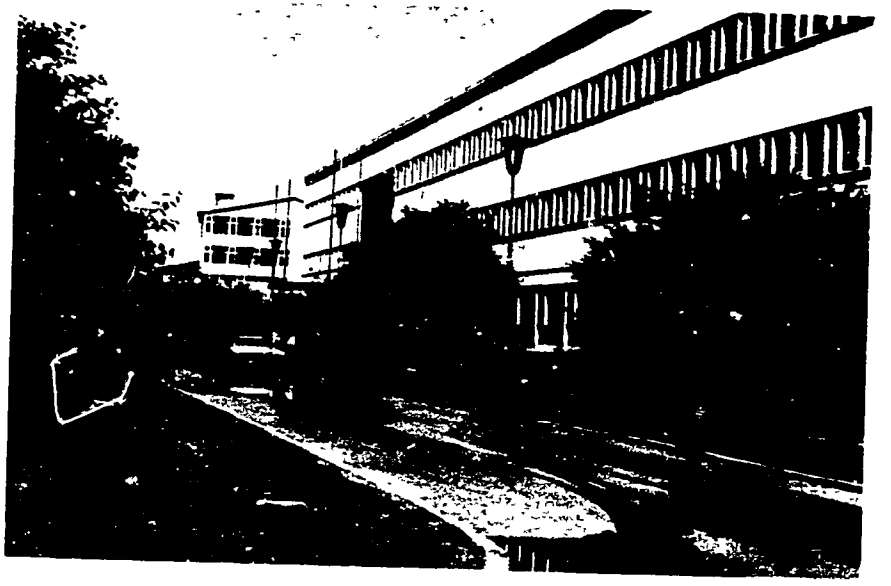
Physiological Processes Associated with High Grain Protein

Research at Nebraska published in 1967 provided evidence that the high protein phenomenon was associated with differential and more complete nitrogen translocation from the plant to its grain during the grain maturation period of development. Differential nitrogen uptake by the roots was not demonstrated.

Subsequently, researchers at the University of Illinois, working with Atlas 66-derived high protein winter wheat lines from Nebraska, implicated the nitrate reductase enzyme system in the high protein phenomenon. Established high protein lines were demonstrated to consistently exhibit higher levels of nitrate reductase activity than ordinary varieties even in the early seedling stages. These findings were later corroborated by research at Oklahoma State University. The possibility of an efficient and rapid method for detection of segregates from hybrid populations with high protein potential was suggested by the Illinois research.

Dr. Lowell Klepper and K. D. Wilhelmi joined the Nebraska wheat research team in 1972 and will pursue investigations of physiological processes - particularly nitrogen metabolism, associated with high protein in wheat. Mr. Wilhelmi has undertaken a field and laboratory study of nitrogen uptake and translocation in four varieties (three of which produce high protein) as a Ph.D. thesis. The varieties are those utilized by Dr. C. L. Lay in 1971 in a genetic study of high protein at Nebraska.

A field experiment has been designed by Mr. Wilhelmi and Dr. Klepper that involves different rates of N-fertilizer and times of application on wheat varieties that are genetically different in grain protein potential. Irrigation will be used to assure immediate availability of nitrogen to the wheat at the time of application. Provisions have been made for measurement of nitrogen uptake by the varieties under the different fertility regimes.



An International Winter Wheat Conference was held at Ankara, Turkey, June 5-9, 1972. Ahmet Demirlicakmak (upper left) chaired one of the sessions. Nursery cooperators who participated in the conference and the conference meeting site in Ankara are shown.

Foliage samples will be taken throughout the growing season for measurement of nitrogen concentration and level of enzyme activity. Finally, grain yields and grain protein content will be determined for the varieties and treatments at maturity.

Enzyme studies on the nitrate nutrition of winter wheat were begun in 1972. In an experiment at Mead, in vivo nitrate reductase activity was determined on the foliage at weekly intervals from May 25 to June 29. Four varieties were tested, three rates of N fertilization (0, 60, 120 #/A) with three dates of fertilizer application. Seasonal patterns of nitrate reductase activity were obtained for each variety. Each showed a large peak at head emergence which slowly decreased until maturity. Largest differences among varieties were detected in their response to increased N fertilization with the early spring application. In this treatment nitrate reductase activity was highest in NE701132 (Atlas 66/Comanche//Lancer) followed by Nebraska Restorer (NE542437), Aniversario derivative (NE69565), and Centurk. Foliage and grain samples also were taken at weekly intervals throughout the season for total-N and nitrate-N analyses. The experiment is being repeated in 1973 before publication of results.

Genetic Studies of High Protein and High Lysine

C. L. Lay Thesis Research -

C. L. Lay (Ph. D. thesis) crossed three high protein winter wheat varieties, NE65313 (Atlas 66-derivative), NE69565 (Aniversario-derivative), and NE542437 (Nebraska Male Fertility Restorer) in all combinations. Objectives of the study were to 1) determine if these three varieties possess common genes for high grain protein, and 2) determine if the high protein capability of NE542437 is cytoplasmic or nuclear controlled. Each of the high protein parent varieties also was crossed to a common low protein variety, NE66403. Parents, F_1, F_2 and backcrosses were grown at Yuma, Arizona and Mead, Nebraska in 1971.

The data indicated that NE65313 and NE69565 possess common major genes that condition high grain protein content. There was evidence of minor gene differences for grain protein content in the two varieties.

Inconclusive evidence was obtained that the genes for high grain protein content of NE542437 are nuclear and linked with a gene for male fertility restoration. The F_3 generation was propagated at Lincoln, Nebraska in 1972 to provide confirmatory evidence for the findings from Yuma and Mead in 1971. Protein analyses of the F_3 material have not yet been completed by the laboratory. Results of the study will be published following analysis of the F_3 protein data.

Estimates of heritability for grain protein content in each F_2 population were not of the same magnitude at Mead as at Yuma -- probably due to apparent genotype x environment interactions for grain protein content. The most consistent estimates were obtained in F_2 populations derived from crosses of NE65313 and NE69565 used as females, with NE542437.

High protein segregates from the Lay study will be evaluated for agronomic worth and utilized in further crosses for high protein breeding purposes. The parent varieties are being studied for physiological differences by Lowell Klepper and K. D. Wilhelmi.

Nap Hal x Atlas 66 Study -

Screening of the World Collection at the University of Nebraska and research at Montana State University have identified Nap Hal (P.I.176217) as an exciting genetic source of both high protein and high lysine. We determined Nap Hal to be consistently superior in protein and lysine to most other varieties in greenhouse tests since 1969. It is compared with the high protein variety Aniversario and the high lysine variety Pearl in table 41. Protein values which are expressed on a dry grain weight basis are very high because of excessively high soil fertility. Even so, both Nap Hal and Aniversario were higher in protein than Pearl by 1.9 and 1.3 percentage points respectively. The lysine content of the high lysine varieties Nap Hal and Pearl was approximately 0.5 percentage points higher than Aniversario.

We crossed Nap Hal with Atlas 66 (high protein) in 1968. The F_2 generation was propagated in the greenhouse and F_2 progeny bulks from Nap Hal x Atlas 66 were grown in the F_3 and F_4 generations at Yuma, Arizona. Each F_2 progeny bulk was analyzed for protein and lysine in each generation. Frequency distributions for protein and adjusted lysine from the 1971 Yuma nursery are shown in figures 12 and 13.

There is strong indication of transgressive segregation for both high and low protein. This is interpreted as evidence for different protein genes in Nap Hal and Atlas 66. If so, there is the opportunity for selections from the cross that are higher in protein content than either parent variety.

Table 41. Protein, lysine, methionine, and threonine contents of Aniversario, Pearl, and Nap Hal grown in a greenhouse at Lincoln, Nebraska in 1969.

Measurement	Aniversario (high protein)	Pearl (high lysine)	Nap Hal (high protein and high lysine)
Protein content[1] (%)	20.7	19.4	21.3
Lysine (% of protein)	2.36	2.83	2.81
" (g/100 g grain)	0.48	0.54	0.60
Methionine (% of protein)	1.32	1.91	1.57
" (g/100 g grain)	0.27	0.37	0.33
Threonine (% of protein)	2.88	3.11	3.18
" (g/100 g grain)	0.59	0.60	0.67

[1]Dry weight basis

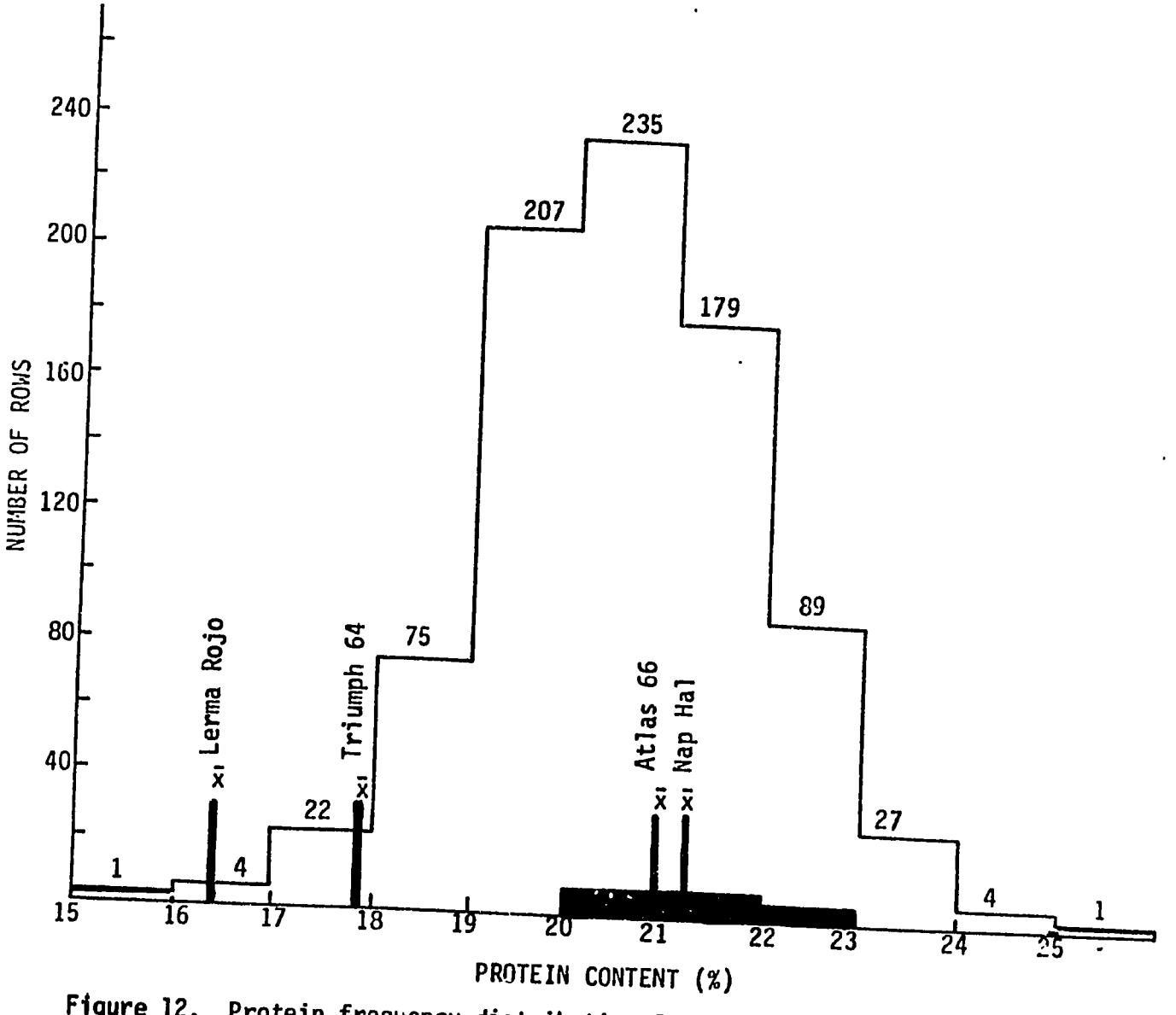


Figure 12. Protein frequency distribution for F_2 progeny bulk rows from a Nap Hal x Atlas 66 cross grown at Yuma, Arizona in 1971.

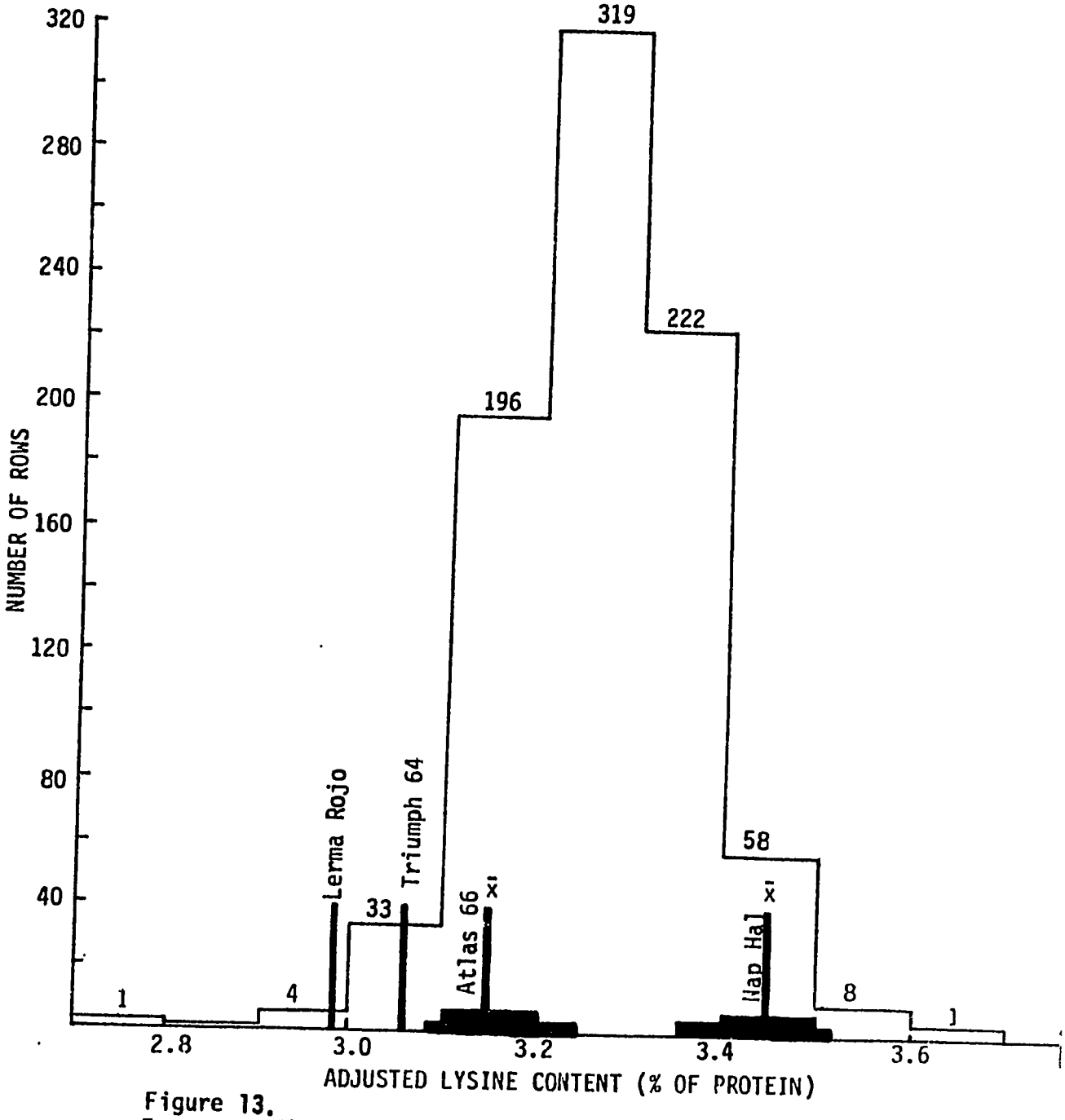


Figure 13.
Frequency distribution for adjusted lysine among F_2 progeny bulk rows from a Nap Hal x Atlas 66 cross grown at Yuma, Arizona in 1971.

The lysine frequency distribution is that which would be anticipated assuming a gene or genes for high lysine from Nap Hal only. The population mean is intermediate to the parents with apparent recovery of the Nap Hal level of lysine in some F_2 progeny bulks but no indication of lysine levels that exceed Nap Hal.

The protein and lysine frequency distributions from the Nap Hal x Atlas 66 F_2 progeny bulks in the F_4 generation grown at Yuma in 1972 are highly similar to those from the previous generation and year (figures 14 and 15). There continued to be strong evidence of transgressive segregation for protein and recovery of the Nap Hal level of lysine. The correlations between 1971 and 1972 protein and lysine values were highly significant.

F_2 progeny bulks with the best combined protein and lysine levels were head selected at Yuma in 1972. The head rows have been seeded again at Yuma for analyses in 1973. Several have been included in our 1972-73 greenhouse planting for use in new hybrid combinations.

A. L. Diehl Thesis Research -

To provide more precise evidence of the genetic relationships for high protein and high lysine between suspect varieties, crosses in all combinations were made between Atlas 66 (high protein), April Bearded (high protein and high lysine), and Nap Hal (high protein and high lysine) in 1971. Parents and F_1, F_2, and backcross populations which were developed from these crosses in 1972 were seeded at Yuma, Arizona in November. The same populations also will be seeded at Fort Collins, Colorado in the spring of 1973. Mr. Diehl will do detailed protein and lysine analyses on the seed crops from the two experimental sites and the inheritance of the two traits will be studied.

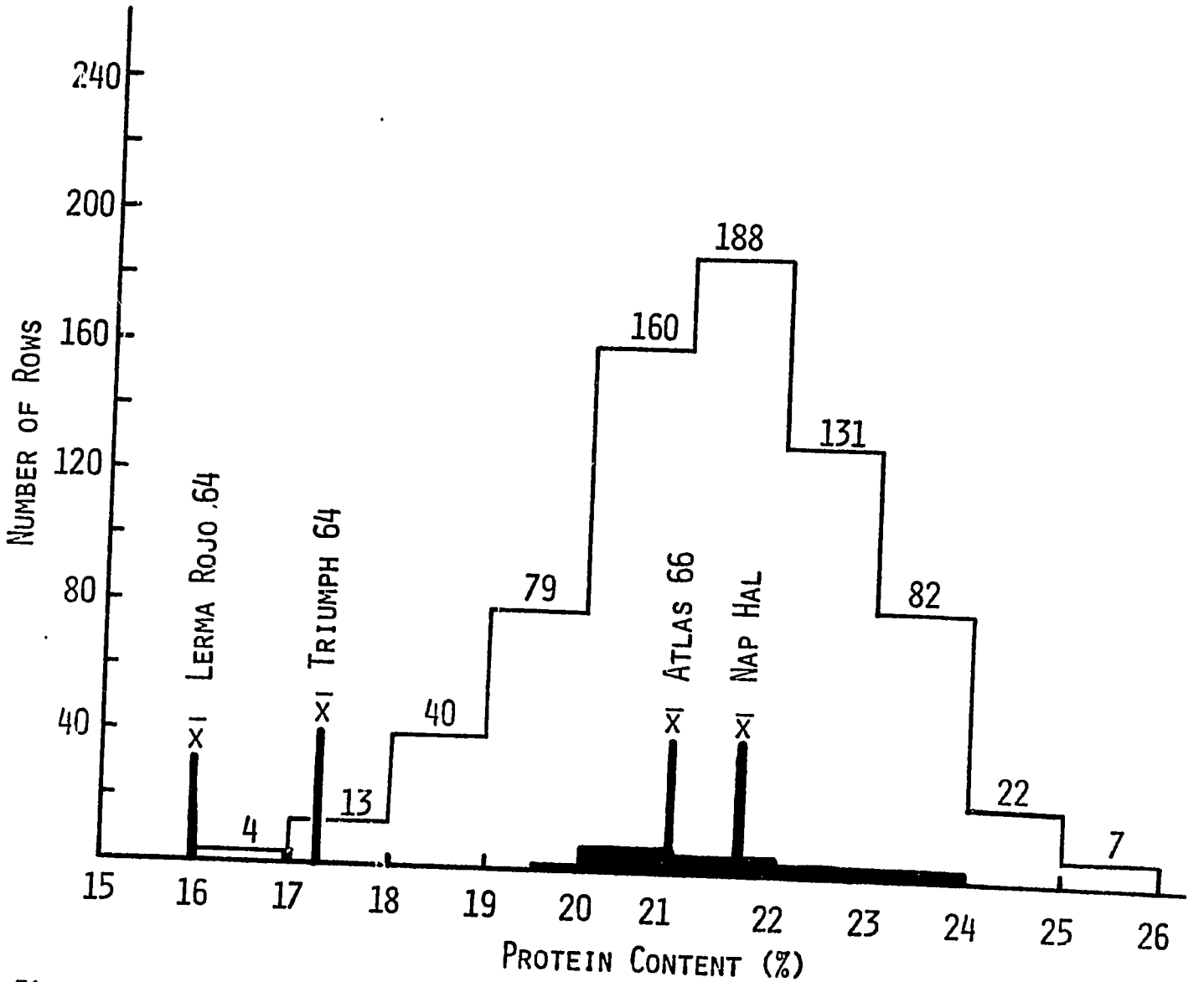


Figure 14.
Protein frequency distribution for F_2 progeny bulk rows (F_4 generation) from a Nap Hal x Atlas 66 cross grown at Yuma, Arizona in 1972.

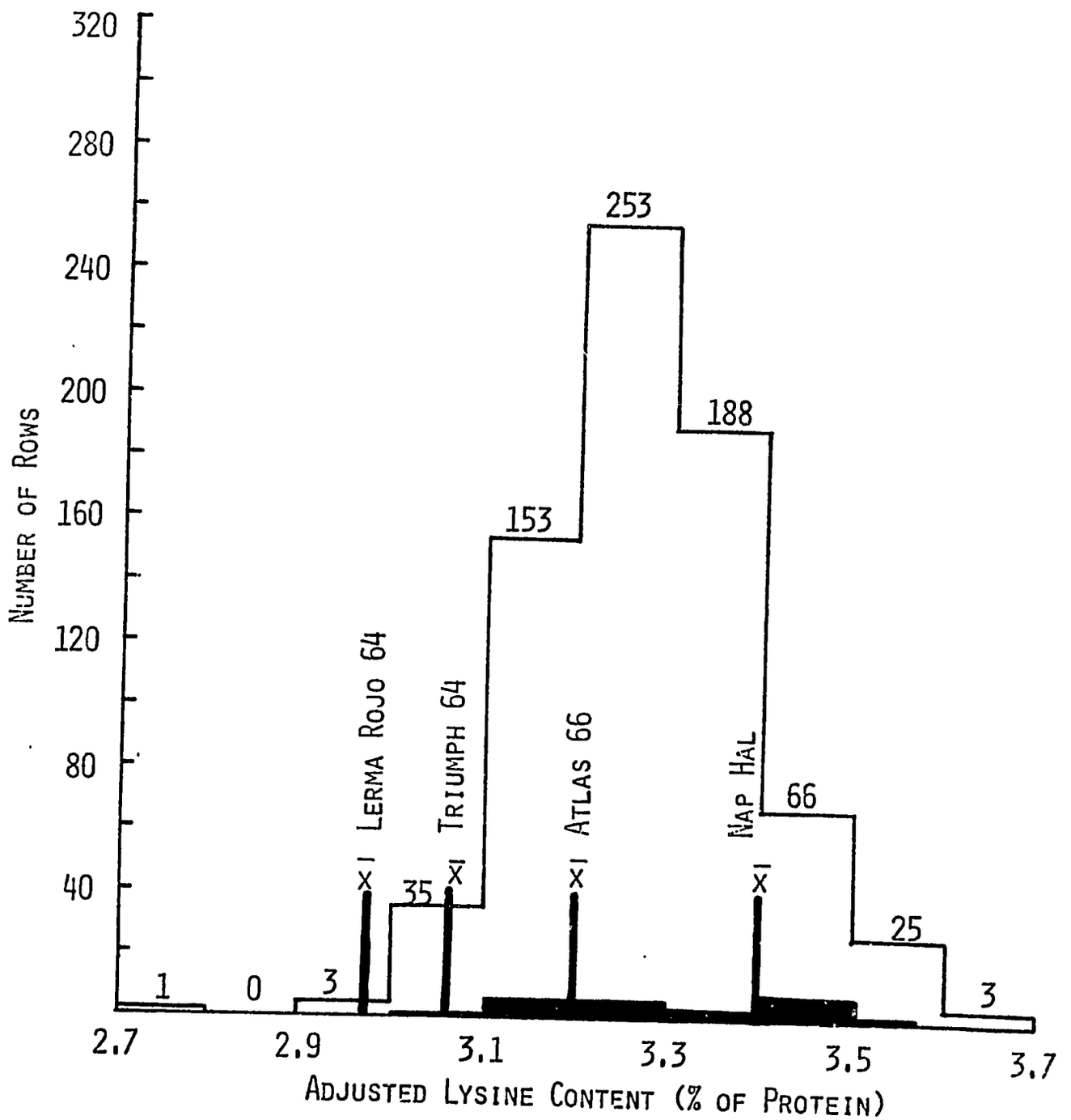


Figure 15.
Frequency distribution for adjusted lysine among F_2 progeny bulk rows (F_4 generation) from a Nap Hal x Atlas 66 cross grown at Yuma, Arizona in 1972.

S. L. Kuhr Thesis Research -

Nap Hal also was crossed with four U. S. and Mexican spring wheat varieties. Parents, F_1 and F_2 populations from these crosses were grown at Fort Collins, Colorado and Bozeman, Montana in 1971. The F_2-progeny rows were propagated at Lincoln in 1972 and will be grown again in 1973. The protein and lysine data will be analyzed by S. L. Kuhr as a M. S. thesis.

Significance of the Findings -

Several different sources of high protein in wheat have been identified.

Preliminary studies of populations derived from Nap Hal x Atlas 66 indicate that these two varieties possess genes for high protein that are different. New high levels of protein in wheat beyond that provided by Atlas 66 appear to be possible.

The Indian variety Nap Hal also possesses high lysine in combination with high protein. The high lysine trait is heritable based on our studies in 1971 and 1972.

Breeding for Improved Nutritional Value

The Agricultural Research Service, USDA and the Nebraska Agricultural Experiment Station have conducted cooperative research on high protein in wheat since 1954. When the AID-supported project on nutritional improvement of wheat was initiated in 1966, there already were available from the ARS-Nebraska research numerous experimental high protein lines derived from crosses of Atlas 66 with the hard winter varieties Comanche and Wichita. Since nothing was known of amino acid variation in the protein of wheat varieties, systematic screening of the World Wheat Collection was the initial area of project research.

It was recognized that varietal differences in lysine level identified in the screening effort were not necessarily genetic and, therefore, would have little if any value in a breeding program. The decision was made to utilize potentially high lysine varieties in crosses before confirmatory data on the nature of the differences was available. Several years would be required for such data and crosses were easily made. Many crosses were subsequently discarded when it was determined that lysine or protein superiority of the parent variety was due to non-genetic factors. The few crosses worthy of retention are being subjected to detailed analyses for protein and/or lysine.

Breeding Approach -

The breeding effort to improve nutritional value fell logically into several categories as follows:

1. Transfer known genes for high protein to commercially acceptable varieties in developing countries.
2. Combine different genetic sources of the high protein trait to achieve new levels of high protein.
3. Combine available high protein lines from Nebraska with World Collection varieties suspected of possessing above-normal lysine.
4. Combine suspect varieties for high lysine to achieve improved levels of high lysine.

A part of the value of the International Winter Wheat Performance Nursery established in 1968 was early identification of superior winter wheat varieties for use as recipient varieties for protein and lysine genes. Additionally, the nursery provides definitive information relative to areas of similarity for winter wheat production. Some winter wheat production areas in developing countries appear to be sufficiently like the central plains of the United States so that varieties developed for the central plains of the U. S. may have potential commercial value in these countries.

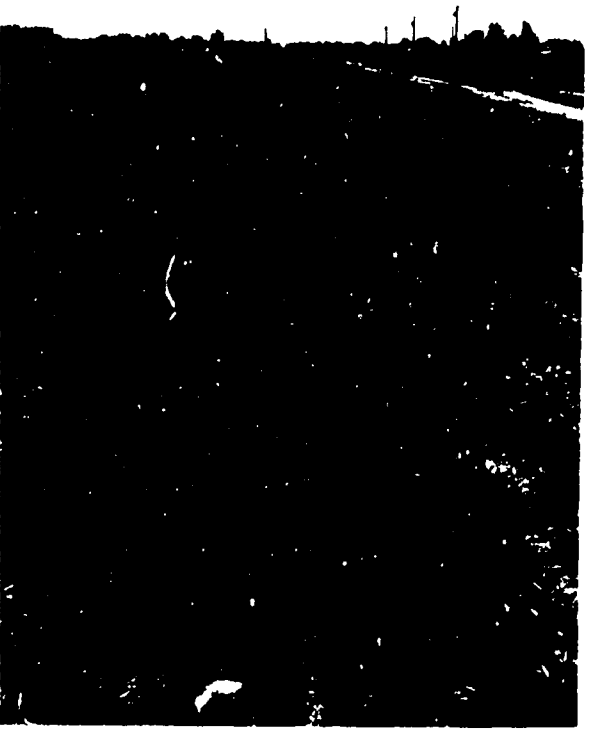
In the first year (1967) crosses were largely restricted to combinations of Nebraska high protein lines with available U. S. and foreign wheats. They can be grouped as follows:

High protein winter lines x agronomically superior U. S. Winter Varieties,
" " " " x available Korean Winter Varieties,
" " " " x Mexican Spring Varieties.

In the second year there was major emphasis on backcrosses of F_1s to recipient varieties with a limited number of new crosses made. Thereafter, emphasis shifted to combinations involving suspected high lysine with high protein and promising foreign varieties.

F_1s of all crosses were propagated in the greenhouse. Winter x winter combinations were advanced to the field at Lincoln in the F_2 generation for evaluation and selection. Winter x spring F_2 populations were propagated from fall plantings at Lincoln where only the winter segregates would survive and in some cases at Yuma, Arizona where both winter and spring segregates could be expected to produce seed. Spring x spring crosses were grown in the F_2 at Yuma, Arizona.

Hybridization of high protein and high lysine wheats is done in the greenhouse during the winter at Lincoln, Nebraska. Graduate students are shown making crosses in lower photo.



Winter wheat candidates for the IWWPN are increased at Yuma, Arizona. Photos show condition of increase planting in February (above) and the varieties Sturdy (USA) and Carifen 12 (Chile) at harvest time (lower left and lower right, respectively.

Results of Breeding for High Protein -

Data from the International Winter Wheat Performance Nursery have provided some evidence that winter wheat varieties adapted to Nebraska also will perform well in the dryland winter wheat production areas of Turkey, Iran, and Afghanistan. We have pursued development of high protein varieties adapted to Nebraska for evaluation and possible production in these countries as well.

High protein lines from our first cycle of breeding were insufficiently productive to be competitive in Nebraska. Performance data from 4 years of testing of five such lines are summarized in table 42. All were consistently higher than Scout in protein but were substantially less productive. It was readily apparent that further breeding was necessary to achieve acceptable levels of productivity. The performance of selections from a second breeding cycle is shown in table 43. Most appear to be comparable or superior to Scout in productivity and have grain protein ranging from 9 to 24% higher than Scout or Scout 66.

Release and Distribution of High Protein Lines -

Public release of 26 high protein lines from the first breeding cycle was made in 1970. These are listed in table 44. Detailed descriptions of these lines may be found in Crop Science 11:141-142. 1971. Seed in germplasm amounts is available from the Nebraska Agricultural Experiment Station. Seed of twelve of the most promising experimental high protein lines from our second cycle of breeding was sent to Turkey, Iran, and Afghanistan in 1971 for local agronomic evaluation. These are listed in table 45.

Table 42. Four-year average yields and protein content of advanced high protein selections from the first breeding cycle grown in Nebraska replicated yield trials, 1966 to 1969.

Variety or pedigree	C. I. or Sel. number	$\bar{x}$ grain yield		$\bar{x}$ protein content	
		bu/a	% of Scout	%	% of Scout
Scout	13546	43.6	--	11.3	--
Atlas 66 x Comanche	65307	38.8	89	12.9	114
"	65317	37.8	87	12.7	112
"	65318	37.1	85	13.3	118
"	65305	34.5	79	13.4	119
Atlas 66 x Wichita	65320	37.3	86	13.2	117

Table 43. Average grain yields and protein content of advanced high protein selections from the second breeding cycle grown in Nebraska replicated yield trials.

Variety or pedigree	C. I. or Sel. number	$\bar{x}$ grain yield bu/a	$\bar{x}$ grain yield % of Scout	$\bar{x}$ protein content %	$\bar{x}$ protein content % of Scout
		3-year Averages, 1967 to 1969			
Scout	13546	42.1	---	11.2	--
Atlas 66-Cmn x Warrior	66548	42.1	100	12.6	113
"	66547	40.5	96	13.0	116
"	66565	40.5	96	12.2	109
"	66550	40.4	96	12.7	113
"	66549	39.5	94	12.5	112
"	66561	37.9	90	12.8	114
		2-year Averages, 1968 to 1969			
Scout	13546	41.4	---	11.1	--
Atlas 66-Cmn x Warrior	66553	41.2	100	13.0	117
"	66551	40.9	99	13.6	123
		3-test averages, 1970			
Scout 66	13996	51.8	---	11.8	--
Atlas 66-Cmn x Lancer	701132	59.4	115	14.4	123
"	701134	55.2	107	14.3	122
"	701137	53.9	104	14.1	120
(Wrr-Atlas 66-Cmn) F_1 x Lancer	701154	53.4	103	14.5	124
Lancer x Atlas 66-Cmn	70654	50.7	98	14.4	123

Table 44. Nebraska high protein wheat germplasm released in 1970.

	Nebr. Sel. No.	C. I. No.
Atlas 66/Comanche	65304	14014
"	65305	14015
"	65307	14016
"	65309	14017
"	65312	13989
"	65313	13990
"	65314	13991
"	65669	14057
"	65670	14058
"	65673	14059
"	67730	14060
"	67786	14061
Atlas 66/Comanche//Warrior	66548	14062
"	66551	14063
"	66553	14064
Atlas 66/Wichita	65321	14065
"	65322	14066
"	67764	14067
"	67765	14068
"	67767	14069
"	67768	14070
"	67769	14071
"	67770	14072
"	67776	14073
"	67785	14074

Table 45. Nebraska high protein experimental lines being evaluated in Turkey, Iran, and Afghanistan in 1972.

(Wrr//Atl 66/Cmn)F_1/3/Cmn/Ott	NB68513
(Wrr//Atl 66/Cmn)F_1/Lancer	NB68510
Cmn/Ott/3/Atl 66/2/Cmn/4/Lancer	NB68570
SS/2/C.I.12500/4/RCh/Cnn/6/Wrr/5/Atl 66/3/Cmn/7/Lcr	NB69559
Atlas 66/Cmn//Lancer	NB701132
"	NB701134
"	NB701136
"	NB701137
"	NB701139
(Wrr//Atl 66/Cmn)F_1/3/Lancer	NB701152
"	NB701154
Lancer//Atl 66/Cmn	NB70654

Sources of High Protein and High Lysine -

The potentially most useful genetic sources of high protein and/or high lysine based upon available evidence from this project are identified in table 46. Several of the high protein sources listed may carry the same genes for protein. This is particularly so in the case of Frondoso and Atlas wheats derived from them. Our data indicate that Frondoso and Aniversario also probably possess a major common gene for protein. The value of the high lysine sources has not been fully established. In no case does their demonstrated lysine superiority over ordinary wheats exceed 0.5 percent. However, all have exhibited a reasonably consistent lysine advantage over other varieties in environmental nurseries and greenhouse tests conducted by the Nebraska group. The Indian variety Nap Hal, P.I.176217, holds particular interest because it has been consistently superior to other varieties in both protein and lysine content in greenhouse tests.

Breeding Crosses Involving Nap Hal -

Nap Hal was crossed with the Korean variety Jang Kwang, and with the Nebraska variety Lancer in 1968. F_3 populations from the crosses were propagated at Yuma, Arizona in 1971. Frequency distributions for protein are shown in figures 16 and 17. Lysine analyses were completed only recently and are not summarized here. Protein distributions for Nap Hal x Yang Kwang and Nap Hal x Lancer are similar. High protein appears to be partially dominant with the modal class of 18 percent protein. Protein distribution ranged from 16 to 21 percent. The dye-binding method of protein analysis does not accurately differentiate protein levels above 20 percent. This is indicated by the broken line portions of figures 16 and 17. All Nap Hal parental values for protein exceeded 20 percent.

Table 46. Useful or potentially useful genetic sources of genes for high protein and/or high lysine.

Variety	Sel., P.I. or C. I. no.	Source	Growth habit	Useful trait
Atlas 50	12534	USA	I	high protein
Atlas 66	12561	USA	I	" "
Atlas-derived lines	(see tables 25 and 26)	USA	W	" "
Frondoso	12078	Brazil	S	" "
Frondoso derivative	Purdue 28-2-1	USA	W	" "
Aniversario	12578	Argentina	S	" "
Aniv.-derived line	NB66565	USA	W	" "
Nebr. Male Fertility Restorer	NB542437	USA	W	" "
Nap Hal	P.I.176217	India	S	" "
Hume^{2} x Nb^{4}-Agrus-Tc^{7}	SD69103	USA	W	high lysine, high protein
April Bearded	7337	England	S	" " , high lysine
Hybrid English	6225	England	W	high protein, high lysine
Pearl	3285	Sweden	S	high lysine (probable)
Fultz x Hungarian	11849	USA	W	" " "
Fultz Sel.-Hungarian x Minturki-Fultz Sel.	12756	USA	W	" " "
Norin 10-Brevor, Sel. 14 x 27-15-Rio-Rex, Sel. 53	13447	USA	W	possible high lysine
Norin 10-Brevor, Sel. 14 x Brevor Sib, 50-3	13449	USA	W	" " "
22A	5484	USSR	S	high lysine (probable)

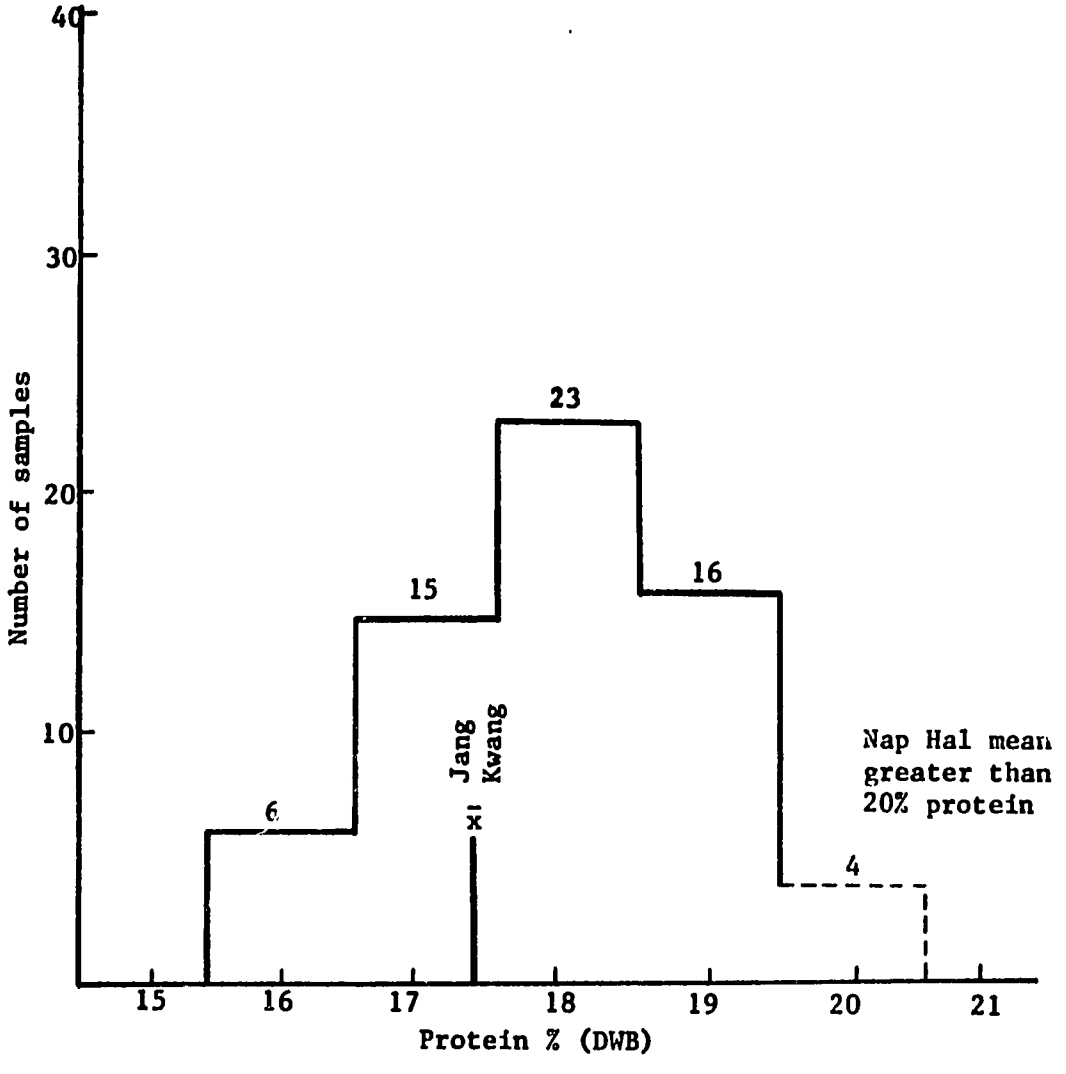


Figure 16. Frequency distribution, P.I.176217 x Jang Kwang, Udy protein (P.I.176217 = Nap Hal).

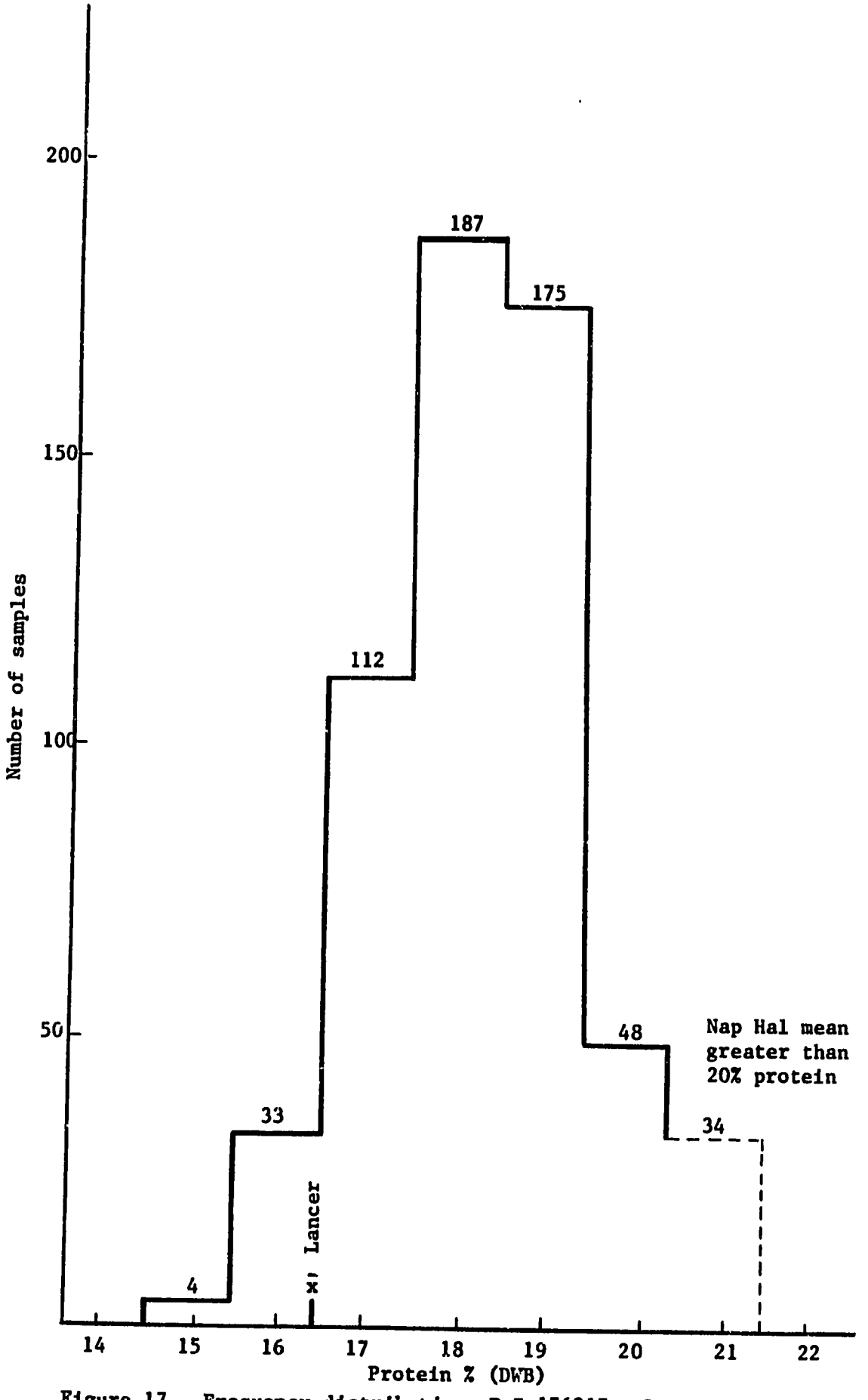


Figure 17. Frequency distribution, P.I.176217 x Lancer, Udy protein (P.I.176217 = Nap Hal).

Availability of high protein-high lysine populations -

Crosses made at Nebraska with potential usefulness for nutritional improvement will be analyzed for protein and lysine in the early generations. Selection pressure for agronomic traits will not be exerted. Unselected F_2 progeny bulk populations demonstrated to be high in protein and/or lysine will be made available to developing and other countries for agronomic evaluation and selection and use in crosses. Several populations derived from Nap Hal crossed with Atlas 66 and other wheats are available for distribution at this time.

Nutritional Evaluation of Experimental Lines -

Plans are being readied for biologic evaluation in 1973 of several hundred experimental lines exhibiting wide differences in levels of protein and lysine. These will be evaluated in our mouse laboratory to determine which are best nutritionally. Those with the highest values will be utilized in further breeding work and made available to developing countries for evaluation. Emphasis will be given to Nap Hal x Atlas 66 and other Nap Hal-derived lines since these possess the best combinations of high protein and high lysine available at this time.

Significance of Breeding Research -

Sufficient knowledge about protein and lysine differences in wheat has accumulated to make feasible a concerted breeding effort for wheat with improved nutritional value.

The high protein trait has been transferred from Atlas 66 to attractive potentially useful lines for dryland production areas. Some of these are being evaluated in Turkey, Iran, and Afghanistan.

Selections with very high protein combined with the Nap Hal level of lysine have been identified among the progeny from Nap Hal crossed with Atlas 66.

These and other populations selected for protein and lysine are available to other countries for local evaluation.

Mouse feeding tests are being employed at Nebraska to identify high protein-high lysine selections with the highest nutritional value.

Several useful genetic sources of high protein and high lysine in wheat have been identified by the AID-supported Nebraska research.

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July 1, 1966-December 31, 1972

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PARTICIPATION IN REGIONAL, NATIONAL, AND INTERNATIONAL CONFERENCES AND INVITATIONAL PAPERS PRESENTED

"Cereal Breeding for Better Protein Impact" -- presented by V. A. Johnson at Symposium on Protein for Food, Soc. Ec. Bot., Miami, Florida, 1967.

"Amino Acid Composition of Selected High Protein Wheats" -- presented by P. J. Mattern at 52nd Annual Meeting AACC, Los Angeles, 1967.

"Screening for High Lysine Content in Wheat" -- presented by P. J. Mattern at 52nd Annual Meeting AACC, Los Angeles, 1967.

"Effect of Protein Content in Winter Wheat on Levels of Essential Amino Acids" -- presented by P. J. Mattern at Fifth National Conference on Wheat Utilization, Fargo, North Dakota, Nov. 2, 1967.

"Nutritional Improvement of Wheat Breeding" -- presented by V. A. Johnson at Third International Wheat Genetics Symposium, Canberra, Australia, 1968.

"Breeding for High Protein Content and Quality in Wheat" -- presented by V. A. Johnson at Panel on New Approaches to Breeding for Plant Protein Improvement, IAEA/FAO, Rostanga, Sweden, 1968.

"Protein Quantity and Quality in Wheat" -- presented by V. A. Johnson at Symposium on Meeting Protein Needs through Plant Breeding, Crop Science Society of America, New Orleans, Louisiana, 1968.

"Current Efforts to Improve the Nutritional Value of Wheat" -- presented by V. A. Johnson at Wheat Quality Conference, Minneapolis, Minnesota, 1968.

"Nutritional Improvement of Wheat" -- presented by V. A. Johnson and P. J. Mattern to AID, U. S. Dept. of State, Washington, D. C., 1968.

"Report on Wheat Laboratory Activities" -- made by P. J. Mattern at Annual Meeting, Nebraska Assoc. of Wheat Growers, Sidney, Nebr., 1968.

"Feeding a Hungry World" -- presented by P. J. Mattern to a High School Conference, North Platte, Nebraska, 1968.

"Efforts to Improve the Nutritional Quality of Wheat" -- presented by P. J. Mattern at Experiment Station Workers Meeting, Peoria, Illinois, 1969.

"Hybrid Wheat and Protein Improvement in Wheat" -- presented by V. A. Johnson at Cornell University, Ithaca, New York, 1969.

"Breeding Wheat and Maize with Improved Protein Content" -- presented by V. A. Johnson at Symposium on Plant Breeding and Nutrition, Cambridge, England, 1969.

"Improving Lysine and Protein Content in Wheat" -- presented by P. J. Mattern at Annual Meeting, AACC, Chicago, Illinois, 1969.

"Role of Wheat Protein Fractions as a Measure of Breadmaking Quality" -- presented by P. J. Mattern at Tri-Section Meeting of AACC, Manhattan, Kansas, 1969.

"High Protein - Lysine Rich Wheats" -- presented by P. J. Mattern at Sixth National Wheat Utilization Research Conference, Oakland, California, 1969.

"Protein Improvement in Wheat" -- presented by V. A. Johnson at 3rd FAO/Rockefeller Foundation Seminar on Wheat, Ankara, Turkey, 1970.

"The International Winter Wheat Performance Nursery" -- presented by V. A. Johnson at 3rd FAO/Rockefeller Foundation Seminar on Wheat, Ankara, Turkey, 1970.

"Cooperative Wheat Research at the University of Nebraska" -- presented by V. A. Johnson at the University of Zagreb, Zagreb, Yugoslavia, 1970.

"International Cooperation in Winter Wheat Improvement" -- presented by V. A. Johnson at the University of Novi Sad, Novi Sad, Yugoslavia, 1970.

"Wheat Nutrition Research at the University of Nebraska" -- presented by V. A. Johnson at U. S. State Department Seminar, Washington, DC, 1970.

"Nutritional Improvement of Wheat by Breeding" -- presented by V. A. Johnson at a Breeding and Fortification Workshop, Annapolis, Maryland, 1970.

"Nutritional Improvement of Cereals" -- presented by V. A. Johnson at Symposium on Protein Synthesis and Deposition in Seeds, American Chemical Society, Los Angeles, California, March 29-April 2, 1971.

"Assaying High Protein Cultivars for Developing Countries" -- presented by P. J. Mattern at AID Workshop, Washington, DC, 1971.

Consultation by P. J. Mattern with FAO/IAEA on Laboratory Techniques for Screening for Improved Protein Quality in Wheat. Vienna, Austria, 1971.

Participation by P. J. Mattern in Annual Review of AID-supported Sorghum Improvement Program, Purdue University, 1971.

"Improvement of Nutritional Quality in Wheat through Increased Protein Content and Improved Amino Acid Balance" -- presented by P. J. Mattern at AID Spring Review of Research, Washington, DC, 1971.

"Screening Approaches for a Program to Improve Protein Nutrition in Wheat" -- presented by P. J. Mattern at Workshop on Development of an International Program for Sorghum and Millet, Washington, DC, 1971.

Attendance by P. J. Mattern at a Cereal Protein Workshop, CIMMYT, Mexico, D.F., 1971.

"Yield versus Quality" -- presented by V. A. Johnson at Interregional Wheat Workers Conference, Stillwater, Oklahoma, 1971.

"Improvement of Wheat Quality and Yield" -- presented by V. A. Johnson at Interdisciplinary Seminar Series, Texas Tech University, Lubbock, Texas, 1971.

"Genetic and Environmental Control of Food Quality" -- presented by V. A. Johnson at Annual Meeting, American Home Economics Association, Denver, Colorado, June 29, 1971.

"Wheat Protein Improvement" -- presented by V. A. Johnson at Symposium on Rice Breeding, International Rice Research Institute, Los Banos, Philippines, 1971.

"Nutritional Research on Wheat at the University of Nebraska" -- presented by V. A. Johnson at First International Winter Wheat Conference, Ankara, Turkey, June 5-9, 1972.

"The International Winter Wheat Performance Nursery: Establishment and Expectations" -- presented by V. A. Johnson at First International Winter Wheat Conference, Ankara, Turkey, June 5-9, 1972.

"Wheat Research Horizons" -- presented by V. A. Johnson at Krasnodar Research Institute of Agriculture, Krasnodar, USSR, 1972.

"Developments in High Protein Wheats" -- presented by P. J. Mattern at Joint Meeting, Assoc. of Op. Millers and Nebr. Section AACC, Kansas City, Missouri, 1972.

"Analytical Procedures and Equipment Requirements to Improve the Nutritional Quality of Wheat" -- presented by P. J. Mattern at 1st International Winter Wheat Conference, Ankara, Turkey, 1972.

"International Winter Wheat Performance Nursery Data Interpretation" -- presented by J. E. Stroike at 1st International Winter Wheat Conference, Ankara, Turkey, 1972.

"Status of Nutritional Improvement in Wheat" -- presented by P. J. Mattern at an International Symposium on Quality of Protein in Maize, CIMMYT, Mexico, D.F., 1972.

Workshop on Genetic Improvement of Protein in Cereals scheduled for 1973. V. A. Johnson invited to serve as chairman of Organizing Committee, Natl. Acad. Science, Washington, D.C.

Invitation accepted by V. A. Johnson to present paper on "Genetic Improvement of Plant Protein" at Symposium on Quality Improvement of Plant Proteins, American Chemical Society, Dallas, Texas, April 8-13, 1973.

Invitation received by V. A. Johnson to participate in an International Wheat Seminar, International Maize and Wheat Improvement Center, Mexico D.F., Mexico, January 22-26, 1973.

Invitation received by V. A. Johnson to present two papers, "International Winter Wheat Yield Nurseries" and "Protein Quality" at the Fourth FAO/Rockefeller Foundation Wheat Seminar, Teheran, Iran, May 21-June 2, 1973.

WHEAT SEED REQUESTS - CONTRACT AID/csd-1208
July 1, 1966 - December 31, 1972

A large number of requests have been received for seed of Nebraska high protein lines and other wheats with potential nutritional value which were identified by the AID-supported wheat research. The requests are identified by country in the listing that follows. They include only the requests received by the Nebraska Agricultural Experiment Station. Many additional seed requests for high protein and high lysine wheats identified by the Nebraska research were received by J. C. Craddock (ARS), Beltsville, Maryland who supervised the World Wheat Collection. We have no record of these so they could not be included in the listing.

Country	No. requests filled
United States	53
Australia	5
Canada	5
Czechoslovakia	2
England	4
Greece	1
Hungary	1
India	7
Italy	2
Korea	1
Lesotho	1
Mexico	2
Nepal	1
Netherlands	1
Pakistan	2
Romania	2
Saudi Arabia	1
Spain	1
Sweden	2
Switzerland	1
Turkey	2
USSR	1
West Germany	1
Yemen	1
	total = 100

REQUESTS FOR REPRINTS OF PROJECT PUBLICATIONS
July 1, 1966 - December 31, 1972

Foreign requests for our wheat protein publications total 327.

Requests from U. S. researchers total 129.