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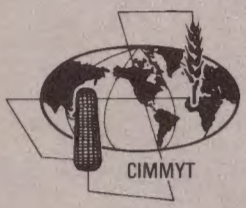

CENTRO INTERNACIONAL DE MEJORAMIENTO DE MAIZ Y TRIGO
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CONTENTS

Variability in the Lysine Content of Wheat, Rye, and Triticale Proteins[1]

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Undernutrition and malnutrition are currently widespread in many areas of the world. The most serious nutritional problem is protein-calorie malnutrition among children in the developing countries. (5, 24).

There now exists a world shortage in production of animal proteins, which are superior in nutritional quality to plant proteins. Animal production can be expanded neither easily nor rapidly to overcome the deficit. The magnitude of the imbalance between production and need for animal protein will become worse as world population increases. It is anticipated that more and more of the world's need for protein will have to be supplied by the plant proteins, as is already the case among low income groups in many densely populated areas of the world.

The total current world protein consumption is estimated to be 120 million metric tons (14). Of this total, only 30,000,000 metric tons or 25 percent is supplied from animal proteins. The remainder, 90,000,000 metric tons or 75 percent, is plant proteins.

Cereals, the principal source of plant protein, represent 60 percent of the world protein supply (14). Unfortunately, cereal proteins are deficient in several amino acids that are required by man for proper growth.

Nutritional qualities of a protein are determined by the amount and the kind of amino acids which become available to the animal organism during digestion. Cereal proteins are lower in nutritional value than animal proteins. Their lower nutritive value results from a poor balance of essential amino acids. The most limiting essential amino acid is lysine (2, 4, 8, 12).

In recent years an ever increasing number of food supplements have been developed (5, 6, 24), which can be used to improve the diets in many underdeveloped countries. Unfortunately, these improvements seldom reach the rural people, who generally constitute from 75 to 90 percent of the total population in such countries, and it is among this sector of the population where the worst nutritional problem exists. Doubtless, this situation could be improved markedly if cereals having improved amino acid balance could be made available.

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In 1964, the mutant gene of corn, opaque-2, was reported to be associated with an increase in the lysine content of corn protein (13). More recently a second mutant gene, floury-2, also has been shown to affect the amino acid pattern of corn protein (18).

These discoveries stimulated genetic biochemical research to find other cereal varieties or mutants with genes for high lysine content.

Improving the amino acid balance in the protein of small grains through genetic manipulation has been handicapped by inadequate and time consuming analytical methods for analyzing a large number of samples.

At present, all methods used for total lysine determination in proteins require previous hydrolysis.

The chemical analysis for lysine in protein hydrolysates using ion-exchange chromatography (15, 16, 17) has been found to be the most reliable and rapid procedure.

Automatic recording devices have opened the way to the routine analyses of protein hydrolysates. The main advantages of the method are: 1) reproducibility, 2) accuracy and 3) automation.

In the present study, lysine variability was investigated among species and varieties of wheat, rye, Triticale and wheat amphiploids. Concurrently with the survey of lysine variability in the above mentioned cereals, a triplicate sample method was developed for the determination of lysine by ion-exchange chromatography on an automated amino acid analyzer.

MATERIALS

D,L-histidine and D,L-lysine used were purchased from Nutritional Biochemical Corporation. An amino acid calibration mixture was obtained from Beckman Instrument, Inc. L-lysine monohydrochloride M. A. Grade which was used as a standard for the lysine analysis was obtained from Mann Research Laboratories, Inc.

Wheat

A. *Triticum aestivum*: Five varieties of hard red spring wheat were grown in North Dakota at Fargo and Minot in 1966, and 7 varieties at Chapingo, Mexico in 1965 from 2 different dates of plantings.

B. *Triticum durum*: Five varieties of durum wheat were grown at Edgeley, Fargo, and Minot, North Dakota in 1966, and 23 lines and varieties at Ciudad Obregón, Sonora, Mexico in 1965-1966.

C. *Triticum species*: Sixty-four samples of diploid, tetraploid and hexaploid species were supplied by the U.S. Department of Agriculture, World Collection of small grains. They were grown in 1964 at Aberdeen, Idaho. Another 26 samples of *Triticum species,* including 6 amphiploids, were grown at Fargo, North Dakota in 1964.

Rye

A collection of 125 varieties and species of *Secale* was supplied by the International Maize and Wheat Improvement Center in Mexico City,

Mexico. These samples were grown in a number of different countries under various ecological conditions.

Triticale

A group of 70 plant and spike selections from 25 varieties or lines, which were developed at the University of Manitoba, were grown at Ciudad Obregón, Sonora, Mexico in 1965-66.

METHODS

Preparation of Samples

Samples that were supplied in spikes were analyzed using 4 kernels from the bottom, 4 kernels from the center, and 4 kernels from the top of the spike, respectively, for each analysis. These data are indicated in the results by the code B, C, and T (Bottom, Center and Top). In some cases only the bottom and center of the spike were analyzed. When the seed was supplied in bulk from 4 to 10 kernels (depending on the amount available) were ground and the necessary analyses carried out.

All samples were stored in a room with controlled temperature of 60°F, and relative humidity of about 50 percent. Sample moisture was maintained between 6 to 9%. Moisture was determined in groups of samples at random, in an air-oven at 130°C for one hour.

All cereal grains were finely ground in a Micro-Wiley mill and passed through a 60 mesh sieve.

Protein

Nitrogen was determined by the Micro-Kjeldahl procedure (1). Protein was calculated from percent nitrogen by the factor 5.7 in the case of wheat and Triticale, and by 6.25 in the case of rye.

Hydrolysis

Thirty mg of ground sample were dispersed in 3 ml of 6N HCl in 25 ml pyrex hydrolysis tubes. The tubes were placed in a dry-ice-alcohol bath and sealed under vacuum. Hydrolysis was carried out for 22 hours in an oven held at 110° ± 2°C. Upon completion of hydrolysis, the tube contents were filtered through a 2 ml fritted-disc funnel, and the humin was washed twice with 1 ml of de-ionized distilled water. The filtrates were collected in 25 ml filtering flasks, which were placed in a vacuum desiccator with a container of sodium hydroxide pellets. The desiccator was connected to a vacuum pump through a sodium hydroxide trap and evacuated overnight to dry the hydrolysates. Thereafter, the dried hydrolysates were dissolved in 4 ml of 0.2N, pH 2.2 sodium citrate buffer (26). All hydrolysates were stored at −20°C until analyzed.

Lysine Analysis

Ion-exchange chromatography

1. The Spackman, Stein and Moore (15, 27) procedure as modified by Benson and Patterson (3) was used. The 15 × 0.9 cm column of the

Beckman Model 120 B amino acid analyzer was packed to a height of 6 cm with Beckman spherical resin type AA-27 in early work. A single sample was analyzed for basic amino acids.

2. A method for triplicate lysine analysis in cereal protein hydrolysates was developed in this study. Stefanye and Spero (28) first used a replicate-sample method for analysis of simple mixture of acidic and neutral amino acids in 1964.

The Benson and Patterson accelerated method for basic amino acids was modified in this study by changing the type of resin for one of different particle size, length of the resin bed, pH and ionic strength of the citrate buffer, and column temperature.

Triplicate lysine analyses of cereal hydrolysates were done using this modified procedure. This method gave similar results as compared with those obtained by the Benson and Patterson method for a single sample. Therefore, because of its advantages the triplicate method was used during this study. The working conditions of both methods are listed in Table 1.

TABLE 1. Conditions for Lysine Analysis.

	Methods of Analysis	
	Accelerated	Triplicate
Buffer, pH	5.28 ± 0.02	5.10 ± 0.02
Buffer, Sodium concentration	0.35 N	0.20 N
Column temperature	55°C	40°C
Resin	AA-27	PA-35
Resin height	6 cm	9 cm

The sodium citrate buffer 0.2N, pH 5.10 was prepared in the same way as described by Benson and Patterson (3), but the amounts of sodium citrate and hydrochloric acid were reduced in order to get the desired sodium concentration and pH as shown in Table 2.

TABLE 2. Composition of Sodium Citrate Buffers.

	Buffer used in	
	Accelerated Analysis	Triplicate Analysis
pH	5.28 ± 0.02	5.10 ± 0.02
Sodium concentration	0.35 N	0.20 N
Sodium citrate $2H_2O$	137.26 g	78.43 g
Concentrated HCl	26.0 ml	23.50 ml
Brij - 35 solution (50 g/100 ml)	8.0 ml	8.0 ml
Caprilic acid	0.4 ml	0.4 ml
Final volume	4.0 liters	4.0 liters

In the triplicate-sample method, one sample aliquot of 1 ml is applied to the column. The sample is pushed into the resin with nitrogen pressure of 30 p.s.i. The column walls are washed thrice with 0.2 ml portions of buffer (0.2N, pH 5.10), which also are pushed into the resin with nitrogen pressure. Addition of sample and wash with buffer require approximately 8.5 min. Thereafter, the buffer is pumped through the column for 3.6 min. at 130 p.s.i. with a flow rate of 68 ml per hr. The pump is then stopped; the column is opened and another sample is applied. After the third sample is added and the column walls washed, the ninhydrin pump is started. Buffer and ninhydrin are introduced into the coil and the recorder is turned on.

The lysine in each sample appears on the chromatogram as a separate peak without overlapping. The histidine which has been slowed in the elution comes under the ammonia peak. The first lysine peak appears on the chromatogram in approximately 44 min.; the third lysine peak is completed on the chromatogram at 57 min. Then the analysis is stopped. Regeneration of the resin is made by pushing sodium hydroxide 0.2N through the column for a period of 7 min. with nitrogen pressure of 30 p.s.i. followed by equilibration with the 0.2N, pH 5.10 buffer for 25 min.

The lysine content of the hydrolysates was calculated from the peak area using the height-width method (26) and standard L-lysine monohydrochloride solution (25 μM per ml.).

RESULTS AND DISCUSSION

Development of the Triplicate-Sample Method for Lysine Analysis in Cereal Hydrolysates by Ion-Exchange Chromatography

In the Benson and Patterson accelerated method for basic amino acids (3) the histidine peak emerges on the chromatogram immediately after the lysine peak ends. Under the conditions of the modified procedure, the histidine elution from the resin is delayed, and the corresponding peak on the chromatogram does not emerge immediately after lysine. The histidine peak appears to come under the ammonia peak. This procedure leaves enough room on the chromatogram for the additional lysine peaks when triplicate samples are applied to the column.

The 0.35N, pH 5.28 sodium citrate buffer used by Benson and Patterson and column temperature of 55°C cause the histidine to be eluted centered between lysine and ammonia. The reduction of the pH to 5.10, the sodium concentration of the buffer to 0.2N, and the temperature from 55° to 40°C cause the histidine to change its position in the chromatogram. The use of the resin PA-35 gave better resolution and sharper peaks than the resin AA-27, and provided an advantage in the triplicate method.

When working under the modified conditions described above, and with a resin bed of 6 cm, good resolution of the 3 lysine peaks was obtained on analyzing either a simple mixture of lysine and histidine or a Beckman amino acid calibration mixture. The latter chromatogram is shown in Figure 1. However, when triplicate samples of whole wheat hydrolysate were analyzed, complete resolution of the 3 lysine peaks was not achieved. The

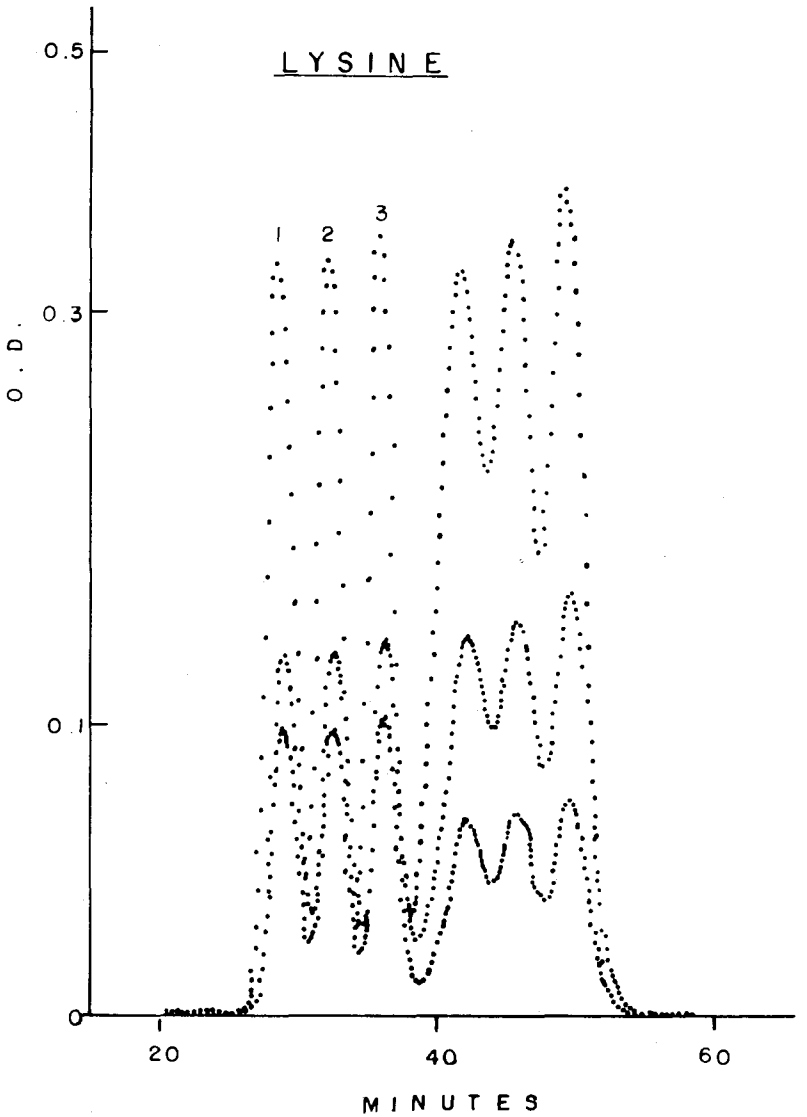

FIGURE 1. Chromatogram obtained from three 1 ml. aliquots of the amino acid calibration mixture with lysine peak 1 from aliquot 1, peak 2 from aliquot 2, and peak 3 from aliquot 3.

3rd peak became largely overlapped by the histidine and ammonia peak. This overlapping is thought to be due to the large amount of ammonia present. The high concentration of ammonia in wheat hydrolysates arises primarily from the cleavage during acid hydrolysis of amide groups of glutamine and asparagine. However, substantial amounts of ammonia may result from breakdown of certain amino acids, notably tryptophan, serine, and threonine.

By changing the resin bed length to 9 cm, good resolution of the 3 lysine peaks was obtained with wheat hydrolysates, and the reproducibility of the results was good. For example, 3 aliquots of 1 ml of a wheat hydrolysate analyzed by the triplicate method gave: 1) 0.173, 2) 0.176, and 3) 0.173 μM. of lysine per ml.

The accuracy of the triplicate-sample method was checked by analyzing different amounts of a whole wheat hydrolysate which were all contained in a volume of 1 ml by appropriately diluting with pH 2.2, 0.2N sodium citrate buffer. The results are shown in Table 3, and the chromatogram of this analysis is shown in Figure 2. The amount of lysine that should have been in each replicate was calculated from the lysine value obtained for 1 ml of hydrolysate by the Benson and Patterson accelerated method. The amount of lysine found in each sample in the triplicate-sample analysis was from 97 to 100% of the calculated value.

TABLE 3. Analysis of Different Amounts of a Wheat Hydrolysate by Triplicate-Sample Method.

Amount of Hydrolysate ml	Number of the Replicate	Lysine Calculated μM.	Lysine Measured μM.
0.500	1st	0.137	0.133
0.667	2nd	0.182	0.183
1.000	3rd	0.274	0.274

Good accuracy by the triplicate method was obtained also on whole wheat hydrolysate to which different amounts of pure lysine (0.5 μM. per ml of solution) were added. The results are shown in Table 4. The total lysine contained in each sample was that calculated from an analysis of 1 ml of the hydrolysate by the accelerated method plus the lysine added. The amount of lysine found in each sample was from 96 to 99% of the expected value.

Reproducible results were also obtained in the triplicate-sample method when the buffer flow rate was increased from 68 ml to 80 ml per hr. (pump pressure 155 p.s.i.). Accuracy as well as resolution of the lysine peaks was maintained, and the analysis time was reduced approximately from 90 min. to 78 min. Analyses of 3 samples using the accelerated method of Benson and Patterson would take approximately 3 hr. The triplicate-sample method with a buffer flow rate of 68 ml per hr requires approximately 90 min. and at buffer flow rate of 80 ml per hr only 78 min. Table 5 shows results obtained of one hydrolysate using the two buffer flow rates.

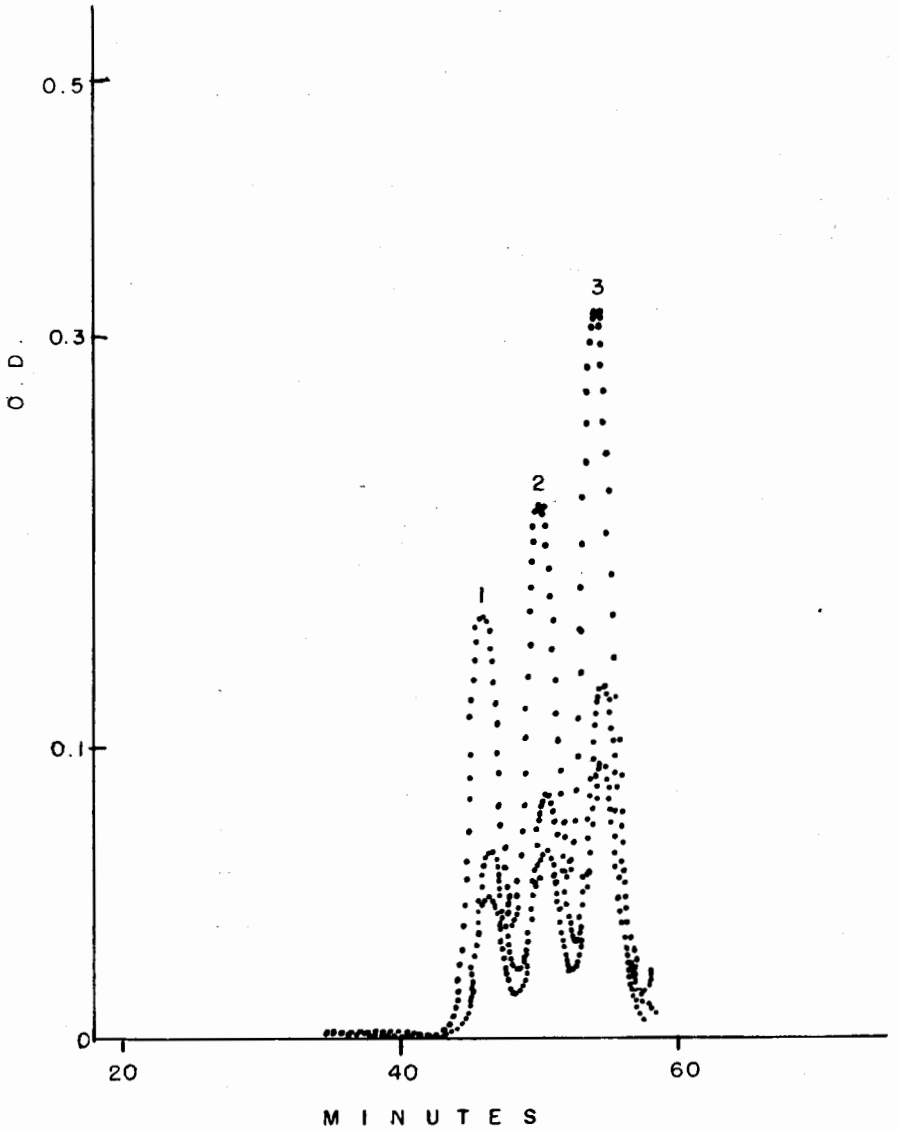

FIGURE 2. Chromatogram obtained from 3 different amounts of a wheat hydrolysate.

TABLE 4. Analysis of Mixtures of Wheat Hydrolysate and Added Lysine by Triplicate-Sample Method.

Amount Lysine Added μM.	Lysine in Hydrolysate μM.	Total Amount Lysine μM.	Lysine Measured μM.
0.250	0.098	0.348	0.338
0.100	0.098	0.198	0.197
0.150	0.098	0.248	0.238

The method used throughout this study was at a buffer flow rate of 68 ml per hr.

During the course of this study, single analysis of each sample was made for lysine content and duplicate analysis for protein.

TABLE 5. Analysis of a Lysine Standard Solution and of a Wheat Hydrolysate by Triplicate-Sample Method at Different Buffer Flow Rates.

Sample	Buffer Flow Rate	
	68 ml/hr	80 ml/hr
	Lysine μM./ml	Lysine μM./ml
Lysine, Standard Solution	0.250	0.250
Hydrolysate Aliquot # 1	0.228	0.222
Hydrolysate Aliquot # 2	0.232	0.233

Variability of Lysine Content in Cereal Grains

1. Triticum durum

The lysine content of durum wheat varieties and lines grown at North Dakota and Mexico were investigated.

The results of analyses for protein and lysine content of 5 varieties of durum wheat grown at 3 locations in North Dakota in 1966 are given in Table 6. The protein content was relatively high in all the varieties at the 3 locations, but the samples from Fargo had a protein content somewhat lower than those from the other locations.

It is customary in the literature to present the results as g. amino acid per 16 g. of nitrogen or in some cases as g. amino acid per 17.5 g. of nitrogen (20) which is equivalent to 100 g. of wheat protein. Expressing amino acids according to Orr and Watt (19) as amount per 1 g. of nitrogen affords the greatest ease of computation, but as yet this method has not been widely accepted (21).

In this study the lysine has been reported as percent of the protein, because the amino acid balance of the protein is most important nutritionally.

The values for the lysine also are given as the percent of the dry sample weight, in order to show clearly that samples with high content of lysine but with low protein content would contribute less lysine than samples with high protein and low lysine in the protein.

TABLE 6. Protein and Lysine Content in Whole Grain of Durum Wheat Grown at 3 Locations in North Dakota in 1966.

Variety	Edgely			Fargo			Minot		
	Protein [a] %	Lysine % [b] in Protein	Lysine % [b] in Sample	Protein [a] %	Lysine % [b] in Protein	Lysine % [b] in Sample	Protein [a] %	Lysine % [b] in Protein	Lysine % [b] in Sample
Mindum	19.18	2.69	0.517	16.06	2.48	0.399	21.43	2.26	0.483
Wells	22.36	2.26	0.505	18.62	2.50	0.466	20.18	2.37	0.478
Lakota	21.99	2.29	0.504	18.87	2.46	0.464	18.75	2.66	0.499
Stewart 63	22.98	2.37	0.544	18.13	2.60	0.472	19.50	2.45	0.478
Leeds	23.16	2.02	0.467	18.57	2.66	0.494	21.68	2.23	0.483

[a] Dry weight basis (N × 5.7).
[b] Lysine expressed as % of protein and of sample on dry weight basis.

The results in Table 6 indicate that the lysine content of the protein varies little among varieties and in the same variety grown in the different locations. The lysine in percent of dry sample weight is higher in the varieties grown in Edgely, because the protein content in general was high.

It has been reported previously (9, 22, 25) that in bread wheat an inverse relationship exists between the protein content of a sample and the lysine content of the protein up to about 14% of protein. Here, statistical analysis showed this inverse relationship with durum wheat even though protein contents were considerably higher than 14%. The correlation coefficient (r) obtained was −0.77 and highly significant.

A group of lines and selected plants in a line, as well as one variety of *T. turgidum* and one variety of *T. dicoccum* (commonly used as progenitors) that were grown in the same location in 1965-1966, were analyzed to evaluate the variability in lysine content due to varietal difference. Data are shown in Table 7.

The kernels from the lateral florets of the spikelets at the bottom and center of the spike were analyzed separately to determine the degree of variability in lysine content among kernels of a single spike. The results showed little variation in lysine content among kernels from the bottom and center of a spike. The small variability observed in some spikes was due to difference in the protein content of the kernels. The lysine percent of dry sample weight showed that the lysine content was maintained almost constant among the kernels at different sites of the spike.

However, the lysine content among lines, and in some cases, even among selections of a given line was variable. Line RD-176 selection 2B had a relatively high amount of lysine and a high content of protein. The percent of lysine in protein of selection 7A was only slightly higher than that of selection 2B, but the protein content was much lower. The same

pattern was observed in other selections of lines. The correlation coefficient for the relationship between lysine content and protein level was —0.33 and not significant.

The lysine content of the progenitor varieties Khapli (*T. dicoccum*) and Barrigon Yaqui (*T. turgidum*) was at the same level as that showed by the durum wheats.

TABLE 7. Protein and Lysine Content in Whole Grain of Durum Wheat Grown at Ciudad Obregon, Sonora, Mexico in 1965-66.

Variety or Line	Generation Seed	Position of Kernels [a]	Protein [b] %	Lysine % [c] in Protein	Lysine % [c] in Sample
RD 173 - 1A	F_4	B	17.52	2.93	0.514
		C	16.91	3.10	0.523
RD 176 - 2A	F_4	B	17.27	2.92	0.504
		C	16.49	3.04	0.502
RD 176 - 2B	F_4	B	19.16	3.14	0.602
		C	19.29	3.14	0.606
RD 176 - 7A	F_4	B	10.91	3.27	0.357
		C	11.16	3.53	0.393
RD 178 - 4A	F_4	B	15.28	2.84	0.435
		C	15.65	2.63	0.412
RD 181 - 3A	F_4	B	17.16	2.65	0.455
		C	17.16	2.65	0.455
RD 181 - 6A	F_4	B	14.24	2.73	0.388
		C	14.55	2.81	0.409
RD 87 - 2A	F_4	B	19.09	2.80	0.534
		C	20.55	2.60	0.535
RD 87 - 2B	F_4	B	17.22	2.68	0.461
		C	15.46	2.88	0.445
RD 101 - 2A	F_3	B	19.65	2.58	0.506
		C	20.19	2.44	0.493
RD 119 - 4A	F_3	B	17.76	2.74	0.487
		C	17.76	2.54	0.452
RD 138 - 3A	F_3	B	14.62	2.81	0.411
		C	13.95	2.85	0.398

TABLE 7. (Cont.)

Variety or Line	Generation Seed	Position of Kernels [a]	Protein [b] %	Lysine % [c] in Protein	Lysine % [c] in Sample
RD 138 - 3B	F_3	B	16.80	2.75	0.461
		C	16.31	2.66	0.438
RD 148 - 1A	F_3	B	18.01	2.64	0.475
		C	18.19	2.53	0.461
RD 182 - 11A	F_4	B	19.09	2.47	0.473
		C	18.37	2.65	0.486
RD 182 - 11B	F_4	B	15.70	2.44	0.383
		C	15.65	2.32	0.363
RD 3 - 2G	F_6	B	13.46	2.66	0.357
		C	13.03	2.49	0.324
(Pi-Th × Tc^2) (Z-B) Wells 21584-A	F_5	B	16.73	2.77	0.463
		C	13.64	3.18	0.433
(Pi-Th × Tc^2) (Z-B) Wells 21584-B	F_5	B	15.04	2.75	0.414
		C	13.70	2.72	0.373
[(Yt N 10-B) BY^2)] Tc^2 18177 - 6Y	F_7	B	14.80	2.71	0.402
		C	14.80	2.75	0.407
[Yt N 10-B) BY^2)] Tc^2 18177 - 13Y	F_7	B	15.52	2.78	0.432
		C	16.55	2.64	0.437
(WtE × TR) Tc^2	F_6	B	16.91	2.98	0.504
		C	15.09	2.78	0.469
(Z × B) Wells		B	18.80	2.77	0.522
		C	19.65	2.93	0.576
Barrigon Yaqui (*T. turgidum*)		B	15.16	2.86	0.433
		C	15.09	3.08	0.464
Khapli (*T. dicoccum*)		B	15.70	2.98	0.467
		C	16.91	2.67	0.461

[a] Kernels from lateral florets of the spikelets at different sites of the spike, B = Bottom, C = Center.
[b] Dry weight basis (N × 5.7).
[c] Lysine expressed as % of protein and of sample on dry weight basis.

2. Triticum aestivum

Two groups of varieties of spring wheat, one grown in Mexico in 1965, and the other in North Dakota in 1966, were analyzed for lysine content in order to evaluate varietal differences.

The results of analysis of 5 varieties of hard red spring wheat grown at two locations in North Dakota are given in Table 8. The lysine contents of these varieties were quite similar. Even though the protein content of the varieties was different in the two locations the lysine level in the protein remained almost constant. The variety Selkirk showed the lowest protein and lysine content. The relation of the lysine content of the protein to the protein level gave a correlation coefficient of −0.4 and not significant. This low correlation may have been due to the high protein level of all the samples.

TABLE 8. Protein and Lysine Content in Whole Grain of Hard Red Spring Wheat Grown at 2 Locations in North Dakota in 1966.

Variety	Fargo			Minot		
	Protein [a] %	Lysine % [b]		Protein [a] %	Lysine % [b]	
		in Protein	in Sample		in Protein	in Sample
Selkirk	17.55	2.62	0.460	19.26	2.49	0.481
Justin	18.37	2.81	0.516	21.93	2.62	0.574
Chris	18.81	2.70	0.507	19.37	2.67	0.517
Manitou	18.19	2.86	0.522	19.49	2.58	0.503
Crim	18.43	2.74	0.506	19.33	2.59	0.500

[a] Dry weight basis (N × 5.7).
[b] Lysine expressed as % of protein and of sample on dry weight basis.

The influence of date of planting and chronology of spike development on the lysine of several varieties of bread wheat grown at Chapingo, Mexico, is shown in Table 9. Kernels from different sites of the primary, secondary, and tertiary spikes were analyzed. In general, the lysine content was more variable among spikes in the first date of planting than in the second. The protein content of the grain was highly variable for the first planting date, and may have caused this variability of the lysine content. This variability may be influence by nitrate and moisture availability, or the temperature during the time of grain formation. The variation in lysine was greater among the primary, secondary and tertiary spike, than among the kernels from different sites of a given spike. The variety Lerma Rojo showed higher variation in the lysine content among kernels from different spikes than the other varieties. The varietal differences in the lysine content were low, not over 0.5%, and can not be considered significant.

TABLE 9. Influence of Date of Planting and Chronology of Spike Development on the Lysine Content of Several Varieties of Bread Wheat.[a]

Variety	Spike Chronology	Kernel Position[c]	First date[b]			Second date[b]		
			Protein[d] %	Lysine % in Protein[e]	Lysine % in Sample[e]	Protein[d] %	Lysine % in Protein[e]	Lysine % in Sample[e]
Chris	1st	B	18.37	2.59	0.476	15.82	2.49	0.394
		C	17.15	3.00	0.515	17.34	2.45	0.425
		T	18.19	2.80	0.510	16.91	2.50	0.423
	2nd	B	17.27	2.87	0.496	17.64	2.43	0.429
		C	16.86	3.19	0.538	17.70	2.48	0.438
		T	17.40	2.67	0.464	17.58	2.41	0.425
	3rd	B	15.52	2.62	0.406	16.55	2.45	0.406
		C	16.06	2.62	0.422	16.43	2.47	0.406
		T	15.70	2.73	0.428	15.83	2.58	0.408
Crim	1st	B	11.63	3.29	0.383	14.24	2.53	0.360
		C	12.48	2.68	0.334	14.30	2.39	0.341
		T				13.88	2.36	0.327
	2nd	B	12.48	2.82	0.353	14.43	2.61	0.377
		C	12.67	2.96	0.375	14.67	2.50	0.365
		T	11.03	3.10	0.341	13.52	2.60	0.361
	3rd	B	13.34	2.61	0.348	13.09	2.82	0.368
		C	12.73	3.21	0.409	12.85	2.91	0.374
		T	12.48	3.19	0.386			
Selkirk	1st	B	16.55	2.56	0.424	17.58	2.40	0.422
		C	17.58	2.62	0.460	17.22	2.69	0.463
		T	16.67	2.57	0.429			
	2nd	B	16.61	2.64	0.438	13.76	2.95	0.406
		C	16.31	2.50	0.408	13.88	3.08	0.427
		T	15.76	2.56	0.403	13.03	3.01	0.392
	3rd	B				12.55	3.02	0.379
		C				13.76	2.89	0.398
		T				13.15	2.85	0.374
Justin	1st	B	18.91	2.43	0.475	19.76	2.22	0.439
		C	22.30	2.12	0.472	20.25	2.21	0.447
	2nd	B	19.70	2.13	0.420	20.01	2.45	0.491
		C	20.85	2.29	0.478	19.21	2.22	0.427
	3rd	B	18.55	2.99	0.555	17.46	2.60	0.454
		C	15.94	3.10	0.495	17.76	2.46	0.436

Variety	Spike Chronology	Kernel Position [c]	First date [b] Protein [d] %	First date [b] Lysine % [e] in Protein	First date [b] Lysine % [e] in Sample	Second date [b] Protein [d] %	Second date [b] Lysine % [e] in Protein	Second date [b] Lysine % [e] in Sample
Lerma Rojo	1st	B	15.34	2.60	0.398	14.85	2.67	0.396
		C	14.30	2.77	0.396	16.85	2.60	0.438
		T	13.34	3.00	0.401	13.76	3.02	0.417
	2nd	B	15.63	2.82	0.442	18.91	2.45	0.463
		C	15.70	2.79	0.438	18.79	2.74	0.516
		T	13.34	3.10	0.413	18.01	2.78	0.501
	3rd	B	13.94	3.28	0.458	14.06	3.13	0.441
		C	13.82	3.31	0.457	15.52	3.19	0.495
		T	11.70	3.60	0.423	14.67	3.55	0.521
Nariño 59	1st	B	17.88	2.41	0.430	15.95	2.66	0.424
		C	16.06	2.71	0.435	15.65	2.98	0.465
		T	16.19	2.69	0.436	16.19	2.71	0.439
	2nd	B	16.91	2.49	0.422	16.86	2.73	0.461
		C	15.83	2.55	0.404	17.28	2.86	0.494
		T	15.46	2.53	0.392	18.01	2.63	0.474
	3rd	B	13.52	2.94	0.398	16.31	2.86	0.467
		C	13.58	2.80	0.380	16.91	2.49	0.422
		T	13.46	2.95	0.397	17.04	2.70	0.461
8156	1st	B	15.65	2.98	0.467	14.24	2.89	0.411
		C	14.62	2.57	0.375	13.64	3.00	0.410
		T	13.70	2.55	0.350			
	2nd	B	15.58	2.71	0.422	13.61	2.15	0.388
		C	16.24	2.76	0.449	14.06	2.73	0.384
		T	14.37	2.66	0.382	16.73	2.15	0.360

[a] Grown at Chapingo, Mexico in 1965.
[b] First date of planting, June 15, Second date June 31.
[c] Kernels from lateral florets of the spikelets at different sites of the spike: B = Bottom, C = Center, and T = Top.
[d] Dry weight basis (N × 5.7).
[e] Lysine expressed as % of protein and of sample on dry weight basis.

It has been reported that soft endosperm portions of the wheat kernel have less total nitrogen but a greater amount of the basic amino acids than the hard endosperm portion (11). Soft endosperm wheats can be expected to contain higher percentages of the water soluble proteins than the hard wheats, with concomitant higher lysine levels in the total protein. However, the protein of the soft endosperm varieties Lerma Rojo, Nariño, and 8156 showed lysine contents similar to those obtained from the hard endosperm wheats. The soft endosperm wheats had an unusually high protein level due to nitrogen fertilization. A highly significant inverse relationship between lysine content of the protein and protein level was observed ($r = -0.68$).

3. Triticum species

Small groups of different species of *Triticum* were investigated to determine if any among them might contain protein with high lysine content.

Table 10 shows the protein and lysine contents of different samples of diploid, tetraploid and hexaploid species of *Triticum*. Variability in the lysine content of these samples is evident. The protein content ranged from 9.96 to 27.00% (dry weight basis) and the lysine of the protein from 2.09 to 3.99%. The negative correlation between the protein content and the lysine level in the protein was highly significant ($r = -0.41$).

Lawrence *et al.* (9) reported that *T. pyramidale, T. sphaerococcum,* and *T. persicum* were the species that have high lysine content among the species analyzed. The lysine values of these species obtained in the present work are similar to those reported by them, except for the *T. sphaerococcum* where lower values were obtained here. No analysis of *T. boeticum* was reported by these workers.

In the present study the species, *T. boeticum,* was an outstanding group. The high values for lysine in percent of dry sample were due to both high lysine in the protein and high protein content.

A group of samples of different species of *Triticum,* grown at Fargo, North Dakota in 1964 was analyzed also and the results are given in Table 11. Only slight variations were observed in the lysine content of these samples, even though large variations were observed in the protein content. The protein content ranged from 12.04 to 28.16% (dry weight basis), and averaged 18.46%. The lysine content of the protein varied from 2.30 to 3.05% and averaged 2.60%.

TABLE 10. Protein and Lysine Content in Whole Grain of Diploid, Tetraploid, and Hexaploid Species of *Triticum,* Grown at Aberdeen, Idaho in 1964.

Species	Code [a] No.	Source	Protein [b] %	Lysine % [c]	
				in Protein	in Sample
Diploid					
T. boeoticum					
	227669		24.08	3.10	0.747
	230133		21.79	3.50	0.763
Rufinnigrum	272519	Hungary	24.46	3.05	0.747
Symbolonense	272520	„	19.40	3.07	0.596
Symbolonense	277121	Germany	21.52	3.12	0.672
T. monococcum					
Einkorn	10474	Germany	**16.84**	2.87	0.483
	94740	Spain	**17.75**	2.59	0.460
	94743	USSR	**15.46**	2.96	0.457
	119422	Turkey	**18.99**	2.62	0.498
	119423	„	**16.73**	2.96	0.496

TABLE 10. (Cont.)

Species	Code [a] No.	Source	Protein [b] %	Lysine % [c] in Protein	Lysine % [c] in Sample
Tetraploid					
T. dicoccoides					
	190919	Spain	12.75	3.25	0.415
	233288	Israel	27.00	2.80	0.757
	266841	England	20.58	2.62	0.540
Tricoccum	272535	Hungary	17.06	2.78	0.475
T. timopheeve					
Wis. D-303-1-A	94760	USSR	12.99	2.91	0.378
	94761		12.90	3.04	0.392
	190974	Spain	13.23	2.89	0.382
	221421	Yugoslavia	15.42	2.86	0.441
	290518	Hungary	20.65	2.88	0.594
T. pyramidale					
Pseudo Capticum	113395	Egypt	11.80	3.28	0.386
Ptolomaeum	113396	,,	13.36	3.59	0.481
White Saidi	113397	,,	12.56	3.39	0.427
Recognitum	113398	,,	12.84	3.34	0.429
	113950	Jordan	11.89	3.44	0.409
T. carthlicum = *T. persicum*					
	7692	USSR	18.30	3.21	0.587
	78812	,,	15.64	3.23	0.506
	94748	,,	12.65	3.29	0.416
	94750	,,	12.81	3.99	0.511
	94751	,,	14.21	3.26	0.462
T. turanicum					
Insigne	113392	England	12.83	3.08	0.395
Notabile	113393	,,	12.32	3.02	0.372
Insigne	115815	Turkey	14.21	2.93	0.417
Devedisa	173482	,,	15.81	2.84	0.449
Gigante Ingles	184526	Portugal	17.24	2.67	0.460
T. polonicum					
Polish	42209	Australia	15.33	3.00	0.460
Gigantil	56261	Portugal	18.28	2.82	0.515
Milagre	56262	,,	17.58	2.97	0.523
Mika	167622	Turkey	17.15	2.80	0.480
	185309	Argentina	16.47	2.84	0.468

TABLE 10. (Cont.)

Species	Code [a] No.	Source	Protein [b] %	Lysine % [c] in Sample	Lysine % [c] in Protein
Tetraploid					
T. turgidum					
Alaska	5988	Oregon	12.83	3.35	0.430
Albidum	7688	USSR	14.67	3.25	0.477
M6-585	13712	Oregon	13.42	3.15	0.423
M7-118	13713	,,	14.30	2.97	0.424
Rubriramosum	277680	Spain	14.82	2.89	0.429
T. dicoccum					
White Spring Emmer	3686	Minnesota	16.06	2.85	0.458
Khapli	4013	India	16.31	2.85	0.465
Garden	12213	,,	17.06	2.09	0.357
Huskie 1	12214	,,	17.00	2.78	0.472
Farrieum	41024	USSR	15.64	2.79	0.438
Hexaploid					
T. sphaerococcum					
	4529	India	14.86	2.76	0.410
	4923	,,	18.60	2.79	0.519
	9054	Iraq	18.70	2.63	0.493
	115818	India	15.92	2.66	0.424
	182115	,,	19.17	2.68	0.513
T. spelta					
Red Winter Spelt	1772	Washington	15.55	3.09	0.480
Alstroun	168682	Virginia	13.23	3.07	0.407
	192724	Spain	12.48	3.37	0.421
Album	272574	Hungary	11.55	3.52	0.407
Duhamelianum	272577	,,	15.71	2.87	0.450
T. macha					
	140191	Iran	16.46	2.77	0.459
	190923	Spain	12.63	3.63	0.458
Palaeo-imereticum	272554	Hungary	12.61	2.91	0.366
Subletschumicum	272555	,,	10.64	3.37	0.359
T. vavilovii					
Vaneum	272598	Hungary	9.96	3.30	0.329

[a] U.S. Department of Agriculture, World Collection of Small Grains.
[b] Dry weight basis (N × 5.7).
[c] Lysine expressed as % of protein and of sample on dry weight basis.

TABLE 11. Protein and Lysine Content in Whole Grain of Diploid, Tetraploid, and Hexaploid Species of *Triticum* Grown at Fargo, North Dakota, in 1964.

Species	Sample[a] No.	Protein[b] %	Lysine % [c] in Protein	Lysine % [c] in Sample
Diploid				
T. boeticum (v. Larionowii)	64-1856	17.81	2.65	0.472
T. boeticum (v. Pancii)	64-1858	21.21	2.39	0.507
T. monococcum (61-B-2601)		16.09	3.05	0.491
Tetraploid				
T. dicoccoides (v. Aarushnii)	64-1861	21.71	2.30	0.500
T. dicoccoides (v. Pseudo-rufavillosum)	64-1862	20.42	2.39	0.489
T. dicoccoides (v. Yaroslove)	64-213	14.37	2.81	0.404
T. dicoccum (v. Khapli)		18.91	2.63	0.498
T. polonicum		18.74	2.48	0.466
T. turgidum (v. Lusitanicum)	64-1865	13.30	2.75	0.365
T. orientale (4B-236)	64-1869	16.42	2.68	0.440
T. orientale (4B-237)	64-1871	17.15	2.57	0.441
T. pyramidale (4B-258)	64-1872	17.87	2.34	0.419
T. pyramidale (4B-265)	64-1875	20.01	2.54	0.509
T. timopheevi (v. Wanatahe)	64-1879	13.52	2.64	0.356
T. timopheevi (v. Typicum)	64-1880	16.66	2.63	0.439
T. timopheevi (v. Typicum)	64-1881	13.58	2.82	0.383
T. timopheevi (v. Nigrum)	64-1883	12.04	2.73	0.329

TABLE 11. (Cont.)

Species	Sample [a] No.	Protein [b] %	Lysine % [c] in Protein	Lysine % [c] in Sample
Hexaploid				
T. zhukovuskyi	64-1885	28.16	2.48	0.699
T. zhukovuskyi		22.16	2.87	0.636
T. paleocolchicum	64-446	16.63	2.52	0.419
Amphidiploids				
(*T. timopheevi* × *Ae. squarrosa*)^2	64-1847	19.71	2.36	0.465
(*T. timopheevi* × *T. durum*)^2	64-1848	23.41	2.62	0.613
(*T. timopheevi* × *T. polonicum*)^2	64-1850	19.59	2.33	0.457
(*T. timopheevi* × *T. pyramidale*)^2	64-1852	19.89	2.64	0.525
(*T. diccocoides* × *S. cereale*)^2 × (*T. durum* "Stewart" × *S. cereale*)^2		19.32	2.89	0.559

[a] Code No. North Dakota Agricultural Experiment Station.
[b] Dry weight basis (N × 5.7).
[c] Lysine expressed as % of protein and of sample on dry weight basis.

The varieties Larionowii and Pancii of the species, *T. boeticum* had lower lysine content than samples of this species showed in Table 10. This difference may be due to varietal difference or the effect of environment. Reliable comparisons cannot be made among these two groups of samples because the varieties of the different species were not the same and they were grown at two different locations.

The lysine values of the *T. pyramidale* reported in Table 11 are also lower than those shown in Table 10, and the one reported by Lawrence *et al.*

4. Secale species

Rye belongs to the genus *Secale* which is closely related to *Triticum*. It is known that rye has better protein quality from the nutritional standpoint than wheat (8).

A group of varieties and species of *Secale* were investigated to determine their variability in the lysine content. The results obtaind from these

analyses are shown in Table 12. High variability in lysine content and protein level was apparent in these samples. The protein content ranged from 7.17 to 20.6% (dry weight basis) and averaged 13.14%. The lysine content varied from 2.42 to 4.26% of protein and averaged 3.72%.

In general, the lysine content of rye has been reported to be higher than that of wheat (4). Rohrlich and Rasmus in 1956 (23) compared the germ, aleurone and endosperm proteins of rye and wheat. These workers used a tetraploid rye, and did not find qualitative differences between the amino acids of the germ and of the aleurone of wheat and rye, but they noted that the wheat endosperm was lower in arginine and lysine than that of rye endosperm.

TABLE 12. Protein and Lysine Content in Whole Grain of Different Varieties and Species of *Secale*.[a]

Variety	Source	Protein[b] %	Lysine % in Protein	Lysine % in Sample
Antelope	Rumania	13.31	3.33	0.443
Vörne	Rumania	17.98	3.16	0.569
Svalöff	Rumania	8.95	4.17	0.373
Meklen burger	Rumania	12.55	3.36	0.422
Hadm stamm 514-48	Rumania	13.45	3.38	0.454
Caribou	Rumania	14.52	3.21	0.466
Gellkorn	Germany	10 76	3.63	0.391
Carsten	Germany	10.72	3.65	0.392
Petk sommer roggen (2n)	Germany	11.04	3.35	0.370
W. roggen kortner	Germany	9.82	3.71	0.364
F.G.P. 1	Germany	11.25	3.81	0.429
Heertvelder	Germany	10.71	3.75	0.401
Heines hellkorn roggen	Germany	8.97	4.13	0.371
Secale montanum CPI 23285	Australia	16.64	3.66	0.609
Secale montanum CPI 22756	Australia	12.48	3.76	0.470
Secale kuprijanoviov CPI 23708	Australia	17.45	3.86	0.673
Secale dalmaticum CPI 22755	Australia	19.18	3.23	0.621
Secale ancestrale CPI 23707	Australia	15.55	3.35	0.521
Secale segetale CPI 23709	Australia	15.51	3.41	0.529
Secale vavilovii 62	Canada	22.26	2.92	0.651
Explorer	Canada	16.42	3.30	0.542
Secale cereale/Hiemeles/ 03.001	Czechoslovakia	8.80	3.96	0.349
Secale cereale dobrenicke 03.011	Czechoslovakia	16.18	3.46	0.560
Rye USDA PI 168136	Turkey	19.87	3.48	0.692

TABLE 12. (Cont.)

Variety		Source	Protein [b] %	Lysine % [c] in Protein	Lysine % [c] in Sample
Rye	USDA PI 168178	Turkey	17.04	3.22	0.549
Rye	USDA PI 168199	Turkey	17.74	3.49	0.514
Rye	USDA PI 173589	Turkey	17.78	3.32	0.591
Rye	USDA PI 220683	Afghanistan	15.23	3.28	0.499
Rye	USDA PI 223895	Afghanistan	15.36	2.93	0.450
Rye	USDA PI 227870	Iran	12.22	3.71	0.453
Rye	USDA PI 228360	Iran	14.60	3.17	0.463
Rye Grand Crovelle	USDA PI 235536	France	14.56	3.35	0.489
Rye	USDA PI 250744	Iran	14.37	3.50	0.504
Rye	USDA PI 251903	USSR	16.68	3.20	0.534
Rye Melker	USDA PI 254815	Australia	14.76	3.36	0.496
Rye	USDA PI 256026	Spain	16.75	3.03	0.507
Rye Khar-kovskaya	USDA PI 260055	USSR	16.46	3.31	0.545
Rye	USDA PI 265470	Finland	14.74	3.48	0.513
Rye Iavamrud	USDA PI 267095	USSR	14.71	3.25	0.478
Rye Ovari	USDA PI 272335	Hungary	15.25	3.33	0.508
Rye Gator		Florida, USA	20.23	3.21	0.649
Prolific spring		Montana, USA	17.32	3.25	0.563
Lundquist line 59-9			16.52	3.25	0.537
Elbon 1965 OFSS			11.48	2.87	0.329
Rosen Sel. 34578			10.98	3.74	0.411
Fairbanks winter			14.72	3.61	0.531
Balbo		USA	13.05	3.63	0.474
Secale ptolemais		Greece	9.39	3.99	0.375
Rodosinski rekord	I-3-17-2	Hungary	17.55	3.17	0.557
Viglasske	I-3-255	Hungary	18.33	3.14	0.575
Chrysanth hanserrogen	I-3-144	Hungary	19.79	3.01	0.595
Detenicke	I-3-275	Hungary	18.62	3.37	0.628
Zhukovsky (England) 9390-5-SC-17		USSR	16.88	3.29	0.556
Secale segetale	1/SS/1	USSR	16.28	2.74	0.446
Secale cereale	5-SC-12	USSR	16.29	3.15	0.513
Secale cereale	5-SC-18	USSR	14.95	3.22	0.482
Secale montanum	23-282	Italy	19.67	3.10	0.611
Secale vavilovii	CPI-23286	Italy	17.25	3.09	0.533
Secale anatolicum	19359	Italy	17.90	2.91	0.521
Secale tuprijanovii	23708	Italy	17.16	3.17	0.545
Secale segetale	23709	Italy	19.96	3.02	0.604
Secale ancestrale	23707	Italy	19.67	2.95	0.580
Secale tuprijanovii		Italy	12.86	3.79	0.488

TABLE 12. (Cont.)

Variety		Source	Protein [b] %	Lysine % [c] in Protein	Lysine % [c] in Sample
Rye Korean *I*		Japan	17.82	3.05	0.544
Rye kotani		Japan	16.23	3.16	0.513
Rye "rust resistant"		New Zealand	16.77	3.04	0.510
Guarda-5038		Portugal	19.03	3.24	0.616
Centeio do alto	5043	Portugal	15.87	3.23	0.513
Marco de Canavezes	5044	Portugal	15.22	3.56	0.543
Dominant	5108	Portugal	12.83	3.80	0.489
Maia barroso	5053	Portugal	12.32	4.26	0.524
Temporao	5058	Portugal	15.78	3.46	0.546
Castelo Branco	5034	Portugal	16.99	3.15	0.535
Estremoz		Portugal	11.64	3.65	0.424
Omka		USSR	16.89	3.07	0.520
Sangaste	9383	USSR	11.25	3.54	0.398
Prickeelskaja	9388	USSR	10.94	3.43	0.375
Manyeskaja		USSR	14.24	3.30	0.470
Volyanka		USSR	13.78	2.91	0.400
Dolinskaja		USSR	13.79	3.39	0.468
Bezencurskaja Zeltozernaja	9573	USSR	15.82	3.11	0.492
Harkovskaja	9577	USSR	14.69	2.79	0.409
Zayersraja	9872	USSR	13.63	3.23	0.441
Baltia	10170	USSR	10.57	3.62	0.383
Sitnikouskaja	9326	USSR	17.37	3.11	0.541
Viatica-2	9441	USSR	12.49	3.38	0.422
Lisicyna	9452	USSR	11.78	3.53	0.416
Spasskaja	9965	USSR	14.28	3.52	0.502
Kalwzskaja 45	10161	USSR	10.94	4.20	0.460
Gibridnaja No. 2	10163	USSR	11.15	3.50	0.391
Dankowskie Sztywnet I		Poland	10.09	3.63	0.366
Wloszanowskie		Poland	10.62	3.63	0.385
Zeelandzkie		Poland	8.71	3.91	0.341
Pulawskie Wczesne		Poland	10.62	3.55	0.377
Wielkopolskie		Poland	16.83	2.81	0.473
Secale montanum	FAO 22350	Turkey	15.58	3.01	0.470
Secale anatolicum	FAO 22351	Turkey	13.51	3.24	0.438
Canadian spring rye	FAO 22358		10.72	3.71	0.397
Afghanistan winter rye	FAO 22359		12.07	3.35	0.405
Perennial rye	FAO 22360	Turkey	15.27	2.85	0.436
Rye Ank. Z. A. 1756	FAO 22364	Turkey	9.91	3.79	0.376
Rye Ank. Z. A. 1761	FAO 22369	Turkey	8.10	3.91	0.317

TABLE 12. (Cont.)

Variety		Source	Protein [b] %	Lysine %[c] in Protein	Lysine %[c] in Sample
Rye Ank. Z. A. 1767	FAO 22375	Turkey	8.62	3.71	0.320
Rye Ank. Z. A. 1778	FAO 22385	Turkey	10.68	3.60	0.385
Rye Ank. Z. A. 1783	FAO 22390	Turkey	11.38	3.56	0.406
Rye Ank. Z. A. 1794	FAO 22401	Turkey	10.78	3.69	0.398
Rye Ank. Z. A. 1799	FAO 22406	Turkey	7.79	4.04	0.314
Rye Ank. Z. A. 1804	FAO 22411	Turkey	7.17	3.90	0.279
Rye Ank. Z. A. 1809	FAO 22416	Turkey	8.39	3.84	0.322
Rye Ank. Z. A. 1814	FAO 22421	Turkey	8.94	3.83	0.343
Rye Ank. Z. A. 1819	FAO 22426	Turkey	9.88	3.71	0.366
Rye Merced A			16.55	3.10	0.513
Rye Merced B			19.62	3.12	0.613
Rye Merced C			20.21	2.83	0.572
Centeno M No. 8A			13.09	3.62	0.474
Centeno M No. 8B			18.55	3.24	0.601
Abruzzi A			11.50	2.42	0.279
Abruzzi B			12.70	3.62	0.459
Explorer A			15.29	3.44	0.525
Explorer B			15.95	3.39	0.540
Centeno Argentino No. 2			17.62	3.45	0.607
Composite USDA	S-31-2M		17.15	3.10	0.531
Prolific	S-28-1M		16.55	3.52	0.583
Petkus C.A.N.	S-27-1M		20.28	2.93	0.594
PI 168133	S-21-1M		20.60	2.98	0.613

[a] Place where these samples were grown is unknown, with exception of the last four, which were grown at Chapingo, Mexico, 1965.
[b] Dry weight basis (N × 6.25).
[c] Lysine expressed as % of protein and of sample on dry weight basis.

From data presented here, it is evident that rye protein is richer in lysine than wheat protein, and that high variability among samples exists in the lysine level. It is impossible to state whether this variability is varietal or mainly environmental, because the samples were grown in many different parts of the world.

As in wheat, the protein content of rye showed an inverse relationship with the lysine level in the protein, and the negative correlation existed

over the entire protein range (7.17 —20.6%). The correlation coefficient was —0.70 and highly significant.

5. Triticale

The new, man-made amphiploid cereal, *Triticale,* is a potentially important food crop. Triticale (hexaploid) is similar to bread wheat in that two thirds of its make-up is tetraploid wheat, but differs in that the other one-third is rye (*Secale* spp.) instead of goat grass (*Aegilops squarrosa*).

Studies from the University of Manitoba have shown that the *Triticale* from durum wheat and rye has a high yield potential. Tests on quality have indicated that the *Triticale* has a high protein content but poor baking quality. Acceptable loaf volume can be produced when the Triticale flour is blended with flour from a hard red spring wheat.

Because of these factors it was interesting to investigate the lysine level and variability of the Triticale protein.

A group of different lines of hexaploid and octaploid Triticales developed by a group of workers of the University of Manitoba, and grown at Ciudad Obregon, Sonora, Mexico, was investigated. In some cases the primary, secondary and tertiary spikes of different selections were analyzed, in other cases only one or two spikes. The results are given in Table 13.

The protein content of these samples ranged from 11.76 to 22.50% (dry weight basis). The lysine content of the protein varied from 2.55 to 3.74%. The averages were 17.48% protein and 3.24% lysine in the protein. In general, the protein level of the samples was high with little variation in the lysine content.

Fox and De Fontaine in 1956 (7) reported that the total lysine content of one *Triticale* analyzed by them was between the rye and wheat parent. In the present study, it is not possible to make a reliable comparison between the lysine content in the protein of durum wheat, rye and *Triticale*, because the durum wheat and rye progenitors used in the development of these lines were not analyzed. However, comparisons of the durum wheats and ryes analyzed in this study indicated that the lysine content of Triticale protein was generally higher than that of wheat protein, and somewhat lower than that of rye protein. The lysine level of *Triticale* analyzed in this study was similar to that reported by Lebedeva in 1965 (10) which only showed value of one sample analyzed.

An inverse relationship between lysine content and protein level was found also in Triticale. The correlation coefficient obtained was —0.52 and highly significant.

To achieve genetic improvement of protein content and nutritional quality in cereal crops, a wide genetic base should be examined for lysine content in each species. The high lysine character, if found, should be incorporated into varieties or lines with high protein and high yield.

The present study includes more than 400 lysine evaluations, however, the number of varieties or species investigated was reduced greatly because in the earlier part of the work the number of analyses for each sample was multiplied when analyzing kernels from different sites on the spike and different spikes of a given variety.

TABLE 13. Protein and Lysine Content in Whole Grain of Triticales Grown at Ciudad Obregón, Sonora, Mexico in 1965-1966.

Variety or Cross	Plant and Spike Selection [a]	Generation Seed	Protein [b] %	Lysine % [c] in Protein	Lysine % [c] in Sample
1593 A × 1620	1-B	F_8	17.88	3.27	0.620
1593 A × 1620	1-C	F_8	16.25	3.50	0.569
1593 B	1-A	F_6	15.88	3.48	0.552
1593 B	1-B	F_6	17.09	3.40	0.581
1593 B	2-A	F_6	11.76	3.43	0.610
1593 B	2-B	F_6	19.89	3.10	0.617
1593 B	4-A	F_6	17.52	3.49	0.611
1593 C	14-A	F_6	19.09	3.36	0.641
1593 D	3-A	F_6	15.34	3.71	0.568
1593 D	3-B	F_6	17.83	3.43	0.611
1594 A	2-A	F_6	18.80	3.21	0.603
1594 A	4-A	F_6	19.16	3.25	0.624
1594 A	9-A	F_6	18.55	3.22	0.597
1594 A	14-A	F_6	18.80	3.39	0.637
1594 A × 1601	1-A	F_3	19.52	3.46	0.676
1594 A × 1601	1-B	F_3	17.22	3.36	0.578
1594 A × 1613	1-A	F_3	19.34	3.67	0.711
1594 A × 1628 A	3-A	F_3	17.22	3.29	0.566
1594 A × 1628 A	4-A	F_3	16.62	3.33	0.553
1605	7-A	F_6	16.98	3.17	0.539
1609 B × 1636	2-A	F_3	19.34	3.20	0.618
1636 A	6-A	F_6	16.25	3.56	0.578
1636 A × 1614	1-A	F_3	16.19	3.44	0.557
1636 A × 1614	1-B	F_3	15.22	3.65	0.555
1636 A × 1614	1-C	F_3	20.31	3.34	0.678
1636 A × 1614	2-A	F_3	18.37	3.37	0.618
1636 A × 1614	2-B	F_3	17.58	3.10	0.544
1636 C	3-A	F_6	16.73	3.32	0.556
1636 C × 1642	1-A	F_3	17.16	3.29	0.565
1636 C × 1642	1-B	F_3	16.67	3.37	0.561
1637 C	4-A	F_6	18.68	3.24	0.605
1637 C	4-B	F_6	18.62	3.17	0.590
1641 A	1-A	F_6	13.46	3.60	0.485
1641 A	2-A	F_6	15.16	3.34	0.507
1641 A	2-B	F_6	14.62	3.46	0.505
1641 A	7-A	F_6	16.55	3.16	0.523
1641 A	7-B	F_6	14.49	3.55	0.514
1641 B	2-A	F_6	17.52	3.21	0.561
1641 B	9-A	F_6	15.16	3.39	0.514
1641 B	9-B	F_6	14.91	3.33	0.497
1641 B	9-C	F_6	13.70	3.40	0.466

TABLE 13. (Cont.)

Variety or Cross	Plant and Spike Selection [a]	Generation Seed	Protein [b] %	Lysine % [c] in Protein	Lysine % [c] in Sample
1641 D	7-A	F_6	16.01	3.52	0.565
6A 250 × 6A 191	Bk.A	F_5	13.76	3.28	0.451
6A 250 × 6A 191	Bk.B	F_5	12.80	3.74	0.478
6A 250 × 6A 191	Bk.C	F_5	16.44	2.69	0.442
6A 250 × 6A 191	Bk.D	F_5	15.76	3.31	0.522
6A 250 × 6A 191	Bk.E	F_5	16.25	3.38	0.550
6A 250 × 6A 191	Bk.G	F_5	18.19	2.95	0.536
6A 250 × 6A 190 (13)	Bk.C	F_6	19.22	2.90	0.556
6A 250 × 6A 190	Bk.E	F_6	19.89	2.82	0.562
6A 250 × 6A 190	A	F_6	19.34	2.73	0.528
6A 250 × 6A 190	B	F_6	18.73	2.55	0.477
6A 250 × 6A 190	C	F_6	16.24	2.96	0.481
6A 250 × 6A 190	D	F_6	18.19	2.70	0.491
6A 250 × 6A 190	E	F_6	17.28	3.49	0.603
6A 250(6A66.12 × 6A20)	Bk.A	F_6	17.47	3.09	0.540
6A 250 × 6A 190	Bk.A	F_6	17.95	3.01	0.540
6A 250 × 6A 190 (19)	Bk.B	F_6	20.19	3.68	0.741
6A 250 × 6A 190	Bk.C	F_6	19.58	3.04	0.595
My 64 × Triticale	2-C	F_4	18.55	3.33	0.618
My 64 × Triticale	2-F	F_4	22.50	2.72	0.613
My 64 × Triticale	2-D	F_4	19.76	2.98	0.590
My 64 × Triticale	2-G	F_4	19.58	3.20	0.628
My 64 × Triticale	2-H	F_4	21.89	2.84	0.622
My 64 × Triticale	2-I	F_4	20.73	2.73	0.566
My 64 × Triticale	2-K	F_4	17.04	3.03	0.516
My 64 × Triticale	2-L	F_4	18.48	3.28	0.608
Triticale S-112	A	F_3	16.06	2.98	0.479
Triticale 6913	A		21.52	2.97	0.639
Triticale 100 C-132-1			17.01	3.11	0.563

[a] Number corresponds to plant selected, A = primary spike, B = secondary spike.
[b] Dry weight basis (N × 5.7).
[c] Lysine expressed as % of protein and of sample on dry weight basis.

Among the varieties and lines of durum wheat analyzed, only the line RD 176 - 7A showed a lysine content in the protein of 3.4% (average), with a protein content of 11.03% (average).

In the bread wheats, 5 varieties of hard red spring wheat and 3 varieties of soft red spring wheat (from Mexico) were analyzed. The highest lysine value obtained was that showed by the variety Lerma Rojo with an average of 3.03% of lysine in the protein with an average protein content of 14.12%.

Among the 16 different species of *Triticum,* 83 varieties were examined. From the 7 varieties of the *T. boeticum* (diploid), 5 varieties high in protein

were high in lysine. The protein content ranged from 19.40 to 24.46% (average 22.25%). The lysine content in the protein ranged from 3.05 to 3.50% (average 3.17%).

Five varieties of the tetraploid *T. pyramidale* showed higher lysine values in the protein (3.28 to 3.59%, average 3.41%) than the varieties of *T. boeticum*. However, the protein contents were lower (11.80 to 13.36%, average 12.49%).

The protein of the hexaploids *T. sphaerococcum, T. spelta, T. macha, T. vavilovii,* and *T. zhukovuskyi,* contained lysine levels similar to those showed by the bread wheats.

The group of 125 varieties and species of *Secale* had protein with high lysine content, except for 13 samples with lysine content below 3.0%. The protein content of this group varied greatly (7.17 — 20.6%), while the lysine content varied in the protein (2.42 — 4.26%).

Among the 70 samples of *Triticale* only 14 samples were found with a lysine content in protein below 3.0%. Some of these are octaploid Triticales in which the wheat progenitor was the hexaploid Mayo 64.

Lines of the different wheats, ryes and Triticales showing a protein content over 12% combined with high lysine content are shown in Table 14. Samples showing over 4.0% lysine in protein were found only among ryes; however, these had low protein content, as shown in Table 15.

Lines indicated in Tables 14 and 15, may be considered potentially valuable as parental material for breeding programs. However, it should be demonstrated first that the high lysine level of these lines is controlled by a genetic factor. Therefore, they must be tested again after being grown under different environmental conditions which will affect the protein content of the samples.

TABLE 14. Varieties or Lines of Wheat, Rye, and Triticale with High Lysine and Protein Content.

Variety		Protein [a] %	Lysine % [b] in Protein
Triticum			
T. boeticum	230133	21.79	3.50
T. pyramidale	113396	13.36	3.59
T. carthlicum	94750	12.81	3.99
T. macha	190923	12.63	3.63
Secale			
S. montanum	CPI 23285	16.64	3.66
S. montanum	CPI 22756	12.48	3.76
S. kuprijanoviv	CPI 23708	17.45	3.86
S. cereale			
Rye (Turkey)	PI 168136	19.87	3.48
Rye (Turkey)	PI 168199	17.74	3.49
Rye (Iran)	PI 227870	12.22	3.71

TABLE 14. (Cont.)

Variety	Protein %	Lysine % [b] in Protein
Fairbanks winter rye	14.72	3.61
Marco de Canavezes 5044	15.22	3.56
Dominant 5108	12.83	3.80
Maia Barroso 5053	12.32	4.26
Temporao 5058	15.78	3.46
Centeno M No. 8A Rye	13.09	3.62
Spasskaja Rye 9965	14.28	3.52
Abruzzi B Rye	12.70	3.62
Prolific Rye	16.55	3.52
Triticale		
1593A × 1620-1C	16.25	3.50
1593B − 1A	15.88	3.48
1593B − 4A	17.52	3.49
1593D − 3A	15.34	3.71
1594A × 1601 − 1A	19.52	3.46
1594A × 1613 − 1A	19.34	3.67
1636A	16.25	3.56
1636A × 1614 − 1B	15.22	3.65
1641A − 1A	13.46	3.60
1641A − 2B	14.62	3.46
1641A − 7B	14.49	3.55
6A 250 × 6A 191-B	12.80	3.74
6A 250 × 6A 190-E	17.28	3.49
6A 250 × 6A 190-B	20.19	3.68

[a] Dry weight basis.
[b] Lysine expressed as % of protein on dry weight basis.

TABLE 15. Ryes with High Lysine and Low Protein Content.

Variety	Source	Protein [a] %	Lysine % [b] in Protein
Svalöff	Rumania	8.95	4.17
Heines Hellkorn	Rumania	8.97	4.13
Kalwzskaja 45-10161	USSR	10.94	4.20
Ank. Z. A. 1799 FAO 22406	Turkey	7.79	4.04

[a] Dry weight basis.
[b] Lysine expressed as % of protein on dry weight basis.

SUMMARY AND CONCLUSIONS

Genetic improvement of cereal crops has been aimed, in the past principally at increasing yields of grain per unit of cultivated area, providing protection against diseases and insects, and improving industrial quality. Recently, with the discovery that a mutant gene in corn increases the content of lysine, genetic-biochemical research efforts have been initiated in order to identify genes of similar action in other cereals. Lysine is nutritionally the most limiting amino acid in cereal protein.

The number of samples which must be investigated in the search for high lysine level is very large. The chemical analysis for lysine using ion-exchange chromatography has been found at present the most reliable procedure. A triplicate-sample method using this technique on an automated amino acid analyzer was developed for the determination of lysine in cereal hydrolysates. This triplicate-sample method is accurate, precise, and more rapid than the single sample analysis. This method was used to investigate many wheats, ryes and Triticales in a search for lines having high lysine content.

Among some lines of durum and bread wheats, analyses were performed on kernels from different sites of a spike and on different spikes of a selected plant. The lysine level in the protein showed little variation among sites and spikes in most cases. The little variation in lysine content was shown to be due to variation in the protein content of the grains. Greater variation in the lysine content of the protein was observed among lines in the durum wheat than in bread wheats. However, this variation was considered insignificant. The highest lysine level in the protein among durum wheats analyzed was 3.4%, and among bread wheats 3.03%.

Different diploid, tetraploid and hexaploid species of *Triticum* were examined. Five varieties of the diploid *T. boeticum* were high in protein (19.48 to 24.8%), and high in lysine content (3.05 to 3.50%) of the protein. Among the varieties of the tetraploid *T. pyramidale,* 5 varieties showed lysine in protein ranging from 3.28 to 3.59%, and protein contents from 11.80 to 13.36%. The lysine level in the protein of the miscellaneous hexaploid species was similar to that of bread wheats (*T. aestivum*).

Rye protein, in general, showed higher lysine level than wheat protein. The protein content of the varieties and species of *Secale* varied from 7.17 to 20.6%, and the lysine content of the protein from 2.42 to 4.26%.

The man-made amphiploid cereal, *Triticale,* showed high protein content and high lysine level in the protein. The protein content of this cereal ranged from 11.76 to 22.50%. The lysine content of the protein varied from 2.55 to 3.74%. The lysine level of the Triticale protein appeared to be intermediate between that of wheat and rye.

The analyses indicated that a few lines among the *Triticum* species, the *Secale* species, and *Triticale* have potential value for breeding programs. If these lines consistently show high lysine in further testing, they should be crossed with high yielding varieties having high protein. If genetic improvement of nutritional quality of cereal protein becomes a reality,

grains containing high lysine level could be produced without additional cost to either the farmer or the consumer. This high quality cereal protein would become available in both rural and urban areas to all groups, irrespective of economic level.

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