Amino Acid Composition of Total Protein and Electrophoretic Behavior of Protein Fractions of Barley[1]

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ABSTRACT

Total protein from 23 barley cultivars of various origins, grown at the Montana Agricultural Experiment Station, was separated into albumin-globulin, hordein, and glutelin fractions. The protein fractions were examined by sodium dodecyl sulfate disc gel electrophoresis. From 13.0 to 19.6% total protein occurred in the barleys, of which 27.5 to 39.8% were albumin-globulin, 17.2 to 36.9% were hordein, and 23.6 to 41% were glutelin. The total protein of most barleys had essentially the same amino acid composition except Hiproly, which had much higher lysine, but low levels of alanine, arginine, glutamic acid, and cystine/2.

The albumin-globulin contained 12 components occurring in six types of electrophoretic combinations. Three of the major components occurred at varying concentrations in all six types of electrophoretic patterns and in all 23 barley cultivars. Hordeins contained seven components at various levels of concentration in five different electrophoretic combinations. Only one of the hordein components occurred in each of the five combinations. Fifteen different glutelin components were found in four types of electrophoretic combinations. Only one of these components, which had the lowest molecular weight (12,400), occurred in three of the four combinations.

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Recently plant breeders have shown increased interest in the development of cereal cultivars with better nutritional qualities. An example is the high lysine barley described by Munck et al (1971). Subsequent development of the Notch mutants (Singh and Sastry 1977) of barley with high protein and high lysine have generated further interest in the nature of genetic changes occurring in these cultivars. Ingversen and Køie (1971) showed interesting differences in the composition of albumin and globulin proteins, while Munck (1972) discovered differences in the hordein proteins of barley. In more recent work, Singh and Sastry (1977) performed solubility fractionation and polyacrylamide gel electrophoresis on the proteins of the cultivar NP-113 and induced mutants of Notch and Notch-2 barleys. They discovered interesting differences in the proteins, the most striking of which were the electrophoretic

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changes in the glutelin and albumin fractions. Less striking differences occurred in the globulin and hordein fractions. El-Negoumy et al (1977) fractionated the protein complex Hiproly (CI 3947) and Hiproly Normal (CI 4362) barleys and found the albumin-globulin fractions accounted for 41.8 and 43.5% of the lysine in Hiproly and Hiproly Normal, respectively. Albumin-globulin and glutelin fractions together accounted for 78.7% of the total lysine in Hiproly and 80.6% in Hiproly Normal.

The present investigation determined the concentration of the albumin-globulin, hordein, and glutelin proteins in 23 barley cultivars. Amino acid composition of the total protein of barleys and electrophoretic composition of the protein fractions obtained are reported.

MATERIALS AND METHODS

Barley source

Twenty-three barley cultivars of various origins were grown at a common location during the same season at the Montana Agricultural Experiment Station, Bozeman. Nineteen cultivars were selected for seed increase on the basis of high lysine potential as predicted by dye-binding capacity. The remaining four barleys were Wapana and the commercial cultivars Compana, Unitan, and Shabet.

Separation of Protein Fractions

Barley grain treatment and recovery of albumin-globulin, hordein, and glutelin have been reported (El-Negoumy et al 1977).

Determination of Protein Content

Nitrogen was determined by micro-Kjeldahl and protein content calculated as N × 6.25. Moisture was determined on ground flour samples and sample weights were corrected accordingly. Corrections were made for nonprotein nitrogen (NPN) in whole flour and the albumin-globulin protein fraction. NPN was determined as soluble nitrogen in 10% trichloroacetic acid.

Electrophoresis and Molecular Weight Determination

Molecular weights were determined using sodium dodecyl sulfate (SDS) disc gel electrophoresis. Molecular weights of barley protein fractions components were determined using molecular weight markers as previously reported by El-Negoumy et al (1977).

Gel Preparation

Standard molecular weight markers and the various barley protein fractions were subjected to SDS disc gel electrophoresis with a Bio-Rad Model 155 electrophoresis cell accommodating 18 tubes. Gel composition, electrode buffer, and gel preparation have been reported (El-Negoumy et al 1977). Sufficient gel for 18 tubes contained 8 ml of Cyanogum-41 solution, 29 ml gel buffer, 0.12 ml N,N,N-tetramethylenediamine, and 0.1 ml freshly prepared 10% ammonium persulfate solution. Sample solutions containing 300 μg albumin-globulin or glutelin proteins and 200 μg hordein were applied to each tube and electrophoresis performed using a current of 8 mA per tube for 3.5 hr until the bromphenol dye marker reached the bottom of the gel. The gels were stained with Coomassie blue according to Koenig et al (1970).

Molecular Weight Determination of Components of the Protein Fractions

Electrophoretic mobilities of standard proteins were plotted against molecular weights on semi-logarithmic scale. Molecular weights of barley protein fractions were estimated from electrophoretic mobilities according to Weber and Osborn (1969). Percentage of each protein component was estimated by densitometry at 590 nm using model R.F.T. scanning densitometer with a linear scale manufactured by Transidyne General Corporation. Contents of the components were estimated from the integrator scale on the scan.

Amino Acid Analysis

Amino acid analysis was performed on duplicate samples, and the analytical values accounted for 87% of the nitrogen in the sample. The chromatograms were quantitated comparatively with standard amino acid mixture. Amino acid composition of whole

TABLE I
Composition of Proteins Recovered from Various Barley Cultivars Obtained from Various Sources

CI Number[a]	Barley Cultivar	Total Protein (%)	Recovered Protein Fractions: Albumin + Globulin (%)	Hordein (%)	Glutein (%)	Residue (%)	Total Protein Recovered (%)
906	Hanna	15.7	39.8	20.0	30.9	9.3	90.7
3383	Bargiers	15.7	34.4	23.5	32.0	10.1	89.9
6400	···	15.6	38.5	19.6	31.8	10.1	89.9
6407	Dornberger Hel Franke	14.0	36.0	22.0	31.2	10.8	89.2
7131	Balder	13.0	36.8	19.3	32.9	11.0	89.0
7622	Lenta	14.4	31.5	20.3	41.0	7.2	92.8
8142	···	14.5	33.4	25.1	30.6	10.9	89.1
10236	···	13.8	34.1	24.9	26.1	14.9	85.1
10328	Italian	16.9	33.3	17.2	34.0	15.5	84.5
10375	···	14.6	29.1	33.7	25.2	12.0	88.0
11201	Weibulls 5573	13.4	35.1	23.3	28.9	13.0	87.3
11308	Bonus	13.9	35.8	22.5	28.1	13.4	86.5
11310	Brage	15.8	32.0	33.9	23.6	10.5	89.5
11315	Primus II	15.2	35.7	20.7	30.4	13.2	86.8
12099	···	13.5	37.8	26.3	23.6	12.4	87.6
12147	Imperial	15.4	39.0	22.6	24.7	13.7	86.3
12171	···	16.7	29.6	28.9	29.7	11.9	88.1
3947	Hiproly	19.6	30.1	24.9	34.5	10.5	89.6
12103	···	13.3	33.7	28.3	25.6	12.3	87.7
5438	Compana	14.8	29.7	34.2	27.8	8.4	91.6
294318*	Wapana	16.2	27.5	36.9	27.3	8.3	91.7
10421	Unitan	13.0	31.7	21.5	36.3	10.6	89.4
13827	Shabet	14.3	29.9	33.1	28.0	8.9	91.1

[a] Montana

protein from each barley cultivar was determined with the hydrolysate prepared at 110° C with 6*N* HCl for 24 hr according to Spackman et al (1958). Analysis was made on a Durrum single column liquid chromatograph. A PDP-8 integrator continuously monitored the analysis and computed the data. Serine was increased by 10% and threonine by 5% to compensate for acid hydrolysis destruction. Cysteine and cystine were oxidized to cysteic acid using performic acid and their values were calculated from the cysteic/alanine ratio and reported as cystine/2 according to Hirs (1967). Tryptophan was determined by a 48 hr alkaline hydrolysis at 135° C and calculated from the tryptophan/histidine ratio according to Hugli and Moore (1972).

RESULTS AND DISCUSSION

Composition of proteins recovered from 23 barley cultivars are given in Table I. Total protein content varied from 13.0% for Balder (CI 7131) and Unitan (CI 10421) to 19.6% for Hiproly (CI 3947). The albumin-globulin protein varied from 27.5% in Wapana (MT 294318) to a maximum of 39.8% in Hanna (CI 906). Hordein proteins varied widely from 17.2% in Italian (CI 10328) to 36.9% in Wapana. Glutelin content ranged from 23.6% in CI 12099 and 11310 to a maximum of 41.0% in Lenta (CI 7622).

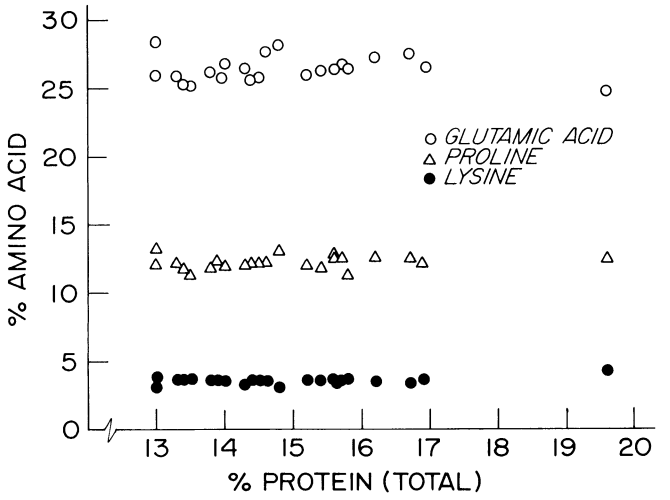

Fig. 1. Percent total protein vs. percentages of glutamic acid, proline, and lysine in 23 barley cultivars from various sources.

Statistical analysis revealed no significant correlation of total protein with contents of different protein fractions within the various barley cultivars. No significant statistical correlation existed among the levels of the different protein fractions within the various barley cultivars. This differs with preliminary results reported by Munck et al (1971) who found a negative correlation between the total protein content and hordein.

Amino acid composition of the total protein is given in Table II. Most barley cultivars contained essentially the same amino acid composition with the exception of Hiproly (CI 3947), which had the highest lysine content accompanied by the lowest alanine, arginine, glutamic acid, and cystine/2. Lack of sufficient available amounts of essential amino acids, especially lysine and methionine, is evident in most of these cultivars. This character, in conjunction with the low total protein level, makes it difficult for animals to obtain maximum growth and feed efficiency from such barleys without supplemental protein. Smith (1972) and Pomeranz and Robbins (1972) showed that glutamic acid, proline, and cysteine increased significantly during barley seed maturation, with a compensating decrease in alanine, lysine, aspartic acid, and threonine. Ivanko (1971) showed that these changes were mainly the result of an increase in hordein concentration of glutamic acid and proline. Brandt (1976) followed the formation of endosperm protein by amino acid analysis in a wild type of barley and in the high lysine mutant Risø 1508. Only minor differences in albumins and globulins of the mutant were observed, but three times more free amino acids were present at all stages of endosperm development compared with the wild type. Hordein formation was severely impaired and the syntheses of two electrophoretic components of the mutant were inhibited, which accounted for its high lysine content.

In Fig. 1, the percentage total protein was plotted against the percentages of glutamic acid, proline, and lysine. Amino acid composition remained relatively constant as protein increased. This is in contrast to results published by Coic et al (1963), Eppendorfer (1968), and Mossé et al (1969), while in agreement with results published by Dingle and McEwan (1972) and

TABLE II
Percentage Amino Acid Composition of Total Acid Composition of Total Protein of Barley Cultivars

	Amino Acids (%)																	
CI No.[a]	Ala	Arg	Asp	Cys/2	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Try	Tyr	Val
906	3.6	4.9	5.8	1.8	26.7	3.4	2.2	3.6	6.9	3.5	1.6	5.8	12.4	4.7	3.6	2.0	2.9	4.6
3383	3.6	5.0	5.7	2.0	26.5	3.3	2.2	3.7	7.1	3.4	1.7	5.8	12.4	4.5	3.5	1.8	3.1	4.7
6400	3.6	5.0	5.8	1.9	26.4	3.4	2.2	3.7	7.2	3.5	1.7	5.8	12.7	4.5	3.4	1.6	2.9	4.8
6407	3.7	4.8	5.9	1.8	26.7	3.4	2.0	3.8	7.3	3.5	1.8	5.7	11.9	4.6	3.4	1.6	2.8	4.7
7131	3.6	5.1	5.9	2.0	26.0	3.6	2.2	3.6	7.2	3.8	1.6	5.8	12.1	4.7	3.5	1.7	2.9	4.8
7622	3.7	5.2	6.0	2.0	25.7	3.5	2.3	3.7	7.2	3.6	1.7	5.8	12.2	4.7	3.6	1.5	3.1	4.8
8142	3.8	5.4	6.2	1.9	25.8	3.4	2.3	3.7	7.0	3.5	1.8	5.6	12.1	4.8	3.7	1.4	3.3	4.8
10236	3.8	5.1	6.3	1.8	26.2	3.4	2.2	3.6	7.0	3.6	1.8	5.8	11.8	4.8	3.7	1.3	3.2	4.7
10328	3.6	5.5	5.7	1.6	26.5	3.2	2.3	3.8	7.0	3.5	1.7	5.7	12.1	4.7	3.5	1.5	3.3	4.9
10375	3.4	4.9	5.9	1.8	27.7	3.3	2.3	3.5	6.8	3.5	1.6	6.0	12.1	4.8	3.5	1.7	2.8	4.6
11201	3.8	5.2	6.1	2.2	25.3	3.5	2.3	3.7	7.0	3.6	1.8	5.6	11.7	4.7	3.6	1.8	3.3	4.9
11308	3.7	5.3	6.0	2.0	25.7	3.5	2.2	3.7	7.2	3.6	1.7	6.0	12.3	4.5	3.5	1.0	3.4	4.8
11310	3.6	5.1	6.1	1.8	26.4	3.6	2.3	3.7	7.1	3.8	1.7	6.1	11.2	4.7	3.7	1.6	2.9	4.7
11315	3.7	5.2	6.1	1.9	26.0	3.3	2.2	3.7	7.1	3.5	1.8	5.9	12.0	4.5	3.4	1.8	3.3	4.9
12099	3.8	5.3	6.4	2.0	25.3	3.5	2.3	3.7	7.0	3.7	1.8	5.7	11.3	4.8	3.8	1.6	3.4	4.7
12147	3.8	5.3	6.3	1.8	26.2	3.4	2.3	3.8	7.0	3.5	1.7	5.7	11.8	4.8	3.6	1.5	2.9	4.8
12171	3.4	5.2	5.6	2.0	27.5	3.3	2.2	3.6	7.0	3.3	1.6	5.7	12.5	4.9	3.3	1.2	2.9	4.7
3947	3.0	4.4	6.8	1.6	24.9	3.4	2.0	3.7	6.7	4.2	1.8	6.1	12.4	4.6	3.6	1.9	2.8	4.8
12103	3.7	5.1	6.3	2.2	25.8	3.4	2.2	3.6	7.2	3.5	1.7	5.8	12.1	4.6	3.6	1.0	3.4	4.8
294318	3.4	4.7	5.3	2.0	28.1	3.4	2.3	3.6	6.7	3.0	1.6	5.8	13.0	4.6	3.4	1.8	3.0	4.5
5438	3.4	4.6	5.4	2.1	28.3	3.0	2.1	3.6	6.7	3.1	1.6	5.8	13.2	4.5	3.3	2.1	3.0	4.4
10421	3.6	4.9	5.8	1.6	27.2	3.4	2.3	3.7	6.9	3.4	1.7	5.8	12.5	4.8	3.5	1.4	3.0	4.7
13827	3.6	5.2	5.9	2.2	26.4	3.4	2.2	3.7	6.9	3.3	1.7	5.4	12.0	4.8	3.5	1.8	3.4	4.9

[a]Montana

McGeown and Maguire (1967).

Protein composition changes of the three major protein fractions in barley cultivars can easily be visualized by electrophoresis. Figures 2, 3, and 4 present photographs of the types of component combinations accompanied by diagrammatic illustrations occurring in the albumin-globulins, hordein, and glutelin fractions, respectively. Data in Table III presents percentage contents of the electrophoretic components in Figs. 2, 3, and 4 as determined by densitometry.

The albumin-globulin fraction showed 12 components occurring in six types of combinations (Fig. 2) found in the 23 cultivars investigated. Several minor components that were not indicated in the diagrammatic illustration also occurred. The main components were identified both by electrophoretic mobility and molecular weight. Components 4, 5, and 12 are major and occurred in all six

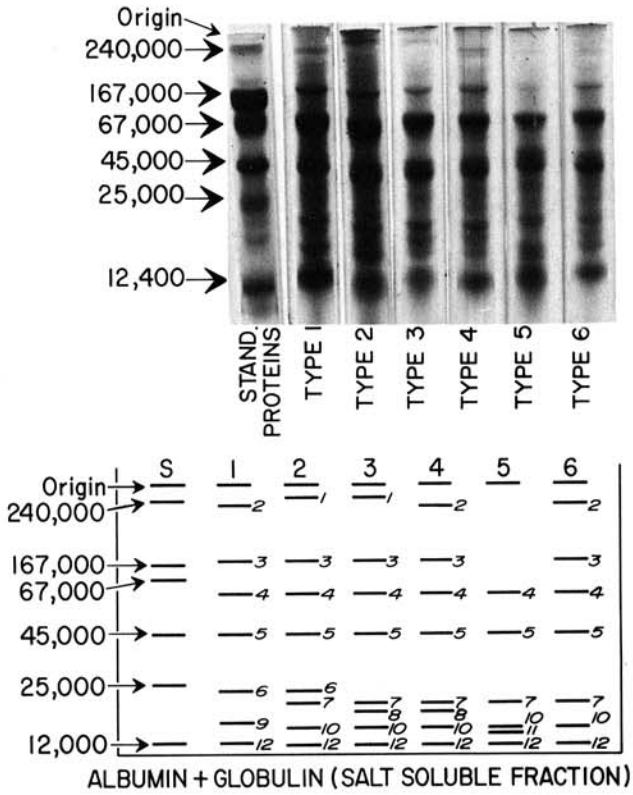

Fig. 2. Electrophoretic patterns and their diagrammatic illustration of six types of component combinations (albumin + globulin) from 23 barley cultivars.

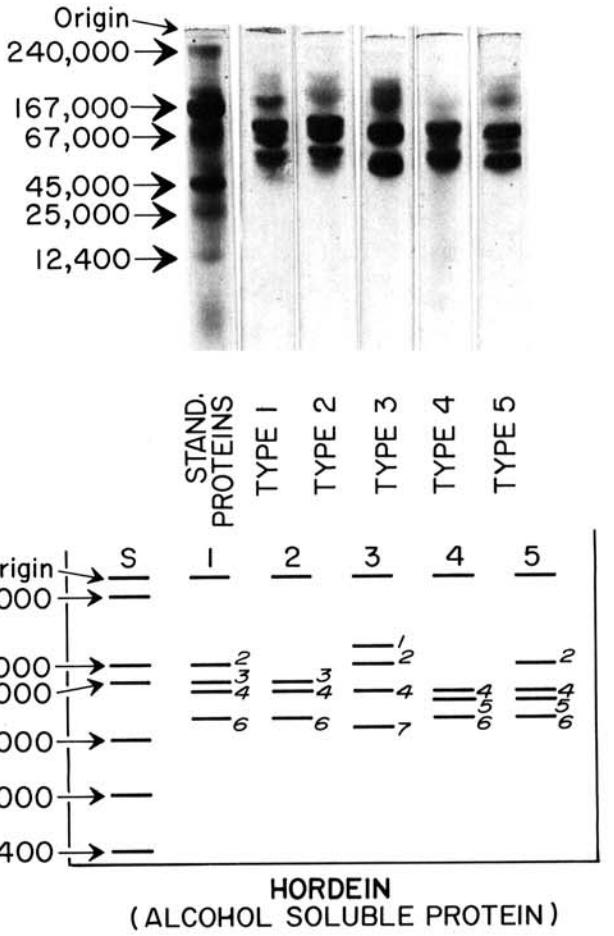

Fig. 3. Electrophoretic patterns and their diagrammatic illustration of five types of component combinations in hordein (alcohol-soluble protein) from 23 barley cultivars.

TABLE III
Percentage Contents of the Electrophoretic Components in the Osborne Fractions of Various Samples of Barley

CI Number	Barley Cultivar	Electrophoretic Components (%)														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Albumin-Globulins																
3383	Bargiers	...	4	7	26	17	13	...	...	13	...	...	20			
7622	Lenta	6	...	7	25	22	4	8	...	...	11	...	17			
10375	...	2	...	4	26	25	...	11	7	...	5	...	20			
12099	...	...	5	8	23	24	...	10	5	...	5	...	20			
3947	Hiproly	...	...	...	22	27	...	8	...	...	11	9	23			
13827	Shabet	...	2	5	23	26	...	9	7	...	6	...	22			
Hordein																
10236	...	...	9	38	25	...	28	...								
3383	Bargiers	...	...	35	34	...	31	...								
10375	...	7	10	...	43	...	...	40								
3947	Hiproly	...	...	...	45	9	46	...								
10421	Unitan	...	4	...	42	14	40	...								
Glutelin																
10421	Unitan	9	...	7	...	...	20	13	...	18	16	...	...	...	17	...
7622	Lenta	7	...	8	...	...	24	...	...	22	13	...	...	7	18	...
10375	...	...	9	...	10	8	...	...	25	...	...	...	21	...	...	27
12147	Imperial	...	9	...	10	8	...	...	24	...	...	27	...	...	22	...

types of combinations. Data for albumin-globulin proteins indicated that those components varied in concentration from 22 to 26%, 17 to 27%, and 17 to 23% for components 4, 5, and 12, respectively (Table III). Corresponding molecular weights were about 60,000, 45,000, and 12,000. Components 3, 7, and 10 occurred in five out of the six types of combinations, while components 1, 2, 6, 8, and 9 occurred in either two or three combinations. Singh and Sastry (1977), working with four different cultivars of barley including Hiproly, reported albumin contained seven distinct and three faint electrophoretic components, whereas globulin contained nine. Concentration differences in these components determined by densitometry have also been reported (Singh and Sastry 1977).

Seven total components were observed in the five types of component combinations found in hordeins of the 23 barley cultivars (Fig. 3). Component 4, a major component, had a molecular weight of about 62,000, occurred in all 23 varieties of barley cultivars, and ranged in concentration from 25% in CI 10236 to 45% in Hiproly (CI 3947) (Table III). Component 6 was another major component with a molecular weight of about 50,000 that occurred in four out of five types and varied in concentration from 28% in CI 10236 to 46% in Hiproly. Component 1 was a very minor component with a molecular weight of about 58,000 and occurred in the two types of component combinations represented by Hiproly and Unitan (CI 10421) (Fig. 3). Component seven was a major component with a molecular weight of 47,000 and occurred in only one type represented by CI 10375. Singh and Sastry (1977) reported the occurrence of eight components in the hordein from four different cultivars of barley, with one component occurring in all cultivars at varied concentrations.

Fifteen electrophoretic components, determined by both electrophoretic mobilities and molecular weight, were found in four types of component combinations of glutelin (Fig. 4). Glutelin showed heavy protein concentration at the electrophoretic pattern origin indicating presence of high molecular weight components that were unable to penetrate the gel. Components 1, 2, 3, 4, and 5 occurred in two types of combinations and ranged in molecular weights from about 185,000 to 250,000. These five components were all minor components as indicated by their percentage concentration (Table III). Component 6 was a major component, had a molecular weight of about 64,000, and occurred in the cultivars Unitan (CI 10421) and Lenta (CI 7622). Components 8, 9, and 10 were major components occurring only in one type of combination represented by CI 10375.

The glutelin fractions illustrated in Fig. 4 were not alkylated and were prepared for electrophoresis in a manner similar to the albumin-globulin and hordein. Singh and Sastry (1977) failed to separate the glutelin fraction into separate components by electrophoresis without alkylation. They reported six components in the alkylated product varying in proportion in the four barley cultivars investigated.

Differences in concentrations of protein components found in the various types of combinations in albumin-globulin, hordein, and glutelin (Table III) may be important indicators of the nutritive value of barley, especially in the case of albumin-globulin and glutelin, which have been shown to possess high contents of essential amino acids (Nelson 1969).

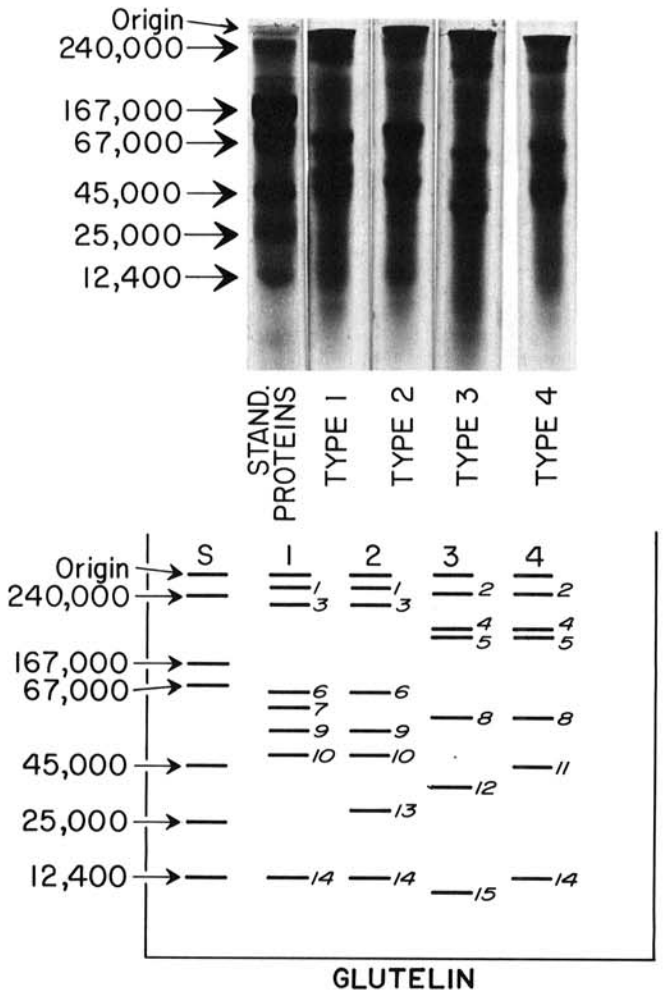

Fig. 4. Electrophoretic patterns and their diagrammatic illustration of four types of component combinations in glutelin (alkaline-soluble protein) from 23 barley cultivars.

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