Amino Acid Composition of Two-Rowed and Six-Rowed Barleys

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Kernel weight, protein content, and amino acid composition were determined in isogenic pairs of 2-rowed and 6-rowed barleys and in 2-rowed and 6-rowed backcrosses to a 2-rowed barley cultivar (Bonneville). The 2-rowed selections were higher both in kernel weight and in protein content than the 6-rowed selections. In addition, the proteins in 2-rowed selections contained more glutamic acid and proline (and less of most of the other amino acids) than the proteins of the 6-rowed selections. Amino acids were determined in proteins of 15 2-rowed and 21 6-rowed cultivars (grown for 2 years), each from two locations which consistently produced barleys varying widely in protein content. The results showed that the amino acid composition in the two types of barleys (2-rowed and 6-rowed) depended on their protein contents. It is concluded that amino acid composition in proteins of 2-rowed and 6-rowed barleys is governed by the total protein content, rather than type of barley. There was a highly significant, positive correlation between the lysine and aspartic acid contents of proteins in all types and groups of barleys that were studied.

The two main types of malting barleys (depending on the arrangement of the grains in the ear) are 2-rowed and 6-rowed. In the U.S.A., approximately 90% of malt is made from the midwestern 6-rowed Manchurian type barley or varieties developed from it. These barleys are relatively small-kerneled, medium high in protein, and produce high enzymatic activities during malting (Kneen and Dickson, 1967). The western 2-rowed barleys have a medium-sized, uniform, plump kernel with a thin hull. The best samples are relatively low in protein and high in starch. Protein content is an important specification in malting barley; high protein levels impair modification of malt, decrease malt extract, and affect beer quality. According to recommendations of the Malting Barley Improvement Association (1971), preferred upper protein limit (dry basis) for midwestern 6-rowed types is 12.5% and for western 2-rowed types is only 12.0%.

Day and Dickson (1957) studied linkage associations between nitrogen percentages and several visible morphological characteristics of known inheritance. They found that nitrogen percentage was associated with the 2-rowed *vs.* 6-rowed character. In a series of Alpha × O.A.C. 21 crosses, the 6-rowed segregates had a mean barley nitrogen percentage significantly lower than that of the 2-rowed segregates. In a survey of 1110 samples of barleys grown in 1969, evaluated by the National Barley and Malt Laboratory, the average grain protein was 11.9 and 12.6% in 6-rowed and 2-rowed barleys, respectively (Standridge *et al.*, 1970).

The present work is concerned with inherent protein content and amino acid composition of 6-rowed and 2-rowed barleys grown in the U.S.A.

MATERIALS AND METHODS

The 2-rowed and 6-rowed barleys used in this investigation are divided into three groups: isogenic lines developed by inbreeding with forced selection at the 2- *vs.* 6-rowed Vv locus; isogenic lines developed by backcrossing; and 2-rowed and 6-rowed cultivars from Uniform Nurseries. A pair of isogenic lines is assumed to differ by a single gene, although more accurately pairs differ by small gene blocks identified by a marker gene—in this case VV (2-rowed) or vv (6-rowed). Some pairs of samples also differed in head morphology (lax and dense types) and ii (small lateral) or II (large lateral) kernels (for description of nomenclature and abbreviations see Nilan, 1964).

Inbred-Derived Isogenic Lines. We used ten pairs of isogenic lines developed by Dr. G. A. Wiebe from crosses of Manchuria/Abed Binder, Manchuria/Valki, Manchuria/CI 5037, Manchuria/Anatolian Black, Manchuria/Poppenheim, Manchuria/Kolter, Manchuria/Nesbit, Manchuria/Plumage, Manchuria/CI 3345, and Manchuria/CI 4254-1. The selfed generation in which the two homozygous lines were selected ranged from F_{28} to F_{31}. The pairs were grown under irrigation at Aberdeen, Idaho, in 1964 and 1968. Kernel weight and protein content were determined in ten pairs from both crop years; amino acids were determined in ten pairs from 1968 only.

Backcross-Derived Isogenic Lines. Bonneville and four pairs of lines developed at Bozeman, Mont., from crosses of Compana/7 × Bonneville were grown at 12 locations in 1971. The genotypes of the eight isogenics were VVii dense, vvii dense, VVii lax, vvii lax, VVII dense, vvII dense, VVII lax, and vvII lax. Samples from Akron and Fort Collins, Colo., Moro, Ontario, and Pendleton, Ore., Aberdeen, Sandpoint, and Twin Falls, Idaho, Tulelake, Calif., Logan, Utah, and Laramie and Sheridan, Wyo., were evaluated. Kernel weight and protein content were determined in the eight isogenics and the Bonneville parent was determined from each of the 12 locations. For determination of amino acids we selected three locations which produced barleys with the highest (Sandpoint, Idaho), lowest (Twin Falls, Idaho), and intermediate (Fort Collins, Colo.) protein contents.

Western 2-Rowed and Mississippi Valley 6-Rowed Uniform Nurseries. Fifteen 2-rowed and 21 6-rowed barley cultivars were grown for 2 years (1969 and 1970) at two locations. The locations were selected on the basis of their producing consistently low- and high-protein barleys. The 2-rowed barleys were from Delta, Colo., (high) and Corvallis, Ore. (low protein); the 6-rowed barleys were from Carrington, N. D. (high) and Morris, Minn. (low protein). All 144 samples from uniform nurseries were analyzed for kernel weight, protein content, and amino acid composition.

Analytical Determinations. Crude protein (by the Kjeldahl method) and amino acid analyses of acid hydrolyzates (on a Beckman 121 automatic amino acid analyzer) were performed as described elsewhere (Robbins *et al.*, 1971). Crude protein was estimated by multiplying the nitrogen concentration by 6.25 and is reported on a moisture-free basis. Amino acid values are reported in grams of amino acid per 100 g of amino acid recovered. Recoveries were at least 90%.

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Table I. Ranges and Means of Kernel Weights and Protein Contents of Ten Pairs of Isogenic Lines Grown in Aberdeen, Idaho, in 1964 and 1968

	1964				1968			
	2-Rowed		6-Rowed		2-Rowed		6-Rowed	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Kernel weight, mg	42.8–60.1	51.5	30.5–40.7	34.6	34.8–53.6	46.5	23.5–36.4	29.3
Protein (N × 6.25, % in dry matter)	16.8–19.3	18.3	14.5–15.8	15.1	17.2–19.8	18.3	14.2–15.8	14.9

RESULTS AND DISCUSSION

Ranges and means of kernel weights and protein contents of ten pairs of isogenic lines grown at Aberdeen, Idaho, in 1964 and 1968 are summarized in Table I. The kernels from 2-rowed barleys were heavier than the kernels from 6-rowed barleys; the 2-rowed barleys also contained about 3% more protein than the 6-rowed barleys. A similar pattern can be observed by comparing the results for the 2- and 6-rowed Bonneville isogenics and Bonneville (Table II). Isogenics with dense spikes averaged higher in kernel weight and were slightly higher in protein content than isogenics with lax spikes.

To simplify presentation of the large volume of data, for all results of amino acid analyses only means of groups of samples and their statistical evaluation are given.

Amino acid compositions of the various isogenic pairs and Bonneville are given in Table III. In both groups, there are consistent and identical patterns of amino acid composition. The protein-rich 2-rowed barleys contained more glutamic acid and proline (and probably phenylalanine) and less of most of the other amino acids than the 6-rowed barleys.

Statistical evaluation of correlation coefficients among the amino acids and between amino acids and protein for the various isogenic lines is given in Table IV. Several parameters are highly correlated in all four groups of barleys. In addition, specific parameters showed either positive or negative significant correlations in the four groups of samples. In several instances, correlation coefficients are lower in the two groups of inbred-derived isogenic lines (AB) than in the two groups of backcross-derived lines (CD). This may have resulted to some extent from the small differences in numbers of degrees of freedom in calculation of statistical results. It seems, however, that the higher correlations for the backcross-derived Bonneville isogenics may be related to their more homogenous parentage than in the case of inbred-derived isogenic lines. In the Bonneville isogenics, there were only two original parents (Compana and Bonneville); in the inbred-derived isogenics, there was one common 6-row parent (Manchuria) but ten different 2-row parents.

If the amino acid composition of the protein was governed by the 2-rowed or 6-rowed character, one would expect significant correlations for the pairs of 2- and 6-rowed barleys (AC and BD in Table IV). However, only the correlations between glutamic acid and protein and between proline and tyrosine were significant for the 6-rowed and insignificant for the 2-rowed barleys. It would seem, therefore, that amino acid composition is not governed by 2- and 6-rowed character. The amino acid composition in the two types depended on their protein contents.

As the 2-rowed and 6-rowed varieties differed consistently in protein content, we examined samples of 2-rowed (15 cultivars grown for 2 years) and of 6-rowed (21 culti-

Table II. Varietal Ranges and Means of Kernel Weights and Protein Contents of Nine Barley Selections Grown at 12 Locations in 1971

	Kernel weight, mg		Protein (N × 6.25, % in dry matter)	
Variety	Range	Mean	Range	Mean
Bonneville	31.3–49.4	43.1	9.3–17.6	13.2
2-Rowed		54.5		15.6
VVii dense	44.9–65.5	56.3	12.1–19.8	15.5
VVii lax	44.7–62.8	54.3	11.1–19.6	15.4
VVII dense	44.7–62.8	55.5	11.4–19.7	16.0
VVII lax	43.6–63.2	51.5	13.1–18.6	15.6
6-Rowed		41.6		13.7
vvii dense	32.0–48.0	43.1	9.3–17.9	14.4
vvii lax	33.6–46.7	40.3	11.6–18.0	14.3
vvII dense	31.8–48.6	42.3	9.4–16.5	13.1
vvII lax	32.2–47.5	40.6	9.6–16.4	13.0

Table III. Mean Kernel Weights, Protein Contents, and Amino Acid Composition of Ten Pairs of Isogenic Lines, Three Bonneville Barleys, and Twelve 2-Rowed and Twelve 6-Rowed Crosses to Bonneville Barleys

	Isogenic barleys		Bonneville crosses		
	2-Rowed	6-Rowed	Parent	2-Rowed	6-Rowed
Kernel weight, mg	46.5	29.3	46.7	59.2	44.7
Protein, % (PCP)	18.3	14.9	13.4	16.3	13.6
Lysine (Lys)	3.4	3.7	3.6	3.2	3.4
Histidine (His)	2.1	2.2	2.3	2.1	2.2
Ammonia (NH_3)	3.2	3.0	3.1	3.2	3.1
Arginine (Arg)	4.2	4.6	4.5	4.2	4.5
Aspartic acid (Asp)	6.0	6.6	6.6	5.8	6.3
Threonine (Thr)	2.8	3.1	3.3	3.1	3.2
Serine (Ser)	3.4	3.6	3.9	3.7	3.7
Glutamic acid (Glu)	27.8	25.8	26.4	28.0	26.9
Proline (Pro)	14.6	13.5	12.5	13.9	13.1
Cystine (Cys)	0.9	0.9	0.9	1.1	1.1
Glycine (Gly)	3.4	3.8	4.2	3.8	4.0
Alanine (Ala)	4.0	4.3	4.0	3.6	3.8
Valine (Val)	4.8	5.2	5.2	4.9	5.1
Methionine (Met)	2.0	2.2	2.2	2.2	2.4
Isoleucine (Ile)	3.5	3.6	3.6	3.5	3.5
Leucine (Leu)	6.6	6.8	6.6	6.4	6.5
Tyrosine (Tyr)	1.7	1.9	1.9	2.1	2.2
Phenylalanine (Phe)	5.4	5.2	5.2	5.3	5.2

Table IV. Statistical Evaluation[a] of Variables in Isogenic Lines and Backcrosses to Bonneville Barleys[b]

	PCP[c]	Lys	His	Arg	Asp	Thr	Ser	Glu	Pro
Lys	-(bCD)								
His	-(CD)	abCD							
Arg	-(D)	aD	D						
Asp	-(bcD)	ABCD	ACD	ACD					
Thr	-(cD)	bCD	CD	D	abCD				
Ser									
Glu	BD	-(ACD)	-(CD)	-(ACD)	-(ACD)	-(aCD)			
Pro	CD	-(aCD)	-(ACD)	-(aCD)	-(aCD)	-(CD)		CD	
Cys	-(C)			b		A	-(CD)		
Gly	-(BCD)	ABCD	abCD	aD	ABCD	BCD		-(abCD)	-(aCD)
Ala	-(CD)	aBCD	CD	CD	bCD	CD		-(CD)	-(CD)
Val	-(cD)	CD	CD	D	bCD	bCD		-(CD)	-(CD)
Met	-(d)	d	d	D	cD	d	-(B)	-(CD)	-(cD)
Ile	-(D)	CD	CD	D	CD	CD		-(CD)	-(cD)
Leu	-(cD)	CD	CD	D	CD	CD		-(CD)	-(cD)
Tyr		d		ABCD	D	d		-(aD)	-(BD)
Phe	D	-(cD)	-(d)	-(CD)	-(CD)	-(CD)		CD	cD

	Cys	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe
Lys									
His									
Arg									
Asp									
Thr									
Ser									
Glu									
Pro									
Cys									
Gly									
Ala		CD							
Val	-(b)	CD	bCD						
Met	A	D	CD	cd					
Ile		CD	cD	CD					
Leu		CD	CD	CD		aCD			
Tyr	ABC	D	D	D	aD	d			
Phe		-(CD)	-(CD)	-(CD)	-(CD)	-(CD)	-(CD)	-(D)	

[a]Significant correlation coefficients; small letters above 5%, capital letters above 1% of significance. [b]A or a, 2-rowed isogenics; B or b, 6-rowed isogenics; C or c, 2-rowed Bonneville backcrosses; D or d, 6-rowed Bonneville backcrosses. [c]For description of abbreviations see Table III.

vars grown also for 2 years). Each type from two locations which consistently produced barleys varying widely in protein contents was examined. The results are summarized in Table V. Coefficients of variation (ranges of averages for the four groups, *i.e.*, 2-rowed and 6-rowed each from two locations) reflect combined varietal differences and accuracy of the assay procedure. The effect of the latter is much larger in the assay of methionine and cystine than in the assay of the other amino acids. The amino acid patterns for the 2-rowed barleys (as well as for the 6-rowed cultivars) depended on the protein content of the samples. High-protein samples (in either type) contained more glutamic acid and proline and less of most of the other amino acids than low-protein samples.

The results of this study indicate that protein content governs amino acid composition in 2-rowed and 6-rowed barleys. This is different from the situation in Hiproly barley, a naked cultivar discovered by Swedish workers and shown to contain high levels of protein and high concentrations of lysine in the protein (Munck *et al.*, 1970). In Hiproly, the genes controlling protein content, lysine concentration in the protein, and kernel development (absence of shrunken grains) are not genetically linked.

As indicated previously, 2-rowed isogenics were higher in protein than 6-rowed barleys. It should be emphasized, however, that the 2-rowed barleys yielded less than the 6-rowed isogenics. The lower yield, probably, contributed to increased protein although the quantitative correlation between these two parameters has not been established. In addition, there is apparently enough variation in germ plasm of 2-rowed varieties to select agronomically desirable cultivars with low-protein content.

Statistical evaluation of amino acid patterns in proteins from all types and groups of barley that were studied pointed to several important correlations. This is illustrated by the results of Table VI, which show amino acids with consistently highest correlations with lysine, the nutritionally limiting amino acid of barley. The results indicate, as expected, a consistently high negative correlation between lysine and the main amino acids of storage proteins in cereals (glutamic acid and proline; the latter not shown in Table VI). The results also show that lysine is very highly correlated with aspartic acid. (The correlations between aspartic acid and either glutamic acid or proline, not shown in Table VI, were negative and similar in magnitude to the correlations between lysine and those two amino acids.) The high and positive correlation between lysine and aspartic acid is of interest, as recent studies from our laboratories have shown that high lysine in Hiproly is associated with high aspartic acid. It is

Table V. Mean[a] Kernel Weights, Protein Contents, Amino Acid Composition, and Coefficients of Variation of 2-Rowed and 6-Rowed Barleys Grown in Uniform Nurseries in 1969 and 1970

	2-Rowed		6-Rowed		Coefficients of variation
	Delta, Colo.	Corvallis, Ore.	Carrington, N. D.	Morris, Minn.	Range of averages for the four groups
Kernel weight, mg	42.9	39.5	30.9	30.0	4.4–6.4
Protein, %	14.1	9.6	14.9	12.4	4.4–7.6
Lysine	3.6	4.2	3.5	3.8	5.5–7.9
Histidine	2.3	2.4	2.3	2.4	5.5–7.9
Ammonia	3.2	2.9	3.3	3.3	4.5–8.9
Arginine	4.8	5.2	4.9	5.2	6.6–8.7
Aspartic acid	7.0	7.5	6.4	6.8	5.4–6.5
Threonine	3.4	3.7	3.2	3.4	3.3–5.4
Serine	3.8	3.9	3.6	3.8	3.8–7.4
Glutamic acid	25.5	23.3	25.8	24.8	3.3–4.4
Proline	11.7	10.2	12.3	10.7	6.8–9.0
Cystine	1.2	1.1	1.3	1.3	12.1–21.0
Glycine	4.1	4.5	3.9	4.2	4.8–6.1
Alanine	4.2	4.6	3.8	4.2	5.2–6.8
Valine	5.2	5.4	5.1	5.3	2.4–4.2
Methionine	2.2	2.6	2.5	2.5	6.2–21.9
Isoleucine	3.7	3.8	3.6	3.7	2.0–3.3
Leucine	6.6	7.1	6.6	6.8	2.6–7.6
Tyrosine	2.3	2.6	2.7	2.6	11.1–12.9
Phenylalanine	5.1	5.0	5.2	5.2	2.9–4.2

[a]For 15 2-rowed and 21 6-rowed barley cultivars, each grown for 2 years.

Table VI. Simple Correlation Coefficients between Lysine and Protein and Lysine and Several Highly Correlated Amino Acids

	Lys *vs.*				
Group of barleys	PCP	Asp	Gly	Ala	Glu
Isogenics					
2-Rowed	−0.271	0.912	0.893	0.672	−0.814
6-Rowed	−0.666	0.869	0.784	0.807	−0.568
Bonneville back-crosses					
2-Rowed	−0.833	0.931	0.972	0.925	−0.852
6-Rowed	−0.963	0.930	0.982	0.886	−0.971
Uniform nurseries					
2-Rowed, Delta, Colo.	−0.378	0.686	0.575	0.701	−0.809
2-Rowed, Corvallis, Ore.	−0.451	0.592	0.526	0.709	−0.588
6-Rowed, Morris, Minn.	−0.545	0.701	0.596	0.653	−0.780
6-Rowed, Carrington, N. D.	−0.450	0.755	0.678	0.538	−0.822

known that aspartic acid is a key intermediate in the biosynthesis of lysine in bacteria, algae, and higher plants (Vogel, 1965).

LITERATURE CITED

Day, A. D., Dickson, A. D., *Agron. J.* **49,** 244 (1957).
Kneen, E., Dickson, A. D., *Kirk-Othmer Encycl. Chem. Technol.* **12,** 861 (1967).
Malting Barley Improvement Association, "Malting barley, protein content," Milwaukee, Wis., 1971.
Munck, L., Karlsson, K. E., Hagberg, A., Eggum, B. O., *Science* **168,** 985 (1970).
Nilan, R. A., "The Cytology and Genetics of Barley," Monograph Supplement No. 3, Washington State University, Pullman, Wash., 1964.
Robbins, G. S., Pomeranz, Y., Briggle, L. W., *J. Agr. Food Chem.* **19,** 536 (1971).
Standridge, N. N., Goplin, E. D., Pomeranz, Y., *Brew. Dig.* **45** (12), 58 (1970).
Vogel, H. J., *Evol. Genes Proteins Symp.* 25–40 (1965).

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