Amino Acid Composition of Selected Strains of Diploid Wheat, Triticum monococcum L.

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ABSTRACT

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Diploid wheat (*Triticum monococcum* L., einkorn) is of interest both as a crop and as a source of genes unavailable in the more widely grown wheat species, *T. aestivum* L. (common wheat) and *T. durum* Desf. (durum wheat). The biochemical constituents of its seeds, however, have been investigated very little. In this study, 15 strains of *T. monococcum* and two modern cultivars of common wheat and durum wheat were compared with regard to grain protein content and amino acid composition of seed proteins. Differences in amino acid composition between these three

Diploid wheat (Triticum monococcum L., einkorn) was one of mankind's earliest plant domesticates and probably also the donor of the A genome to common wheat (T. aestivum L., genome AABBDD) and durum wheat (T. durum Desf., genome AABB) (Feldman 1976, Kerby and Kuspira 1988). At present, this ancient wheat is cultivated only in marginal farmlands of Yugoslavia, Turkey, and Italy (Vallega 1992, Ohta and Furuta 1993). However, recent reports suggest that it may still play a significant role in modern agriculture, both as a source of useful genes (Multani et al 1989) and as a crop in its own right (Vallega 1979, 1992; Waines 1983; Multani et al 1992; D'Egidio et al 1993). T. monococcum appears especially valuable for detecting recessive endosperm mutants (for high lysine, high amylose, etc.) already identified in diploid cereals such as maize and barley but as yet unavailable in the polyploid wheat taxa (Vallega 1977, 1978). Detection of such genes is expected to widen the scope of utilization of all the cultivable Triticum species and to enhance their nutritional value (Vallega 1978, Waines 1983, D'Egidio et al 1993). Einkorn has been surveyed for a number of agrobiological and technological characteristics (The 1975; Vallega 1978, 1979, 1992; Sharma et al 1981; Multani et al 1989; D'Egidio et al 1993; D'Egidio and Vallega 1994) as well as with respect to variation in grain protein and grain lysine content (Johnson and Mattern 1975; Vallega 1977, 1978; Sharma et al 1981; Blanco et al 1990), flour β -carotene content (D'Egidio et al 1993), and electrophoretical patterns of seed proteins (Waines and Payne 1987, Galili et al 1988). Data on the amino acid composition of einkorn's seed proteins have not been published.

The grain proteins of common wheat and durum wheat are notoriously rich in glutamine and proline, but low in amino acids considered essential for the human diet: especially lysine and threonine, possibly also tryptophan, methionine, and isoleucine (Wrigley and Bietz 1987). In these wheat species, as in other cereals, variation in amino acid composition is limited and very closely correlated with variation in grain protein percentage (Johnson and Mattern 1975; Mossé et al 1985, 1988; Martin del Molino et al 1989). Increases in grain protein percentage are normally accompanied by lower levels of lysine and other essential amino acids (EAA) per unit protein (Johnson and Mattern 1975, Mossé et al 1985). Major genes capable of substantially altering the nutritional adequacy of wheat proteins have been sought without

¹Istituto Nazionale della Nutrizione, Via Ardeatina 546-00179 Rome, Italy. ²Istituto Sperimentale per la Cerealicoltura, Via Cassia 176-00191 Rome, Italy. species of wheat and among diploid wheat strains were minimal, especially after amino acid values were adjusted to a common protein level. One of the einkorn accessions examined, however, presented a deviant amino acid profile. This strain may be of use for investigating the biosynthetic pathway of wheat proteins. Among einkorns, correlations between grain protein content and amino acid values were positive for glutamine and proline and negative for threonine, 1/2 cystine, valine, isoleucine, leucine, asparagine, serine, glycine, and alanine.

success in various *Triticum* species, including *monococcum* (Johnson and Mattern 1975; Vallega 1977, 1978; Sharma et al 1981).

The main goals of the present work were to determine the amino acid composition of T. monococcum's grain proteins, examine its relationship with grain protein percentage, and seek diploid wheats with a more balanced amino acid composition.

MATERIALS AND METHODS

The wheats examined comprised a free-threshing diploid wheat strain (T. monococcum ssp. sinskajae Filat. et Kurk., accession WIR 48993), 14 tenacious-glumed einkorns (T. monococcum ssp. monococcum), and two modern cultivars of bread wheat (cv. Centauro) and durum wheat (cv. Latino). Diploid wheat accessions were chosen on the basis of some favorable agronomical characteristic (Vallega 1992) and included strains with reportedly disparate grain protein contents (Johnson and Mattern 1975, Sharma et al 1981). Diploid and polyploid wheat entries were grown together in a field trial near Rome during 1988-89 in 10-m² plots distributed according to a completely randomized block design with three replicates. Data on their agronomical performance (Vallega 1991, 1992) and on their rheological and technological properties (D'Egidio et al 1993, D'Egidio and Vallega 1994) were presented elsewhere. All entries were threshed mechanically, and residual invested einkorn seeds were dehulled by hand. Samples for amino acid determinations were obtained by mixing 3 g of clean seed from each of three replicates. Grains were ground with a 0.129-mm sieve Cyclotec mill (Tecator, Höganäs, Sweden). Protein content of meals was determined by the Kjeldahl method (% N \times 5.7 dm). Hydrolyzates were prepared according to the procedure described by Satterlee et al (1982), using 250-mg meal subsamples. Meals were dispersed in 25 ml of 6N HCl for 24 or 72 hr at 110°C under vacuum, and then derivatized with ninhydrin. Performic acid oxidation, followed by acid hydrolysis, was used for cystine-cysteine and methionine determinations. Amino acid analyses were made by ion-exchange chromatography on a Liquimat III Automatic Analyser (Kontron-Labotron, Munich, Germany) using a single column (30 cm \times 4 mm i.d.) and an ion exchange Dionex DC-6A resin. Tryptophan was not determined. Results, expressed as grams of amino acid per 100 g of protein corrected to 100% recovery, are the average of three replicates. To permit direct comparisons between wheats with disparate grain protein contents, linear regression, as described by Steel and Torrie (1980), was utilized to adjust the amino acid composition of all entries to a common (16.7%) protein level. Only data collected on monococcums were used to compute regression equations. Outlying amino acid values were sought by the robust

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statistical procedure described by Miller (1993), using computer software supplied by M. Mecozzi.

RESULTS AND DISCUSSION

Amino acid values and protein contents for the 17 wheats examined are given in Tables I and II. Grain protein content of einkorns (mean: 16.7% dm) was extremely variable (from 14.1 to 25.2%). On average, about 3-4 percentage points higher than that recorded on cvs. Centauro and Latino. Despite this conspicuous protein content difference, the average amino acid composition of monococcum proteins was analogous to that of the two polyploid wheat controls and to that generally reported for common wheats and durums (Baroccio and Alessandroni 1978,

TABLE I
Amino Acid Composition ^a of Grain Proteins of Fifteen Diploid Wheats and Two Polyploid Wheats, Part 1

	Thr	Cys/2	Val	Met	Ile	Leu	Tyr	Phe	Lys
Diploid wheats									.
CI 13962 ^b	2.28	2.49	2.90	1.64	2.71	5.15	2.22	4.74	2.14
CI 13961	2.48	2.92	4.18	1.88	4.16	6.80	2.46	4.90	2.64
CI 13963	2.56	2.89	4.15	1.67	3.90	6.19	3.19	5.08	2.12
PI 167526	2.52	2.92	4.15	1.70	3.87	6.25	2.54	5.60	2.78
PI 290508	2.77	3.02	4.24	1.89	3.72	5.92	2.62	4.36	2.65
VV 319	2.71	2.73	4.29	1.71	3.75	6.12	2.93	5.06	2.03
WIR 8365	2.73	2.65	4.55	1.64	3.94	6.98	3.74	5.84	2.27
PI 277133	2.42	2.83	4.38	1.78	4.13	8.10	4.09	4.21	2.77
VV 281	2.70	2.99	4.02	2.00	3.65	5.95	2.65	4.14	2.63
WIR 48993	2.69	2.77	3.98	1.73	3.80	6.43	3.50	4.44	2.69
CI 2433	2.69	2.92	3.94	1.58	3.81	6.73	3.97	5.55	2.45
PI 277123	2.62	2.93	4.41	1.78	4.05	6.51	2.84	4.76	2.77
PI 277130	2.72	2.97	3.83	1.66	3.57	6.17	4.54	5.00	2.74
PI 221413	2.62	2.89	4.48	1.83	3.90	6.39	2.73	6.03	2.86
PI 306545	2.40	2.94	4.40	1.77	3.96	6.64	2.95	4.52	2.69
Mean $(n = 15)$	2.59	2.86	4.13	1.75	3.79	6.42	3.13	4.95	2.60
SEM	.038	.037	.103	.029	.088	.166	.177	.152	.061
CV ^c	5.61	5.00	9.67	6.47	9.03	9.98	21.86	11.90	9.14
r ^d	-0.573*	-0.597*	-0.732**	-0.171	-0.662**	-0.484	-0.506	-0.083	-0.642**
Polyploid wheats							0.000	0.005	0.042
Latino	2.91	2.99	4.45	1.89	3.65	6.70	3.02	4.75	2.85
Centauro	2.81	2.75	4.11	1.74	3.52	6.60	2.98	4.32	2.56

^aGrams of amino acid per 100 g of protein corrected to 100% recovery, protein basis.

^bPrefixes WIR, CI or PI, and VV refer, respectively, to accessions from the N.I. Vavillov All-Union Institute of Plant Industries (Russia), the USDA Small Grains Collection, and the Cereal Research Institute (Italy).

^cCoefficient of variation.

^d Simple correlation coefficients between amino acid values and protein content; * and ** are significant at the 0.05 and 0.01 probability level, respectively.

	EAAs ^b	Asp	Ser	Glu	Pro	Gly	Ala	His	A	NIT	~ N D (Protein
	EAAS	Азр	50	Giu	110	GIY	Ala	riis	Arg	NH ₃	% N Rec ^c	% d.m.
Diploid wheats												
CI 13962 ^d	26.27	3.88	3.57	35.40	15.94	2.74	2.66	2.91	4.18	2.48	116.40	25.17
CI 13961	32.42	4.66	4.07	34.42	9.04	3.08	3.02	2.16	3.96	3.17	106.33	18.86
CI 13963	31.75	4.40	4.07	34.49	10.86	2.90	2.92	2.17	3.65	2.79	109.46	18.72
PI 167526	32.33	5.40	3.95	32.30	9.01	3.43	3.26	2.21	4.98	3.13	106.21	18.12
PI 290508	31.19	5.38	4.13	33.35	9.81	3.38	3.34	2.26	4.28	2.88	106.03	17.03
VV 319	32.03	5.35	4.10	32.33	9.59	3.41	3.37	2.43	4.33	3.06	105.79	16.23
WIR 8365	34.34	4.60	3.96	27.79	10.31	3.26	3.58	2.83	6.73	2.60	107.51	16.12
PI 277133	34.71	5.32	3.78	28.97	10.47	3.37	3.36	2.38	4.57	3.07	110.14	16.02
VV 281	30.73	5.42	4.07	32.08	11.63	3.43	3.41	1.98	4.37	2.88	110.19	15.99
WIR 48993	32.03	5.54	4.44	32.03	9.62	3.26	3.32	2.17	4.21	3.38	106.89	15.36
CI 2433	33.64	5.31	4.02	31.28	9.96	3.22	3.20	2.18	3.99	3.20	110.78	15.23
PI 277123	32.67	5.48	4.36	31.58	9.15	3.41	3.32	2.42	4.38	3.23	102.43	14.48
PI 277130	33.20	5.23	4.00	28.36	9.77	3.28	3.21	2.33	5.73	4.89	111.09	14.16
PI 221413	33.73	5.85	3.93	30.27	9.01	3.74	3.46	2.23	4.77	3.01	103.91	14.12
PI 306545	32.27	5.53	4.01	30.62	10.66	3.42	3.28	2.39	4.38	3.44	103.21	14.05
Mean $(n = 15)$	32.22	5.16	4.03	31.68	10.32	3.29	3.25	2.34	4.57	3.15	102.22	16.66
SEM	.513	.136	.053	.577	.447	.062	.059	.064	.199	.142	.964	.734
CV ^e	6.16	10.24	5.11	7.05	16.76	7.30	7.07	10.63	16.87	17.48	3.47	17.08
r	-0.788**	-0.832**	-0.554*	0.712**	0.743**	-0.782**	-0.794**	0.429	-0.267	-0.514*	0.627*	17.00
Polyploid wheats								0.747	0.207	0.514	0.027	
Latino	33.21	4.92	4.41	28.70	11.50	3.61	3.53	2.30	4.70	3.10	100.34	13.42
Centauro	31.39	4.77	4.40	30.45	11.99	3.85	3.38	2.03	4.44	3.30	100.34	13.42

 TABLE II

 Amino Acid Composition^{*} of Grain Proteins of Fifteen Diploid Wheats and Two Polyploid Wheats, Part 2

^aGrams of amino acid per 100 g of protein corrected to 100% recovery, protein basis.

^bTotal essential amino acids.

^cPercentage of nitrogen recovered by amino acid analysis.

^dPrefixes WIR, CI or PI, and VV refer, respectively, to accessions from the N.I. Vavillov All-Union Institute of Plant Industries (Russia), the USDA Small Grains Collection, and the Cereal Research Institute (Italy).

^eCoefficient of variation.

^fSimple correlation coefficients between amino acid values and protein content; * and ** are significant at the 0.05 and 0.01 probability level, respectively.

Wrigley and Bietz 1987, Martin del Molino et al 1989). Total EAA content was also nearly identical for diploid wheats (mean 32.2%) and for the two controls (mean 32.3%). Variation in amino acid values among monococcums was relatively low, except for tyrosine (CV 21.9%), arginine (CV 16.9%), and proline (CV 16.8%). Among these wheats, simple correlation coefficients between grain protein percentage and amino acid values (Table I) were mostly significant (P = 0.05); positive for glutamine and proline, and negative for threonine, 1/2 cystine, valine, isoleucine, lysine, asparagine, serine, glycine, alanine, amide, and total EAA

content. Analogous correlations were reported for common wheat (Mossé et al 1985, Martin del Molino et al 1989). As might be expected, accession CI 13962, a low-yielding diploid wheat (Vallega 1992) with a grain protein as high as 25%, had a relatively low total EAA content (26.3%) and either the highest or lowest values for 13 of the 17 amino acids considered. Accessions PI 277133 and WIR 8365, on the contrary, exhibited relatively high values for both grain protein content (16.0–16.1%) and total EAA content (34.1–34.4%). Fifteen of the amino acid values recorded on monococcums were classed as outliers; eight of these deviant

TABLE III Amino Acid Composition^a of Grain Proteins of Fifteen Diploid Wheats and Two Polyploid Wheats Adjusted to a Common Protein Level (16.7%), Part 1

	Adjusted to a Common Protein Level (16.7%), Part 1													
	Thr	Cys/2	Val	Met	Ile	Leu	Tyr	Phe	Lys					
Diploid wheats	······································													
CI 13962 ^b	2.53	2.75	3.76	1.70	3.39	6.08	3.26	4.89	2.60					
CI 13961	2.54	2.99	4.41	1.89	4.33	7.04	2.73	4.94	2.76					
CI 13963	2.62	2.95	4.37	1.68	4.06	6.41	3.44	5.12	2.24					
PI 167526	2.56	2.97	4.30	1.71	3.98	6.41	2.72	5.63	2.87					
PI 290508	2.78	3.03	4.28	1.89	3.75	5.96	2.67	4.37	2.68					
VV 319	2.69	2.72	4.25	1.71	3.71	6.07	2.88	5.05	2.71					
WIR 8365	2.71	2.64	4.50	1.64	3.89	6.92	3.67	5.83	2.25					
PI 277133	2.40	2.81	4.32	1.78	4.08	8.03	4.01	4.20	2.74					
VV 281	2.68	2.97	3.96	1.99	3.59	5.88	2.57	4.13	2.60					
WIR 48993	2.65	2.73	3.85	1.72	3.69	6.29	3.34	4.42	2.63					
CI 2433	2.64	2.88	3.80	1.57	3.69	6.57	3.80	5.53	2.38					
PI 277123	2.55	2.87	4.19	1.76	3.87	6.27	2.58	4.72	2.66					
PI 277130	2.64	2.90	3.58	1.64	3.37	5.90	4.24	4.96	2.61					
PI 221413	2.54	2.82	4.22	1.81	3.69	6.11	2.42	5.99	2.73					
PI 306545	2.32	2.86	4.14	1.75	3.75	6.36	2.63	4.48	2.56					
Mean $(n = 15)$	2.59	2.86	4.13	1.75	3.79	6.42	3.13	4.95	2.60					
SEM	.031	.029	.070	.028	.066	.145	.152	.152	.047					
CV ^c	4.59	3.98	6.61	6.28	6.74	8.73	18.85	11.86	6.99					
Polyploid wheats														
Latino	2.81	2.90	4.12	1.87	3.39	6.35	2.63	4.70	2.68					
Centauro	2.68	2.63	3.68	1.71	3.18	6.14	2.47	4.25	2.34					

^aGrams of amino acid per 100 g of protein corrected to 100% recovery, protein basis.

^bPrefixes WIR, CI or PI, and VV refer, respectively, to accessions from the N.I. Vavillov All-Union Institute of Plant Industries (Russia), the USDA Small Grains Collection, and the Cereal Research Institute (Italy).

^cCoefficient of variation.

 TABLE IV

 Amino Acid Composition^a of Grain Proteins of Fifteen Diploid Wheats and Two Polyploid Wheats

 Adjusted to a Common Protein Level (16.7%), Part 2

	Adjusted to a Common Protein Level (10.1%), Part 2													
	EAAs ^b	Asp	Ser	Glu	Pro	Gly	Ala	His	Arg	NH ₃	% N Rec			
Diploid Wheats														
CI 13962 ^d	30.96	5.20	3.91	30.63	12.08	3.30	3.21	2.59	4.80	3.33	109.38			
CI 13961	33.64	5.01	4.16	33.18	8.04	3.23	3.16	2.08	4.12	3.39	104.50			
CI 13963	32.89	4.72	4.15	33.32	9.92	3.04	3.06	2.10	3.80	3.01	107.75			
PI 167526	33.14	5.63	4.01	31.47	8.34	3.53	3.36	2.16	5.09	3.28	104.99			
PI 290508	31.40	5.44	4.14	33.13	9.63	3.41	3.37	2.25	4.31	2.92	105.71			
VV 319	31.80	5.29	4.08	32.56	9.78	3.38	3.35	2.45	4.30	3.02	106.13			
WIR 8365	34.05	4.52	3.94	28.08	10.55	3.23	3.55	2.85	6.69	2.55	107.94			
PI 277133	34.37	5.23	3.75	29.31	10.75	3.33	3.32	2.41	4.53	3.01	110.65			
VV 281	30.37	5.32	4.04	32.44	11.92	3.39	3.37	2.01	4.33	2.82	110.73			
WIR 48993	31.32	5.34	4.39	32.74	10.20	3.18	3.24	2.22	4.12	3.25	107.95			
CI 2433	32.86	5.09	3.96	32.07	10.60	3.13	3.11	2.24	3.89	3.06	111.94			
PI 277123	31.48	5.15	4.27	32.79	10.13	3.27	3.18	2.50	4.23	3.02	104.21			
PI 277130	31.83	4.85	3.90	29.74	10.89	3.12	3.05	2.43	5.55	4.65	113.13			
PI 221413	32.34	5.46	3.83	31.68	10.15	3.57	3.30	2.33	4.59	2.76	105.99			
PI 306545	30.84	5.13	3.91	32.07	11.83	3.25	3.12	2.49	4.19	3.18	104.35			
Mean $(n = 15)$	32.22	5.13	4.03	31.68	10.32	3.29	3.25	2.34	4.57	3.15	107.69			
SEM $(n - 15)$.316	.076	.044	.406	.298	.038	.036	.058	.191	.122	.752			
CV ^e	3.80	5.67	4.25	4.96	11.20	4.51	4.32	9.53	16.24	15.01	2.70			
Polyploid wheats	5.00	2.57			=-									
Latino	31.44	5.90	4.28	30.50	12.96	3.40	3.33	2.42	4.67	2.78	102.99			
Centauro	29.09	4.13	4.23	32.78	13.88	3.58	3.12	2.19	4.14	2.89	112.45			

^aGrams of amino acid per 100 g of protein corrected to 100% recovery, protein basis.

^bTotal essential amino acids.

^cPercentage of nitrogen recovered by amino acid analysis.

^dPrefixes WIR, CI or PI, and VV refer, respectively, to accessions from the N.I. Vavillov All-Union Institute of Plant Industries (Russia), the USDA Small Grains Collection, and the Cereal Research Institute (Italy).

^eCoefficient of variation.

values were detected in the amino acid profile of the high protein strain CI 13962.

Tables III and IV presents the amino acid composition of all entries following adjustment to a common protein level. Removal of the regression contribution resulted in somewhat higher total EAA content values for monococcums (mean 32.2%) than for the controls (mean 30.3%). The substantial analogy between the amino acid profiles of diploid and polyploid wheat entries, however, was not impaired. On the other hand, variation in amino acid values among diploid wheats decreased considerably and remained relatively high only for arginine (CV 16.2%) and tyrosine (CV 18.9%). Outlying values also diminished. Among einkorns, deviant data were observed only for accessions PI 277130 (5.55% arginine), PI 277133 (8.03% leucine), and WIR 8365 (6.69% arginine). The corrected amino acid profiles of the first two of these accessions were otherwise similar to those observed for other monococcums. Accession WIR 8365, in contrast, presented not only a deviant value for arginine, but also the highest or lowest absolute values for seven other protein components (including amide) and extreme values for threonine, methionine, leucine, phenylalanine, lysine and total EAA content. Because of its deviant amino acid composition, the latter accession will be of use for investigating the biosynthetic pathway of wheat proteins.

CONCLUSIONS

The amino acid composition of diploid wheat seed proteins, as well as their presumptive nutritional adequacy and association with total seed protein content were found to be practically identical to those previously reported for the cultivated polyploid wheats. Differences in amino acid composition among the 15 einkorn strains assayed were generally small, and the differences became minimal after adjustment to a common protein level. One strain, however, exhibited a clearly disparate amino acid profile, involving 8–13 protein components. Wheats with a deviant amino acid profile had not been reported before.

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