Variability in Gliadin Electrophoregrams and Hardness of Individual Wheat Kernels Selected from Foundation Seed on the Basis of Grain Morphology

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ABSTRACT

Sixty wheat kernels from foundation seed, representing one soft red winter, two hard red spring, and six hard red winter wheat cultivars, were each characterized three ways: 1) by the Federal Grain Inspection Service according to morphological and apparent textural characteristics, 2) according to hardness on the basis of instrumental crushing characteristics, and 3) according to polyacrylamide gel electrophoretic (PAGE) patterns of gliadin proteins. On the basis of grain morphology and apparent texture, 24 kernels differed from typical kernels of certified wheat cultivars. Seven kernels had intermediate hardness characteristics and seven kernels had hardness characteristics different from those expected of the typical cultivars. PAGE patterns of 15 kernels deviated from typical PAGE patterns run on bulk samples. Deviations from expected hardness characteristics and PAGE patterns occurred both for kernels differing in morphology and among kernels considered typical of a cultivar on the basis of grain morphology and texture.

Key words: Wheat cultivar purity

Previous studies from our laboratories (Pomeranz et al 1984) have pointed to the limitations in testing for hardness in bulk wheat samples to determine the composition of commercial mixtures of hard and soft wheats. Four hardness methods were useful, in varying degrees, for determining admixtures of large amounts of one wheat class to another, provided that hardness of the individual wheats used for blending was known. Methods for hardness determination of bulk samples, however, are of little value for analysis of small amounts of unknown wheats of various classes. The usefulness of the tests for bulk samples is further limited since 3, 5, and 10% of wheat of classes (other than the designated one) are allowed by the U.S. Grain Standards for wheat for grades 1, 2, and 3, respectively.

To determine the composition of blends of wheat classes, hardness can be measured on single kernels (Lai et al 1985), but such determinations are subject to a large sampling error and are time-consuming. In addition, the varietal purity of samples must be known before valid conclusions about admixtures of other wheat kernels can be established.

This study determined the heterogeneity of reference foundation seed samples, obtained from plant breeders, which had been classified by the Federal Grain Inspection Service (FGIS-USDA) on the basis of accepted morphological characteristics. Gliadin electrophoregrams and hardness parameters of individual wheat kernels, identified by FGIS-USDA as typical and atypical of a variety based on apparent hardness and grain morphology, were used as the criteria of heterogeneity.

MATERIALS AND METHODS

Wheat Samples

Samples of nine cultivars, approximately 10 g each, were obtained from individual plant breeders as reference foundation seed wheats and were separated and classified according to grain morphology and apparent hardness (hard or soft) by FGIS. The wheats are listed in Table I.

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Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others not mentioned.

Hardness Testing

Four kernels, picked at random from each classified sample (Table I), were tested for hardness by the method of Lai et al (1985). The tester measures the compression force as a function of time; characteristics of the crushing curve are used to differentiate hard and soft wheats.

In the experimental procedure, a wheat sample is placed in the vibrating feeder, which aligns one kernel at a time to be picked up by a vacuum head, which places the kernel in the testing cup on a rotating disc. The kernel is crushed, and the crushing energy signal is recorded on a floppy disk for further data analysis.

Electrophoresis

Wheat samples (four single kernels of each classified sample) previously crushed during the hardness testing were individually ground in a mortar and pestle. Gliadins were extracted with 100 μl of 70% ETOH and electrophoresed at 10°C for 6½ hr. Polyacrylamide gel electrophoretic (PAGE) patterns were determined by the method of Lookhart et al (1982).

RESULTS AND DISCUSSION

PAGE

Typical PAGE patterns of bulk wheat samples (Jones et al 1982, Lookhart et al 1983) were compared to the single kernel pattern of each classified sample to determine heterogeneity. FGIS inspectors found only one phenotype in four of the cultivars tested (Sage, Arthur, Newton, and Eagle), and more than one phenotype in each of the other five cultivars (Arkan, Ute, Guard, Stoa, and Ram).

Three of the wheat samples listed in Table I, Arkan and its parents, the hard red winter (HRW) wheat Sage and the soft red winter (SRW) wheat Arthur, have been the subject of recent controversy. Arkan samples in commerce have been visually graded as mixtures of soft wheat and hard wheat by FGIS. As mixtures, price reductions up to $0.30/bu were given, even though Arkan had the good milling and baking quality of HRW wheats. Interestingly, no soft kernel types were picked from the Arkan sample.

Comparison of four kernels each, of the cultivars Arkan, Sage, and Arthur, and of the Arkan variant with Sage characteristics (ARK-SA), required 16 slot positions. Because gels we use normally have eight slots (Lookhart et al 1982), two gels were run (Figs. 1 and 2), each characterizing two of the four kernels of the classified samples Arkan, ARK-SA, Sage, and Arthur. The patterns of Arkan 1 and 4 are identical to patterns found in other Arkan samples. One minor variation in the banding patterns was found in Arkan 2 and 3: specifically, an additional light band slightly lower in mobility than the light fifth band (counting from

top). That band is also present in the Sage patterns. Less than 4% of the HRW variety Arkan classified by FGIS possessed atypical morphological characteristics (Table I).

Arkan kernels selected by FGIS, having morphological characteristics of Sage (ARK-SA), exhibited three different patterns. The patterns of ARK-SA 2 and 4 were similar to the typical Sage patterns Sage 2, 3, and 4. Among the differences were the absence of the light fifth band (mobility 24) in ARK-SA 2 and 4 that was present in the Sage pattern and two bands in the high mobility region (70–85 relative mobility [RM] units) where the ARK-SA types had a band at 74 RM that was absent in Sage and the dark band in Sage at 76 RM that was absent in ARK-SA types.

TABLE I
Wheats Used in the Study

Class Variety	Weight (g) Major	 Minor
Soft red winter		
Arthur	9.89	...
Hard red spring		
Stoa	10.76	0.32-HRW[a] (2.88)
Guard	10.93	0.76-HRW (6.50)
Hard red winter		
Sage	9.98	...
Ram	10.21	0.31 HRS, 0.54 SRW (2.80) (4.88)
Ute	11.42	1.56 HRS (12.02)
Newton	9.91	...
Eagle	10.00	...
Arkan	10.16	0.42 (HRW-Sage) (3.97)

[a] Value in parentheses is percent of minor class on the basis of morphological characteristics.

Another difference in band patterns of ARK-SA kernels was found in ARK-SA 1, where the upper fourth of the gel, 0–25 RM (low mobility), had Arthur type bands instead of Arkan or Sage bands and the lower fourth exhibited some bands that were not present in Arkan, Arthur, or Sage.

A third pattern type in ARK-SA was found in ARK-SA3, which has a pattern identical to bulk Arkan. Thus, kernel morphology seems effective (in three of four cases) in separating ARK-SA kernels having major electrophoretic differences, because the four ARK-SA kernels exhibited three different PAGE patterns, one of which was identical to Arkan but none to Sage.

Patterns of the four Sage kernels appear identical except for the presence of a minor band in Sage 1, at 38 RM units. The patterns of Sage 2, 3, and 4 were identical to Sage electrophoregrams previously analyzed (Jones et al 1982). The four Arthur patterns were all identical and were typical of previously analyzed Arthur patterns.

Single seed electrophoregrams of four seeds each of Newton and Eagle are shown in Figure 3. Newton patterns 1 and 4 appear identical to each other but different from Newton 2 or 3, which are also different from each other. All of the differences among Newton samples were in the top half (10–25 RM region) of the gel. None of these single seed electrophoregrams was identical to the typical (bulk) pattern of Newton (Jones et al 1982). The gliadin patterns of all four Eagle kernels (Fig. 3) appear identical to each other and to the typical pattern of other bulk Eagle samples previously analyzed in our laboratory (Jones et al 1982). Eagle was expected to be very homogeneous, as it was originally selected from an F_{16} single plant, whereas most of the other cultivars studied were selected from F_4 or F_5 single plants.

The electrophoretic patterns of four single kernels each of the major Ute fraction, Ute M, and of Ute kernels with morphological characteristics of hard red spring wheat (Ute-HRS) are show in Figure 4. All patterns of the individual kernels of the HRW wheat Ute, including kernels with HRS morphological characteristics,

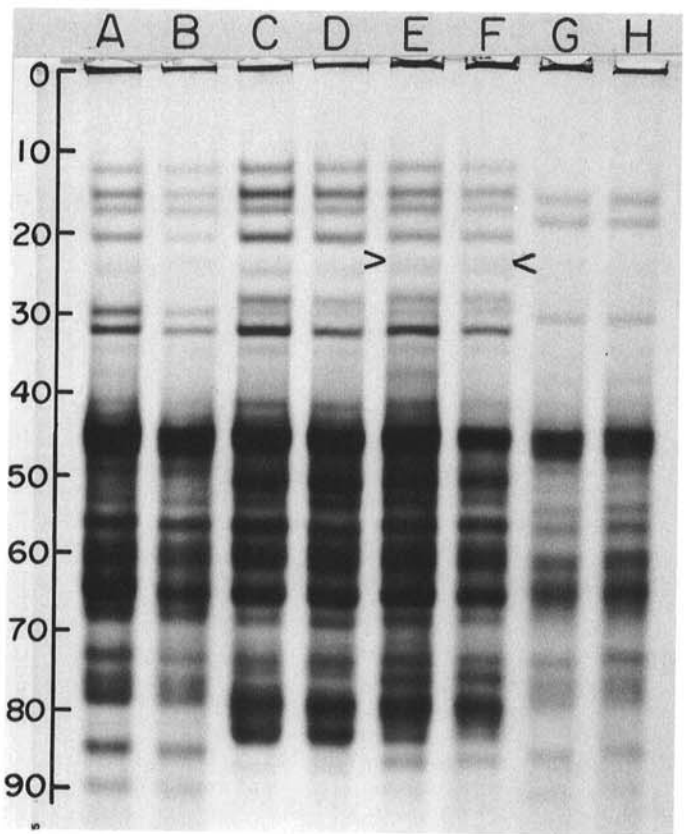

Fig. 1. Gliadin electrophoregrams of **A,** Arkan 1; **B,** Arkan 3; **C,** Arkan with Sage characteristics (ARK-SA 2); **D,** ARK-SA 4; **E,** Sage 1; **F,** Sage 3; **G,** Arthur 2; **H,** Arthur 3. Carets indicate light bands not easily seen.

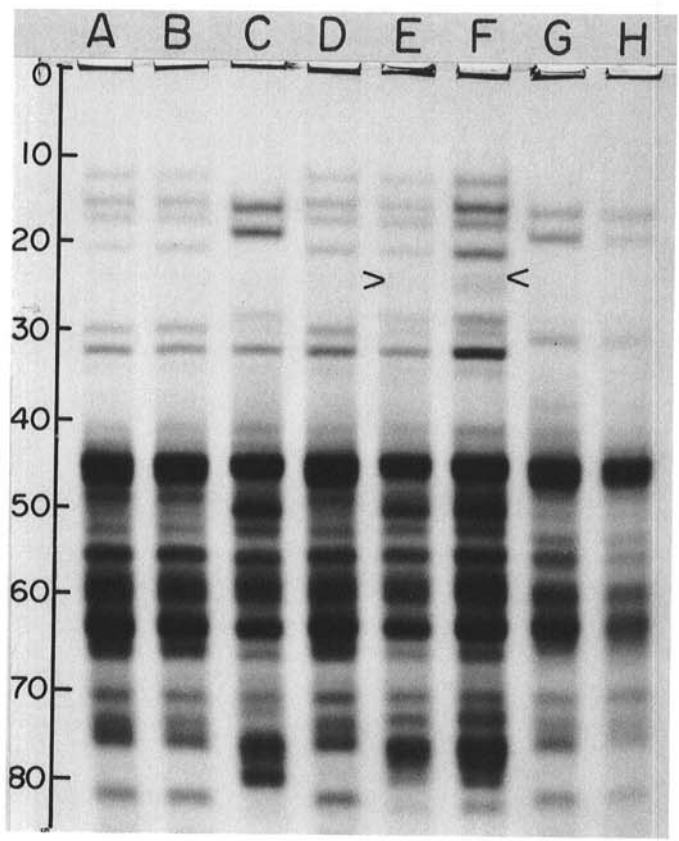

Fig. 2. Gliadin electrophoregrams of **A,** Arkan 2; **B,** Arkan 4; **C,** Arkan with Sage characteristics (ARK-SA 1); **D,** ARK-SA 3; **E,** Sage 2; **F,** Sage 4; **G,** Arthur 1; **H,** Arthur 4. Carets indicate light bands not easily seen.

exhibited gliadin patterns identical to each other and to bulk Ute samples previously analyzed.

Gliadin patterns of four kernels of the major kernel type in the HRS wheat Guard, Guard M, and of four of the kernels with morphological characteristics of hard red winter, Guard HRW, are shown in Figure 5. The HRS variety Guard contained 6.5% of the HRW type kernels (Table I). All patterns are identical to the typical Guard bulk pattern except for Guard M-3, in which the third fastest band has higher mobility (the normal 74 RM band appears at 77 RM). Because the gliadin patterns of the Guard kernels having typical HRW morphology appear identical to the typical PAGE patterns of the major fraction, differences in kernel morphology appear unrelated to the electrophoregrams of Guard.

Electrophoretic patterns of four single kernels each of the major kernel type, in the HRS wheat Stoa (Stoa M) and of kernels having HRW characteristics (Stoa HRW) are shown in Figure 6. All gliadin patterns are identical to the typical pattern of a bulk Stoa sample previously analyzed. Thus, Stoa kernels having HRS and HRW morphology do not differ electrophoretically.

Gliadin patterns of four kernels each of the major HRW wheat fraction of the cultivar Ram (Ram M) and kernels having HRS characteristics (Ram HRS) and soft red winter (Ram SRW) characteristics are shown in Figure 7. Gliadin patterns of eight of the 12 kernels are identical to the typical bulk electrophoretic pattern of Ram. The electrophoretic patterns of the other four kernels, Ram M-1, Ram HRS-1, Ram HRS-4, and Ram SRW-1, exhibit a slight change in the mobility of a single band (marked by an arrow) near the center of the gel, which is the only difference between their patterns and the typical pattern of a bulk sample. Again, differences in kernel morphology were not expressed in the gliadin patterns. This effect is particularly striking because these gliadin patterns were nearly invariant for kernels having HRW, SRW, and HRS morphological characteristics. These data support the concept that the gliadin proteins, which are coded for by only six complex loci (Payne et al 1984), are inherited independently from other genotypic and phenotypic characteristics.

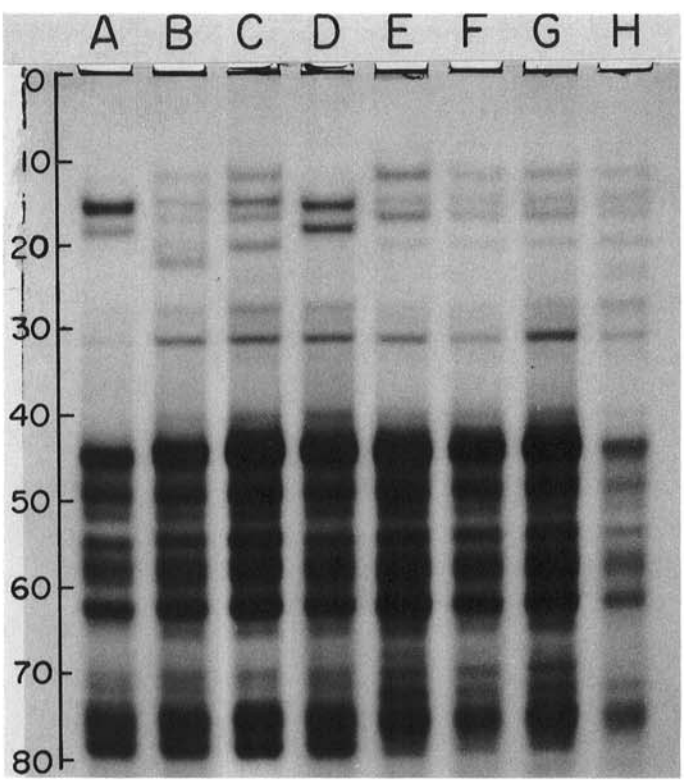

Fig. 3. Gliadin electrophoregrams of **A,** Newton 1; **B,** Newton 2; **C,** Newton 3; **D,** Newton 4; **E,** Eagle 1; **F,** Eagle 2; **G,** Eagle 3; **H,** Eagle 4.

Hardness

The method for determining hardness of individual kernels of hard and soft wheat was described by Lai et al (1985). For a hard wheat, there is a distinct drop in crushing force after the first peak, whereas for a soft wheat, the drop is gradual and relatively small. To establish the characteristic pattern of each curve, we measured the magnitude of the first peak and first valley and calculated the ratio of first valley over first peak (Lai et al 1985). The results of those measurements on each of the 60 kernels are given in Table II. The predicted hardness value (PHV) is determined from a combination of the ratio of the first peak to the first valley and the magnitudes of each. If the ratio is less than 0.25 or greater than 0.45, the PHV is determined as hard or soft, respectively. However, if the ratio is between 0.25 and 0.45, additional criteria, including the magnitude of the first peak and first valley, are considered. We found that soft wheats normally have a first peak to first valley ratio greater than 0.4 and hard wheats less than 0.3.

Relation of Electrophoretic and Hardness Patterns

All kernels of Arkan and Arkan (Sage type) were predicted to be hard. The ratios for Arkan kernels 2 and 3 were higher than for kernels 1 and 4 (Table II); they were also grouped by similar electrophoretic patterns.

The PHV for Sage kernel 1 was soft. The hardness pattern for that kernel was distinctly different from the patterns of the other Sage kernels. The size of the first peak was unusually high, which caused the ratio to be high and therefore to grade soft. The overall intensities of the bands in the electrophoretic pattern of Sage 1 were greater than those in the other Sage kernels' patterns, which implies a higher protein content. Therefore, high protein content may affect hardness determination of single kernels.

The PHV of Arthur kernel 1 was hard. The first valley was low compared to those of the other three kernels, which may have affected the PHV. The intensities of the electrophoretic bands of

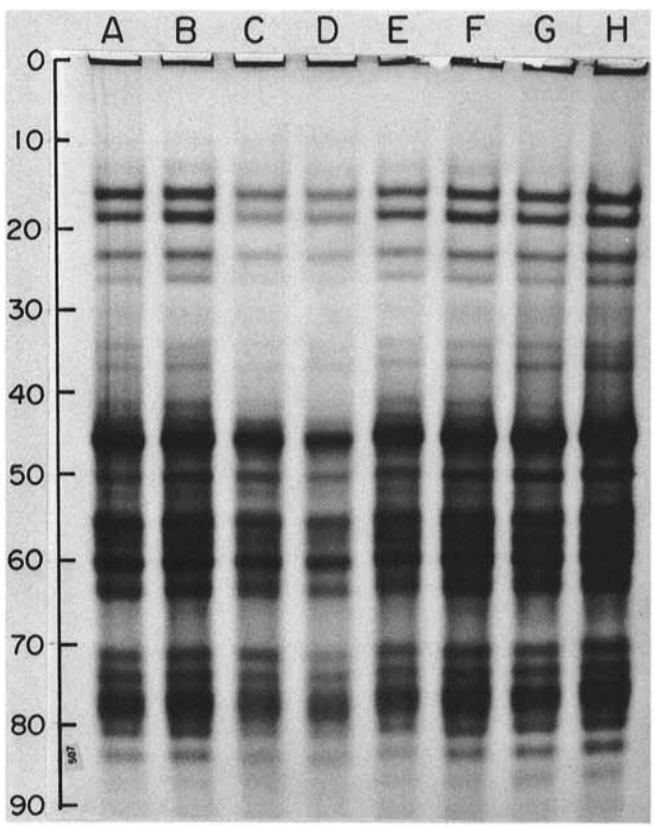

Fig. 4. Gliadin electrophoregrams of **A,** Ute major fraction (Ute M-1); **B,** Ute M-2; **C,** Ute M-3; **D,** Ute M-4; **E,** Ute with hard red spring wheat morphological characteristics (Ute HRS-1); **F,** Ute HRS-2; **G,** Ute HRS-3; **H,** Ute HRS-4.

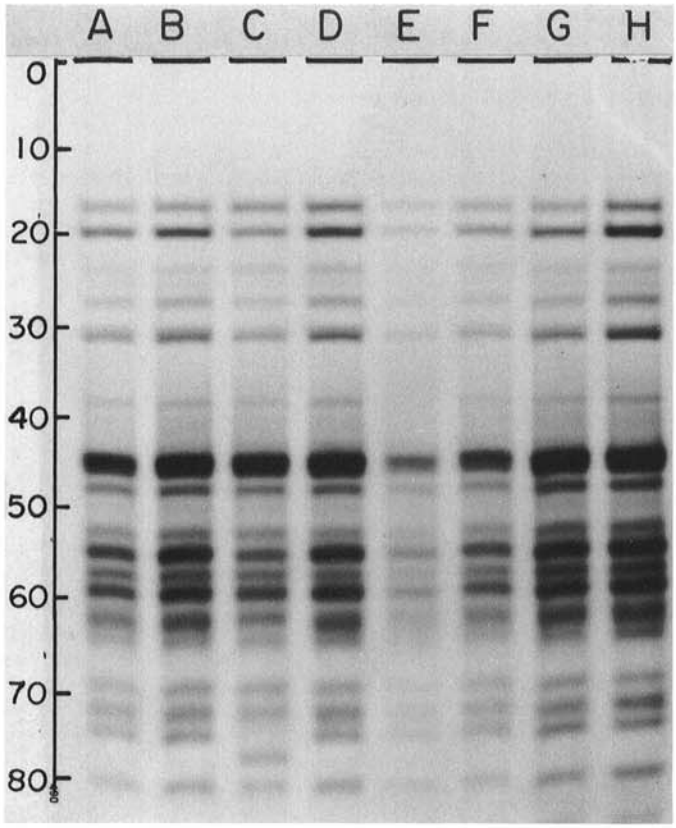

Fig. 5. Gliadin electrophoregrams of **A,** Guard major fraction (G M-1); **B,** G M-2; **C,** G M-3; **D,** G M-4; **E,** Guard with hard red winter wheat morphological characteristics (G HRW-1); **F,** G HRW-2; **G,** G HRW-3; **H,** G HRW-4.

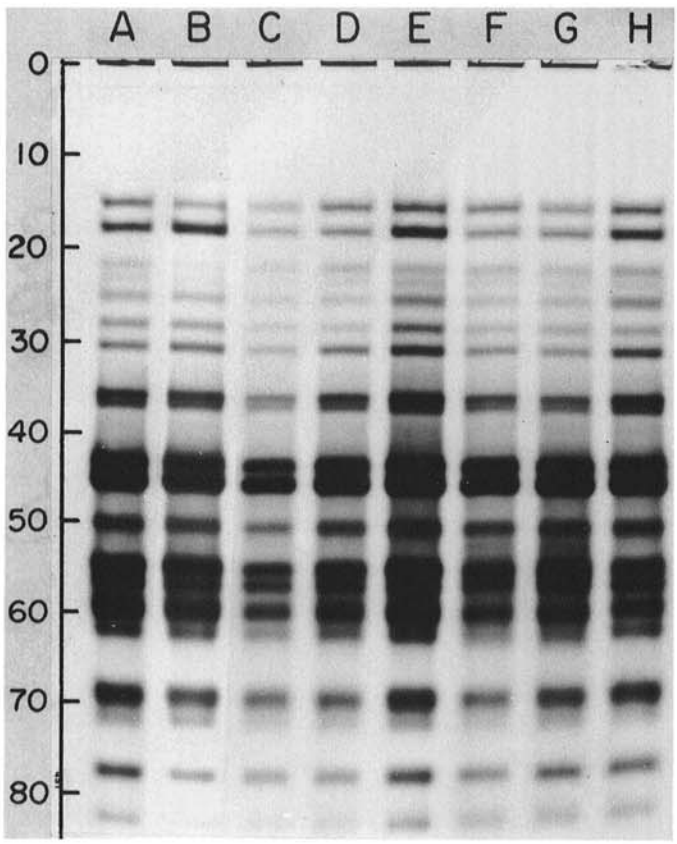

Fig. 6. Gliadin electrophoregrams of **A,** Stoa major fraction (Stoa M-1); **B,** Stoa M-2; **C,** Stoa M-3; **D,** Stoa M-4; **E,** Stoa with hard red winter wheat morphological characteristics (Stoa HRW-1); **F,** Stoa HRW-2; **G,** Stoa HRW-3; **H,** Stoa HRW-4.

kernel 1 were higher than those of the other Arthur kernels, which implies a higher protein content. Thus, in Arthur 1 and Sage 1, higher protein contents may have affected the ability of the hardness test to accurately predict hardness in individual kernels, even though in bulk samples protein content does not have a consistent effect on hardness characteristics (Miller et al 1982).

The PHV for Newton segregates the kernels into soft types (1, 4) and hard types (2, 3). The soft types (1, 4) were previously found to exhibit electrophoretic band patterns in the top fourth of the gel (Fig. 4) similar to patterns of Arthur (Fig. 3), a soft wheat. Thus, in the Newton samples examined, a correlation exists between PHV and electrophoretic pattern.

Eagle kernel 1 was predicted as intermediate and kernel 4 was soft by the PHV. PAGE, however, showed all Eagle kernels to be identical.

All Ute and Ute HRS kernels were predicted hard except for HRS-3, which was intermediate. All Ute and Ute HRS kernels appeared identical upon PAGE.

All Guard samples were predicted hard except Guard M-3 and Guard HRW-1, which were intermediate in hardness. The electrophoretic pattern of Guard M-3 (Fig. 6), which showed a minor band change, and the light gliadin band pattern of Guard HRW-1 (Fig. 6) are the only electrophoretic pattern differences found with the kernels of intermediate hardness. Therefore no consistent interaction of electrophoretic pattern with intermediate hardness was found.

The PHVs for Stoa kernels (major HRS fraction) and those of its HRW subset are all hard, consistent with their identical electrophoretic patterns (Fig. 7). Because the intensities of the bands in the Stoa kernels varied from dark in M-2 to light in M-3 and no difference in hardness value was found, hardness is probably not directly related to intensity of gliadin patterns.

Ram major kernels were predicted hard. Ram HRS-1 was predicted intermediate and Ram HRS-2 was soft; Ram HRS-3 and Ram HRS-4 were predicted hard. The electrophoretic bands of HRS-1 are very intense, implying a high protein content. The effect of possibly higher protein content affecting the PHV of individual kernels was noted previously for Arthur 1, Sage 1, and Guard HRW-1. The PHV of Ram SRW-1 was soft, of SRW-3 intermediate, and of SRW-2 and SRW-4 hard. PAGE patterns, however, appeared nearly identical for all 12 Ram kernels; only in Ram HRW-1, HRS-1, HRS-4, and SRW-1 were minor band

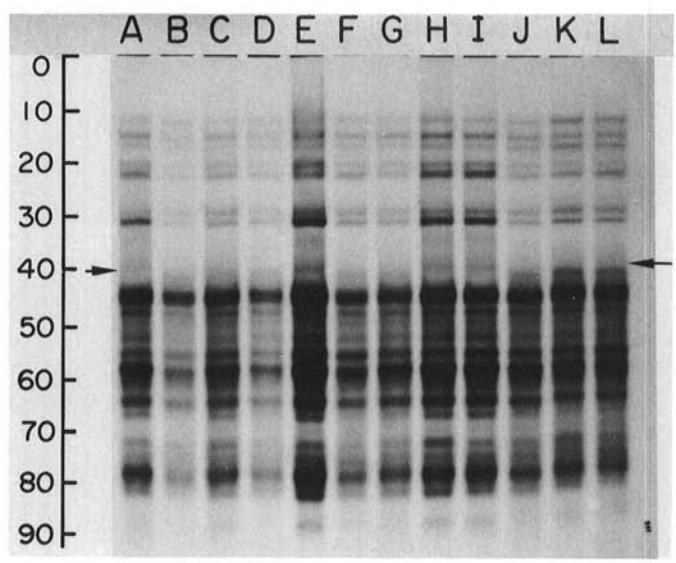

Fig. 7. Gliadin electrophoregrams of **A,** Ram major fraction (Ram M-1); **B,** Ram M-2; **C,** Ram M-3; **D,** Ram M-4; **E,** Ram with hard red spring wheat morphological characteristics (Ram HRS-1); **F,** Ram HRS-2; **G,** Ram HRS-3; **H,** Ram HRS-4; **I,** Ram with soft red winter wheat morphological characteristics, Ram SRW-1; **J,** Ram SRW-2; **K,** Ram SRW-3; **L,** Ram SRW-4. Arrows indicate light bands not easily seen.

differences found. Again, minor variations in PAGE patterns appear unrelated to hardness as detected by the hardness tester.

Variability in hardness characteristics, electrophoretic patterns of individual kernels, and the fact that the destructive crushing tests preclude replicated analyses must all be considered in interpreting these data. It should be noted, however, that electrophoretic patterns and hardness tests on bulk samples of pure varieties and on individual kernels from a single wheat head show consistent patterns (Lookhart et al 1984).

Each of 60 kernels representing one SRW, two HRS, and six HRW wheat cultivars were analyzed for PAGE and hardness characteristics. Of these, 24 kernels appeared to differ, on the basis

TABLE II
Characteristics of Wheat Samples

		Hardness				PAGE[b]
Variety	Kernel no.	First Peak	First Valley	Ratio	PHV[a]	Band Patterns
Arkan (HRW)	1	5.85	0.30	0.051	H	I
	2	4.20	0.45	0.107	H	S
	3	7.45	0.90	0.121	H	S
	4	4.70	0.20	0.042	H	I
Arkan (Sage)	1	7.34	0.64	0.087	H	N[c]
Type	2	3.99	0.09	0.022	H	S[d]
	3	8.44	0.39	0.046	H	S[d]
	4	8.04	0.24	0.029	H	I[e]
Sage (HRW)	1	10.16	7.71	0.759	S	S
	2	3.91	0.66	0.168	H	I
	3	5.86	1.91	0.326	–	I
	4	3.66	0.26	0.071	H	I
Arthur (SRW)	1	4.75	0.70	0.148	H	I
	2	4.85	1.95	0.403	S	I
	3	3.65	1.25	0.343	S	I
	4	3.10	1.85	0.577	S	I
Newton (HRW)	1	6.92	2.87	0.414	S	S
	2	5.37	1.32	0.245	H	N
	3	6.17	0.52	0.084	H	N
	4	5.52	3.72	0.674	S	S
Eagle (HRW)	1	6.16	1.96	0.318	–	I
	2	9.16	0.21	0.023	H	I
	3	5.31	0.41	0.077	H	I
	4	2.66	1.61	0.605	S	I
Ute (HRW)	1	5.63	0.38	0.008	H	I
	2	5.63	1.38	0.245	H	I
	3	6.13	0.43	0.070	H	I
	4	7.13	0.23	0.032	H	I
Ute (HRS)	1	7.30	1.45	0.198	H	I
Minor	2	5.15	0.10	0.019	H	I
	3	4.80	1.45	0.302	–	I
	4	4.40	0.25	0.056	H	I
Guard (HRS)	1	10.05	2.20	0.219	H	I
	2	9.35	1.20	0.129	H	I
	3	6.35	2.10	0.331	–	S
	4	9.00	0.15	0.017	H	I
Guard (HRW)	1	5.96	1.86	0.312	–	I
Minor	2	6.76	0.46	0.069	H	I
	3	8.26	0.31	0.038	H	I
	4	6.91	0.61	0.089	H	I
Stoa (HRS)	1	4.34	0.48	0.112	H	I
	2	7.84	0.18	0.024	H	I
	3	3.43	0.73	0.214	H	I
	4	5.24	1.43	0.274	H	I
Stoa (HRW)	1	4.42	0.07	0.015	H	I
Minor	2	6.02	1.07	0.177	H	I
	3	3.92	0.12	0.030	H	I
	4	3.32	0.07	0.020	H	I
Ram (HRW)	1	8.23	0.98	0.119	H	S
	2	5.63	0.43	0.077	H	I
	3	7.08	0.23	0.033	H	I
	4	8.23	0.43	0.052	H	I
Ram (HRS)	1	6.67	2.52	0.378	–	S
Minor	2	3.42	1.72	0.503	S	I
	3	6.97	0.72	0.104	H	I
	4	4.27	0.02	0.005	H	S
Ram (SRW)	1	13.75	11.25	0.818	S	S
Minor	2	4.00	0.35	0.086	H	I
	3	9.10	3.00	0.329	–	I
	4	6.90	1.70	0.246	H	I

[a] Predicted Hardness Value. "–" indicates intermediate hardness.
[b] Polyacrylamide gel electrophoresis patterns: I = identical, S = similar, and N = not similar.
[c] Not similar to Arkan, Sage, or Arthur.
[d] Similar to Sage.
[e] Identical to Arkan.

of morphological characteristics and apparent texture from expected typical kernels of the certified wheat cultivars. Seven kernels appeared to have intermediate (soft-hard) kernel characteristics as predicted from a hardness test. In seven kernels, hardness characteristics were different from that expected of the cultivar. Thus, significant differences in both hardness and PAGE characteristics were found among kernels identified as morphologically pure and among kernels identified as morphologically different.

CONCLUSIONS

This study established how morphologically equal and morphologically different kernels (as evaluated by FGIS) of foundation seed (as provided by plant breeders) compare in hardness characteristics and electrophoretic patterns. The number of kernels analyzed was not sufficient to allow a statistically significant determination of the percentage of biotypes in certified seed of pure cultivars; that would be a comprehensive project of its own and should be done in the future. However, the manner in which the samples were selected and graded lends appreciable credence to our results.

The gliadin patterns of Arkan seed with Sage morphological characteristics were more similar to Sage than to Arkan, which implies that gliadin patterns are related to grain morphology as well as to genetic background or that considerable genotypic heterogeneity also exists.

Four of the Ram kernels exhibited gliadin patterns different from the other eight kernels and the typical bulk pattern. Because those four kernels included members of each class, no clear-cut conclusions can be derived. No differences in gliadin patterns of the other cultivars of mixed morphological characteristics were found. The data from Ute, Guard, and Stoa also imply no correlation of kernel type to electrophoretic pattern. Finally, the lack of consistent electrophoretic difference between the kernels of Ram with HRW, HRS, or SRW morphological characteristics is of interest and implies again that kernel morphology is not necessarily related to grain hardness or gliadin electrophoretic patterns. A possible effect of high protein content on hardness characteristics of individual kernels (unlike bulk samples) was also noted.

The relative protein content seems to be related to the energy required to crush a single seed (first peak height) and the intensity of the electrophoretic bands. Moreover, a high protein content, as indicated by intense electrophoregram bands, seems to affect borderline (intermediate) hardness values.

Our results seem to demonstrate that gliadin patterns are not necessarily related to hardness, that hardness measurements are not necessarily related to phenotype, that many cultivars are significantly heterogeneous, and that standards for grading on the basis of morphological characteristics alone may relate to the genetic background of some varieties but were of limited value for most of the varieties included in this study.

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