Gliadin Electrophoretic Variations in Foundation Arkan Wheat Grown at 16 Kansas Agricultural Experiment Stations in 1983[1]

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ABSTRACT

Gliadin electrophoretic patterns of composite samples of foundation Arkan wheat grain grown at 16 Kansas branch experiment stations and experimental fields in 1983 were compared with patterns of two other hard red winter wheats, Newton and TAM 105, grown at the same locations, and with patterns of Arkan's parents, Sage and Arthur. Gliadin polyacrylamide gel electrophoretic patterns were also determined for single hard- and soft-appearing kernels. Electrophoregrams of the single seeds at each location were identical, or nearly so, to bulk Arkan patterns at the respective locations, and to typical Arkan patterns previously established. Therefore, differences in electrophoretic patterns are not the result of varietal mixtures, but more likely of biotypes. Extra bands found in some Arkan samples were usually present in one of its parents. Gliadin patterns of bulk extracts of Newton and TAM 105 from some locations also exhibited patterns different from normal. The number of locations where electrophoretic differences were found was highest for Arkan and lowest for TAM 105.

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Key words: Kernel characteristics, Kernel hardness, PAGE, Wheat class

Classification of wheat by the United States Department of Agriculture, Federal Grain Inspection Service (FGIS) allows commercial enterprises to buy and sell wheat based on intended end uses; for example, hard wheats are used for pan breads, soft wheats for pastries, and durum wheats for pastas. The classification scheme has been under constant scrutiny and revision since its inception in 1916. Recently, additional pressure has been put on this classification system with the use of new exotic germ plasms and interclass breeding in varietal development, which may improve disease resistance, yield, and protein content. However, such improvements may also introduce problems, the major one being variability of kernel characteristics for a cultivar. Because FGIS identifies and determines purity of wheat cultivars by kernel characteristics (USDA-FGIS 1977) and sample homogeneity, respectively, new methods must now be developed to aid in identifying and classifying wheat cultivars, as long as classification standards remain unchanged.

Arkan is a recently introduced wheat cultivar that resulted from crossing a hard red winter (HRW) wheat, Sage, with a soft red winter (SRW) wheat, Arthur. Arkan exhibits milling and baking properties typical of HRW wheat, but exhibits nonhomogeneous kernel characteristics. As a result, Arkan foundation-quality seed has been graded by FGIS as a mixture of SRW and HRW wheats.

Two instrumental methods (polyacrylamide gel electrophoresis [PAGE] and high-performance liquid chromatography [HPLC]) have been reported that can identify wheat cultivars by analyzing their gliadin proteins (Bushuk and Zillman 1978, Lookhart et al 1982, Wrigley et al 1982, Bietz 1983). This paper describes variations in electrophoretic patterns of bulk Arkan and of the soft- and hard-appearing kernels of Arkan. It also compares their patterns with those of its parents, Arthur and Sage, and with two other HRW wheats, Newton and TAM 105.

MATERIALS AND METHODS

Chemicals and Reagents

Acrylamide, *N,N'*-methylenebisacrylamide, ascorbic acid, Coomassie Brilliant Blue R-250, methyl green, trichloroacetic acid

[1]Mention of firm names or trade products does not constitute endorsement by the USDA over others not mentioned.

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(TCA), and trifluoracetic acid (TFA) were from Sigma Chemical Company, St. Louis, MO; lactic acid (USP grade) and ferrous sulfate heptahydrate (AR grade), from Mallinckrodt Chemicals, St. Louis, MO; and LC-grade ethanol and acetonitrile from Burdick and Jackson Laboratories, Muskegon, MI. Hydrogen peroxide (3% practical grade) was purchased in small plastic bottles from a local pharmacy. Water was purified by passage, in series, through a membrane filter, a charcoal filter, and two mixed-bed ion-exchange filters. Aluminum lactate was from K & K Laboratories, Plainview, NY.

Samples

All wheat samples (Table I) except Arthur, Sage, and Marquis were foundation seed from Rollin Sears, who requested and received them from agronomists at 16 Kansas branch experiment stations and experimental fields (KAES). Samples of Arthur and Sage were obtained from Y. Pomeranz, USDA; the Federal Grain Inspection Service; and from Carl B. Overly, Kansas State University. A sample of Marquis was obtained from W. Bushuk, University of Manitoba, Winnipeg, Canada (see Acknowledgment).

Sample Preparation

Each wheat sample was segregated manually by visual characteristics into kernels appearing hard or soft. A single kernel was selected at random from those subsamples. The two kernels and a bulk group of seeds (about 20) from each sample were ground and extracted as previously described (Lookhart et al 1982).

PAGE

The electrophoresis apparatus, power supply, and staining and destaining procedures have been described previously (Lookhart et al 1982). The gel temperature (10° C) and electrophoresis time (5 hr) were modified to optimize prolamin separations.

RESULTS

Electrophoretic patterns of gliadins from bulk samples of Marquis (hard red spring, HRS), and two samples each, from different sources, of Arkan (HRW) and its parents, Sage (HRW) and Arthur (SRW) are shown in Figure 1. The Marquis patterns are used to compare gels and to standardize the relative mobility 50 positions (Bushuk and Zillman 1978). The typical Arkan gliadin pattern has a protein band at the same mobility as Marquis reference band 50; consequently, Marquis was deleted from subsequent gels, since mobility of other bands could be determined by comparison to band 50 of Arkan. Both accessions of each cultivar (Fig. 1) appear to exhibit identical bulk gliadin patterns. The bulk gliadin pattern (electrophoregram) of Arkan appears more similar to that of Sage in the top half of the gel (mobility ≤ 50) and in the lower half of the gel more similar to that of Arthur (mobility >50). Bulk samples of pure cultivars usually exhibit unique gliadin electrophoretic patterns (Jones et al 1982, Wrigley et al 1982, Lookhart et al 1983).

Gliadin electrophoregrams of bulk Arkan samples from the branch experiment stations or experimental fields are shown in Figure 2. All light bands that are difficult to see and are discussed in the text are denoted by a carat in the figures. Electrophoregrams of the samples from Manhattan and Ottawa have an electrophoretic band at 22 relative mobility units (MU) not typically present in Arkan patterns (Lookhart et al 1983). The electrophoregram of the Belleville sample (F) has an extra band at 17 MU and the Parsons sample (D) has extra bands at 17, 22, 36, and 37 MU. The sample from Parsons (D) appears to have the most differences from the typical Arkan pattern (A, E, G, H, and Lookhart et al 1983). The bulk Arkan samples from Colby I (irrigated, L), Tribune D (dry, M), and Tribune I (N) each possess a gliadin band in addition to those of the typical bulk Arkan at 17, 48, and 48 MU, respectively.

The number of Arkan kernels with apparent hard (H) and soft (S) characteristics, which had been manually segregated for each of the 16 sites, varied with the location. Electrophoresis of single kernels was done to eliminate the possibility that mixtures of cultivars (impurities) were responsible for the extraneous bands. Gliadin electrophoregrams of individual H and S kernels from each KAES are shown in Figures 3 and 4. PAGE patterns of Arkan H-appearing kernels (Fig. 3) are very similar to the bulk Arkan patterns (Figs. 1 and 2). Sites where bands were found in addition to the typical pattern were: Belleville (B), 18 MU; Hesston (C), 28 and 49 MU; St. John I (F), 23 and 26 MU; Colby I (H), 18 and 23 MU; Powhattan (I), 18 MU; Parsons (L), 18, 23, 36, and 39 MU; Tribune D (M), 18, 28, and 73–81 MU; and Tribune I (N), 18, 36,

TABLE I
Location and Cooperators of Bulk Samples of Arkan, Newton, and TAM 105 from the 16 Kansas Agricultural Experiment Stations (1983)

Name	Kansas Location[a]	Cooperators
Powhattan	NE	Marvin Lindquist
Manhattan		Rollin Sears
Ottawa	EC	Keith Janssen
Parsons	SE	Kenneth Kelley and Richard Dougherty
Hays	NC	T. Joe Martin
Belleville		Robert Raney
Hesston		Mark Claasen
Hutchinson		George Mueller-Warrant
St. John D[b]	SC	James Long and George TenEyck
St. John I[c]		James Long and George TenEyck
Colby D	NW	John Lawless
Colby I		
Tribune D	WC	Mike Bourne and Roy Gwin
Tribune I		
Garden City D	SW	Merle Witt
Garden City I		

[a] Geographic area of Kansas, where SE = southeast, NE = northeast, NC = north central, NW = northwest, WC = west central, and SC = south central.
[b] D = Dryland.
[c] I = Irrigated.

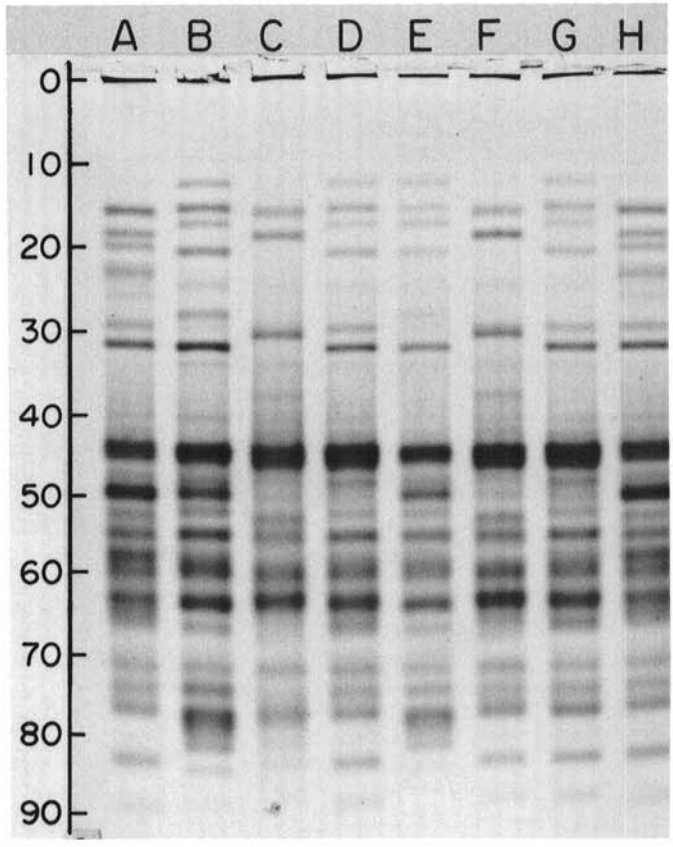

Fig. 1. Electrophoregrams of gliadin extracts of Marquis (**A**), Sage (**B**), Arthur (**C**), Arkan (**D**), Sage-P (**E**), Arthur-P (**F**), Arkan-P (**G**), and Marquis (**H**). Origin at top is cathode. The P designates samples obtained from Y. Pomeranz.

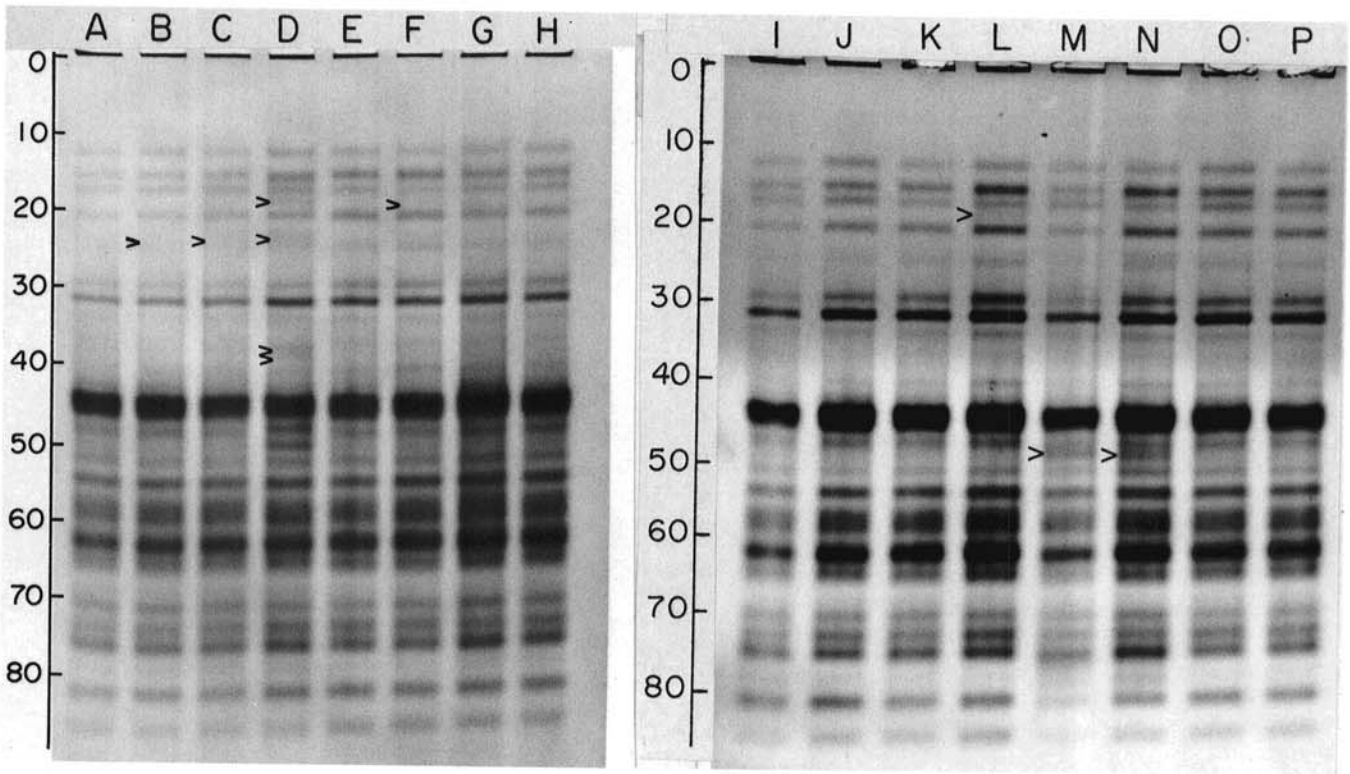

Fig. 2. Electrophoregrams of gliadin extracts of bulk Arkan foundation seed from the Kansas Agricultural Experiment Stations: Powhattan (**A**), Manhattan (**B**), Ottawa (**C**), Parsons (**D**), Hays (**E**), Belleville (**F**), Hesston (**G**), Hutchinson (**H**), St. John D (**I**), St. John I (**J**), Colby D (**K**), Colby I (**L**), Tribune D (**M**), Tribune I (**N**), Garden City D (**O**), and Garden City I (**P**).

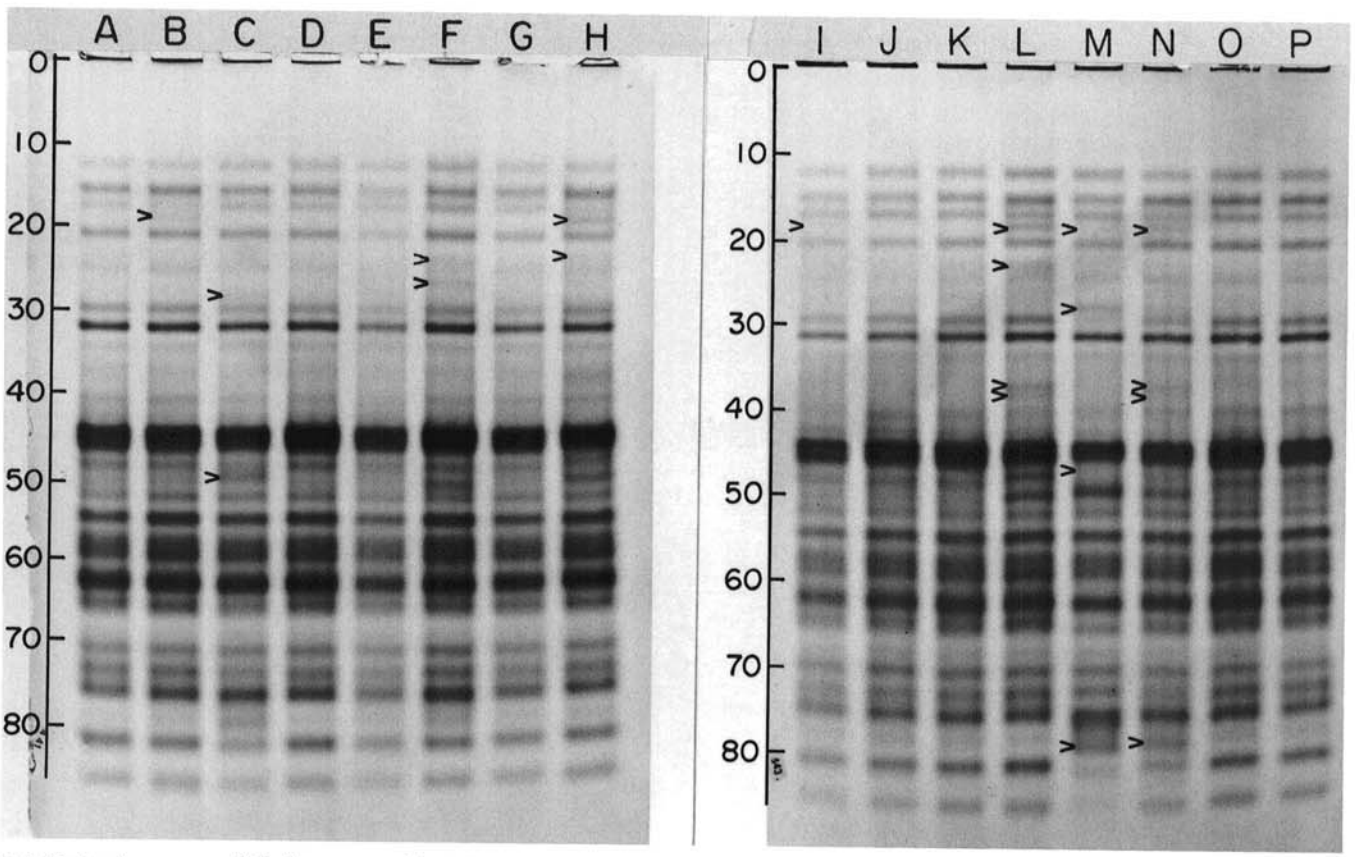

Fig. 3. Electrophoregrams of gliadin extracts of single hard-appearing seeds of foundation Arkan from the Kansas Agricultural Experiment Stations: Hays (**A**), Belleville (**B**), Hesston (**C**), Hutchinson (**D**), St. John D (**E**), St. John I (**F**), Colby D (**G**), Colby I (**H**), Powhattan (**I**), Manhattan (**J**), Ottawa (**K**), Parsons (**L**), Tribune D (**M**), Tribune I (**N**), Garden City D (**O**), and Garden City I (**P**).

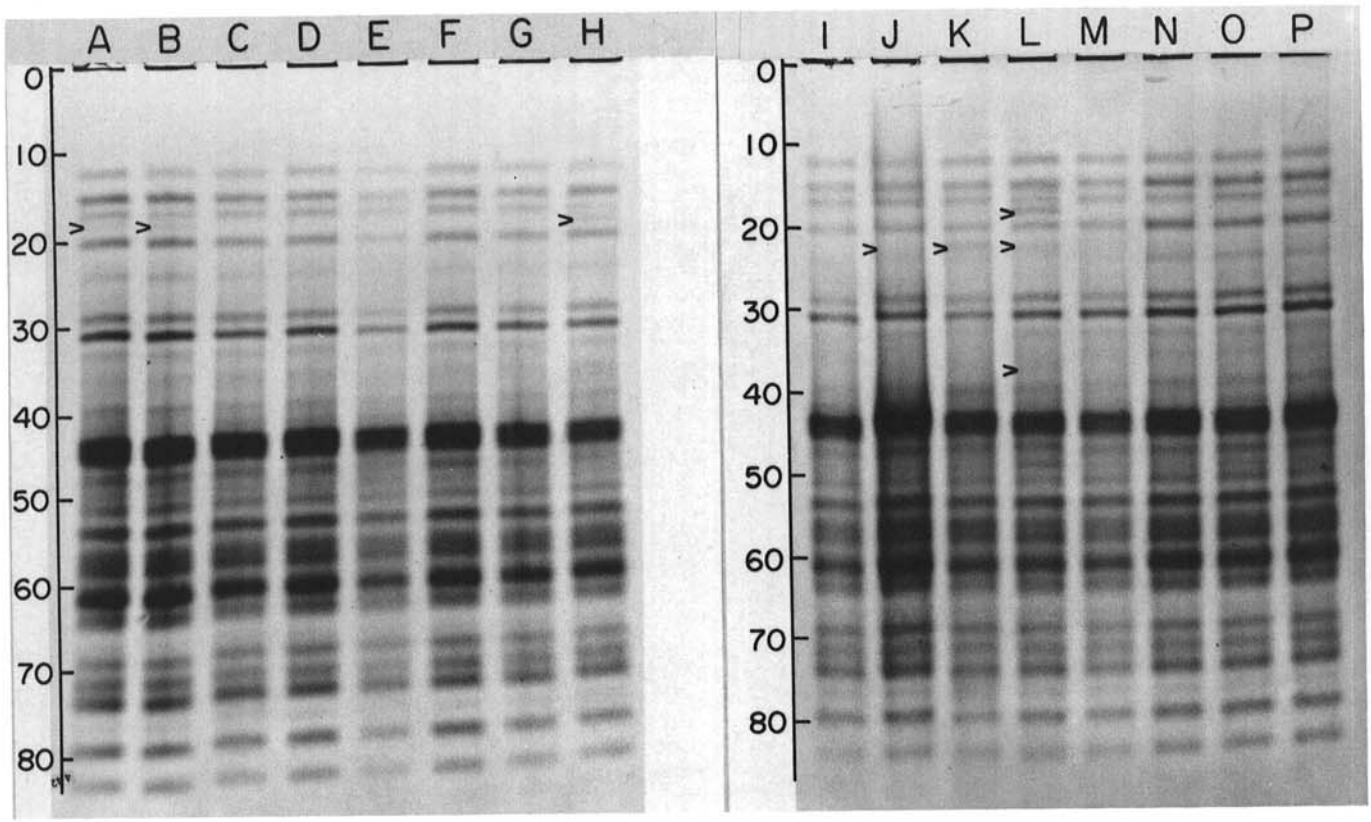

Fig. 4. Electrophoregrams of gliadin extracts of single soft-appearing seeds of foundation Arkan from the Kansas Agricultural Experiment Stations: Hays (**A**), Belleville (**B**), Hesston (**C**), Hutchinson (**D**), St. John D (**E**), St. John I (**F**), Colby D (**G**), Colby I (**H**), Powhattan (**I**), Manhattan (**J**), Ottawa (**K**), Parsons (**L**), Tribune D (**M**), Tribune I (**N**), Garden City D (**O**), and Garden City I (**P**).

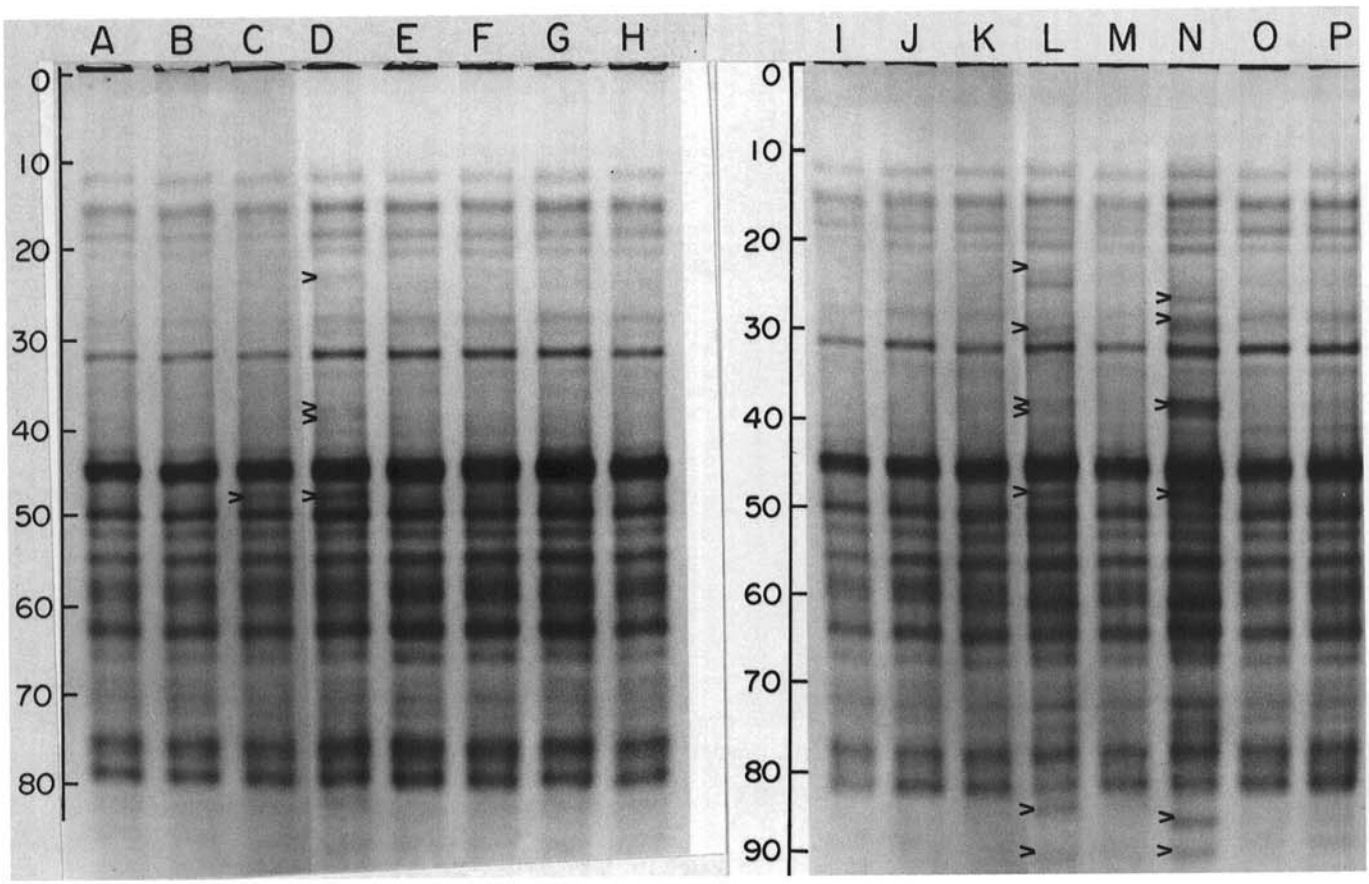

Fig. 5. Electrophoregrams of gliadin extracts of bulk Newton foundation seed from the Kansas Agricultural Experiment Stations: Powhattan (**A**), Manhattan (**B**), Ottawa (**C**), Parsons (**D**), Fort Hays (**E**), Belleville (**F**), Hesston (**G**), Hutchinson (**H**), St. John I (**I**), St. John D (**J**), Colby I (**K**), Colby D (**L**), Tribune I (**M**), Tribune D (**N**), Garden City I (**O**), and Garden City D (**P**).

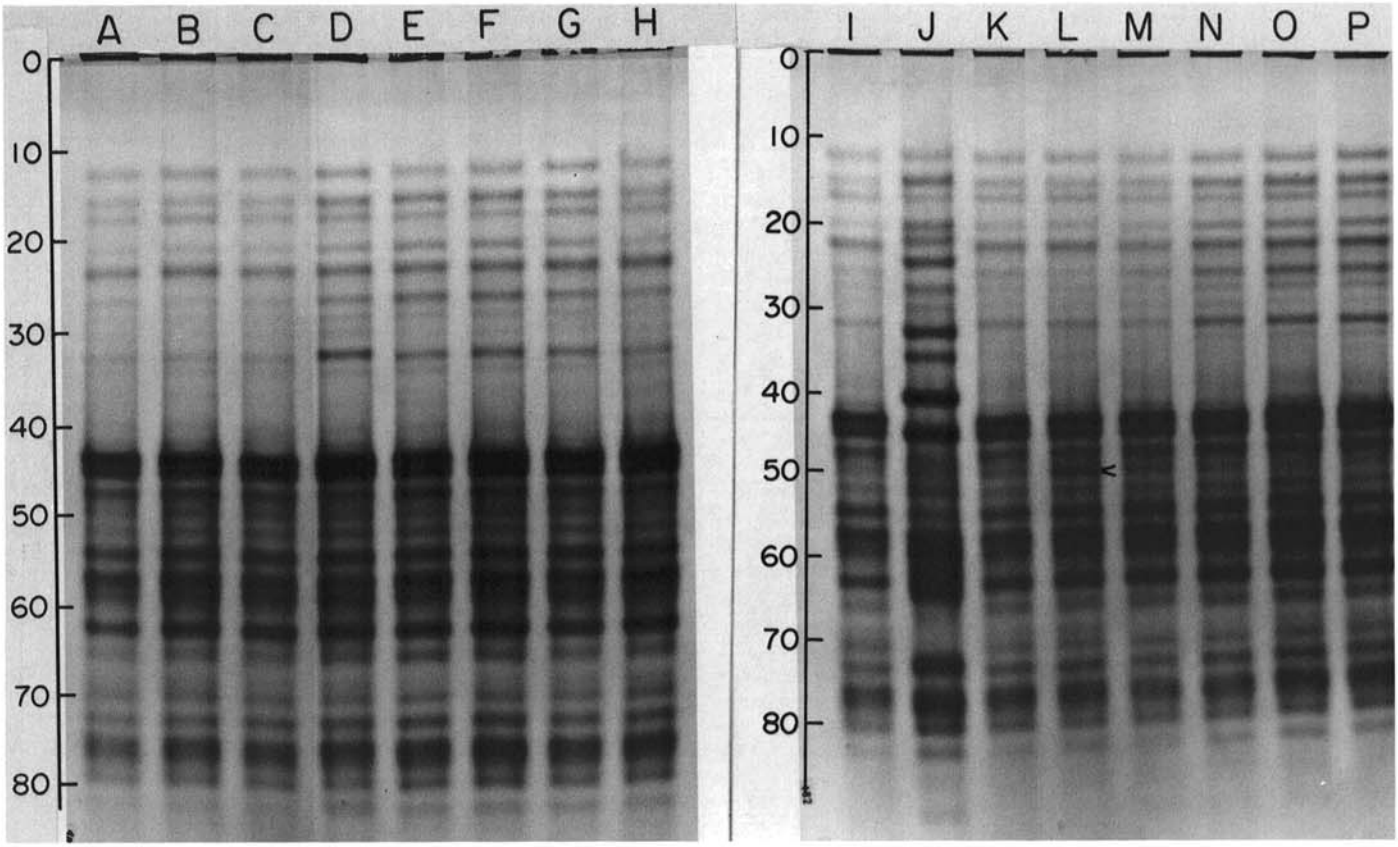

Fig. 6. Electrophoregrams of gliadin extracts of bulk TAM 105 foundation seed from the Kansas Agricultural Experiment Stations: Powhattan (**A**), Manhattan (**B**), Ottawa (**C**), Parsons (**D**), Fort Hayes (**E**), Belleville (**F**), Hesston (**G**), Hutchinson (**H**), St. John D (**I**), St. John I (**J**), Colby I (**K**), Colby D (**L**), Tribune I (**M**), Tribune D (**N**), Garden City I (**O**), and Garden City D (**P**).

TABLE II
Electrophoretic Differences in Wheat Samples from 16 Kansas Agricultural Experiment Stations (1983 Crop)

Variety	Kansas Location						No. of Differences
	Southeast	Northeast	North Central	Northwest	West Central	South Central	
Bulk							
Newton	Parsons	Ottawa		Colby D[b]	Tribune D		4
TAM 105				Colby D		St. John I[d]	2
Arkan	Parsons	Ottawa Manhattan	Belleville	Colby I	Tribune D Tribune I		7
Single seeds							
Arkan S	Parsons	Ottawa Manhattan	Belleville Hays	Colby I			6
Arkan H	Parsons	Powhattan	Belleville	Colby I	Tribune D Tribune I	Hesston St. John I	8

[a] Number of locations where electrophoretic band differences were found. The total number of locations was 16.
[b] D = Dryland.
[c] I = Irrigated.
[d] Mismarked; later identified as Triumph 64.

39, and 79 MU. The electrophoretic pattern of the Tribune D-H (M) kernel possesses the bottom bands (73–81 MU) characteristic of Sage (Fig. 1).

Electrophoregrams of the S-appearing Arkan kernels (Fig. 4) are also very similar to the bulk Arkan patterns (Fig. 1). Locations where the electrophoretic patterns of the S-appearing kernels exhibited additional bands (indicated by carats) were: Hays (A), 18 MU; Belleville (B), 18 MU; Colby I (H), 18 MU; Manhattan (J), 23 MU; Ottawa (K), 23 MU; and Parsons (L), 18, 23, and 38 MU. The location where the largest number of additional bands was noted was Parsons. However, positions of those additional bands were the same as from other locations; in fact, both the 18- and 38-MU bands are identical in mobility to bands in Arthur and Sage, respectively, which are not present in typical Arkan. The band at 23 MU is not found in either parent or the typical Arkan pattern.

To examine the extent of electrophoretic heterogeneity among locations for other HRW wheats, bulk samples of Newton and TAM 105 from the 16 sites were extracted. The resulting electrophoregrams are shown in Figures 5 and 6. For Newton (Fig. 5) bands not found in typical bulk Newton samples (Lookhart et al 1983; marked with a >) were found at Ottawa (C), Parsons (D), Colby D (L), and Tribune D (N). The patterns of Colby D and Tribune D are very dark because of their higher protein content.

The electrophoretic patterns of bulk samples of TAM 105 (Fig. 6) were more nearly identical. Only in electrophoregrams of TAM 105 samples from St. John I (J) and Colby D (L) were differences found. In the pattern of Colby D only a band intensity difference (marked with <) was found; the band marked is much darker than the respective band in samples from other locations. The pattern of the St. John I sample is completely different from TAM 105. It does

not correspond to TAM 105, because several intense TAM 105 bands are absent. Electrophoretic analysis tentatively identified this sample as Triumph 64, evidently mismarked in the field. Since we previously found 4% of samples obtained to be mismarked (Jones et al 1982), finding one mismarked sample out of the 87 obtained is less than expected.

DISCUSSION

Table II summarizes electrophoretic differences among wheat samples from the 16 sites. The number of locations where the electrophoregrams of bulk samples of Newton, TAM 105, and Arkan appeared to differ from typical samples were 4, 2, and 7, respectively. No pattern correlating electrophoretic differences with state locations could be established.

It was very evident that wheat breeders do not breed for electrophoretic purity; there has been no incentive to do so until recently, when interclass breeding and use of exotic germ plasms has begun to cause significant variations in kernel characteristics, resulting in problems in classification.

Single-kernel analysis of Arkan wheat segregated into H- and S-appearing kernels indicates that kernel appearance is not related to electrophoretic variation among kernels. The percentage of S-appearing kernels varied with location. In locations where patterns had additional bands in only S- or H-type kernels, the extra band differences were not found in the bulk pattern, which may be due to the relative number of those kernels. However, when differences were found in the patterns of both H and S single kernels, for example, Parsons, Belleville, and Colby I, the same band differences were found in bulk Arkan patterns.

In summary, this paper has shown that heterogeneity sometimes found in pure cultivars represents biotypes and is not the result of outcrossing or mixtures. In addition, each of the cultivars examined had some inherent variability in band patterns among locations and among single kernels, the extent of which may differ for cultivars. Finally, these results suggest that electrophoretic variability is not necessarily related to apparent kernel hardness.

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