Identification of Wheat Cultivars by Gliadin Electrophoresis: Electrophoregrams of the 88 Wheat Cultivars Most Commonly Grown in the United States in 1979[1]

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ABSTRACT

The polyacrylamide gel electrophoretic patterns (electrophoregrams) of the gliadins from 88 U.S. wheat cultivars were determined and cataloged. The cultivars were those grown on the largest acreages—each cultivar on 130,000 acres (0.2% of the total U.S. wheat acreage) or more—in 1979. The 88 cultivars comprised 89.3% of the 1979 acreage. The following classes and numbers of wheats were investigated: 37 hard red winter, 17 hard red spring, 12 soft red winter, 14 common white, one white club, and seven durum. Most of the cultivars were readily differentiated by their electrophoregrams. Some very closely related cultivars gave identical patterns and were thus not uniquely identifiable by polyacrylamide gel electrophoresis.

A method that can quickly and accurately identify unknown wheat cultivars is needed in the United States. Such a method would allow people in the wheat industry to ensure that the cultivars they obtain are suitable for their intended uses and permit researchers to verify that their experiments are conducted with correct varieties. As one example of the need for such a method, we found that eight (about 4%) of 193 samples sent to us for analysis were apparently incorrectly identified.

Several groups have shown and confirmed that the gliadin compositions of wheats are genetically determined and are not altered by environmental factors (Zillman and Bushuk 1979a). Zillman and Bushuk (1979a) extended those findings by showing that gliadin polyacrylamide gel electrophoretic (PAGE) patterns are normally not affected by many experimental factors. We used an improved PAGE method (Lookhart et al 1982) to determine the electrophoretic patterns of gliadins extracted from the 88 most commonly grown (1979) U.S. wheat cultivars. Those patterns are reported in this article.

MATERIALS AND METHODS

Wheat Samples

The goal of this study was to investigate each of the wheat cultivars grown on 130,000 or more acres in the United States in 1979. To determine which cultivars met that criterion, Crop and

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TABLE I
Wheats Examined by Polyacrylamide Gel Electrophoresis

Cultivar	Pedigree[a]
	Hard Red Winter
Agent	Triumph/ / *Triticum* spp./ *Agropyron elongatum*[b]
Baca	Selection from Scout
Buckskin	Scout/4/Quivira/ /Tenmarq/3/Marquillo/Oro
Caddo	Wichita/ /Marquis/Oro
Centurk	Kenya 58/ /Newthatch/3/Hope/2* Turkey/4/ Cheyenne/5/Parker
Cheyenne	Selection from Crimean (CI 1435)
Concho	Blackhull/Hard Federation
Danne	Super Triumph/Western Prince
Eagle	Selection from Scout
Gage	Ponca/3/Mediterranean/Hope/ /Pawnee[c]
Homestead	Scout/4/Kenya 58/Newthatch/ /Cheyenne/ Tenmarq/Mediterranean/Hope/3/Pawnee/ Cheyenne
Improved Triumph	Danne Beardless/Blackhull/3/Kanred/ Blackhull/ /Florence
Jeff	Itana/ /Kiowa/PI 178383
Lancer	Turkey/Cheyenne/ /Hope/2* Cheyenne
Lancota	Atlas 66/Comanche/ /Lancer
Larned	Scout *5/Ottawa[d]
Newton	Pitic 62/Chris sib/ /2* Sonora 64/3/Klein Rendidor/4/Scout[d]
Osage	5* Scout/Agent
Palo Duro	Tascosa *4/Norin derivative
Parker	Quivira/3/Kanred/Hard Federation/ /Prelude/ Kanred/4/Kawvale/Marquillo/ /Kawvale/ Tenmarq
Roughrider	NB63265/Hume/3/Yogo/Frontana/ /2* Minter
Sage	Agent/4* Scout
Satanta	Tascosa *4/Norin 10 derivative
Scout	Nebred/ /Hope/Turkey/3/Cheyenne/Ponca[c]
Scout 66	Composite of 85 Selections of Scout
Sturdy	Crockett sib/Seu Seun 27
TAM W-101	Norin 16/3/Nebraska 60/ /Mediterranean/ Hope/4/Bison[d]
Tascosa	Mediterranean/Hope/ /Kanred-Hard Federation-Tenmarq/3/Cimarron
Trison	Triumph/Bison
Triumph	Florence/ /Kanred/Blackhull/3/Kanred/ Blackhull
Triumph 64	Purification of Danne's "Rust Resistant" Triumph[d]
Vona	II 21183/CO652643/ /Lancer/KS62136[d]
Wanser	Burt/Itana
Warrior	Pawnee/Cheyenne
Wichita	Early Blackhull/Tenmarq
Winalta	Minter/Wichita
Winoka	Mixture of six lines of Winalta
	Hard Red Spring
Anza	Lerma Rojo/ /Norin 10/Brevor/4/Yaktana 54/ / Norin 10/Brevor/3/3* Andes
Butte	ND480/Polk/ /Wisconsin 261[e]
Ellar	Waldron/ND140
Era	II-50-10/4/Pembina/II-52-329/3/II-53-38/III-58-4/ /II-53-546
Fortuna	Rescue/Chinook/4/Frontana/3/ Thatcher/ /Kenya 58/Newthatch
Kitt	MNII-55-14/MNII-60-15
Lew	Fortuna/S6285
Newana	Sheridan/ /CI 13253/5* Centana
Olaf	Waldron/Semidwarf line including Justin, Conley and Norin 10
Prodax	Tezanos Pinto Precoz/Sonora 64/3/Lerma Rojo 64/Tezanos Pinto Precoz/ /Andes Dwarf/4/ 2* Jaral/ /Mengavi/8156[e]
Protor	Tobari 66/Ciano 67 sib
Solar	Selected from crosses involving Sonora 64 and Tezanos Pinto Precoz[f]
Tioga	Fortuna/3/ND4/Rescue/ /II-50-17/51-3349
Waldron	Justin/4/Lee/3/Kenya 338A/ / Lee/Mida (ND81)
Wared	II-55-10/4/Pembina/II-52-329/3/II-53-38/ II-58-4/ /II-53-546[e]
World Seeds 1809	Sonora 64/Pitic 62/ /Chris sib[g]
Yecora Rojo	Ciano 67/ /Sonora 64/Klein Rendidor/3/II 8156
	Soft Red Winter
Abe	Arthur *4/3/Purdue 6028A-15-9-2/2/Riley *2/Riley 67
Arthur	Stadler/Redcoat
Arthur 71	Arthur *5/3/Purdue 6028A2-15-9-2/ /Riley sib *2/Riley 67
Beau	Arthur *3/3/Purdue 6028A2-15-9-2/ /Riley *2/Riley 67[h]
Coker 68-15	Coker 57-6 *2/Purdue 4946A4-18-2-10-1[i]
Coker 747	Arthur/Coker 68-15[i]
Doublecrop	Selection from Arthur
Hart	Etoile de Choisy/Thorne-Clarkan/ /Pawnee/ CI 12454
Logan	Vermillion/Lucas
Oasis	Arthur 71 *5/5/Arthur *3/3/Purdue 6028A2-15-9-2/ /Riley *2/Riley 67 *2/4/Arthur *2/3/Riley 67 *2/ /Riley/ Bulgaria 88[j]
Pioneer S-76	Purdue 4946-A4-18-2/Missouri W 7510[k]

(continued on next page)

[a] Unless noted differently, pedigrees are from Zeven and Zeven-Hissink (1976) with (X) indicating backcrosses changed to (*).
[b] Pedigree from Smith et al (1968).
[c] Pedigree from Walter (1977).
[d] Pedigree from Walter and Fjell (1980).
[e] Pedigree from Wheat Variety Handbook, USDA (1976).
[f] Pedigree from Anonymous (1980).
[g] Pedigree courtesy World Seeds, Inc., Carlsbad, CA.
[h] Pedigree from Patterson et al (1978a).
[i] Pedigree courtesy of H. Harrison, Coker Pedigreed Seed Co., Hartsville, SC.
[j] Pedigree from Patterson et al (1975).
[k] Pedigree courtesy of M. Iwig, Pioneer Hi-Bred Seed, Tipton, IN.
[l] Pedigree from Patterson et al (1978b).
[m] White club wheat. Other cultivars are common white wheats.

Livestock Reporting Service representatives in the various states were polled. This poll resulted in a list of 80 cultivars that were investigated (Jones et al 1980). As the experiments on the original 80 varieties were being finished, we received a copy of the official U.S. Department of Agriculture wheat-acreage report. That report confirmed that the 80 previously investigated cultivars had been grown on the requisite 130,000 acres and listed eight additional cultivars that met the criterion. Samples of the eight cultivars were obtained and investigated, making a total of 88 cultivars examined.

Duplicate samples of each of the wheats were obtained from separate researchers located throughout the United States (see Acknowledgments). If the duplicate samples did not give identical gliadin PAGE patterns, another sample of the cultivar was obtained from a third source. The cultivars and their pedigrees are listed in Table I.

Sample Preparation and Electrophoresis

Wheat samples were ground, extracted, electrophoresed, stained, and destained as reported by Lookhart et al (1982).

Determination of the Relative Mobilities of Gliadin Bands

The destained PAGE gels were photographed, and reversal negatives of the gels were produced as reported previously (Lookhart et al 1982). The final reversals (consisting of black bands on a transparent background) were scanned with 500-nm light by using a Kratos SD 3000 spectrodensitometer operated in the transmittance mode. The output from the densitometer was plotted as absorbance versus time by a Hewlett-Packard 3385 printer-plotter, which automatically printed the time each peak of absorbance (dark band) was encountered. For best results, the

TABLE I (*cont.*)

Cultivar	Pedigree[a]
Redcoat	Supresa PI 103,833/Fultz sel CI 11,845/7/ Kaw vale/5/Fultz/Hungarian/2/I11 No. 1, W 38/3/Wabash/4/Fairfield/6/Trumbull *3/2/Hope/Hussar[l]
Ruler	L494A1-8-5-5/Lucas
	White
Daws	CI 14484//CI 13645/PI 178383
Fielder	Yaktana 54A *4//Norin 10/Brevor/3/2* Yaqui 50/4/Norin 10/Brevor//Baart/Onas
Fieldwin	Yaktana 54A *4//Norin 10/Brevor/3/2* Yaqui 50/4/Norin 10/Brevor/ /Baart/Onas
Gaines	Norin 10/Brevor//Orfed/Brevor sib/3/Burt
Hyslop	Nord Desprez/2* Pullman Sel. 101 (CI 13438)
Luke	PI 178383/Burt/ /Sel. 101 (CI 13438)
Marfed	Marquis/Florence//Federation
McDermid	Nord Desprez/2* Pullman Sel. 101
Moro[m]	PI 178383/2* Omar
Nugaines	Sibling of Gaines
Sprague	PI 181268/Gaines
Stephens	Nord Desprez/Pullman Sel. 101[e]
Techumseh	Minhardy/Wabash/5/Fultz Sel./Hungarian/ 2/W38/3/Wabash/4/Fairfield/6/ Redcoat sib/WI 245/7/Vigo/4/Trumbull/ 2/Hope/Hussar/3/Fulhio/Purkoff *3/5/Kenya Farmer[e]
Twin	Norin 10/Brevor//3* Lemhi 53/3/Lemhi 62/4/ Lemhi 53 *5/3/Lee *7/ /Chinese/ *Aegilops umbellulatum*
Yorkstar	Genesee *5/3/Yorkwin//Norin 10/Brevor
	Durum
Botno	Langdon/3/Langdon Sel. 357/ /CI 7780/ Langdon Sel. 362/4/Br 180/Wells[e]
Cando	Lakota/5/Willet sib//Norin 10/ Brevor/3/Langdon/4/Langdon/6/Leeds/7/ Br 180/Wells
Crosby	Langdon *2/ST 464/ /Leeds[e]
Rolette	LD 393/2* Yuma/3/LD 398//LD 357 *2/ST 464
Rugby	Langdon/3/Langdon Sel. 357//CI 7780/ Langdon Sel. 362/4/Br 180/Wells[e]
Ward	Langdon/3/Langdon Sel. 357//CI 7780/ Langdon Sel. 362/4/Br 180/Wells[e]
Wells	Sentry//LD 379/LD 357

reversals were scanned at 25 mm/min and the printer-plotter was run at the same speed. That gave a density-versus-distance plot that was the same size as the reversal, making the bands on the reversals and the peaks on the printer-plotter scan easy to match up. The density plot gave semiquantitative information about the density of each band and a precise measure of how far down the gel each protein band had run. Because the scans were run at 25 mm/min and the printer-plotter labeled the peaks in minutes, the distance (in centimeters) that a band had moved into the gel was calculated by multiplying the plotter time by 2.5. Conversely, a ruler marked in "units" of 25 mm could be used to measure the distance, in minutes, that a band had moved. That was necessary in some cases because, for calculating relative migration distances, we used the time at which a peak was detected, as determined from the densitometer trace, as the distance that the peak had moved. Because some bands that showed up on the densitometer traces were not integrated separately (shoulders on large peaks, etc.), the distances those bands had moved had to be measured manually. In each electrophoregram, some protein material did not enter the gel but remained at the origin of the electrophoregram and served as an indicator of the point of sample application.

Eight-slot gels were run with a standard, Marquis, which was normally applied in slots 1, 4, and 8. Five "unknown" samples were placed in the remaining sample wells. This ensured that no unknown samples were run in the slots adjacent to the edge of the gel, where "edge effects" were sometimes noticed. By comparing the Marquis patterns across the gel, an indication of the uniformity of conditions across the gel was obtained.

The distance from the origin to the center of the Marquis reference band (the heavy single band that ran slightly farther down the gel than the heavy doublet) was assigned a relative distance value of 50 units, following the practice of Bushuk and Zillman (1978). For each band of an electrophoregram, the distance (in minutes) from the origin to the center of that band was determined by the densitometer tracing and, after substraction of the distance from scan start to the point where the sample had been applied, was taken as the band migration distance. This migration distance value was divided by the migration distance of the Marquis standard band, and the result was multiplied by 50 to give the relative migration distance of the band. The precision of the relative mobilities was generally ±0.5 units. The mobilities of each band, calculated from the duplicate electrophoretic runs, were averaged and rounded off to the nearest whole unit.

Determination of Band Densities

The reversal negatives (positives) of the gels were placed on a light box (Lookhart et al 1982), and each band was subjectively assigned a numerical value from 1 (very light) to 5 (very dark), according to the method of Zillman and Bushuk (1979b). The precision of the band density assignment was ±1 unit. Three people assigned density values to all bands, and the numbers assigned by the various investigators were identical in more than 95% of the cases. We apparently gave bands of comparable densities smaller values than did Zillman and Bushuk (1979b), because they assigned densities of 3 to many of the Marquis bands that we regarded as having densities of 2. Originally, an attempt was made to assign band densities on the basis of the areas under the density peaks as determined by the scanning densitometer traces. That method was not reliable, however, because of problems with bands that showed up as shoulders on large peaks and because the background varied at different positions along the gel. Also, the reversals being scanned varied somewhat because of differences in exposure times and other factors. The exposure and developing times varied among the gels because the gels were sometimes stained and/or destained for different times. Some of the problems in assigning band densities from integrator data could probably be resolved by complicated computer massaging of the data, but assigning the densities as reported above was found to be much simpler and more reliable.

RESULTS AND DISCUSSION

Cultivars Analyzed

For this study, 88 cultivars were analyzed, including each variety grown on more than 130,000 acres in the United States in 1979.[4] Because 130,000 acres amounted to less than 0.2% of the total 1979 U.S. wheat acreage, we examined all American wheat varieties that would normally be encountered in commerce. The 88 cultivars together were grown on a total of 63.9 million acres and thus comprised 89.3% of the total 1979 wheat acreage (71.5 million acres).

Gels

A typical gel, loaded with gliadins extracted from five different cultivars and with three samples of the standard Marquis gliadin is shown in Fig. 1. The samples placed in the outside sample wells characteristically showed some distortion of their leading bands. The outer wells normally contained the Marquis controls because they were used only to determine the distance the standard 50-unit band had run. Because this band did not run near the front, it was normally not distorted. The unknown samples on this gel were hard red winter wheats commonly grown in the southern part of the

[4]L. W. Briggle, USDA, personal communication.

Great Plains, and all of these cultivars were easily differentiated from each other by their electrophoretic patterns.

To ensure against misidentification of samples, duplicate samples of all 88 cultivars were extracted and analyzed. The fact that the duplicate samples were grown in different parts of the United States, under different climatic conditions, did not affect their gliadin electrophoresis patterns. One example of this is shown in Fig. 2. In this gel, slots 2, 3, 4, and 6 were loaded with gliadins extracted from the durum wheat cultivar Botno. The gliadins run in slots 2 and 4 were extracted from wheat obtained from South Dakota; the material in slots 3 and 6 was from Maryland. The only discernible difference between the South Dakota and Maryland samples was that a little less protein seemed to have been extracted from the Maryland wheat; it yielded lighter protein bands. The positions of the bands along the gel, and even the densities of the bands relative to each other, were essentially identical. This gel also shows that the gliadin samples were stable to storage; the samples run in slots 2 and 3 had been stored six months (at 4° C) before analysis, whereas the samples in slots 4 and 6 were freshly extracted.

Of the original 176 samples obtained for this study, seven pairs gave gliadin electrophoretic patterns that were not identical. In these cases, a third sample was obtained from a third source. Each of the seven new samples, when analyzed, was identical to one of the original samples. Thus about 4% of the samples sent to us for analysis were apparently mislabeled. We could not find any evidence for the presence of different biotypes in our samples because we did not analyze any single-seed samples but worked only with 3-g bulked samples.

Densitometry

Comparing the PAGE patterns of the various cultivars directly, using either fresh gels or photographs, was a tedious procedure. A much easier method was to scan the patterns, getting a plot of density versus distance from the origin, and to compare these scans with each other. We first attempted to scan the stained gels directly. That proved technically infeasible because of the physical properties of the gel and the fact that blemishes within and at the surfaces of the gels caused artifacts. When photographs of the gels were made and reversals were made from the photograph negatives, the reversals were easily scanned and did not cause artifacts. The densitometer was sensitive enough to detect bands barely observable by eye. In Fig. 3, which is a photograph of the densitometer scan of the Tascosa gliadin electrophoregram from slot 5 of the gel shown in Fig. 1, the numbers printed above the peaks correspond to the distance the band had moved into the gel (where 1 unit = 25 mm). These distances correspond to the centers of the bands and, after calculating the distance from that point to the origin, we used these values to calculate the distance that particular band had moved down the gel. The quickest and most reliable way of comparing the gliadin electrophoregrams of two wheat cultivars was to place the densitometer scan of one variety upon a light box and superimpose the scan of the second cultivar upon the first. The differences and similarities between the two were then obvious.

Catalog of Cultivar Electrophoregrams

Each cultivar was characterized by a formula that consisted of the relative distances traveled into the gel and the densities of all of the protein bands associated with the cultivar, following the method of Bushuk and Zillman (1978) and Zillman and Bushuk (1979b). The formulas determined for each of the 88 cultivars

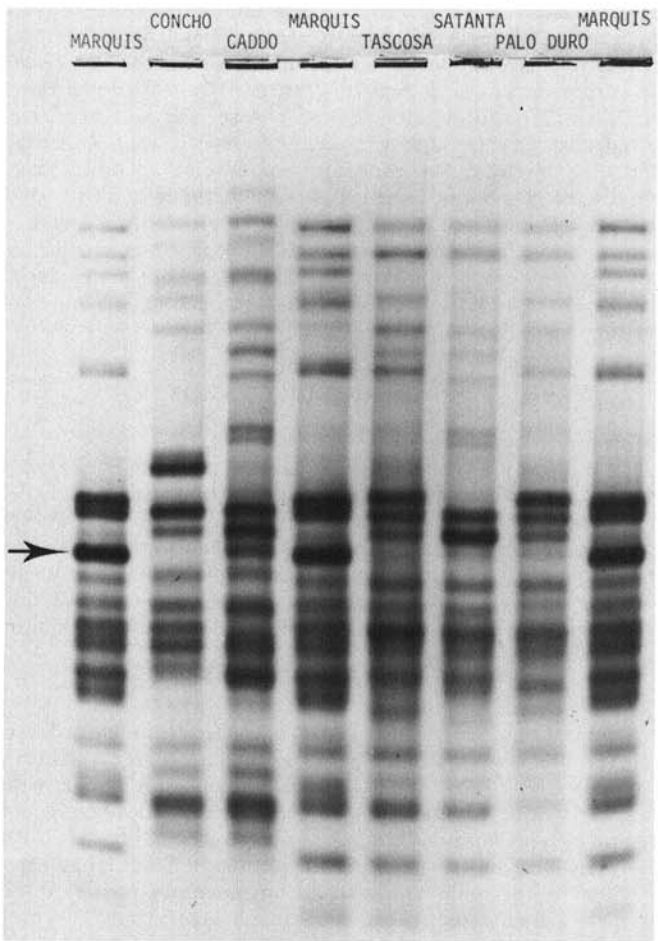

Fig. 1. Typical gliadin electrophoresis gel. The arrow indicates the Marquis band defined as having moved 50 units into the gel.

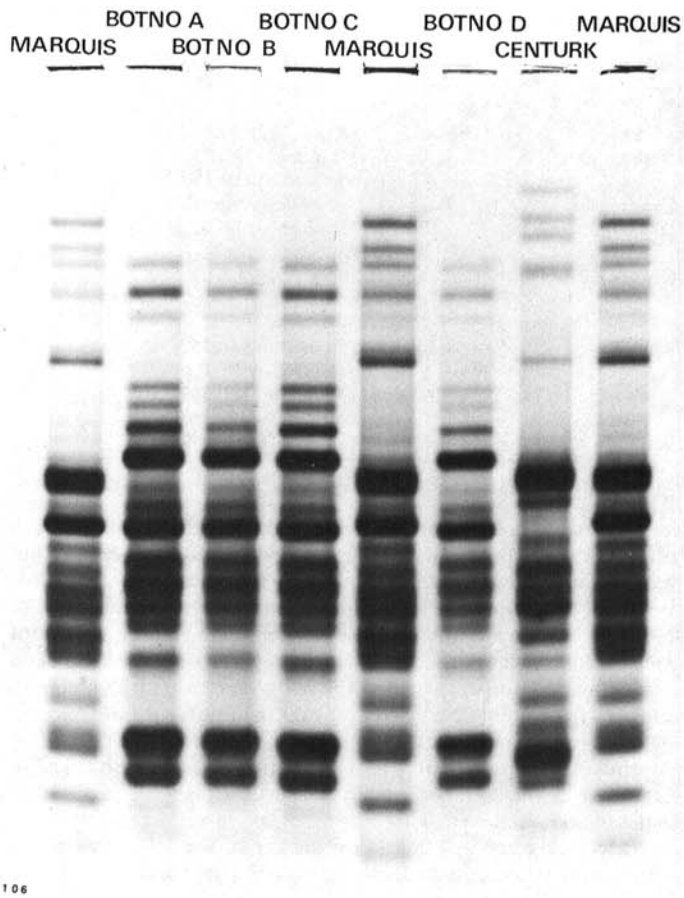

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Fig. 2. Reproducibility of electrophoregrams with gliadins from wheat obtained from different sources and with gliadins stored for six weeks. **Botno A**, gliadins from Botno (durum) wheat from South Dakota, stored six weeks (4° C) before analysis; **Botno B**, wheat from Maryland, stored six weeks before analysis; **Botno C**, wheat from South Dakota, freshly extracted; **Botno D**, wheat from Maryland, freshly extracted. The second Marquis standard was run in slot 5 instead of slot 4 to facilitate comparisons of the Botno B and C samples.

cataloged in Table I are shown in Tables II–VI.

Generally, the mobility values listed were obtained by averaging the values obtained from the duplicate runs and rounding those values to the nearest whole unit. However, in several cases two or more cultivars gave patterns in which most or all of the protein bands occupied nearly identical positions. In those cases, the similar cultivars were reexamined by electrophoresing their gliadins in adjacent wells of a gel, which allowed us to determine precisely which bands were similar in relative mobility and which were identical.

The biggest problem in constructing the catalog was deciding whether or not very faint bands (present in many of the gels) were real. If the band could be seen in duplicate gels, we included it in the catalog. However, if data from Tables II–VI are compared with data from gels that are poorly destained or streaked or that have been loaded with insufficient protein, some of the bands listed as "1" in density may not be apparent.

Hard Red Winter Wheats

Most of the hard red winter (HRW) wheats were readily differentiable by gliadin PAGE (Table II). Their gliadin bands fell between 13 and 87 relative units, with most of the heavily staining bands occurring 40–60 relative units into the gel. Some of the closely related HRW cultivars yielded gliadin electrophoregrams that were similar or identical. For example, the varieties Scout and Scout 66 gave identical patterns, as did Larned, Sage, and Eagle. In addition, the patterns of the Scout and the Larned groups were

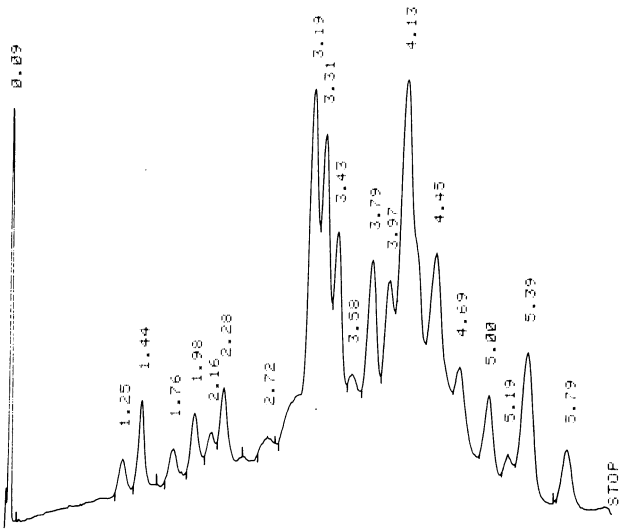

Fig. 3. Densitometer scan of a reversal negative of the Tascosa gliadin electrophoregram of Fig. 1. The numbers above the peaks indicate the times, in minutes, that bands were encountered by the densitometer. The reversal was scanned at 25 mm/min, so 1 min = 25 mm along the photo. The peak at 0.09 min represents the position at which the sample was applied to the gel.

TABLE II.

Electrophoretic Formulas of Gliadins of Hard Red Winter Wheats

CULTIVAR	Mobility of Band Relative to Marquis Standard Band (10 20 30 40 50 60 70 80 90)*
AGENT	2 2 2 1 2 1 1 5 4 1 3 22 22 2 2 1 2 3 2 1 1
BACA	1 2 1 2 1 2 1 1 5 2 1 3 4 3 1 1 1 3 1 1
BUCKSKIN	2 2 1 2 2 3 1 2 1 1 5 1 3 1 3 1 3 2 1 2 2 3 1 1 1
CADDO	1 2 1 2 1 2 1 22 5 4 3 2 3 3 4 1 1 1 3 1
CENTURK	1 1 1 2 2 1 5 2 2 3 2 3 2 1 1 1 4 2
CHEYENNE	1 1 1 1 1 2 44 2 1 3 3 2 1 1 1 4 2
CONCHO	1 2 1 2 1 2 1 34 4 3 2 2 3 32 1 2 3 2 1
DANNE	1 2 1 1 2 1 1 5 4 1 1 2 1 22 1 1 3 3 2
EAGLE	1 2 1 2 1 1 2 1 1 1 5 12 1 3 1 4 3 1 1 2 3 1 1
GAGE	1 2 1 2 1 2 1 1 5 12 1 3 4 4 1 2 1 3 2 2
HOMESTEAD	2 2 2 2 2 2 1 1 5 1 2 1 2 4 4 2 2 1 3 2
IMP. TRIUMPH	2 2 2 2 2 1 2 2 1 1 1 5 1 4 1 1 2 3 2 2 2 2 2 1
JEFF	1 2 1 2 2 2 3 1 15 2 1 2 4 3 3 3 2 2 2 3 2 1
LANCER	1 2 1 12 2 11 41 1 4 1 3 2 1 2 2 2 2 2 2 1
LANCOTA	1 1 1 1 1 1 1 1 5 1 3 1 3 3 2 2 2 2 2 2 1
LARNED	1 2 1 2 1 1 2 1 1 1 5 12 1 3 1 4 3 1 1 2 3 1 1
NEWTON	2 2 1 1 2 1 1 1 5 13 2 3 4 3 1 1 1 3 1
OSAGE	1 2 1 2 2 1 21 1 5 1 11 2 3 4 1 1 1 4 3 1
PALO DURO	1 2 2 2 2 1 2 1 1 1 1 4 3 3 1 2 2 2 1 2 1 2 1 2 2 1
PARKER	1 2 1 2 2 1 1 11 1 4 5 2 3 2 3 2 2 2 3 2 1
ROUGHRIDER	1 1 1 2 1 2 1 1 5 1 3 3 2 3 3 11 2 2 2 2 1
SAGE	1 2 1 2 1 1 2 1 1 1 5 12 1 3 1 4 3 1 1 2 3 1 1
SATANTA	2 2 1 1 1 1 22 1 4 5 1 3 2 3 1 3 1 2 1 3 3 1 1
SCOUT	1 2 1 2 1 1 2 1 1 1 5 12 1 3 4 3 1 1 1 3 1
SCOUT 66	1 2 1 2 1 1 2 1 1 1 5 12 1 3 4 3 1 1 1 3 1
STURDY	2 2 1 1 1 2 1 22 4 5 5 1 2 22 2 1 1 2 2 1
TAM W-101	1 2 3 2 1 2 2 3 1 2 55 2 3 3 3 22 2 1 2 2 2 12
TASCOSA	1 2 3 2 2 1 2 1 1 1 1 4 3 31 2 2 3 1 2 1 2 1 3 2 1
TRISON	1 21 2 2 1 2 2 1 22 14 4 2 3 3 3 2 2 2 4 2 1 1 1
TRIUMPH	1 1 1 1 1 1 2 1 1 1 5 1 4 1 1 1 1 1 3 1 2 2 3 1
TRIUMPH 64	1 2 1 3 2 1 1 2 1 1 1 5 1 4 1 1 1 1 2 3 1 2 3 3 1 1 1
VONA	1 2 1 12 2 1 1 21 1 5 3 1 2 2 2 2 2 2 2 1
WANSER	2 2 1 1 2 1 5 1 1 5 1 3 2 3 32 2 2 1 2 2 3 3 1
WARRIOR	2 2 2 2 2 2 1 5 2 1 4 4 3 2 2 1 3 2 1
WICHITA	1 2 1 3 1 22 14 5 3 3 23 3 1 2 2 4 2 1 1
WINALTA	2 2 2 2 2 2 1 1 5 2 1 4 4 3 2 2 1 3 2 1
WINOKA	2 2 2 2 2 2 1 1 5 2 1 4 4 3 2 2 1 3 2 1

*Relative band intensity, 1 is lightest, 5 is darkest.
Position of band on gel is indicated by scales at top and bottom of table.
All gliadin bands were found between 10 and 90 units.

nearly identical. The electrophoregrams of Baca and Homestead, though not identical to either Scout or Larned, were very similar to both. All cultivars with very similar electrophoregrams were closely related (Table I). All except Homestead were either direct selections from Scout or had arisen via multiple backcrosses with Scout. The final cross leading to Homestead involved Scout as the female partner; hence a large proportion of the genetic material in the endosperm of Homestead came from Scout.

One other pair of HRW wheats, Winoka and Winalta, had identical gliadin electrophoregrams. These two cultivars have essentially identical genetic complements because Winoka is a reselection of several lines of Winalta (Table I).

Hard Red Spring Wheats

Two of the hard red spring wheats, Era and Solar, gave identical gliadin electrophoregrams (Table III). The pedigrees for these two cultivars are very different (Table I). In all other cases encountered during this study in which two cultivars were found to give identical electrophoregrams, the two had virtually identical genetic backgrounds (ie, were from reselections or multiple backcrosses or were sibs). The electrophoregrams of Era and Solar thus indicated that the two cultivars are probably genetically very similar, suggesting that one or both of the reported pedigrees may be in error.

The only other two hard red spring cultivars that gave similar (but still readily differentiable) electrophoregrams were Ellar and Olaf. These two cultivars share a common parent, Waldron.

Soft Red Winter Wheats

Most of the 13 soft red winter wheats investigated yielded quite

TABLE III.

Electrophoretic Formulas of Gliadins of Hard Red Spring Wheats

Mobility of Band Relative to Marquis Standard Band

CULTIVAR	10	20	30	40	50	60	70	80	90
ANZA	1*	2 2 1 1 22	1 1 5 2 1 3 2 3 4 3	1 1 3 2 1					
BUTTE	2 2 1 2 11	22	3 5 2 1 1 1 2 1	2 11 2 2 1					
ELLAR	2 3 12 2 22	1 1 43 2 1 2 2 4 1 2 2	2 1 1 2 2	1					
ERA	2 2 2 3 2 2 11 1	22 1 4 5 5 1 1 1 3 3 2	2 2 3 2						
FORTUNA	1 2 1 1 2 2 2 2	1 5 4 2 1 2 1 3 1 11 1	2 1 2 2						
KITT	1 2 1 2 2 1	22 5 5 1 3 1 4 3 3 3	2 2 2 1						
LEW	1 2 1 2 1 1 12	1 5 2 2 2 3 2 1 2	1 11 1						
MARQUIS	2 1 1 1 1 12	1 144 1 5 2 2 2 2 2 2	1 1 2 2 1						
NEWANA	1 2 1 3 2 11 2	22 5 4 1 1 1 2 3 4 2	2 2 2 2 1						
OLAF	2 2 1 1 1 2	1 1 54 2 1 2 1 4 1 2 2	1 1 2 1 1						
PRODAX	1 2 1 1 1 2	1 11 4 5 4 3 1 3 2 2	1 2 1 3 1						
PROTOR	1 2 1 2 2 1 1 2	1 11 4 4 1 4 2 22 3 2	2 1 2 2 2 1						
SOLAR	2 2 2 3 2 2 11 1	22 1 4 5 5 1 1 1 3 3 2	2 2 3 2						
TIOGA	1 2 2 1 1 2	5 2 3 1 3 2 1 3	2 1 2 2 1						
WALDRON	1 2 2 1 11 2 3	1 1 43 2 1 2 1 2 1 1 12	1 1 1 1 2 2 1						
WARED	2 2 2 2 2 1 1	11 1 5 4 1 1 3 3 5 2	2 21 2 1						
WORLD SEED 1809	1 2 2 11 1 22	1 5 3 3 2 3 2 231	2 1 3 2 2						
YECORA ROJO	1 2 2 1 1 2	1 22 1 1 4 4 32 2 22 32	2 2 3 2 2						

*Relative band intensity, 1 is lightest, 5 is darkest.
Position of band on gel is indicated by scales at top and bottom of table.
All gliadin bands were found between 10 and 90 units.

TABLE IV.

Electrophoretic Formulas of Gliadins of Soft Red Winter Wheats

Mobility of Band Relative to Marquis Standard Band

CULTIVAR	10	20	30	40	50	60	70	80	90
ABE	1* 2 2 2	1 1 1 55 1 1 1 2 3 4 1	2 3 2 1						
ARTHUR	1 2 2 1 2 1	1 15 5 1 1 1 2 3 4	2 1 3 1 1 11						
ARTHUR 71	1 2 2 1 2 1	1 1 5 5 1 1 1 2 3 4 1	2 3 2 1						
BEAU	2 2 2	1 1 55 1 1 2 2 3 4 2 1	2 1 3 2 1						
COKER 68-15	1 2 1 2 1	5 1 2 2 2 3 3 1 1 1	3 1 1						
COKER 747	2 2 1 2	1 1 55 1 1 2 2 4 4 1 1	2 1 3 2 1						
DOUBLECROP	1 2 2 12	1 1 5 5 1 1 1 1 2 3 4 1	2 1 3 2 1						
LOGAN	2 2 1 2 1 11	1 4 3 1 1 2 22 3 1	21 3 2 1						
OASIS	1 2 2 1 2	1 1 1 5 5 1 1 1 2 3 4 1	2 3 2 1						
PIONEER S-76	1 1 1 1 2	1 1 54 1 3 2 3 3 41 1 2 2	3 1 1						
REDCOAT	1 2 2 1 2	1 1 1 5 5 1 1 1 2 3 4 1 2	1 3 2 1						
RULER	1 2 2 1 1	1 1 5 5 1 1 1 1 3 4	2 1 13 2						

*Relative band intensity, 1 is lightest, 5 is darkest.
Position of band on gel is indicated by scales at top and bottom of table.
All gliadin bands were found between 10 and 90 units.

similar electrophoregrams (Table IV). The gliadin patterns of Abe, Arthur, Arthur 71, Redcoat, Oasis, Beau, and Doublecrop were especially similar, although all except Arthur 71 and Oasis could be differentiated from each other. The final crosses in the synthesis of Oasis were four backcrosses with Arthur 71 (Table I) so we were not surprised that Oasis and Arthur 71 contained identical gliadins. In contrast, cultivars Abe and Arthur 71, both of which contain multiple backcrosses with Arthur in their pedigrees (Table I), gave gliadin electrophoregrams distinguishable from Arthur's.

White Wheats

The gliadins from two white wheat cultivars, Gaines and Nugaines, produced identical electrophoregrams. That was not surprising because both cultivars were selected from the same cross (Table I). On the other hand, McDermid, Hyslop, and Stephens, which were synthesized by crossing the same parents, Nord Desprez and Pullman Selection 101, gave electrophoregrams dissimilar enough that each could be easily identified, although they had several bands in common (Table V). Fielder and Fieldwin, two cultivars selected from the same cross (Table I), had identical electrophoretic patterns except for one band (Table V), which electrophoresed a distance of 65 units in Fielder but 66 units in Fieldwin. A difference of 1 unit in 65 is not sufficient to differentiate two cultivars electrophoresed on separate gels, but is large enough to allow a preliminary identification of an unknown electrophoresed between Fielder and Fieldwin standards on a common gel.

Durum Wheats

The electrophoregrams of the durum wheats are clearly different from those of the other wheat classes (Table VI). None of the durum cultivars contained any gliadins that migrated less than 18 units into the gels, whereas all of the nondurum wheats had at least one band that moved 17 units or less. That the durums are very different from the other wheats is not surprising because the D genome is not present in the durum wheats and some of the chromosomes of the D genome are known to control the synthesis of several gliadins (Konzak 1977).

The electrophoregrams of the various durum cultivars were quite similar to each other, indicating little genetic diversity among the

TABLE V.

Electrophoretic Formulas of Gliadins of White Wheats

Mobility of Band Relative to Marquis Standard Band

CULTIVAR	10	20	30	40	50	60	70	80	90
DAWS	1* 1		11	11	4 5 4	2 2 33	3	1 1	3 11
FIELDER	2 3	1 2 2		1	1 5	3 1 2 3	43	3 21	2 2 3 2 1
FIELDWIN	2 3	1 2 2		1	1 5	3 1 2 3	43	3 2 1	2 2 3 2 1
GAINES	2 2	2	1	11	1 5 5 3	1 2 1 3	3 3 1	1 1 1	2 1
HYSLOP	1 1 1		1	11	1 5 5 2	1 2 2 1	2	1 1	22 1 1
LUKE	2 2		2	22	1 4 4 3	2 2 4 2 2 2		2 2	3 2 1
MARFED	2 2 2	2 11 2	2 2 1		1 1 5 3 1	2 3 33	4 3	2 1	3 2 1
MCDERMID	1 2 2		1	11	1 5 5 2	1 2 1 3	2	1 1	2 2 1
MORO	2 2	1 1	2	1	1 5	4 2 3	3 3	1 1 1	2 2 1
NUGAINES	2 2	2	1	11	1 5 5 3	1 2 1 3	3 3 1	1 1 1	2 1
SPRAGUE	2 2		1 1	11	1 5	4 2 2 3	2 2 4 2	2 1 1	2 2 1
STEPHENS	1 2 2	2 1	1 3 1	22	1 5 5	2 3	3 2 21	2 2	3 3 1 1
TECUMSEH	1 2 2	1	1 2	2	5 5 1	1 3 3	3 4 1	2 1	3 2 1
TWIN	1 1 1	1 1 1	2 1	1	1 5	2 1 1	1 2 1	3 1	1 2 1 2 1
YORKSTAR	2 2	1 2 1	2 1	1 1 4	5	1 1 2 2	3 4	2 1	2 2 1

*Relative band intensity, 1 is lightest, 5 is darkest.
Position of band on gel is indicated by scales at top and bottom of table.
All gliadin bands were found between 10 and 90 units.

TABLE VI.

Electrophoretic Formulas of Gliadins of Durum Wheats

Mobility of Band Relative to Marquis Standard Band

CULTIVAR	10	20	30	40	50	60	70	80	90
BOTNO	1* 2 2 1	1	2 2 2 1 5	1 2 4	2 2	2 2 1	3	3 1 1 1	
CANDO	1 2 2 1	11	2 2 2 2 5	1 2 4	2 2	2 2 1	3	2 1 1	
CROSBY	1 2 2 1	1	2 2 2 2 5	1 2 4	2 21	2 2 1	1 3	2 1 1	
ROLETTE	1 2 3 21	11	3 2 3 1 5	1 2 4	3 3	2 2 2	2	5 2 1 1	
RUGBY	1 2 2 1	1	2 2 2 1 5	1 2 4	2 2	2 2 1	3	3 1 1 1	
WARD	1 2 2 1	1	2 2 2 1 5	1 2 4	2 3	2 2 21	1 3	3 1 1 1	
WELLS	1 2 3 1	11	2 2 2 1 5	1 2 4	2 3	2 2 21	1 1 1 2	1 1 1	

*Relative band intensity, 1 is lightest, 5 is darkest.
Position of band on gel is indicated by scales at top and bottom of table.
All gliadin bands were found between 10 and 90 units.

commonly grown durum cultivars, at least for the gliadin genes. All durums could be differentiated by their electrophoretic patterns except for the pair Botno and Crosby, two cultivars selected from a single cross (Table I). A third cultivar selected from the same cross, Ward, had a similar electrophoregram but could be differentiated from Botno and Crosby by a band that moved 67 units into the gel.

CONCLUSIONS

This work shows that most of the wheat cultivars grown on appreciable acreages in the United States can be differentiated from each other by PAGE, using the method of Lookhart et al (1982). The same results have been reported for Canadian wheats (Zillman and Bushuk 1979b), although a somewhat different method was used to analyze the gliadins of the Canadian wheats (Bushuk and Zillman 1978).

Of the 88 U.S. varieties investigated in this study, all but 15 had unique electrophoregrams. Of those 15 cultivars, 12 were identical with only one other cultivar (making 6 pairs); the remaining three cultivars all shared a common electrophoregram. Several of the cultivars yielded electrophoregrams that were quite similar to those of other cultivars. In some cases in which two cultivars were found to have very similar electrophoregrams, the two cultivars had to be electrophoresed in adjacent positions on a gel to determine that they were, in fact, different.

In all cases (except one) in which cultivars had identical gliadin electrophoretic patterns, the cultivars were very closely related. They were cultivars that were either a) developed from multiple backcrosses, in which the cultivar and the parent used for backcrossing were identical; b) reselections, in which the original cultivar and the cultivar selected from it contained the same gliadins; or c) cultivars that were sibs, selected from crosses involving identical parents. In other instances, however, electrophoregrams of sibs were similar but readily differentiated from each other. We also found cases in which progeny cultivars could be differentiated from cultivars used in their pedigrees for final multiple backcrosses (eg, Abe and Arthur 71 versus Arthur). Finally, some cultivars that had been "reselected" gave electrophoregrams significantly different from those of the cultivar from which they had been selected (eg, Doublecrop and Arthur).

Era and Solar, a pair of cultivars reportedly having very different pedigrees (Anonymous 1980), yielded identical electrophoregrams. From this identity and because their biological and agronomic characteristics are very similar (Anonymous 1980), the listed pedigrees of one of these cultivars may be incorrect and they are probably genetically closely related, possibly sibs.

While conducting the research reported in this article, we found that about 4% of the samples sent to us as known cultivars were apparently mislabeled, in that duplicates of the cultivars gave very different gliadin patterns. The fact that all samples were obtained from people either doing breeding work or supplying germ plasm material to those conducting breeding indicates that the method should be of considerable use to wheat breeders, as well as to persons who want to more carefully control their variety protection rights, and to wheat consumers who need to purchase specified varieties appropriate to the end-use they want to achieve. We did not examine any single-kernel samples and thus did not see any evidence for multiple biotypes.

One can use the data reported in Tables II–VI as an indication of the identity of an unknown cultivar or to confirm or reject the identity of questionable wheat varieties. For absolute proof of the identity of an unknown wheat sample, however, one should electrophorese the gliadins of the unknown sample next to appropriate standard samples. For groups of cultivars that give identical gliadin patterns, some agronomic or other characteristic must be used to differentiate the group members.

The densitometer tracings of the electrophoregrams of various cultivars are much easier to compare than the actual gels or photographs of the gels, especially if several cultivars must be cross-compared. A computer program has been written that can compare the similarities of different gliadin electrophoregrams. That program and examples of its use will be described in a future article.

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