A Study of Gliadins of Soft Wheats from the Eastern United States Using a Modified Polyacrylamide Gel Electrophoresis Procedure

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ABSTRACT

The conventional continuous aluminum lactate-lactic acid buffered polyacrylamide gel electrophoresis system was modified by omitting aluminum lactate from the gel and lower (cathode) reservoir, giving a discontinuous system with the gel buffered with lactic acid only. Omission of aluminum lactate (usually contaminated) eliminates a potential source of impurities that affect gel formation and properties. Gliadins were extracted with ethylene glycol, giving high-density extracts that permit direct application to gels without additives. Using 10% acrylamide gel slabs, gliadin patterns of more than 120 soft red and soft white winter wheat cultivars were obtained. Patterns fell into four categories ("types") based on configuration of bands in the central region of the pattern: type I, a single heavy band; type II, two closely spaced heavy bands; type III, two widely spaced bands of moderate to heavy intensity; type IV, three (or more) bands of moderate intensity. About half of the cultivars studied were "Arthur type" (type II). Most soft white wheat cultivars were type III. Patterns of several cultivars (especially among types II and III) appeared to be very similar, if not identical.

Key words: prolamin, protein

Since it was first shown that cereal prolamin patterns are genotypical (Coulson and Sim 1964, Lee and Ronalds 1967), polyacrylamide gel electrophoresis (PAGE) has become established as a practical means for identifying cereal varieties. Many variations in apparatus, formulations, and procedures have been described, and techniques and applications have been summarized in several reviews (Autran et al 1979, Cooke 1984, Khan 1982, Lookhart et al 1985, Wrigley et al 1982). However, systematic comparisons of gliadin patterns of eastern U.S. soft wheats are lacking, and although some studies have included representative eastern soft wheat cultivars (Jones et al 1982; Sapirstein and Bushuk 1985a,b,c; Tkachuk and Metlish 1980), those studies were concerned with wheats in general or with differences among classes.

Because of their unique milling and baking properties, the soft red and white wheats have come to be regarded as a separate commodity in U.S. agriculture (Yamazaki and Greenwood 1981). In 1984, almost 100 soft red cultivars were grown in the United States. With minor exceptions, the acreage devoted to those wheats was in the eastern and southern regions. Thirteen soft white cultivars were grown exclusively in the eastern United States (Siegenthaler et al 1986). From a listing of recorded U.S. wheat varieties (Anonymous 1985), an estimated total of about 250 soft red and about 50 soft white varieties have been grown in those areas to date. Varieties grown in the eastern and southern states in the past, like those presently grown, were grown only to a limited extent in other areas (Patterson and Allen 1981).

The USDA Soft Wheat Quality Laboratory at Wooster, OH, processes several thousand experimental lines for quality evaluation each year. Because many entries represent early generations, sample size is often limited, and testing must be restricted to preliminary screening tests. A number of workers have suggested PAGE as a potential medium for prediction of quality characteristics of genotypes. Efforts directed toward use of gliadin profiles as predictors of hard and durum wheat quality have been reviewed by Cooke (1984), Feillet (1980), and Khan (1982). If correlations can be established between gliadin patterns of soft wheat genotypes and particular soft wheat quality traits, the technique could become invaluable for early generation testing because tests can be performed on a fraction of a kernel.

The objectives of this study were to obtain and compare gliadin patterns of soft wheat cultivars grown in the eastern and southern United States, primarily to provide a means for cultivar identification in the Wooster laboratory, but also to serve as a basis for projected studies of gliadin patterns as possible quality

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predictors. Because of problems encountered in attempts to apply published procedures, methods were modified. This report describes the modified procedure, presents patterns of selected soft wheat cultivars, and classifies cultivars according to similarities in banding patterns.

MATERIALS AND METHODS

Wheat

Wheats were from specimens on hand at the Wooster laboratory. Most samples represent specific crop years and locations, but some samples were pooled from more than one crop year or location. Generally, identities are based on identification provided by breeders when samples were submitted for quality testing. For a comparison of wheats grown at different locations, gliadin patterns were obtained from wheats grown at Wooster and at Custar, Ohio, in 1985 as part of a yield study by the Ohio State University Department of Agronomy. For the maturation study, six cultivars grown at Wooster by the Department of Agronomy in 1982 were sampled at three, two, and one week before harvest (July 15). The heads were freeze-dried immediately after picking, and then hand threshed and cleaned. Grain obtained at all samplings was full size but at the first sampling was still green in color.

Reagents

Acrylamide was from three sources: Research Organics, Inc., Cleveland, OH ("99+%, 2× crystallized"), Serva Fine Biochemicals, Inc., Westbury, NY ("2× crystallized"), and Sigma Chemical Co., St. Louis, MO ("suitable for electrophoresis"). Acrylamide was not further recrystallized, but stock solutions (Table I) containing acrylamide and bis-acrylamide (*N,N'*-methylenebisacrylamide, from Fisher Scientific Co., Fair Lawn, NJ) were treated with a mixed-bed ion-exchange resin (Amberlite MB-1, from Mallinckrodt, Inc., Paris, KY) by adding 5 g of resin to 250 ml of solution, stirring 1–2 hr, and filtering (Anonymous 1981). Lactic acid was 85% reagent grade (J. T. Baker Chemical Co., Phillipsburg, NJ), aluminum lactate was a "98%" grade (Pfaltz and Bauer, Waterbury, CT), and Coomassie Brilliant Blue R-250 was a "Min. 85%" grade (Serva Blue R, from Serva Fine Biochemicals). Ethylene glycol (Fisher Scientific Co.) and other reagents were reagent grade. Water was from a central deionizer but further purified by passage through two mixed-bed ion-exchange cartridges in series (IWT research grade model 2, manufactured by Illinois Water Treatment Co., Rockford, IL). Conductivity of the treated water was negligible (less than 0.1 $\mu S/cm$ at 25°C).

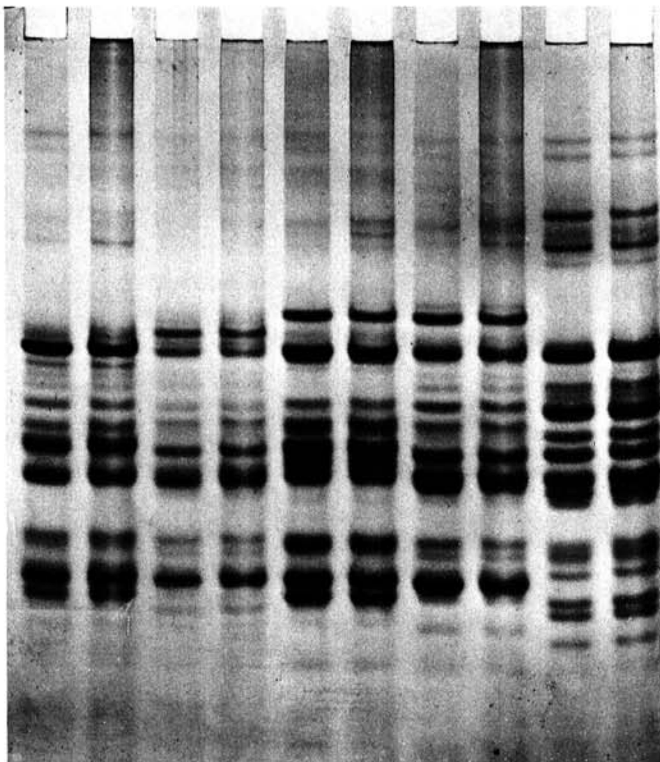

Fig. 1. Gliadin patterns from 70% ethanol extracts and ethylene glycol extracts (respectively, in each pair) of five wheat cultivars. The polyacrylamide gel eletrophoretic system (10% T, 3% C) was discontinuous, with lactic acid in the gel (pH 2.6) and lower reservoir (pH 2.6) and aluminum lactate-lactic acid buffer (pH 3.1) in the upper reservoir. Left to right: Adena, Augusta, Comanche, Hillsdale, Veery. Load, 15 μl; running time, 8 hr.

TABLE I
Preparation of Polyacrylamide Gel (10% T, 3% C) and of Reservoir Solutions[a]

Substance	Quantity
Gel (pH 2.6)	
Acrylamide stock solution	25 ml
Acrylamide, 32%	
Bisacrylamide, 1%	
Ferrous sulfate ($FeSO_4 \cdot H_2O$) 0.032%	10 ml
Water	45 ml
Lactic acid, 85%	0.4 ml
Ascorbic acid	80 mg
Hydrogen peroxide 0.6%	0.2 ml
Reservoirs	
Upper (pH 3.1)	
Stock solution	100 ml
Aluminum lactate, 10.0 g	
Lactic acid, 85% 16.0 ml	
Water, to 1,000 ml	
Water	600 ml
Lower (pH 2.6)	
Lactic acid 85%	16 ml
Water, to 4,100 ml	

[a]T, Total acrylamide; C, bisacrylamide concentration as percent of total acrylamide.

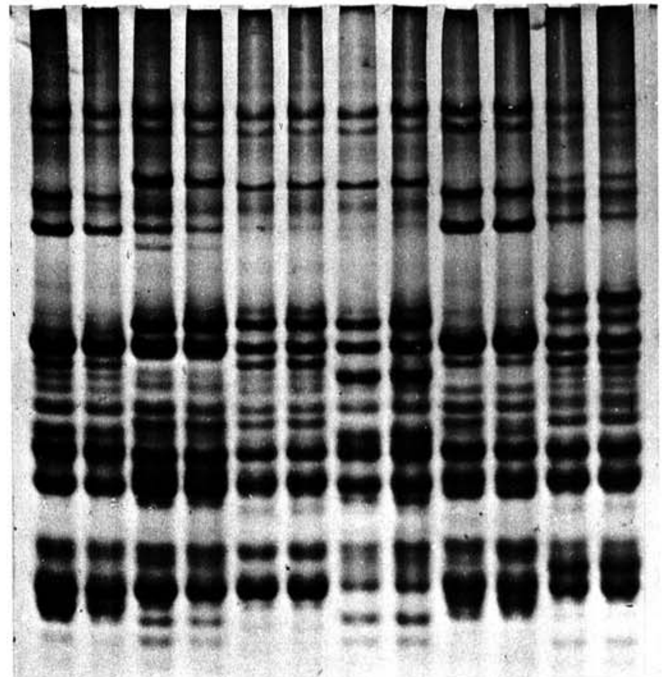

Fig. 2. Gliadin patterns of six soft red winter wheat cultivars grown at Wooster and at Custar, Ohio, in 1985. Pairs, from left to right: Adena, Arthur, Becker, Caldwell, Cardinal, Hart.

Protein Extraction

Gliadins were extracted from meals by suspending 1 g of meal in 2 ml of ethylene glycol. Suspensions were placed in a vacuum desiccator and the chamber was evacuated with an aspirator for 10–15 min. The suspensions were removed, agitated at intervals for 2–3 hr, and centrifuged at 25,000 × g. Supernatants were decanted and stored at 4° C. Extractions with 70% ethanol were performed in a similar manner but without vacuum infiltration. Sucrose solution (80%) was added to ethanol extracts (1:2, v/v) to increase density for sample application.

Electrophoresis

Gels, buffered with lactic acid, contained 10% acrylamide, with 3% cross-linkage (10% T, 3% C) (Table I). Electrophoresis was performed using 16 × 18 cm plates separated by 3-mm spacers (Hoefer model SE 600, Hoefer Scientific Instruments, San Francisco, CA) with temperature maintained at 20° C. Gels were cast with either 10 or 15 wells using combs provided, but well depth was reduced to 1 cm by taping stops to the combs. Before assembly, the inner surface of one plate in each pair was coated with a static preventive designed for fabrics (Static Guard, Alberto Culver Co., Melrose Park, IL) to promote release. Solutions for each gel were prepared individually (Table I), deaerated under vacuum with magnetic stirring, and cooled in a refrigerated bath (−50° C) until partially frozen. The solution was gently swirled at room temperature (and warmed with the hand, if necessary), and as the last traces of ice disappeared, the hydrogen peroxide was added (with swirling). The solution was poured rapidly into the precooled sandwich, and the comb was inserted. Gels were cast 1–3 days before use and stored at 4° C. Usually, dye marker (methyl green) only was added to outside wells. Electrophoresis was carried out with the lower electrode as cathode and with current limiting (30 mA per gel, or about 7 mA per cm^2), and unless otherwise noted, was terminated 1.5 hr after the slow (magenta) band passed off the gel. Voltage increased from about 160 to 250 V during an electrophoretic run (about 8 hr).

Gels were fixed in 350 ml of 12% trichloracetic acid for 30 min before addition of 5 ml of aqueous 0.5% Coomassie Brillant Blue R 250 and were allowed to stain for at least 24 hr. (For maximum density for photography, gels were stained 5–7 days with daily addition of 5 ml of stain solution.) Gels were destained with two short (1–2 min) rinses in aqueous 50% methanol/10% acetic acid followed by agitation in aqueous 7% acetic acid/5% methanol (Laemmli 1970) for 1–4 hr, and were photographed immediately (Clements 1970). An extract of the cultivar Veery, a hard red winter wheat with a distinctive gliadin pattern (relative to soft wheat patterns), was included on most gels to serve as a check on the procedure and to assist in identifying gels.

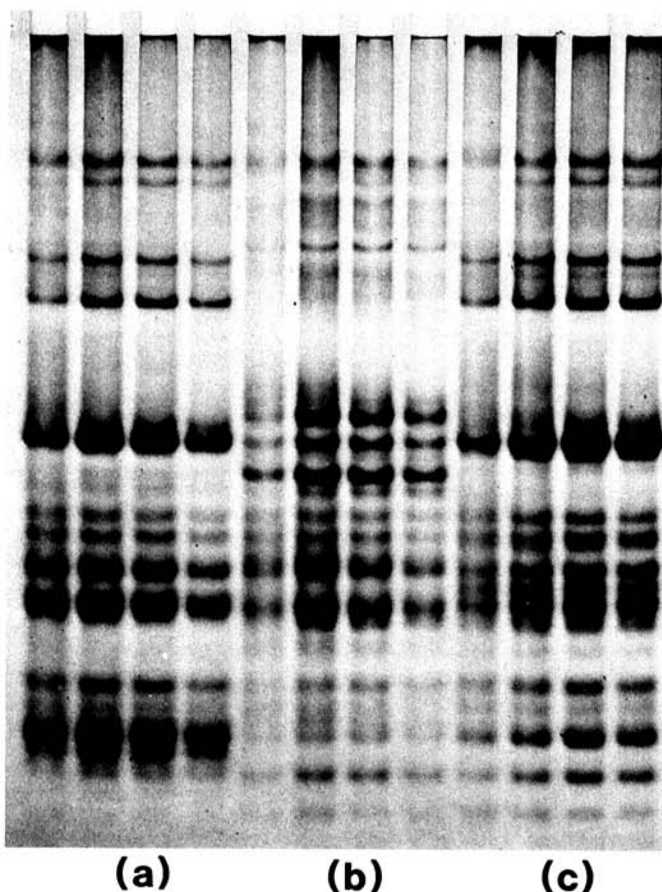

Fig. 3. Gliadin patterns of three soft red winter wheats sampled at three, two, and one week before harvest, and at harvest (July 15). From left to right: **(a)** Logan, **(b)** Argee, **(c)** Blackhawk.

RESULTS AND DISCUSSION

Ethylene Glycol as an Extraction Medium

In an early review, Bailey (1944) noted that a number of solvents are more efficient than 70% ethanol for extraction of gliadin. However, the classical medium has been 70% ethanol as a result of the pioneering work of Osborne (1907) and general acceptance of a definition of prolamins based on this medium (Kasarda et al 1976; Osborne 1924). In most electrophoretic studies of gliadins, 70% ethanol has been employed for extraction. Among exceptions, however, have been studies in which dimethyl formamide (Lafiandra and Kasarda 1985), aqueous urea (du Cros and Wrigley 1979), water-dioxane (Popineau and Godon 1982), and chloroform-methanol (Redman and Ewart 1973) were used.

Ethylene glycol, used in this study, gave patterns that were essentially the same as patterns from 70% ethanol extracts (Fig. 1) and offers several advantages (high density, permitting pipetting onto gels without inclusion of additives; low volatility; convenience). Extracts remain clear after prolonged storage, and appear to contain only small amounts of glutenin. Air entrapment (particularly in small samples) caused by high viscosity can be eliminated by vacuum infiltration. The very low volatility of ethylene glycol has been an asset in a simplified PAGE procedure developed for single kernels (Clements, *unpublished*).

Electrophoretic Conditions

Attempts to use conventional gel formulations buffered with aluminum lactate-lactic acid resulted in erratic gelling, but when aluminum lactate was omitted, polymerization occurred without failure. Inclusion of aluminum lactate in the upper (anode) buffer was found to be necessary, however, to achieve acceptable migration rates, and this discontinuous system was employed for the study. Although gel pH (2.6) is lower than pH of aluminum lactate-lactic acid gels commonly used (pH 3.0–3.3), the systems give essentially identical patterns when other conditions are the same.

Acrylamide concentration (10%) was higher than commonly employed (6–8%), but the gels were easier to handle than the softer gels. Concentrations of ascorbic acid, ferrous sulfate, and hydrogen peroxide are conventional (Bushuk and Zillman 1978, Khan et al 1985, Lookhart et al 1982). and result in polymerization in 1–2 min.

Soft Wheat Patterns

Gliadin patterns of cultivars grown at two locations confirmed the genotypical nature of the gliadin proteins (Fig. 2). Moreover, gliadin patterns of six genotypes sampled at weekly intervals during the three weeks prior to harvest exhibited their characteristic patterns throughout the period (Fig. 3). However, intensities of patterns were greatest at 1–2 weeks before harvest, presumably due to gliadin synthesis followed by dilution by endosperm starch. This qualitative stability could be of importance in considerations of gliadin patterns of preripe harvested wheat (Yamazaki 1976).

The 47 gliadin patterns shown in Figure 4 are representative of patterns from soft red winter wheats. Patterns of 12 soft white winter wheats from the eastern United States are shown in Figure 5.

Pattern Types

Application of the described procedures to more than 120 soft wheat cultivars has shown that gliadin patterns from these wheats can be divided into four categories according to distribution of gliadin bands in the central (intermediate mobility) region of the electrophoregram. This classification permits immediate sorting and facilitates comparisons and cataloging. Gliadin patterns representative of the four types are compared in Figure 6. The cultivars Pike and Roy (1 and 2 in Fig. 6) exemplify type I (a single heavy band); FL302 (3) and Arthur (4) are representative of type II (two heavy closely spaced bands). Frankenmuth (Michigan) (6), a soft white wheat, like most white wheats studied to date, is type III (two widely spaced bands). Hillsdale (Michigan) (7), also type III is the only soft red wheat found in this category thus far. Compton (Indiana) (5) appears to be unique in that the pattern falls between types II and III, i.e., two bands of intermediate spacing (but considered type III). Massey (Virginia) (8) and Pioneer S 76 (9) are characterized by three bands (type IV), but in the pattern from the latter cultivar the leading band is weaker than the other two bands (which correspond to the Arthur doublet). Hart (Missouri) (10) is unique thus far in that the pattern exhibits four bands in the central area.

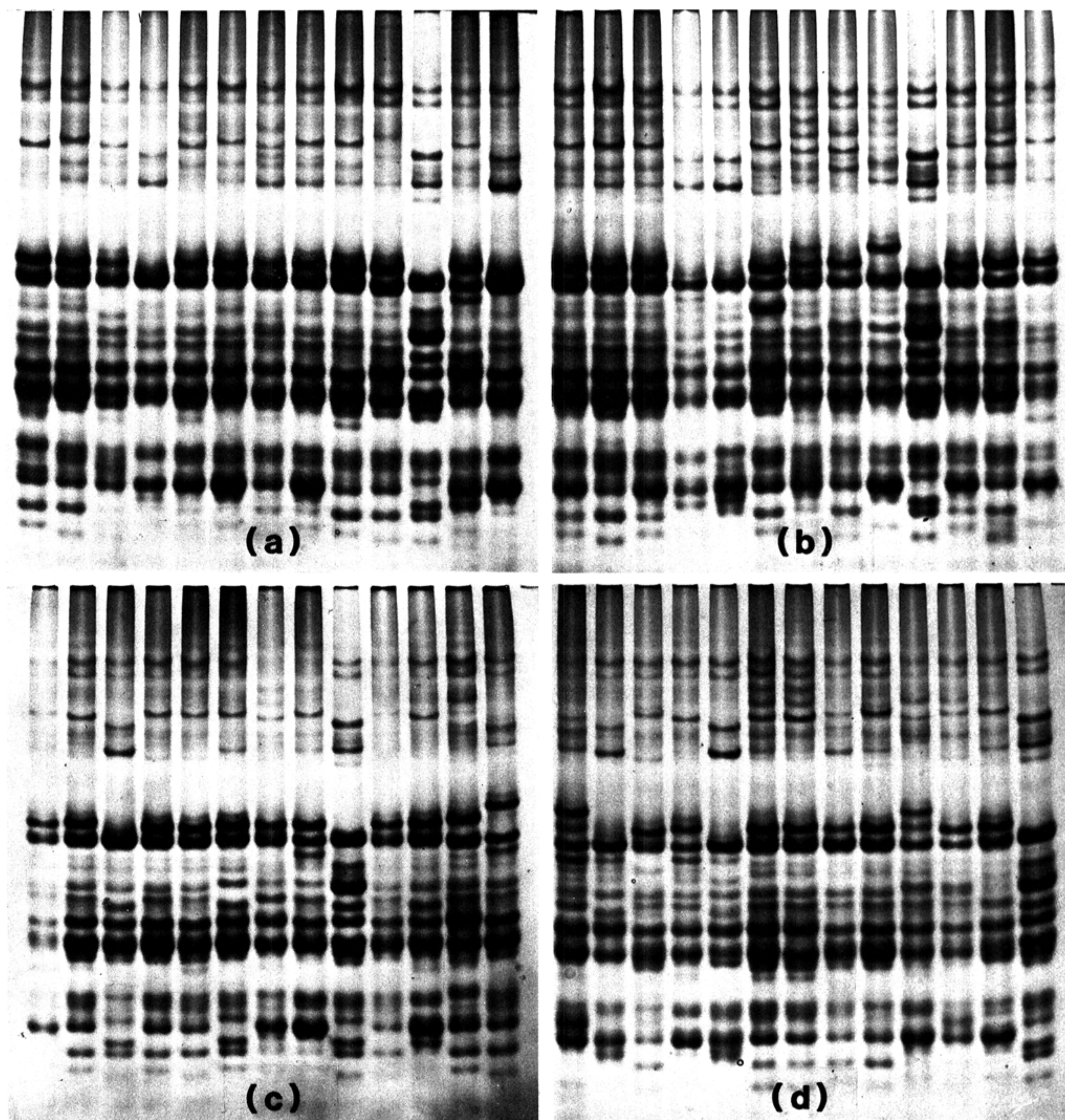

Fig. 4. Gliadin patterns of 47 soft red wheat cultivars (with hard red wheat Veery included as check). From left to right: (**a**) Monon, Severn, Titan, Tyler, Vigo, Stacy, McNair 1813, Redcoat, Omega 78, Hunter, Veery, Rosen, Pike; (**b**) Sullivan, FL 301, Scotty, Blueboy, Logan, Adder, Thorne, Trumbull, Hillsdale, Veery, Holley, Southern Belle, Delta Queen; (**c**) Delta Queen, Doublecrop, Roy, Abe, Saluda, FL 302, Kawvale, Pioneer S 76, Veery, Wheeler, Ruler, Compton, Pioneer 2550; (**d**) Hart, Adena, Bradford, Becker, Cardinal, Forward, Nured, Magnum, Nelson, Clarkan, Pioneer 2553, Roland, Veery.

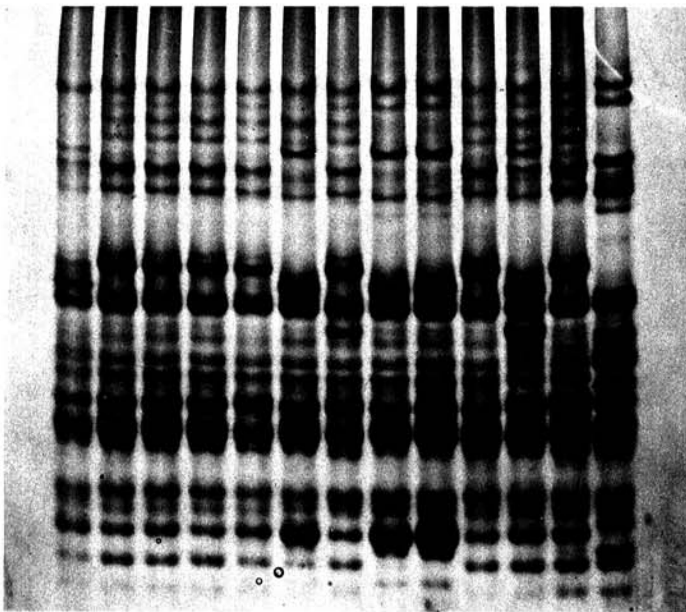

Fig. 5. Gliadin patterns of 12 soft white winter wheat cultivars from the eastern United States (with hard red winter wheat Veery included as check). From left to right: Ticonderoga, Frankenmuth, Genesee, Ionia, Dawson (American Banner), Augusta, Purcell, Frederick, Tecumseh, Yorkstar, Houser, Honor, Veery.

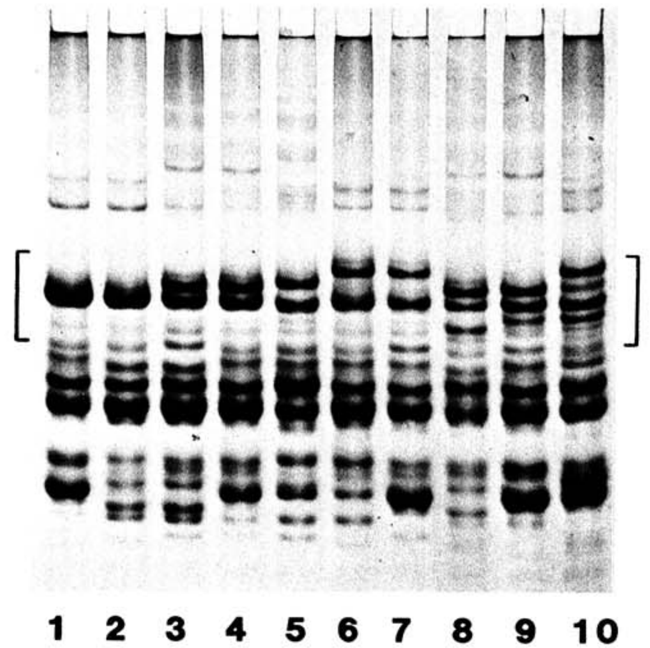

Fig. 6. Range of gliadin patterns shown by soft wheats from the eastern United States. From left to right: **(1)** Pike, **(2)** Roy, **(3)** FL 302, **(4)** Arthur, **(5)** Compton, **(6)** Frankenmuth, **(7)** Hillsdale, **(8)** Massey, **(9)** Pioneer S 76, **(10)** Hart.

TABLE II
Soft Wheat Cultivars of the Eastern United States Classified According to Electrophoretic Patterns of Gliadins[a]

Type I	Type II			Type III	Type IV
Soft red winter wheats					
Adena	Abe	Florida 301	Redcoat	Hillsdale	Adder
Blackhawk	Arthur	Florida 302	Roland		Argee
Blueboy	Arthur 71	Forward	Ruler		Becker
Blueboy II	Auburn	Fultz	Saluda		Caldwell
Butler	Bailey 4287	Georgia 1123	Scotty		Clarkan
Cardinal	Beau	GR-855	Seneca		Coker 916
Charmany	Bradford	Holley	Severn		Coker 833
Coker 9323	Callahan 115	Hunter	Southern Belle		Dancer
Kenosha	Callahan 654	Kawvale	Stacy		Fulcaster
Lincoln	Chancellor	Knox	Sullivan		Hart
Logan	Coker 747	Knox 62	Terral 81-17		Jones Fife
Lucas	Coker 762	Magnum	Thorne		Massey
McNair 1003	Coker 797	McNair 1813	Titan		Pioneer S 76
Pioneer 2550	Coker 983	Monon	Todd		Rosen
Pike	Coker 9227	Nelson	Trumbull		Rudy
Racine	Coker 68-15	Nured	Valprize		
Roy	Compton	Oasis	Vigo		
Stadler	Delta Queen	Omega 78	Wakeland		
Stoddard	Double Crop	Pennoll	Wheeler		
Trio	Downy	Pioneer 2553			
Twain	Fairfield	Pioneer S 78			
Tyler	Felland	Poole			
Voris 8088	Fillmore	Potomac			
Soft white winter wheats					
...	Augusta			American Banner	Houser
	Frederick			Arrow	Purcell
	Tecumseh			Avon	
	Ticonderoga			Cornell 595	
	Yorkwin			Favor	
				Frankenmuth	
				Genesee	
				Geneva	
				Gordon	
				Honor	
				Ionia	
				Talbot	
				Yorkstar	

[a]Classified according to dominant bands in the central region of the electrophoregram: type I: one heavy band; type II, two closely spaced heavy bands; type III, two widely spaced heavy bands; type IV, three or more dominant bands.

The four types are clearly evident among the specimens illustrated in Figures 1–6. The relative distribution of pattern types among cultivars studied to date (Table I) is also evident. Type II ("Arthur" type) predominates among soft red wheat cultivars; type III predominates among soft white cultivars (Table II).

Ten representative patterns of each of the four types are shown in Figure 7 to permit comparison of gliadin patterns within these categories. These examples suggest that within types I (a), II (b), and III (c), the classifications are based on common gliadin components. However, type IV (d) represents a heterogeneous group based only on the number of bands (and not necessarily coincidence of bands).

These comparisons also suggest that identification of some cultivars cannot be made on the basis of gliadin patterns alone. This appears to be especially true of type II cultivars (Fig. 7b), which predominate among the soft red wheats. It is noteworthy that eight of the 10 cultivars illustrated are Indiana releases; exceptions are Roland (Illinois) and Saluda (Virginia). Close

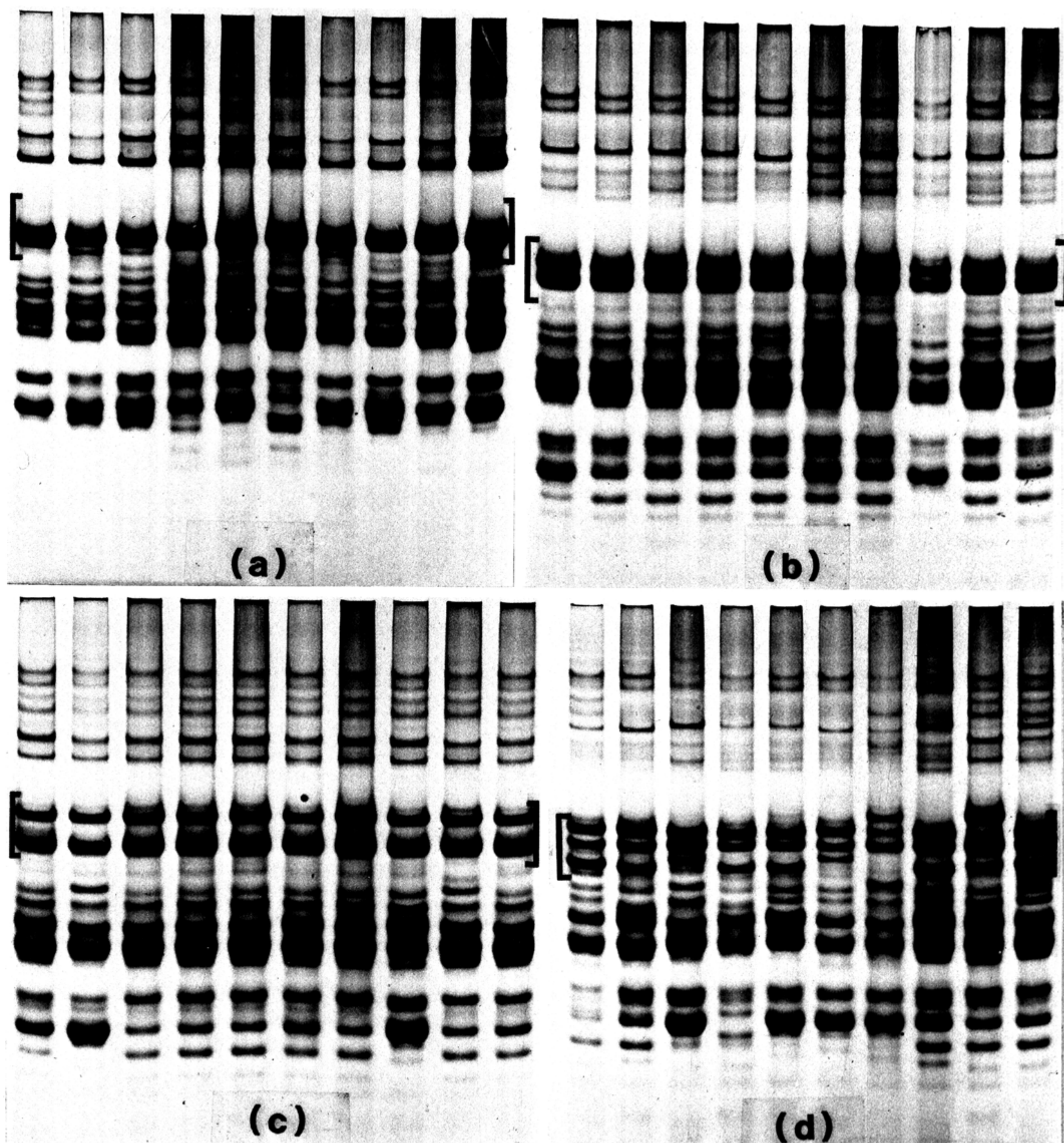

Fig. 7. Gliadin patterns of 40 soft wheat cultivars from the eastern United States grouped according to similarities. From left to right: (**a**) type I: Charmany, Adena, Cardinal, McNair 1003, Kenosha, Roy, Blueboy, Logan, Tyler, Pike; (**b**) type II: Arthur, Beau, Arthur 71, Auburn, Fillmore, Knox 62, Oasis, Roland, Abe, Saluda; (**c**) type III: Cornell 595, Hillsdale, Frankenmuth, Genesee, Ionia, Dawson (American Banner), Yorkstar, Arrow, Geneva, Favor; (**d**) type IV: Argee, Caldwell, Pioneer S 76, Massey, Dancer, Purcell, Clarkan, Adder, Becker, Houser.

similarities were also noted among several Eastern soft white cultivars, most of which are type III (Fig. 7c). Among type IV soft white cultivars are New York releases Purcell and Houser (Fig. 7d).

Several laboratories have adapted computers to gliadin fingerprinting (Jones et al 1982; Lookhart et al 1983; Sapirstein and Bushuk 1985a,b,c,). Computerization could be very helpful for systematic cataloging and sorting of patterns for genotype identification, but such an approach leaves little room for variation in methodology. As Sapirstein and Bushuk (1985a) pointed out, minimizing error in band mobility is crucial in adapting the computer to such applications. Electrophoresis, however, by its nature is subject to variability and is difficult to standardize. This is especially true when the technique is not used on a continuous, daily basis. Reagents, conditions, and techniques must be rigidly controlled, and the operator is an important factor.

The classification described here represents a preliminary effort to categorize the gliadin patterns of the many soft wheat cultivars grown in the eastern United States (and that are proliferating at a rapid rate). The approach does not require precise reproducibility, and some variation in reagents and conditions can be tolerated. Such sorting can simplify comparison and identification by narrowing the field of possible identities of a pattern in question, and when used in conjunction with other information (e.g., kernel shape, color and texture, source, quality tests) has been found to be very helpful. Preliminary attempts to relate pattern type to specific quality parameters suggest the approach may be useful, but the cultivars within a given type exhibit a wide range of milling and baking behavior. Further subdivision based on pattern characteristics should be possible, however, and is being explored as collection of patterns continues. Division into smaller subgroups based on specific families of bands may reveal associations that are not evident in the broad classifications.

Changes in methodology have proved to be helpful in this laboratory. It has been suggested that standard methodology be adapted (Autran et al 1979, Cooke 1984, Lookhart et al 1982), and certainly availability of a standardized procedure is desirable. However, as pointed out by du Cros and Wrigley (1979), the methods and materials best suited to a particular laboratory will depend on several factors, and a specific procedure may not be practical in all situations. The modifications introduced here are examples of additional variations possible in approaches to gliadin fingerprinting and may suggest useful alternatives to other workers.

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