Fast Horizontal Sodium Dodecyl Sulfate Gradient Polyacrylamide Gel Electrophoresis for Rapid Wheat Cultivar Identification and Analysis of High Molecular Weight Glutenin Subunits[1,2]

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Cereal Chem. 66(3):186-192

ABSTRACT

Fast horizontal sodium dodecyl sulfate gradient polyacrylamide gel electrophoresis (SDSGPAGE) with silver staining in conjunction with the PhastSystem was used to separate wheat storage proteins (gliadin and glutenin). Resolution achieved with miniature 8–25% gradient PhastGels (50 × 43 × 0.45 mm) was almost equal to that of conventional SDSGPAGE and was superior to that of 10–15% gradient PhastGels. Wheat cultivars distinguishable by conventional electrophoresis also were distinguishable by fast horizontal SDSGPAGE using either 8–25% or 10–15% gradient PhastGels. The 10–15% gradient format had the shortest analysis time (45 min electrophoresis and 44 min staining) and was used for the analysis of 70 wheat cultivars. The majority of cultivars could be distinguished by qualitative differences in protein patterns with the exception of three groups of cultivars comprised of seven red spring, two white winter, and two red winter wheats. Identification of high molecular weight glutenin subunits by fast horizontal SDSGPAGE also was possible with the limitation that subunits 2* and 5 were not resolved and 1 and 2 were partially resolved. An overall evaluation of the advantages of fast horizontal SDSGPAGE and silver staining in conjunction with the PhastSystem suggests that this technique could be used effectively in situations where smaller numbers of samples would be analyzed on an intermittent basis as contrasted to daily analysis of large numbers of samples.

Wheat cultivars may be identified by various techniques including gliadin analysis by starch gel electrophoresis (Autran and Bourdet 1975) and acid polyacrylamide gel electrophoresis (acid PAGE) (Bushuk and Zillman 1978, Tkachuk and Mellish 1980, Jones et al 1982). Analysis of wheat storage proteins (gliadins and glutenins) by sodium dodecyl sulfate gradient polyacrylamide gel electrophoresis (SDSGPAGE) also was shown to be useful for cultivar identification (Du Cros et al 1980, Marchylo 1987b). Although this technique provides excellent resolution of the storage proteins, some disadvantages such as gradient gel preparation and analysis time requirements place limitations on its usefulness, especially in a routine cultivar identification program.

The electrophoretic identification of individual high molecular weight (HMW) glutenin subunits is of importance because of the demonstrated relationship of these subunits to breadmaking quality (Payne et al 1979, Payne et al 1984, Moonen and Zeven 1985). Electrophoretic analysis of these subunits typically is carried out by conventional SDSGPAGE but, although subunits generally are well resolved, this technique may require more than 24 hr to perform (Payne et al 1980, 1981; Ng and Bushuk 1987).

A microprocessor-controlled electrophoresis and staining system (PhastSystem) was introduced in 1986 that can perform fast horizontal SDSGPAGE and silver staining. This system, which uses precast miniature SDS gradient gels, reportedly provides excellent resolution in a short time (30 min) and rapid, automatically controlled silver staining (1–2 hr) (Olsson et al 1988a,b). A recent report furnished evidence that this system may be used for oat cultivar identification (Hansen et al 1988), and preliminary work suggests that it also may be used for wheat cultivar identification (Marchylo and Mellish, *in press*).

The purpose of this study, therefore, was to evaluate the separation of wheat storage proteins with the PhastSystem and to determine its potential for the identification of wheat cultivars and quality-related HMW glutenin subunits.

MATERIALS AND METHODS

Wheat Cultivars

Protein patterns were obtained, using the PhastSystem, for 68 wheat cultivars registered in Canada as of 1988, listed below alphabetically by grade eligibility, and two spring wheat cultivars not registered in Canada, Len and Solar (from the United States).

Spring Wheats

Canada Western Red Spring (CWRS). Benito, Canuck, Chinook, Columbus, Conway, Katepwa, Kenyon, Lancer, Laura, Leader, Manitou, Marquis, Napayo, Neepawa, Park, Pembina, Roblin, Saunders, Selkirk, Sinton, and Thatcher.

Canada Eastern Red Spring (CERS). Ankara, Belvedere, Casavant, Concorde, Dundas, Max, Messier, Milton, Mondor, Opal, and Vernon. These are all regionally registered.

Canada Prairie Spring (CPS). HY320 and Oslo.

Canada Eastern/Western Utility (CEU/CWU). Bluesky, Glenlea, and Wildcat.

Canada Western Soft White Spring (CWSWS). Fielder and Owens, which has been given an interim regional registration.

Canada Western Feed (CWF). Garnet and Pitic 62.

Canada Eastern Feed (CEF). Laval-19.

Winter Wheats

Canada Western Red Winter (CWRW). Norstar, Norwin, and Sundance.

Canada Eastern Red Winter (CERW). Absolvent and Borden, registered regionally, and Lennox, Monopol, Valor, and Vuka.

Canada Eastern White Winter (CEWW). Augusta, Frankenmuth, Fredrick, Gordon, Harus, Houser, and Yorkstar.

Amber Durum

Canada Western Amber Durum (CWAD). Arcola, Coulter, Hercules, Kyle, Macoun, Medora, Pelissier, Sceptre, Wakooma, Wascana.

Glutenin Subunits

Reference wheat cultivars and their HMW glutenin subunit compositions, according to Payne and Lawrence (1983) included Chinese Spring (2, 7, 8, 12), Hobbit (3, 6, 8, 12), Champlein (4, 7, 8, 12), Hope (1, 5, 6, 8, 10), Flinor (1, 2, 7, 11), Danchi (2.2, 7, 8, 12), Bezostaya (2*, 5, 7, 9, 10), Federation (1, 5, 20, 17, 10), Lancota (2*, 2, 13, 16, 12), Sappo (2, 14, 15, 12), and Gabo (2*, 2, 17, 18, 12).

Extraction and Preparation of Storage Proteins

Storage proteins were extracted from single kernels and

[1]Paper 627 of the Grain Research Laboratory, Canadian Grain Commission, Winnipeg, Manitoba.

[2]Presented in part at the Third International Symposium on the Biochemical Identification of Varieties in Leningrad, September, 1987.

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prepared for conventional SDSGPAGE as described previously (Marchylo 1987b). To reflect PhastSystem requirements (Pharmacia 1986), changes were made to the extraction solvent (50% 1-propanol containing 1% [w/v] dithiothreitol and 41 m*M* Tris-HCl, pH 8.0), alkylating solution (50% 1-propanol containing 0.14*M* 4-vinylpyridine and 41 m*M* Tris-HCl, pH 8.0), and sample buffer (41 m*M* Tris-HCl, pH 8.0, containing 2% [w/v] SDS and 0.02% [w/v] bromophenol blue), and extraction volume was increased to 1 ml to minimize protein overloading.

SDSGPAGE Analysis of Storage Proteins

Conventional SDSGPAGE was carried out as described previously (Marchylo 1987a,b) with some modifications. An 11.5–17% T + 2% C linear polyacrylamide gradient (T is the total weight of acrylamide and cross-linking agent (bis) per 100 ml of solution; C is the percentage concentration of cross-linking agent relative to the total concentration T) and a 28-well sample comb (Hoeffer Scientific Instruments, San Francisco, CA) were used, and the lower tank buffer was cooled with circulating coolant at 10° C for an electrophoresis time of 1.75 hr.

Fast horizontal SDSGPAGE was carried out with the PhastSystem (Pharmacia-LKB Biotechnology AB, Bromma, Sweden), which is described in detail elsewhere (Pharmacia 1986, Olsson et al 1988a). Separation of wheat storage proteins was tested using PhastGels (50 × 43 × 0.45 mm) with linear polyacrylamide gradients of 8–25% and 10–15%. Electrophoresis programs that provided optimum resolution are shown in Table I.

TABLE I
PhastSystem Running Programs for the SDSGPAGE[a] Analysis of Wheat Storage Proteins

Gradient/ Step	Stage	Voltage (V)	Current (mA)	Power (W)	Temperature (° C)	Volt Hours (Vhr)
8–25%	Gradient					
1	Separation[b]	400	5.0	2.5	20	200
10–15%	Gradient					
1	Prerun[c]	400	5.0	1.0	20	3
2	Separation	400	10.0	2.5	20	97

[a] Sodium dodecyl sulfate gradient polyacrylamide gel electrophoresis.
[b] Sample applicator down at 0 Vhr and up at 5 Vhr.
[c] Sample applicator down at 0 Vhr and up at 2 Vhr.

TABLE II
Silver Staining Protocol for Wheat Storage Proteins Separated in 8–25% Gradient PhastGels

Step	Solution[a]	Time (min)	Temperature (° C)
1	50% (v/v) Methanol (MEOH)/12% glacial acetic acid (HAc)	5	50
2	50% MEOH/12% HAc	5	50
3	50% MEOH/12% HAc	5	50
4	10% Ethanol (ETOH)/5% HAc	2	50
5	10% ETOH/5% HAc	2	50
6	3% (w/v) NaOH + 0.015% (w/v) $NaBH_4$	2	40
7	0.12*M* Sodium acetate buffer, pH 5.6	2	50
8	0.12*M* Sodium acetate buffer, pH 5.6	2	50
9	0.12*M* Sodium acetate buffer, pH 5.6	2	50
10	H_2O	2	50
11	0.015*M* $AgNO_3$	5	40
12	H_2O	2	30
13	0.75% (w/v) Na_2CO_3 + 0.75% (v/v) formaldehyde[b]	0.5	30
14	0.75% (w/v) Na_2CO_3 + 0.75% (v/v) formaldehyde[b]	3	50
15	10% HAc/5% glycerol	3	50
Chamber filling and emptying		15.0	···
Total		57.5	···

[a] HPLC-grade deionized water prepared with a Barnstead Nanopure II water purification system was used throughout.
[b] This solution was prepared just prior to use.

For reference purposes, a mixture of standard proteins (low molecular weight calibration kit, Pharmacia Fine Chemicals, Uppsala, Sweden) including phosphorylase b (molecular weight [MW] 94,000), bovine serum albumin (MW 67,000), ovalbumin (MW 43,000), and carbonic anhydrase (MW 30,000) were prepared essentially as described previously (Marchylo 1987b) and analyzed along with the wheat proteins. An eight-well sample applicator (0.5 μl sample volume) was used in the majority of cases, but a 12-well sample applicator (0.3-μl sample size) also was tested, and PhastGels were analyzed two at a time.

Silver Stain Procedures

Conventional SDSGPAGE gels were stained essentially as described before (Marchylo 1987a) except that the trichloroacetic acid fixation step was deleted and the length of the silver addition step was decreased from 30 min to 15 min.

PhastGels were silver stained in the PhastSystem development unit using protocols shown in Tables II and III for 8–25% and 10–15% gradient PhastGels, respectively. Stained gels were dried in an oven at 50° C for about 30 min and then were photographed and mounted in 40 × 40 mm slide mounts for examination with a viewer projector.

RESULTS AND DISCUSSION

Extraction of wheat proteins was carried out under reducing conditions; therefore, both HMW and low molecular weight (LMW) glutenin subunits and gliadins were analyzed. Reduced proteins were alkylated with 4-vinylpyridine to prevent reoxidation and provide sharper protein bands (Marchylo 1987b, Heukeshoven and Dernick 1988). PhastSystem running conditions (Table I) were chosen to provide optimum resolution in a minimum time of about 2 hr for 8–25% and 45 min for 10–15% gradient PhastGels. Run times varied up to 10 min for 8–25% and 5 min for 10–15% gradient PhastGels even though electrophoresis was carried out for a preset number of volt hours. This variability may be a result of some loss in electrical contact between buffer strips and electrodes in conjunction with the longer running times and higher volt hours necessary to obtain optimum resolution of the wheat proteins. Unacceptable losses in resolution were incurred at lower volt hours, whereas further attempts to minimize run times by increasing power conditions were not successful because of overheating of gels or buffer strips concomitant with problems associated with the depletion of SDS in buffer strips and gels (Olsson et al 1988a).

TABLE III
Silver Staining Protocol for Wheat Storage Proteins Separated in 10–15% Gradient PhastGels

Step	Solution[a]	Time (min)	Temperature (° C)
1	50% (v/v) Methanol (MEOH)/12% glacial acetic acid (HAc)	7	50
2	50% MEOH/12% HAc	5	50
3	50% MEOH/12% HAc	3	50
4	3% (w/v) NaOH + 0.015% (w/v) $NaBH_4$	1.5	50
5	0.12*M* Sodium acetate buffer, pH 5.6	0.5	50
6	0.12*M* Sodium acetate buffer, pH 5.6	3.5	50
7	H_2O	1	50
8	0.015*M* $AgNO_3$	5	50
9	H_2O	1	50
10	0.75% (w/v) Na_2CO_3 + 0.75% (v/v) formaldehyde[b]	0.5	30
11	0.75% (w/v) Na_2CO_3 + 0.75% (v/v) formaldehyde[b]	2	50
12	10% HAc/5% glycerol	2	50
Chamber filling and emptying		12.0	···
Total		44.0	···

[a] HPLC-grade deionized water prepared with a Barnstead Nanopure II water purification system was used throughout.
[b] This solution was prepared just prior to use.

Proteins separated in PhastGels were silver stained automatically with the PhastSystem development unit described in detail by Olsson et al (1988b). Two protocols, which are modifications of the conventional gel silver staining procedures (Materials and Methods), were designed for 8–25% (Table II) and 10–15% (Table III) gradient PhastGels. Total staining time, including the time necessary for filling and emptying the development chamber, was 57.5 min and 44 min for 8–25% and 10–15% gradient PhastGels, respectively. Staining time for the 10–15% gradient PhastGels essentially was equal to electrophoresis running time, thus providing for efficient concurrent scheduling of electrophoresis and silver staining. The background color of silver-stained PhastGels was a light orange that contrasted well with stained bands. Protein bands stained a range of colors with the 8–25% gradient PhastGel stain, which is consistent with previous work (Marchylo 1987b), whereas with the 10–15% gradient PhastGel stain bands stained red to reddish brown.

Typical storage protein electrophoregrams obtained by PhastSystem SDSGPAGE analysis of seven CWRS wheat cultivars (with an eight-well sample applicator and 8–25% and 10–15% gradient PhastGels) are illustrated in Figure 1A–C. Visualization and comparison of protein patterns were enhanced by enlarging electrophoregrams as shown in Figure 1A and B for original sized and enlarged reproductions, respectively, of separations achieved with an 8–25% gradient PhastGel. For routine comparison of protein patterns, dried PhastGels were mounted in slide mounts and examined with a viewer projector, which facilitated discrimination of closely spaced bands.

Plots of log (MW) versus log (R_f) for standard proteins resolved by 8–25% and 10–15% gradient PhastGels provided linear regression curves with correlation coefficients ($r^2 = 0.993$, Fig. 1B; $r^2 = 0.996$, Fig. 1C) comparable to those reported for conventional SDSGPAGE (Marchylo 1987b).

As indicated by the number of protein bands resolved, resolution achieved with 8–25% gradient PhastGels (Fig. 1B) was almost equivalent to that of conventional SDSGPAGE (Fig. 2) and was superior to that of the 10–15% gradient PhastGels (Fig. 1C). For example, 29 protein bands were resolved for the cultivar Columbus by conventional SDSGPAGE (Fig. 2, track 4) compared with 26 by 8–25% (Fig. 1B, track 4) and 19 by 10–15% (Fig. 1C, track 4) gradient PhastGels. The anomolous band (or group of bands), which was resolved just preceding the HMW glutenin subunits by conventional (Fig. 2) as well as PhastSystem SDSGPAGE, was not considered as part of the HMW glutenin

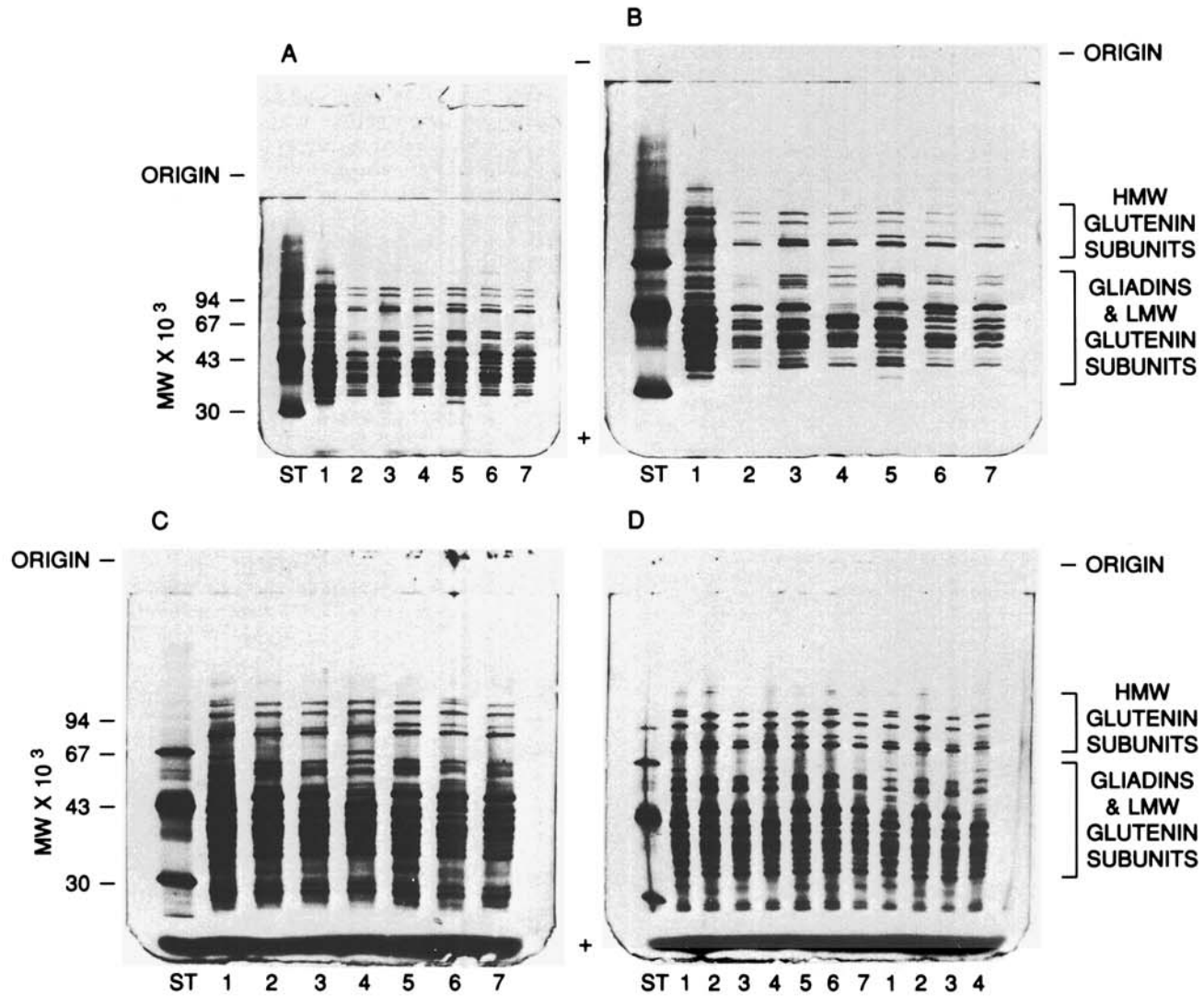

Fig. 1. Fast horizontal sodium dodecyl sulfate gradient polyacrylamide gel electrophoretic separation of wheat storage proteins using PhastGels. **A,** 8–25% gradient PhastGels photographically reproduced approximately to actual gel dimensions (i.e., 5.0 × 4.3 cm); **B,** enlargement of A (about 1.5 times) to facilitate visualization of protein bands; **C** and **D,** 10–15% gradient PhastGels (enlarged about 1.5 times). Samples were loaded in C with an eight-well sample applicator and in D with a 12-well sample applicator for comparison. The Canada Western Red Spring wheat cultivars analyzed include: Marquis (**1**), Neepawa (**2**), Conway (**3**), Columbus (**4**), Lancer (**5**), Laura (**6**), and Roblin (**7**). **ST** denotes a standard reference mixture of proteins of phosphorylase b (molecular weight [MW] 94,000), bovine serum albumin (MW 67,000), ovalbumin (MW 43,000), and carbonic anhydrase (MW 30,000). Storage proteins were divided for simplicity into the two groups: high molecular weight (HMW) glutenin subunits; and low molecular weight (LMW) glutenin subunits and ω-, α-, β-, and γ-gliadins.

fraction. As discussed previously (Marchylo 1987b), this band (or group of bands) may consist of oligomers of HMW glutenin subunits (Lawrence and Payne 1983) or may be an artifact of the extraction or electrophoresis procedure.

It also should be noted that resolution by both PhastGel media was sensitive to protein load. In this study, extraction conditions were chosen so that all HMW glutenin subunits could be detected while minimizing overloading of the LMW glutenin subunits and gliadins. Variability in protein content among cultivars and/or single kernels, however, did influence protein load as illustrated for Marquis (Fig. 1B and C, track 1).

Six of seven CWRS wheat cultivars were distinguishable on the basis of protein patterns obtained using 8–25% PhastGels (Fig. 1B). Neepawa and Conway (Fig. 1B, tracks 2 and 3, respectively) exhibited identical protein patterns, but this also was the case with conventional SDSGPAGE (Fig. 2, tracks 2 and 3). A similar result also was obtained by 10–15% gradient PhastGel analysis in combination with eight-well (Fig. 1C) or 12-well (Fig. 1D) sample applicators, despite the somewhat poorer resolution achieved in this gel medium.

Cultivar Analysis

Although 8–25% provided superior resolution to 10–15% gradient PhastGels, the 10–15% PhastGel SDSGPAGE and staining procedure appeared able to differentiate cultivars equally well while offering the advantage of significantly shorter analysis times. Thus, in a practical cultivar identification program, the 10–15% gradient PhastGels would be more useful and, consequently, this medium was used for further cultivar analysis.

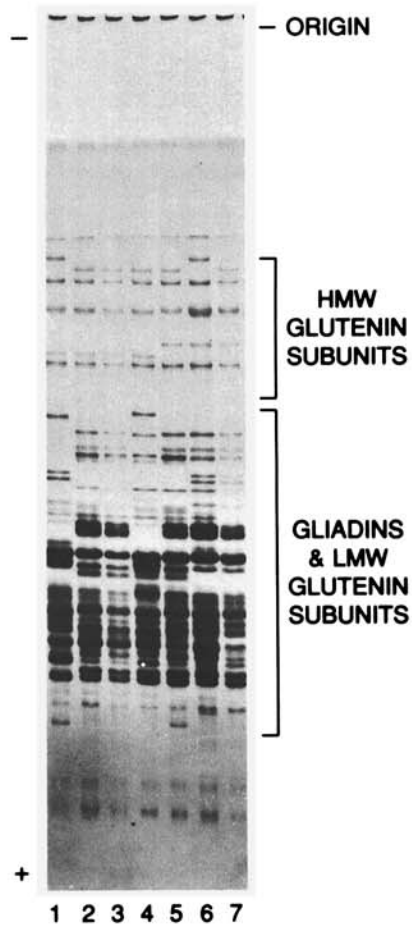

Fig. 2. Conventional sodium dodecyl sulfate gradient polyacrylamide gel electrophoretic separation of wheat storage proteins. The Canada Western Red Spring wheat cultivars analyzed include: Marquis (**1**), Neepawa (**2**), Conway (**3**), Columbus (**4**), Lancer (**5**), Laura (**6**), and Roblin (**7**). Storage proteins were divided into the two groups: high molecular weight (HMW) glutenin subunits; and low molecular weight (LMW) glutenin subunits and ω-, α-, β-, and γ-gliadins.

In addition, the eight-well, in preference to the 12-well, sample applicator was used for easier visualization of protein patterns.

Typical electrophoregrams obtained by 10–15% gradient PhastGel analysis are illustrated in Figure 3A–D for 27 of the 70 cultivars analyzed. Protein bands, in general, migrated evenly across the gel but uneven migration occasionally occurred (Fig. 3C). Protein patterns, however, were consistent from run to run as illustrated for the CWRS wheat cultivar Conway (Fig. 1B, track 3, and Fig. 3A, track 3).

The majority of cultivars were distinguishable on the basis of qualitative differences in banding patterns. Three groups of cultivars, however, were indistinguishable, including the CWRS wheat cultivars Neepawa, Katepwa, Conway, Benito, Napayo, Manitou, and Thatcher; the CEWW cultivars Gordon and Yorkstar, and the CERW cultivars Lennox and Valor. In addition, the CERS cultivars Ankra, Milton, Opal, and Vernon were difficult to distinguish since only minor differences in proteins patterns were observed. It must be realized, however, that these cultivars are indistinguishable or difficult to distinguish by other conventional electrophoretic procedures such as acid PAGE (Tkachuk and Mellish 1980; Mellish and Tkachuk, *unpublished results*; Sapirstein and Bushuk 1985).

Some cultivars were polymorphic for electrophoretic patterns as illustrated for HY320 (Fig. 3B, tracks 8 and 9) and Glenlea (Fig. 3B, tracks 13 and 14), which is in agreement with previous reports (Tkachuk and Mellish 1980, Sapirstein and Bushuk 1985, Marchylo 1987b). Information on the polymorphic nature of a cultivar is important for identification purposes (Wrigley et al 1982) since, for example, the occurrence of polymorphic patterns may be mistakenly attributed to impurity. For the purpose of this study, however, a thorough examination of polymorphism was not undertaken.

Analysis of HMW Glutenin Subunits

The HMW glutenin subunits in 11 reference cultivars were resolved by 10–15% (Fig. 4) and 8–25% (results not shown) gradient PhastGel SDSGPAGE and for comparative purposes by conventional SDSGPAGE (Fig. 5). Resolution of the HMW subunits was comparable for 10–15% and 8–25% gradient PhastGels and, therefore, only the 10–15% gradient PhastGel results are shown. Separations using the eight- as compared to the 12-well sample combs (results not shown) facilitated comparative identification of subunits. Some variation was observed among individual HMW subunits in band coloration following silver staining. In general, the x-type HMW glutenin subunits stained a reddish color, and the y-type subunits stained a darker reddish-brown. Similar results were observed using silver staining protocols for both 10–15% and 8–25% gradient PhastGels. Band coloration may be related in part to differences in amino acid composition of proteins (Nielsen and Brown 1984, Merril et al 1988). Therefore, the observed variation in band coloration of the HMW subunits may be reflective of the reported difference in cysteine composition (Payne 1987) between x- and y-type HMW glutenin subunits. It also is of note that, in some instances, certain HMW subunits appeared to silver stain negatively, as illustrated for subunit 7 present in the cultivar Wildcat (Fig. 3, track 11, second major protein band closest to the origin). This behavior, however, was coincident with higher protein loads and may be ascribed to the seeding of too many silver grains at the nucleation site(s) on the protein, as suggested by Merril et al (1988).

In general, most components found in any one cultivar were resolved clearly by PhastGel SDSGPAGE with the exception of 2* and 5 and 1 and 2 (nomenclature according to Payne and Lawrence [1983]) which were not separated. As shown in Figure 4 (track 8) for Bezostaya, components 2* and 5 migrated as one band whereas by conventional SDSGPAGE (Fig. 5, track 7), they were well-resolved. Similarly, PhastGel SDSGPAGE analysis of Canadian wheat cultivars such as Neepawa, Conway, and Columbus (Fig. 1B, tracks 2, 3, and 4 [8–25%]; Fig. 1C, tracks 2, 3, and 4 [10–15%]) did not resolve 2* and 5, while conventional SDSGPAGE (Fig. 2, tracks 2, 3, and 4) provided excellent resolution of these components. Components 1 and 2 (cultivar Flinor) also were well-

resolved by conventional SDSGPAGE (Fig. 5, track 5) but not by PhastGel SDSGPAGE (Fig. 4, track 5); however, band broadening was indicative of partial resolution. HMW glutenins 2 and 2*, although closely spaced and difficult to see in the illustration, were resolved by 10–15% gradient PhastGel analysis of Gabo, Sappo, and Lancota (Fig. 4, tracks 10, 11, and 12). These components also were resolved by conventional SDSGPAGE (Fig. 5, tracks 10, 11, and 12) but, whereas subunit 2 was the more mobile component by conventional SDSGPAGE, it exhibited the lesser mobility by 10–15% gradient PhastGel analysis. This variation in the relative mobility of 2 and 2* may be attributed to differences in gel composition as previously reported (Payne et al 1981).

Mobilities of some components present in different cultivars also were similar, making comparative identification by PhastGel or conventional SDSGPAGE more difficult. For example, subunits 10 and 12, found in Federation and Lancota, respectively, exhibited similar mobilities by PhastGel (Fig. 4, tracks 12 and 14) and conventional (Fig. 5, tracks 9 and 10) SDSGPAGE. Small differences in mobilities of some components were detected by conventional SDSGPAGE but were not observed by PhastGel SDSGPAGE. For example, a subunit present in Bezostaya (Fig. 5, track 7) originally identified as subunit 7 (Payne and Lawrence 1983) exhibits a slightly greater mobility than subunit 7 in the reference cultivar Chinese Spring (Fig. 5, track 8), indicating that these are different proteins. Despite these limitations, identification of HMW subunits was possible with side-by-side comparative analysis of unknowns and reference cultivars. For example, comparative PhastGel analysis of the CWSWS cultivar Fielder (Fig. 4, track 13) suggested an HMW glutenin composition of 2, 20, 17, and 12 that was confirmed by conventional SDSGPAGE (Fig. 5, track 13).

CONCLUSIONS

The results of this study indicate that fast horizontal SDSGPAGE and silver staining in conjunction with the PhastSystem can be used for wheat cultivar identification and, with some limitations, for the analysis of HMW glutenin subunits. Resolution of storage proteins on miniature 8–25% gradient PhastGels was superior to that of 10–15% gradient PhastGels and almost equal to that of conventional SDSGPAGE. Cultivars that could be distinguished by conventional electrophoresis also were distinguishable using 8–25% or 10–15% gradient PhastGels. The

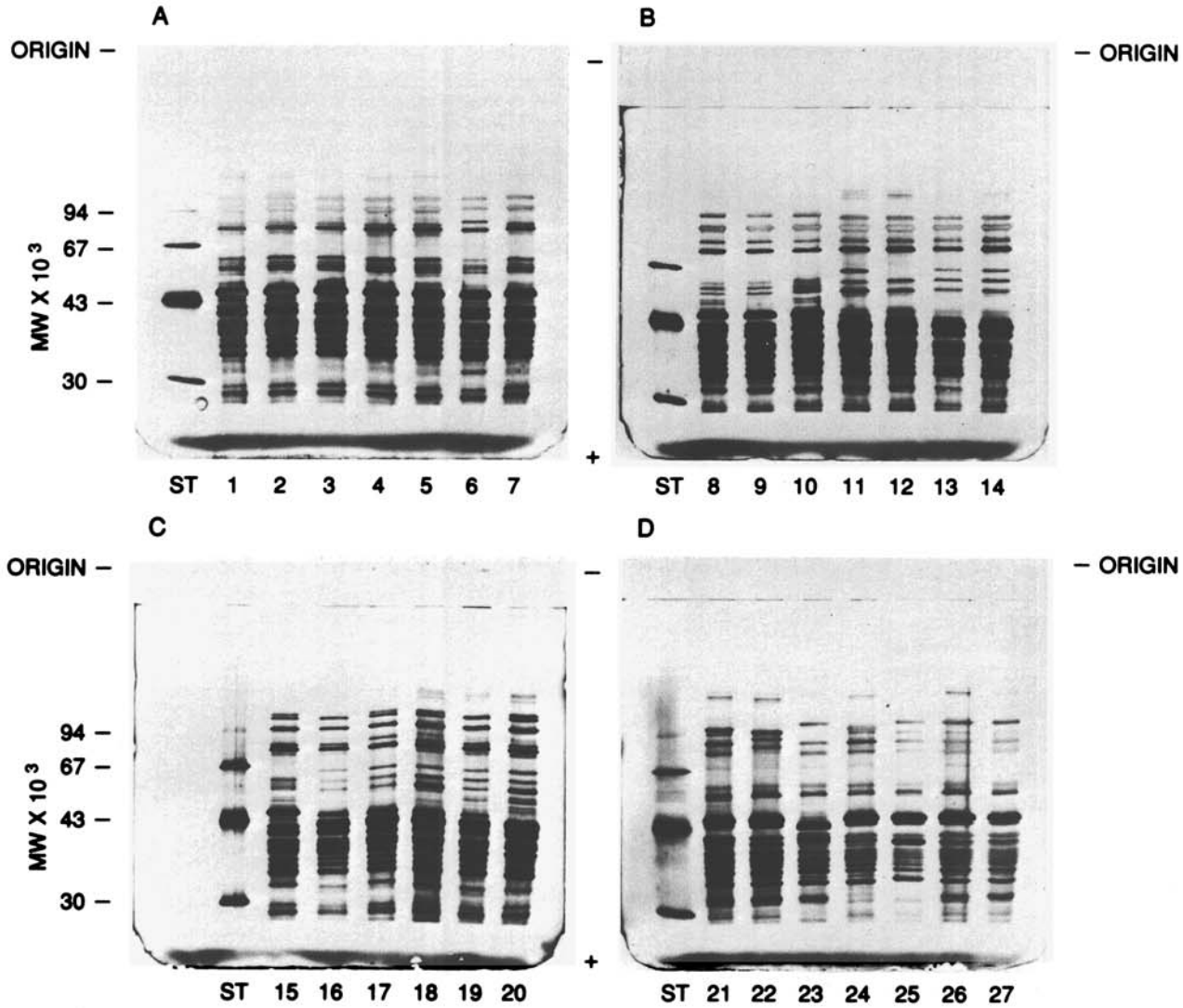

Fig. 3. Fast horizontal sodium dodecyl sulfate gradient polyacrylamide gel electrophoretic analysis, using 10–15% gradient PhastGels, of wheat storage proteins from the following wheat cultivars: **A,** Canada Western Red Spring: Benito (**1**), Canuck A (**2**), Conway (**3**), Katepwa (**4**), Kenyon (**5**), Leader (**6**), Manitou (**7**); **B,** Canada Prairie Spring: HY320 A (**8**), HY320 B (**9**), Oslo (**10**); Canada Eastern/ Western Utility: Wildcat (**11**), Bluesky (**12**), Glenlea A (**13**), Glenlea B (**14**); **C,** Canada Western Red Winter: Sundance (**15**), Norwin (**16**), Norstar (**17**); Canada Eastern Red Winter: Valor (**18**), Absolvent (**19**), Monopol (**20**); **D,** Canada Western Amber Durum: Arcola (**21**), Pelissier (**22**), Wascana (**23**), Hercules (**24**), Medora (**25**), Kyle (**26**), Wakooma (**27**). **ST** denotes standard reference mixture of proteins, and a letter following a cultivar name designates one of the polymorphic forms or "off-types" comprising that cultivar.

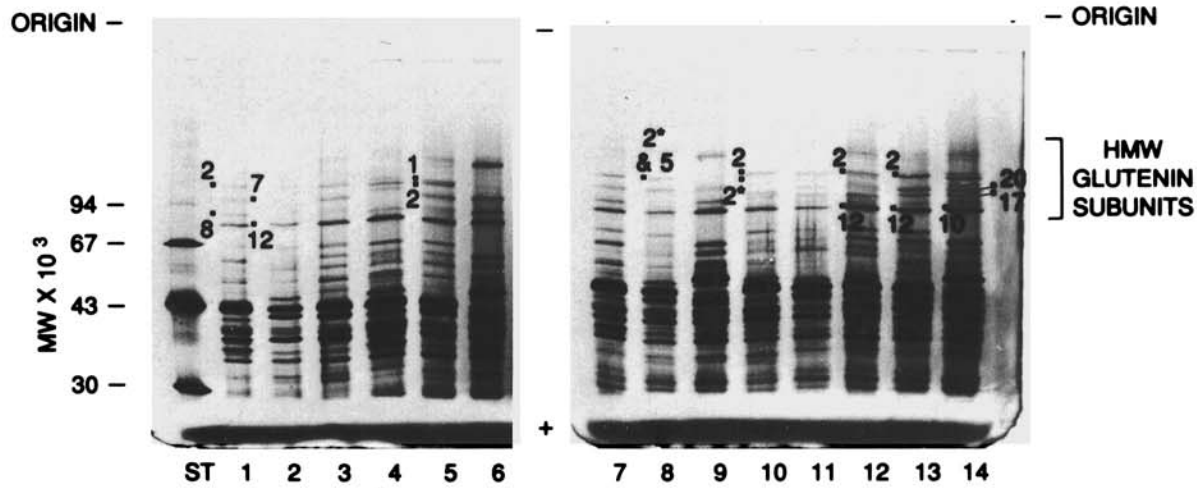

Fig. 4. Fast horizontal sodium dodecyl sulfate gradient polyacrylamide gel electrophoretic analysis using a 10–15% gradient PhastGel of high molecular weight (HMW) glutenin subunits from the following wheat cultivars (11 reference [Payne and Lawrence 1983] and one Canada Western Soft White Spring [CWSWS] wheat cultivar): Chinese Spring (**1,7**), Hobbit (**2**), Champlein (**3**), Hope (**4**), Flinor (**5**), Danchi (**6,9**), Bezostaya (**8**), Gabo (**10**), Sappo (**11**) Lancota (**12**), Fielder (CWSWS, **13**), and Federation (**14**). **ST** denotes standard reference mixture of protein. HMW glutenin subunit nomenclature is according to Payne and Lawrence (1983).

10–15% gradient format, however, is preferable since its analysis time requirements (45 min electrophoresis and 44 min staining) are much shorter, thus providing greater throughput. The identification of HMW glutenin subunits also can be quickly carried out with the PhastSystem with the limitation that subunits 2* and 5 are not resolved while subunits 1 and 2 are partially resolved.

The PhastSystem offers a number of advantages relative to conventional electrophoretic procedures. As noted previously, (Hansen et al 1988), the system is compact, easy to operate and, since premade gradient gels are employed, technical and time requirements for preparation of conventional gels are not necessary. The use of premade gels also provides for reproducible protein patterns, but some uneven migration of bands across a gel can occur and interfere with the identification of HMW glutenin subunits, in particular. Electrophoresis time for resolution of wheat storage proteins (45 min for 10–15% gradient PhastGel) is substantially shorter than conventional SDSGPAGE (1.75 hr) or acid PAGE (70 min; Mellish and Tkachuk, *unpublished results*), while silver staining requires 44 min as compared to about 1.5 hr for conventional silver staining. It should be realized, however, that the PhastSystem electrophoresis and staining can be carried out concurrently to maximize the number of gels and single kernels analyzed per day. A further increase in throughput also is possible by using the 12-well sample applicator but resultant protein patterns are more difficult to visualize, making it particularly unsuitable for analysis of HMW glutenin subunits.

The PhastSystem exhibits two major disadvantages when compared to conventional procedures. Firstly, a significantly larger number of samples can be analyzed per gel by conventional means. For example, a 28-well sample comb is used for preparation of conventional SDSGPAGE gels and a 40-well sample comb has been used with acid PAGE gels (Clements 1988). Thus, large numbers of single kernels can be analyzed side-by-side, facilitating comparison of protein patterns. Secondly, conventional electrophoresis procedures can be designed for more economical processing of large numbers of single kernels (Clements 1988), whereas buffer strip and PhastGel costs result in high PhastSystem analysis costs.

An overall evaluation of the relative advantages and disadvantages of the PhastSystem suggest that this electrophoresis system is not as well suited as conventional electrophoresis to the daily analysis of large numbers of samples for either wheat cultivar identification or the identification of HMW glutenin subunits. However, the PhastSystem could be used effectively in a situation where smaller numbers of samples are analyzed on an intermittent

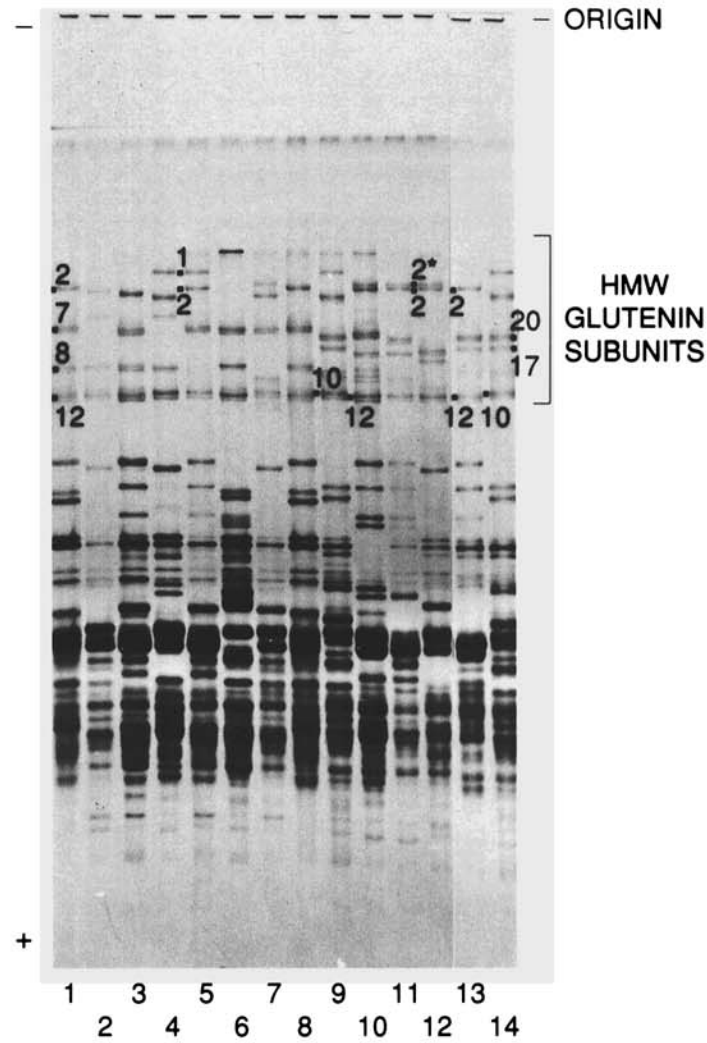

Fig. 5. Conventional sodium dodecyl sulfate gradient polyacrylamide gel electrophoretic analysis of high molecular weight (HMW) glutenin subunits from the following wheat cultivars (11 reference [Payne and Lawrence 1983] and one Canada Western Soft White Spring [CWSWS] wheat cultivar): Chinese Spring (**1,8**), Hobbit (**2**), Champlein (**3**), Hope (**4**), Flinor (**5**), Danchi (**6**), Bezostaya (**7**), Federation (**9,14**), Lancota (**10**), Sappo (**11**), Gabo (**12**), and Fielder (CWSWS, **13**). HMW glutenin subunit nomenclature is according to Payne and Lawrence (1983).

basis. In such cases, results could be provided much more rapidly as compared to conventional procedures with their attendant solution and gel preparation time requirements. In addition, the PhastSystem, unlike conventional electrophoresis procedures, could be used in locations where limited laboratory facilities and technical assistance are available because of its compact nature and ease of operation.

ACKNOWLEDGMENTS

The authors would like to thank P. I. Payne for supplying samples of the cultivar standard for the HMW glutenin subunits and K. Jarrin for technical assistance.

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[Received August 8, 1988. Accepted December 12, 1988.]