NOTE

Polyacrylamide Gel Electrophoresis of Gliadin Proteins for Wheat Variety Identification—Procedural Modifications and Observations[1]

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Polyacrylamide gel electrophoresis (PAGE) of gliadin proteins is being widely investigated as a method for wheat variety identification (Bushuk and Zillman 1978, DuCross and Wrigley 1979, Jones et al 1982, Tkachuk and Metlish 1980, Wrigley et al 1982). A major aim is to standardize the PAGE technique for wheat variety identification so that reproducible intra- and interlaboratory results may be obtained. Autran et al (1979) and Lookhart et al (1982) reported on some standardization problems associated with PAGE for wheat variety identification. The need for standardizing the PAGE technique for wheat variety identification culminated in the formation of a study group on variety identification by electrophoresis by the International Association for Cereal Chemistry.

We report certain modifications to the Bushuk and Zillman (1978) PAGE procedure. We also compare the separation of gliadin proteins with aluminum (Al)- and sodium (Na)-lactate buffer systems with the apparatus of Bushuk and Zillman (1978) and with that used by Lookhart et al (1982). This note, therefore, provides additional information that can be useful in standardizing the PAGE procedure for wheat variety identification.

MATERIALS AND METHODS

Wheat Samples

Marquis (Canadian variety), Diplomat (French variety), and Halberd (Australian variety) wheats were supplied by W. Bushuk, Department of Plant Science, University of Manitoba, Winnipeg, Canada. The other wheat varieties used in this study were supplied by A. Allan Mann, Leader of Seeds Stock Project, Department of Agronomy, North Dakota State University, Fargo.

Polyacrylamide Gel Electrophoresis

The PAGE procedures of Bushuk and Zillman (1978) and Lookhart et al (1982) were used in this study. In addition, the Na-lactate buffer system reported by Bushuk et al (1981) was compared to the Al-lactate buffer system. However, to improve the resolution of gliadin components on polyacrylamide gels, a few modifications were made to the original procedure of Bushuk and Zillman (1978).

The slot former of Bushuk and Zillman (1978) was modified to improve the resolution of protein components. The 3/16-in. long prongs (projections) of the slot former were cut back to 3/32 in., resulting in the formation in the gel of shallower sample wells. Because the sample wells were so shallow, a smaller sample volume (5 μl) containing approximately the same amount of protein as the original procedure of Bushuk and Zillman (1978) was applied (ground grain or flour was extracted 1 g:1.5 ml with 70% aqueous ethanol, and the extracts were diluted 1:1 (v/v) with electrophoresis buffer before the samples were applied to the gel).

To obtain uniform and purified chemicals, both the acrylamide and *N,N*-methylenebisacrylamide were recrystallized before use for this study according to the procedure of Loening (1967), which was modified as follows. 1) Acrylamide (35 g) was dissolved in 500 ml of 50° C chloroform, stirred for 10 min, and filtered on a Buchner funnel under vacuum on Whatman no. 3 paper. 2) The filtrate was cooled to room temperature and then placed in the freezing compartment of a refrigerator. The dissolved acrylamide crystallized in about 1 hr. 3) The crystallized acrylamide was filtered under vacuum on Whatman no. 3 paper and finally washed through on the Buchner funnel with 100 ml of cold chloroform. 4) The crystals were spread into a thin layer in an appropriate container and dried under a gentle stream of air in a fume hood. (NOTE: The crystals are loose and fluffy and can be blown away when the current of air is too strong. Acrylamide dust should not be inhaled, as acrylamide is poison). The yield was approximately 75–80%.

Bisacrylamide was recrystallized as follows: 5.0 g of bis was dissolved in 500 ml of 40–50° C acetone and vacuum-filtered on Whatman no. 3 paper. Steps 2, 3, and 4 above were then followed as for recrystallization of acrylamide, except acetone was used in place of chloroform in step 3. The yield was approximately 55–60%.

A consistently firm gel was obtained when dissolved oxygen in the gel solution was removed under vacuum (aspirator attached to water tap) for at least 5 min. Also, 0.5 ml of hydrogen peroxide instead of 1.0 ml, when added to the deaerated solution to initiate polymerization, gave a firmer gel. If the gel solution is not deaerated, gel firmness fluctuates widely from day to day.

We also found that a smaller applied constant current of 90 mA instead of 100 or 110 mA gave better results with the Na-lactate system and the Bushuk and Zillman (1978) apparatus. With the E-C 470 apparatus, we used the PAGE procedure of Lookhart et al (1982) with their modified Al-lactate (pH 3.1) buffer system. The E-C 470 apparatus was also used with the Na-lactate (pH 3.1) buffer at a constant current of 75 mA. Unlike the Bushuk and Zillman (1978) and Lookhart et al (1982) procedures, the electrophoresis run was not allowed to proceed after the second tracking dye band had migrated off the gel. Instead, all runs were terminated when the second tracking dye band had migrated to a fixed distance on the gel, ie, about 0.5 cm from the end of the gel. In order for the second tracking dye band in the lower part of the gel on the E-C 470 apparatus to be visible, the amount of methyl green recommended by Bushuk and Zillman (1978) must be doubled. Electrophoresis to a fixed distance is advantageous in that the origins of gels from different runs or their photographs can be easily aligned and their protein components compared. Another advantage of a fixed-distance run with gliadin proteins is that the α-gliadins are retained on the gel.

Apparatus for PAGE

The resolution of gliadin components was compared on two apparatuses that were available in our laboratory. The first was the Bushuk and Zillman (1978) horizontal gel apparatus, which has a gel bed 21–22 cm long. The second was the E-C 470 vertical gel cell (E-C Corporation, St. Petersburg, FL), which has a gel bed 17 cm long.

Photography of Gels

Gels were photographed with a 35-mm camera according to the procedure of Bushuk and Zillman (1978) with Kodak technical pan film 2415 (ESTAR-AH Base) at an ASA value of 100. The 35-mm camera was also set at ASA 100.

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Scanning of Gels

Gels stained with Coomassie brilliant blue R250 were scanned at 600 nm with an SD 3000 Schoeffel Spectrodensitometer (Schoeffel Instrument Division, Westwood, NJ) equipped for scanning a slab gel up to 17 × 17 cm in dimension. The scan was monitored with a Fisher Record All 5000 Series recorder (Fisher Scientific Company, Springfield, NJ). The stage speed of the densitometer was set on fast, and the chart speed of the recorder was 10 cm/min. Background interference from the gel was greatly diminished when the upper gel surface was layered with distilled water before scanning.

Chemicals

Acrylamide, methylenebisacrylamide, sodium lactate lactic acid, and ascorbic acid were from Sigma Chemical Co. Aluminum lactate (odorless white powder) was from K and K laboratories (ICN Pharmaceuticals). Coomassie brilliant blue and trichloroacetic acid were from MCB Chemicals. Ferrous sulfate was from Fisher Scientific. Hydrogen peroxide solution (3%) was purchased at a local pharmacy.

RESULTS AND DISCUSSION

We encountered numerous difficulties during our investigations on the suitability of the Bushuk and Zillman (1978) PAGE procedure for wheat variety identification. Our greatest problem was that the gliadin bands (protein components) sloped on the gel in the direction of the cathode end. We also noticed that gels were not always of the same consistency but varied widely in firmness from very soft to relatively firm. Softer gels showed greater band sloping than firmer gels. Figure 1A shows the gliadin components of some hard red spring and durum wheats of North Dakota separated according to the original procedure of Bushuk and Zillman (1978) on their horizontal apparatus, ie, with nonrecrystallized acrylamide, nondeaerated gel solution, 1.0 ml H_2O_2, and their original slot former. The patterns are blurry when viewed from directly above, especially in the mid-mobility region where the protein components are more intensely stained and are close to each other. The blurry patterns are due to the sloping of the protein bands: the proteins migrate faster in the upper part than in the lower part of the gel, which results in nonvertical bands within the gel. At the beginning of the electrophoretic run, we noticed that the tracking dye always migrated upwards from the sample well as it began to enter the gel and sloped towards the cathode. The protein components, therefore, presumably would also assume the same slope as the tracking dye. We concluded that the sample wells were perhaps too deep, since the tracking dye had to migrate upwards before proceeding across the gel. To reduce the depth of the sample well, we modified the slot former as described.

Figure 1B shows the electrophoregrams of the gliadins of the variety Marquis (pattern 12) and those of some durum wheat varieties electrophoresed according to our modified PAGE procedure with the shallower sample well (modified slot former) on the horizontal Bushuk and Zillman (1978) apparatus. The better resolution and clarity of the patterns in Fig. 1B as compared to the patterns in Fig. 1A are evident. The bands in B are straighter, and the separation between bands, when viewed from directly above, is clearer, especially in the mid-mobility region of the gel. The firmer 6% gels also showed enhanced resolution because the firmness further reduced band sloping. Improved resolution (straighter, sharper bands) facilitated more accurate measurement of relative mobility values of gliadin components for use in wheat variety identification.

Figure 2A compares the separations obtained with the E-C 470 vertical apparatus of the gliadins of some hard red spring and

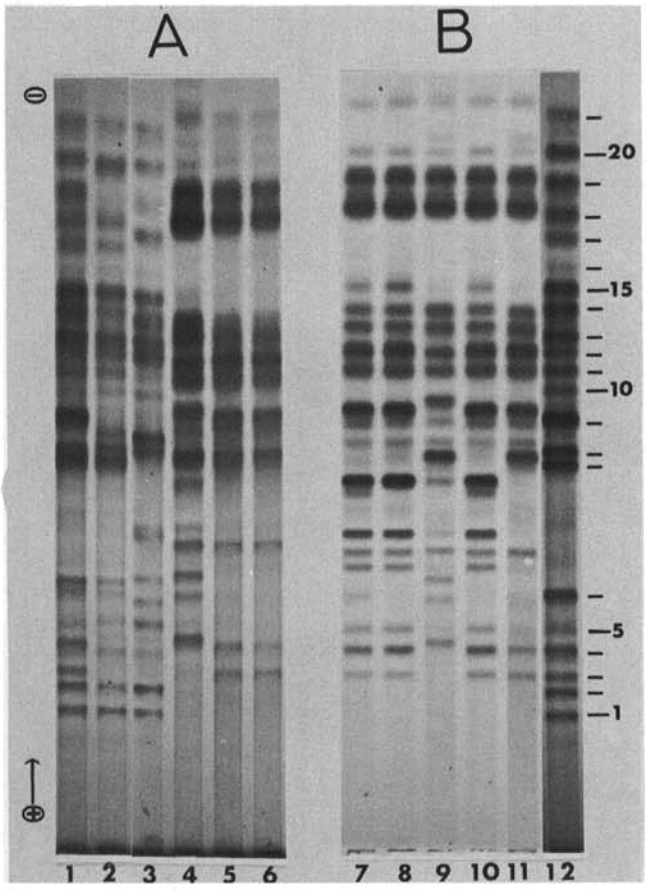

Fig. 1. Comparison on the horizontal gel apparatus of the resolution of gliadin components obtained with the original PAGE procedure (gel A) of Bushuk and Zillman (1978) and the modified PAGE procedure (gel B) used in this study. Gel A: patterns 1–3, the hard red spring wheat varieties Marquis, Alex, and Coteau; patterns 4–6, the durum varieties Coulter, Edmore, and Vic. Gel B: patterns 7–11, the durum varieties Calvin, Botno, Coulter, Crosby, and Edmore; pattern 12, the hard red spring wheat Marquis.

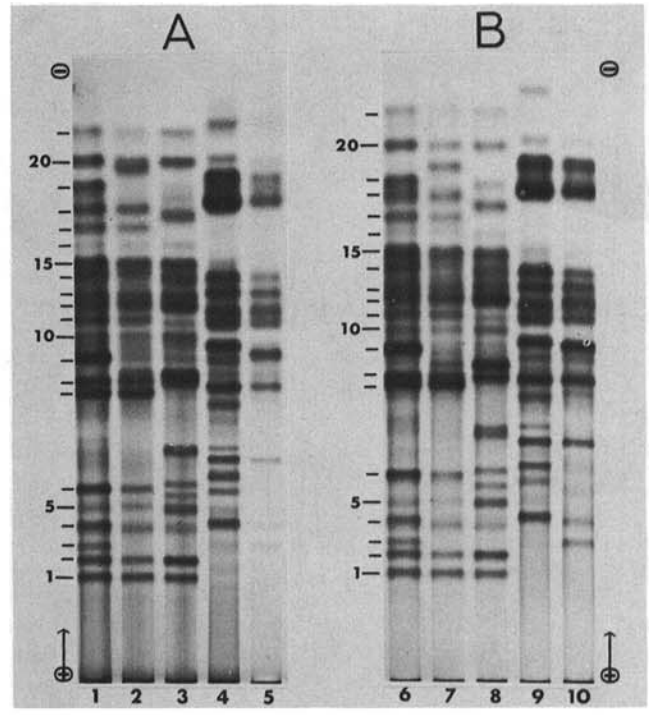

Fig. 2. Comparison of Na-lactate (gel A) and Al-lactate (gel B), pH 3.1, buffer systems with the E-C 470 apparatus. Gel A: patterns 1–3, hard red spring varieties Marquis, Alex, and Coteau; 4–5, the durum varieties Coulter and Vic. Gel B: 6–8, Marquis, Alex, and Coteau; 4–5, Coulter and Vic.

durum varieties, using the Na-lactate buffer system. Examination of Figs. 1B and 2A shows that approximately 21 major components are separated from the variety Marquis with both the Bushuk and Zillman (1978) and the E-C 470 apparatus. However, the relative mobility (Rm) values (not shown) for the protein components of Marquis (calculated according to Bushuk and Zillman 1978, from the photographs of Figs. 1B and 2A) show variations between the two apparatuses. Some of the gliadin components migrate differently on the horizontal (Bushuk and Zillman 1978) than on the vertical (E-C 470) apparatus. We also noticed, as is evident in the gels of Jones et al (1982; Fig. 1) and of Lookhart et al (1982; Fig. 1), that the gliadin patterns obtained with the E-C 470 apparatus show a slight curvature, ie, the migration of components in the middle slot is faster than the same material in the slots to the left or the right of the middle. On the other hand, the gliadin patterns obtained with the Bushuk and Zillman (1978) apparatus do not show any curvature (Fig. 1B). The Bio-Rad vertical apparatus (Bio-Rad Laboratories, Mississauga, Ontario, Canada) used by Tkachuk and Metlish (1980) sometimes showed a slight curvature, as noted with the vertical E-C 470 apparatus. However, the PAGE patterns obtained by Tkachuk and Metlish (1980; Fig. 2) showed greatly improved resolution over other reported gliadin patterns obtained on vertical gels. The Bio-Rad PAGE apparatus and the PAGE procedure of Tkachuk and Metlish (1980) may be the procedure of choice for separating gliadin proteins on vertical gel systems.

Figure 2A and B compares the separation obtained with the Na-lactate and Al-lactate buffer systems using the E-C 470 apparatus. Only the clearly visible bands of Marquis are numbered. The same number of gliadin components are separated for Marquis with both buffer systems. Therefore, the Na-lactate (pH 3.1) buffer system can also be used with the E-C 470 vertical apparatus. However, the migration of gliadin components in the two buffer systems are different, especially in the mid-mobility region and beyond. This is shown by the densitometric scans (Fig. 3A and B) of the electrophoregrams of the variety Marquis from Fig. 2A and B. The Rm values of most of the bands of Marquis are identical with both buffer systems except for bands 6, 11, 14, 18, and 19, which differ by one mobility unit (Rm values were measured from the scans of Fig. 3A and B, according to Bushuk and Zillman 1978, and values were rounded off to the nearest whole number according to Jones et al 1982).

Figure 4A and B shows that better resolution of gliadin components is also obtained with the Na- than with the Al-lactate buffer system using the Bushuk and Zillman (1978) apparatus and our modified PAGE procedure. However, the Na-lactate gels have darker backgrounds and contain more material at the origin (point of sample application). As shown earlier with the E-C 470 apparatus, the Rm values of some of the gliadin components separated with the Na- and Al-lactate buffer systems also show differences in the Bushuk and Zillman (1978) apparatus.

We also noticed that the first and last slots of both the Bushuk and Zillman (1978) and E-C 470 apparatuses are prone to edge effects, which cause gliadin bands to be skewed. Therefore, we no longer use the two end slots (slots 1 and 10) in our electrophoresis of gliadin proteins for wheat variety identification.

CONCLUSION

Good resolution of gliadin components is necessary in order to obtain accurate Rm values for wheat variety identification. We recommend the latter system because Na-lactate is readily available, and its composition among lots is more consistent than that of Al-lactate. Na-lactate also gives better resolution of some

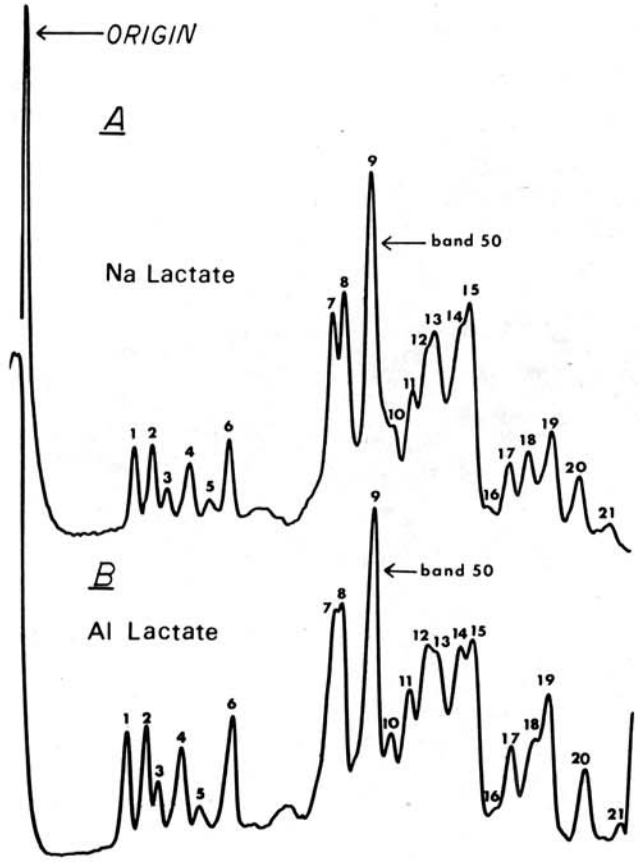

Fig. 3. Densitometric scans of Marquis gliadin electrophoresed on the E-C 470 apparatus as shown in Fig. 2A (pattern 1) and B (pattern 6). A: Scan from the Na-lactate (pH 3.1) buffer system. B: Scan from the Al-lactate (pH 3.1) buffer system. Band 50 is the reference band of Bushuk and Zillman (1978) used to calculate relative mobility values.

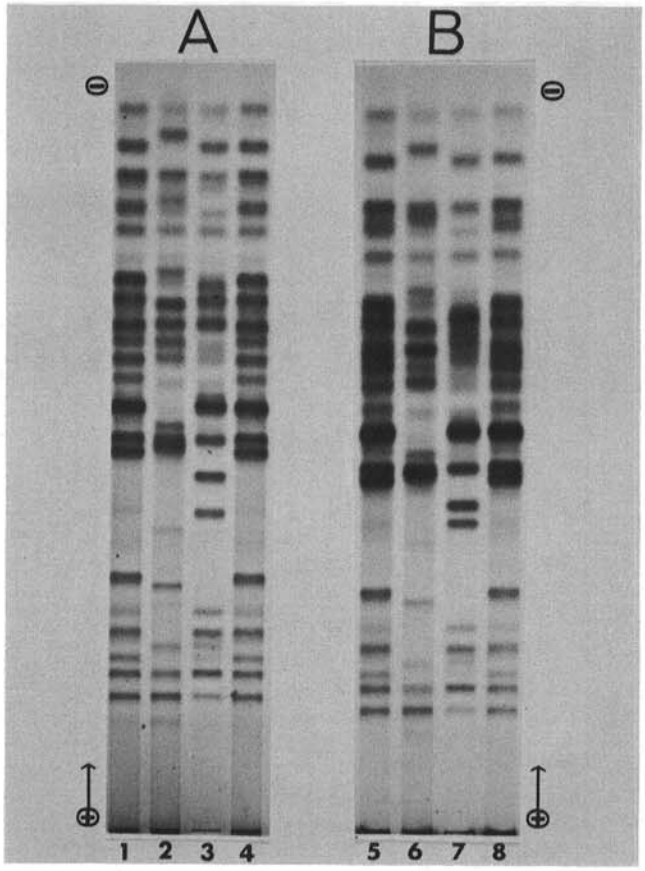

Fig. 4. Comparison of the resolution of gliadin components obtained with the Na-lactate (gel A) and Al-lactate (gel B) pH 3.1 buffer systems on the Bushuk and Zillman (1978) PAGE apparatus. Gel A: patterns 1 and 4, Marquis; 2, Diplomat; and 3, Halberd. Gel B: patterns 5 and 8, Marquis; 6, Diplomat, and 7, Halberd.

gliadin components than the Al-lactate system. DuCross and Wrigley (1979) also noted that Na-lactate gave better resolution of gliadin components than Al-lactate buffer. Our comparison of apparatuses for gliadin electrophoresis shows that the Bushuk and Zillman (1978) apparatus, because of its longer gel bed, results in a clearer separation of the gliadin components in the mid-mobility region of the gel. However, use of the Bushuk and Zillman (1978) apparatus is much more difficult to master than that of the E-C 470 apparatus. The Bushuk and Zillman (1978) apparatus requires a great deal of skill and dexterity to obtain a satisfactory gel. The E-C 470 apparatus, on the other hand, requires less skill and dexterity to operate. An ideal apparatus would be one that is designed like the E-C 470 apparatus but with a gel bed about 25 cm in length. Such an apparatus would incorporate the resolving features of the Bushuk and Zillman (1978) apparatus and the simplicity of operation of the E-C 470 apparatus.

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