Comparison of Polyacrylamide Gel Electrophoresis and High-Performance Liquid Chromatography Analyses of Gliadin Polymorphism in the Wheat Cultivar Newton[1]

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ABSTRACT

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Polyacrylamide gel electrophoresis (PAGE) and reversed-phase high-performance liquid chromatography (HPLC) are highly effective and complementary techniques for differentiation of wheat genotypes through gliadin analysis. To compare directly their relative abilities to detect kernel-to-kernel variation within a variety, 100 individual kernels of the wheat variety Newton were cut in two, and gliadins were extracted from each half and analyzed by the two methods. Two major genotypes were observed in a ratio of 42:58. In addition, there was a perfect correlation between the methods for genotype identification of each individual kernel. The PAGE patterns of the isolated HPLC peaks that were different for the two genotypes were identical to the band patterns that were characteristic of each genotype, demonstrating that the same proteins were responsible for the major differences, which were readily apparent by either method.

One of the most important decisions a wheat farmer makes each year is choosing what cultivar to grow. Each variety has specific genetic potential for quality and protein content, geographic adaptability, resistance to pests, and ability to withstand stresses. The Federal Grain Inspection Service (FGIS) has been charged by Congress with identifying and classifying grain in marketing channels. A visual classification procedure, used since 1917, has generally worked well. Lately, however, changed breeding strategies, such as crosses between wheat classes, have made visual identification nearly impossible. However, instrumental methods, including polyacrylamide gel electrophoresis (PAGE) and high-performance liquid chromatography (HPLC), have been developed that can identify wheat cultivars by "fingerprinting" their gliadin proteins (Lookhart et al 1982, Burnouf et al 1983, Bietz et al 1984).

Recently, Lookhart et al (1985a) prepared and analyzed gliadin electrophoregrams of single seeds of several cultivars from each wheat class. These were certified pure by breeders and were found by FGIS to have appropriate physical characteristics. In that study, differences in the PAGE gliadin patterns among single kernel and bulk extracts of a given cultivar (genotypes) were found for some cultivars. These included the hard red winter wheats Arkan, Newton, and Sage and the hard red spring wheats Guard and Ram. The presence of gliadin polymorphisms in western U.S. soft wheats was recently shown by Mecham et al (1985). The sample purities, degrees of pattern heterogeneity, and percentages of each pattern type require analysis of 100 kernels of each cultivar for determination with a 95% confidence limit (Wrigley and Baxter 1974). Both studies (Lookhart et al 1985a, Mecham et al 1985) analyzed small numbers of samples as they were mainly interested in showing that genotypes existed in those varieties.

Reversed-phase HPLC can also detect gliadin polymorphisms within wheat varieties. Initially, gliadin differences among individual kernels were demonstrated from a bulk (multi-kernel) sample of the land race Nap Hal (Bietz et al 1984). Subsequently, using an improved, more rapid methodology (Bietz and Cobb 1985), significant differences in gliadin compositions were found among individual kernels of recently released U.S. hard red winter wheat cultivars, such as Arkan and Newton.

In this study, we used two independent methods (PAGE and HPLC) in separate laboratories to determine the number of major gliadin patterns (heterogeneity), the percentage of each pattern type, the purity of samples, and the effect of environment on the patterns by analyzing 25 individual seeds of the cultivar Newton from each of four separate sources (a total of 100 seeds). This study is the first systematic and direct comparison of HPLC and PAGE methods for analysis of gliadin polymorphism within a cultivar.

MATERIALS AND METHODS

Chemicals and Reagents

Acrylamide, *N, N'*-methylenebisacrylamide, ascorbic acid, Coomassie Brilliant Blue R-250, methyl green, and trichloroacetic acid were from Sigma Chemical Co., St. Louis, MO. Trifluoroacetic acid (TFA) was from Sigma or Pierce (Rockford, IL). Lactic acid (USP grade) and ferrous sulfate heptahydrate (AR grade) were from Mallinckrodt Chemicals, St. Louis, MO. LC-grade ethanol and acetonitrile were from Burdick and Jackson Laboratories, Muskegon, MI, Mallinckrodt, or Fisher. Hydrogen peroxide (3% practical grade) was purchased from a local pharmacy. Water was purified by passage, in series, through two mixed-bed ion-exchange cartridges, a charcoal bed, and a membrane filter. Aluminum lactate was from K & K Laboratories, Plainview, NY.

Wheat Samples

Foundation Newton seed samples were obtained from the Illinois Foundation Seed Company, Champaign, IL (Richard Denhurt); Foundation Seed Division, University of Nebraska, Lincoln, NE (Richard Mills); and Foundation Seed Center, Kansas State University, from the Manhattan and Hutchinson locations (Carl Overly).

Sample Preparation

Single kernels (25) from each location were split longitudinally and labeled so that one-half could be analyzed by HPLC and the other half by PAGE. Each half kernel was ground in a mortar with a pestle.

Polyacrylamide Gel Electrophoresis

The electrophoresis apparatus used was a Hoefer SE600 (18 cm × 16 cm) vertical slab gel system (3-mm thick gel). Gel acrylamide concentration (6.0%), temperature (20° C), electrophoresis voltage

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(500 V), and time (2 hr) were those that optimized band resolution and minimized electrophoresis times (Tkachuk and Mellish 1980, Khan et al 1985, Lookhart et al 1985b). The gel recipe was a combination of those of Lookhart et al (1985b) and Khan et al (1985): 100 ml of gel solution (1 gel) contained 0.25 g of aluminum lactate, 0.024 g of ascorbic acid, 0.25 g of bis-acrylamide, 6.00 g of acrylamide, 0.2 mg of ferrous sulfate heptahydrate, lactic acid to pH 3.1, water to 100 ml, and 100 μl of 3% hydrogen peroxide. The power supply, staining, and destaining procedures were described previously (Lookhart et al 1982). Each half kernel was extracted with 50 μl of 70% ethanol for 1 hr at room temperature. Methyl green tracking dye and glycerol were added to extracts for PAGE (Lookhart et al 1985b).

High-Performance Liquid Chromatography

The HPLC procedure was that of Bietz and Cobb (1985). Half kernels were extracted with 0.5 ml of 70% ethanol for 30 min at room temperature and centrifuged at 20,000 rpm (Beckman L8-M) for 10 min at 20° C. The supernatant was decanted and stored at room temperature until analysis by reversed-phase (RP) HPLC. Extracts (45 μl) were analyzed at 2 ml/min on a Beckman/Altex RPSC column (no. C37-4A; C_3) at 70° C using a linear 25–45% acetonitrile (+0.1% TFA) gradient during 15 min, with a total run time of 17 min, and a 3-min reequilibration between runs. HPLC results were confirmed by extending gradient times to 50–60 min at 1.0 ml/min; although total resolution improved considerably (Bietz and Cobb 1985), no major differences were observed from the 15-min gradient separations. Detection was at 210 nm (0.2 absorbance units full scale). A data point was stored every 30 milliseconds on a Modcomp computer for subsequent integration, replotting, and comparison. Blanks were run periodically to ensure that all peaks represented sample components. Aliquots of a Newton standard, extracted from four kernels of a bulk sample of foundation seed, were periodically analyzed to monitor the column and system performance and ensure that samples did not change appreciably upon storage. All samples were analyzed within four days of extraction.

The HPLC system used to collect selected peaks was a Hewlett-Packard 1084B equipped with a fraction collector. A SynChrom RP-P C_{18} (6.5 μm) column was eluted at 1 ml/min and 70° C using a binary gradient of solvents A (acetonitrile plus 0.1% TFA) and B (water plus 0.1% TFA). The gradient, optimized to improve separation of the peaks of interest, consisted of the following series of linear binary gradients: 75% B at time 0 to 65% B at 8 min, 62% B at 12 min, 50% B at 20 min, and then to initial conditions at 25 min.

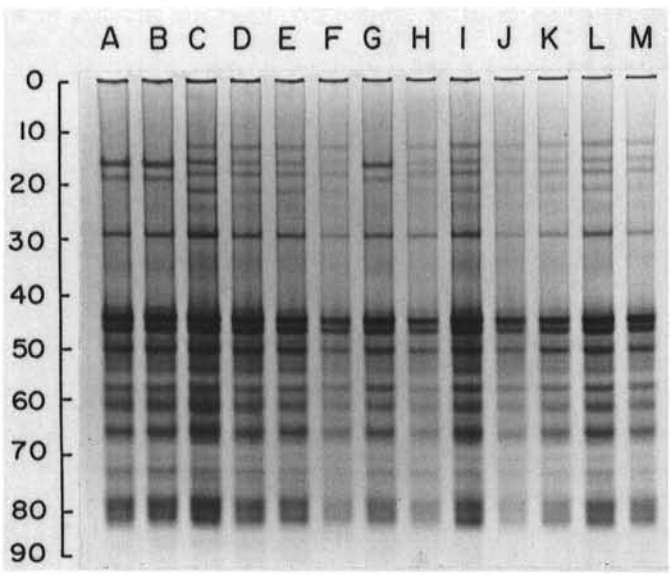

Fig. 1. Gliadin polyacrylamide gel electrophoretic patterns of 13 half kernels (**A–M**) of Newton wheat from Hutchinson, KS.

RESULTS AND DISCUSSION

PAGE

Bulk extracts of Newton wheat gave consistent gliadin PAGE patterns (Jones et al 1982). However, gliadins extracted from single kernels of Newton produced heterogeneous PAGE (Lookhart et al 1985a) and HPLC (Bietz and Cobb 1985) patterns, none of which were identical to that of a normal bulk sample (Jones et al 1982). Consequently, gliadins were extracted from one-half of each of 100 kernels (25 from each of four locations), and the extracts were analyzed by PAGE. Typical PAGE patterns are shown in Figure 1. Significant differences occurred, especially in the relative mobility range from 10 to 30. Two major kernel types were distinguished on the basis of differences in the ω-gliadin (relative mobility <37) region: a two-band type I (lanes a, b, and g) and a four-band type II. The ratio of genotypes I:II in the 100 kernels was 42:58. The percentages of genotype I were 32, 40, 36, and 60% for the Illinois, Nebraska, and Manhattan and Hutchinson, Kansas locations, respectively. Because the confidence intervals for each of the four subsamples overlap (Wrigley and Baxter 1974) with a confidence coefficient of 0.95, they can all be averaged as a single sample. Similar results have been found using two-dimensional gel electrophoresis (Norman Anderson, *personal communication*).

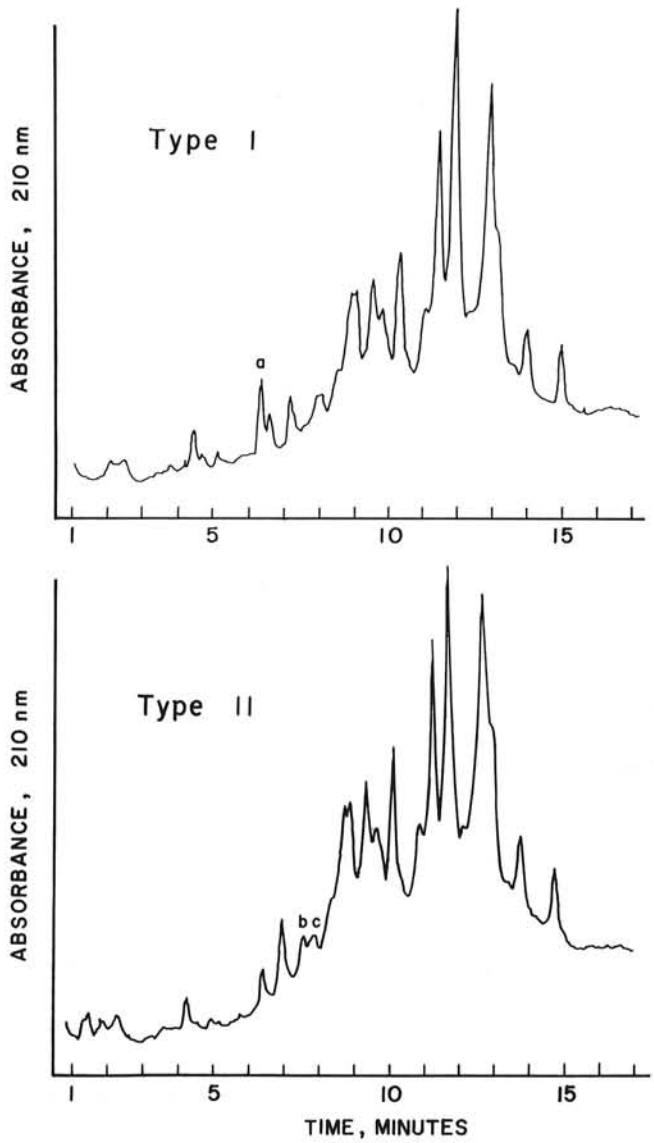

Fig. 2. Gliadin high-performance liquid chromatographic patterns of half kernels of Newton wheat from Hutchinson, KS, exhibiting two major kernel patterns (I and II). Peaks characteristic of the different pattern types are indicated by **a, b,** and **c**.

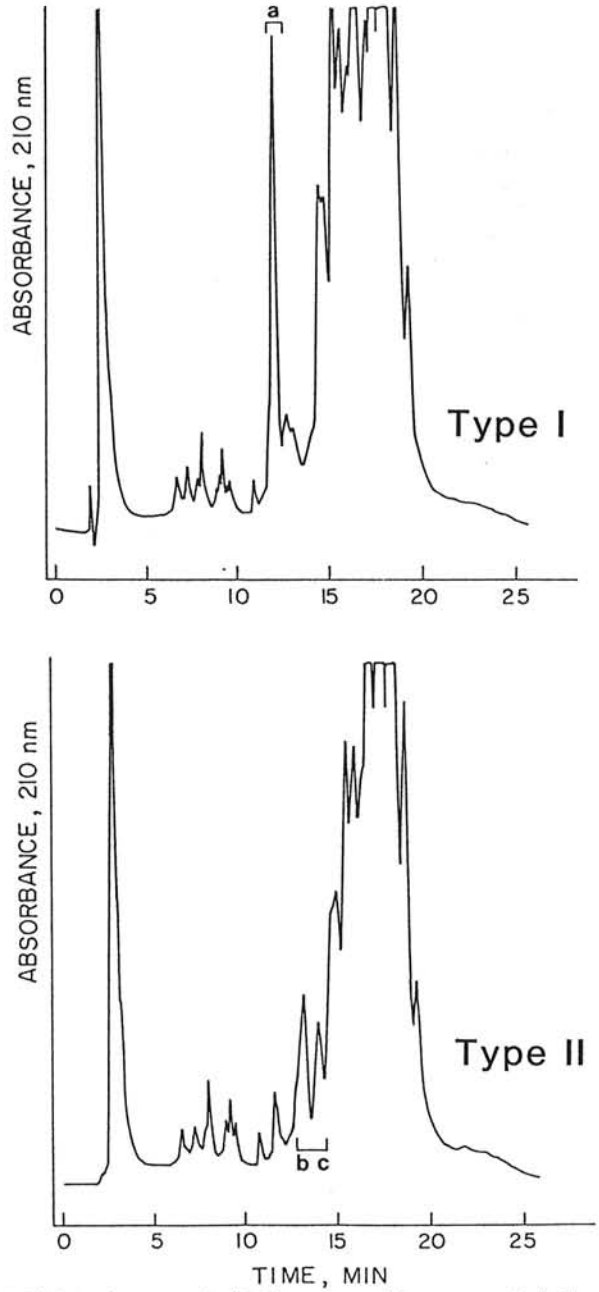

Fig. 3. High-performance liquid chromatographic patterns of gliadins of the two major kernel genotypes (I and II). Collected fractions **a** and **b–c** correspond to peaks a, b, and c in Fig. 2.

HPLC

HPLC elution patterns of gliadins are genotype-specific and can be used to identify wheat varieties (Bietz et al 1984). HPLC elution patterns of gliadin extracts of single kernels of Newton wheat showed the gliadins were heterogeneous (Bietz and Cobb 1985). To compare the abilities of PAGE and HPLC to differentiate genotypes, the second half of each kernel, previously analyzed by PAGE, was extracted and analyzed by HPLC. Typical elution patterns are shown in Figure 2. Major differences were observed among different seeds in early-eluting (5–9 min) components; such components are generally ω-gliadins (Bietz 1983, Bietz and Burnouf 1985). Two major pattern types were present: one (I) had a peak "a" at 6.2 min that was absent in II; peaks "b" and "c" in II, eluting at 7.0 and 7.6 min, respectively, were increased (or new) relative to I. Of the 100 kernels analyzed, the ratio of types I to II was 42:58, the same as found for the two major PAGE patterns. Both methods revealed a few additional minor qualitative and quantitative differences, as is evident from Figure 1 and in HPLC studies (Bietz and Cobb 1985).

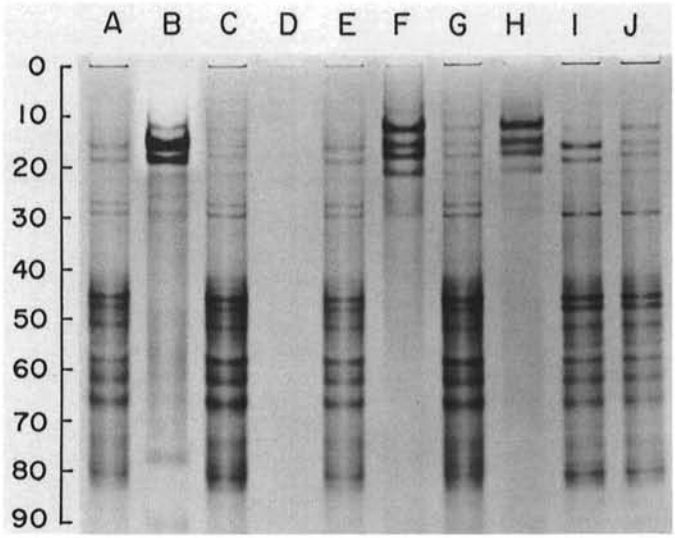

Fig. 4. Polyacrylamide gel electrophoresis patterns of collected gliadin high-performance liquid chromatographic peaks a (lanes **B** and **D**) and b + c (lanes **F** and **H**). Typical type I electrophoresis patterns are shown in **A, E,** and **I,** and typical type II patterns in lanes **C, G,** and **J**. Fractions analyzed in **B** and **H** were dialyzed against water, fraction **D** was dialyzed against 70% ethanol, and fraction **F** was not dialyzed.

Peaks characteristic of HPLC genotypes I (12.2 min) and II (13.0 and 14.1 min) were collected from 10 separate separations of 50 μl injections of extracts of each pattern type (Fig. 3). Changes in the column and elution conditions made the small differences seen in Figure 2 into more visible differences in Figure 3, which aided in collecting the fractions. Pooled peak "a" and peaks "b + c" (from genotypes I and II, respectively) were each divided into two equal portions; one portion of peak "a" was dialyzed overnight against water, the other against 70% ethanol. The first portion of peaks "b + c" was dialyzed against water, and the other portion was refrigerated overnight (no precipitates were seen in any of the fractions). All portions were then frozen, lyophilized, and dissolved in 20 μl of 70% ethanol plus 10 μl of glycerol and 1 μl of tracking dye for PAGE (Fig. 4).

HPLC peak "a" from genotype I contained gliadins having electrophoretic mobilities identical to those bands characteristic of the two-band electrophoresis type I (Fig. 4, lanes B and D). The portion of peak "a" (type I) dialyzed against 70% ethanol (lane D) showed no bands, possibly caused by adherence to the dialysis bag. Peaks "b + c", characteristic of type II, gave PAGE patterns (Fig. 4, lanes F and H) corresponding to the four bands typical of type II (Fig. 4, lanes C, G, and J).

These results demonstrate that HPLC and PAGE are both capable of separating and differentiating major gliadin proteins in a polymorphic wheat cultivar; each technique may be advantageous for such purposes under specific conditions. HPLC and PAGE separate proteins by totally different criteria (surface hydrophobicity versus charge plus size, respectively), and are thus complementary techniques. Our study clearly shows, for the first time, that for differentiating the major Newton genotypes HPLC and PAGE are equally capable.

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LITERATURE CITED

BIETZ, J. A. 1983. Separation of cereal proteins by reversed-phase high-performance liquid chromatography. J. Chromatogr. 255:219-238.

BIETZ, J. A., and BURNOUF, T. 1985. Chromosomal control of wheat gliadin: analysis by reversed-phase high-performance liquid chromatography. Theor. Appl. Genet. 70:599-609.

BIETZ, J. A., BURNOUF, T., COBB, L. A., and WALL, J. S. 1984. Wheat varietal identification and genetic analysis by reversed-phase high-

performance liquid chromatography. Cereal Chem. 61:129-135.
BIETZ, J. A., and COBB, L. A. 1985. Improved procedures for rapid wheat varietal identification by reversed-phase high performance liquid chromatography of gliadins. Cereal Chem. 62:332.
BURNOUF, T., BIETZ, J. A., COBB, L. A., and WALL, J. S. 1983. Reversed-phase high-performance liquid chromatography of gliadins from hexaploid wheat (*Triticum aestivum* L.) varieties cultivated in France and possibility of varietal identification. C. R. Acad. Sci. Paris, Ser. 3 297:377-382.
JONES, B. L., LOOKHART, G. L., HALL, S. B., and FINNEY, K. F. 1982. Identification of wheat cultivars by gliadin electrophoresis: Electrophoregrams of the 88 wheat cultivars most commonly grown in the U.S. in 1979. Cereal Chem. 59:181.
KHAN, K., HAMADA, A. S., and PATEK, J. 1985. Polyacrylamide gel electrophoresis for wheat variety identification: Effect of variables on gel properties. Cereal Chem. 62:310.
LOOKHART, G. L., JONES, B. L., HALL, S. B., and FINNEY, K. F. 1982. An improved method of standardizing polyacrylamide gel electrophoresis of wheat gliadin proteins. Cereal Chem. 59:178.
LOOKHART, G. L., LAI, F. S., and POMERANZ, Y. 1985a. Variability in gliadin electrophoregrams and hardness of individual wheat kernels selected from foundation seed on the basis of grain morphology. Cereal Chem. 62:185.
LOOKHART, G. L., ALBERS, L., and POMERANZ, Y. 1985b. Stability of electrophoregrams of heat-treated oat groats. (Abstr.) Cereal Foods World 30:545.
MECHAM, D. K., KASARDA, D. D., and QUALSET, C. O. 1985. Identification of western U.S. wheat varieties by polyacrylamide gel electrophoresis of gliadin protein. Hilgardia 53:1.
TKACHUK, R., and MELLISH, J. 1980. Wheat cultivar identification by high voltage gel electrophoresis. Ann. Tech. Agric. 29:207.
WRIGLEY, C. W., and BAXTER, R. I. 1974. Identification of Australian wheat cultivars by laboratory procedures: Grain samples containing a mixture of cultivars. Aust. J. Exp. Agric. Anim. Husb. 14:805.

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