Rapid Differentiation of Oat Cultivars and of Rice Cultivars by Capillary Zone Electrophoresis[1]

GEORGE L. LOOKHART[2] and SCOTT R. BEAN[3]

Cereal Chem. 72(3):312-316

ABSTRACT

Capillary zone electrophoresis (CZE) of endosperm storage proteins was used to differentiate cultivars of both oats and rice in less than 12 min. This is the first report that proteins of these two cereals have been separated by CZE. Cultivars were chosen for the difficulty of differentiating them by other means, electrophoretic or chromatographic. Ethanol (70%) extracts of the oat samples were separated on a 20-μm i.d. untreated fused-silica capillary, whereas rice samples were extracted with 60% 1-propanol, and the solubilized proteins were separated on a 50-μm i.d. untreated fused-silica capillary. The CZE separation buffer was 0.1*M* phosphate, pH 2.5, containing 0.05% hydroxypropylmethylcellulose (HPMC). Most cultivars were differentiated quickly and easily. Only the patterns of two rice cultivars, IR36 and IR50, were nearly identical. There were no differences between IR36 and IR50 extracts by high-performance liquid chromatography (HPLC) or acid (A)-polyacrylamide gel electrophoresis (PAGE). CZE is a faster method of separating endosperm storage proteins than A-PAGE and separates as least as well and better in most cases than either A-PAGE or reversed phase (RP)-HPLC.

The ability to correctly differentiate and identify cultivars of cereal grains is an important aspect of cereal science. Polyacrylamide gel electrophoresis (PAGE) and high-performance liquid chromatography (HPLC) are the laboratory methods of choice. Gel electrophoresis methods, usually acid (A)-PAGE or sodium dodecyl sulfate (SDS)-PAGE, are the established techniques for separation of proteins. However, they have several drawbacks that include the use of toxic reagents, long analysis times, and data that are difficult to quantify and interpret. The electrophoretic methods and extraction conditions used to differentiate cultivars of all major cereal crops were recently reviewed by Lookhart (1990). Lookhart and Wrigley (1995) reviewed electrophoretic methods for varietal identification. The methods varied widely in extraction procedures, in proteins analyzed, and in the type of electrophoresis. Analysis times ranged from 1 to 12 hr (Lookhart 1990, Lookhart and Wrigley 1995).

Reversed-phase (RP)-HPLC has also been used to differentiate cereal cultivars, alone and as a complement to A-PAGE, for rice (Lookhart et al 1987, Lookhart et al 1991, Huebner et al 1991, Lookhart and Juliano 1994), oats (Lookhart 1985, Lookhart and Pomeranz 1985, Bakhella et al 1992), barley (Marchylo and Laberge 1980, Heisel et al 1986), and wheat (Bietz 1983, Lookhart et al 1986, Lookhart and Albers 1988, Bakhella et al 1991).

Recently, wheat proteins were characterized by a new technique, capillary zone electrophoresis (CZE) (Bietz and Schmalzried 1992, 1993; Bietz and Lookhart 1994; Lookhart et al 1994; Werner et al 1994; Lookhart and Bean 1995). An aluminum lactate buffer, in conjunction with capillaries coated with Microcoat (a patented coating by Perkin-Elmer that reversed the charge on the inside of the capillary), was used by Werner et al (1994) to separate gliadins. The charge reversal on the capillary walls reduced the interaction with, and improved the separation of, the positively charged gliadins in the aluminum lactate buffer. In addition, glutenins were separated by an entangled polymer, ProSort (Perkin Elmer), after extraction with SDS (Werner et al 1994). The other authors (Bietz and Schmalzried 1992, 1993; Bietz and Lookhart 1994; Lookhart et al 1994; Lookhart and Bean 1995) used a low pH (2.5) commercial (Bio-Rad) phosphate buffer to separate wheat gliadins. The method of Lookhart and Bean (1995) optimized the time, resolution, and reproducibility for the separation of those proteins. It allowed the separation of wheat gliadins in less than 10 min, while providing a relative standard deviation of 0.6% for elution times.

For uncoated capillaries, phosphate offers a distinct advantage over other buffer systems. Phosphate forms complexes with silanol groups present on the inside of silica capillaries, thus providing capillaries that have been partially deactivated (Mitsyuk 1972, McCormick 1988). As pH is decreased, the charge on the inside wall of the capillary also decreases. At pH 2.5, the charge on the capillary wall is almost zero (McCormick 1988, Zhu et al 1989, Kuhn and Hofstetter-Kuhn 1993). This reduces electroosmotic flow (EOF) (the bulk movement of solvent in a capillary toward the cathode) due to the negative charged silanol groups and reduces protein attraction to the wall. Therefore, we have tested the ability of CZE using low pH phosphate buffers to separate rice and oat prolamins. Hydroxypropylmethylcellulose (HPMC) was added to the buffer to further reduce EOF (Kuhn and Hofstetter-Kuhn 1993) and reduce attraction of proteins to the wall, which may lead to improved reproducibility. In separations that depend on EOF, minor perturbations in pH, ionic strength, and capillary-wall conditioning cause reproducibility problems. On the other hand, separations that depend on electrophoretic mobility for transport through the capillary are not subject to those variations (McCormick 1988).

While not a new technology, CZE has recently undergone considerable development and offers a rapid technique that is easily automated and requires little sample or reagents to operate (Chen 1991). CZE is thus well suited for rapid cultivar identification of rice and oats, alone and as a complement to older techniques. This study demonstrates the use of CZE for rapid separation and differentiation of rice and oat cultivars, and establishes the use of CZE as an effective technique for rapid cultivar identification.

METHODS AND MATERIALS

Oat Samples

Eight oat cultivars (Dal, Garry, Kelsey, Lang, Lodi, Nodaway, Nodaway 70, and Rodney) and two recent releases (Gopher and Troy) were obtained from breeders of five major oat-producing states (Lookhart 1985). A-PAGE was used to segregate the 23 major U.S. oat cultivars into three groups with identical electro-

[1]Cooperative investigations: USDA-ARS and the Department of Grain Science and Industry, Kansas State University. Contribution 95-204-J, Department of Grain Science and Industry, Kansas State Agricultural Experiment Station, Manhattan. Mention of firm names or trade products does not constitute endorsement by the U.S. Department of Agriculture over others not mentioned.

[2]Research chemist, USDA-ARS, Grain Marketing Research Laboratory, Manhattan, KS.

[3]Research assistant, Department of Grain Science and Industry, Kansas State University, Manhattan.

phoretic patterns (group 1: Benson, Dal, Garry, Lodi, Lyon, Moore, and Wright; group 2: Harmon, Kelsey, Lang and Rodney; and group 3: Nodaway and Nodaway 70) and 10 cultivars with unique A-PAGE patterns. RP-HPLC subdivided group 1 into two groups of similar patterns: group 1A (Benson, Dal, Lyon, and Wright) and group 1B (Garry, Lodi, and Moore). The cultivars chosen as examples for CZE differentiation were three cultivars (Dal, Garry, and Lodi) from groups 1A and 1B; three cultivars (Kelsey, Lang, and Rodney) from group 2; both cultivars in group 3; and the two new releases (Gopher and Troy).

Rice Samples

The rice cultivars tested for CZE differentiation were six rice lines from B. O. Juliano (International Rice Research Institute [IRRI], Philippines) and five U.S. rice cultivars from Bill Webb (USDA Rice Quality Lab, Beaumont, TX). Those cultivars were previously analyzed by HPLC (Lookhart et al 1991). The IRRI lines included IR28, IR30, IR36, IR42, IR50, and IR62. The U.S. rice cultivars consisted of three long-grain cultivars (Lemont, L202, and Newbonnet); a medium-grain cultivar (Mars); and a short-grain cultivar (S201).

Extraction of Avenins

Avenins were extracted from crushed whole oats by 70% ethanol as previously described for wheat prolamins (Lookhart et al 1986) or ground oats (Lookhart 1985). All samples were analyzed within 24 hr of extraction. Extracts were stirred constantly with micro stir bars (3 × 10 mm) on a Corning (PC-353) stirrer plate.

Extraction of Rice Prolamins

Ground rice or rice flour (200 mg) was placed in 3-dram (12-ml) glass vials (Kimble 60975-L) and extracted with 60% 1-propanol (2.5 × weight) for 1 hr at room temperature. This method was similar to that reported by Lookhart et al (1991) for HPLC analysis of rice. Minor peak pattern variations were found when the sample was not continuously stirred during extraction with 60% 1-propanol. Other alcohol solvents (ethanol, methanol, and 2-propanol) and percentages (30–70%) caused minor CZE peak pattern variations.

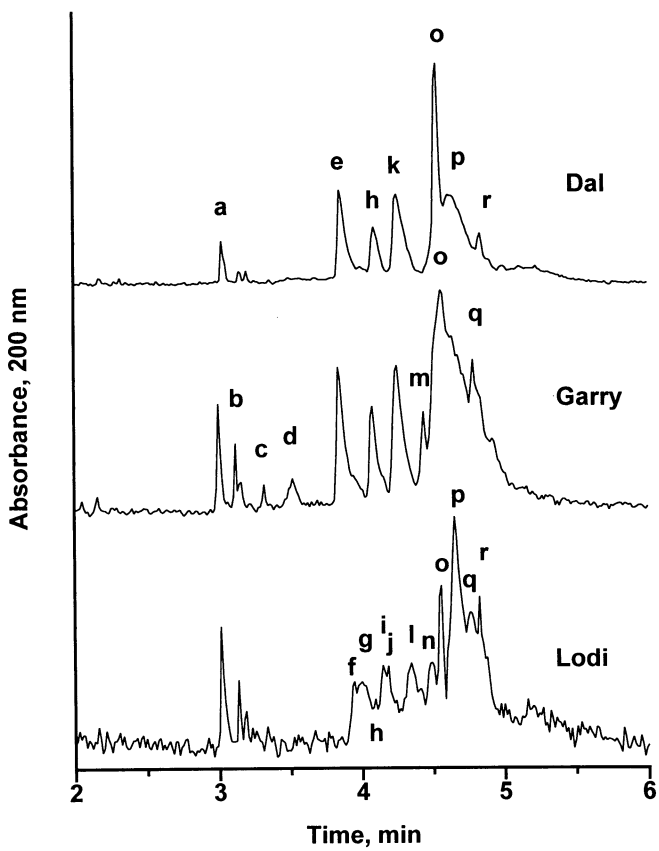

Fig. 1. Capillary zone electrophoresis patterns of 70% ethanol extracts (avenins) of oat cultivars Dal, Garry, and Lodi, whose polyacrylamide gel electrophoresis and high-performance liquid chromatography patterns were nearly identical. Extracts were pressure-injected (8 sec) into a 20-μm i.d. fused-silica capillary and separated at 45°C and 22 kV using 0.1*M* phosphate buffer (pH 2.5) containing 0.05% hydroxypropylmethylcellulose. Capillary was 27-cm long (20-cm, inlet to detector). Detection wavelength was 200 nm.

Capillary Zone Electrophoresis

A Beckman P/ACE 2100 system was used to characterize all extracts. Capillary cleaning protocols were identical to those reported by Lookhart and Bean (1995) for wheat, except that phosphoric acid (1.0*M*) was substituted for nitric acid. All capillaries used in this experiment were uncoated fused-silica and were 27-cm long (20 cm, inlet to detector). A 0.1*M* phosphate buffer at pH 2.5 (1.839 ml of concentrated phosphoric acid and 8.47 g of sodium monobasic phosphate [MW = 120] to 1L) containing 0.05% HPMC (Sigma Chemical, Co., H-7509, viscosity of 2% solution 4,000 cp) (0.500 g/L) was used for all separations. It was as reproducible as the similar commercial phosphate buffer from Bio-Rad. Detection was by absorbance at 200 nm. The total run time including analysis, washing, and equilibration was 24 min.

Oats

All avenin separations were conducted in a 20-μm i.d. untreated fused-silica capillary (27-cm long; 20 cm, inlet to detector). The separation conditions for avenins were the same as those used by Lookhart and Bean (1995) for wheat gliadins (45°C and 22 kV), except that avenins were loaded by pressure injection for 8 sec (0.504 nl, Lookhart and Bean 1995).

Rice

Rice prolamins were separated using a 50-μm i.d. fused-silica capillary. Rice extracts were pressure-injected for 5 sec (12.2 nl) (Lookhart and Bean 1995) and separated at 40°C and 10 kV. The larger capillary i.d. permitted use of larger volumes of extract (12.2 nl), necessary because of the limited amount of prolamin in rice (<3%) (Lookhart 1990).

RESULTS AND DISCUSSION

Oats

Excellent separations of oat storage proteins were achieved by CZE (Fig. 1). At least 12 peaks were found in the CZE patterns of the alcohol extracts of cultivars Garry and Lodi. Using the same extraction conditions, the storage proteins of oat cultivars Garry and Lodi exhibited 11 peaks and 4 bands by RP-HPLC and A-PAGE, respectively (Lookhart 1985). Clearly, CZE has an advantage over A-PAGE in speed and resolution. CZE and RP-HPLC techniques are similar in automation capabilities but differ slightly in analysis time and resolution. CZE and RP-HPLC techniques are complementary, since free-zone CZE separates on the basis of charge (like A-PAGE), whereas RP-HPLC separates on the basis of hydrophobicity.

Protein peaks eluting in the 3–4 min range using a 20-μm i.d. capillary from the oat extracts may be albumins or globulins, since Lookhart and Bean (1995) found that 30% ethanol extracts of ground wheat exhibited albumins and globulins eluting at 3–4 min. The difference in selectivity between 30% ethanol and 70% ethanol, for water or salt soluble proteins, would result in more albumins and globulins extracted in 30% ethanol than in 70% ethanol. And, since up to 80% of oat proteins are albumins and globulins (Lookhart 1990), compared to 15% in wheat, some oat albumins and globulins would be extracted. The HPLC patterns of the prolamin extracts (with the same solvent) would also be expected to contain the same amount of albumins and globulins, and those patterns have been used to diferentiate oat cultivars (Lookhart 1985). Therefore, the CZE patterns, with the ability to separate the albumins and globulins from the prolamins, are even more powerful for cultivar differentiation.

Reproducibility of replicated (5×) CZE patterns of avenins extracted from the cultivar Garry showed relative standard devia-

tions in retention time of 0.6% (data not shown). This is similar to that for wheat gliadins (0.6%) (Lookhart and Bean 1995). CZE patterns of avenin extracts of Dal, Garry, and Lodi are shown in Figure 1. The CZE patterns of Dal and Garry each exhibited three large peaks (Fig. 1e, h, and k) between 3.9 and 4.5 min that were either minor peaks or nonexistent in the pattern of the Lodi extract. The Dal extract pattern contained a large sharp peak (o) at 4.6 min that appeared as a large, blunt peak in the Garry pattern. The Lodi pattern contained a peak at 4.5 min (n) not found in either the Dal or Garry pattern. As a result, Dal, Garry, and Lodi were readily differentiated by CZE (Fig. 1). The CZE patterns of Dal Garry, and Lodi also exhibited several minor differences (peaks a, b, c, and d) in the 3–4 min range. Those minor and major changes were readily seen, even though their RP-HPLC patterns were nearly identical (Lookhart 1985).

Kelsey, Lang, and Rodney (another set of oat cultivars with nearly identical HPLC patterns) (Lookhart 1985) were also successfully differentiated by CZE of their avenins (Fig. 2). Kelsey and Rodney both contained a large sharp peak (o) at 4.6 min that appeared as a smaller peak in the Lang CZE pattern. The CZE avenin pattern of Rodney contained a large peak (i) at 4.1 min that was only a small peak in Lang and almost nonexistent in Kelsey. The CZE patterns of Kelsey, Lang, and Rodney were missing the 3.8 min (e) peak of Dal and Garry (Fig. 1), as well as the f and g peaks of Lodi, and were thereby differentiated from them as well.

Avenin CZE patterns (Fig. 3) of Nodaway and Nodaway 70 were differentiated from each other by several peaks between 3.5 and 5 min. The Nodaway CZE pattern contained peaks g, h, k, and o that were much larger than in the Nodaway 70 pattern. On the other hand, Nodaway 70 contained a larger peak at 4.5 min (n) than did Nodaway. Other minor differences were also readily apparent. The CZE patterns of Nodaway or Nodaway 70 contain peak n not found in patterns of Dal, Garry, Kelsey,

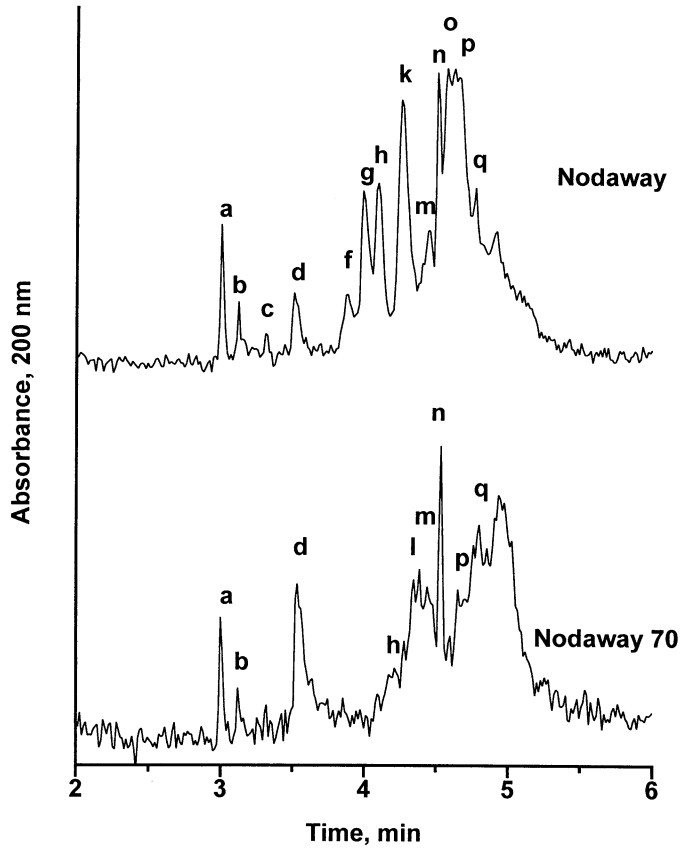

Fig. 3. Capillary zone electrophoresis patterns of 70% ethanol extracts (avenins) of oat cultivars Nodaway and Nodaway 70, whose polyacrylamide gel electrophoresis and high-performance liquid chromatography patterns were nearly identical. Extraction conditions as given in Fig. 1.

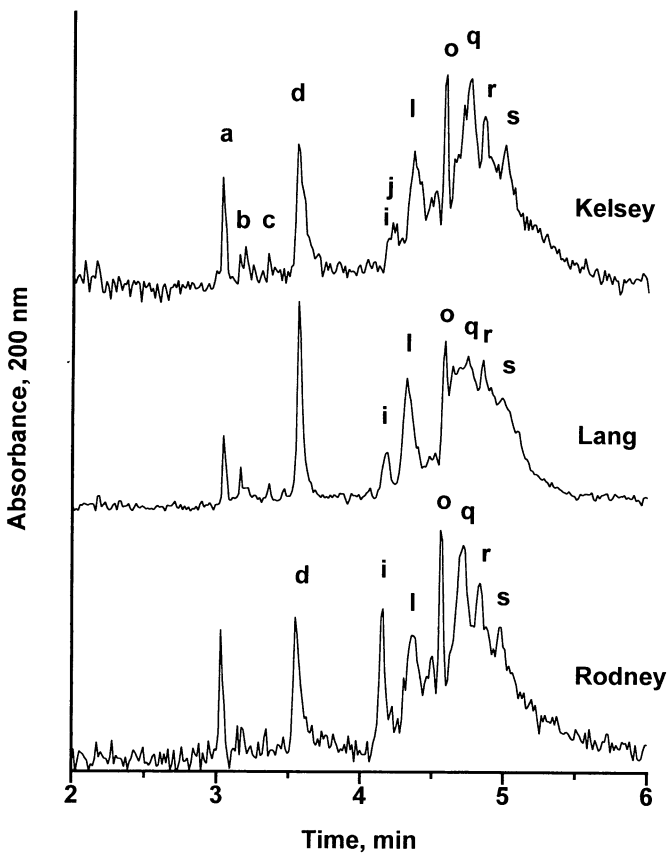

Fig. 2. Capillary zone electrophoresis patterns of 70% ethanol extracts (avenins) of oat cultivars Kelsey, Lang, and Rodney, whose polyacrylamide gel electrophoresis and high-performance liquid chromatography patterns were nearly identical. Extraction conditions as given in Fig. 1.

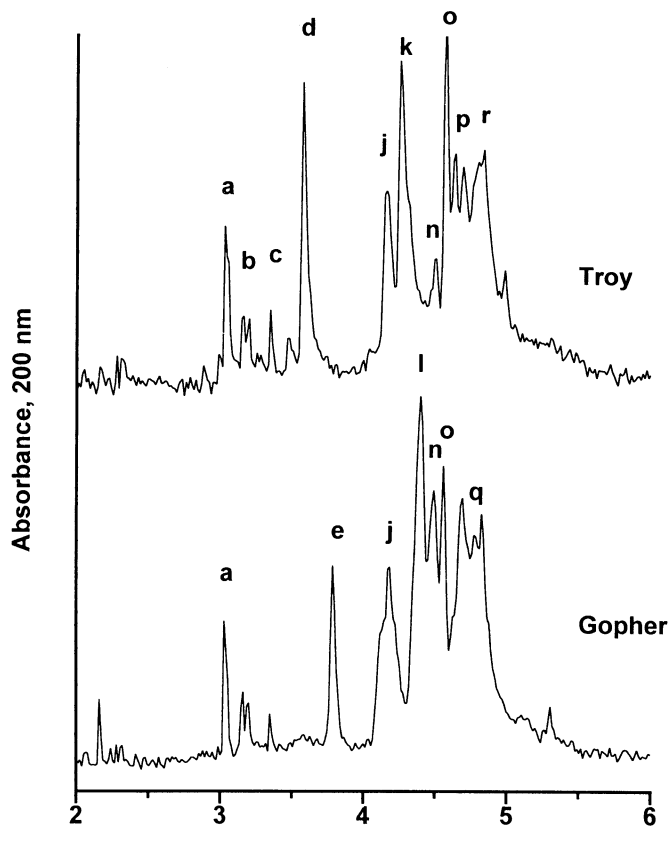

Fig. 4. Capillary zone electrophoresis patterns of 70% ethanol extracts (avenins) of oat cultivars Gopher and Troy. Extraction conditions as given in Fig. 1.

Lang, or Rodney and also have peak d not found in Lodi. HPLC and A-PAGE patterns of those eight cultivars were reported by Lookhart (1985).

The CZE patterns of Troy and Gopher (Fig. 4) were easily differentiated from each other, the major differences being the presence of peaks d and k in Troy (absent in Gopher) and peaks e and l in Gopher (absent in Troy). Several other qualitative and quantitative differences also exist. The CZE patterns of Troy and Gopher were different from the patterns of the other oat cultivars examined; peak j is a medium size peak in both Troy and Gopher and found only in Kelsey, Lodi, Nodaway 70, and Rodney as a very minor peak. The e, j peak combination was found only in Gopher, whereas peaks d, j, k were found only in Troy.

Rice

Good-to-excellent separations of rice storage proteins were achieved by CZE (Fig. 5). The average number of peaks exhibited by cultivars in Figure 5 by CZE and in a previous report by RP-HPLC (Lookhart et al 1991) on similarly extracted rice flours were 10 and 6, respectively. Clearly, CZE patterns exhibit more peaks than noted by RP-HPLC. The peaks eluting between 4 and 5 min in the 50-μm capillary may be albumins and globulins that are partially soluble in the 60% 1-propanol extraction solvent, since Lookhart and Bean (1995) found that 30% ethanol extracts of ground wheat exhibited albumins and globulins eluting at 3–4 min using a 20-μm i.d. capillary. The small difference in selectivity between 60% 1-propanol and 70% ethanol, for water or salt soluble proteins, would result in similar amounts of albumins and globulins extracted, especially since similar amounts (15%) of albumins and globulins are present in wheat and rice (Lookhart 1990). The HPLC patterns of the prolamin extracts (with the same solvent) would also be expected to contain the same amount of albumins and globulins, and those patterns have been used to differentiate rice cultivars (Lookhart et al 1987, 1991). Therefore, the CZE method with the ability to separate the albumins and globulins from the prolamins is even more powerful for cultivar differentiation. CZE is complementary to RP-HPLC because of differences in separation modes, but exhibits faster analysis times with similar reproducilibity and ease of automation.

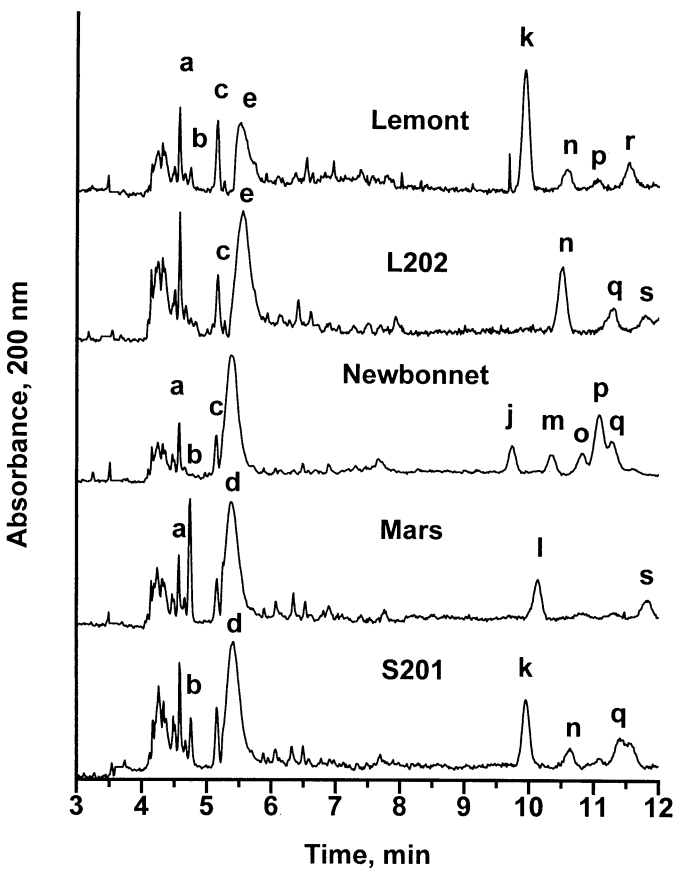

Fig. 5. Capillary zone electrophoresis patterns of 60% 1-propanol extracts (prolamins) of U.S. long-grain rice cultivars Lemont, L202, and Newbonnet, which have nearly identical polyacrylamide gel electrophoresis and high-performance liquid chromatography patterns, and of medium-grain rice cultivar Mars, and of short-grain rice cultivar S201. Extracts were pressure-injected (5 sec) into a 50-μm i.d. fused-silica capillary and separated at 40°C and 10 kV using 0.1*M* phosphate buffer (pH 2.5) containing 0.05% hydroxypropylmethylcellulose. Capillary was 27-cm long (20 cm, inlet to detector). Detection wavelength was 200 nm.

Reproducibility of replicated CZE rice prolamin patterns established relative standard deviations in elution time (0.4%) similar to that of the relative standard deviation of elution time reproducibility of the oat avenins (cited above) and the wheat gliadins (0.6%, Lookhart and Bean 1995). The U.S. long-grain rice cultivars Lemont, L202, and Newbonnet exhibited nearly identical HPLC patterns (Lookhart et al 1991); HPLC patterns of Mars and S201 were different from each other and from the long-grain cultivars. CZE prolamin patterns of these U.S. rice cultivars (Fig. 5) showed consistent and major differences. The prolamin patterns of the individual cultivars of U.S. rice samples examined in this study from five environments were conserved by HPLC (Lookhart et al 1991) and by CZE (data not shown). The HPLC patterns of L202 and Newbonnet were identical but several differences in their CZE patterns were found; the Newbonnet CZE pattern included peaks d and m not found in the L202 pattern and the L202 CZE pattern exhibited large peaks e and n not found in the Newbonnet pattern. The presence of peak k in the CZE pattern of Lemont differentiated it from the other long-grain rices, since k was not found in the prolamin patterns of the other long-grain cultivars. Many other minor differences were also apparent among these cultivars. The prolamin CZE pattern of Mars is distinguished from the others by the presence of a large peak b and the peak l that were not found in the CZE patterns of the other U.S. rice cultivars examined. For comparison of HPLC peaks or patterns, see Lookhart et al (1991).

Rice cultivars from the Philippines (IR28, IR30, IR36, IR42, IR50, and IR62) were also readily differentiated by their CZE prolamin patterns (Fig. 6). All IR lines exhibited a large peak a. However, peak b was found only in CZE patterns of IR30 and IR62, which were differentiated from each other by the presence of peak d (present in IR30 but not in IR62). CZE peak k was found only in lines IR28 and IR42, which were differentiated from each other by the presence of peaks h and q in IR42. The CZE patterns of IR36 and IR50 were nearly identical. Their patterns differed only in the relative intensity of peak x at 4.1 min. Closely related lines usually exhibit similar patterns (Lookhart et al 1987). IR36 is the male parent of IR50 (Lookhart et al 1991) and their HPLC patterns of 60% 1-propanol extracts were also identical. However, IR36 was a sister line of IR42 and their 60% 1-propanol extracted protein patterns by CZE and HPLC were quite different. The HPLC patterns of the IRRI rice cultivars were reported previously (Lookhart et al 1991, Huebner et al 1991).

CONCLUSIONS

In this study, CZE was successfully used for the first time to differentiate oat cultivars and rice cultivars. Although RP-HPLC was able to distinguish oat cultivars with identical A-PAGE patterns, the different separation modes enabled more differences to be detected with CZE than with RP-HPLC. In addition, CZE analysis required considerably less time (24 min) to analyze the storage proteins than A-PAGE (2–4 hr) and were consistently shorter than RP-HPLC analysis times (30–60 min), even when equilibration was considered. CZE is complementary to RP-HPLC, where it has the advantage of speed and resolution with the similarity of ease of automation, and differs in mode of separation, charge versus hydrophobicity. Oat avenins were separated in less than 6 min by free-zone capillary electrophoresis using a low pH phosphate buffer. CZE pattern differences allowed

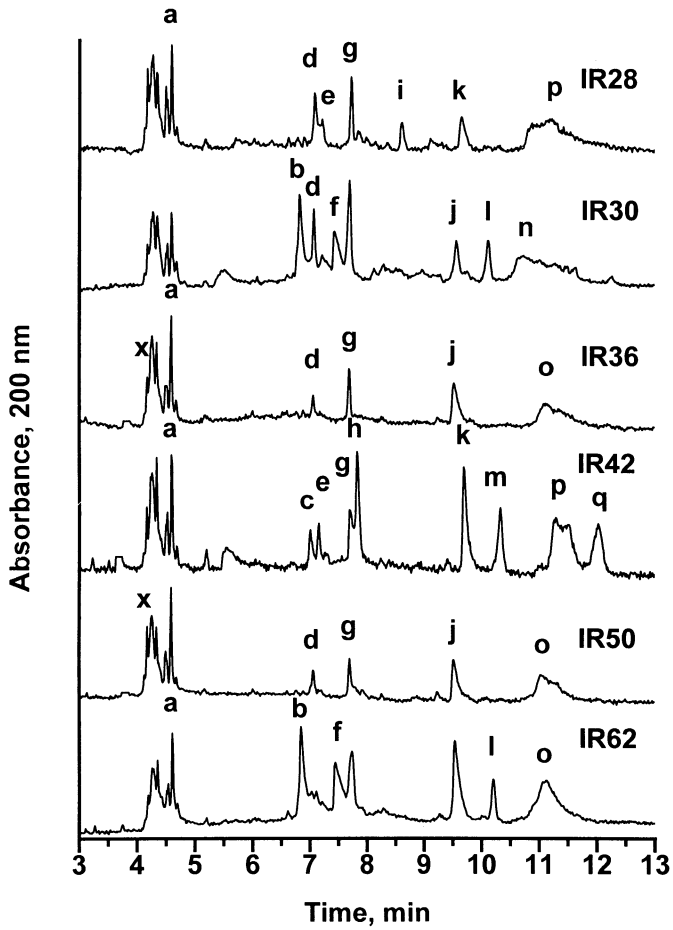

Fig. 6. Capillary zone electrophoresis patterns of 60% 1-propanol extracts (prolamins) of rice cultivars IR28, IR30, IR36, IR42, IR50, and IR62. Extracts were pressure-injected (5 sec) into a 50-μm i.d. fused-silica capillary and separated at 40°C and 10 kV using 0.1*M* phosphate buffer (pH 2.5) containing 0.05% hydroxypropylmethylcellulose. Capillary was 27-cm long (20 cm, inlet to detector). Detection wavelength was 200 nm.

differentiation of all oat cultivars studied. Rice cultivars, both from the U.S. and from the IRRI, were consistently differentiated by CZE in less than 12 min. U.S. long-grain cultivars L202 and Newbonnet, which had identical HPLC patterns, were also readily differentiated by CZE. Cultivars with close genetic relationships may exhibit similar or identical prolamin patterns by CZE, as they do by A-PAGE or RP-HPLC.

The fast speed, high resolution, and complementary nature of CZE make it well suited to quickly differentiate oat and rice cultivars, even those with similar or identical A-PAGE and RP-HPLC patterns.

LITERATURE CITED

BAKHELLA, M., LOOKHART, G. L., and HOSENEY, R. C. 1991. Identification of Moroccan wheats by reversed-phase high performance liquid chromatography and electrophoretic procedures. Actes Inst. Agron. Vet. (Maroc) 11:5-19.

BAKHELLA, M., LOOKHART, G. L., and HOSENEY, R. C. 1992. Identification of Moroccan oat cultivars by electrophoresis of their prolamins and total proteins. Actes Inst. Agron. Vet. (Maroc) 12:19-28.

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

BIETZ, J. A., and LOOKHART, G. L. 1994. Wheat varietal identification by capillary electrophoresis: An inter-laboratory comparison of methods. (Abstr.) Cereal Foods World 39:603.

BIETZ, J. A., and SCHMALZRIED, E. 1992. Capillary electrophoresis of wheat proteins: Optimization and use for varietal identification. (Abstr.) Cereal Foods World 37:555.

BIETZ, J. A., and SCHMALZRIED, E. 1993. Improved wheat varietal identification by acidic capillary electrophoresis. (Abstr.) Cereal Foods World 38:615.

CHEN, F. T. 1991. Rapid protein analysis by capillary electrophoresis. J. Chromatogr. 559:445-543.

HEISEL, S. E., PETERSON, D. M., and JONES, B. L. 1986. Identification of United States barley cultivars by sodium dodecyl sulfate polyacrylamide gel electrophoresis of hordeins. Cereal Chem. 63:500-505.

HUEBNER, F. R., HUSSAIN, A., LOOKHART, G. L., BIETZ, J. A., BUSHUK, W. A., and JULIANO, B. O. 1991. Discrimination of sister-line IR rice varieties by polyacrylamide gel electrophoresis and reversed-phase high-performance liquid chromatography. Cereal Chem. 68:583-588.

KUHN, R., and HOFFSTETTER-KUHN, S. 1993. Capillary Electrophoresis: Principles and Practice. Springer-Verlag: Berlin.

LOOKHART, G. L. 1985. Identification of oat cultivars by combining polyacrylamide gel electrophoresis and reversed-phase high-performance liquid chromatography. Cereal Chem 62:345-350.

LOOKHART, G. L. 1990. Cereal proteins: Composition of their major fractions and methods for identification. Pages 441-468 in: Handbook of Cereal Science and Technology. K. J. Lorenz and K. Kulp, eds. Dekker: New York.

LOOKHART, G. L., and ALBERS, L. D. 1988. Correlations between reversed-phase high-performance liquid chromatography and acid- and sodium dodecyl sulfate-polyacrylamide gel electrophoretic data on prolamins from wheat sister lines differing widely in baking quality. Cereal Chem. 65:222-227.

LOOKHART, G. L., and BEAN, S. R. 1995. A fast method for wheat cultivar differentiation using capillary zone electrophoresis. Cereal Chem. 72:42-47

LOOKHART, G. L., and JULIANO, B. O. 1994. RP-HPLC for varietal identification in cereals and legumes: Rice. Pages 175-184 in: High-Performance Liquid Chromatography of Cereal and Legume Proteins. J. E. Kruger and J. A. Bietz, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

LOOKHART, G. L., and POMERANZ, Y. 1985. Characterization of oat species by polyacrylamide gel electrophoresis and high-performance liquid chromatography of their prolamin proteins. Cereal Chem. 62:162-166.

LOOKHART, G. L., and WRIGLEY, C. W. 1995. Variety identification by electrophoretic analyses. Page 55 in: Identification of Food Grain Varieties. C. W. Wrigley, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

LOOKHART, G. L., ALBERS, L. D., and BIETZ, J. A. 1986. Comparison of polyacrylamide gel electrophoresis and high-performance liquid chromatography analyses of gliadin polymorphism in the wheat cultivar Newton. Cereal Chem. 63:497-500.

LOOKHART, G. L., ALBERS, L. D., POMERANZ, Y., and WEBB, B. D. 1987. Identification of U.S. rice cultivars by high-performance liquid chromatography. Cereal Chem. 64:199-206.

LOOKHART, G. L., JULIANO, B. O., and WEBB, B. D. 1991. Effect of solvent extraction, environment, and genetic background on differentiating rice by reversed-phase high-performance liquid chromatography. Cereal Chem. 68:396-400.

LOOKHART, G. L., BEAN, S. R., and AKKINA, S. K. 1994. High-performance capillary electrophoresis optimization for wheat gliadin analyses and comparison of individual protein peak positions to high performance liquid chromatography. Cereal Foods World 39:627.

MARCHYLO, B. A., and LABERGE, D. E. 1980. Barley cultivar identification by electrophoretic analysis of hordein proteins. Extraction and separation of hordein and environmental effects. Can. J. Plant Sci. 60:1343-1350.

McCORMICK, R. M. 1988. Capillary zone electrophoretic separation of peptides and proteins using low pH buffers in modified silica capillaries. Anal. Chem. 60:2322-2328.

MITSYUK, B. M. 1972. Mechanism of the reaction of silica with phosphoric acid in aqueous solutions. Russian J. Inorganic Chem. 17:471-473.

WERNER, W. E., WICTOROWICZ, J. E., and KASARDA, D. D. 1994. Wheat varietal identification by capillary electrophoresis: Gliadin and high molecular weight glutenin analyses. Cereal Chem. 71:397.

ZHU, M., HANSEN, D. L., BURD, S., and GANNON, F. 1989. Factors affecting free zone electrophoresis and isoelectric focusing in capillary electrophoresis. J. Chromatogr. 480:311-319.

[Received November 2, 1994. Accepted January 24, 1995.]