Identification of Oat Cultivars by Combining Polyacrylamide Gel Electrophoresis and Reversed-Phase High-Performance Liquid Chromatography[1]

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ABSTRACT

Prolamins were extracted from 23 United States oat cultivars consisting of the top seven commercially grown varieties from each of the five major oat-producing states (Iowa, Minnesota, North Dakota, South Dakota, and Wisconsin). Ten of these cultivars were readily differentiated by polyacrylamide gel electrophoresis of their prolamins. The other 13 cultivars segregated into three groups: the members of each group had identical electrophoregrams and generally had very similar pedigrees. Prolamin extracts of the cultivars from each group were also subjected to reversed-phase high-performance liquid chromatography. Differences in the resulting chromatograms allowed differentiation of all members in each group.

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The most important decision of farmers in buying seed is selection of the appropriate cultivar. That decision is based largely on economic factors; the return the farmer receives on his investment is a function of yield, maturity, disease resistance, lodging, and other characteristics. Cultivar purity is also important. To ensure purity within a lot, certification programs have been established to maintain and make available high-quality, genetically pure seed. Morphological characteristics such as seed size, color, rachilla shape, and presence or absence of awns were first used to differentiate some oat (*Avena sativa*) cultivars (Hervey-Murray 1970, Baum and Thompson 1976). However, some of these traits are influenced by environment, and many new cultivars are phenotypically similar and closely related.

The nearly identical morphological characteristics of some genotypes have led to the development of sophisticated technologies for cultivar identification. Laboratory tests utilizing polyacrylamide gel electrophoresis (PAGE) and high-performance liquid chromatography (HPLC) to identify cereal cultivars have been reported. Some of these tests are both independent of the environment (Zillman and Bushuk 1979) and sensitive to genetic background (Zillman and Bushuk 1979, Bietz 1983, Robert et al 1983b). Some oat cultivars have been identified by isoelectric focusing or electrophoresis of various protein fractions including prolamins (Lauriere and Mosse 1982, Robert et al 1983b), globulins (Robert et al 1983a, Burgess et al 1983), and isozymes (Williamson et al 1968, Murray et al 1970, Singh et al 1973, McDonald 1982). The purpose of this study was to develop a quick and reliable procedure to uniquely identify the major United States oat cultivars by combining two powerful methods, PAGE and HPLC.

[1]Mention of firm names or trade products does not constitute endorsement by the USDA over others not mentioned.

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MATERIALS AND METHODS

Chemicals and Reagents

Acrylamide, *N,N'*-methylene-bis-acrylamide, ascorbic acid, Coomassie Brilliant Blue R-250, methyl green, trichloroacetic acid (TCA), and trifluoroacetic acid (TFA) were from Sigma Chemical Company, St. Louis, MO; lactic acid (USP grade) and ferrous sulfate heptahydrate (AR grade) from Mallinckrodt Chemicals, St. Louis, MO; and LC-grade ethanol and acetonitrile from Burdick and Jackson Laboratories, Muskegon, MI. Hydrogen peroxide (3% practical grade) was purchased in small plastic bottles from a local pharmacy. Water was purified by passage, in series, through a membrane filter, a charcoal filter, and two mixed-bed ion-exchange filters. Aluminum lactate was from K & K Laboratories, Plainview, NY.

Oat Samples

Oat breeders (see acknowledgment) at state universities of the five major oat-producing states in the United States (Minnesota, South Dakota, North Dakota, Wisconsin, and Iowa) were polled, and they furnished appropriate samples of the top seven oat cultivars in each state (1982 crop, Table I). Twenty-three cultivars, each from two or more sources, were examined. Each cultivar was grown on at least 10,000 acres.

Samples Preparation

Oat samples (Table I) were dehulled, ground, and extracted as described for wheat (Lookhart et al 1982).

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Electrophoresis apparatus, power supply, and staining and destaining procedures have been described previously (Lookhart et al 1982). An EC-490 gel former (24 × 17 cm) that accommodated 24 samples was used in addition to the EC-470 gel former (12 × 17 cm) normally employed. The gel acrylamide concentration (7.5%), temperature (10° C), and electrophoresis time (5 hr) were modified to optimize separations.

TABLE I
Oat Samples (*Avena sativa*)

Oat Cultivars	States of Commercial Production (<10,000 Acres/State in 1982)
Benson	MN
Burnett	SD
Chief	SD, MN, IA
Dal	WI
Froker	WI, SD, ND, MN
Garry	WI, ND
Harmon	ND
Hudson	ND
Kelsey	ND, MN
Lancer	SD, MN
Lang	IA
Lodi	WI, ND, MN
Lyon	WI, SD, ND, MN
Moore	MN
Multiline E	SD, MN, IA
Noble	WI, SD, MN, IA
Nodaway	IA
Nodaway 70	SD
Otana	ND
Otee	IA
Rodney	WI, SD, ND, MN
Stout	WI, IA
Wright	WI, SD

TABLE II
Gradient Program for Separating Oat Cultivar Prolamins by High-Performance Liquid Chromatography[a]

Solvent	Time (min) 0	20	50	55	57
%A[b]	20	40	47	60	20
%B[c]	80	60	53	40	80

[a] Using SynChropak RP-P column 25 cm × 4.1 mm.
[b] Solvent A = 80% acetonitrile/20% water plus 0.1% trifluoroacetic acid.
[c] Solvent B = 20% acetonitrile/80% water plus 0.1% trifluoroacetic acid.

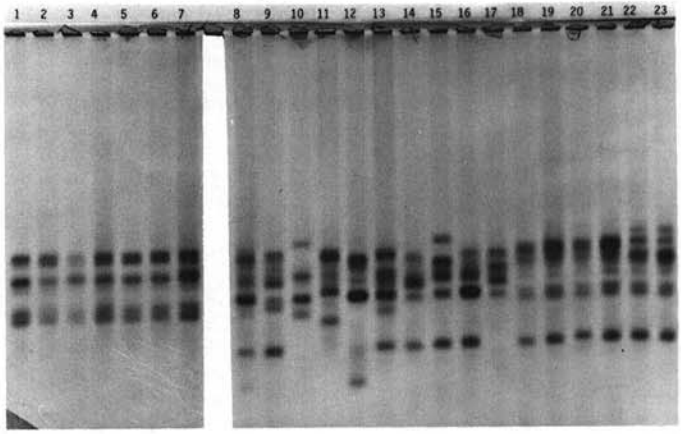

Fig. 1. Avenin polyacrylamide gel electrophoresis patterns of 23 commercially grown oat cultivars: 1–23 are Benson, Dal, Garry, Lodi, Lyon, Moore, Wright, Burnett, Chief, Froker, Hudson, Lancer, Multiline E, Noble, Otana, Otee, Stout, Harmon, Kelsey, Lang, Rodney, Nodaway, and Nodaway 70 (analyzed on the EC-490 system).

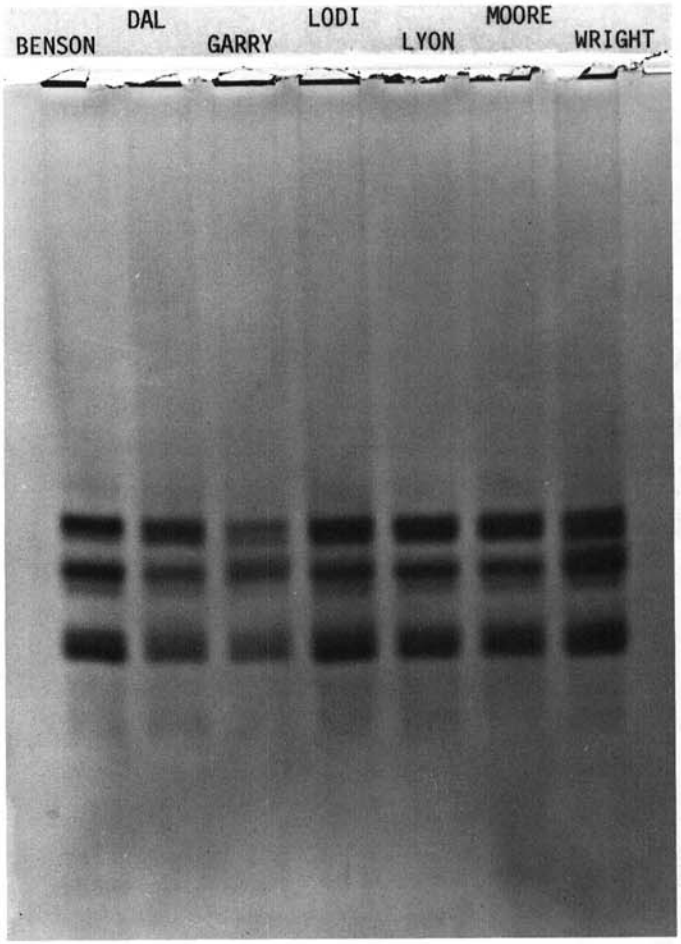

Fig. 2. Avenin polyacrylamide gel electrophoresis patterns of Benson, Dal, Garry, Lodi, Lyon, Moore, and Wright oat cultivars (group 1, EC-470 system).

HPLC

A modification to the HPLC procedure for wheat reported by Bietz (1983) was used. The HPLC system was composed of a Varian Associates (Walnut Creek, CA) model 5060 microprocessor controlled pump, a Rheodyne 7125 manual injection valve (50-μl loop), a Tracor model 970 variable wavelength detector (set at 210 nm), a SynChrom, Inc. (Linden, IN) SynChropak RP-P (C_{18}) 6.5-μm particle column (250 × 4.1 mm id), and a Hewlett-Packard 3388 printer-plotter automation system. Injections of 20–50 μl were made (depending on protein concentration), and the prolamin peaks were eluted at room temperature (23° C), using the gradient in Table II, at 1.0 ml/min. The solvents were (A) 80% acetonitrile/20% water and (B) 80% water/20% acetonitrile, each with 0.1% TFA. All samples were extracted and analyzed at least twice.

RESULTS AND DISCUSSION

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The prolamin fraction contains only up to 15% of the total protein in *A. sativa* (Peterson and Smith 1976, Kim et al 1978). Even so, Lookhart and Pomeranz (1985) reported that PAGE and HPLC prolamin patterns can be useful to differentiate oat species.

Prolamin PAGE patterns, produced with the EC-490 gel former (24 × 17 cm), for each of the 23 major U.S. oat cultivars (Table I) are shown in Figure 1. Direct comparison of prolamin bands of all cultivars allowed segregation of the following three groups of apparently identical PAGE patterns: 1) slots 1–7, Benson, Dal, Garry, Lodi, Lyon, Moore, and Wright; 2) slots 18–21, Harmon, Kelsey, Lang, and Rodney; and 3) slots 22–23, Nodaway and Nodaway 70. PAGE patterns of Burnett, Chief, Froker, Hudson, Lancer, Multiline E, Noble, Otana, Otee, and Stout (slots 8–17) were each unique and easily identified.

Further discrimination of the three segregated groups was accomplished by electrophoresing the prolamin extract of each of the cultivars on the smaller gel former EC-470 (12 × 17 cm). The identical prolamin patterns of group 1 are shown in Figure 2, and those of groups 2 and 3 are shown in Figure 3. The smaller gel former consistently gave clearer and more highly resolved band patterns, but the need for overall comparison and grouping required the use of the large (EC-490) gel former.

HPLC Patterns

Aliquots of prolamin extracts of oat cultivars with identical electrophoretic patterns were subjected to HPLC. HPLC analysis of oat prolamins appears to produce at least three times as many bands as PAGE analysis. The avenin HPLC patterns of four different samples of the oat cultivar Moore are shown in Figure 4. The nearly identical nature of these chromatograms demonstrates the repeatability of the technique as well as the homogeneity of the Moore cultivar.

Typical HPLC patterns for Nodaway and Nodaway 70, the two group 3 cultivars which could not be differentiated by PAGE, are shown in Figure 5. Minor differences (marked with a V) in the chromatograms could differentiate cultivars from within a group. In Figure 5, Nodaway (B) and Nodaway 70 (A) were differentiated by a band at 42 min in A that was absent in B. Similar differences in HPLC patterns permitted differentiation of members of groups 1 and 2 having identical PAGE patterns (Figs. 6–8).

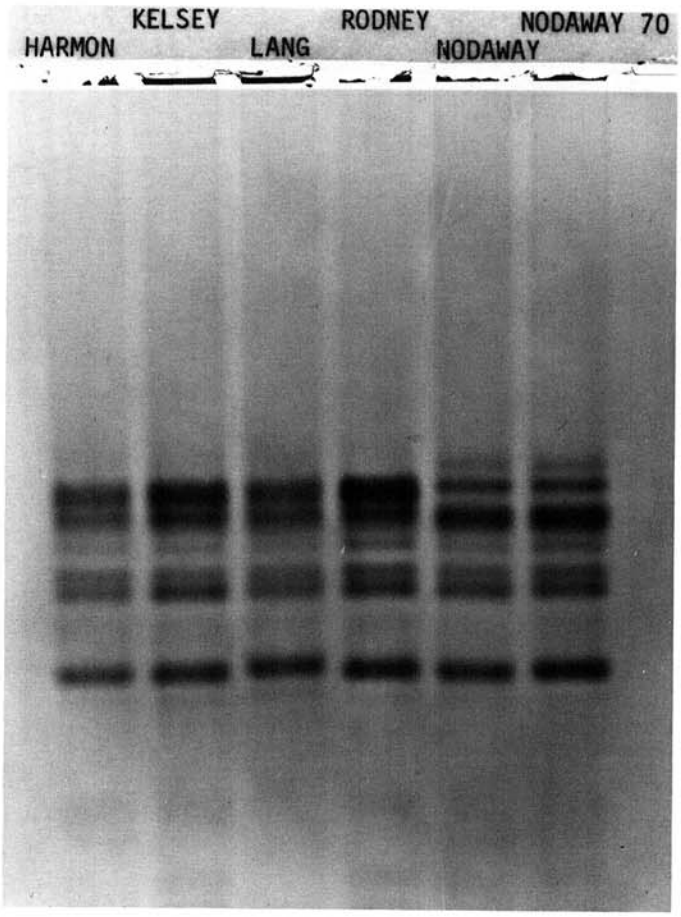

Fig. 3. Avenin polyacrylamide gel electrophoresis patterns of oat cultivars: Harmon, Kelsey, Lang, and Rodney (group 2); and Nodaway and Nodaway 70 (group 3, EC-470 system).

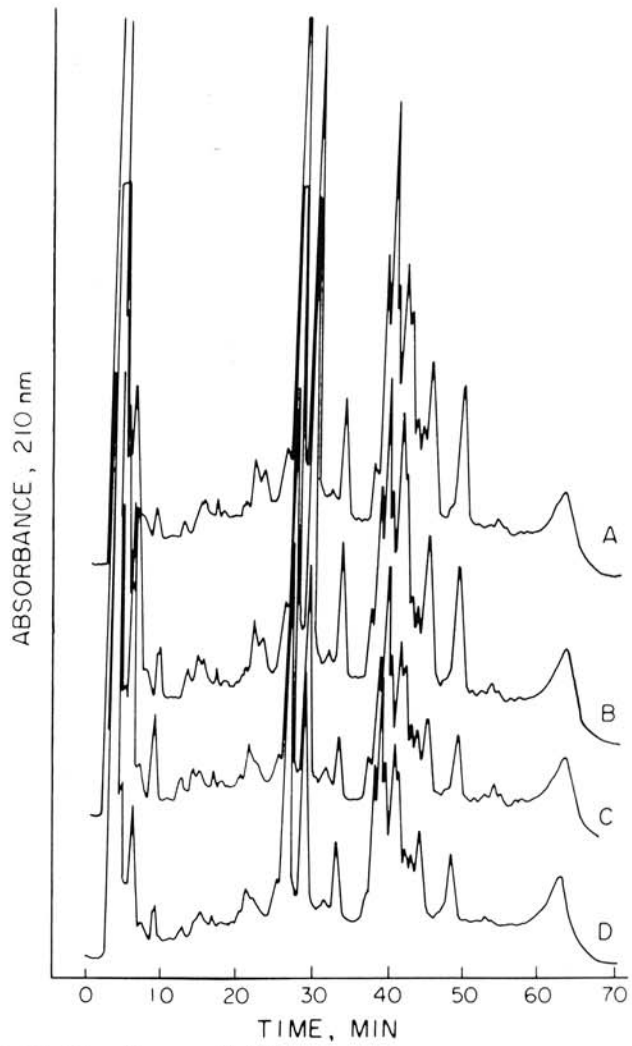

Fig. 4. High-performance liquid chromatography patterns of avenins from four accessions (**A-D**) of the oat cultivar Moore using conditions of Table II.

The group 2 varieties Rodney, Harmon, Lang, and Kelsey (Fig. 6) can be differentiated by HPLC. Rodney has a characteristic double peak at 48 min that is absent in the other varieties. Kelsey has a characteristic doublet peak at 43 min not apparent in the other varieties. Lang and Harmon are differentiated by the differences in their patterns between 11 and 15 min and 51 and 55 min.

The group 1 oat cultivars, which appeared identical by PAGE, were clearly divided into two subgroups by HPLC: Benson, Dal, Wright, and Lyon (Fig. 7) were very unlike Lodi, Moore, and Garry (Fig. 8). Lyon and Wright (Fig. 7, D and C) are easily differentiated by their band patterns in the 40–45 min range, where Lyon is missing a 42-min band, and Wright's band is more complex than the corresponding band for Benson or Dal (Fig. 7, A and B). Benson and Dal exhibit several differences in the 10–23 min range.

Garry (Fig. 8, G) differs from Lodi and Moore (E and F), which have a split peak at 40 min. Lodi and Moore are differentiated by a shoulder band three-fourths of the way up the band at 43 min, which was not seen in Moore (F) or in any of the Moore samples (Fig. 4).

Coefficients of Parentage

Coefficients of parentage (Kempthorne 1969) calculated by the method of T. S. Cox and D. M. Rodgers (*personal communication*) were determined for each pair of cultivars. The coefficient of parentage between two cultivars is defined as the probability that a random allele in one cultivar is identical by descent to a random allele at the same locus in the other cultivar. Those coefficients, computed from pedigree data, are quantitative estimates of genetic similarity. For example, the coefficient of parentage between a given homozygous cultivar and itself is 1.0, whereas those between full siblings, half-siblings, and unrelated cultivars are 0.50, 0.25, and 0, respectively.

Recently, genetic similarity coefficients were reported for 43 hard red winter wheat cultivars (Cox et al 1985a) and for modern soybean (*Glycine max* (L.) Merrill) cultivars (Cox et al 1985b). The more closely related cultivars were clustered with coefficients greater than 0.40. The average coefficient of parentage between oat cultivars exhibiting identical electrophoretic patterns was 0.32. The average coefficient between oat cultivars with different electrophoretic patterns was 0.11. Thus, similarity of electrophoregrams was associated with similarity of genetic backgrounds; the higher the coefficient of parentage between two cultivars, the more likely their patterns were to be identical. Efficiency of breeding programs could be improved by emphasizing crosses between parents with diverse patterns.

Varietal identification of cereal cultivars has been clearly demonstrated by PAGE (Zillman and Bushuk 1979, Lookhart et al 1982, Jones et al 1982, Robert et al 1983a) and HPLC (Bietz et al 1984, Marchylo and Kruger 1984). This paper presents the combined use of these powerful techniques to identify cultivars of a cereal grain. Using the two techniques together simplifies the identification process in that only visual comparisons of a small number of PAGE and/or HPLC patterns are required. The PAGE patterns identified 10 of the 23 cultivars directly and segregated the remaining 13 cultivars into three groups (of 7, 4, and 2 members each) that were easily differentiated by visual comparison of their HPLC pattern. These techniques could be useful in other cereals where a single technique does not provide satisfactory differentiation and characterization or when large numbers of samples would preclude visual comparisons.

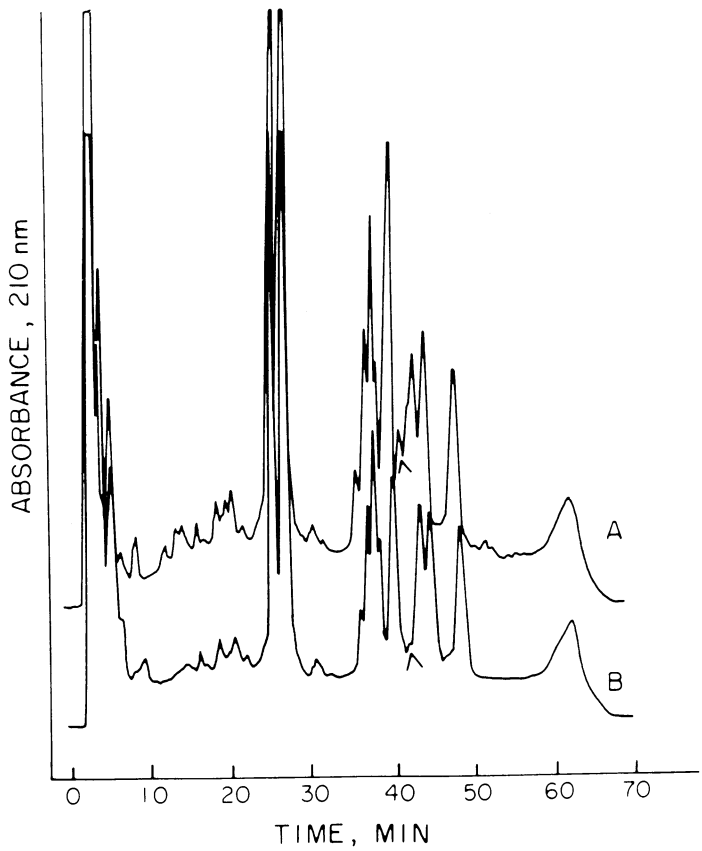

Fig. 5. High-performance liquid chromatography patterns of avenins from the oat cultivars Nodaway 70 (**A**) and Nodaway (**B**) (group 3) using conditions of Table II.

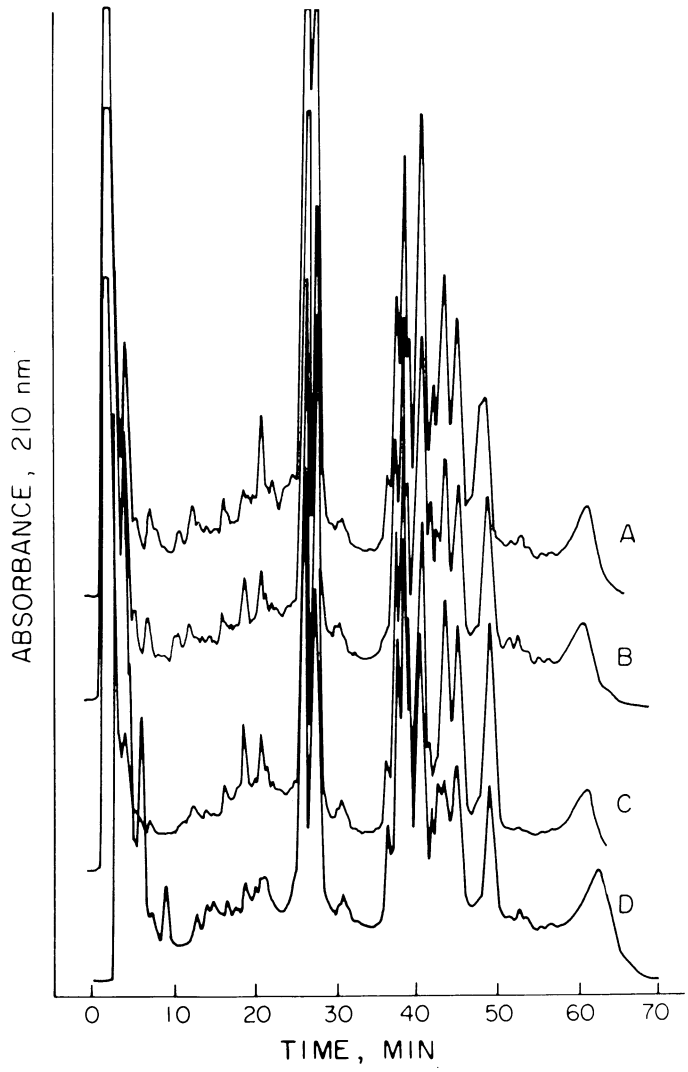

Fig. 6. High-performance liquid chromatography patterns of avenins from the group 2 oat cultivars Rodney (**A**), Harmon (**B**), Lang (**C**), and Kelsey (**D**) using conditions of Table II.

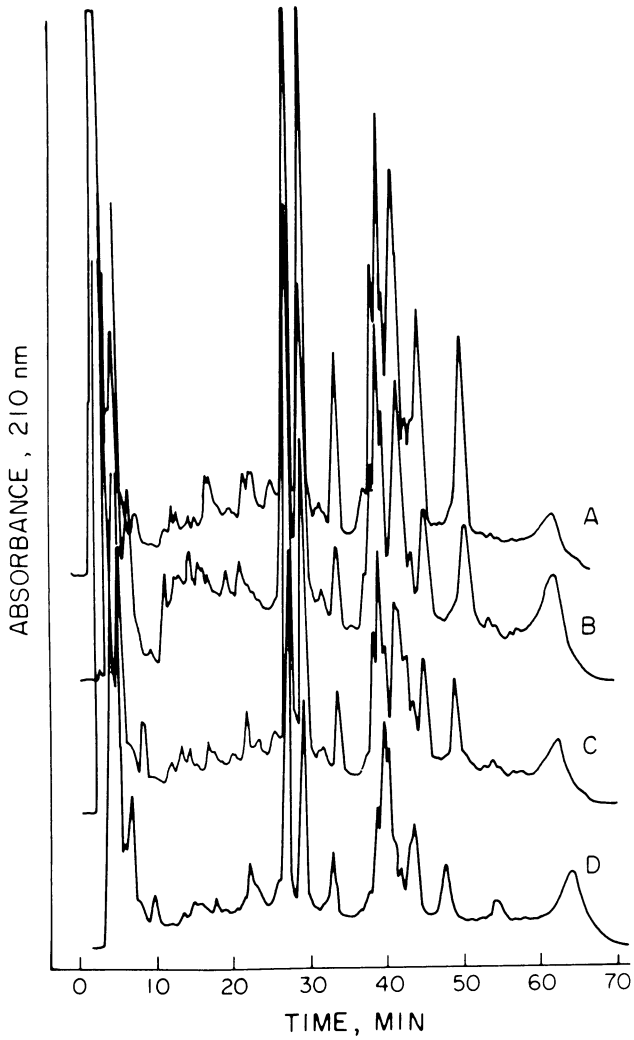

Fig. 7. High-performance liquid chromatography patterns of avenins from the group 1 oat cultivars Benson (**A**), Dal (**B**), Wright (**C**), and Lyon (**D**) using conditions of Table II.

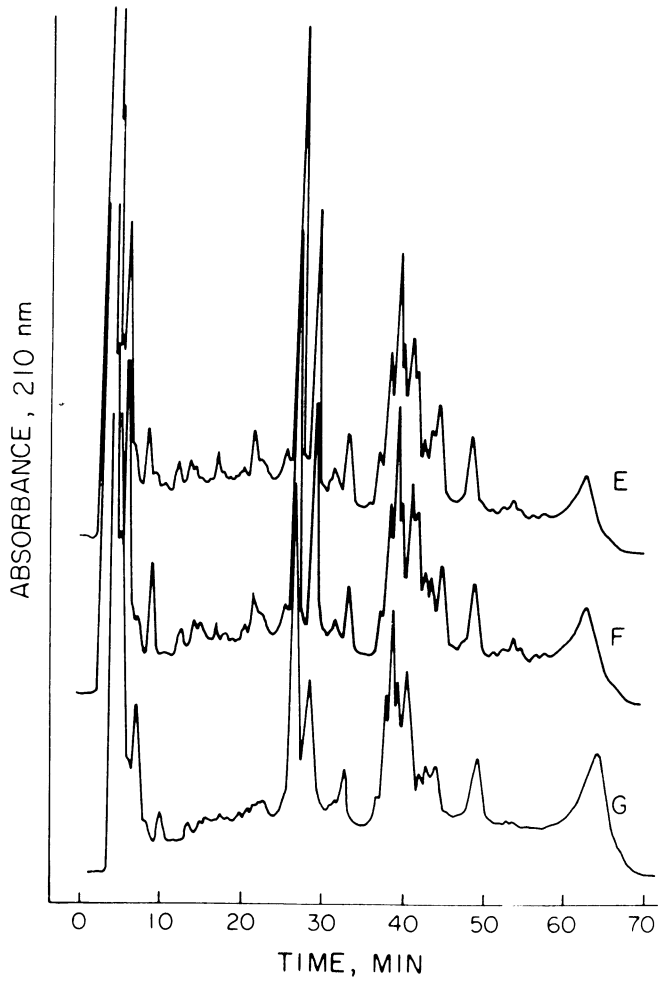

Fig. 8. High-performance liquid chromatography patterns of avenins from the group 1 oat cultivars Lodi (**E**), Moore (**F1**), and Garry (**G**) using conditions of Table II.

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