Characterization of Oat Species by Polyacrylamide Gel Electrophoresis and High Performance Liquid Chromatography of Their Prolamin Proteins

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ABSTRACT

Cereal Chem. 62(3):162–166

Prolamin proteins (avenins) in the groats of 14 oat (*Avena*) species were characterized by polyacrylamide gel electrophoresis (PAGE) and high performance liquid chromatography (HPLC). Generally, complexity of PAGE and HPLC patterns increased as ploidy of the selections increased from diploid (five species), to tetraploid (four species), and to hexaploid (five species). PAGE was more powerful than HPLC in differentiating among species.

The prominence of oats as a cereal grain is due to its high protein content and the excellent nutritional score of oat amino acids (Robbins et al 1971). Oat breeders are consistently increasing the groat-to-hull ratio, with a concomitant increase in protein content.

Pomeranz et al (1973) reported greater variability in protein concentration of groats in 11 oat species than in 289 cultivars of *Avena sativa*. Since genetic variation within a population is a prerequisite for progress through selection for a specific trait, the crossing of *A. sativa* to the broad spectrum of *Avena* species should yield lines with increased variation in protein content. In fact, Campbell and Frey (1972) reported increases in protein content and disease resistance in progeny of *A. sativa* with *A. sterilis* crosses. Luby and Stuthman (1983) reported improved grain yield and groat oil content in several *A. sativa* × *A. fatua* crosses compared to the parent *A. sativa*.

The limited variation in protein content of *A. sativa* cultivars (Pomeranz et al 1973) and prolamin electrophoretic patterns of the commercially important oat cultivars (Lookhart 1984) led us to characterize the prolamin fractions of the major oat species by polyacrylamide gel electrophoresis (PAGE) and high performance liquid chromatography (HPLC). The prolamin fraction consists of only up to 15% (Peterson and Smith 1976, Kim et al 1978) of the total protein in oats (*A. sativa*). Even so, Lookhart (1984) showed differences in the PAGE and HPLC patterns of the prolamins for the commercially important oat cultivars. Globulins, the major protein storage fraction in oats, were electrophoretically homogeneous, which did not allow for PAGE identification (Lookhart 1984).

Species with large variations in PAGE or HPLC patterns might be useful in oat breeding, because large differences in electrophoretic patterns are usually associated with high genetic diversity (Lookhart et al 1983, Jones et al 1982). Bietz (1983) has shown that HPLC can be an effective tool in identifying wheat

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TABLE I
Oat Species Gradient Program

	Time (min)					
Solvent	**0**	**5**	**30**	**32**	**33**	**34**
% A[a]	28	35	45	45	75	28
% B[b]	72	65	55	55	25	72

[a]Acetonitrile with 0.1% trifluoracetic acid.
[b]Water with 0.1% trifluoracetic acid.

TABLE II
Oat Species Characteristics

ID No.[a]	Species	Seed Color	Chromosome No.[b]	Genome	Protein Content (%)	1,000 Kernel Weight[c] (g)
PI258542	*A. brevis*	light	14	AA?	13.1	17.50
PI266826	*A. brevis*	dark	14	AA?	11.1	11.22
CI9048	*A. hirtula*	dark	14	ApAp	21.1	6.20
CI337795	*A. hirtula*	light	14	ApAp	10.4[d]	2.40
PI282730	*A. longiglumis*	light	14	AlAl	19.1	14.92
PI287314	*A. nuda*	tan	14	AA	19.1	6.80
PI401822	*A. nuda*	light	42	AACCDD	14.5	21.00
CI9052	*A. pilosa*	light	14	CpCp	29.4	3.22
PI194201	*A. strigosa*	dark	14	AsAs	13.0	17.72
PI292226	*A. strigosa*	dark	14	AsAs	13.1	17.94
PI234842	*A. abyssinica*	light	28	AABB	11.1	13.98
PI411340	*A. abyssinica*	dark	28	AABB	13.9	21.87
PI268213	*A. barbata*	light	28	AABB	21.6	9.00
PI282715	*A. barbata*	light	28	AABB	21.3	4.14
CI8330	*A. magna*	tan	28	AACC	24.2	17.20
CI8331	*A. magna*	dark	28	AACC	24.0	15.50
PI412753	*A. vaviloviana*	light	28	AABB	19.6	10.00
PI412763	*A. vaviloviana*	dark	28	AABB	16.0	10.47
PI258586	*A. byzantina*	light	42	AACCDD	12.6	34.01
PI411439	*A. byzantina*	light	42	AACCDD	12.3	34.88
PI203450	*A. fatua*	light	42	AACCDD	10.6	34.72
PI219574	*A. fatua*	dark brown	42	AACCDD	11.4	19.71
PI411500	*A. sterilis*	light	42	AACCDD	23.5	12.60
PI432474	*A. sterilis*	light	42	AACCDD	24.2	14.37
Cv. Burnett	*A. sativa*	light	42	AACCDD	12.4	30.00

[a] Identification number for U.S. Small Grains Collection.
[b] Baum (1977).
[c] Protein content and 1,000 kernel weight on groats, as received basis.
[d] Very small sample size (0.07 g).

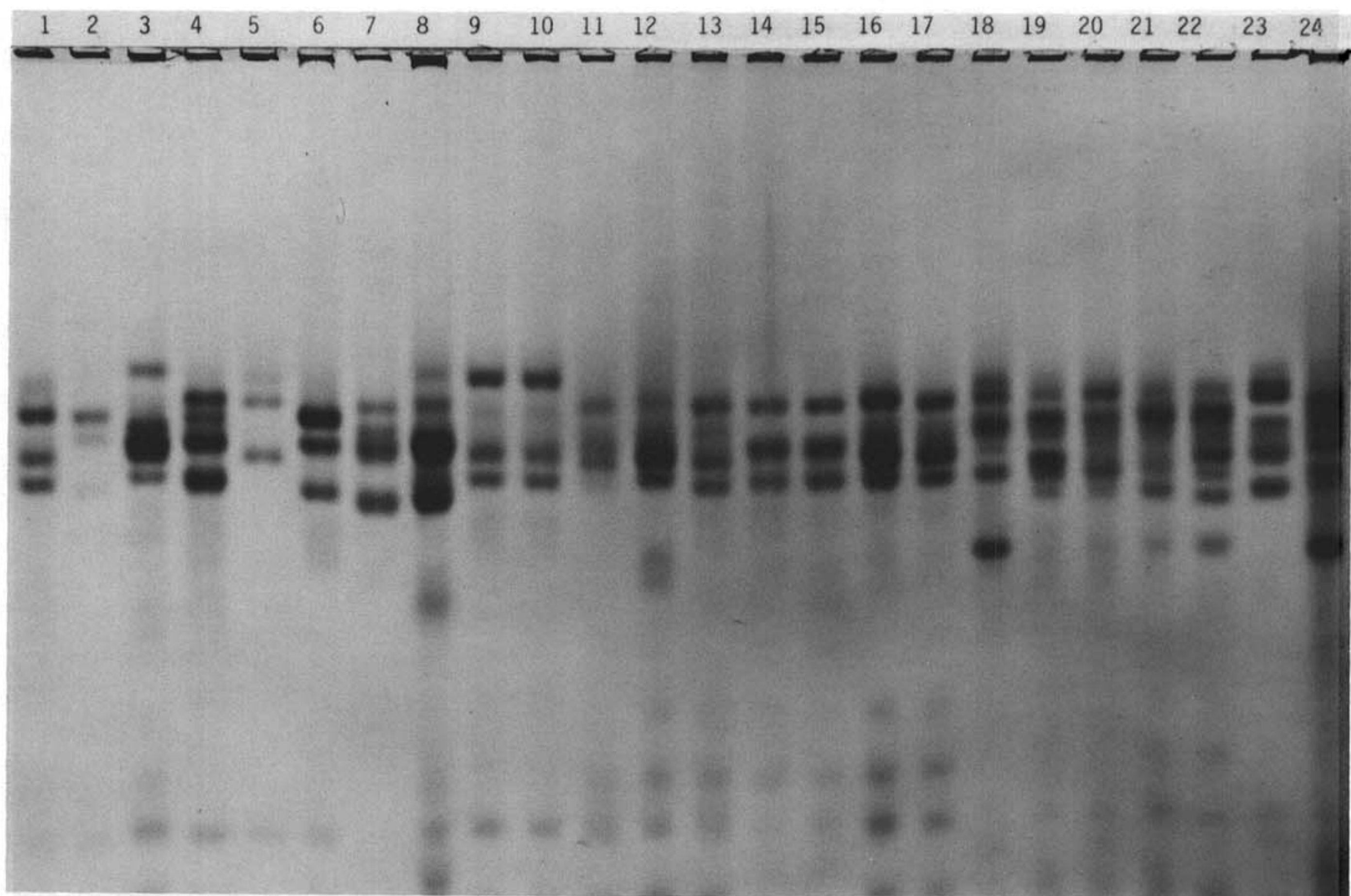

Fig. 1. Prolamin electrophoretic patterns of oat species on a 24× 17 cm gel. **1–2,** *Avena brevis*; **3–4,** *A. hirtula*; **5,** *A. longiglumis*; **6–7,** *A. nuda*; **8,** *A. pilosa*; **9–10,** *A. strigosa*; **11–12,** *A. abyssinica*; **13,** *A. barbata*; **14–15,** *A. magna*; **16–17,** *A. vaviloviana*; **18–19,** *A. byzantina*; **20–21,** *A. fatua*; **22–23,** *A. sterilis*; **24,** *A. sativa* (cv. Burnett).

cultivars. This technique yields results which are as definitive as PAGE but less labor intensive and time-consuming.

The purpose of this study was to develop PAGE and HPLC methods to characterize the prolamin fraction of *Avena* species so that species with widely divergent germ plasm could be selected to broaden the gene pool, resulting in improved cultivars.

MATERIALS AND METHODS

Chemicals and Reagents

Acrylamide, *N,N'*-methylenebisacrylamide, ascorbic acid, Coomassie Brilliant Blue R-250, methyl green, trichloracetic acid (TCA), and trifluoracetic acid (TFA) were obtained from Sigma Chemical Company; lactic acid (USP grade) and ferrous sulfate heptahydrate (AR grade) from Mallinckrodt, Inc.; LC-grade ethanol and acetonitrile from Burdick and Jackson Laboratories; and aluminum lactate from K & K Laboratories. Hydrogen peroxide (3% practical grade) was purchased 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.

Oat Samples

Fourteen oat species (Table I) were obtained from D. H. Smith, Curator, USDA-ARS Plant Genetics and Germplasm Institute, Beltsville, MD.

Protein Content

Protein content was determined by AACC approved method 46-10 (expressed as N $\times$ 6.25, %, as is moisture basis).

Sample Preparation

Oat samples were dehulled manually (except for the hull-less *A. nuda*), and the groats were ground and extracted as previously described for wheat by Lookhart et al (1982).

PAGE

The electrophoresis apparatus, power supply, and the staining and destaining procedures were as described previously (Lookhart et al 1982). An EC-490 gel former (24 $\times$ 17 cm) that accommodated 24 samples (EC Apparatus Corp., St. Petersburg, FL) was also used. Acrylamide concentration was 7.5%, temperature was controlled at 10° C, and prolamins were electrophoresed for 5 hr, conditions that gave optimal protein separations.

HPLC

The HPLC system was composed of a Varian Associates 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., SynChropak RP-P 6.5-μm particle column (250 $\times$ 4.1 mm i.d.), 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) with the gradient in Table I at 1.0 ml/min. The solvents were A, acetonitrile, and B, water, each with 0.1% TFA. All samples were extracted and analyzed at least two times.

RESULTS AND DISCUSSION

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The protein content of groats of the 14 *Avena* species examined ranged from 10.4% for *A. hirtula* to 29.4% for *A. pilosa* (Table II) which is in agreement with that reported by Pomeranz et al (1973). The protein contents of different accessions of most species were similar; only in *A. hirtula* and *A. nuda* were the protein contents quite different.

Electrophoregrams of the prolamin fractions of all oat species examined (Table II) showed at least three major bands (Fig. 1) and were different for each species. The first six species (PAGE patterns 1–10) were diploids, the next four species (PAGE patterns 11–17) were tetraploids, and the last four species (PAGE patterns 18–24) were hexaploids. Because *A. nuda* (PAGE patterns 6 and 7) has

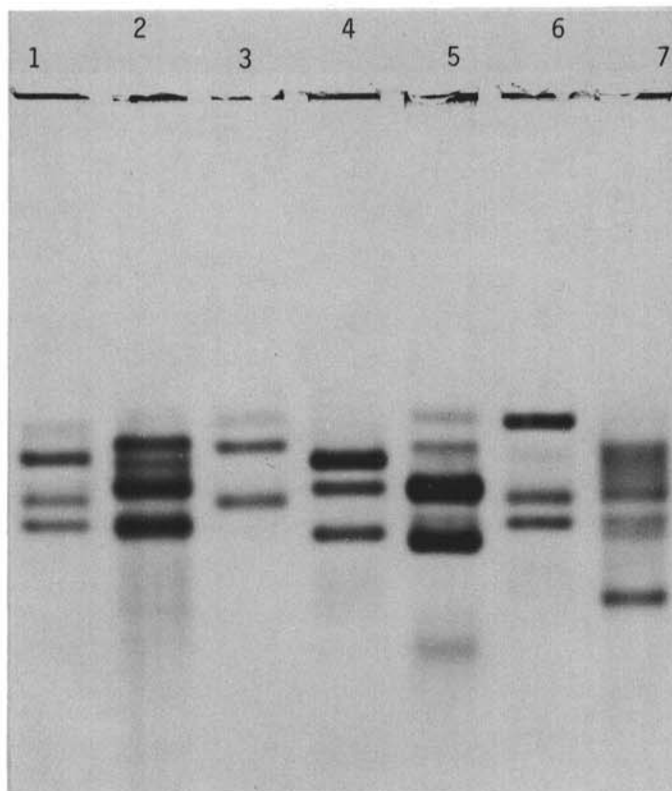

Fig. 2. Prolamin electrophoretic patterns of oat diploid species (1–6) on a 12 $\times$ 17 cm gel. **1,** *Avena brevis*; **2,** *A. hirtula*; **3,** *A. longiglumis*; **4,** *A. nuda*; **5,** *A. pilosa*; and **6,** *A. strigosa*. **7,** *A. sativa* (cv. Burnett) for comparison.

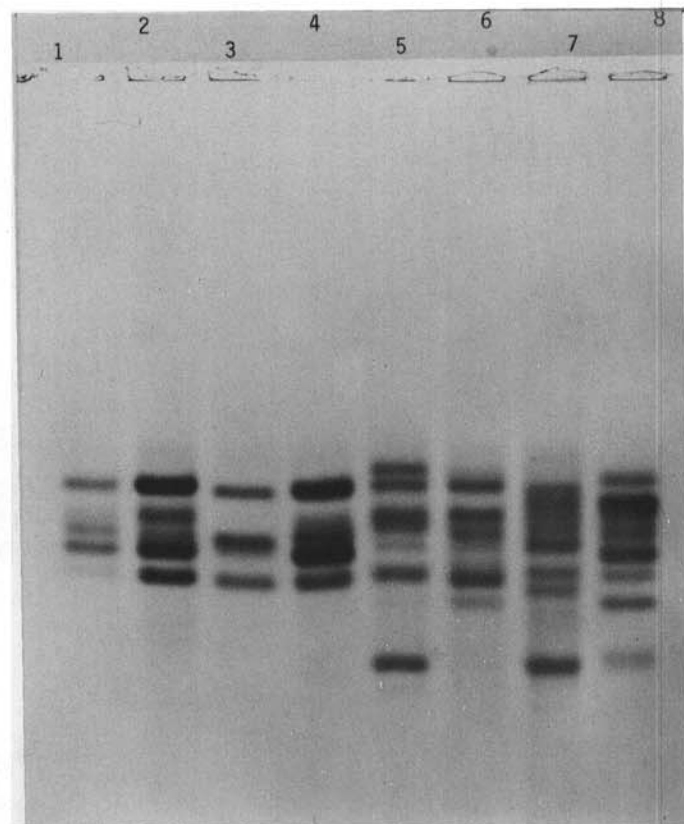

Fig. 3. Prolamin electrophoretic patterns of oat tetraploid (1–4) and hexaploid (5–8) species on a 12 $\times$ 17 cm gel. **1,** *Avena abyssinica*; **2,** *A. barbata*; **3,** *A. magna*; **4,** *A. vaviloviana*; **5,** *A. byzantina*; **6,** *A. fatua*; **7,** *A. sativa* (cv. Burnett); **8,** *A. sterilis*.

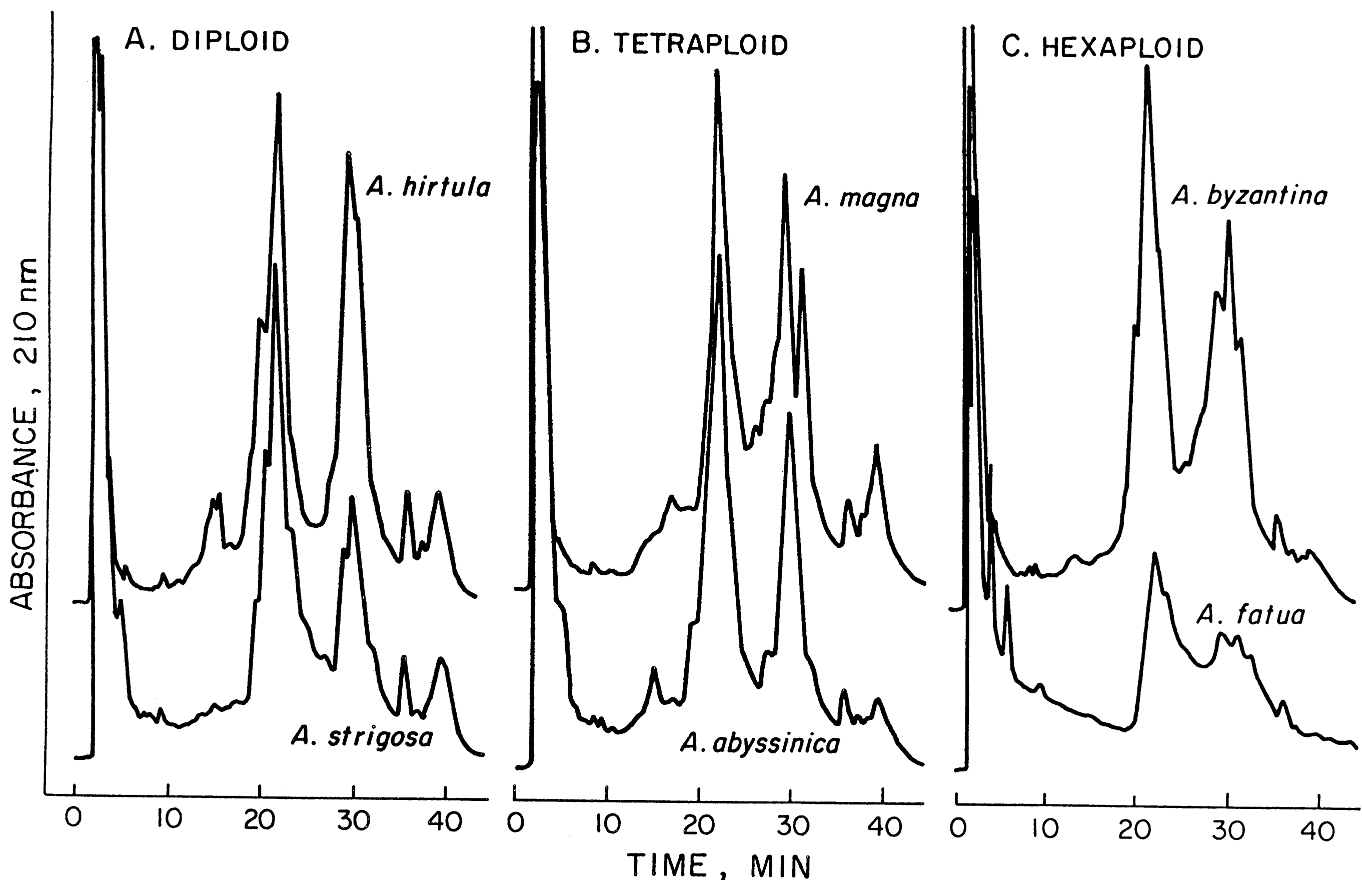

Fig. 4. Prolamin HPLC patterns of typical **A**, diploid; **B**, tetraploid; and **C**, hexaploid oat species.

been reported as both a diploid (Baum 1977) and a hexaploid (Coffman 1977), two *A. nuda* accessions were electrophoresed side by side with the diploids and chromosomes were counted. Counts taken from the root tips of seed corresponding to patterns 6 (PI287314) and 7 (PI401822) were 14 and 42, respectively, indicating that the accession with pattern 6 is a diploid and that with pattern 7 is a hexaploid. The complexity of the patterns tended to increase with ploidy level. Different accessions of a given species usually did not exhibit identical PAGE patterns; only in *A. strigosa* (9 and 10) and *A. magna* (14 and 15) were the patterns identical. Similar, but not identical, PAGE patterns were found for both accessions of *A. abyssinica* (11 and 12), *A. fatua* (20 and 21), and *A. vaviloviana* (16 and 17) species, which may indicate the lack of polymorphism in those species.

The diploid species show great diversity in their electrophoretic patterns, which is to be expected from their chromosomal differentiation (Baum 1977). In tetraploids, similar electrophoretic patterns were exhibited by *A. abyssinica, A. barbata,* and *A. vaviloviana*, which may indicate the similarity of their genetic backgrounds. In fact, all three species are tetraploids with AABB genome character, with similar head height, and erect juvenile growth (Baum 1977).

The hexaploids showed as much variation in electrophoretic patterns among accessions of one species as among species. Therefore, identification of a given species from its prolamin electrophoretic pattern would only be possible on homogeneous species, where different accessions have identical patterns. Of the species studied, the most likely to be identified by electrophoretic pattern analysis is *A. magna*, since both accessions had identical patterns. It was impossible to check 20 or 30 accessions of a single species for homogeneity, because the accessions studied are the world collection of *Avena* species, except for *A. sativa.* In contrast, the ability to select species with quite different electrophoretic patterns makes possible the use of heterogeneous germ plasms in a breeding program. Even in the cultivated oat cultivars, Williamson et al (1968) reported different esterase patterns for 61% of Orbit, 30% of Nodaway, and 4% of Putnam 61 plants.

Single accessions of each species were electrophoresed on the regular (12 × 17 cm) gel former. Electrophoretic patterns of the diploid oat species *A. brevis, A. hirtula, A. longiglumis, A. nuda, A. pilosa*, and *A. strigosa*, shown on Figure 2, correspond to the diploids on lanes 1, 4, 5, 6, 8, and 9 of Figure 1, respectively. Similarly, the electrophoretic patterns of the tetraploids, *A. abyssinica, A. barbata, A. magna*, and *A. vaviloviana*, and the hexaploids, *A. byzantina, A. fatua, A. sativa*, and *A. sterilis*, are shown on Figure 3 and correspond to lanes 11, 13, 14, 16, 18, 20, 24, and 22 of Figure 1, respectively. The regular gel apparatus (12 × 17 cm) consistently gave better resolution than the larger apparatus (24 × 17 cm), but direct comparison of all samples required use of the larger one.

HPLC

Similar, but not identical, HPLC patterns were found for each of the *Avena* species and accessions, except for *A. strigosa* and *A. magna* accessions where the patterns were identical. In general, HPLC patterns for all species showed effects similar to the PAGE data; accessions of those species with idential PAGE patterns also had identical HPLC patterns. Typical HPLC results of two different diploid (*A. hirtula* and *A. strigosa*), tetraploid (*A. magna* and *A. abyssinica*), and hexaploid (*A. fatua* and *A. byzantina*) species are shown in Figure 4. In each species examined two major bands were found, 23 and 30 min, and several bands of minor intensity eluting more than 34 min after injection. The complexity of the peaks eluting at 30 ± 3 min increased as the ploidy level increased, just as previously discussed in the PAGE section.

Species identification by PAGE or HPLC could be achieved if a catalog of all patterns of a species and of all species were available for comparison, as reported by Lookhart et al (1983); they cataloged gliadin PAGE patterns to aid in identifying wheat cultivars.

The ability to characterize the prolamin fractions of various species and pick out the most diverse of them for breeding purposes may be useful to oat breeders in the future. Furthermore, if associations between certain prolamin patterns and desirable or

undesirable trait complexes are found in polymorphic species, electrophoretic screening of accessions or plants before crossing could make breeders' work more efficient.

ACKNOWLEDGMENTS

We thank Leisa Albers for the photography and the electrophoresis work and Nora Lapitan for the chromosome counting.

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[Received November 2, 1984. Accepted December 7, 1984.]