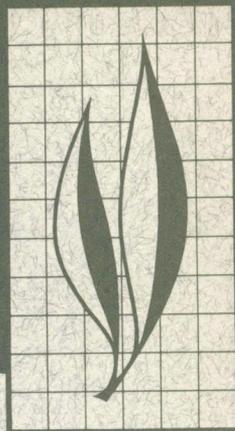


HILGARDIA

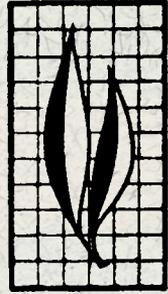
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Identification of Western U.S. Wheat Varieties by Polyacrylamide Gel Electrophoresis of Gliadin Proteins

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ABSTRACT

Polyacrylamide gel electrophoresis (PAGE) patterns of gliadin endosperm proteins were obtained for 52 western U.S. wheat varieties, using foundation seed stocks or stocks maintained by wheat breeders. Two PAGE procedures were used; one, based on a uniform concentration of polyacrylamide, was somewhat superior in the number of bands separated; the other, based on a polyacrylamide concentration gradient, required much less time. Their combined use appeared advantageous, although patterns obtained by either method could be used to distinguish and identify most of the varieties examined. Exceptions were the pairs Gaines and Nugaines, Manning and Hansel, Fielder and Fieldwin, Hatton and McCall, and possibly, Cajeme 71 and Yecora Rojo. More than one pattern among individual seeds within a variety sample indicated two or more biotypes (genetic variants) in 17 of the varieties. These variations ranged from the occurrence of about equal numbers of two quite different patterns to the occurrence of one predominant pattern with biotypes of slightly different patterns in minor proportions.

The presence of biotypes in some varieties complicates the determination of the varietal composition of wheat samples, but at present, no other techniques are available that approach the resolution given by PAGE for the purpose. Photographs of the gliadin patterns included in this report can serve as reference documentation for future work on gliadin proteins in western U.S. wheats.

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INTRODUCTION

BREEDING AND RELEASE of new varieties of wheat have increased manyfold over the past 50 years, and a progressively wider selection of parental material has been used in breeding commercial types. Yields, disease resistance, and other characteristics have been much improved, but a secondary effect has been that visually identifying varieties has become more and more uncertain. At present, reliable assignment by visual identification of some samples to U.S. market classes, such as Hard Red Spring and Hard Red Winter, is not possible; furthermore, admixture of unwanted varieties in seed stocks often cannot be recognized.

Precise selection of market lots of wheat with specific processing characteristics is necessary to convert them satisfactorily into a great diversity of end products. As stated by Akroyd and Doughty (1970), "Wheat is grown to be eaten. Its whole history, and indeed its present position in the world, turn on the methods which have been evolved for transforming harvested grains into forms suitable for daily consumption, i.e., on food technology in the broadest sense of that term." Nine principal types of products are listed: parched or roasted grains, porridges, unleavened or flat breads, leavened breads, bulgur, paste or pasta products, breakfast foods, cookies, and cakes. Innumerable versions of these forms are consumed.

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When these foods are prepared in the home or in small commercial operations, adjustments to variations in the wheat or flour supply can be made continually by skillful preparation; when they are prepared in large amounts in automated operations, as is now common, such adjustments cannot be made; the raw material supply must be uniform to meet production line requirements.

Processing characteristics of wheats and flours are sometimes influenced critically by variety, and are nearly always affected by it (Finney and Barmore 1948; Finney and Yamazaki 1967), so identification of varieties and control of their proportions in wheat and flour mixtures have become more important in processing. At the same time they have become more difficult to accomplish. Because a major portion of the U.S. crop is exported, the technological demands of foreign markets, sometimes as exacting as those of the domestic market, must be considered. Particularly for exports to Japan and Europe, there is need for reliable and rapid procedures for varietal identification.

Scientists in many countries have attacked the problem of variety identity, although sometimes with limited objectives. For example, an early goal was to detect the admixture of common wheats in durum wheats in pasta manufacture. Some simple tests have been useful, such as treating red-and-white wheat mixtures with sodium hydroxide solution to enhance the coloration of red wheat's bran; otherwise, color differences are not always apparent because of variations in grain hardness. Treatment with alkaline 1 percent phenol solution to develop a brown color also exposes varietal differences, but distinguishing degrees of coloration, to permit classification into more than three or four categories, is difficult. Methods based on gel electrophoresis² of the gliadin proteins have had the most success. High-performance liquid chromatography (HPLC) procedures have been developed recently (Bietz and Cobb 1985) and will likely become important in the future. Electrophoretic methods have sufficient resolving power to distinguish many wheat varieties, and have been used to check the presence or absence of specific varieties in commercial shipments in Australia and France (Wrigley 1980; Autran 1979). The gliadins, originally defined by their solubility in aqueous 70 percent ethyl alcohol, are storage proteins in the endosperm of the wheat kernel. Early in the development of gel electrophoretic methods of separation and characterization of proteins, the patterns of bands into which gliadins separated were recognized to differ among cultivars (Elton and Ewart 1962; Bourdet, Feillet, and Mettavant 1963; Graham 1963) and to be largely unaffected by environmental variations during the growth of the plant and development of the seed (Coulson and Sim 1964; Lee and Ronalds 1967; Doekes 1968).

The large number of gliadins separable by electrophoresis results in many unique patterns. With presently used one-dimensional polyacrylamide gel electrophoresis (PAGE) or starch gel (SGE) methods, 20 to 25 bands usually are produced from a single variety, and the bands may differ in intensity as well as mobility and number; therefore, many different varietal patterns are possible. The gliadins also have relatively low mobilities so that they

²Electrophoresis is the movement of particles in a liquid, induced by a voltage gradient across the liquid. In the present work, the particles of interest are molecules of the gliadin proteins extracted from wheat kernels; the movement in a voltage gradient implies the presence of electrostatic charges on the molecules; the separation into bands shows the presence of gliadin molecules differing in the ratio of number of charges to molecular size. Separations can be shown by optical means in solutions of proteins, but the fluidity of liquids makes the patterns unstable and technique difficult. Gel electrophoresis uses a supporting material (polyacrylamide, starch, or agar) that produces a gel that is usually 85 percent or more liquid, but sufficiently solid so that the pattern of separated bands is not so easily disrupted and the gel may be stained to show the number, location, and relative intensity of protein bands. The network structure of the supporting material also has a screening effect, which aids in separating molecules of differing size and shape.

separate from other classes of wheat proteins during electrophoresis, eliminating any need for selective extraction or for removal of other protein classes before electrophoresis. Single seeds supply more than enough material for examination; this is a requirement if the presence of biotypes (genetic variants) within a variety is to be detected. Biotypes have been found by gel electrophoresis in Soviet wheat varieties (Konarev 1983; Konarev, Gavrilyuk, Gubareva, and Peneva 1979; Sozinov 1985), in Australian varieties (Wrigley and Shepherd 1974; Appleyard, McCausland, and Wrigley 1979), and in some samples of the variety Marquis in Canada (Kosmolak and Kerber 1980). Biotypes have also been noted in HPLC chromatograms of wheat grain proteins (Bietz and Cobb 1985).

A recent publication (Wrigley, Autran, and Bushuk 1983) extensively reviews several laboratory procedures. In addition to the Australian and French applications noted, wide interest in such methods is shown by extensive Russian and Canadian studies and by reports from several other countries. In contrast, only four reports concerned with varieties grown in the United States have been noted, and these have not considered the possible occurrence of biotypes within a variety. Qualset and Wrigley (1979) used SGE and gradient PAGE to examine varieties grown in California and were able to distinguish all varieties, with the exception of Lerma Rojo 64 and its mutant Lerma Blanco 70. By combining electrofocusing and electrophoresis in a two-dimensional procedure, even these two varieties were distinguished. Lookhart et al. (1982, 1983) and Jones et al. (1982) examined ground samples of the 88 varieties grown most extensively in the United States, accounting for 89 percent of the 1979 acreage. Most of the varieties were readily identified by PAGE, but six pairs of identical patterns and one case of three matching varietal patterns were found.

All market classes are found in the wheats produced in the western United States; more than one market class is grown in each state, and a wide range of processing characteristics suits them for many different markets and end products. In California, varieties properly classed as Hard Red Spring are marketed as Hard Red Winter, thereby adding to the complexity of wheat production and marketing in the western U.S. This is in contrast with the Great Plains, Midwest, and eastern United States, in which the wheats produced over large areas fall almost entirely into one or two market classes, e.g., Hard Red Spring and Durum in the northern Great Plains, Soft Red Winter and Soft White Winter in the eastern U.S., and predominantly Hard Red Winter in the southern Great Plains.

In this paper, we report PAGE patterns for 52 common (*Triticum aestivum* L.) and durum (*T. turgidum* L. durum variety group) wheat varieties grown in the western United States; 20 of these were included in studies by Lookhart and co-workers (1982; 1983).

Individual seeds were examined to determine the presence of biotypes. Other objectives were to obtain patterns for all important wheat varieties grown in the western U.S., to provide a readily available "handbook" of the patterns, to determine whether any bands or banding patterns could be used to identify a market class of wheat and to develop a reliable, fast, easy-to-use method.

MATERIALS AND METHODS

Wheat Samples

Table 1 lists the wheat varieties from the western U.S. that were assayed. Stocks were supplied by:

R. K. Thompson, University of Arizona, Agricultural Experiment Station, Mesa Farm, Box 1308, Mesa, AZ 85201.

H. E. Vogt, Department of Agronomy and Range Science, University of California, Davis, CA 95616.

D. W. Sunderman, USDA-University of Idaho Research Center, Aberdeen, ID 83210.

W. K. Pope, Department of Plant and Soil Sciences, University of Idaho, Moscow, ID 83843.

H. F. Bowman, Department of Plant and Soil Science, Montana State University, Bozeman, MT 59717.

W. E. Kronstad, Agronomic Crop Science Department, Oregon State University, Corvallis, OR 97331.

Wade Dewey and S. A. Young, Department of Agronomy, Utah State University, Logan, UT 84332.

T. Wagner, Department of Agronomy and Soils, Washington State University, Pullman, WA 99164.

Samples from research stocks were received from:

C. J. Peterson, Jr., and R. E. Allan (USDA), Department of Agronomy and Soils, Washington State University, Pullman, WA 99164.

Samples of several varieties, each grown at different locations, were provided by G. Rubenthaler, Western Wheat Quality Laboratory, U.S. Department of Agriculture, Pullman, WA 99164, from samples submitted to that laboratory for evaluation of milling and baking properties.

Justin, a hard red spring wheat obtained from North Dakota State University, Fargo, North Dakota, and Inia 66R, from the California foundation seed stock, were used as reference varieties. In some runs, flour milled from the Justin wheat on a Brabender Quadrumat Jr. mill was used in place of a whole seed; no differences were seen in gliadin patterns between flour and seeds.

Additional reference varieties (Marquis, Diplomat, Halberd) were obtained from W. Bushuk, University of Manitoba, Winnipeg. These had been collected for use as reference varieties by an International Association for Cereal Chemistry Study Group.³

Electrophoresis

A-PAGE

The apparatus and procedures were similar to those of Nimmo, O'Sullivan, and Bernardin (1968), Nimmo et al. (1963), and Kasarda, Bernardin, and Qualset (1976), but differed in many details. A gel slab with 6 percent polyacrylamide, 27×12×0.6 cm, containing 10 sample slots or wells, each 7×4×1.6 mm, was polymerized in a plastic mold, using 14.4 g acrylamide, 0.6 g N, N'-methylene-bis-acrylamide, 0.15 ml N, N, N', N'-tetramethylethylenediamine, and 150 mg ammonium persulfate in 250 ml water. The gel was soaked overnight in 4 L of water and then equilibrated overnight in 4 L of 0.0085 M aluminum

³Personal communication, W. Bushuk.

lactate-lactic acid buffer, pH 3.1 ± 0.1 . The buffer was prepared as a 0.17 M stock solution (Jones and Cluskey 1963; Ballou and Smithies 1977). Commercial aluminum lactate from Crescent Chemical Company, Hauppauge, New York, has been used in the latter part of the work; 30 g aluminum lactate and 40 ml 85 percent lactic acid were made to 500 ml with water for a stock solution; 100 ml was made to 3 L for electrophoresis. We found marked variations in mobility of the gliadins in early runs; we think these resulted from variations in buffer composition.

To extract gliadins, a single seed was weighed, ground dry in a mortar, and 1.5 M dimethylformamide (DMF) added at 12.5 to 25 μ l per mg of seed. Ratios in the lower part of the range were used with soft wheats or those known to be low in protein, and higher ratios with hard or high-protein wheats. The seed-extractant mixture was ground about 5 seconds, let stand for 2 or 3 minutes, and again ground. The slurry was transferred (with a Pasteur pipet) to a 3 ml plastic centrifuge tube and centrifuged 10 minutes in a clinical centrifuge (3500 rpm, 1775 g).

A 100- μ l portion of each supernatant extract was pipetted into a small vial containing 15 to 20 mg sucrose or, preferably, sucrose-methyl violet mixture (30 g sucrose and 10 mg methyl violet, mixed thoroughly), and each vial was rotated until a uniform solution was obtained. Slots in the gel were loaded with 30 μ l of the extract-sucrose-dye mixtures placed at the bottom of a slot without first removing buffer. (An exact weight of the sucrose-dye mixture is not required; its purpose is to increase the density, viscosity, and visibility of the extract to make loading the sample slots easier. The increased density and viscosity keep the sample solution in place during loading. The visibility of the dye band during electrophoresis indicates irregularities in migration arising from poor sample loading, uneven gel polymerization, and inadequate cooling of the gel. The movement of the dye band can be used to control the distance of migration of the gliadin bands of high mobility, i.e., to determine the time to terminate a run.)

Runs were made with a current of 50 milliamperes. The gel was not covered or cooled for the first 15 minutes; a strip of polyethylene plastic was then placed over the sample slots, the top cooling plate was placed on the gel, and the flow of cold tap water was begun. Runs were for 6.5 hours, or in later work, until the methyl violet marker had migrated 14.25 to 15 cm—its mobility is slightly greater than that of the fastest gliadin band. (In the apparatus used, a 50 milliamperes current gave a voltage drop of about 12 v/cm in the gel.)

Gels were stained with Coomassie Brilliant Blue R 250 for at least 48 hours (1 g dye extracted with 250 ml absolute ethanol to provide a stock solution; 25 ml dye stock added to 500 ml 12 percent trichloroacetic acid to stain the gel). Gels then were rinsed, washed for 20 to 30 minutes with dilute household dishwashing detergent solution to remove any dye that had precipitated on the gel surface, rinsed with water, destained in 12 percent trichloroacetic acid overnight, and photographed.

AG-PAGE

The procedures of Wrigley and McCausland (1977) and Du Cros et al. (1980) were followed with minor changes. A single seed was weighed, crushed with pliers, placed in a 3-ml plastic centrifuge tube, and 1 M urea solution added (6 μ l per mg seed). The mixture was stirred two or three times with a rod or spatula while standing at room temperature for 1 hour, then centrifuged 10 minutes at 1775 g. The supernatant extracts were decanted into the wells of a spot plate and enough methyl violet was added to produce a strong color.

A two-cell electrophoresis unit and the prepared gradient gels for use in it were purchased

from Isolab, Inc., Akron, Ohio. The gels were 70 mm wide, 75 mm long, and 3 mm thick, cast between glass plates 80 mm wide and 88 mm long. Polyacrylamide concentration ranged linearly from 3 percent at the upper loading end to 10 percent at the lower end. The gels, as purchased, had been polymerized and stored in a slightly alkaline buffer; use, therefore, of a pH 3.1 buffer necessitated a pre-run of the unloaded gel of about 1.5 hours at 300 v. A 10× stock of sodium lactate-lactic acid buffer was prepared by dissolving 2.25 g sodium carbonate in 900 ml water, adding lactic acid to attain pH 3.1, making to 1 L with water, diluting tenfold for use.

Samples of 10 μ l taken from the spot plates were loaded onto the gels and runs were made at 200 v, running for 30 minutes after the violet dye spot had migrated to the bottom of the gel. Staining and destaining were carried out as for the large gels; however, the time required for each step was less because the gradient gels were thinner; overnight staining and a few minutes washing with detergent and no destaining usually were adequate.

Reliability of the Detection of Biotypes

Any of the following were taken as evidence of the presence of biotypes in a variety, rather than as an admixture of a second variety: (1) A second pattern was not recognized as that of another variety; (2) seed from more than one source contained the same second pattern; (3) the pattern observed could have arisen as a combination of bands from the known parents of the variety; or (4) the seeds were from foundation or certified seed stocks which were inspected for off-type features of the plants during growth.

By probability analysis (Wrigley and Baxter 1974; Wrigley and McCausland 1977), when 10 grains are sampled from a large lot of seed and these are found to give identical patterns, the large lot, at 95 percent probability, could contain from 0 to 31 percent grains giving other (unobserved) patterns. When 20 and 30 grains give identical patterns, the probability ranges are 0 to 17 percent and 0 to 11.5 percent, respectively. In the present work, at least 11 seeds of each variety were examined by AG-PAGE. With 11 identical patterns, the sample could contain, at 95 percent probability, from 0 to 27 percent of seeds of differing pattern(s). In many cases, 20 to 30 seeds were examined, but we considered 11 identical patterns sufficient to show that a predominating pattern was present. A minimum of two additional seeds of each variety were examined by A-PAGE also. The A-PAGE and AG-PAGE patterns were compared and their corresponding bands usually could be identified by considering intensity as well as mobility and the total number of bands separated. Thus, at least thirteen seeds of each variety were examined.

When one or two seeds of the thirteen gave a nonpredominant pattern additional seeds were examined (table 1) until at least three seeds with a nonpredominant pattern had been found. This appeared to be sufficient to indicate whether a biotype was present in more than minor proportions. However, estimates of proportions at the 95 percent confidence level would have wide limits unless many seeds were examined. For example, du Cros et al. (1980) give values as follows: One grain with A pattern in 10 grains examined (10 percent of A found) indicates only that A patterns occur in the whole sample in 0 to 45 percent of the kernels, using a 95 percent confidence interval. With 10 A patterns from 100 seeds, the limits are 4 to 16 percent; with 20 A patterns from 200 seeds, they are 6 to 14 percent.

Our principal interest was to show whether a reasonable percentage of kernels gave a pattern differing from the predominant one, rather than to establish precisely actual proportions. The number of seeds examined is given for all the variety samples. For convenience, distinct patterns for any given variety have been identified by Roman numerals (I, II, III, and so forth) within each variety in order of decreasing frequency.

RESULTS

General Features of PAGE Patterns: Reference Varieties

A-PAGE patterns given by the gliadins of six varieties from widely separated sources are shown in figure 1. In general, the patterns show groupings of bands that have been designated α , β , γ , and ω gliadins (Jones, Taylor, and Senti 1959). The groupings are indicated to help refer to particular bands. Difficulty in reading the A-PAGE patterns in some figures occurs because the migration front tends to curve, with patterns near the edge of the gel migrating more slowly than the center ones. The extent of this curvature apparently depends largely on the degree of heating that occurs in the gel (or on a lack of effective cooling), and the 6-mm thickness of the large gels requires that the current be kept low to allow for dissipation of heat. The curvature of the front is slight in figure 1, but noticeable in lane 9 in that bands in the α and β sections are not quite perpendicular to the edge of the gel. Variations in the temperature of the tap water used in the cooling plates and variations in the conductivity of the aluminum lactate buffer may account for appreciable curvature in other cases, and is most pronounced in the outer lanes.

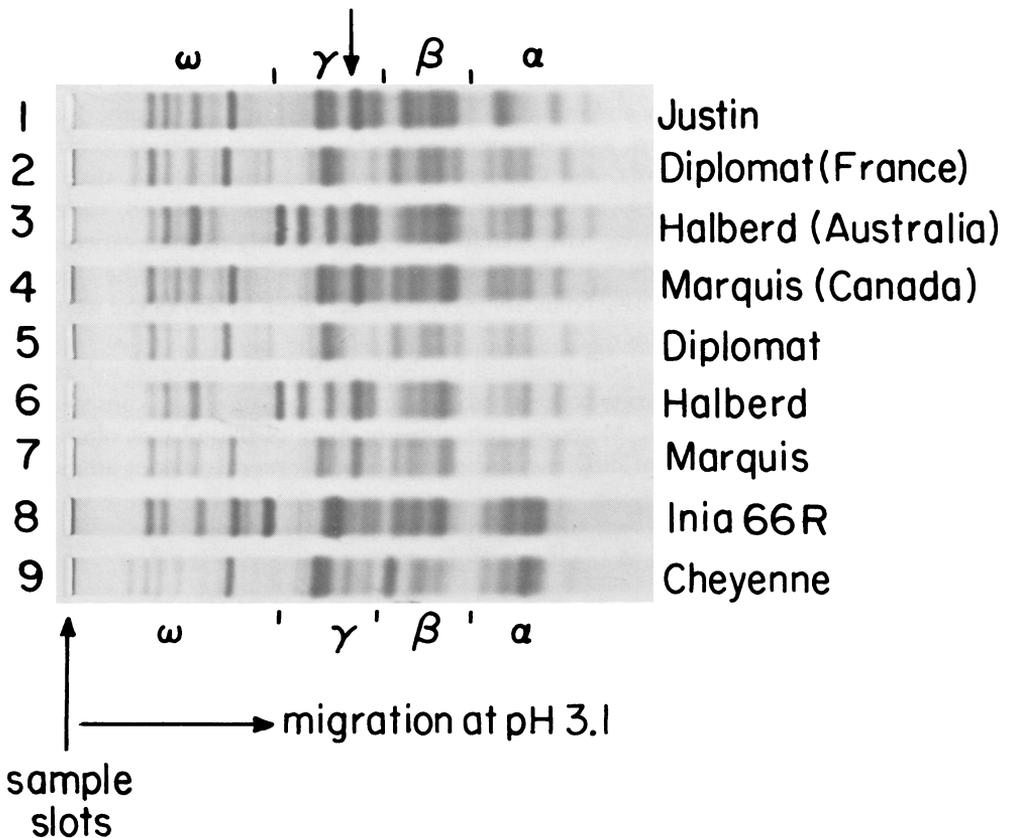


Fig. 1. Acidic PAGE (A-PAGE) patterns of gliadin proteins from single seeds of wheat varieties used for reference. Patterns in lanes 5, 6, and 7 were obtained with one-half the volume of extract used in lanes 2, 3, and 4. Extract placed in slots at left, migration of proteins occurred to the right. Regions are labelled α , β , γ , and ω as an aid in referring to individual bands.

Extracts of Justin and Inia 66R hard red spring wheats have been included in most of the gels to provide reference patterns. Their A-PAGE patterns (lanes 1 and 8, fig. 1) differ in many respects and provide numerous different bands with which to compare other patterns and to indicate the degree of separations of bands achieved in different runs. Also shown are the patterns of Cheyenne, which has been used extensively in gliadin studies, and of Diplomat, Halberd, and Marquis, which have been proposed as reference varieties from France, Australia, and Canada, respectively, for collaborative studies.⁴

The α regions show five different patterns from these six varieties. The grouping of bands in Justin is relatively simple. Compared with it, the Diplomat pattern has two more bands and the mobility of the second fastest band is somewhat greater than that of Justin. Halberd and Marquis give identical α -region patterns, but differ from Diplomat in that the mobility of their second fastest band matches that of Justin. Inia 66R and Cheyenne contain relatively greater amounts of gliadins in the α bands of intermediate mobilities, but their patterns, although similar, are not identical as can be seen more readily with longer migration or in two-dimensional runs.

The β regions of these varieties all appear to differ, although the β region characteristically contains several components not well resolved in most one-dimensional runs.

The γ -region bands are usually better resolved, and, at least in most of the older U.S. varieties we have examined, they are less complex. The most prominent band in Marquis (arrow, fig. 1) has been used as a reference band considered to have an arbitrary mobility of 50 and the mobilities of all other bands were related to this one in defining a varietal pattern (Bushuk and Zillman 1978). The same band occurs in the Justin and Halberd patterns with equal prominence and in the Cheyenne pattern less intensely. In the Justin, Marquis, and Cheyenne patterns, a prominent pair of γ bands occurs (referred to earlier as γ_5 and γ_6 ; Mecham, Kasarda, and Qualset 1978) migrating more slowly than the Marquis reference band. These γ components have been present in most U.S. varieties released before about 1960 that we have examined. In more recently released varieties, a γ pair as found in Inia 66R (the γ_4 and γ_5 pair) has been found as often. The type of pattern seen in Halberd in the ω - γ boundary region was not given by any of the western U.S. wheats examined in this study.

The ω region provides more well separated bands than the other regions, and small differences in mobility and intensity between varieties and biotypes are shown consistently in this region in different runs. Moreover, two, three or more of these bands often are genetically closely linked so that differences of several bands between varieties are fairly common.

A-PAGE and AG-PAGE Patterns Compared

Some differing characteristics of the patterns obtained by the two PAGE procedures can be compared in figures 2 and 3 (California varieties) and figures 4 and 5 (Arizona varieties). An advantage of the AG-PAGE patterns is the small separation between lanes, so that judgment as to the coincidence of bands in adjacent lanes is made more rapid and certain. For example, in figure 3 there is no question that one ω band is common to all ten patterns, and that in the top five lanes, another ω band is present in all five. This "lining up" of bands in different lanes across the gel makes it easy to see a variant pattern when examining seeds from a sample that contains a low percentage of a biotype or admixture of a different variety. A disadvantage is the lack of clear separation into groups, especially of the β and

⁴Personal communication, W. Bushuk.

γ groups, in many patterns. This makes it more difficult to compare patterns section by section from different runs, i.e., on different gels. Because the mobilities of all components are high in the part of the gel of low polyacrylamide concentration, the pattern tends to be concentrated in half the gel. On the other hand, the movement of components into gel of increasing polyacrylamide concentration tends to counteract diffusion and provide

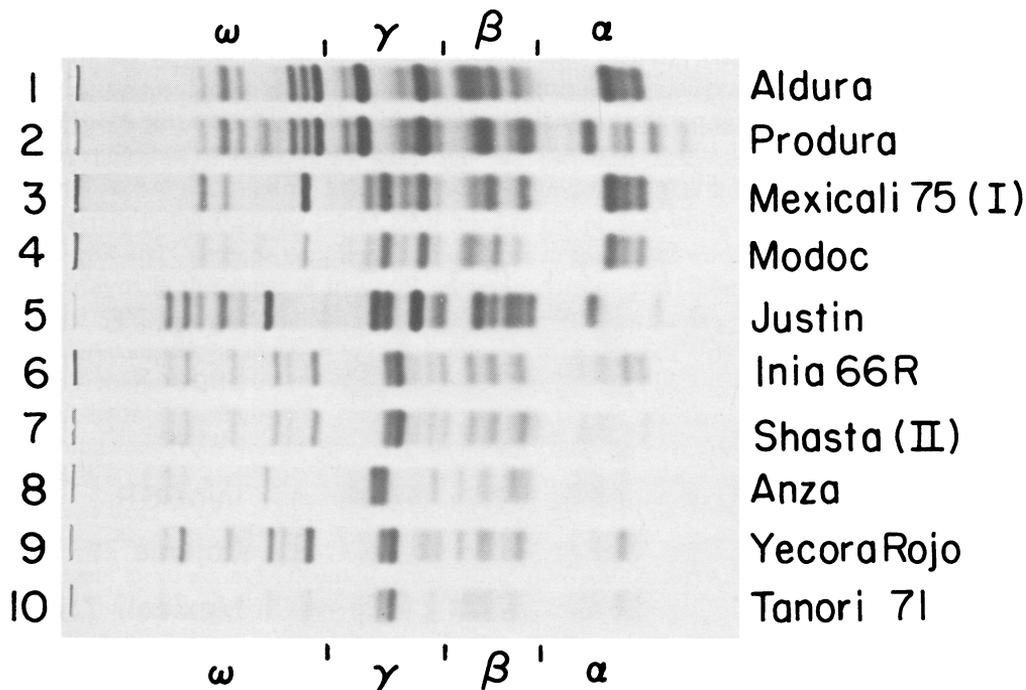


Fig. 2. A-PAGE patterns of gliadin proteins from California wheat varieties. Roman numerals refer to pattern identification code when more than one pattern was found in a variety sample.

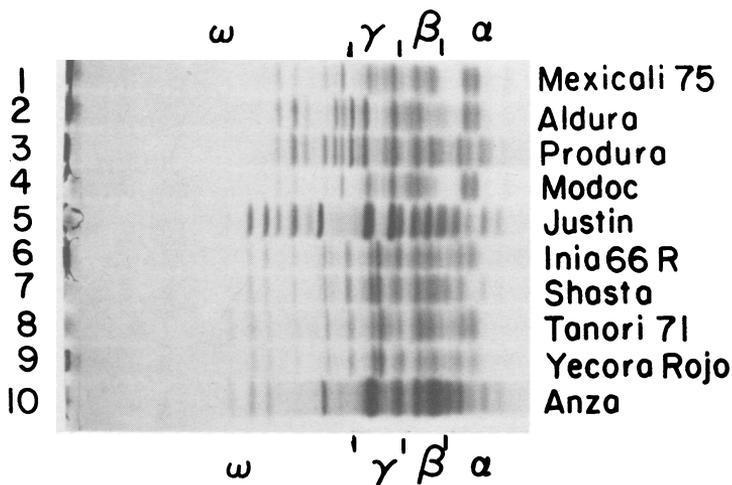


Fig. 3. Acidic gradient PAGE (A-PAGE) patterns of gliadin proteins from California wheat varieties. Migration is from left to right.

a sharpening of bands, especially those of higher mobilities, sometimes enabling faint α bands to be seen more clearly than in the large nongradient gel systems.

An advantage of the large-gel A-PAGE is the spread of the patterns over most of the length of the lane and the separation in most cases into α , β , γ and ω sections, so that patterns can be compared section by section. In some cases, the A-PAGE system also resolved more bands, which was needed to identify certain varieties. Also, most laboratories have used nongradient gels; varietal patterns from the A-PAGE system consequently are more easily compared with patterns published by others.

For these latter reasons, we have used the A-PAGE patterns for most of the figures presented. For overall purposes of the study, we believe use of both procedures was advantageous. To examine large numbers of individual seeds in a reasonable time, the AG-PAGE procedure had a great advantage; in those cases in which greater resolution and comparison among patterns made at different times were required, the A-PAGE procedure was useful.

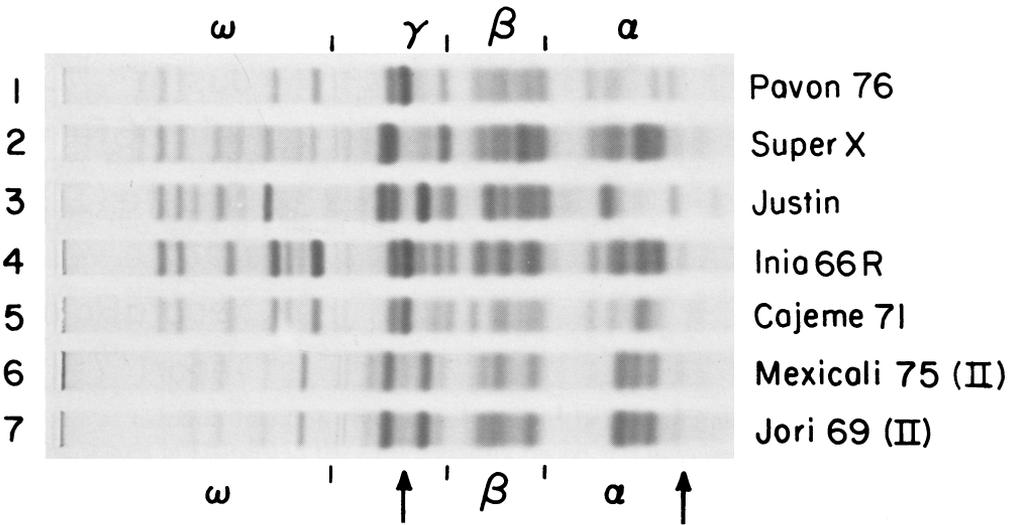


Fig. 4. A-PAGE patterns from Arizona varieties

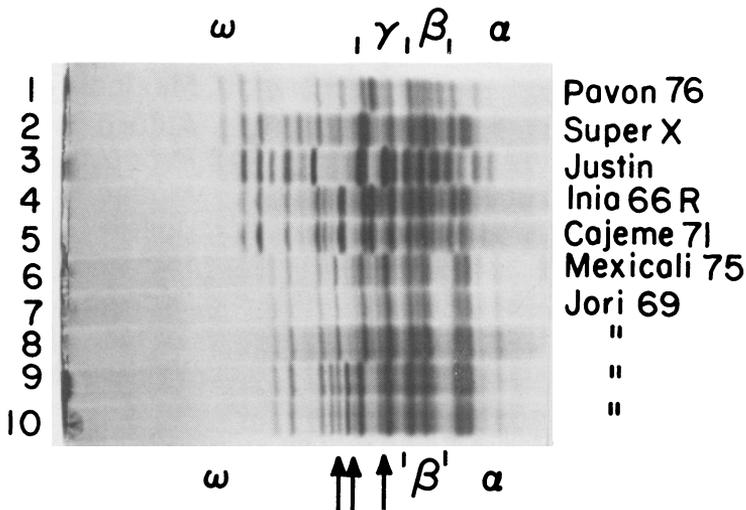


Fig. 5. AG-PAGE patterns from Arizona varieties

Stability of Varietal Patterns

In the course of work on the protein composition of wheats and on genetic relationships among wheat and related species, we have repeatedly used as reference varieties Cheyenne, Scout 66, Justin, and Chinese Spring. Our experience with seeds of these varieties grown at different locations and in several crop years has been that differences in location and crop year do not affect the gliadin patterns obtained from mature seeds by PAGE in any significant way not explainable by variations in the electrophoresis procedures used. Justin patterns in large gels from seeds grown in North Dakota in 1979 are shown in several figures in this paper; they may be compared with Justin patterns from seed grown at Davis, California in 1974 shown in an earlier paper (Mecham, Kasarda, and Qualset 1978). Distribution and intensity of the bands agree well; a darker background and more "streaking" in the earlier work can be attributed to the aluminum lactate buffer used as the gliadin extractant rather than the 1.5 M DMF used in the present work. Similarly, patterns from Inia 66R and Cheyenne samples grown in Davis in the earlier work show the same features as patterns in this paper of Inia 66R grown in Arizona and Cheyenne grown in Montana in 1979. These observations agree with those in many laboratories that show storage protein composition in wheat to be a genotypic characteristic (Wrigley, Autran, and Bushuk 1983) that is unaffected by environment.

There is a possibility that immaturity at harvest may occasionally affect patterns. Seed of Nugaines from Washington and Idaho certified seed gave identical patterns, with no evidence of the presence of biotypes when 38 seeds were examined. Also, the pattern of Gaines from Washington certified seed was identical to that of Nugaines, and no biotype patterns were found in 40 seeds. When seeds from several samples of Nugaines grown at different locations and submitted to the Western Wheat Quality Laboratory were examined, some different patterns were found. The differences appeared to reflect the presence of small proportions of other varieties with one exception. One pattern differed in the slower α bands from the usual Nugaines pattern (lane 1 vs. lanes 2 through 6, fig. 6) in that one heavier band is present in place of two lighter bands in the other patterns. This

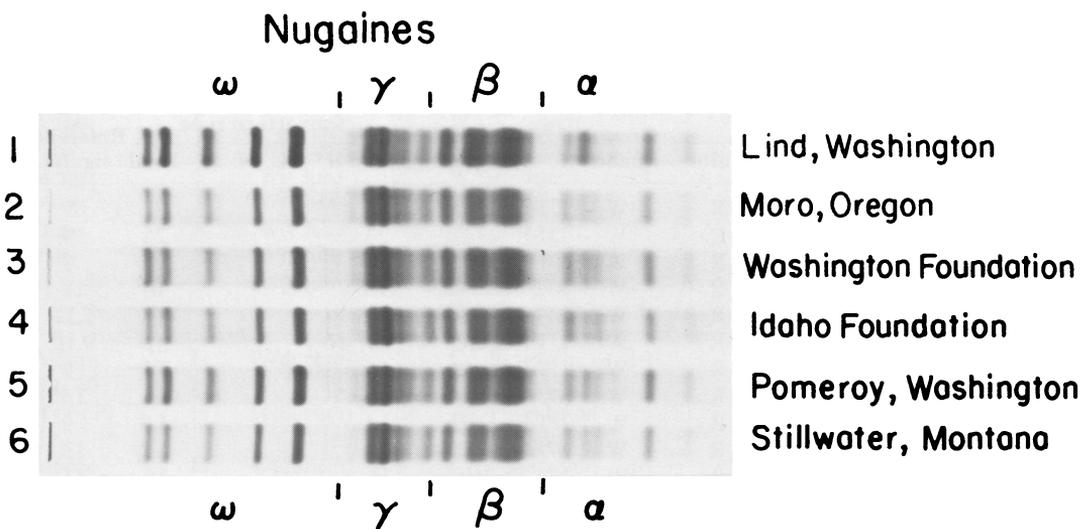


Fig. 6. A-PAGE patterns of Nugaines variety from different locations

pattern difference is the same as observed in other work (Mecham, Fullington, and Greene 1981) in which extracts of immature Nugaines seeds (20 days after anthesis) were compared with those of fully mature seeds, and an additional band in the α region was found in the mature seed. Electrophoresis conditions were not identical (8 vs. 6 percent gels), but would not be expected to alter separations noticeably. Substitution of 60 percent ethanol for 1.5 M DMF was found in the earlier work to produce a pattern from the immature seeds identical to that with mature seeds.

Western U.S. Wheat Varieties

Table 1 shows the origin, grain type, and growth habit of the 52 varieties examined. The varieties are grouped into their most appropriate market class and listed alphabetically within each group. The total number of seeds examined and the number found for each pattern are given. Seventeen of the varieties were polymorphic for gliadin patterns. Of these, only five showed a predominant pattern in 83 percent or more of the seeds examined. The remaining 12 varieties were distinctly polymorphic. At least one of the less frequent patterns occurred at 20 percent or higher frequency in each of these 12 varieties except Wanser, but four variants in addition to the principal Wanser pattern were found. Varieties, for the most part, were uniquely identified by their A-PAGE patterns, including those with multiple patterns. Four pairs of varieties showed identical patterns: the hard red winter varieties Hatton and McCall, Hansel and Manning; soft white winter varieties Gaines and Nugaines; and soft white spring varieties Fielder and Fieldwin. Within each pair, these varieties are closely related in their breeding histories and would be expected to be very similar. Likewise, their end-use properties are similar. On the other hand, two pairs of closely related varieties, Hyslop-McDermid and Wanser-McCall, had distinctly different patterns.

In the following sections, the varieties are discussed in groups related to the geographic origin of samples; this is by states, except in the case of Washington, Oregon, and Idaho, in which there is considerable overlap in use of the varieties.

TABLE 1. WHEAT VARIETIES ASSAYED ON SINGLE SEEDS FOR GLIADIN PATTERNS BY A-PAGE AND AG-PAGE

Variety	Grain		Growth habit	Seed sample origin	No. of seeds for pattern type					Reference fig. no.	
	Texture	Color			I	II	III	IV	V		Total
Bridger	H	R	W	UT-Research	22					22	9
Cache	H	R	W	UT-Research	24					24	9
Cardon	H	R	W	UT-Research	14					14	9
Centurk	H	R	W	MT-Certified	16					16	11
Cheyenne	H	R	W	MT-Registered	17					17	1,11
Delmar	H	R	W	UT-Research	29	10				39	9,10
Hansel	H	R	W	UT-Research	14					14	9
Hatton	H	R	W	WA-Foundation	16					16	13
Manning	H	R	W	UT-Research	14					14	9
McCall	H	R	W	WA-Foundation	14					14	13
Redwin	H	R	W	MT-Registered	16					16	11
Wanser	H	R	W	WA-Research	18	3	2	2	2	27	13,14
Winalta	H	R	W	MT-Registered	20	2	1			23	11,12

(continued)

TABLE 1. (continued)

Variety	Grain		Growth habit	Seed sample origin	No. of seeds for pattern type					Reference fig. no.	
	Texture	Color			I	II	III	IV	V		Total
Anza	H	R	S	CA-Foundation	9					9	2
				CA-Research	55					55	2
Cajeme 71	H	R	S	AZ-Foundation	28					28	4
Fremont	H	R	S	UT-Research	14					14	9
Inia 66R	H	R	S	AZ-CA-Found.	68					68	1,2,4
Lew	H	R	S	MT-Registered	16					16	11
Newana	H	R	S	MT-Foundation	31	2	1			34	11,12
Powell	H	R	S	UT-Research	15	14				29	9,10
Shasta	H	R	S	CA-Foundation	10	5	3	2		20	2,7
Super X	H	R	S	AZ	14					14	4
Tanori 71	H	R	S	CA-Foundation	20					20	2
Wampum	H	R	S	WA-Foundation	9	5				14	13,15
Wared	H	R	S	WA-Foundation	35					35	13
Yecora Rojo	H	R	S	CA-Foundation	13					13	2
Daws	S	W	W	WA-Foundation	21	10	3			34	19,20
Gaines	S	W	W	WA-Foundation	23					23	17
				WA-Research	17					17	17
Hyslop	S	W	W	WA-Foundation	14					14	13
				OR-Research	12					12	13
Luke	S	W	W	WA-Research	16					16	17
McDermid	S	W	W	WA-Foundation	13					13	17
Nugaines	S	W	W	ID, WA-Found.	38					38	6
Raeder	S	W	W	WA-Research	26	18				44	17,18
Sprague	S	W	W	WA-Foundation	20					20	17
Stephens	S	W	W	WA-Foundation	19					19	13
				OR-Research	16					16	13
Yamhill	S	W	W	OR-Research	20	4				24	13,16
Pavon 76	H	W	S	AZ-Foundation	13					13	4
Fielder	S	W	S	ID, WA-Found.	27					27	17
Fieldwin	S	W	S	ID-Foundation	14					14	17
Walladay	S	W	Facultat	WA-Foundation	16					16	17
Barbee	S	W(Club)	W	WA-Research	11	3				14	19,23
Faro	S	W(Club)	W	WA-Research	33	3				36	19,21
Jacmar	S	W(Club)	W	WA-Research	14					14	19
Moro	S	W(Club)	W	WA-Research	14					14	19,21
Omar	S	W(Club)	W	WA-Research	54	16				70	19,22
Paha	S	W(Club)	W	WA-Research	40	6				46	19,22
Tyee	S	W(Club)	W	WA-Foundation	14	6				20	19,20
				WA-Research	9	14				23	19,20
Aldura	H	W(durum)	S	CA-Research	14					14	2
Jori 69	H	W(durum)	S	AZ-Foundation	11	9				20	4,8
Mexicali 75	H	W(durum)	S	AZ-Foundation	13	11	1			25	4,8
				CA-Research	18	7	2			27	2
Modoc	H	W(durum)	S	CA-Foundation	13					13	2
Produra	H	W(durum)	S	CA-Research	13					13	2

Abbreviation: H = hard, S = soft texture or spring growth habit, R = Red, W = white color or winter growth habit. Standard abbreviations are used for states.

Varieties grown in California

Extracts of single seeds produced the patterns shown in figure 2 (A-PAGE procedure) and figure 3 (AG-PAGE procedure). Identical groupings of two or more bands in more than one varietal pattern occur frequently and large portions of some patterns are identical, but no two patterns are identical overall.

Inia 66 and Anza are the parents of Shasta, and Inia 66R (lane 6) has an identical gliadin pattern to Inia 66. Most of the ω bands of the Shasta pattern (fig. 2) clearly are inherited from Inia 66. In other parts of the Shasta pattern, Inia 66 bands also predominate, but contributions from the Anza parent are present, e.g., the fastest α band. The α bands can be seen better in the patterns of figure 3 (AG-PAGE). The ω bands and the major pair of γ bands of Yecora Rojo (fig. 2, lane 9) match those of Inia 66R and Shasta. Tanori 71 (lane 10) appears to contain the same pair of γ components, but while the bands appear to be of equal intensities in lanes 6, 7, and 9, the band of higher mobility is much more intense than the trailing band of the pair in lane 10.

When separate patterns were obtained from 13 or more seeds of each of the California varieties, variations in patterns indicating the presence of biotypes were found in Shasta. These variations are shown in figure 7 (A-PAGE procedure). In the γ region, three seeds (lanes 2, 6, and 8) have the higher-mobility γ pair present in the Inia 66 parent (lane 1). Lane 6 lacks a minor band, just ahead of the slower-moving γ pair, which is present in

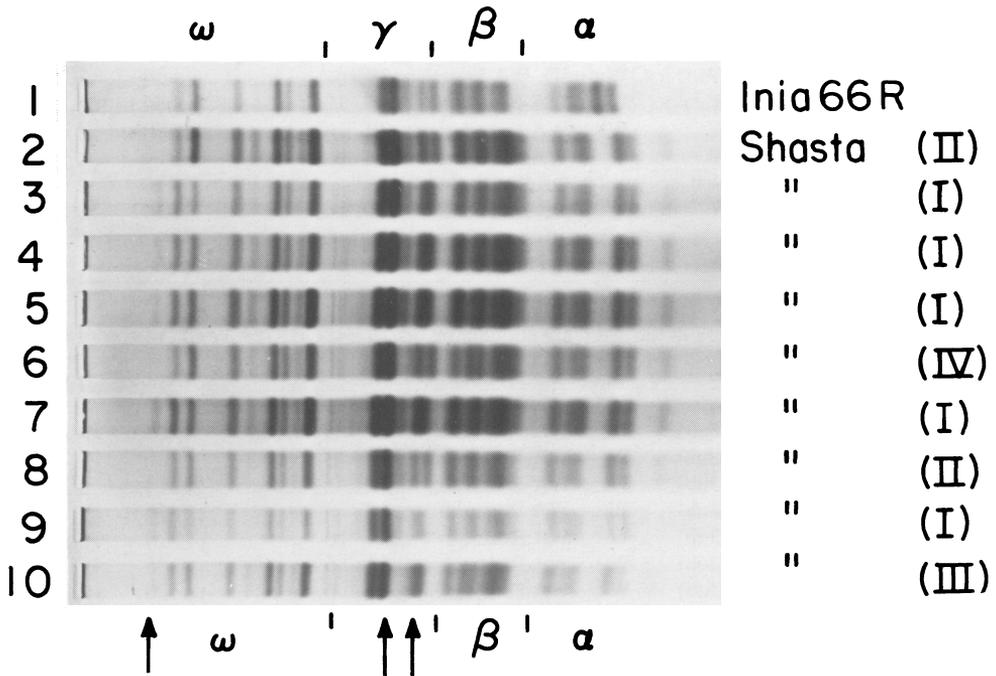


Fig. 7. A-PAGE patterns showing biotypes in Shasta variety. Arrows indicate locations of pattern differences referred to in text. In patterns I and III, a broad band occurs on the right (higher-mobility) side of the γ region; Pattern I also contains a minor band, just to the right of the major γ_4, γ_5 pair of bands, that is absent from pattern III. In patterns II and IV, a pair of bands occurs in place of the broad band in the γ region; pattern II contains the minor γ band also present in pattern I. The Shasta II and Inia 66R patterns are identical in the γ region. Patterns II and IV lack a slow ω band present in Anza patterns.

2 and 8. The other six seeds of Shasta (lanes 3, 4, 5, 7, 9, and 10) gave a single, but broad band of about the same mobility as the faster pair of γ bands of Inia 66R. This broad band may consist of one band from each parent (Inia 66 and Anza) that are not separated completely because they differ only slightly in mobility. In these six patterns, only the one in lane 10 lacks the minor γ band, just ahead of the slower γ pair (also missing from lane 6). Of 20 Shasta seeds examined, 13 gave the single broad band; 10 of these had the minor band (type I) and 3 did not (type III); 7 gave the faster γ doublet of the Inia 66 parent, with the minor γ band present in 5 (type II) and absent from 2 (type IV). The type I and type III patterns (with the single broad γ band) in all 13 cases contained the slowest, very weak ω band found in Anza and not in Inia 66R, which is indicated by an arrow in figure 7 also.

Plant height variants occur in Anza (Qualset et al. 1973). Strains were selected with five different mature plant heights. Seeds from each strain were examined, but no variations in PAGE patterns were found; thus, the data were pooled with other Anza analyses, giving a total of 64 seeds showing a single pattern.

The durum wheat patterns (lanes 1 through 4, figs. 2 and 3) suggest that Aldura and Produra are closely related, with many common bands. Mexicali 75 and Modoc also have many bands in common. All the durum patterns contain some groups of bands that do not occur in the common wheat patterns, e.g., in the α -section patterns in lanes 1, 3, and 4, (fig. 2) and the γ -section bands of low mobility in lanes 1 through 4. Biotype patterns were found in Mexicali 75 seeds.

Varieties grown in Arizona

Patterns from single seeds by A-PAGE are shown in figure 4 (common wheats, lanes 1 through 5, durum wheats, lanes 6 and 7). The patterns of Pavon 76 and Super X differ in several respects from other common wheat patterns in this set, making them easy to distinguish. Inia 66R and Cajeme 71 patterns contain many bands matching in mobilities, but they show considerable differences in intensities in the α and β regions. However, Inia 66R had a γ -region band of moderate intensity (migrating just ahead of the two major γ bands with its position indicated by an arrow in figure 4) that is not present in Cajeme 71. This is the same Inia 66R γ band present or absent in Shasta biotypes. Also, the fastest α -region band of Inia 66R migrates definitely more slowly than the fastest α -region band of Cajeme 71 (arrow, fig. 4). Cajeme 71 is very close in pattern to Yecora Rojo (fig. 2) and the two varieties may not be distinguishable on this basis.

AG-PAGE patterns (fig. 5) showed again that the separation into groups of bands is less apparent than in the A-PAGE gel, but differences among varieties are evident in most cases. The differences in the γ -band patterns between Inia 66R and Cajeme 71 seen in the large gel also can be seen here; but the α -region differences were barely distinguishable in the gel and are not visible in the figure. No evidence of the presence of biotypes was found when 13 or more seeds of Pavon 76, Super X, Inia 66R, and Cajeme 71 were examined.

The durum varieties Mexicali 75 and Jori 69 have patterns with marked similarities to patterns of other durums grown in California (figs. 2 and 3), particularly in the α and γ sections. Biotype patterns were found in both Mexicali 75 and Jori 69. Those of Jori 69, as obtained by AG-PAGE, are shown in the last four lanes of figure 5; marked differences are present in the region of higher mobility ω and slower γ bands. The least complex pattern of Jori 69 (lane 7) appears here to be identical with the Mexicali 75 pattern (lane 6).

When patterns from four seeds of each of these varieties were obtained by A-PAGE, the two Jori 69 patterns were markedly different (fig. 8) in the groupings of the major bands

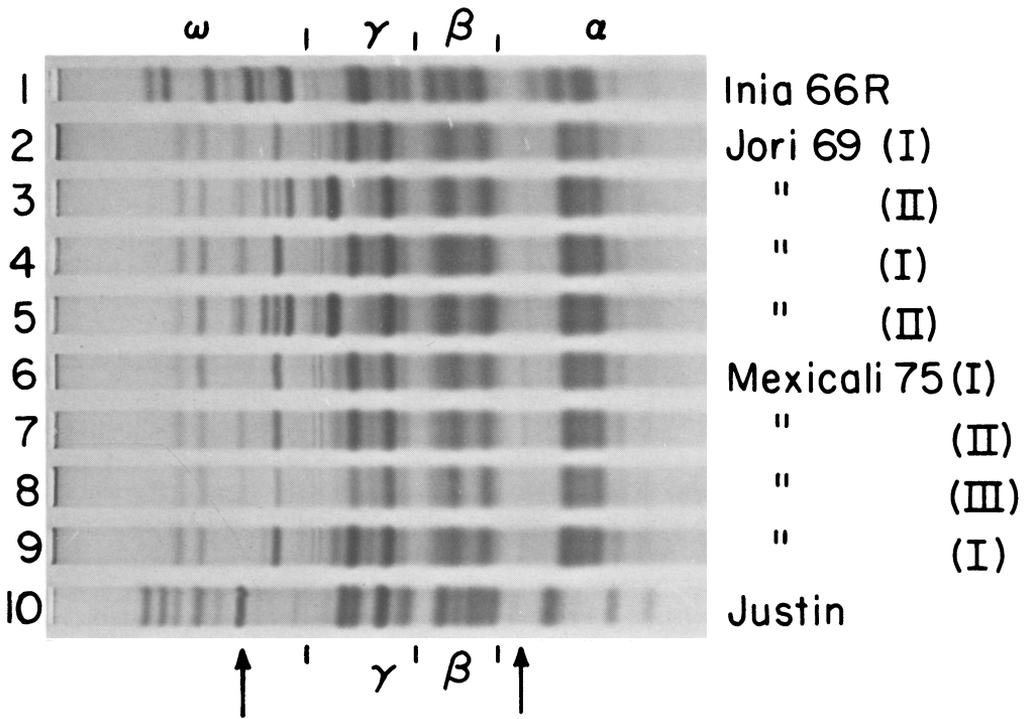


Fig. 8. A-PAGE patterns showing biotypes in Jori 69 and Mexicali 75 varieties. The type I pattern of Jori 69 is simpler in the ω and γ regions than the type II pattern. The types I and II patterns of Mexicali 75 have a slow α band (arrow) absent from the type III patterns; the type II and III patterns contain a weak ω band absent from type I.

in the γ and ω regions (lanes 2 and 4, type I, vs. 3 and 5, type II). Differences in Mexicali 75 are less prominent, but three patterns occur in the four seeds involving the presence or absence of a weak ω and a weak α band (positions indicated by arrows, fig. 8). Patterns in lanes 6 and 9 (type I) contain the α band, but not the ω band; in lane 7 (type II) both are present; and in lane 8 (type III), the ω band is present, but not the α band. The Type II Mexicali 75 pattern with both bands (lane 7) is very similar to the Type I Jori 69 patterns (lanes 2 and 4), but the position of the ω band of the Mexicali 75 pattern is occupied in all the Jori 69 patterns by a broader band which appears to represent at least two gliadin components not completely separated, i.e., the band is somewhat diffuse, but more intense in the leading portion than in the trailing portion.

Of 20 Jori 69 patterns, 11 contained the additional ω bands, 9 did not. With 25 Mexicali 75 seeds from Arizona, 12 gave a weak ω band (with one pattern without the weak α band). The weak ω band was not present in the other 13 patterns. With 27 Mexicali 75 seeds from California, nine gave a weak ω band not found in the other 18 seeds; two of the former also showed a slight variation in the β region. All differences in the Mexicali 75 patterns were minor.

Varieties grown in Utah

A-PAGE patterns (fig. 9) show that Cardon gave a similar pattern to the Hansel-Manning patterns, except in the α region and with an additional minor β component. The other varietal patterns were easily distinguished. AG-PAGE also distinguished all varieties except Hansel and Manning.

Delmar and Powell produced two patterns each (fig. 10). The Delmar patterns differed in the α region with one biotype (type II) containing a heavy concentration of α gliadins of intermediate mobilities, possibly A-gliadins (Platt, Kasarda, and Qualset 1974); the second (type I) contained proportionately less α gliadin in bands of different mobilities. Of a total of 39 seeds, 10 gave the patterns with a heavy concentration of α gliadins. The Powell patterns differed in the presence or absence of a fast ω band and in the slow γ doublet bands. When the fast ω band was present (type II), the γ doublet bands were of the same mobilities as those found in Inia 66R (compare lanes 1 and 6, fig. 9, and lanes 1 and 6, fig. 10); in the absence of the fast ω band (type I), the γ doublet bands were of the same mobilities as those of Justin (patterns 7 to 10, fig. 10). Of 29 seeds examined, 14 contained the fast ω band; 15 did not.

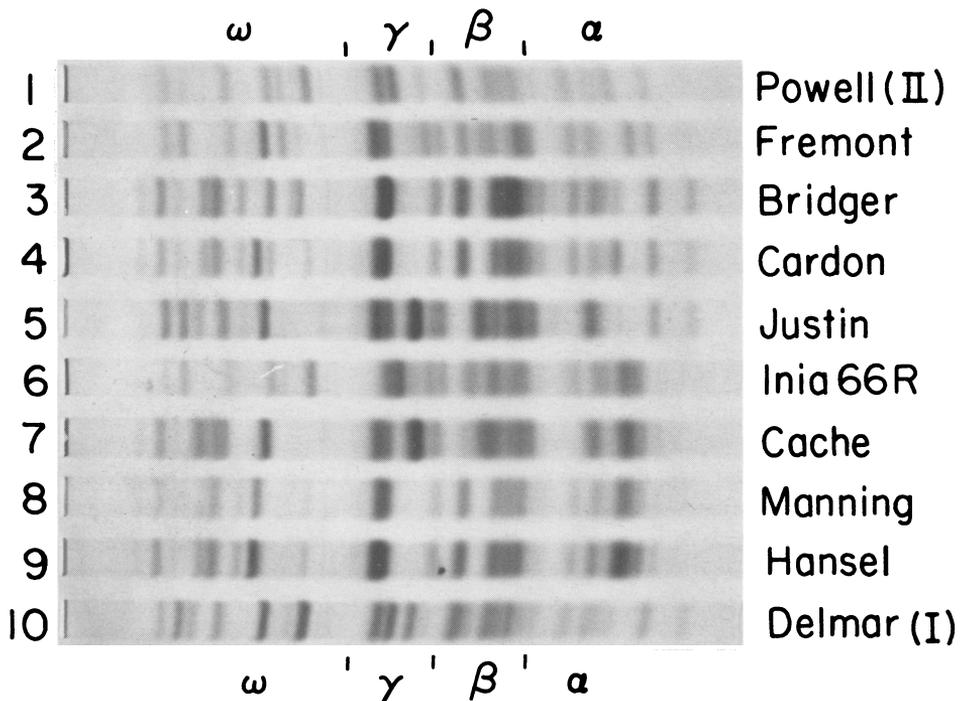


Fig. 9. A-PAGE patterns from Utah varieties

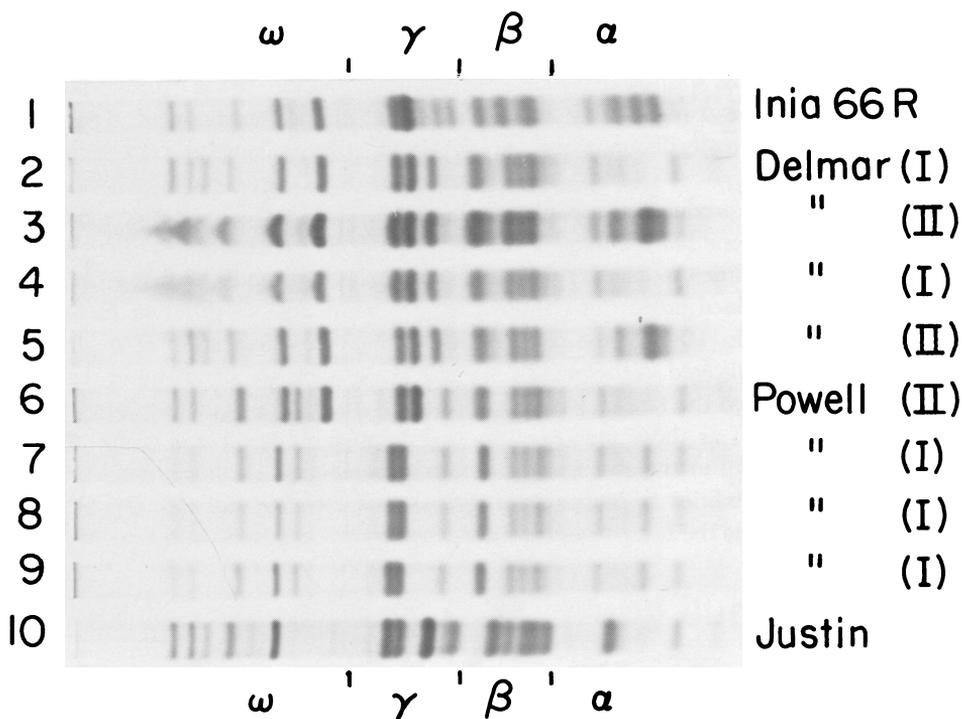


Fig. 10. A-PAGE patterns showing biotypes in Delmar and Powell varieties. The Delmar type I pattern lacks the heavy intermediate-mobility α bands present in the type II pattern. The Powell type I pattern contains the γ_5 , γ_6 doublet found in Justin (lane 10); the type II pattern contains the γ_4 , γ_5 doublet matching that found in Inia 66R (lane 1) and also contains a heavy ω band not found in the type I pattern.

Varieties grown in Montana

Cheyenne and Winalta A-PAGE patterns (fig. 11) were similar in the β and γ sections, but there are differences in the ω region, and the Winalta α band of highest mobility traveled slightly less far than the leading Cheyenne band. However, this is a biotype pattern (type II) of Winalta that was found infrequently; in figure 12, the four seeds examined gave a different α -region pattern and an additional β component, differing appreciably from the Cheyenne pattern. Of 23 Winalta seeds examined, 20 gave the pattern shown in figure 12 (type I). In the case of Newana, a variant was found (fig. 12, lane 5) in which a heavy ω band was missing and a major γ band was much reduced in intensity. Another variant, not shown, but found in two kernels, had an extra ω band. Of 34 seeds, 31 gave the predominant pattern.

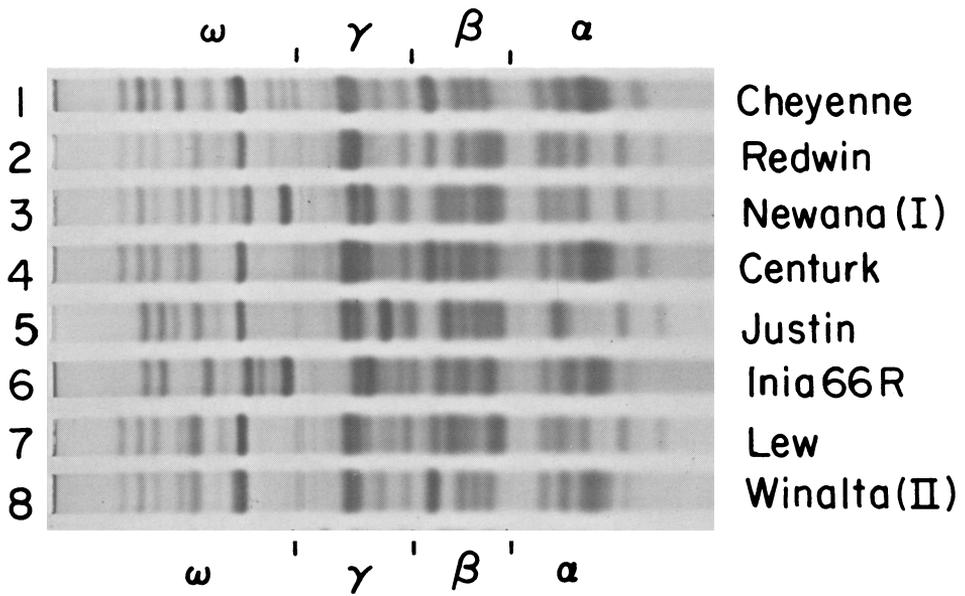


Fig. 11. A-PAGE patterns from Montana varieties

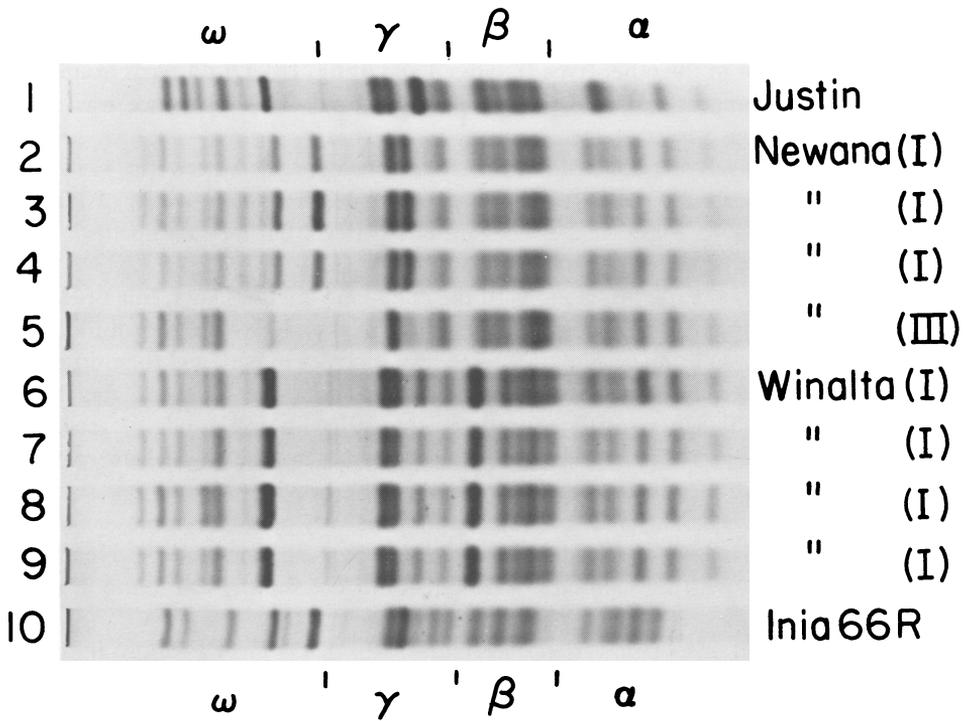


Fig. 12. A-PAGE patterns showing two biotypes in Newana variety and the predominant pattern in Winalta variety. The type I pattern of Newana contains a heavy ω band missing from the type III pattern; one of the major γ bands is much weaker in the type III pattern also.

Varieties grown in the Pacific Northwest (figs. 13-23)

Of 25 varieties examined, at least minor differences were found between any two varietal patterns with the exception of three pairs mentioned earlier (Hatton-McCall, fig. 13; Gaines-Nugaines, figs. 6 and 17; and Fielder-Fieldwin, fig. 17).

Biotype patterns were found in the red wheats Wanser and Wampum. The predominant Wanser pattern (type I) is that in figure 13, lane 4, and figure 14, lanes 4 and 5, but variant patterns involving all sections of the patterns were found. Two (types II and III) are shown in lanes 2 and 3, figure 14, both differing from the predominant pattern in the α region, and lane 3 in the ω and γ regions as well. Of 27 Wanser seeds examined, 18 gave the predominant pattern, but the remaining seeds gave four different patterns.

In the Wampum patterns (fig. 15), the minor variant (type II, lanes 3 and 4) found with five seeds yielded an additional band in the β region that was not found in the predominant pattern (type I, lanes 1 and 2; nine seeds). The variant was detected more readily with AG-PAGE than with A-PAGE; the band-sharpening effect of the gradient sometimes separates bands that differ little in mobility in nongradient gels.

Biotype patterns were found in Yamhill and Raeder among the white wheats. The dominant Yamhill pattern (type I) is shown in figure 16, lane 1, and its less frequent form (type II) in figure 13, lane 3, and figure 16, lane 2. A band of moderate intensity migrating slightly more slowly than the principal γ band occurs in the major pattern; in the minor pattern, two less intense bands are present. Of 24 seeds, 20 contained the single trailing band.

Raeder seeds yielded two patterns showing a small difference in the ω bands. In one case, a single band occurs (type I, lanes 1 and 2, fig. 18) in the area in which a wider more diffuse band is present in the other (type II, lanes 3 and 4, fig. 18). Of 44 seeds examined, 26 gave the more compact band, 18 the more diffuse band. This difference was more readily seen in the large gels.

Patterns from two seeds of Sprague differed from the predominant pattern (fig. 17) found with 20 seeds, but the minor pattern was indistinguishable from the pattern of Hatton and McCall. Similarly, 13 McDermid seeds gave one pattern, but a second pattern (two seeds) appeared to be that of Nugaines or Gaines.

Patterns of club wheat varieties, along with the common varieties Daws, Inia 66R, and Justin, are shown in figure 19. Additional patterns of Daws and Tyee are shown in figure 20 because they differ so little, despite the presence of biotypes in both and their differing head type. Three patterns were found in Daws. One of these (type II, lane 2, fig. 20) is identical to the type II pattern of Tyee (lane 8, fig. 20) with five bands of moderate intensities in the α region. The type I Tyee pattern differs from the type I Daws pattern only in the occurrence of a minor band just ahead of the heaviest α band, not resolved completely from it (lanes 6, 7, 9, and 10, fig. 20) that is very faint or not present in the type I Daws pattern (lanes 1, 3, 4, and 5). In addition, a type III Daws pattern resembling the type I pattern but with an additional γ band (pattern not shown) was found in three seeds of 33 examined.

Tyee differs from all other club varieties in figure 19 in that it gives a pattern in which the two principal slow γ bands match those of Inia 66R in mobility. Also, unlike the other club varieties, the variation between biotypes is in the α region of the patterns. These are shown in figure 20; one pattern (type II, lane 8) contains the five bands of moderate intensities found in the α region of Barbee patterns; the other Tyee pattern (type I, lanes 6, 7, 9, and 10) lacks the two faster of those bands, but the bands of lower mobility

are much heavier. Of 43 seeds examined, 23 gave the heavy α bands; 20 gave the faster α bands.

The patterns from single Moro and Faro seeds are identical in figure 19. However, a pair of bands in the center of the β region was found in patterns (type I) of four of five additional Faro seeds (figure 21). The faster band of the pair was missing (type II) from one Faro seed (lane 2) as in the figure 19 Faro pattern. The faster band was missing from all Moro patterns. Of 36 Faro seed patterns, 33 contained the pair of bands; the other 3 gave the Moro pattern.

The patterns of Omar, Paha, and Jacmar in figure 19 resemble one another and differ appreciably from Moro and Faro in the γ and ω regions. The Jacmar pattern lacks a band in the center of the β region, which distinguishes it from the Omar and Paha patterns. The Omar and Paha patterns of figure 19 are the predominant ones. Variations do occur, however, as shown in figure 22. A heavy γ band is missing from one Paha pattern (type II). In Omar, a fast γ band is relatively light in the type I pattern and very heavy in the type II pattern. Of 46 Paha seeds examined, 40 gave the predominant pattern; of 70 Omar seeds, 54 gave the predominant pattern.

Two patterns also were found in Barbee seeds; one (type II) resembled the predominant Faro pattern, with slow γ bands of nearly the same mobilities as those of Justin (lanes 3 and 5, fig. 23). Another biotype (type I) has an appreciably different pattern in the faster ω bands (lanes 1, 2, and 4, fig. 23). This Barbee pattern has some features of Omar and Paha patterns, but is not identical with either. In the γ section, the second fastest band (arrow, fig. 23) is slightly, but consistently, less dark than the fastest, while in the biotypes of Omar, the bands differ appreciably in intensity. The Barbee pattern of lanes 1, 2, and 4 appears to be the predominant one, occurring in 11 of 14 seed extracts.

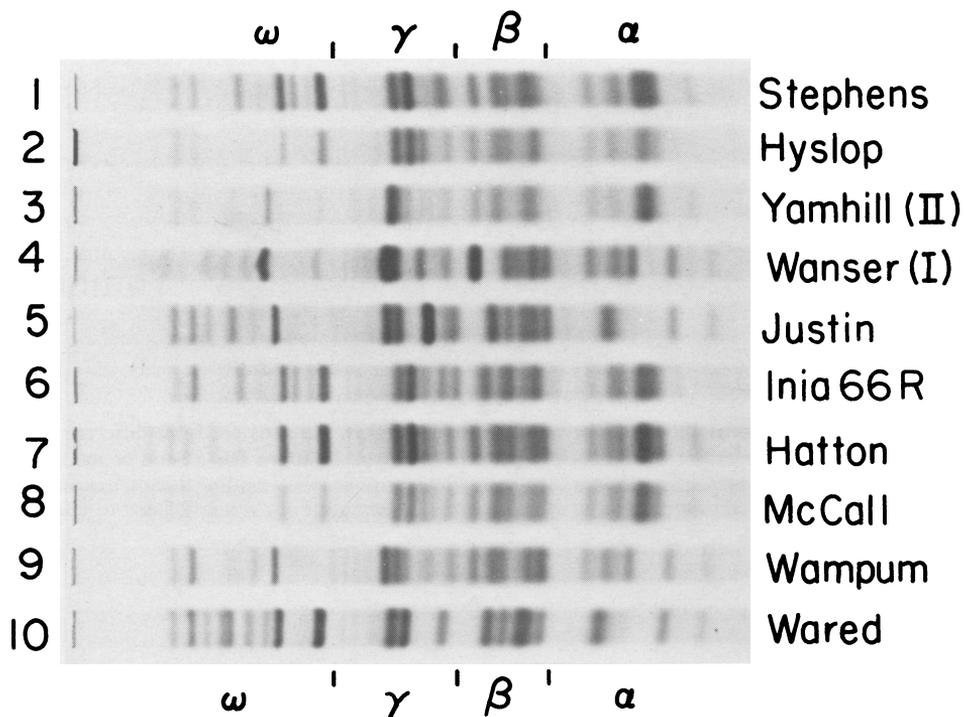


Fig. 13. A-PAGE patterns from Pacific Northwest varieties

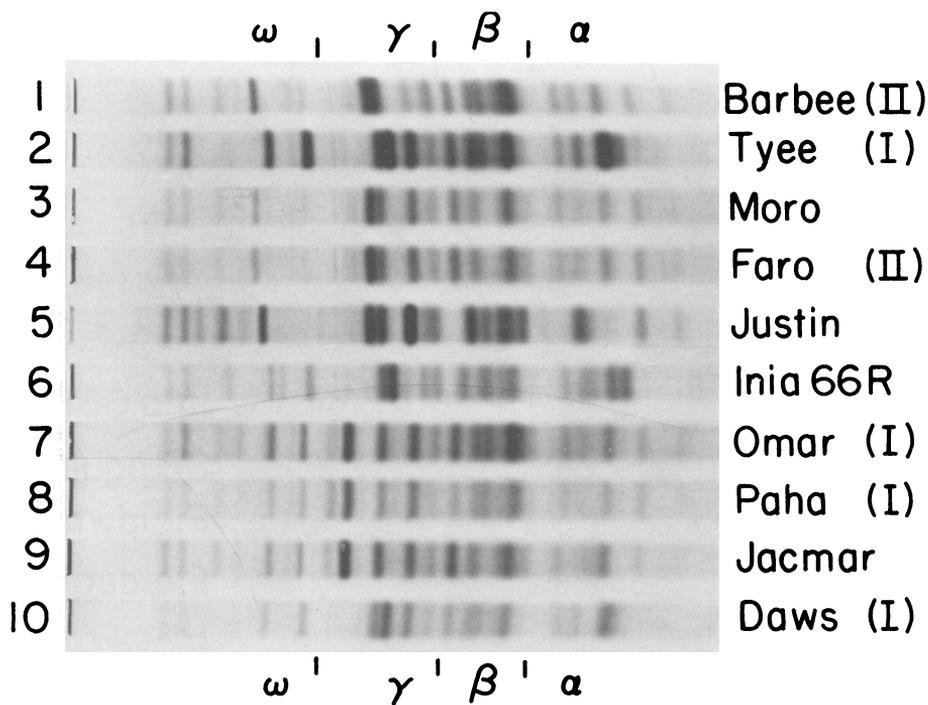


Fig. 19. A-PAGE patterns from additional Pacific Northwest varieties, including club wheats

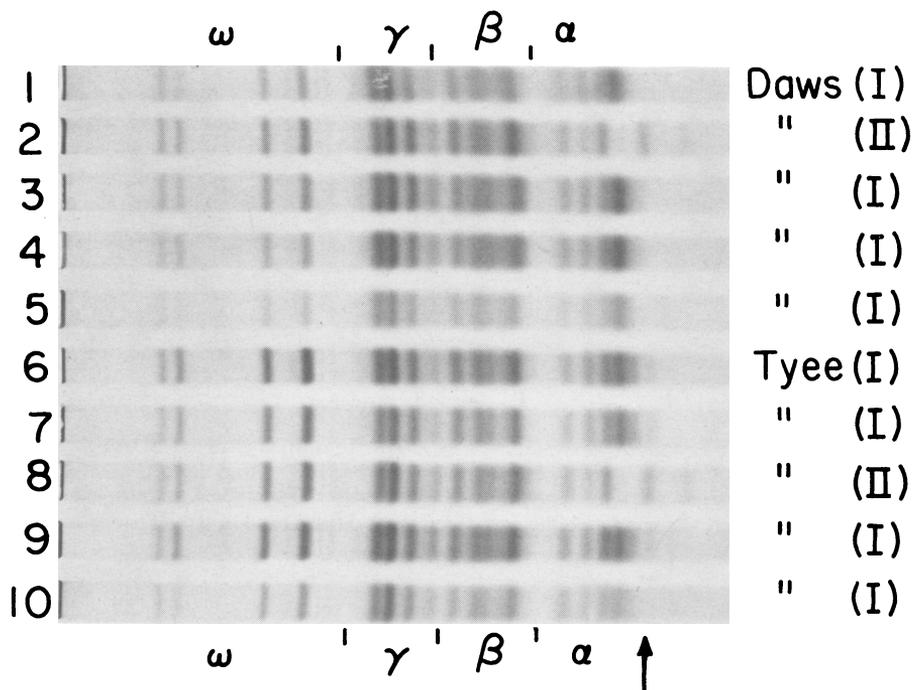


Fig. 20. A-PAGE patterns showing biotypes in Daws and Tye varieties. In both varieties, the type I patterns lack the two bands of highest mobilities in the type II patterns, but the next two bands are much more intense.

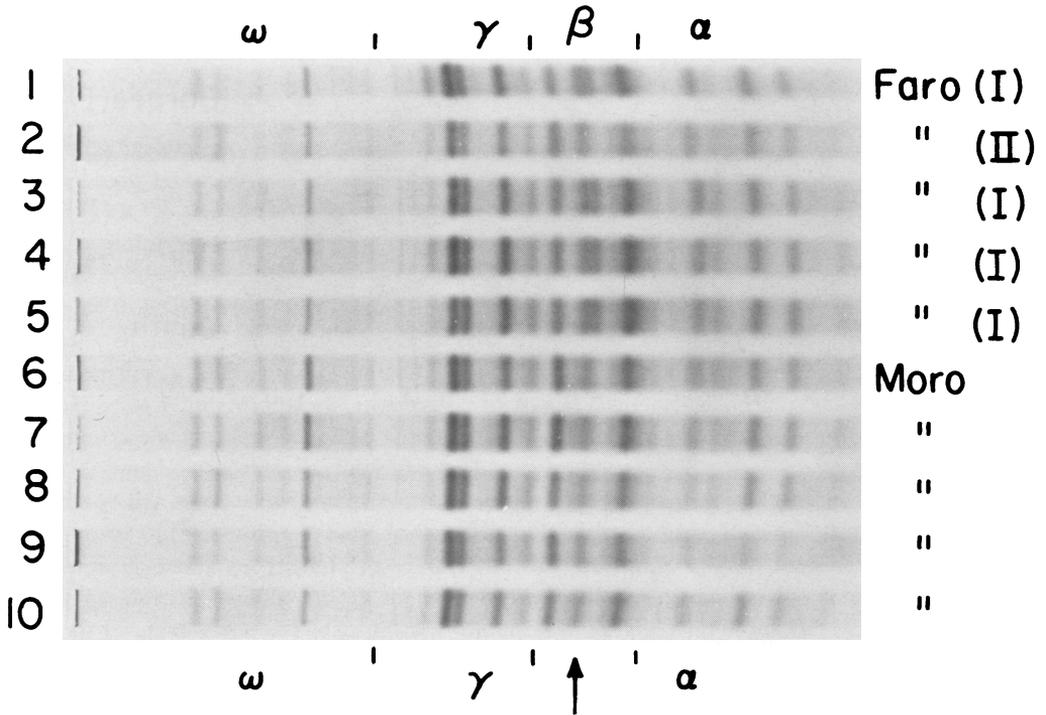


Fig. 21. A-PAGE patterns comparing Faro and Moro varieties. Conditions are as for figure 1, except migration has extended by about 2 cm to give better separation of components. The type II Faro pattern lacks a band in the β region present in the type I pattern.

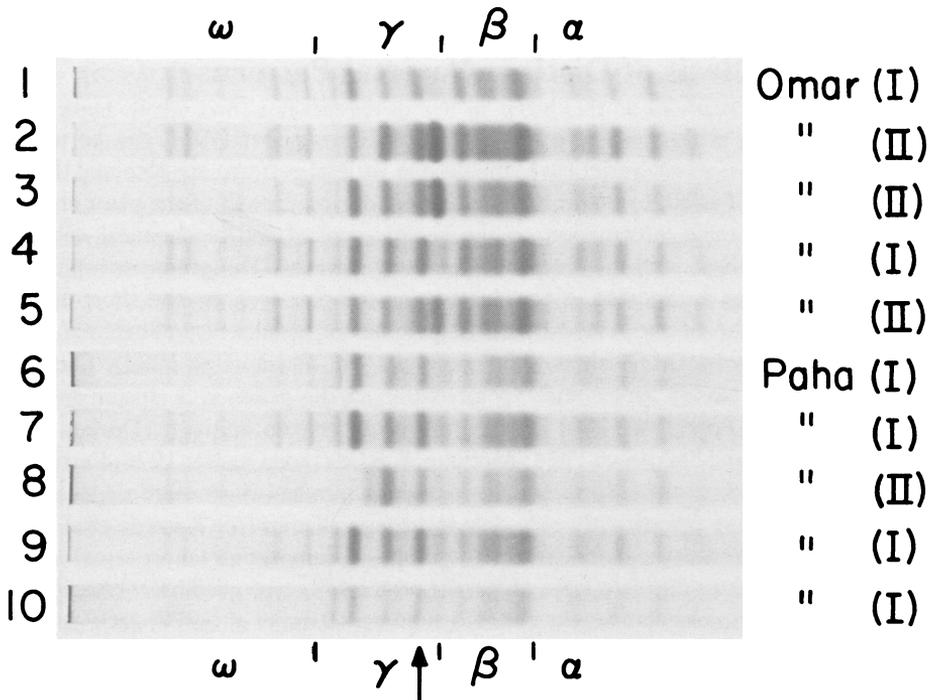


Fig. 22. A-PAGE patterns showing biotypes in Omar and Paha varieties. In the type I Omar pattern, the leading γ -region band is weak and much less intense than the next following band; in the type II pattern, the leading γ -region band is much more intense and stronger than the following band. In the Paha patterns, one of the slower γ -region bands of the type I pattern is absent from the type II pattern.

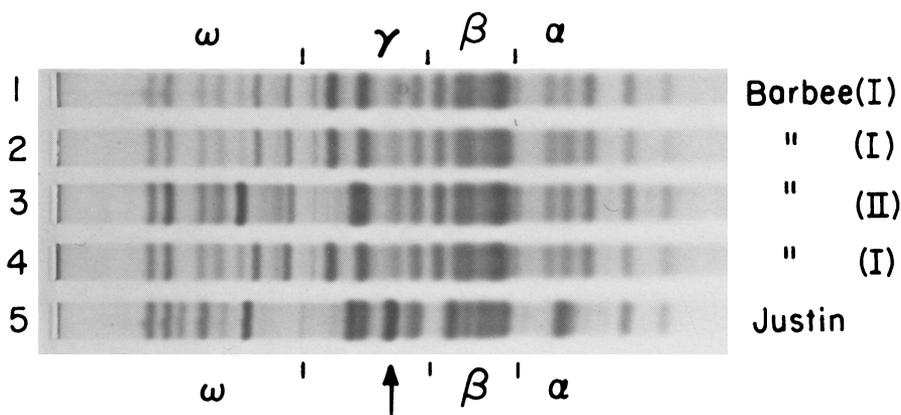


Fig. 23. A-PAGE patterns showing biotypes in Barbee. The type I and II patterns differ in the lower-mobility bands of the γ section.

GENERAL DISCUSSION

Basis of Distinct Varietal Patterns

Our results agree with those of many others in showing that PAGE can be used to identify some or most wheat varieties. Many unique varietal patterns are obtained that are affected minimally, if at all, by environmental factors during growth of the plant and seed production. Considerable information on the genetic control and biochemical nature of the gliadins is consistent with the observance of many patterns. The genes controlling the formation of gliadins are located in six clusters, or complex loci, on the short arms of chromosomes 1 and 6 of the A and B and D genomes of bread wheat (Shepherd 1968; Wrigley and Shepherd 1974; Sozinov and Poperelya 1980; Payne et al. 1984). The genes in each cluster are closely linked and recombination of the genes in a cluster or allelic block (Sozinov and Poperelya 1980) is rare in crosses between cultivars (Doekes 1973; Sozinov, Poperelya, and Stakanova 1974; Mecham, Kasarda, and Qualset 1978; Payne et al. 1984). Although each complex locus usually includes a number of genes, those located on chromosomes of group 6 apparently code only for α -type gliadins (see Kasarda et al. 1983, for terminology), whereas the complex loci on group 1 chromosomes (short arms) include genes that code for three different types of storage proteins, ω -type gliadins, γ -type gliadins, and some of the low-molecular-weight glutenin subunits (Bietz et al. 1977; Jackson, Holt, and Payne 1983; Shewry, Mifflin, and Kasarda 1984). The electrophoretic patterns of different wheat cultivars represent various combinations of these allelic blocks.

Many different "blocks" or stable combinations of genes have been recognized (Sozinov and Poperelya 1980; Lafiandra, Kasarda, and Morris 1984), although many of the variations

found for any single complex locus are similar—differing qualitatively in only one or a few components. These differences could result from rare recombinations of the genes in a block during crossing, or from mutational changes in genes (such as point mutations, unequal crossing over, or gene conversion) that result in differences in charge or size of the equivalent protein components (Kasarda et al. 1984a, 1984b). Such differences undoubtedly contribute to the wide range of patterns we have observed for different wheat varieties.

In addition, we have occasionally noted differences in intensity for apparently equivalent protein components in the patterns of different varieties. Such intensity differences might result from differences in the frequency of gene transcription as a consequence of differences in noncoding DNA that has a controlling function, e.g., promoter sequences. Alternatively, although most gliadin genes probably code for proteins that are separable by one- or two-dimensional electrophoretic methods, there remains the possibility that some gene copies are identical or so similar that the resulting proteins are not distinguishable by electrophoretic analysis. Differences in the number of such copies among varieties could change the amount of protein synthesized during endosperm development, thus affecting intensity of the equivalent band in the electrophoretic patterns. Recombinant DNA studies of wheat gliadin genes (Rafalski et al. 1984; Kasarda et al. 1984b; Anderson et al. 1985) are progressing rapidly and, in the near future, will very likely provide definitive explanations for qualitative and quantitative differences among gliadin proteins.

PAGE Methodology

The two PAGE procedures were chosen because they differed in many details, e.g., gel size, uniform gel concentration vs. a gradient, buffer composition, horizontal vs. vertical positioning. The characteristic differences in the banding patterns given by the two procedures were discussed earlier.

The AG-PAGE procedure, described by Wrigley and McCausland (1974), is much the more rapid of the two, and the advantage in speed can be increased by the purchase of prepared gradient gels. It was especially useful for initial screening of a fairly large number of seeds; in addition to its speed, an occasional variant pattern was easy to detect among several identical ones.

Nevertheless, the A-PAGE procedure was useful in two ways: (1) Patterns from most other laboratories have been obtained in gels of uniform polyacrylamide concentration, so that the A-PAGE patterns were more easily compared with those in most published reports than were AG-PAGE patterns. (2) The clearer resolution of more bands in some cases was useful in examining polymorphic seed lots, particularly those in which differences in patterns were small, or in identifying some varieties when two or three had similar patterns. The extra resolving power was needed, for example, with the club wheat varieties.

Improvements in both speed and resolution over the procedures we used can be expected; in fact, some have been made in the resolution obtained with A-PAGE procedures since this work was begun. Also, the use of high-performance liquid chromatography to separate gliadin components for varietal identification has been proposed recently (Bietz and Cobb 1985) and may prove advantageous in some ways. Aside from the suitability of methods for separation of gliadin components, methods for varietal identification could be improved markedly by automated and computerized scanning of patterns and comparisons with known varietal patterns. Such procedures already have been developed and used to some extent by others (Wrigley, Robinson, and Williams 1981; Bushuk, Saperstein, and Zillman 1978; Lookhart et al. 1983) with considerable success.

Polymorphic Varieties

The varieties we examined were from production areas not well represented in earlier reports, and, by analyzing single seeds, biotypes were found in one-third of the seed stocks—more frequently than expected from other reports. The most probable source of the variants appears to be the presence in the varietal samples of a segregant from the original cross in addition to the one giving the predominant patterns. Appleyard, McCausland, and Wrigley (1979) were concerned with off-types in the Australian varieties Condor and Egret, and Kosmolak and Kerber (1980) with off-types in the Canadian variety, Marquis. The latter authors listed as sources of off-types “variability that is indigenous or inherent at the time of release by the breeder; that which is due to mechanical mixture; and that which may arise through spontaneous mutation.” Appleyard, McCausland, and Wrigley (1979) list essentially the same sources, and point out that if two or more segregants of the original cross are present in a variety, all should show characteristics of the parents, while off-types from outcrossing could introduce new characteristics and give different patterns among the seeds of one plant or head. We examined only threshed wheat and so could not have identified the latter cases. In most cases, the variants we found yielded the same pattern from several seeds, indicating that their source was another segregant of the original cross rather than outcrossing. In Wanser, however, nine variant seeds gave at least four different patterns; and in others, e.g., Newana and Yamhill, so few variant seeds were found that no inference can be made.

Any part of the PAGE patterns could be involved in the difference responsible for a biotype, as noted with the discussion of the various patterns; and the differences ranged from obvious to barely detectable with the procedures we used. In the sample of Jori 69, the biotype patterns are distinctly different, involve intense bands, and seem to occur in roughly equal numbers. In such a case, the presence of variants can hardly be questioned; rather, it might be expected that plant characteristics would give some indication, too. The sample was from the 1979 crop, and the variants were present in about equal numbers; it appears that they must survive and reproduce about equally well to maintain the equal numbers 10 years after release. However, 20 seeds, divided 11 and 9, are not enough for more than a very rough estimate of proportions in the whole sample.

In contrast to the case with Jori 69, some of the varietal differences and variants reported here may appear to be based on such slight differences as to raise a question whether a unique varietal pattern or biotypes should be considered to exist. However, weak bands have been found to provide just as consistent indication of varieties and biotypes as strong bands (although more difficult to show in photographic reproductions). The different Mexicali 75 patterns are examples; they differ from one Jori 69 variant only in that the Jori 69 pattern has a broader weak ω band (probably formed by two weak components not completely resolved), where the Mexicali 75 variants have either a narrow band or no band. Raeder variants also yielded two patterns with minor differences in ω bands, and those differences also could be seen consistently in the gel patterns.

A pattern variation that is difficult to pick up consistently is the presence or absence of a weak band just ahead of or behind a heavy band. If good resolution of such bands is not obtained, the number of bands present is not clear; even with fairly good resolution, a too heavy loading, e.g., from a seed of higher than usual protein content, will obscure the presence of weaker bands. An example is the leading band just ahead of the heavy α band in the Tyee variant pattern, which is absent from the otherwise similar pattern of Daws.

Finding reliable pattern differences in the club varieties as a group was the most difficult assignment, and differences in band intensity were relied upon, in addition to number and mobility of bands. The club wheats appeared to fall into two groups of closely related varieties, with biotypes of nearly every variety (in some cases these gave the same pattern as the predominant pattern of another variety).

Identifying Market Classes by PAGE

Wheat usually is classified and handled in commerce by market classes rather than by variety. The presence of common wheats in durum or club classes is not always easy to detect, however, raising the question whether the presence or absence of specific bands in PAGE patterns might be used to check samples of different market classes for the presence of improperly classified wheat. For such purposes, the identification of specific varieties would not be necessary.

Such a possibility occurs in the case of the five California- and Arizona-grown durums. They lack ω bands that migrate as slowly as the two slowest bands in Justin and Inia 66R and the two or three slowest bands in all the other nondurum wheats used in this work. Bands of such low mobilities from a sample of wheat classified as durum would indicate the presence of common or club wheats. The bands are well separated and fairly heavy, suggesting that such a test could show low levels of nondurum kernels. Examination of ground bulk sample would be more efficient than examination of individual kernels, if sensitivity for the specific bands is high enough. The possibility of distinguishing durum from common wheats in this way was pointed out by Wrigley and Shepherd (1974) on the basis of similar observations, i.e., absence of the slowest two or three ω bands in patterns from two Australian durum varieties. Durum wheats are tetraploid, lacking the D genome. The genes coding for the ω gliadins of lowest mobility in common (hexaploid) wheat are on chromosome 1D (Payne et al. 1984; Kasarda, Bernardin, and Qualset 1976; and Shepherd 1968).

The Western White market class must have at least 10 percent club wheat blended with white common wheat (Anonymous 1978). It is difficult to determine the 10 percent frequency in a commercial sample by visual means. If the banding patterns of the club and common white varieties were distinct, it would be a simple matter to verify by PAGE that 10 percent or more club varieties occurred in the sample. Unfortunately, the club winter wheats showed considerable variability among varieties and no band or group of bands could be uniquely used in verifying the Western White class. However, it may be possible to introduce a "club wheat band" into future club varieties for ease of identification of the Western White class.

Patterns of the common wheats were so varied that no indication of pattern features characteristic of other market classes, such as hardness, red or white seed coat color, or spring or winter habit, were noted.

RECOMMENDATIONS

Earlier mention of the possibility for improvements in varietal identification methods was not meant to imply that use of the PAGE procedures now available should be delayed. Present techniques are reliable and sensitive enough to distinguish many gliadin patterns; it seems useful to install some system to determine and record gliadin patterns at the time of release of new wheat varieties. In view of the relatively large number of polymorphic varieties, it would be useful to reselect them to a uniform type, if there are no adverse effects after "cleaning up" such varieties. Gliadin patterns then would provide a sensitive method for detecting the intrusion of any off-types into a seed supply and the appearance of additional segregants. The PAGE procedures could also provide a means of checking the composition of the Western White market class and of multiline varieties in which biotypes resistant to different strains of diseases are to be maintained in definite proportions.

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