COMPUTER-BASED QUANTIFICATION, WHEAT CULTIVAR IDENTIFICATION AND COMPARATIVE ANALYSIS OF GLIADIN ELECTROPHOREGRAMS

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HARRY D. SAPIRSTEIN

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To my parents

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ABSTRACT

Sapirstein, Harry Daniel, Ph.D., The University of Manitoba, July 1984. Computer-Based Quantification, Wheat Cultivar Identification and Comparative Analysis of Gliadin Electrophoregrams

Major Professor: Dr. Walter Bushuk.

A computerized wheat cultivar identification system is described based on gliadin electrophoregrams. This research is divided into two sections:

I. Automatic quantification of electrophoregrams by minicomputer processing of densitometric scanning profiles.

In order to eliminate the subjective and tedious manual calculation of electrophoretic mobility and band density values required for cultivar identification, algorithms were developed and implemented as FORTRAN programs to operate on sets of replicate absorbance profiles acquired by a linear scanning densitometer. The polyacrylamide gel electrophoresis (PAGE) system of Bushuk and Zillman (1978) was used to prepare a representative range of gliadin electrophoregrams for analysis.

Data analysis and processing was performed on a laboratory scale PDP-11/40 minicomputer. Densitometric profiles were digitized and protein bands found by a procedure based on first derivatives which detects components as relative maxima or as inflection points on peak slopes. The sensitivity of this procedure was such that virtually all

major and minor electrophoregram components visible on film or photographic prints were detected by the program.

Internal gel reference proteins were used to scale raw peak position and height coordinates to form a standardized list of normalized relative mobility and band density parameter values termed a cultivar signature array. This permits comparisons to be made with band patterns from other gel slabs, a process which was facilitated by computer-generated electrophoregram graphics.

A replicate analysis program was also developed to combine individually computed gliadin band patterns from replicate densitometric profiles into a single mean signature array. This task functions to improve data precision and to eliminate spurious information which is identified by the program as unmatched relative peak coordinates.

Computed relative mobility and band density data were found to be at least equivalent in precision and accuracy to results based upon manual measurement procedures, the latter obtainable only by tedious migration distance measurements and subjective and possibly erroneous estimates of band density. It was concluded that the facility of a densitometer and minicomputer can be successfully implemented to quantify gliadin electrophoregrams in an optimal format for cultivar identification.

II. Computer-based wheat cultivar identification system.

Vertical flatbed PAGE was used to establish a data base of gliadin electrophoregrams from bulk and single kernel samples of 107 Canadian common and durum wheat cultivars and nine U.S. common spring wheat cultivars. Approximately 20% of the total were found to possess

composite electrophoregrams with varying degrees of admixture. Many offtype patterns were incorporated into the computerized catalog of cultivar formulas.

A new gliadin nomenclature is introduced, substantially modifying the single reference band method of Bushuk and Zillman (1978). In addition to "Marquis band 50" which serves as a primary reference, the nomenclature uses two additional reference bands with low and high mobilities respectively in electrophoregrams of the standard cultivar patterns used for each PAGE run. The three reference bands are used in a weighted nearest neighbor algorithm to compute relative mobilities for cultivar formulas. This method significantly improved the precision of results compared to the single reference band approach. The average uncertainty in the relative position of gliadin bands was reduced more than three-fold with mean standard deviations falling below 0.1 relative mobility units. The effect to increase the discrimination power of electrophoregram data for cultivar identification is discussed.

A computerized system of wheat cultivar identification is described. The system is comprised of three programmed procedures which are dedicated to different aspects of the comparative analysis problem. The common task is to compare a numerically encoded unknown or sample electrophoregram with all reference patterns in the data base taken one at a time, to obtain a measure used to assess the degree of pattern resemblance and on this basis prepare a ranked list of cultivars. Programs were written in FORTRAN and were tested on an AMDAHL 470/580 computer system (IBM 470 compatible). The following components of the cultivar identification system are discussed:

1. Data base organization of cultivar names, pedigrees and compact integer representation of gliadin electrophoregrams as cultivar signature arrays which additionally encode attributes for quality, class and primary growing region.
2. The equation used to compute percent pattern homology (%PH) scores. Included as parameters in the formula are both matching and non-matching band counts, and bands which differ significantly on a density basis alone. This fully quantified the heterogeneity of gliadin band patterns for comparative analysis, and provided a sensitive measure for discrimination. Also examined is the effect of weighting the number of matched and unmatched electrophoregram components by their band densities.
3. Detail of the short list ranking program output which generates a list of possible cultivars ordered by declining PAGE pattern homology with the unknown electrophoregram. A user defined threshold value for %PH controls entry into the list. Tabulated data includes cultivar names, pedigrees, summary attributes and an accounting of the number of matched bands and the distribution of unmatched gliadin bands for compared electrophoregrams.
4. For the list of ranked cultivars, a graphic analysis program permits the user to visualize the gliadin band patterns for matching and non-matching components which are respectively isolated in separate plots. An alternate pattern homology analysis program can also be invoked to provide a similar type of output focused on selected pairs of electrophoregrams of special interest.

5. A third program element of the cultivar identification system complements other analyses by using positional differences in electrophoregrams as the criterion for ranking. It also provides an output result which is extended to include the entire data base population in which each member is explicitly identified in a frequency distribution which comprises the printout. This program assists in evaluating the uniqueness of the unknown or test electrophoretic pattern, and identifies cultivars which are of diverse genotype.
6. The use of regression analysis to stretch or compress the scale of relative mobilities in one electrophoresis system to permit accurate registration with counterpart data in a different system.

In addition to cultivar identification methodology, the heterogeneity of gliadin PAGE patterns in the data base was studied by computing the frequency distribution of protein bands as a function of fine scale relative mobility. The resulting "PAGE map" of gliadin composition confirmed the existence of more than 90 individual gliadin components among a population of 98 common spring and winter wheat cultivars. The map is proposed as a reference spectrum to facilitate the classification of gliadins for the purpose of multivariate analysis and inter-laboratory comparisons.

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I

INTRODUCTION

The alcohol soluble proteins of the wheat kernel (gliadins) may well be the single most heterogeneous class of plant proteins known. Moreover, the number and distribution of these proteins, commonly separated by polyacrylamide gel electrophoresis (PAGE) to yield a gliadin electrophoretic pattern or electrophoregram, is a genotypic character virtually unaffected by the environment in which the crop is grown. Accordingly, the electrophoregram can be considered a taxonomic signature at the subspecies level of the grain from which the gliadin was derived.

While electrophoretic methods have been applied extensively in gluten protein research and patterns interpreted widely for genetic and quality relationships, the utility of results for broad-based comparisons has been limited in large part by the inherent multiplicity of the gliadin protein fraction and resolution by different electrophoretic systems. Efforts in recent years to standardize the latter for identification of cereal varieties (Autran and Bourdet, 1975; Bushuk and Zillman, 1978) resulted in the first numerical catalogs of wheat protein composition by starch gel electrophoresis (SGE) and PAGE respectively for French and Canadian cultivars. A catalog of U.S. and Italian cultivars derived by PAGE has subsequently been reported by

other workers. However, aspects of precision and the problem of identifing common bands within and among catalogs minimizes their individual and combined value. Elsewhere, subjective nomenclature formats and/or classification procedures has restricted the interpretation of multivariate analysis results of gliadin composition to localized gene pools.

Given the steady accumulation of gliadin electrophoregrams, the need of a uniform nomenclature for gliadin electrophoresis has been recognized (Autran et al., 1979). Systematic treatment of electrophoregram data with emphasis on reproducibility and precision is also indicated by the increasing demands for procedures of cultivar identification in commerce, the introduction of plant breeders' rights, and related documentation of wheat genetic resources. As more knowledge is gained about gluten proteins in relation to functionality, screening of breeding populations based on protein composition will become commonplace.

The objectives of the present study are basically twofold: (i) to evaluate computer-based densitometric scanning as an automatic method to quantify electrophoretic data required for cultivar identification and (ii) to present a computerized system of cultivar identification based on gliadin electrophoregram mobility and density parameters. The effect of using multiple reference bands to compute relative mobilities was examined, and on this basis a a high-resolution computerized catalog of electrophoregrams for Canadian and some U.S. wheat cultivars was prepared. Various comparative analysis schemes for cultivar identification are described and numerous examples of program output are

given. Regression analysis was investigated as a potential method to accurately cross-reference between electrophoregram data base catalogs. The research was also extended to develop a strategy that could quantitate the overall level of gliadin heterogeneity commonly observed in a large series of electrophoretic runs. The resulting PAGE "map" of gliadin composition was also examined as a means to classify gliadin bands for the purpose of multivariate analysis.

Throughout the text of this study, wheats of the species Triticum aestivum L. (hexaploid wheats with the genome formula AABBDD) will be referred to as common wheats, while wheats of the species Triticum turgidum L. s.sp. durum (tetraploid wheats with the genome formula AABB) will be referred to as durum wheats.

II

LITERATURE REVIEW

INTRODUCTION

The major focus of this thesis project was to investigate and extend current methods for comparative electrophoretic analysis of wheat gliadins and to characterize further the heterogeneity in its composition. Accordingly, this review will cover a range of publications on gliadin which document the affirmation of the protein fraction's multiplicity, its progressive resolution by electrophoresis and some related techniques, and the variable approaches taken within the separation methods employed. Also reviewed will be the irregular nature of gliadin nomenclature and the application of electrophoretic pattern data for the comparative analysis of wheats especially for cultivar identification.

DEFINITION OF WHEAT GRAIN PROTEIN CLASSES

The scientific watershed for the study of wheat proteins derives from the comprehensive fractionation scheme developed by Osborne (1907). Wheat proteins were classified into four major fractions based on their differential solubility by sequential solvent extraction:

i) albumins - soluble in water

ii) globulins - soluble in salt solutions

iii) gliadins - soluble in 70% to 90% alcohol

iv) glutenins - soluble in dilute acid or alkali

Gliadin and glutenin are Triticum specific names that correspond to broader categories of cereal proteins termed prolamin and glutelin respectively. Prolamin was chosen as the generic name for the alcohol soluble group (Osborne, 1908) as all related proteins (e.g. wheat=gliadin, barley=hordein, maize=zein, rye=secalin) yielded relatively large quantities of proline and amide nitrogen upon hydrolysis.

That the classification of proteins based on solution properties suffers from certain inadequacies was recognized by Osborne (1924) himself. The perspective provided by current electrophoretic methods reveals a heterogeneity of wheat protein composition that 60 years earlier would have been considered fantastic. Notwithstanding problems relating to overlapping solubility which are commonly cited in the literature, it is universally accepted that "classical" gliadin and glutenin are structurally distinct in the unreduced state, are the main constituents of gluten, and dominate the physical and chemical nature of functional properties of wheat flour. However, what constitutes the boundary between the chemically reduced forms of these complex protein fractions is at present an issue of some disagreement. The contrasting positions are reflected in the reviews by Kasarda et al. (1976), Miflin and Shewry (1979) and Miflin et al. (1983).

EARLY PHYSICAL EVIDENCE FOR GLIADIN HETEROGENEITY AND MOVING BOUNDARY ELECTROPHORETIC RESOLUTION

The term gliadin was originally proposed by Taddei (1820) to describe the gross fraction of wheat gluten soluble in aqueous alcohol. The composition of this protein was discussed by Ritthausen (1872) who provided evidence for 3 distinct fractions: gliadin, mucedin and glutenfibrin, which were soluble in varying concentrations of alcohol. Osborne (1907), while not discounting the possible fractionability of gliadin at some future date, maintained that differences in solubility as reported by Ritthausen, could not be made a basis for characterizing different individual proteins. Osborne, who first formally defined the fraction, reaffirmed the individuality of gliadin as "the protein of the wheat kernel insoluble in neutral aqueous solutions, but distinguished from all others by its ready solubility in neutral 70% alcohol".

Following Osborne's work, the literature concerned with the nature of wheat gluten components is characterized by a protracted period of frustrated attempts to achieve clear-cut and quantitative fractionations. During this period, many studies call into question the identity of protein material extractable from wheat flour by alcohol (Bailey and Blish, 1915; Gottenberg and Alsberg, 1927; Gortner et al., 1929; Blish and Sandstedt, 1929; Herd, 1931; Sandstedt and Blish, 1933; McCalla and Rose, 1935; Kuhlman, 1937). While these workers raised questions that could not be answered by the fractional precipitation and solution techniques available at the time, evidence began to accumulate in support of heterogeneous gliadin upon examination of the physical properties of individual subfractions.

Haugaard and Johnson (1930) used precipitation temperature (Dill and Alsberg, 1925) as an index to characterize different gliadin preparations. They showed that gliadin could be fractionated by cooling a solution in 60% ethanol first to 0°C and then to -11°C. Optical rotation and viscosity measurements were used to associate varying physical properties with the three resulting fractions. Cook (1931) confirmed this result, finding that gliadin preparations with a high maximum solubility defined by a low precipitation temperature displayed lower viscosities than fractions of lower maximum solubility with higher peptization temperatures. He concluded that gliadin consists of a number of fractions.

Krejci and Svedberg (1935) reported in a sedimentation equilibrium study that gliadin was inhomogeneous with regard to molecular weight, consisting of a mixture of light and heavy molecules. The latter increased in proportion in fractions of declining solubility as defined by the method of Haugaard and Johnson (1930). Similar conclusions were reached by Burk (1938) based upon osmotic pressure measurements on different cold-labile fractions of gliadin, and by Lamm and Polson (1936) who found gliadin to be inhomogeneous by a diffusion method.

McCalla and Gralen (1942) applied sedimentation equilibrium and diffusion measurements on gluten dispersed in sodium salicylate solutions. They agreed with observations made in previous studies which showed that the existence of homogeneous gliadin was incompatible with experimental evidence. They concluded from their data that gluten was a multicomponent protein system varying progressively in chemical and physical properties.

The separation of ionic mixtures which depends solely upon differences of free electrophoretic mobilities among constituents forms the basis of the moving boundary or free electrophoresis procedure of Tiselius (1937). The technique was first applied to wheat proteins by Schwert et al. (1944) and resulted in the identification of at least two electrophoretically separable components of gliadin. It was recognized that a low pH, low ionic strength buffer system would provide the best separations. Their study and the subsequent use of this technique for the fractionation of gliadin (Mills, 1954; Holme and Briggs, 1959) and gluten proteins (Laws and France, 1948), to display heterogeneity, yielded patterns of poor definition due to gross asymmetry between the ascending and descending boundaries, a condition believed to be caused by protein-protein and protein-buffer ion interactions. As a result, Holme and Briggs (1959) described their own and previous results in support of heterogeneity as inconclusive.

The imposition of pattern symmetry in free electrophoresis as an important precondition to characterize the minimum number of gluten components was used by Jones et al. (1959) to evaluate a number of suitable buffers for the fractionation of gluten proteins. Sodium and aluminum buffers, notably aluminum lactate - lactic acid pH 3.1, at nominal ionic strength of 0.05-0.1 (8.5-17 mM), were used to resolve gluten dispersed in 0.01 N acetic acid, from a hard red winter wheat cultivar Ponca. Six protein components revealed in Schlieren diagrams were classified as $\alpha 1$, $\alpha 2$, $\beta 1$, $\beta 2$[1], γ and ω, in order of decreasing

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[1]Whereas the α-components of cv. Ponca were resolved only in aluminum chloracetate buffer, the β-components were resolved only in the aluminum lactate counterpart.

migration distance from the initial boundary. A significant portion of the high mobility α component was shown to consist of glutenin protein, with the remaining components identified as gliadins.

ZONE ELECTROPHORESIS AND GLIADIN RESOLUTION

The elucidation of gluten protein composition was significantly improved with the application of zone electrophoresis in a starch gel support medium introduced by Smithies (1955). Smithies recognized the capacity of the gels to sieve high molecular weight (MW) substances and the utility of starch gel electrophoresis (SGE) to separate mixtures of proteins according to size as well as charge differences.

In the first reported use of this method for wheat proteins, Elton and Ewart (1960) employed a 12% starch gel with the aluminum lactate buffer system of Jones et al. (1959) to separate a preparation of gluten dispersed in acetic acid. SGE for 2-4 hr at a potential gradient of 4-6 V/cm was found to resolve gluten of four cultivars (Flamingo, Rescue, Bison, Witchita[1]) into at least eight bands of "similar" mobility. The authors described the difficulty in achieving reproducibility in terms of absolute mobility, which was observed to vary by as much as 20% between successive runs. No further characterization of the gluten protein separation was made. The possibility that some bands (of the acetic acid dispersed gluten prepared from distilled water-washed flour) might correspond to albumin and globulin protein was noted elsewhere (Simmonds and Winzor, 1961).

[1]Bison and Witchita are two hard red winter cultivars very similar in ancestry.

The incorporation of concentrated urea (3M) to the aluminum lactate SGE buffer (urea-SGE) was used by Woychik et al. (1961) to increase protein solubility and thereby improve the detection of minor bands. Gluten prepared by the method of Jones et al. (1959) was electrophoresed in 18% gels at 8 V/cm for a reported 24 hours. Eight protein components that migrated in the gel were considered to be gliadins.

Protein that remained at the origin unable to penetrate the starch gel due to molecular size and/or shape contraints was characterized as being identical to the α1 gluten (glutenin) component in free electrophoresis. This component was reported by Jones et al. (1961) to have a weight average molecular weight (MW) of 1.5 - 3 million by sedimentation equilibrium in aluminum lactate buffer. In contrast, β and γ-gliadins were shown to have MW's of 42,000 and 47,000 respectively. Thus zone electrophoresis provided a means at the time, for distinguishing between gliadin and glutenin components possessing similar mobilities in free electrophoresis. SGE could therefore be used to effectively separate gliadins from the structurally different glutenin fraction in spite of any overlapping solubility of the gluten proteins in the extracting solvent.

Early studies using SGE commonly applied to separate ethanol extracts of flour or gluten dispersions in acetic acid often described sub-optimal resolution of gliadin proteins. Most authors were seemingly unaware that a longer period of electrophoresis could expedite the resolution of the gliadins in sample preparations that often contained overlapping protein fractions.

Coulson and Sim (1961), using the buffer system of Jones et al. (1959), identified more than 20 components by 10% SGE (8-10 V/cm, 2 hr.) of acetic acid soluble gluten prepared from flour washed successively with butanol and sodium pyrophosphate according to the procedure of Simmonds and Winzor (1961). This extraction procedure included a significant proportion of albumin/globulin proteins as only the slower moving bands were reported to correspond to ethanol soluble gliadin. The authors also noted that the addition of up to 8M urea to the protein solution prior to electrophoresis did not affect the resulting patterns.

Incomplete electrophoretic resolution of gliadins was similarly obtained by Elton and Ewart (1962) and later by Kaminski (1962) who used SGE for direct comparison of different flour protein extracts on individual gel slabs. Only partial separation of 70% ethanol soluble gliadin was achieved in order to accomodate resolution of higher mobility albumin and globulin proteins. These were shown to be present in electrophoregrams of gluten dispersed in acetic acid (Elton and Ewart, 1962) and aluminum lactate buffer (Kaminski, 1962), however only traces were found in patterns of the ethanol soluble extracts. Thus of 21 bands detected in the SGE pattern of buffer dispersed gluten, 10 major components of low mobility were attributable to gliadins.

During this period, the first attempt to specifically optimize the electrophoretic resolution of gliadins was made by Bourdet et al. (1963). Direct 60% ethanol extracts of wheat flour was shown to yield preponderantly gliadin components by urea-SGE (10% starch gel, Al lactate buffer + 0.5 M urea, pH 3.25). By separating the gliadins over the entire gel, the authors obtained an average of 15 bands for each of

three cultivars studied. A combined total of 27 different components in terms of mobility were detected. Graham (1963) and Elton and Ewart (1964) likewise improved the resolution of acetic acid soluble proteins identified as gliadins by extending the electrophoresis time period.

The incorporation of polyacrylamide (Raymond and Weintraub, 1959; Raymond and Wang, 1960) as a homogeneous and stable support medium, greatly improved the overall flexibility of the zone electrophoresis technique as a set of gel parameters were introduced that could be controlled to selectively modify the sieving effect on protein separations. Polyacrylamide gel electrophoresis (PAGE) of acetic acid and water soluble wheat proteins was first reported by Lee (1963) and Nimmo et al. (1963) respectively. Lee used an alkaline buffer system (pH 8.6) containing 2M urea in 5% gels to effect a gluten protein separation. While the resolution of components in this study by PAGE was limited, Lee utilized the transparent quality of the support medium to scan the gel with a densitometer in order to obtain an objective quantitation on the amount of protein in a band, as well as to identify minor components not readily visible in the gel.

Chemical reduction of gliadin in conjunction with electrophoresis was first undertaken by Woychik et al. (1964). They showed that a gliadin preparation, treated with the disulphide bond reducing agent mercaptoethanol, and resolved by urea-SGE gave no increase in the number or density of bands compared to an unreduced treatment, although mobilities were reduced by about 20%, presumably caused by an increase in resistance to migration for the unfolded gliadin polypeptides. In contrast, a glutenin fraction normally unable to penetrate the gel

during electrophoresis, yielded as many as 20 bands following reduction. These results were interpreted as deriving from the different nature of disulphide bonding in gluten proteins, being largely intra-molecular for the gliadin fraction. This conclusion was subsequently supported by viscosity and sedimentation equilibrium measurements (Nielsen et al., 1968) and by sodium dodecyl sulphate PAGE (SDS-PAGE; method is discussed later) which was first applied to gluten proteins by Bietz and Wall (1972) to convincingly show that most gliadins are single-chain polypeptides stabilized by intra-molecular disulphide bonds.

The manner in which heterogeneity could be masked in a whole gliadin separation by gel electrophoresis was reported in a study by Huebner et al. (1967). They found that the γ-gliadin component of the cultivar Ponca, resolved by urea-SGE [buffer: 3M urea, 5mM aluminum lactate, 0.02M lactic acid, after Beckwith et al. (1966)] as a single dense band, could be fractionated by cation exchange chromatography to yield 3 distinct components. These were subsequently shown to have slightly different relative mobilities by extending the electrophoresis period. Further analysis revealed that the individual proteins possessed significant differences in amino acid composition with smaller differences detected in γ2- and γ3-gliadin. N-terminal amino acid sequence data of Bietz et al. (1977) confirmed this finding. More positional homology in the sequence of approximately 25 amino acids was detected between γ2 and γ3 proteins with γ1-gliadin having a greater resemblance with other β-gliadins. These results also indicated potential problems in the nomenclature of gliadins resolved by gel electrophoresis.

Recognition that mobilities could be used as a meaningful parameter for characterizing the gliadin electrophoregram spectrum of a given cultivar was exploited by Autran and Bourdet (1975). Using 9% starch gels and a buffer system containing 0.5M urea and aluminum lactate at pH 3.2, these workers devised a numerical mobility format relative to a γ-gliadin band common to all the cultivars that they examined in their study. The precision gained by using relative mobilities aided in the observation of 43 possible positions of gliadin bands (a maximum of 25 in any one cultivar) for 73 spring and winter wheats grown in France.

In contrast, Australian workers at the Commonwealth Scientific and Industrial Research Organization (CSIRO) Wheat Research Group reported only 34 band positions in electrophoregrams obtained by urea-SGE (12% starch gel containing 12% urea, aluminum lactate buffer, pH 3.1) of 1M urea extracts of gliadin from 78 cultivars (du Cros et al., 1980).

The French classification format and one-dimensional SGE methodology for gliadin separation was further refined by Bushuk and Zillman (1978) for flatbed PAGE (6% gels) with 8.5 mM aluminum lactate buffer, pH 3.1. These workers incorporated the fast polymerization system developed by Jordan and Raymond (1969) for use with polyacrylamide gels in acid medium. With this method, resolution of up to 30 gliadin bands for a single cultivar (e.g. Red Fife) was reported in a catalog of PAGE patterns for Canadian cultivars (Zillman and Bushuk, 1979). A composite count of gliadin components among the 88 wheats that were studied was not given.

An alternative to uniform concentration polyacrylamide gels for electrophoretic resolution of proteins is represented by the

gradient-PAGE methodology in which average gel porosity decreases along a gel bed of continuously increasing polyacrylamide concentration (Margolis and Kenrick, 1968; Slater, 1969). In gradient-PAGE, a mixture of proteins driven by an electric potential migrates down a gradient of increasing frictional resistance which limits diffusion. The pattern which results can be one of extremely sharp bands where differences in charge between proteins are minimized, and estimates of molecular size may be obtained in single electrophoretic runs.

Separation of gliadins by this technique was first reported by Wrigley and McCausland (1977), who used a gradient of 3-27% polyacrylamide in sodium lactate buffer pH 3.1. They found the method to be superior in comparison with SGE for distinguishing between Australian wheats for the purpose of cultivar identification. du Cros and Wrigley (1979) narrowed the range of the polyacrylamide gradient to 2-16% to limit the loss of resolution for leading bands in the high acrylamide concentration zone of the gel. The shallower gradient also shortened the electrophoresis period for wheats to 1h at 400 V. Sodium lactate buffer was also found to give a better resolution in this system compared to aluminum lactate. A gradient range of 2.5-13% polyacrylamide was subsequently recommended as optimal for the gliadins (du Cros et al., 1980).

While the method of gradient-PAGE has been used as a resolution device, its application for determining MW's of gliadins or other wheat proteins has been rarely reported in the literature; the comparative electrophoretic study by Hussein and Stegemann (1978) is a notable exception. This situation is contrasted in the use of the contemporary

technique of SDS-PAGE which was introduced as an analytical tool by Shapiro et al. (1967) and elaborated by Weber and Osborne (1969) regarding its reliability to measure MW's of proteins. SDS-PAGE methodology involves the elimination of charge differences among reduced proteins which is achieved via the binding of the anionic detergent SDS. The migration of the complexed proteins by PAGE in the presence of SDS will normally be in the direction of the anode at a rate which is a function of their molecular size.

For wheat gluten proteins, the method has been extensively applied to examine the subunit composition and molecular weight distribution of glutenin which is only minimally resolved by electrophoresis without prior chemical reduction to disrupt the disulphide bonding which cross-link and stabilize this high molecular weight complex of proteins[1]. SDS-PAGE studies of gluten proteins until recently, have commonly used the continuous buffer system methodology of SDS-Tris-borate in 5% polyacrylamide gels as described by Koenig et al. (1970). Compared to reduced glutenin, reduced gliadin has been shown to be a protein fraction relatively homogeneous in molecular size distribution, narrow in range and hence limited in electrophoretic resolution. In a "whole gliadin" preparation from cv. Ponca, Bietz and Wall (1972) were able to resolve a total of seven gliadin subunits, three of which were present in only trace amounts. The dominant electrophoregram component had a MW of approximately 36,000. This subunit along with a second of 44,000 MW corresponded to most of the α-, β- and γ-gliadins. Subunits which were identified as ω-gliadins,

[1]The reader is referred to Kasarda et al. (1976) for an excellent discussion on the characterization of glutenin.

constituted a separate pair of bands corresponding to MW's of 69,000 and 78,000. Other workers using similar electrophoretic methods have obtained results in general agreement with those cited by Bietz and Wall (e.g. Hamauzu et al., 1972; Ewart, 1973).

A different approach to SDS-PAGE that often produces improved electrophoregram resolution was introduced by Laemmli (1970) who adapted the discontinuous buffer methodology of Davis (1964) described for analytical PAGE. This system, which employs an apparatus of vertical design, emphasizes the production of thin starting zones into which a solution of protein will concentrate at the initiation of electrophoresis. Concentration will occur at the boundary established between a high mobility solution of buffer ions and a lower mobility counterpart stacked with the sample protein in a relatively larger pore acrylamide gel section above it. A discussion on the background and theory of discontinuous PAGE is given by Ornstein (1964).

Use of such a system for the resolution of cereal proteins was first reported by Shewry et al. (1977) for electrophoresis of barley hordeins, in a separating gel formed using the SDS-Tris-borate pH 8.9 buffer of Koenig et al. (1970) but with 12.5 % acrylamide. The stacking gel consisted of 3% acrylamide in the same buffer at pH 6.7.

Gliadins were first separated using this approach by Shewry et al. (1978) in 17.5% polyacrylamide slabs in an otherwise identical SDS-PAGE procedure as described above. Gliadins were extracted in a solution containing propan-2-ol plus mercaptoethanol. The electrophoresis period was 3h at 20 mA. This method was recommended as an alternative to SGE for the purpose of variety identification (section to follow) in

instances where SGE patterns were not distinguishable between cultivars. Shewry observed that the region corresponding to high MW gliadins provided the best discrimination, while components of lower MW i.e. those commonly separated by 10% SGE or 6% PAGE were "less clearly" resolved. It was also found that resolution of the latter was improved by alkylation.

Payne et al. (1979) used a similar SDS-PAGE procedure but with Tris-HCl buffer (Laemmli, 1970) and 17% polyacrylamide separating gels to study the subunit composition of glutenin. Very long electrophoresis times of 18h at 8 mA were reported. Resolution of a total protein extract by this method (Payne and Corfield, 1979) gave a superior result in comparison with total protein separations obtained by continuous buffer SDS-PAGE (Fullington et al., 1980).

The use of discontinuous buffer SDS-PAGE by other workers has confirmed the relative superiority of the technique to resolve and monitor the distribution of gluten proteins in various solubility and chromatographic fractions (Field et al., 1982, 1983b). However, while small differences in MW's of purified gliadins can be detected by SDS-PAGE (Bietz and Wall, 1972), it was acknowledged that the large number of gliadin proteins precluded these differences from being recognized in patterns of the mixture. A similar position was taken by Field et al. (1983a) who, working with Laemmli's method, observed that because so many α-, β- and γ-gliadins as well as reduced subunits of "aggregated high MW gliadin", had apparent MW's of 36,000 and 44,000, the mixture could not be clearly resolved by SDS-PAGE.

The apparent comigration of discrete gliadin components with inadequate charge and/or size differences to significantly affect their respective mobilities continues to underscore the complex nature of the gliadins and limitations of the prevailing electrophoretic methods to fully characterize their composition. Perhaps the most accurate measure of gliadin heterogeneity to date was obtained by means of a novel analytical approach which was introduced by Wrigley (1970). Two dimensional protein maps of gliadin, extracted from single grains with 2M urea, were produced by a combined technique of gel isoelectric focusing (IEF) between pH 5-9 and starch gel electrophoresis in aluminum lactate buffer as described by Graham (1963). This procedure was shown to typically double the number of components resolved by either one-dimensional method which individually yielded a different sequence of fractionated bands.

In this way, 46 gliadin components were revealed in the protein map for the cultivar Chinese Spring (Wrigley and Shepherd, 1973). Because of the novel analytical approach and the greater than two-fold increase in the number of gliadins previously reported for a given cultivar, aspects of experimental anomalies were considered by the authors as contributing factors. However, no evidence for artifact formation in the IEF step could be found. Likewise, the possibility of protein modification caused by cyanate produced from urea, was discounted. The demonstration of specific genetic control for most of the proteins resolved for cv. Chinese Spring was further confirmation that the observed level of gliadin heterogeneity was real.

An isolated report of artifact-related heterogeneity of gliadins was made by Charbonnier (1974). It was observed that relatively homogeneous preparations of ω-gliadins stored at pH 2.1 for over three days, would yield a series of multiple bands upon electrophoresis at alkaline pH. The effect was attributed to random deamidation of glutamine residues explained by only a 5% difference in the degree of amidation of total ω-gliadins. The effect was reproduced by Wrigley (1977) who obtained artifactual components with both electrophoresis and IEF methods. It was reasoned that if partial deamidation was a factor in gliadin heterogeneity it would likely only occur by an in vivo process.

ENVIRONMENTAL EFFECTS ON GLIADIN ELECTROPHOREGRAMS

It has been well documented that the gliadin electrophoregram represents a stable genetic signature of a wheat cultivar. Initial evidence on the state of the relative contributions of genetic and environmental factors to gliadin electrophoretic composition was forwarded by Lee and Ronalds (1967) and Feillet and Bourdet (1967). In both studies, the location or conditions under which a crop is grown was shown to have a minimal effect on band intensities and no effect on band positions. Doekes (1968) obtained a similar result on the effect of different levels and time of application of nitrogen fertilizer on the gliadin electrophoregram. Combined gel electrofocusing and electrophoresis was applied by Wrigley (1970) to a cultivar sample varying in protein content from 10-26%. Within this range, no

significant modification of the highly resolved 2-dimensional protein map of gliadin composition was reported.

In contrast to the relative constancy of gliadin electrophoregrams to conditions of varying growth location and nitrogen content, low grain sulphur has been shown to have a marked effect on protein electrophoretic composition. Wrigley et al. (1980) demonstrated that the composition of albumin, gliadin and glutenin proteins were severely altered for plants grown in sand culture with nutrient sulfur supplied at an extremely low level. Gradient-PAGE on gliadin extracts revealed a dramatic drop in the proportion of high:low mobility components by 60% in one Australian cultivar and 80% in another. Analysis elsewhere of purified low mobility gliadins by PAGE i.e. ω-gliadins, have shown these proteins to have very low levels of sulfur-containing amino acids cysteine and methionine (Booth and Ewart, 1969; Bietz and Wall, 1972; Charbonnier, 1974). Electrophoretic pattern data obtained by Wrigley and coworkers thus indicated a specific and preferential shift in the synthesis of proteins with relatively low sulfur requirements.

In a separate study on the effects of sulfur and nitrogen fertilizer on grain quality, Moss et al. (1981) provided similar electrophoretic evidence of changes in protein composition caused by low sulfur supply. Trends in the proportion of extracted albumin:gliadin proteins and in the ratio of low:high mobility gliadin by gradient-PAGE were most marked at flour sulfur levels below 0.11% and abundant nitrogen supply. Highly significant effects on parameters important to breadmaking quality were also observed.

While sulfur deficiency in wheat to the degree reported above is not a common phenomenon, Wrigley et al. (1980) noted the potential to manipulate the proportions of "sulfur poor" and "sulfur rich" proteins by breeding and selection to affect flour end-use quality. A similar observation was made by Miflin et al. (1983) who postulated that the relative proportion of sulfur-rich, sulfur-poor and high MW polypeptides were responsible for the different processing properties of wheat, rye and barley.

CULTIVAR IDENTIFICATION BY GLIADIN PAGE

The high level of gliadin heterogeneity revealed by PAGE and the stable nature of its electrophoretic signature in response to a variable environment have contributed to the gradual evolution of PAGE of gliadin as the most widely accepted diagnostic tool for identification of common and durum wheat cultivars. This cultivar identification process however, is not restricted to the PAGE technique or to the prolamin fraction in wheat and other cereals. Most noteworthy is the systematic approach developed by Australian workers (Wrigley and McCausland, 1977; du Cros et al., 1980) which reveals the characteristic tradeoffs in speed and discrimination power of the various testing procedures employed. A recent report by Wrigley et al. (1982a) gives an overview on the various electrophoretic methods and complimentary procedures of cultivar identification in cereal crops.

Studies using electrophoretic methods as the basis to discriminate between wheat varieties have traditionally been guided by the aim to

uncover specific variation in protein composition that could account for the ranging quality differences of gluten. While this original objective still prevails, increasing demands for quality control in the commerce of wheat has also placed significant emphasis on electrophoresis as a means to identify undesireable cultivars that are otherwise indistinguishable.

Due in large measure to the marginal resolution of gluten proteins by moving boundary electrophoresis, this technique was limited in its application and provided equivocal success as a method to differentiate functional classes or cultivars of wheat according to protein pattern. Jones et al. (1959) compared electrophoregrams of acetic acid extracts of gluten for four common wheats of different baking quality and two durum cultivars. It was observed that the bread wheat patterns, which were uniform in profile, could be distinguished as a group from the durum patterns, the latter being far less consistent in appearance. It is interesting to note that of the six cultivars used in the study, Golden Ball, which was unique in terms of pedigree and nationality (Zeven and Zeven-Hissink, 1976), correspondingly gave the most distinct electrophoregram even compared to its durum wheat counterpart. This feature was not commented upon by the authors.

In an early and isolated application of a quantitative strategy to compare electrophoretis data, Cluskey et al. (1961) studied compositional differences in free electrophoresis patterns of gluten (dispersed in acetic acid) among three hard and eight soft wheats. The concentration of schlieren diagram components was determined and the data was then subjected to a statistical analysis. While no obvious

differences were observed, it was tentatively concluded on statistical evidence that hard and soft wheats had different gluten composition. The level of α-gluten component [= glutenin + α-gliadin, after Woychik et al. (1961)] was higher in the hard wheats. Class affiliation was found to be a more significant source of this variation than protein content.

A similar approach was taken by Kelley and Koenig (1963) who identified gluten components in terms of mobility and percent distribution in moving boundary profiles of 15 wheats. It was found that the method provided a reasonable basis for discrimination of wheats at the class level, but not for individual cultivars.

Low mobility acetic acid-soluble proteins (gliadin) by SGE at acid pH in aluminum lactate buffer, have been shown to contain differences in the number and distribution of migrating components between cultivars. Graham (1963), in comparing seven common and one durum wheat cultivars found differences to be less marked for cultivars of related genotypes. It was speculated (Elton and Ewart, 1962) that the observed variation in protein patterns for eight common wheats that were studied, could contribute to differences in protein quality.

Bourdet (1963) obtained striking differences in gliadin band patterns for two common and one durum cultivar by resolving the protein fraction over the full length of the starch gel to the exclusion of higher mobility albumin and globulin components that were extracted along with the gliadins in 60% ethanol. It was concluded that the number and mobility of gliadin components was a cultivar characteristic.

Densitometer curves were used by Lee and Wrigley (1963) to compare gluten proteins resolved by PAGE at alkaline pH for twelve cultivars

incuding common, durum and primitive tetraploid species. Gluten profiles were found to be characteristic for each cultivar and appeared to be independent of variations in protein content or location of growth. Genetically related cultivars displayed some common features in the patterns of respective densitometric profiles. A similar result was obtained by Coulson and Sim (1964) who examined, by densitometry, SGE patterns of acqueous extracts of flour from thirty-four common and a number of tetraploid wheat cultivars. They noted the potential of electrophoresis as a new method to classify wheat proteins and to provide accurate varietal identification.

The emphasis however, on visual discrimination of differences in gliadin electrophoretic composition continued to limit meaningful analysis to a relatively small number of protein samples that could be electrophoresed on a single gel slab. A basis for inter-gel comparison of a large population of wheat genotypes was yet to be established. As a consequence, only consistent and obvious differences in gliadin composition among a small sample of cultivars could prove useful for cultivar identification or comparative studies to associate specific variation in protein pattern composition with functional properties. If any relationship existed between electrophoretic composition of the gliadin proteins and functional quality of flour, the complexity of electrophoretic data precluded such an association from being established at the time (Lee and Wrigley, 1963; Elton and Ewart, 1964; Coulson and Sim, 1965; Kaul, 1967; Huebner and Rothfus, 1968).

The first attempt to standardize wheat protein electrophoretic data to a common format for the purpose of comparative analysis was reported

by Doekes (1968) in a densitometric study of urea-starch gel electrophoregrams of sequential water soluble extracts of flour. This procedure yielded sample solutions containing a mixture of albumin, globulin and gliadin proteins, with the latter predominating by the third extraction cycle. Densitometric profiles thus detected a range of electrophoregram components with each assigned a mobility value, precise to a half-unit, relative to a leading albumin/globulin peak which was arbitarily given a mobility of 100.

On this basis, each wheat variety was assigned a different distribution of relative mobility numbers corresponding to densitometric profile data which was reported to be highly reproducible both qualitatively and quantitatively (Doekes, 1969). The gliadins were also formally defined for SGE in aluminum lactate buffer pH 3.1 as segregating between the origin and a distinct absorption minimum in the profile at a relative mobility of 31. More detailed curves for the gliadins could be obtained by longer electrophoresis.

Despite the numerical emphasis on the use of normalized data, densitometric profiles were employed only as visual aids to compare Triticum species with regards to genome relationships (Doekes, 1969). Generalized shape features of gliadin profiles were also used to develop a tentative classification of eighty diverse cultivars into five main groups representing a complex "morphological series" for gliadin bands. Group I cultivars possessed compact densitometric profiles (densitomegrams) and were predominantly soft winter wheat (SWW) types. Group V comprised mainly hard spring wheats which were characterized by "well spread" profiles. Group II, III and IV densitomegrams were intermediate in nature with cultivars varying in class and origin.

Variations in number, density and position of a few major slow moving bands (gliadins) in starch gel electrophoregrams also containing albumin/globulin components, formed the basis of a key devised by Ellis (1971) to distinguish thirty-two predominantly commercial English cultivars. A range of twelve different types of patterns for the low mobility gliadin bands were defined. These patterns were then used to identify a majority of the samples.

An comparable approach to cultivar identification was applied by Wrigley and Shepherd (1974) to identify Australian wheats by the presence of gliadin components noted to be specific for certain cultivars. Gliadin patterns for standard samples of thirty-five cultivars were studied and a table was prepared documenting the major distinctive features for each cultivar. Specific bands in electrophoregrams were identified in regions of common mobility as α,β,γ or ω-gliadins according to the nomenclature convention of Woychik et al. (1961). The identity of a majority of cultivars could be established on the basis of specific protein patterns in their gliadin electrophoregrams. A similar band notation and procedure was used for the identification of eight New Zealand cultivars (Coles and Wrigley, 1976).

More recently, du Cros et al. (1980) have published a diagnostic key of 78 gliadin electrophoregrams by SGE to assist in the systematic identification of Australian wheats. The key is based on a set of 32 bands known to vary among their population of cultivars.

Gradient polyacrylamide gel electrophoresis has also been proposed as a means to routinely distinguish Australian wheat cultivars (Wrigley

and McCausland, 1977; du Cros and Wrigley, 1979). Compared to their SGE system, gradient-PAGE offered improved resolution of the gliadin fraction in a shorter time period. Variation in relative mobilities was reported to be less than 1% in 2.5-13% gels (Wrigley et al., 1982) for the most recent version of the method described by du Cros et al. (1980).

The Australian workers using precast gels, presented a catalog of simplified gradient gel diagrams for 28 Australian wheats, several of which had indistinguishable patterns. To identify an unknown sample, a stepwise procedure was recommended which first involved direct comparison of the unknown electrophoregram to suitable standard patterns. If this strategy was not successful, the catalogued reference gradient-PAGE diagrams were then used to find a match, and if necessary, the more complete list of SGE diagrams was employed to compare with major corresponding bands in the gradient-PAGE pattern of the unknown.

Elsewhere, the gradient gel method (3-27% acrylamide) was applied to discriminate between a set of 12 California wheat varieties (Qualset and Wrigley, 1979) and except for two closely related genotypes, clear pattern differences were found.

The results of gliadin separations by gradient-PAGE in comparison with the PAGE system of Bushuk and Zillman (1978), were judged in a collaborative study carried out by Autran et al. (1979) to be less suitable for wheat cultivar identification on the basis of discriminating ability particularly for fast moving bands. Redman et al. (1980) also preferred the uniform PAGE method for the comparison of English wheat varieties.

A significant advance in the research of comparative methods to analyze electrophoretic data for cultivar identification was achieved by Autran (1973). The major elements of this work were further elaborated in a subsequent publication (Autran and Bourdet, 1975) in which a standardizing methodology was proposed to handle the variation in gliadin composition obtained by urea-SGE of 73 soft spring and winter French wheat cultivars.

The authors exploited the ubiquitous occurrence of a prominent γ-gliadin component in all cultivars that were examined. This band was adopted as a reference standard, and arbitrarily assigned a mobility of 65 to which other bands in each electrophoregram could be compared. The intensities of individual bands were scaled based on absorbance data as 0, trace, +, ++, +++ and a relative mobility (RM) range of 21-100 was established to classify gliadin components in terms of 43 integer mobility positions, of which 14 were contiguous assignments. If any problems existed in allocating gliadin bands to these discrete RM zones, they were not discussed. To determine the identity of an unknown sample, the gliadin pattern could be systematically translated into its relative mobility formula and identified by direct comparison to a key for French cultivars which was formulated by the authors.

Autran and Bourdet (1975) also proposed an index of relative dissimilarity (IRD) to evaluate pairwise comparisons of RM-normalized gliadin electrophoregrams by using an adaptation of a taxonomic measure of pattern similarity as described by Dedio et al. (1969). For 12 cultivars that were examined in this manner, IRD values ranged from 0 (patterns indistinguishable) to 67 and agreed with subjective assessments made by the authors.

The French nomenclature system and scheme of chemotaxonomic keys was subsequently adopted for the identification of 80 soft wheat cultivars involved in the grain commerce of eight European Common Market countries (Autran, 1975), and to identify 25 principal UK wheat cultivars (Ellis and Beminster, 1977).

The improved resolution of gliadin components derived from the PAGE system introduced by Bushuk and Zillman (1978) undermined a predominantly historical advantage held by hydrolyzed starch as the support medium for electrophoresis. It was found that this PAGE system could easily differentiate several French cultivars of commercial importance (e.g. Capitole and Ducat) that could not be previously distinguished in starch gel (J.C. Autran, personal communication).

Bushuk and Zillman (1978) proposed a modification of the Autran and Bourdet system for the identification of gliadin components. This came as a result of the difficulty in locating the French reference γ-gliadin band amid several contiguous and prominent gliadin components in Canadian cultivars, or in instances where band 65 was clearly not to be found at all, particularly for durum wheats.

A readily identifiable gliadin band in electrophoregrams of the historic Canadian cultivar Marquis was selected as a reference and was assigned a mobility of 0.50. A 70% ethanol gliadin extract derived from a standard sample of cv. Marquis was run in duplicate as an internal reference for each PAGE slab. For each gliadin band in an electrophoregram, a mobility value was determined precise to the nearest 1/2 integer relative to the migration distance of the reference band. To quantify relative intensities, bands were subjectively scaled

numerically from 0 to 5. The normalized data for each gliadin pattern was then formatted as an array of integer band density values with relative mobilities denoted by the position of each density element.

Using this procedure, catalogs of normalized "electrophoregram formulas" have been published for 88 Canadian (Zillman and Bushuk, 1979) and 88 U.S. wheat cultivars (Jones et al., 1982), and for 29 Italian wheat cultivars without band density assignments (Dal Belin Peruffo et al., 1981). A manual method was described by Zillman and Bushuk (1979) for determining the identity of an unknown sample by direct comparison of its gliadin formula to the compendium of tabled array data. Although an attempt was not made to test this system, the application of a computer to manage the problem was suggested.

A computerized approach to expedite the cultivar identification process in wheat was first reported by Bushuk et al. (1978) who replaced the subjective assessment of gliadin band intensity and relative mobility parameter values by scanning electrophoregrams with a microdensitometer interfaced with a computer to automatically produce "signature arrays" that encode gliadin electrophoregram formulas. Subsequent application of digitized electrophoregram formulas for wheat cultivar identification was described by Sapirstein et al. (1980) who outlined a system of computer programs to assist in the comparative analysis of gliadin PAGE data.

Reference to identification of Australian varieties by means of a computer was made by Wrigley (1980) and Wrigley et al. (1981) which involved the application of one program of a system's package of programs designed to solve problems in taxonomy. Comparison of an

electrophoregram from an unknown variety to reference patterns was based on a standard set of 32 gliadin bands known to be present among Australian wheat cultivars resolved by SGE (du Cros et al., 1980). In this scheme, gliadin bands ordered in sequence from the origin were considered to be multi-state characters possessing four levels of density (0-3, 0=absent). As no objective method was applied to classify gliadin bands detected in electrophoregrams to character set data, it was acknowledged that the transformation of SGE patterns to the numerical form required some subjective interpretation.

Details of a computer program to facilitate the identification of wheat cultivars using gliadin electrophoregrams transformed into numerical arrays has recently been reported (Lookhart et al., 1983). Despite the computerized environment, only integer precision was used to code relative mobility as unit values from 10 to 90. The basis for pattern discrimination involved the calculation of a unique form of a similarity coefficient, derived from pairwise comparison of band arrays, in which contribution from non-matching gliadin bands was ignored. Program evaluation was limited to a computer plot which traced the declining distribution of data base cultivars as a function of "relative percent similarity" from 100% for a perfect match, to a minimum value of about 35% for data base electrophoregrams least similar to tested unknown band patterns.

NOMENCLATURE FOR IDENTIFICATION OF GLIADINS

The application of the Greek character/numerical system of nomenclature, now commonly used to classify gliadin electrophoregram components, originated in a study by Jones et al. (1959) to identify gluten components that were consistently resolved by moving boundary electrophoresis into four mobility groups possessing modest boundary definitions. These were designated as α, β, γ and ω in order of decreasing mobility. The denominational labelling of gliadin components in this manner, was further justified by Jones et al. (1963) who cited the carboxymethyl-cellulose (CMC) chromatographic fractionation of acetic-acid dispersed gluten from cv. Ponca reported by Woychik et al. (1960). These workers obtained an elution profile displaying four peaks, which when isolated, yielded the characteristic α,β,γ, and ω schlieren pattern by moving boundary electrophoresis. In contrast, Simmonds and Winzor (1961), also used a CMC column to fractionate acetic acid extracts of flour for several Australian cultivars. However, a different gradient elution procedure resulted in eleven separated fractions "as a lower limit to the number of resolvable entities". Therefore, it appeared as if ion-exchange chromatography provided an arbitrary basis for the grouping of gliadin components.

Woychik et al. (1961) adopted the α-β-γ-ω notation for gliadins separated by starch gel electrophoresis in an experiment which resolved the gliadin fraction of the cultivar Ponca into eight components. Bands within each grouping were numbered beginning with "1", in order of decreasing mobility. This SGE result was compared in nomenclature to the moving boundary separation of Jones et al. (1959) as follows:

Jones et al. (1959)	Woychik et al. (1961)
a1 - gluten	glutenin
a2 - gluten	a1 & a2 gliadin
B - gluten	B1 - B4 gliadin
Y - gluten	Y - gliadin
w - gluten	w - gliadin

While the electrophoretic pattern mobility zones corresponding to W- and A-gliadins, in this study and elsewhere, are respectively more and less distinct, no apparent boundary was demonstrated to distinguish bands belonging to the B-Y intermediate mobility groups. This division appears to be arbitrary as no biological, chemical or physical basis for such a division has been reported in the literature.

Studies which have used the A-B-Y-W system to label their own results (e.g. Wrigley and Shepherd, 1974; Mecham et al., 1978; Konarev et al., 1979), or those which have attempted to establish correspondences between band groupings in the A, B, Y, and W system and proposed relative mobility formats (e.g. Doekes, 1973; Autran and Bourdet, 1975; Bushuk and Zillman, 1978) have yielded certain inconsistencies when for example, relative proportions of some groups are examined (Table 1).

While some agreement in the scale of gliadin band assignments exists in the PAGE studies cited in Table 1, less uniformity is demonstrated in results by SGE. Autran and Bourdet's result is clearly inconsistent. Autran and Bourdet (1975) related their nomenclature system to the U.S. scheme of Woychik et al. (1961) as follows:

TABLE 1

Relative size of beta and gamma-gliadin mobility zones by SGE and PAGE as reported in the literature

γ/ω	β/ω	Electrophotetic System	Gel Support Medium
43%	57%	Doekes (1973)	12% SGE + urea
48%	55%	Wrigley & Shepherd (1974)	12% SGE + urea
29%	22%	Autran & Bourdet (1975)	9% SGE + urea
35%	35%	Bushuk & Zillman (1978)	6% PAGE
40%	40%	Mecham et al. (1978)	7% PAGE
39%	42%	Konarev et al. (1979)	7.5% PAGE

Woychik et al. (1961)	Autran and Bourdet (1975)	
	Relative mobility	No. of Bands
α-gliadins	88 - 100	7
β-gliadins	75 - 87	6
γ-gliadins	60 - 74	7
ω-gliadins	21 - 59	23

By this SGE approach, almost 60% of the total electrophoretic pattern field is dedicated to the resolution of "ω-gliadins". Not surprisingly the number of proteins assigned to this grouping is greater than α-, β- and γ-gliadins combined.

The difficulties in attempting to cross-reference within and among different gel support media, in terms of number and distribution of gliadin bands were discussed in a collaborative study of major electrophoretic systems described by Autran et al. (1979) who postulated that apparatus design could partly account for different results.

It would then appear that the lack of a standardized methodology and ambiguous qualitative designation of gliadins, has limited meaningful exchange of results in the literature, as the correspondence between laboratories with respect to the identity of individual component proteins in compared cultivars is unreliable.

Despite the equivocal precision associated with expressing the identity of electrophoresed gliadin bands in a qualitative format which effectively privatizes research results, two current Soviet systems of nomenclature are designed with unique alphanumeric notation. Gubareva et al. (1975) used the term varietal formula to characterize gliadin electrophoregrams (resolved in 7.5% polyacrylamide gel cylinders, 0.013N acetic acid buffer at pH 3.1) in terms of a complex adaptation of α, β, γ and ω mobility group parameters of Woychik et al. (1961). In this system, most recently discussed by Konarev et al. (1979), a "standard" gliadin pattern is prepared from the electrophoregram of a composite mixture of gliadin extracts derived from a sample of three typical wheat genotypes representing the putative progenitors of the A,B and D genomes: T. boeoticum, Aegilops speltoides and Ae. squarossa respectively.

It was further proposed that the standard pattern contained 29 bands which represented all the major protein components of gliadin. The positional distribution of bands in the standard pattern was given by its assigned formula as: α 1 to 7, β 1 to 5, γ 1 to 5, ω 1 to 12. As an example, the gliadin formula for cv. Ponca was written as:

$$\alpha_{567} \quad \beta_{23_{1}45} \quad \gamma_{234} \quad \omega_{\overline{\underline{26}8\dot{9}\dot{1}\dot{0}}}$$

Additional notations were used to accommodate five types of deviation from the standard pattern:

(i) higher intensity - band number underlined
(ii) lower intensity - band number overlined
(iii) slightly greater mobility - subscript 1 to the band number
(iv) slightly lesser mobility - subscript 2 to the band number
(v) band doublet - double mark over the band number

In this fashion, the Soviet workers catalogued the gliadin electrophoregram formulas for 130 common spring and winter wheats grown in the U.S.S.R. as a basis for determining the cultivar identity of unknown samples (Gubareva et al., 1975).

A second Soviet nomenclature system, involves the classification of gliadin electrophoregram components (resolved in 3M urea-starch gel cylinders, aluminum lactate buffer at pH 3.1) using genetic keys. The "allelic block system", recently described by Sozinov and Poperelya (1980), is based upon genetic studies which showed that gliadin components were inherited in linked groups or blocks of bands that are controlled by allelic loci (Sozinov et al., 1974). The concept of linked allelic loci originated in early reports of the genetic determination of the expression of gliadin electrophoregram bands (e.g. Solari and Favret, 1967).

Sozinov and Poperelya (1980) observed that gliadin electrophoregrams were composed of discrete combinations or blocks of bands which were inherited in qualitatively unaltered states. Bands within a block could vary in number and did not necessarily have contiguous mobilities. For example, it was reported that a group of four gliadin components of the variety Odesskaya 26, controlled by chromosome 1D was allelic to a group of three bands possessing similar,

but not identical mobilities for the variety Bezostaya 1 because no recombinant patterns were found in F2 seeds derived from crossing the two cultivars. The block of bands from Odesskaya 26 was given the designation "Gld 1D4". It was assumed that the apparently allelic block of bands from Bezostaya 1 was also controlled by the 1D chromosome and was given the designation "Gld 1D1". Thus the last number in the designation represented an arbitrary key for the designated block of components (controlled by chromosome 1D) which was registered in a "catalog of blocks". For example, the cultivars Bezostaya 1 and a biotype of Odesskaya 16 were given the following block formulas:

Bezostaya 1: 1A4, 1B1, 1D1, 6A1, 6B1, 6D1
Odesskaya 16: 1A1, 1B1, 1D5, 6A3, 6B2, 6D1

The potential of this nomenclature procedure for plant breeding purposes was demonstrated when the Soviet workers were able to correlate the presence of different block phenotypes with various agronomic and utilization quality attributes possessed by the cultivars. While this result affirmed the relevance of gliadin composition to functional quality, comprehensive genetic studies are necessary if the allelic block system is to be reproduced elsewhere. Additionally, aspects of accurate band classification to blocks, a subject not addressed by the authors, needs to be fully explored.

A simpler and quantitative approach for the designation of gliadin bands was proposed by Autran and Bourdet (1975). Their nomenclature system (see preceeding section) based on mobilities relative to a band common to all varieties by SGE, had the pure objective to discriminate and identify wheat genotypes grown in France.

The principle of relative mobilities to identify gliadin bands was adapted for PAGE by Bushuk and Zillman (1978). These workers proposed a mobility system relative to a designated band in the gliadin fraction of a reference cultivar (cv. Marquis band "50") to be run as an internal gel standard thus controlling the precision of results, a necessary precondition for the cultivar identification process. Relative mobility values obtained with this method were reproducible to within 0.5 units for measurements taken from different gels (Zillman and Bushuk, 1979). Lookhart et al. (1983), using a commercial vertical PAGE apparatus, also found the precision of relative mobilities to be generally ± 0.5 units, but that variations of ± 1 units would sometimes occur especially for bands with high mobilities.

In an attempt to reach a consensus on an electrophoretic system which could potentially be standardized, Autran et al. (1979) collaborated to compare the resolution and reproducibility of a variety of published SGE, uniform and gradient-PAGE systems for cultivar identification. The study concluded that the 6% horizontal PAGE system of Bushuk and Zillman (1978) was most suitable as a reference procedure.

The pending adoption of a modified version of this PAGE system by the International Association of Cereal Chemistry Study Group 6 (A-2320 Schwechat, Schmidgasse 3-7, Austria) will no doubt facilitate the production of uniform gliadin composition data and place needed emphasis on cultivar identification and analysis strategies to which this present study addresses itself.

III

MATERIALS AND METHODS - I - AUTOMATIC QUANTIFICATION OF GLIADIN ELECTROPHOREGRAMS BY MINICOMPUTER PROCESSING OF DENSITOMETRIC SCANNING PROFILES

WHEAT CULTIVARS

Wheat samples were supplied by the Research Branch of Agriculture Canada. Cultivars chosen for study in this segment of the research project are listed along with their class type and origin in Table 2. These common and durum wheat cultivars were selected to provide a representative range of gliadin electrophoregrams for analysis. This list represents a small portion of a larger group of samples (refer to Tables 4-6, pages 67-71) from which was derived an extensive data base of gliadin PAGE patterns for the system of computerized cultivar identification and electrophoregram analysis to be described later.

TABLE 2

Wheat cultivars analyzed by densitometric scanning of gliadin electrophoregrams

Cultivar	Class	Nationality
Glenlea	hard red spring	Canada
Marquis	hard red spring	Canada
Neepawa	hard red spring	Canada
Pembina	hard red spring	Canada
Lemhi 62	soft white spring	USA
Fredrick	soft white winter	Canada
Talbot	soft white winter	Canada
Stewart 63	durum	Canada
Wascana	durum	Canada

6 MM HORIZONTAL PAGE ELECTROPHORESIS SYSTEM

Apparatus and Gel Preparation

Polyacrylamide gel electrophoresis (PAGE) was carried out in a horizontal flatbed apparatus according to the method of Bushuk and Zillman (1978). The apparatus (Figure 1) accomodated 200 ml of gel solution to form a slab approximately 6 mm in thickness. Recipes for the gel solution and tank buffer are given in Table 3. All chemicals used were of reagent grade or better. Distilled and deionized water was used in preparing solutions for gliadin extraction and electrophoresis.

A 6% polyacrylamide gel was prepared by dissolving acrylamide (12.0 g), bisacrylamide (0.6 g), ascorbic acid (0.2 g), ferrous sulfate (0.005 g) and aluminum lactate (0.5g) in distilled/deionized water to yield a final volume of 200 ml. Lactic acid was added to achieve a final pH of 3.1. For polymerization, hydrogen peroxide (3%, 1 ml) was added to the

Figure 1. Bench configuration of horizontal 6 mm electrophoresis apparatus, power supply and circulating water bath.


BIO-RAD Laboratories
MODEL 500 POWER SUPPLY
POWER
101886
VOLTAGE
CURRENT
SCALE
SCALE

TABLE 3

Recipes for gel and tank buffer solutions - 6 mm PAGE

	Amount required for 200 ml final volume
Gel solution	
Acrylamide	12.0 g
N,N'-methylene-bis-acrylamide	0.6 g
Ascorbic acid	0.2 g
Ferrous sulfate	0.005 g
Aluminum lactate[1]	0.5 g
Lactic acid	to pH 3.1
Catalyst solution	
Hydrogen peroxide, 3%	1.0 ml
Tank buffer solution	
Aluminum lactate	0.50 g
Lactic acid	to pH 3.1

[1]Aluminum lactate was obtained from Research Organic/Inorganic Chemical Corp. (11686 Sheldon St., Sun Valley, Calif. 91352, U.S.A.).

gel solution which has been cooled to 4°C. The mixture was gently swirled for 3-5 sec and poured into the electrophoresis apparatus. A ten-place slot former was quickly positioned and was removed after 5 min by which time polymerization was complete. The tank buffer reservoirs were subsequently filled to a combined capacity of 500 ml.

Gliadin Extraction from Bulk Wheatmeal Samples

The gliadin solution for electrophoresis was prepared by extracting a sample (0.5 g) of ground grain (Udy Cyclone Mill) with 70% ethanol (1.5 ml) in a stoppered centrifuge tube. The mixture was agitated

periodically on a vortex mixer during the extraction interval (60 minutes at room temperature). The contents were then centrifuged (10 min, at 20,000 x g). The supernatant was decanted and mixed with twice its volume (2 ml) of tank buffer. Sucrose (0.5 g) was added to the sample solution to increase the density and thereby facilitate sample loading onto the gel prior to electrophoresis. Methyl green dye (0.02g) was added to serve as a tracking marker during electrophoresis. The sample solution could then be sealed and stored at 0°C for an extended interval (1-2 months) or electrophoresed directly.

Gliadin extracts were prepared in triplicate for each cultivar listed in Table 2 excluding Marquis[1], and the sample solutions were electrophoresed on separate polyacrylamide gel slabs.

Sample Application and Electrophoresis

Prior to the start of electrophoresis, 20 ul of sample solution was carefully deposited into each slot (max. capacity = 30 ul) using a microsyringe (e.g. 50 ul Drummond disposable bore micropipet). The flanking slots of the gel were reserved for the reference gliadin sample from the cultivar Marquis. The remaining eight slots held the test samples.

The apparatus was held at 21°C by a circulating water bath (e.g. GCA/Precision Scientific Model 256). A constant current of 80 ma was applied to the system. This corresponded to a voltage drop of approximately 320 V (16 V/cm) at the beginning of electrophoresis which declined to about 280 V (14 V/cm) at the end of the run. It was found

[1]Marquis, as the reference cultivar for PAGE, was present in duplicate on each gel run.

that the apparatus could dissipate approximately 28,000 mW before ohmic heating effects became noticeable.

The progress of electrophoresis was monitored with methyl green dye which was used as a tracking agent added to the sample solution. This tracking dye possesses two bands of different relative mobility: a green band of high mobility and its slow and fainter purple counterpart which was used to time the electrophoretic run. In order to optimize gliadin separation over the full gel slab, electrophoresis was continued for 30 min after this marker band had migrated off the end of the gel. The duration of electrophoresis was usually in the range of 6 to 6.5 hr.

Gel Staining and Photography

To fix and visualize the protein bands, the gel was immersed for 48 hr in a filtered staining solution containing 0.1 g Coomassie Brilliant Blue R (dissolved in 10 ml of 95% ethanol) in 250 ml of 12% trichloroacetic acid (TCA). The staining tray (e.g. 17 x 25 cm polyethylene Frig-O-Seal brand food container) was covered during this period and gently aggitated in order to prevent the accumulation of precipitated dye adhering in spots to the gel surface. Precipitated Coomassie Blue detracts from the appearance of the gel for photography and is subsequently observed as non-replicating peaks in the densitometric profiles. This "noise" was eliminated as a matter of course during the digital processing of the absorbance traces.

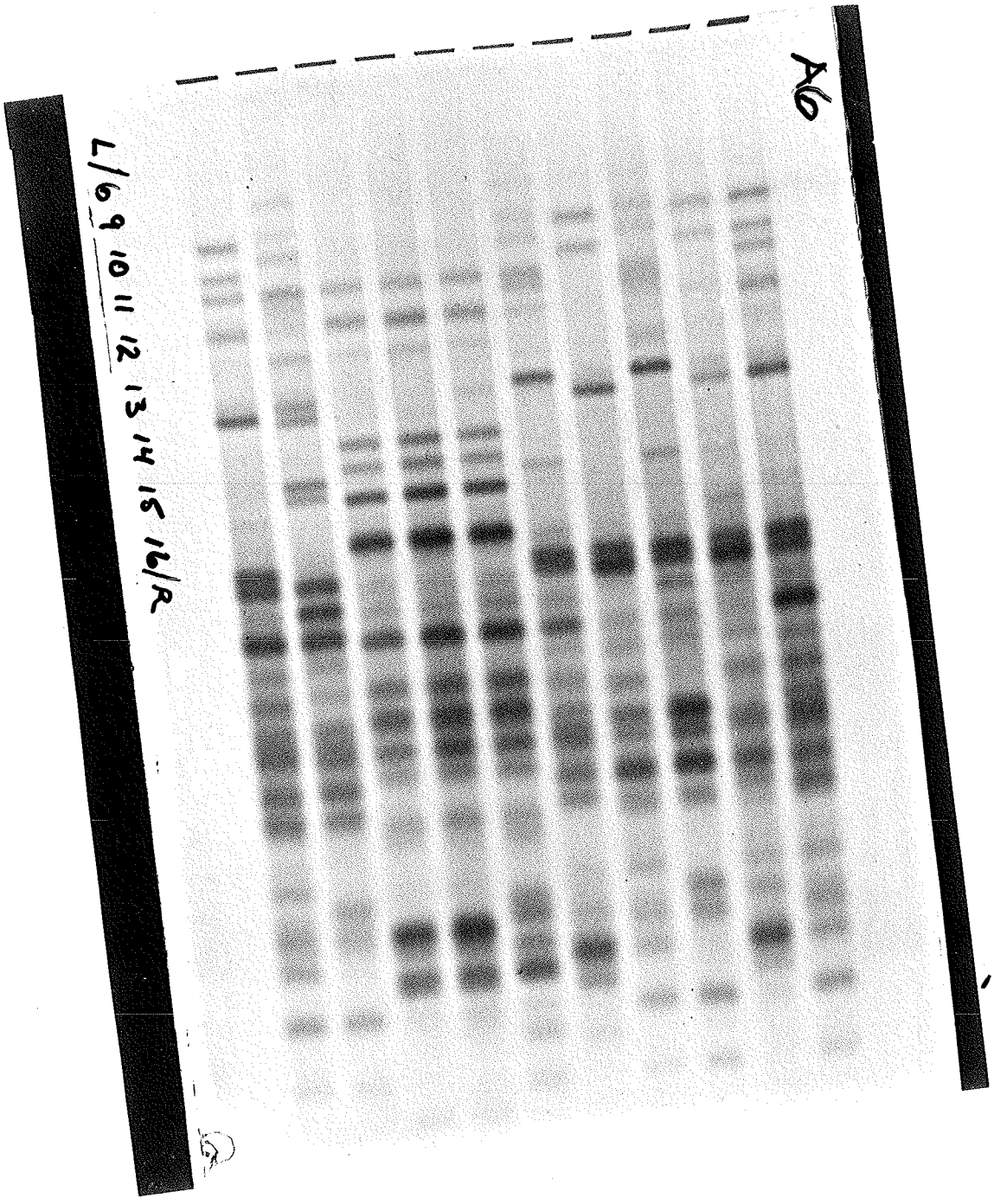
To optimize resolution of the stained gliadin bands, the gel was destained for a period of 24 hours in 250 ml of 12% trichloroacetic acid. Cotton swab was used to gently remove any precipitated stain still

adhering to the surface of the gel. At the end of the destaining period, the gel was rinsed thoroughly with water and transferred onto a clear glass plate in preparation for photography.

A fluorescent light box was used to illuminate the gel from below. The gel was photographed using Kodak 4 x 5 format Tri-X film. The film was developed with DK50 developer. The 4 x 5 negatives were then exposed on 8 x 10 format Plus X film to produce a positive film transparency (Figure 2) which represented a permanent dry record of gliadin electrophoregrams and the corresponding gel slab. These positive film transparencies were the source images for densitometric scanning and computer processing. Apart from convenience, the use of film avoided potential innacuracies arising from linear distortion of polyacrylamide gel slabs upon handling.

An 8x10 format photographic print was additionally prepared for each gel slab. Kodak Ektamatic SC type F paper was used for printing. Exposure conditions were adjusted depending on the density of the negative. The photographs were used for manual determination of relative mobilities described below.

Figure 2. Positive photographic film transparency of electrophoregrams in a polyacrylamide gel slab. Direction of migration is from left to right. Film was cut at the point of sample application for each electrophoregram to facilitate electronic tagging of the origin by the densitometric profile recording system described in the text.


A6
L/6 9 10 11 12 13 14 15 16/R

CALCULATION OF RELATIVE MOBILITIES AND CODING ELECTROPHOREGRAM DATA FOR 6 MM PAGE REFERENCE CULTIVAR DATA BASE

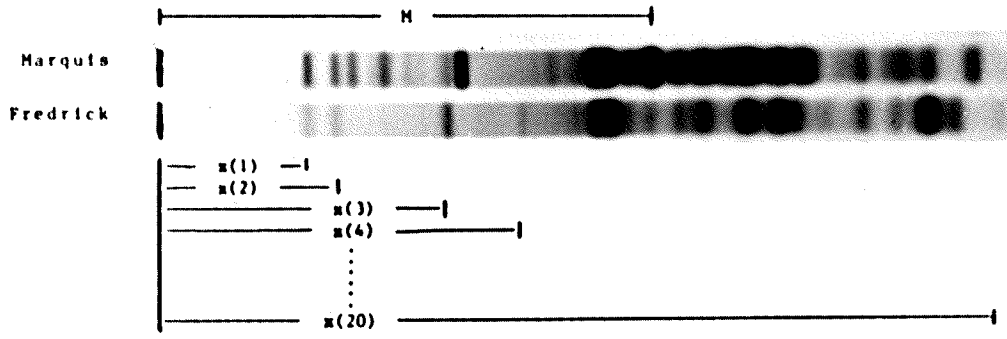
Relative mobility data for reference cultivar formulas were manually derived from 8 x 10 format photographic prints of gel slab results. The procedure used to normalize electrophoregrams is similar to that described by Bushuk and Zillman (1978) and is illustrated in Figure 3A for the soft white winter wheat cultivar Fredrick.

For each band in the electrophoregram, migration distances (mm) were measured from the leading edge of the origin to the centre of the band[1]. In the adjacent electrophoregram for the cultivar Marquis, the gliadin reference band was identified[2] and its migration distance was similarly determined. Relative mobilities were then calculated by dividing the migration distance for a specific Fredrick band by the migration distance of the Marquis reference band (on the same photograph) and multiplying the result by 50.0 (or 500 for integer format representation of relative mobility data). Band intensities were quantified by a number from 1 to 8 for the computerized application.[3] Bands of intermediate intensity were assigned values based on comparison of peak heights in the corresponding densitomegram.

To encode cv. Fredrick data for computer input, relative mobility and band density values were ordered in a pairwise fashion to form a list of 64 numbers. Cultivar signature array (CSA) is the term that

[1]These measurements were accurate to ± 0.25 mm with gliadin bands usually resolved along a 230 mm axis in photographic prints.
[2]The Marquis reference band was readily distinguished by its intensity and by being preceded in lower mobility by a distinctive doublet.
[3]The hardware capability of the minicomputer system described in the text conveniently permitted the dynamic display of 8 intensity levels for graphic data produced on the computer's video display screen.

Figure 3. Preparation of a cultivar signature array used to encode the gliadin electrophoregram for the soft white winter wheat cv. Fredrick. See text for explanation.



$$\text{Band(\#) Relative Mobility} = \frac{\text{band migration distance: } x(\#)}{\text{reference band migration: } M} * 50.0$$

$$RM(1) = \frac{x(1)}{M} * 50.0 = 15.3$$

$$RM(2) = \frac{x(2)}{M} * 50.0 = 18.0$$

$$\vdots \qquad \vdots \qquad \vdots$$

$$RM(20) = \frac{x(20)}{M} * 50.0 = 84.8$$

cv. Fredrick

Cultivar Signature Array [one dimensional - 64 elements]

Element position*								
1 - 16	15.3(2)	18.0(2)	29.4(4)	37.0(2)	42.3(3)	44.5(7)	46.0(7)	47.6(3)
17 - 32	49.8(2)	52.7(3)	55.2(4)	59.5(5)	62.8(6)	64.4(4)	67.4(4)	71.2(3)
33 - 48	74.5(3)	77.8(6)	80.5(4)	84.8(1)	....	....	....	....
49 - 64	....	....	....	....	....	....	....	1(0)

* Odd array element positions encode relative mobility values for gliadin electrophoregram band components, effective range = 10.0 - 95.0 . Even array element positions encode band density parameter values, scale = 1 - 8, determined from cultivar electrophoregram densitometric profiles.

will be used to describe these standardized numerical records of gliadin electrophoretic patterns. Each CSA, as illustrated in Figure 3 could potentially code relative mobility and band density data for a maximum of 30 gliadin bands. The remaining four positions in the array were reserved for tag purposes or index numbers to encode particular attributes of the cultivar.

Manually prepared signature arrays which encoded mean electrophoregram data, typically from three separate gliadin extractions run on different gel slabs, were used as checks to evaluate the precision of computed gliadin PAGE patterns derived from the analysis of corresponding densitometric scanning profiles.

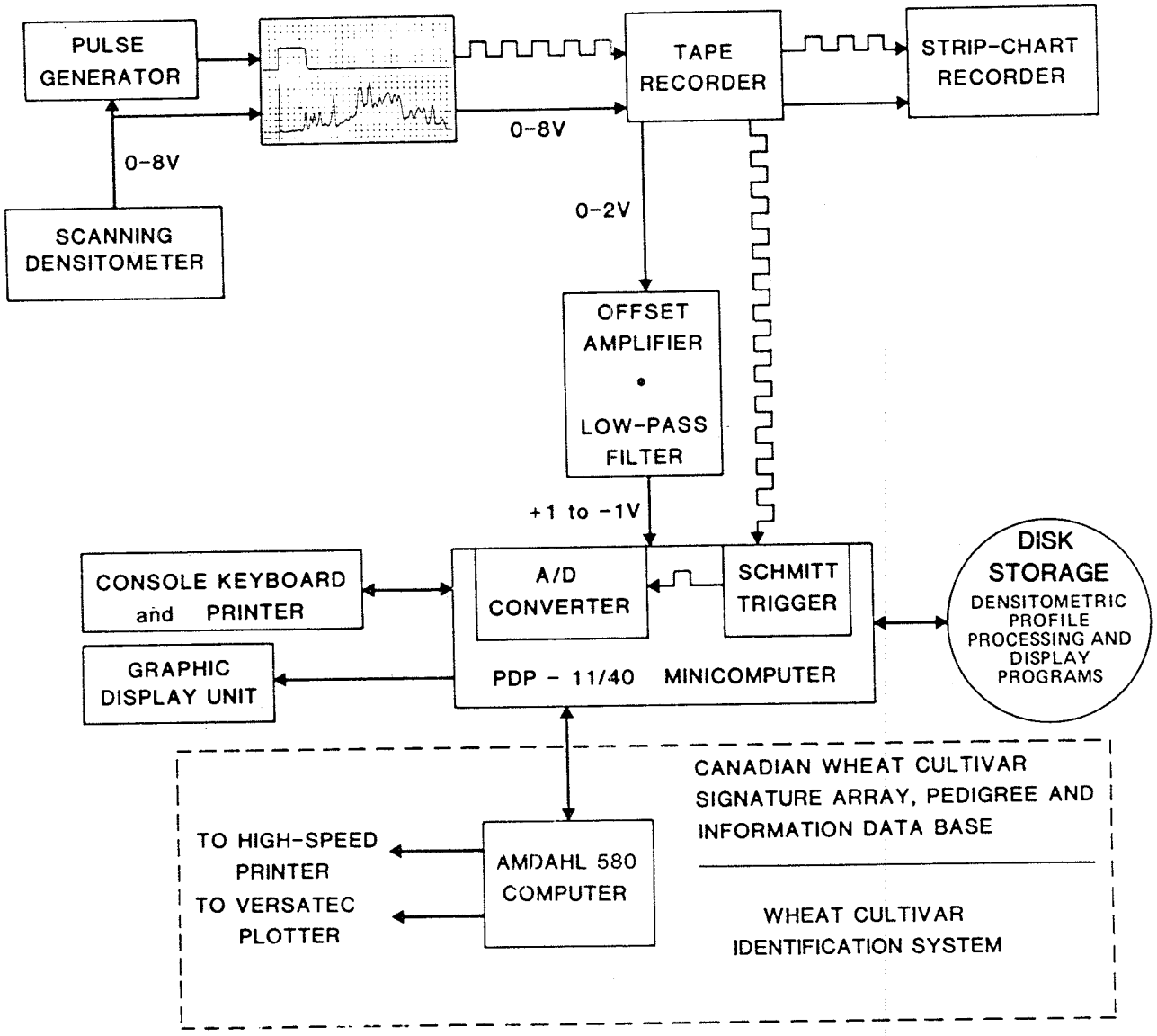
DENSITOMETER SYSTEM AND RECORDING OF ANALOG PROFILES

The instrumentation shown in Figure 4 was used to scan, monitor and record densitometric profiles of the gliadin electrophoregrams in preparation for the digitization process. The accompanying block diagram (Figure 5) indicates the direction of information flow for this system.

Positive film transparencies of slab gels (e.g. Figure 2) were scanned on an Ortec Model 4310 densitometer with a 100 x 5,000 um aperture slit. A constant scanning speed of approximately six mm/sec was used. The densitometer provided a logarithmic analog voltage (0-12V) proportional to the optical density (0-1). Due to input voltage contraints of the digitizer (see below) the densitometer was operated with a sensitivity gain on the photometer to yield a maximum of 8 volts of output for the highest gliadin peak in any given trace.

Figure 4. Bench configuration of instrumentation to acquire and record densitometric profiles. From left to right: oscilloscope, densitometer, instrumentation tape-recorder, pulse generator (top) and two-channel strip-chart recorder.

Figure 5. Block diagram of information flow for the acquisition of densitometric profile data. Graphical inset illustrates a two-channel channel strip chart record of analog data for the Marquis gliadin electrophoregram. The lower portion traces the optical density profile, the top gives the trigger pulse, coincident with the origin which was used to initiate the analog to digital conversion process.


PULSE GENERATOR
0-8V
SCANNING DENSITOMETER
0-8V
TAPE RECORDER
STRIP-CHART RECORDER
0-2V
OFFSET AMPLIFIER
•
LOW-PASS FILTER
+1 to -1V
CONSOLE KEYBOARD and PRINTER
GRAPHIC DISPLAY UNIT
A/D CONVERTER
SCHMITT TRIGGER
PDP - 11/40 MINICOMPUTER
DISK STORAGE
DENSITOMETRIC PROFILE PROCESSING AND DISPLAY PROGRAMS
CANADIAN WHEAT CULTIVAR SIGNATURE ARRAY, PEDIGREE AND INFORMATION DATA BASE
TO HIGH-SPEED PRINTER
TO VERSATEC PLOTTER
AMDAHL 580 COMPUTER
WHEAT CULTIVAR IDENTIFICATION SYSTEM

A common baseline for all densitometric profiles was established by calibrating the light source to 100% transmittance against the background density of the film. The region of each electrophoretic pattern which best specified this background was located in the broad mobility zone ahead of the slot which is uniformly devoid of gliadin protein bands. All profiles were thus referenced to a standard level.

Because the densitometer and minicomputer used in this study were located separately, a tape recorder (e.g. Hewlett Packard Model 3960 Instrumentation Tape Recorder) was employed to read the analog densitomegrams on magnetic tape (e.g. Ampex Audio Mastering Tape, Type 407). The instrument was subsequently interfaced directly with the computer for digitization. Magnetic tape storage also serves a useful function as a backup and permanent file for over 160 densitomegrams maintained on both sides of a single 1,800 ft reel recorded at 7.5 ips.

The tape recorder further conditioned each densitometric profile by attenuating the signal 4-fold, from 0-8V, to capture recorded data within an analog range of 0-2 volts (refer to Figure 5). This was made necessary to satisfy the input voltage requirement of the analog to digital (A/D) converter on the minicomputer, otherwise densitomegram peaks with an analog signal exceeding 2 volts would be clipped upon digitization with the result that corresponding protein band data would be lost from further processing and analysis.

A pulse generator (e.g. Hewlett Packard Model 8003A) was used in conjunction with the recording of densitomegrams to later facilitate precise initiation of the A/D conversion process for each profile. A T-connector was used to split signal output from the densitometer which

was fed simultaneously to the tape recorder (channel 1) and separately to the input side of the pulse generator. The latter was adjusted to trigger a 5 volt pulse which was recorded (channel 2) coincident with the first sharp peak of the densitomegram corresponding to the point of sample application on the gel. Thus each recorded gliadin densitomegram acquired an electronically tagged origin to standardize subsequent digitization and processing steps.

A two-channel strip chart recorder (e.g. Hewlett Packard Model 7402, low-gain preamplifier) permitted verification that densitomegram and trigger pulse data were correctly read onto the magnetic tape with absence of extraneous noise and within the desired voltage range. The top portion of the strip chart record (inset of Figure 5) represents the signal which provides the external trigger for the A/D conversion of the accompanying densitometric data. The recorder was also used to monitor the A/D conversion process and an oscilliscope (e.g. Tektronix Model 453) (see Figure 4) provided an overall monitor for the tape recording system.

An offset amplifier and low-pass filter supplied the final massaging of densitometric data prior to the A/D conversion process. The recorded data was filtered to remove extraneous noise above 100 Hz, and was additionally offset -1 volt (from the 0-2 volt range) to fall within the ± 1 volt input range of the A/D converter on the computer which operated in offset binary code.

Typically 10 gliadin electrophoregrams/gel slab (8-experimental + 2-reference samples) were scanned as a single set of data and recorded serially on tape. Following the digitization step these 10 densitometric

records comprised one of several "SCAN Files" (e.g. SCAN01 in Figure 9) in a cultivar data base of replicate gliadin electrophoregrams on a computer storage disk.

COMPUTER HARDWARE AND THE DIGITIZATION PROCEDURE

The data processing equipment used in the acquisition, storage and analysis of densitometric data is shown in Figure 6. The machine is a Declab 40 [Digital Equipment Corporation (DEC)] minicomputer. This computer consisted of (i) a PDP-11/40 central processing unit (CPU) with 24K (K=1,024) words of core memory (1 word is 16 bits) and an RT-11 operating system, (ii) an LA-36 Decwriter (printer and keyboard console), (iii) an LPS-11 Laboratory Peripheral System, (iv) a VR-14L graphics display unit, and (v) two RK05 disk drives.

The display unit has an effective screen resolution of 1,023 (X-axis) by 767 (Y-axis) data points and can generate eight separate intensity levels. Laboratory application hardware on the computer consists of several devices. The two that were used in the present application are the A/D converter and the Schmitt trigger. Each device had separate phono connectors for input of analog data (densitomegrams) and pulse signals (one per profile) used to drive the Schmitt trigger which initiates A/D conversion.

The firing of the Schmitt trigger is governed by slope and threshold controls on the face panel of the processor. Each time a pulse signal crosses the preset voltage in the direction indicated by the slope switch, the trigger fires and A/D conversion begins on the analog densitomegram concurrently applied to an A/D input channel.

Figure 6. Minicomputer and periferal devices comprising a PDP-11/40 central processor, an LPS-11 Laboratory Periferal System, a LA-36 Decwriter terminal, a VR-14 graphics display unit, and two RK05 disk drives.


decpack
laboratory peripheral system
decpack
0
decpack
1
11/40
decpack
WHEAT CULTIVAR IDENTIFICATION
PROJECT - PLANT SCIENCE DEPT.
H.D. SAPIRSTEIN W. BUSHUK

The A/D converter has a 12-bit resolution. Thus a sample input analog voltage proportional to optical density could acquire a digital value between 0-4,095 (i.e. 2^{12} = 4,096 discrete levels) corresponding to analog input range of ± 1.0 volts. This wide range of digital values for gliadin band densities was ultimately scaled to yield an optimized range of band densities from 1-8 in accordance with the hardware capability of the graphics display unit.

Digitization and storage of taped densitomegram data was effected by executing a system program. This program was initiated by means of a simple protocol which establishes sampling parameters[1] for the A/D conversion process and allows the operator to designate a file name to permit subsequent retrieval of the data from computer memory. Digitization was performed at a rate of 17 samples per second to accumulate 512 data points within the approximate 30 second scanning interval for each densitomegram record. The RK05 disk units which were used to store program, instruction and data files had a storage capacity of approximately 2.5 megabytes.

COMPUTER SOFTWARE

The detail of computer programs developed for this thesis is presented in the Results and Discussion chapters. Commented FORTRAN program listings may be obtained from the author on request.

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[1]The operator must specify (i) the number of densitomegrams to be digitized and stored, (ii) the sampling rate i.e. the number of digitizations per second of tape recorder playback and (iii) the total number of samples, in powers of 2, for each densitomegram (e.g. 2^8=256, 2^9=512 and 2^{10}=1,024 data points respectively per densitomegram).

IV

MATERIALS AND METHODS - II - COMPUTER-BASED WHEAT CULTIVAR IDENTIFICATION SYSTEM

WHEAT CULTIVARS

Wheat samples used to establish a data base of gliadin electrophoregrams for computerized cultivar identification are listed along with their pedigrees in Tables 4-6. These samples represent a comprehensive collection of varieties that have been previously licensed in Canada prior to 1983. The list includes cultivars both of commercial and historic importance as well as varieties which possess regional or restricted licenses. Several U.S.A. registered HRS bread wheat cultivars were also included in the data base.

Pedigree information for cultivars licensed prior to 1976 are mainly from Zeven and Zeven-Hissink, (1976). The balance of pedigree data was obtained primarily from varietal description reports supplied by the Production and Marketing Branch, Plant Products Division, Agriculture, Canada, Ottawa, Ontario. Pedigrees for USA cvs. Alex and Coteau were kindly provided by Dr. R.C. Frohberg at North Dakota State University.

The major suppliers of wheat samples for this project include Dr. R. Loiselle, Central Office for the Plant Gene Resources of Canada, Ottawa Research Station, Agriculture Canada; Dr. M. Grant, Lethbridge

Research Station, Agriculture Canada. U.S.A. registered cultivars Coteau and Waldron and Eastern Canada region material: Dundas, Laval 19, Milton, Opal, Vernon, Monopol, Valor, Vuka, Favor, Gordon and Houser and cv. Chester were obtained from Dr. R. Tkachuk, Grain Research Laboratory, Canadian Grain Commission, Winnipeg, Canada. Durum wheat cultivars Coulter and Carleton were provided by Dr. D. Leisle, Winnipeg Research Station, Agriculture Canada.

Where possible, the cultivars listed in Tables 4-6 were divided into functional, class or pedigree groupings. The organization of gliadin electrophoregrams in the data base reflects these divisions, as well as computer results generated by the cultivar identification programs to be discussed. The numbers assigned to each cultivar in this tabulation, while mainly sequential, correspond only to sample numbers in the 3 mm PAGE results presented in Figures 20-34.

Winter wheat cultivars were conveniently split into hard red winter (HRW), soft white winter (SWW) and soft red winter (SRW) categories. Compared to their winter counterparts, Canadian wheats of spring habit represent a commercially more important segment of cultivars and were given a more complex structure. The "Pre-Marquis" group of hard red spring (HRS) wheats (Table 4) are those that were licensed before the introduction (1910) of the historic Canadian wheat cultivar Marquis. From then to the present, the majority of HRS bread wheats licensed for production in Western Canada can be traced in pedigree to a parentage dominated by either cv. Marquis or cv. Thatcher.

Other functional or type-specific groupings of Canadian spring wheats include HRS bread wheats possessing resistance to sawfly damage

and soft white wheat (SWW) cultivars. Spring wheats listed as miscellaneous represent a diverse collection of cultivars possessing unique pedigrees (e.g. Kota and Red Bobs 222), non-standard kernel characteristics (e.g. Glenlea - very large semi-hard kernels; Bishop, Norquay and Quality A - hard white wheats; Laval 19 - purple grain). Other wheats in this category include the semi-dwarf Pitic 62, and cultivars recommended for production in Eastern Canada (e.g. HRS cv. Huron). The HRS feed wheats cvs. Concorde, Opal and Milton and SRS feed cvs. Dundas and Vernon are restricted by license for production outside the Western prairie region because they possess kernel characteristics which are indistinguishable from the class of HRS bread wheat cultivars.

TABLE 4

Spring wheats used for computerized cultivar identification

	Pre-Marquis HRS wheats	Pedigree and Nationality
1	EARLY RED FIFE	selection of Red Fife, Canada
2	GARNET	Preston A/Riga M, Canada
3	PIONEER	Riga/Preston, Canada
4	PRELUDE	Downy Gehun/Fraser, Canada
5	PRESTON	Ladoga/Red Fife, Canada
6	RED FIFE	? Introduced from S. Poland into Canada
7	RUBY	Downy Riga/Red Fife, Canada
	Marquis related HRS wheats	**Pedigree and Nationality**
8	ACADIA	Marquis/Pentad//Canus, Canada
9	APEX	H-44-24/Double Cross//2*Marquis, Canada
10	CANUS	Marquis/Kanred, USA & Canada
11	CERES	Marquis/Kota, Canada
12	CORONATION II	Pentad/Marquis, Canada
13	LAKE	Regent/Canus, Canada
14	LEE	Hope//Bobin*2/Gaza, USA
15	MARQUIS	Hard Red Calcutta/Red Fife, Canada
16	REDMAN	Regent/Canus, Canada
17	REGENT	H-44-24/Reward, Canada
18	RELIANCE	Kanred/Marquis, USA
19	RENFREW	selection of Marquis, Canada
20	RENOWN	H-44-24/Reward, Canada
21	REWARD	Marquis/Prelude, Canada
22	SELKIRK	McMurachy/Exchange/3*Redman, Canada
	Thatcher related HRS wheats	**Pedigree and Nationality**
23	BENITO	Neepawa/3/RL4255*4//Manitou/CI7090, Canada
24	CANTHATCH	Thatcher*6/Kenya Farmer, Canada
25	COLUMBUS	Neepawa*6/RL4137, Canada
27	KATEPWA	Neepawa*6/RL2938/3/Neepawa*6//C.I.8154/ 2*Frocor, Canada (RL2938=Lee*2/Kenya Farmer)
28	MANITOU	Thatcher*7/Frontana//Canthatch/3/PI 170925/ 6*Thatcher, Canada
29	NAPAYO	Manitou*2/4/Thatcher*5/Lee/3/Thatcher*7/ Frontana//Thatcher*6/Kenya Farmer, Canada
30	NEEPAWA	Thatcher*7/Frontana//Thatcher*6/Kenya Farmer /3/Thatcher*2//Frontana/Thatcher, Canada

TABLE 4

Spring wheats used for computerized cultivar identification (cont'd)

	Thatcher related HRS wheats	Pedigree and Nationality
31	PARK	Mida/Cadet//Thatcher, Canada
32	PEMBINA	Thatcher/3/McMurachy/Exchange//3*Redman, Canada
33	SAUNDERS	Hope/Reward//Thatcher, Canada
34	SINTON	Manitou/3/Thatcher*6/Kenya Farmer//Lee*6/ Kenya Farmer, Canada
35	THATCHER	Marquis/Iumillo/Marquis/Kanred, Canada
	Sawfly resistant HRS wheats	**Pedigree and Nationality**
36	CANUCK	Canthatch/3/Mida/Cadet//Rescue, Canada
37	CHESTER	Renown/S-615//Rescue/3/Kendee/4/Mida/Cadet, Canada
38	CHINOOK	Thatcher/S-615-11, Canada
39	CYPRESS	Rescue/Chinook, Canada
40	LEADER	Fortuna/Chris, Canada
41	RESCUE	Apex/S-615, Canada
	USA registered HRS wheats	**Pedigree and Nationality**
42	ALEX	ND507/ND496
43	BUTTE	ND480/Polk//Wisconsin 261, USA
44	CHRIS	Frontana/3*Thatcher/3/Kenya 58/Newthatch/ 2*Thatcher, USA
45	COTEAU	ND496 sib//ND487/Fletcher
46	ERA	II-50-10/4/Pembina/II-52-329/3/II-53-38/ III-58-4//II-53-546, USA
47	LEN	N/A
48	OLAF	N/A
49	POLK	Thatcher/Suprenza/3/Kenya 58/Newthatch// Frontana, USA
50	WALDRON	Justin/4/Lee/3/Kenya 338A//Lee/Mida(ND81), USA
	Miscellaneous spring wheats	**Pedigree and Nationality**
51	BISHOP	Ladoga/Gehun, Canada
52	CONCORDE	Sel. of CI 13931 (Penjamo/Yaqui 54), Canada
53	DUNDAS	Opal/Inia 66, Canada
54	GLENLEA	Pembina*2/Bage//CB 100, Canada
55	HURON	White Fife/Ladoga, Canada

TABLE 4

Spring wheats used for computerized cultivar identification (cont'd)

	Miscellaneous spring wheats	Pedigree and Nationality
56	KOTA	? found in USSR durum, USA
57	LAVAL 19	F.W.606-A/Opal//Opal, Canada
58	MILTON	Kentville selection*6/Pompe, Canada; (Kentville sel.=awned plant sel. from Opal)
59	NORQUAY	Lerma Rojo/Sonora 64//Justin, Canada
60	OPAL	Triesdorf Stamm 21/40 x von Romke Erli; pedigree includes Garnet, Origin N/A
61	PITIC 62	Yaktana 54//Norin 10/Brevor 26-1C, Mexico
71	QUALITY A	selection of Florence, USA
62	RED BOBS 222	selection of Early Triumph, Canada
65	VERNON	Opal*4/Pompe, Canada
SWS wheats		Pedigree and Nationality
66	CASCADE	Quality A/Pacific Blue Stem//C26-59-2D/3/ Onas, Canada
67	FIELDER	Yaktana 54A*4//Norin 10/Brevor/3/2*Yaqui 50 /4/Norin 10/Brevor//Baart/Onas, USA
68	KENHI	Kenya 338 AC2E3/2*Lemhi, Canada
69	LEMHI 53	California 3098/5*Lemhi, USA
70	LEMHI 62	Lemhi 53*5/3/Lee*7//Chinese/Ae. Umbellata, USA
72	SPRINGFIELD	Norin 10/Brevor//3*Lemhi 53/3/Lemhi 62, USA

H-44-24= Marquis/Yaroslav emmer
RL4255 = Manitou*4//Africa 43/3*Thatcher/3/Manitou*5//Exchange/ 3*Thatcher/5/L4204*2/3/Manitou*3//Webster/4*Thatcher /4/Manitou*5//Centennario/6*Thatcher
RL4204 = RL4125*5//Thatcher*2/Red Egyptian/4/RL4125//Thatcher*6/ PI 170925/3/RL4125*4//Thatcher*3/Transfer
RL4137 = Frontana/3/McMurachy/Exchange//2*Redman/4/Thatcher*6/ Kenya Farmer
RL4125 = Thatcher*7/Frontana//Thatcher*6/Kenya Farmer
ND 496 = Waldron/ND269
ND 487 = ND259/Conley//Conley/ND122/3/Justin/ND142
ND 507 = Waldron/RL4205

TABLE 5

Winter wheats used for computerized cultivar identification

	HRW wheats	Pedigree and Nationality
73	KHARKOV 22 M.C.	selection of Kharkov, Canada
74	LENNOX	selection, Mironovskaja, USA
75	MONOPOL	Panthus/Admiral, West Germany
76	NORSTAR	Winalta/Alabaskaya, Canada
77	RIDIT	Turkey/Florence, USA
78	SUNDANCE	Cheyenne/Kharkov 22 M.C., Canada
79	VALOR	Kent/Sangaste (rye), Canada
80	VUKA	Toeering 2/Merlin//Carsten 8, FRG
81	WASATCH	Relief/Ridit, USA
82	WESTMONT	Rio/Rex//Nebred, USA
83	WINALTA	Minter/Wichita, USA
84	YOGO	Minturki/Beloglina//Buffum, USA
	SWW wheats	Pedigree and Nationality
85	CORNELL 595	Honor/Forward//Nured/3/Honor, USA
86	DAWBUL	Dawson's Golden Chaff/Bulgarian, Canada
87	DGCHAFF	selection of Clawson, Canada
88	FAVOR	Diga//Gabo/New Zealand 496.01, Canada
89	FREDRICK	Washington 1//Genesee/Capelle, Canada
90	GAINES	Norin 10/Brevor//Orfed/Brevor sib./3/Burt, USA
91	GENESSEE	Yorkwin//Honor*2/Forward, USA
92	GORDON	CD7561(related to Etoile de Choisy)/ Genessee/2/CD7561/Kent/3/7453-4-2-4 (Fredrick sib)/4/2*Yorkstar, Canada
93	HOUSER	Brevor/Norin 10//NY wheat rye sel./3/ Hope Hussar/Yorkwin/4/Genessee// CT12658/Alaskan/3/Avon, USA
94	JR.NO.6	as Goldcoin, selection of Redchaff or Redchaff Bald, USA
95	NUGAINES	sib. of Gaines, USA
96	OAC104	Dawson's Golden Chaff/Bulgarian, Canada
97	RICHMOND	Dawson's Golden Chaff*2/RIDIT, Canada
98	RIDEAU	Kharkov 22 M.C./Dawson's Golden Chaff, Canada
99	TALBOT	Trumbull//Hope/Hussar/3/Dawson's Golden Chaff*2/Ridit//Cornell 595, Canada
100	YORKSTAR	Genesee*5/3/Yorkwin//Norin 10/Brevor, USA

TABLE 5

Winter wheats used for computerized cultivar identification (cont'd)

	SRW WHeats	Pedigree and Nationality
101	EGYPTIAN AMBER	Fultz/Lancaster, USA
102	FAIRFIELD	Purkof/Fulhio, USA
103	JONES FIFE	Fultz/?/Mediterranean/?/Russian Velvet, USA
104	KENT	Caldwell 10/Dawson's Golden Chaff, Canada
105	SUN	as Sol, selection of local variety/ English Standup, Sweden
106	THORNE	Portage/Fulcaster, USA

TABLE 6

Durum wheats used for computerized cultivar identification

		Pedigree and Nationality
107	CARLETON	Vernal Emmer/Mindum, USA
108	COULTER	D.T.188/D.T.224/D.T.182, Canada
109	GOLDENBALL	? from S. Africa
110	HERCULES	RL 3097/RL 3304//Stewart/RL 3380, Canada
111	MACOUN	RL 3607/ DT 182, Canada
112	MEDORA	Ward/Macoun, Canada
113	MINDUM	? found in bread wheat field, USA
114	NUGGET	Mindum/Carleton//Heiti/Stewart, USA
115	PELISSIER	? introduced from Algeria into USA
116	RAMSEY	Carleton/PI 94701, USA
118	STEWART 63	ST 464/8*Stewart, Canada
119	WAKOOMA	Lakota*2/Pelissier, Canada
120	WASCANA	Lakota*2/Pelissier, Canada

3 MM VERTICAL PAGE ELECTROPHORESIS SYSTEM

For the wheat cultivar identification section of this project, several modifications in the PAGE system of Bushuk and Zillman (1978) were incorporated to improve electrophoregram resolution, the precision of results and to accomodate the scale of procedures for single kernel analysis. These modifications are described below.

Apparatus and Gel Preparation

The flatbed apparatus used to prepare a data base of gliadin PAGE patterns for cultivar identification (Figure 7), represents a vertical version of the 6 mm PAGE apparatus used previously. This apparatus accomodated 100 ml of gel solution to form a slab approximately 3 mm thick. Gel dimensions are 200 mm long and 150 mm wide. Recipes for the gel solution and tank buffer are given in Table 7.

A 6% polyacrylamide gel was prepared by dissolving acrylamide (6.0 g), bisacrylamide (0.3 g), ascorbic acid (0.1 g), ferrous sulfate (0.0016 g) in aluminum lactate buffer (pH 3.1) to achieve a final volume of 100 ml adjusted to pH 3.1 with lactic acid. The amount of ferrous sulfate used in the preparation of 3 mm thick gel slabs was reduced disproportionately, from 0.0025% w/v used in the 6 mm gel bed horizontal apparatus, to 0.0016% w/v. It was found that this concentration significantly improved the firmness of polymerized gels to facilitate ease of handling.

The procedure for gel polymerization was essentially unchanged, except that an 11-place slot former was used. Once polymerization was

Figure 7. Vertical 3 mm flatbed PAGE apparatus as modified from the 6 mm horizontal flatbed design described by Bushuk and Zillman (1978).

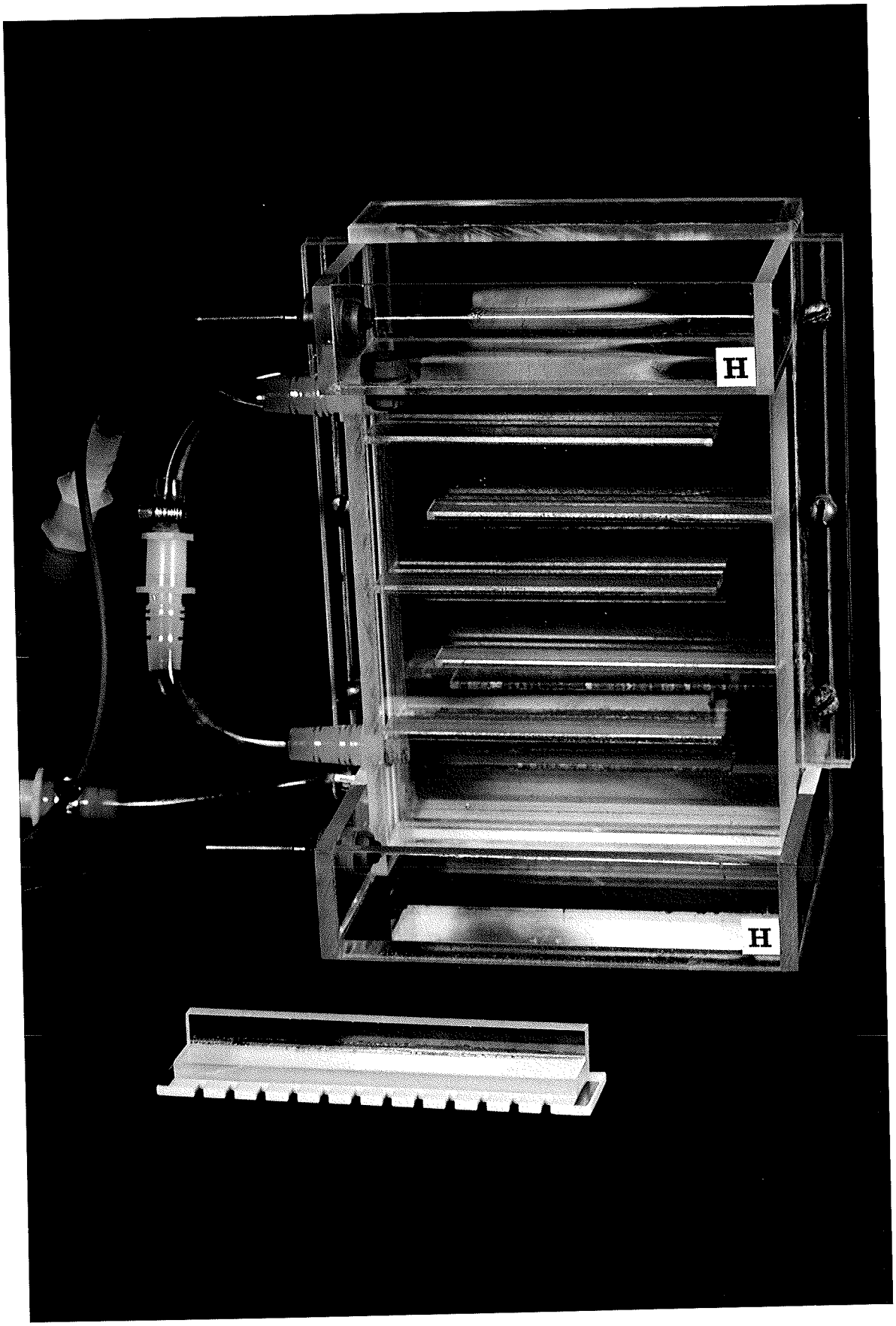

H
H

TABLE 7

Recipes for gel and tank buffer solutions - 3 mm PAGE

	Amount required for 100 ml final volume
Gel solution	
Acrylamide	6.0 g
N,N'-methylene-bis-acrylamide	0.3 g
Ascorbic acid	0.1 g
Ferrous sulfate	0.0016 g
Catalyst solution	
Hydrogen peroxide, 3%	0.5 ml
Tank buffer solution	
Aluminum lactate[1]	0.25 g
Lactic acid	to pH 3.1

[1]Aluminum lactate was obtained from Fluka Chemical Corp. (255 Oser Ave., Hauppauge, N.Y. 11787, U.S.A.)

complete, the apparatus was oriented in the vertical position and tank buffer reservoirs were filled to a combined volume of 580 ml.

Gliadin Extraction Procedure

For each cultivar listed in Tables 4-6, a minimum of two gliadin extracts were prepared for electrophoresis. These were derived from at least one single kernel and one ground sample of grain; for the first 50 numbered cultivars, a minimum of three extracts was used. All replicates were run on separate gel slabs.

If the electrophoregram from the bulk wheatmeal source precisely matched its single kernel counterpart(s), then the cultivar sample was

assumed to be homogeneous and mean relative mobility (Rm) data was subsequently determined from the replicate electrophoretic patterns. If on the other hand, the two patterns were found not to correspond, usually where the electrophoregram from the ground sample contained bands which complemented the single kernel pattern but not vice versa, then a series of single grains was examined (typically 9-27) to twice account for the dominant type patterns contributing to the composite electrophoregram of the mixture. The aim of this strategy was to identify heterogeneous cultivar samples and not determine the proportion of genotypes therein. The latter is not a trivial undertaking and requires the sampling of hundreds of single grains to obtain reliable estimates (Wrigley and Baxter, 1974).

Gliadin was extracted from single kernels by crushing and macerating the dry seed between a folded sheet of weighing paper with a hammer; the meal was then transferred to a 1.5 ml microcentrifuge tube and was extracted with 70% ethanol (2 ul/mg grain) by agitating briefly on a Vortex mixer. The mixture was left to stand at room temperature for 30 min and the contents were then centrifuged for 5 min at 8,000 x g at room temperature using a tabletop microcentrifuge (e.g. Beckman Microfuge B).

The 30 min standing interval was used as a matter of convenience, as the mixture of meal and ethanol could be centrifuged directly after mixing, with no detectable difference in the quality of the PAGE results (refer to Appendix A). When bulk wheatmeal samples were extracted, a 50 mg subsample of ground grain (4 g) was substituted for single kernels in the extraction procedure.

After centrifugation, the clear supernatant (e.g. 30 ul) was removed with a micro-pipettor and was diluted with 1.5 times its volume of a gliadin extract dilution solution and stored in a sealed vial. The extract dilution solution consisted of tank buffer containing 40% w/v sucrose and 0.6% w/v methyl green dye. Typically 10 ml of this solution was sufficient to complete the preparation of over 175 gliadin samples.

Electrophoresis Using Three Standard Gliadin Patterns Per Gel Slab

Prior to electrophoresis, a sample solution of 8 ul was applied to each gel slot using a micro-pipettor. To improve the precision of PAGE results, the number of reference gliadin samples was increased to three from two used previously. As with the 6 mm PAGE system, the flanking slots of the gel were reserved for reference gliadin samples from the cultivar Marquis and eight slots held the test samples. Additionally, the center slot was designated for a reference sample from cv. Neepawa. In this way, the ratio of test to reference gliadin samples was increased and flanking reference samples were only used to calculate relative mobilities for electrophoregrams in their respective half of the gel slab.

Neepawa was chosen as a supplementary PAGE reference cultivar for a number of reasons. Neepawa is a commercially important Canadian HRS wheat and possesses a gliadin band pattern complementary to its Marquis counterpart covering a wider range of the electrophoregram field in the low mobility region. Neepawa also contains the three reference gliadin bands common to Marquis which were subsequently used to compute relative mobilities (refer to following section). No offtype patterns for cv.

Neepawa could be found in the PAGE analysis of over 150 single kernels from two different sources.

Due to the thinner gel slab thickness with the vertical apparatus, the electrophoresis period was reduced to 4h (from 6.5h with the 6 mm horizontal PAGE system) at a constant current of 60 ma which produced a potential of 360 V (17 V/cm) across the gel.

Gel Staining and Photography

Thinner gel slabs also decreased the time required to produce a suitably stained gel for photography. The staining and destaining period was reduced respectively from 48h and 24h (6 mm PAGE) to 18h and 6h. The photographic procedure was modified by using Kodak Technical Pan film to produce both prints and positive film transparencies. The use of Technical Pan film in photographing gel slabs was found to give a satisfactory result, with good resolution and minimal loss of extremely faint bands observed in the stained electrophoregrams (refer to Figures 20 to 34). HC110 developer was used at medium dilution (9:1). The film format for densitometry was reduced to 4 x 5 inch positives replacing the more expensive 8 x 10 format transparencies used for scanning the 6 mm PAGE results.

Determination of Gliadin Band Densities

The intensity of gliadin bands in photographic prints of gel slabs was subjectively assigned a numerical integer value according to the procedure of Zillman and Bushuk (1979) except that the range of band density was expanded from 1 to 5 in their system to 1 (very faint)

through 9 (very dense) in the present study. Band density assignments were aided by visual reference to densitometric profile data. The precision of this method is considered to be ±1 unit.

Calculation of Gliadin Band Relative Mobilities

The procedure employed to calculate electrophoretic pattern mobility data relative to the Marquis reference band as described by Bushuk and Zillman (1978) was substantially modified for the wheat cultivar identification system developed in this study. The aim was to improve the precision associated with these calculations and hence to increase overall data base accuracy. The proposed method, apart from applying an electronic digitizing tablet to acquire band migration distances, involves the application of three reference protein components, designated as "R24", "R50" and "R79" on the basis of their respective mobilities relative to the Marquis reference band (R50). A characterization of these reference bands and complete description of the computational procedure will be deferred to the Results and Discussion section.

Data Base Organization and Coding of Gliadin Electrophoregrams

The 120 wheat cultivars listed in Tables 4-6 were used to derive over 180 distinct gliadin PAGE patterns which comprised the data base for the computerized system of cultivar identification. The discrepancy in numbers reflects an effort to document all off-type patterns including instances where source of material contributed to

disagreements in electrophoregram composition. Over 85% of the material analyzed by electrophoresis was apparently homogeneous or nearly so. The balance represented cultivar samples possessing varying degrees and type of admixture. The latter, insofar as it was possible, was designated by an extension appended to the name of the data base member. A characterization of off-type electrophoretic patterns is presented in Appendix C.

Each reference PAGE pattern entry in the data base is represented sequentially by three character and six numerical records of information in a set structure (refer to Figure 8) constrained by the input and output (I/O) formats of the cultivar identification system programs. Character records specify the cultivar name and pedigree. The numerical portion comprises a 110 element one-dimension cultivar signature array (row vector) of integer data type. The first 100 element positions contain paired relative mobility and density values, in order of increasing mobility, for up to 50 gliadin bands per electrophoregram. The remaining 10 element positions are used to store ancillary data on the cultivar and PAGE result which includes the total number of gliadin bands in the pattern, the number of replicates averaged in computing Rm values, the data base identification number (DBIN), and three index codes specifying the class of grain, functional quality, and production region. These codes are subsequently used to print an attribute summary along with the name and pedigree for each cultivar listed by the ranking program of the cultivar identification system to be described.

Figure 8 shows the standard format of a data base reference PAGE pattern entry for cv. Neepawa. Because the cultivar name appears

NEEPAWA[1]
THATCHER*7/FRONTANA//THATCHER*6/KENYA FARMER/3/THATCHER
*2//FRONTANA/THATCHER, CANADA[2]

```
 121 2 152 3 173 2 183 1 206 4 220 4 225 1 239 4 264 4 278 2
 293 2 305 5 318 5 372 5 383 5 437 3 457 7 478 8 500 9 520 6
 543 5 570 8 583 5 594 7 618 6 638 6 644 3 680 1 708 4 723 3
 736 3 750 2 789 4 805 1 812 1 821 1 836 2   0 0   0 0   0 0
   0 0   0 0   0 0   0 0   0 0   0 0   0 0   0 0   0 0   0 0
   0 0  3713   0 2   4 2  56 0
    |     | |   | |   | |   | |
    4     5 6   7 8   9 10  11 12
```

[3] Signature array element positions (SAEP) 1-100 comprise paired Rm and band density parameter values in odd and even array element locations respectively. Rm values are in integer data type with the decimal point implicit after the second digit.

[1] Cultivar name; maximum 16 characters including extension.
[2] Pedigree; 110 characters, 55/record maximum.
[4] SAEP 101-102 set=0.
[5] SAEP 103 = gliadin bands encoded for electrophoregram.
[6] SAEP 104 = replicates averaged to compute mean Rm values.
[7] SAEP 105 = blank, not assigned.
[8] SAEP 106 = kernel class code.
[9] SAEP 107 = general functional quality or utility code.
[10] SAEP 108 = production region code.
[11] SAEP 109 = data base identification number.
[12] SAEP 110 = blank, not assigned.

Figure 8: Standard data base coding format for the gliadin electrophoregram of cultivar Neepawa

without an extension, an electrophoretically homogeneous cultivar sample is implied. The additional numbers specified by the signature array (element positions 103-109) indicate that the electrophoregram encodes 37 gliadin bands whose Rm values were averaged using 13 replicate PAGE patterns and that the cultivar is a hard red spring wheat, superior to Marquis quality, grown in Western Canada and represents entry number 056 in the data base. A full listing of attribute summary codes (array

TABLE 8

Cultivar signature array attribute summary index codes and definitions

Attribute index no.	Signature array element position: 106 Class code	definition	107 Quality code	108 Region code
1	blank		blank	blank
2	HRS	hard red spring	NEMQ[1]	W.CAN
3	SHRS	semi-hard red spring	EMQ[2]	SAWFLY
4	HWS	hard white spring	SMQ[3]	S.ALTA
5	SHWS	semi-hard white spring	FEED	BC
6	SWS	soft white spring	PASTRY	ONTARIO
7	SRS	soft red spring	BW[4]	QUEBEC
8	SHPS	semi-hard purple spring	PASTA	ATLANTIC
9	HRW	hard red winter	GHP[5]	ATL/BC
10	SHRW	semi-hard red winter	nd[6]	R/W.CAN.[7]
11	SWW	soft white winter	nd	USA
12	SRW	soft red winter	nd	UTILITY
13	DURM	durum	nd	E.CAN
14	nd		nd	E.CAN/BC

[1]NEMQ - HRS wheat not equal to Marquis in milling and baking quality
[2]EMQ - HRS wheat equal to Marquis in milling and baking quality
[3]SMQ - HRS wheat superior to Marquis in milling and baking quality
[4]BW - non-HRS bread wheat
[5]GHP - general household purpose
[6]nd - not defined
[7]R/ W.CAN - restricted from W.CAN region by kernel characteristics

Source of attribute data: In "Handbook of Canadian Varieties of Barley, Field Beans, Field Peas, Flax, Oats, Rye, and Spring, Durum, and Winter Winter Wheat". Prepared by Research Branch, Canadian Department of Agriculture; Varietal description reports prepared by The Production and Marketing Branch, Plant Products Division, Agriculture Canada, Ottawa, Ontario.

element positions 106-108) and their respective definitions is presented in Table 8.

V

RESULTS AND DISCUSSION

AUTOMATIC QUANTIFICATION OF GLIADIN ELECTROPHOREGRAMS BY MINICOMPUTER PROCESSING OF DENSITOMETRIC SCANNING PROFILES

This section will examine results of the system of programs developed to compute gliadin electrophoregram data from sets of replicate densitometric profiles. The objective was to eliminate the tedious and subjective determination of relative mobility and band density values required for cultivar identification. A flow diagram, which can be used as a guide for the data acquisition and processing system is shown in Figure 9.

Outline of Computer Programs

The recovery of gliadin electrophoregram data by computer analysis of densitomegrams could involve a maximum of three replicate traces. The process was executed in four sequential program steps:

1. Raw digitized densitometric profile records are retrieved from a PAGE sample-gel slab data base library stored on disk (program: SELECT). Accessed data can be initially viewed as a superimposed graphic display of normalized densitometric profiles (program: GSELEC).

2. A first derivative trace is computed for each replicate densitomegram (program: DISTIL, subroutine PRIME). Slope values are then used as the basis to detect protein bands represented in the absorbance profile as peaks and shoulders. X and Y-axis

coordinate positions for peak maxima and leading and trailing shoulder inflection points are respectively normalized to relative mobility and band density values and the data is transcribed to the standard cultivar signature array numerical format (program: DISTIL, subroutine PEAK).

3. Pairwise combinations of replicate cultivar signature arrays are analyzed to delete non-replicating gliadin band data. The problem is one of identifying homologous gliadin components which fall within a predefined threshold for differences in Rm and band density parameter values. A mean electrophoregram cultivar signature array is then computed for each pair of densitometric profiles (program: FILTER).

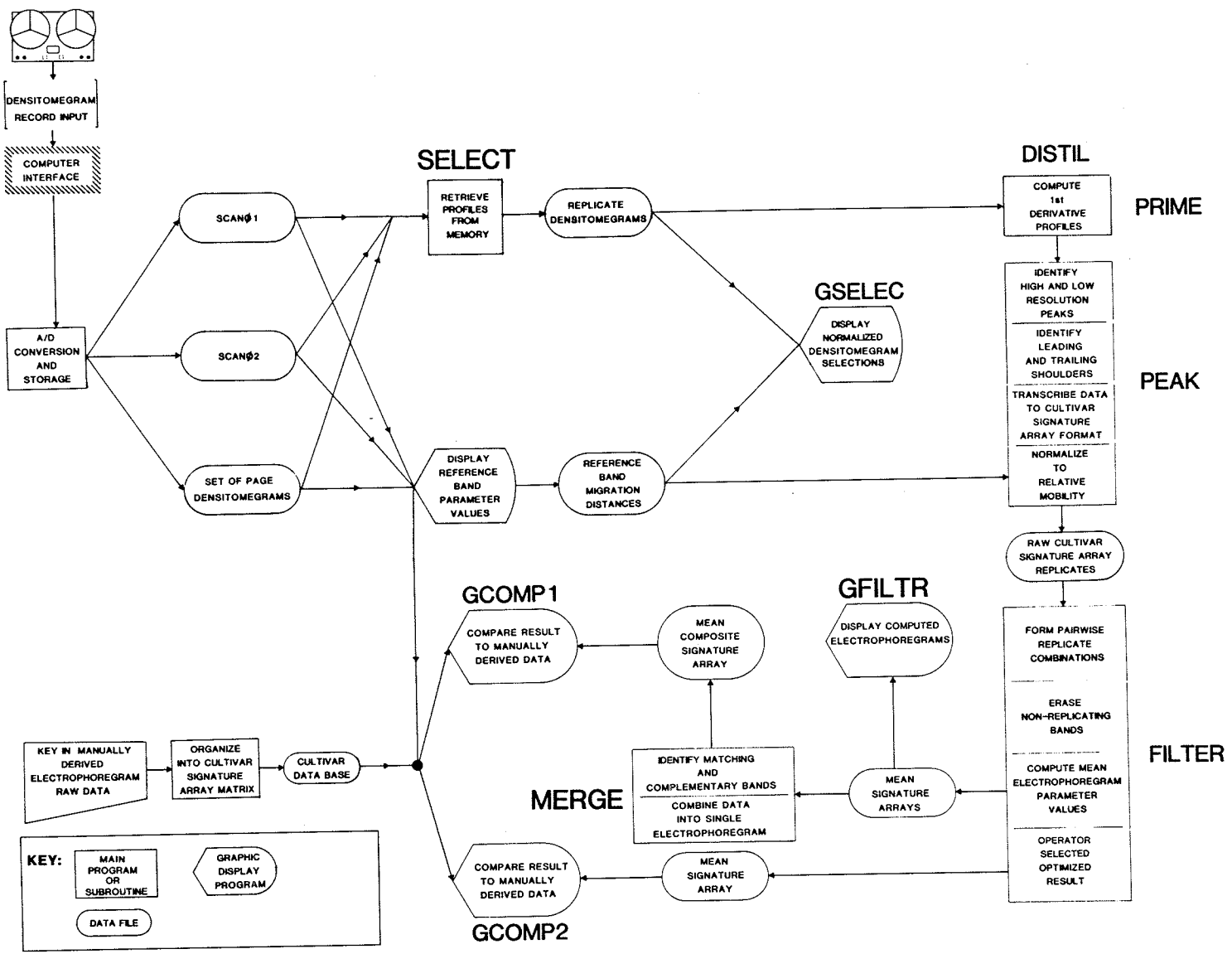
4. If three densitometric profiles are involved in the analysis, then homologous bands may be identified in one paired replicate combination but not in another. The task is then to identify this type of event and compute a single mean composite signature array which combines gliadin components in one replicate array which complement the pattern of bands in another (program: MERGE).

To permit the operator to assess the numerical result after various stages of program analysis, several display programs (GFILTR, GCOMP1, GCOMP2, GMERGE; refer to Figure 9) were developed which produce PAGE pattern graphics from cultivar signature array data. The last three additionally compare the computed gliadin electrophoregram against its manually derived counterpart which represents averaged relative mobilities calculated from triplicate electrophoretic runs. Programs GCOMP1 and GCOMP2 include densitometric profiles as part of the display.

All the programs referenced above were written using standard FORTRAN and were executed under the DEC RSX-11S operating system. Input and output routines are typically machine dependant. With the exception of the initial record selection step and graphic display programs with interactive capability, execution flow was automatic.

The four processing steps (programs: SELECT, DISTIL, FILTER and MERGE) to achieve a typical numerical result will be discussed using densitometric profile data for the soft white winter wheat cultivar

Figure 9. Flow diagram of program execution to compute gliadin electrophoregrams from densitometric scanning profiles.


DENSITOMEGRAM RECORD INPUT
COMPUTER INTERFACE
A/D CONVERSION AND STORAGE
SCANϕ1
SCANϕ2
SET OF PAGE DENSITOMEGRAMS
SELECT
RETRIEVE PROFILES FROM MEMORY
REPLICATE DENSITOMEGRAMS
DISPLAY REFERENCE BAND PARAMETER VALUES
REFERENCE BAND MIGRATION DISTANCES
GSELEC
DISPLAY NORMALIZED DENSITOMEGRAM SELECTIONS
DISTIL
COMPUTE 1st DERIVATIVE PROFILES
PRIME
IDENTIFY HIGH AND LOW RESOLUTION PEAKS
IDENTIFY LEADING AND TRAILING SHOULDERS
TRANSCRIBE DATA TO CULTIVAR SIGNATURE ARRAY FORMAT
NORMALIZE TO RELATIVE MOBILITY
PEAK
RAW CULTIVAR SIGNATURE ARRAY REPLICATES
GFILTR
DISPLAY COMPUTED ELECTROPHOREGRAMS
FORM PAIRWISE REPLICATE COMBINATIONS
ERASE NON-REPLICATING BANDS
COMPUTE MEAN ELECTROPHOREGRAM PARAMETER VALUES
OPERATOR SELECTED OPTIMIZED RESULT
FILTER
GCOMP1
COMPARE RESULT TO MANUALLY DERIVED DATA
MEAN COMPOSITE SIGNATURE ARRAY
MERGE
IDENTIFY MATCHING AND COMPLEMENTARY BANDS
COMBINE DATA INTO SINGLE ELECTROPHOREGRAM
MEAN SIGNATURE ARRAYS
GCOMP2
COMPARE RESULT TO MANUALLY DERIVED DATA
MEAN SIGNATURE ARRAY
KEY IN MANUALLY DERIVED ELECTROPHOREGRAM RAW DATA
ORGANIZE INTO CULTIVAR SIGNATURE ARRAY MATRIX
CULTIVAR DATA BASE
KEY:
MAIN PROGRAM OR SUBROUTINE
GRAPHIC DISPLAY PROGRAM
DATA FILE

Talbot. Also described are the results of five graphic display programs (GSELEC, GFILTR, GCOMP2, GCOMP1 and GMERGE) which aid in evaluating the performance of the system. An assessment was additionally based upon the result of comparing precision of the computed gliadin electrophoregram against counterpart pattern data derived using the manual reference procedure of Bushuk and Zillman (1978).

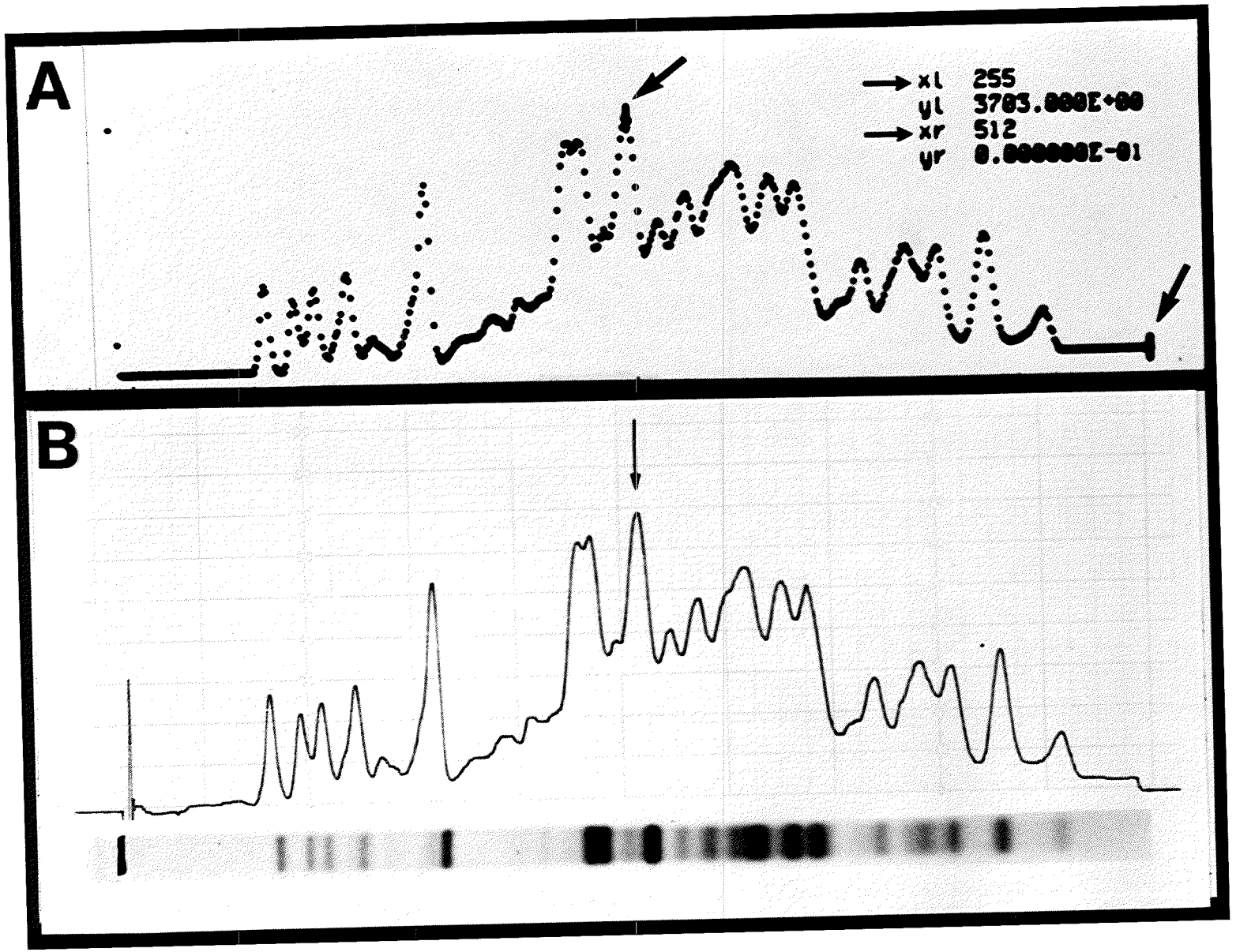
Acquisition of Marquis Reference Band Migration Distance Values

The pair of migration distance values for cv. Marquis gliadin band "50", the internal gel slab reference protein, represent critical normalization factors which permit the precise comparative analysis of densitometric profiles and numerically encoded electrophoregrams. To obtain data on the Marquis reference band for each gel slab, a minicomputer system graphics program[1] was invoked which allows the operator to readout the Cartesian coordinates of graphic cursors which can be positioned along display profile envelopes by manipulating a joystick-like control.

A typical result of this process is illustrated in Figure 10 which shows the computer display of a digitized densitometric profile for cv. Marquis and its analog counterpart. The Marquis reference band is readily identified by its relative high density in the center of the pattern (Figure 10B), being preceeded in mobility by a characteristic doublet. In Figure 10A, the leftmost cursor is positioned at the reference protein peak maximum with its X and Y-coordinates displayed in odometer fashion (x1=255; y1=3703). The abscissa value was recorded and

[1]SPARTA, in: Lab Applications-11 System Reference Manual, 1974. Digital Equipment Corporation. Maynard, Massachusetts.

Figure 10. Digitized (A) and analog (B) densitometric profiles for the Marquis electrophoregram. Two operator-controlled cursors are shown (large arrows in A) positioned respectively on the reference peak maximum, and the last data point of the curve which was imaged on the video display monitor. X and Y-axis coordinates for the left (l) and right (r) cursors are displayed in odometer fashion. As shown, each sample electrophoregram was resolved into 512 discrete mobility positions. The abscissa value (xl) was used in an algorithm to normalize experimental gliadin band position data in the gel slab to relative mobilities according the procedure of Bushuk and Zillman (1978).


A
xl 255
yl 3703.000E+00
xr 512
yr 0.000000E-01
B

combined with counterpart data, for other PAGE runs to comprise a file of Marquis reference band parameter values to be used as input for programs GSELEC and DISTIL (refer to Figure 9) which normalize densitometric profiles and derived gliadin electrophoretic pattern data.

Retrieval of Densitomegram Data from Computer Memory

The execution of program SELECT initiates an interactive dialog between the computer and operator in order to access densitomegrams that were stored on disk during the digitization procedure. In this procedure, the computer prompts the operator to solicit up to three replicate densitometric profiles from the data base in seven terminal input steps. The dialog is illustrated in Figure 11. The initial entry "2", which specifies cv. Talbot, corresponds to the identification number arbitrarily assigned to each cultivar in the data base. Three pairs of PAGE slab and gliadin sample numbers specify the scan file and record respectively for the selected profiles which carry the labels "R1", "R2" and "R3" in subsequent program printouts and graphics. As densitomegrams are retrieved from disk, each is tagged as to its source (i.e. gel slab and sample number) and cultivar identification number. This tagging process satisfies program requirements as the data is passed on through different stages of processing.

Once the retrieval operation is completed, program GSELEC can be invoked to normalize the profiles to a relative mobility basis and display the data in selected sequence, and in superimposed fashion, on the graphics monitor (Figure 11B-D). This step provides a visual check for the operator to ensure that the desired traces have been accessed

Figure 11. Computer display of replicate densitomegrams for cv. Talbot. Replicate selection display program SELECT result (A) illustrates the interactive dialog which serves to retrieve densitometric profile data (cv. Talbot) from disk storage. The selected replicate densitomegrams were normalized to relative mobility and displayed by program GSELEC (B-D) in a superimposed graphic format which depicts the degree of variability existing in the data.

A

```
Program: SELECT
Retrieval of densitomegram replicate data from disk storage.
.....................................................................
ENTER: Cultivar identification number.
  2

ENTER: PAGE slab number (SCAN## FILE) for replicate ## #1 ##
  1
ENTER: Gliadin sample number (RECORD) on gel.
  3

ENTER: PAGE slab number for replicate ## #2 ##
  2
ENTER: Gliadin sample number on gel.
  3

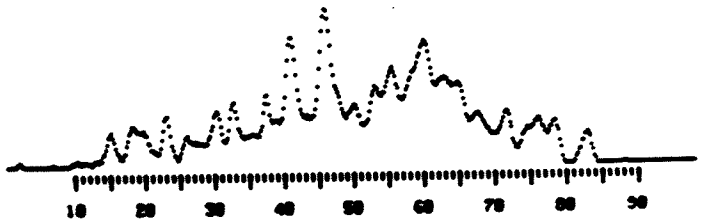
ENTER: PAGE slab number for replicate ## #3 ##
  3
ENTER: Gliadin sample number on gel.
  3
.....................................................................
STOP --

.B
```

B

```
Program: GSELEC
Graphic display of replicate densitomegrams retrieved from memory.

Data for cultivar:  TALBOT
Scan for PAGE slab no. 1   -   sample no. 3          Ref. band = 252
```

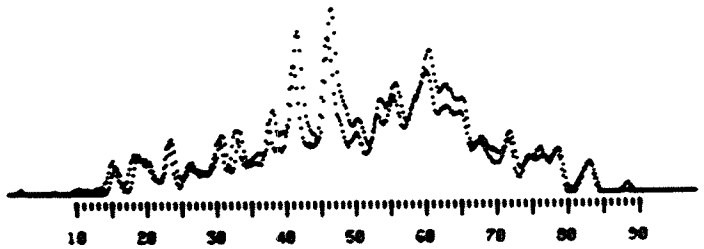


Electrophoretic Mobility Relative To The Marquis Reference Band

C

```
Program: GSELEC
Graphic display of replicate densitomegrams retrieved from memory.

Data for cultivar:  TALBOT
Scan for PAGE slab no. 1   -   sample no. 3          Ref. band = 252
Scan for PAGE slab no. 2   -   sample no. 3          Ref. band = 253
```

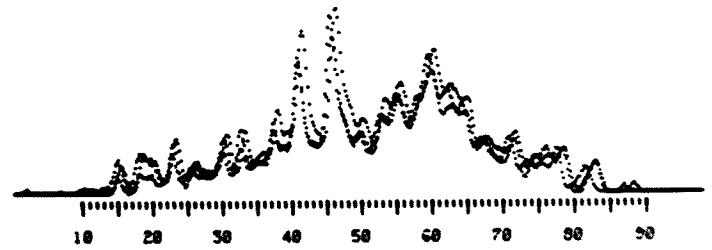


Electrophoretic Mobility Relative To The Marquis Reference Band

D

```
Program: GSELEC
Graphic display of replicate densitomegrams retrieved from memory.

Data for cultivar:  TALBOT
Scan for PAGE slab no. 1   -   sample no. 3          Ref. band = 252
Scan for PAGE slab no. 2   -   sample no. 3          Ref. band = 253
Scan for PAGE slab no. 3   -   sample no. 3          Ref. band = 259
```



Electrophoretic Mobility Relative To The Marquis Reference Band

correctly as well as to view the level of variation which exists in the data. The near congruency of the replicate densitomegrams especially the Talbot replicates derived from PAGE slabs 1 and 2 (Figure 11C) reflects the level of reproducibility that can be achieved by careful adherence to a standard electrophoretic procedure.

The decrease in densitometric profile homology with inceasing mobility for Rm > 50 (Figure 11D) is typical of the variability which can be encountered. A similar result has been reported elsewhere (Lookhart et al., 1983) when calculating Rm values from measurements of different gels. The larger absolute error associated with Rm values for proteins migrating the greatest distance into the gel slab is clearly reflected in the statistical data presented in Table 13. As will be later demonstrated, this variability can be effectively minimized by calculating mobility data relative to a second reference gliadin band positioned in the high mobility electrophoregram region.

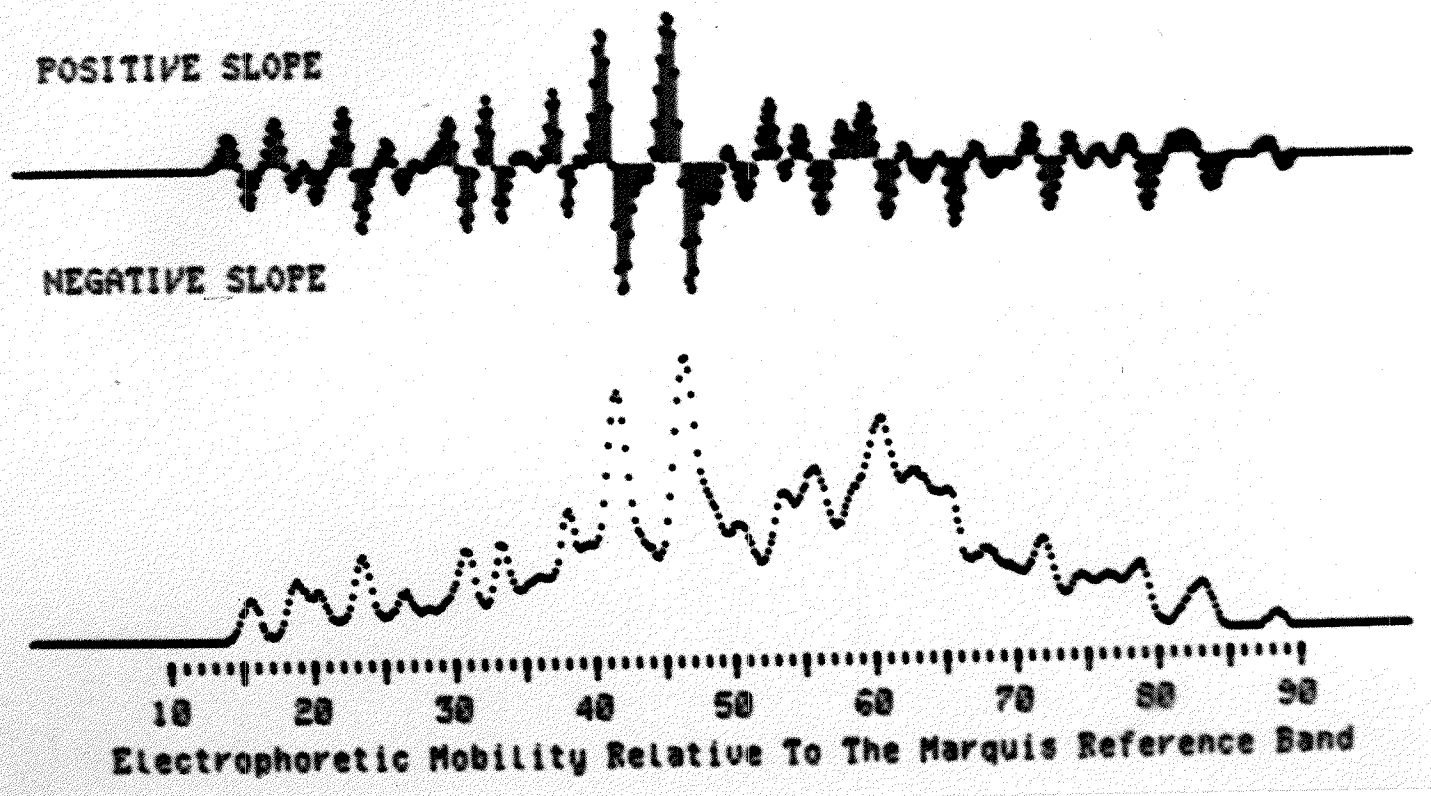
Peak Detection and Normalization of Position Coordinates to Relative Mobility and Band Density Values

The procedure of reducing densitometric profiles to peak coordinates and transcribing the list of feature parameters to a standard format of relative mobility and band density data involved the preliminary task of peak detection by a procedure based on first derivatives. The initial subroutine segment PRIME of program DISTIL (refer to Figure 9) calculates the first smoothed derivative for each replicate densitomegram. The computation was based on the form tabulated by Savitzky and Golay (1964) for a second-degree polynomial least squares fit over five points.

Figure 12 Densitometric scanning profile for cv. Talbot and its smoothed 1st derivative computed according to the method of Savitzky and Golay (1964) for a second-degree polynomial least squares fit over five data points.

Program: GPRIME
Graphic display of 1st derivative transformation data
for gliadin densitomegram of wheat cultivar - TALBOT

Scan for PAGE slab no. 2 - sample no. 3 Ref. band = 253



A typical result is shown in Figure 12 which illustrates the string of positive and negative slope values representing peaks and valleys along each densitomegram. The identification of electrophoregram components across 1st derivative spectra was accomplished by subroutine PEAK of program DISTIL. A gliadin band was found if one of the three following conditions was satisfied with respect to the slope (dy/dx) of the densitometric profile.

1. Positive to negative change in dy/dx across a zero baseline denotes a peak maximum.
2. A minimum positive value in dy/dx denotes a shoulder on the leading edge of a peak.
3. A maximum negative value in dy/dx denotes a shoulder on the trailing edge of a peak.

An operational threshold for peak detection was defined as the minimum number of data points preceeding and following the change in sign of the first derivative. Two iterations are used to scan the data for peak maxima. The operator, by setting a higher detection threshold in the first scan than in the second, can differentiate components based on relative peak resolution.

This strategy provided a safeguard in the cultivar identification application where the unknown sample was represented by only one replicate densitometric profile. Because a change in sign of the first derivative is the key factor in detecting peaks, the operator may choose to include only those peaks with a clear and consistent signature thus guarding against false gliadin band assignments. When two or more replicate densitometric profiles are processed, the decision to accept or reject both high and low peaks is programmed to depend on a replication test which is described in the following section. As will be

shown, the greater proportion of non-replicating components possess low resolution peak envelopes.

Threshold values were selected using empirical guidelines and depend mainly upon the sampling density of densitometric profile data points. In the present application, high resolution peaks were found using a window of five positive and five negative data points about the first derivative zero baseline ("Peak ID sensitivity" in Figure 13). Additional peaks found in the second iteration by selecting the lowest threshold that could be applied, i.e. ±1 data points, were arbitrarily defined as low resolution components and were allocated to a separate array list encoding detected peaks.

Each time a band is detected, X and Y-coordinates of the peak maximum or inflection point of a shoulder are tranformed to relative values. A standardized mobility scale in accord with that described by Bushuk and Zillman (1978) is established within the range 10.0 to 95.0. For a single band, "i", its relative mobility, Rm(i), at coordinate, Xi, is given by

$$Rm(i) = 50.0 * (Xi/Xref)$$

where Xref specifies the mean coordinate position of the Marquis reference band for the corresponding gel slab. Similarly, a measure of relative band density, Rd(i), within the range 0.1 to 8.0 is obtained by comparing the coordinate Yi with the profile component possessing the largest peak height, Ymax, where

$$Rd(i) = 8.0 * (Yi/Ymax)$$

Band density is then rounded up to the nearest integer for signature array encoding. This procedure establishes an optimized relative band

density scale which serves to minimize the variability in band densities which may arise from variation in the protein content of gliadin extracts (or grain samples) prepared for electrophoresis.

Once relative mobility and band density values are determined, the paired data is appended to a lengthening list termed a cultivar signature array as the computer continues to acquire more data. The end result for three density curves is shown in Figure 13 which illustrates the printout report of program DISTIL. Each densitomegram replicate was reduced to a set of two cultivar signature arrays (CSA), [I] and [II] which encode the list of Rm and band density feature parameters for high and low resolution densitomegram peaks, plus shoulders respectively. Low resolution densitometric profile components are tagged with asterisks. Leading and trailing shoulders are specified with characters "L" and "T" respectively.

Filter Process to Eliminate Non-Replicating Peak Data

Figure 13 shows that a total of 28, 27 and 26 gliadin bands were detected in each of the respective densitometer tracings for cv. Talbot. Because the number of detected peaks and their corresponding mobilities can vary between replicates, processing constraints are imposed (program FILTER) to pair together only homologous bands which are identified in different profiles. In addition to the variability associated with band mobilities, anomalous peaks may arise from gel slab "noise" which commonly originates from absorbance of precipitated Coomassie Blue dye adhering as random spots on the surface of the polyacrylamide gel slab. Spurious information in general, is detected by the software as

Figure 13. Computer printout of relative mobility and band density data for detected peak maxima and shoulders from densitometric profiles of cv Talbot. Peak identification by program DISTIL, was performed on individual replicates of gliadin densitomegrams (A, B and C), by analysis of the respective 1st derivative data. Computed Rm and density parameter values for detected components were ordered in pairs to form a cultivar signature array. For a single densitomegram, the program generates two such arrays. Each corresponds to bands detected above and below an operator-selected threshold level for peak detection (see text for details).

Program: DISTIL

Densitomegram processing data
for replicate gliadin electrophoregrams of wheat cultivar: TALBOT.

A REPLICATE R1: PAGE slab 1 sample 3 - reference band position = 252.5

HIGH resolution scan
Signature array [I]

Peak ID sensitivity = +5/-5 data points.
18 peaks detected.

14.9(2)	18.3(3)	23.0(3)	26.0(2)	30.1(3)	32.7(4)	37.4(4)
41.0(7)	45.9(8)	50.0(4)	55.3(6)	59.9(7)	62.6(5)	67.5(3)
71.5(3)	76.0(3)	78.3(3)	82.7(2)			

LOW resolution scan
Signature Array [II]

Peak ID sensitivity = +1/-1 data points.
8 additional (*) peaks detected.
2 shoulders detected: (L)leading,(T)trailing.
28 = total number of gliadin bands encoded.

11.2(1)*	14.9(2)	18.3(3)	19.5(2)*	23.0(3)	26.0(2)	28.0(2)*
30.1(3)	32.7(4)	35.5(2)*	37.4(4)	38.8(3)*	41.0(7)	45.9(8)
50.0(4)	52.9(5)*	55.3(6)	59.9(7)	62.6(5)	64.6(5)*	67.5(3)
71.5(3)	76.0(3)	78.3(3)	82.7(2)	88.2(1)*	58.2(5)L	47.7(4)T

B REPLICATE R2: PAGE slab 2 sample 3 - reference band position = 253.0

HIGH resolution scan
Signature array [I]

Peak ID sensitivity = +5/-5 data points.
16 peaks detected.

15.5(2)	18.8(2)	23.4(3)	26.3(2)	30.7(3)	33.4(3)	37.9(4)
41.5(8)	46.4(8)	55.5(5)	60.2(7)	67.7(3)	71.6(3)	78.5(2)
83.0(2)	88.2(1)					

LOW resolution scan
Signature Array [II]

Peak ID sensitivity = +1/-1 data points.
9 additional (*) peaks detected.
2 shoulders detected: (L)leading,(T)trailing.
27 = total number of gliadin bands encoded.

15.5(2)	18.8(2)	20.2(2)*	23.4(3)	26.3(2)	28.1(1)*	30.7(3)
33.4(3)	36.2(2)*	37.9(4)	41.5(8)	46.4(8)	50.1(4)*	53.4(5)*
55.5(5)	60.2(7)	62.5(5)*	64.8(5)*	67.7(3)	71.6(3)	74.5(2)*
76.4(2)*	78.5(2)	83.0(2)	88.2(1)	58.3(5)L	48.2(5)T	

C REPLICATE R3: PAGE slab 3 sample 3 - reference band position = 259.5

HIGH resolution scan
Signature array [I]

Peak ID sensitivity = +5/-5 data points.
17 peaks detected.

14.9(1)	22.8(2)	25.5(2)	30.1(2)	32.6(2)	37.4(3)	41.0(7)
45.6(8)	54.9(5)	59.5(7)	62.1(5)	66.8(3)	70.8(3)	73.2(2)
77.4(2)	81.5(2)	86.8(1)				

LOW resolution scan
Signature Array [II]

Peak ID sensitivity = +1/-1 data points.
7 additional (*) peaks detected.
2 shoulders detected: (L)leading,(T)trailing.
26 = total number of gliadin bands encoded.

14.9(1)	18.2(1)*	19.5(1)*	22.8(2)	25.5(2)	27.0(1)*	30.1(2)
32.6(2)	37.4(3)	41.0(7)	45.6(8)	49.7(3)*	52.7(4)*	54.9(5)
59.5(7)	62.1(5)	64.2(5)*	66.8(3)	70.8(3)	73.2(2)	75.5(2)*
77.4(2)	81.5(2)	86.8(1)	57.6(5)L	47.4(4)T		

non-replicating relative peak coordinate data and is deleted from cultivar signature arrays before mean band values are determined. Program logic to obtain a typical result (Figs. 14 and 15) is described below.

The analysis begins by forming pairwise combinations of electrophoretic pattern data generated by program DISTIL (i.e. R1/R2, R1/R3, R2/R3). Each combination is handled separately but in an identical fashion. Gliadin components were designated as homologous if replication occurs within a narrow relative mobility and band density window. The patterns are first evaluated for positional agreements. If the Rm difference between two bands in replicate arrays is not greater than a threshold value a potential matching event is flagged, and is either confirmed or rejected by a comparison of band densities.

The difference threshold for band density can be selected by the operator in unit increments and a match is rejected if this limit is exceeded. For typical program runs, a threshold value of 2 was chosen which represents more than four standard deviations of mean relative peak height (for three replicates) averaged over 200 peaks derived from 10 separate cultivar analyses.

For comparison of band mobilities, it was found that the application of a fixed difference threshold equivalent to approximately four standard deviations of relative mobility units (RMU) i.e. 1.6 RMU, resulted in a low but significant number of false matches involving groups of closely contiguous gliadin bands. Consequently, the evaluation of electrophoretic pattern positional agreements was handled in an iterative fashion using a range of difference threshold values.

The computer repeatedly scans each combination of paired replicate patterns searching for homologies. The difference threshold, which can be described as a moving window with an initial size of 0.1 RMU, is incremented by 0.1 mobility units for each iteration up to a maximum established by the operator (typically 1.5 mobility units). When two gliadin components (one/pattern) appear in the window, a replication event is flagged. The pair of bands are tagged so that they cannot be found in a subsequent scan. Mean mobility and band density values are then computed and the data is recorded into a list termed a mean signature array (MSA). As the MSA increases in size with appended data, the number of bands remaining untagged declines. In this way, the potential for error in identifying homologous bands possessing greater variation in mobility is effectively minimized as the average distance between contiguous untagged components increases.

This process ultimately yields three mean signature arrays which encode the replicating components of three densitometric profiles for cv. Talbot. The result is elaborated in the computer printout of program FILTER presented in Figure 14. Analysis of densitomegrams R1 and R2 (Figure 14A) has identified a total of 26 gliadin components (15 high and 11 low resolution), replicating within a range of 0.7 relative mobility units, the highest difference threshold reached. Corresponding band densities, which appear directly below component mobilities, show that only one pair of matching bands, in all three replicates, [R1: 41.0(6), R2: 41.5(8)] differs by more than one density unit. While a greater level of variation is indicated (by higher Rm difference threshold levels) for densitometric profile replicate pairs R1/R3

Figure 14. Computer printout of FILTER program processing to isolate and delete non-replicating gliadin "bands" found by the peak detection program. The analysis erases non-replicating peak data encoded into the cultivar signature arrays generated by program DISTIL. Three pairwise combinations of replicates were formed (A, B and C) and scanned separately using a moving window technique (see text) to detect matching gliadin components. Replicate bands are identified and listed in order of increasing absolute difference in Rm value. For each paired replicate combination, a mean signature array (MSA) was computed. At the end of program execution the operator can tag a particular MSA (see bottom of figure) to graphically display a computer-composed electrophoregram representing the encoded data (refer to Figure 16).

```
Program:  FILTER

Erasure of non-replicating gliadin bands (detected by program DISTIL)
in paired combinations of densitomegram data for cultivar:   TALBOT.
Scanning window width (Delta) = 0.1 to 1.5 relative mobility units.
Program DISTIL - peak ID resolution parms. = 5/1 - shoulder detect on.
.....................................................................

Paired input data: DENSITOMEGRAM REPLICATES - R1/R2 - integer mobility

A
 High resolution scan: 15 matching gliadin bands identified.
  Delta = 0.1 0.2 0.2 0.2 0.3 0.3 0.3 0.4 0.5 0.5 0.5 0.5 0.6 0.6 0.7
          --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    R1:   715 553 675 783 260 599 827 230 183 374 410 459 149 301 327
    R2:   716 555 677 785 263 602 830 234 188 379 415 464 155 307 334
          --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
  Dens. = 3/3 6/5 3/3 3/2 2/2 7/7 2/2 3/3 3/2 4/4 7/8 8/8 2/2 3/3 4/3

 low resolution scan: 11 matching gliadin bands identified.
  Delta = 0.1 0.1 0.1 0.1 0.1 0.2 0.4 0.5 0.5 0.7 0.7
          --- --- --- --- --- --- --- --- --- --- ---
    R1:   280 500 582 626 882 646 760 477 529 195 355
    R2:   281 501 583 625 882 648 764 482 534 202 362
          --- --- --- --- --- --- --- --- --- --- ---
  Dens. = 2/1 4/4 5/5 5/5 1/1 5/5 3/2 4/5 5/5 2/2 2/2

          2 unmatched bands isolated in replicate R1
             11.2(1)  38.8(3)
          1 unmatched band isolated in replicate R2
             74.5(2)

 Mean Signature Array  -  [1]  -  Number of bands encoded = 26

 15.2(2)  18.5(2)  19.8(2)  23.2(3)  26.1(2)  28.0(2)  30.4(3)
 33.0(3)  35.8(2)  37.6(4)  41.2(7)  46.1(8)  47.9(4)  50.0(4)
 53.1(5)  55.4(5)  58.2(5)  60.0(7)  62.5(5)  64.7(5)  67.6(3)
 71.5(3)  76.2(2)  78.4(3)  82.8(2)  88.2(1)   0.0(0)   0.0(0)
  0.0(0)   0.0(0)   0.1(2)   0.2(*)
.....................................................................

Paired input data: DENSITOMEGRAM REPLICATES - R1/R3 - integer mobility

B
 High resolution scan: 15 matching gliadin bands identified.
  Delta = 0.1 0.1 0.1 0.1 0.1 0.2 0.3 0.4 0.4 0.5 0.5 0.7 0.7 0.9 1.2
          --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    R1:   149 301 327 374 410 230 459 553 599 260 626 675 715 783 827
    R3:   149 301 326 374 410 228 456 549 595 255 621 668 708 774 815
          --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
  Dens. = 2/1 3/2 4/2 4/3 7/7 3/2 8/8 6/5 7/7 2/2 5/5 3/3 3/3 3/2 2/2

 low resolution scan: 10 matching gliadin bands identified.
  Delta = 0.1 0.1 0.2 0.3 0.3 0.4 0.5 0.6 1.0 1.4
          --- --- --- --- --- --- --- --- --- ---
    R1:   183 195 529 477 500 646 760 582 280 882
    R3:   182 195 527 474 497 642 755 576 270 868
          --- --- --- --- --- --- --- --- --- ---
  Dens. = 3/1 2/1 5/4 4/4 4/3 5/5 3/2 5/5 2/1 1/1

          3 unmatched bands isolated in replicate R1
             11.2(1)  35.5(2)  38.8(3)
          1 unmatched band isolated in replicate R3
             73.2(2)

 Mean Signature Array  -  [2]  -  Number of bands encoded = 25

 14.9(2)  18.2(2)  19.5(2)  22.9(3)  25.7(2)  27.5(1)  30.1(3)
 32.6(3)  37.4(4)  41.0(7)  45.7(8)  47.5(4)  49.8(3)  52.8(4)
 55.1(5)  57.9(5)  59.7(7)  62.3(5)  64.4(5)  67.1(3)  71.1(3)
 75.7(2)  77.8(3)  82.1(2)  87.5(1)   0.0(0)   0.0(0)   0.0(0)
  0.0(0)   0.0(0)   0.1(3)   0.2(*)
.....................................................................

Paired input data: DENSITOMEGRAM REPLICATES - R2/R3 - integer mobility

C
 High resolution scan: 15 matching gliadin bands identified.
  Delta = 0.5 0.5 0.6 0.6 0.6 0.6 0.7 0.8 0.8 0.8 0.8 0.9 1.1 1.4 1.5
          --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
    R2:   379 415 155 234 307 555 602 263 334 464 716 677 785 882 830
    R3:   374 410 149 228 301 549 595 255 326 456 708 668 774 868 815
          --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
  Dens. = 4/3 8/7 2/1 3/2 3/2 5/5 7/7 2/2 3/2 8/8 3/3 3/3 2/2 1/1 2/2

 low resolution scan: 11 matching gliadin bands identified.
  Delta = 0.4 0.4 0.6 0.6 0.7 0.7 0.7 0.8 0.9 1.1 1.3
          --- --- --- --- --- --- --- --- --- --- ---
    R2:   501 625 188 648 202 534 583 482 764 281 745
    R3:   497 621 182 642 195 527 576 474 755 270 732
          --- --- --- --- --- --- --- --- --- --- ---
  Dens. = 4/3 5/5 2/1 5/5 2/1 5/4 5/5 5/4 2/2 1/1 2/2

          1 unmatched band isolated in replicate R2
             36.2(2)
          No unmatched bands isolated in replicate R3

 Mean Signature Array  -  [3]  -  Number of bands encoded = 26

 15.2(1)  18.5(2)  19.8(2)  23.1(3)  25.9(2)  27.5(1)  30.4(3)
 33.0(3)  37.6(4)  41.2(7)  46.0(8)  47.8(4)  49.9(3)  53.0(4)
 55.2(5)  57.9(5)  59.8(7)  62.3(5)  64.5(5)  67.2(3)  71.2(3)
 73.8(2)  75.9(2)  77.9(2)  82.2(2)  87.5(1)   0.0(0)   0.0(0)
  0.0(0)   0.0(0)   0.2(3)   0.2(*)
.....................................................................

ENTER: Number (1,2 or 3) designating MSA for graphics by program GCOMP2
  2
```

(Figure 14B) and R2/R3 (Figure 14C), a comparable number of replicating components (25 and 26 respectively) have been identified.

With respect to non-replicating components, the report of program FILTER (Figure 14) lists a total of six unmatched bands in the individual pairings of densitometric profile replicates:

R1: 11.2(1) 35.5(2) 38.8(3)
R2: 36.2(2) 74.5(2)
R3: 73.2(2)

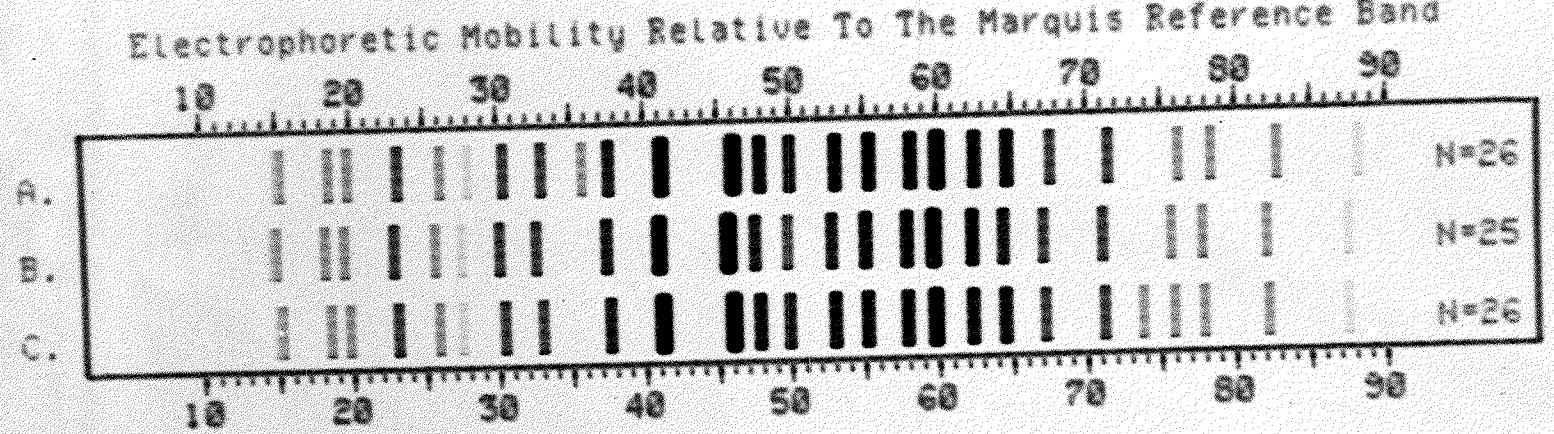
Only one of these six [R3: 73.2(2)] was originally detected as a high resolution peak; and only two components [R1: 11.2(1) 38.8(3)] both of low resolution type (refer to "*" tagged bands in Figure 13), remain in an unmatched state when homologous counterparts could not be found in both pairs of replicate analyses involving a common profile. It is clear then, that the use of peak detection thresholds in program DISTIL is an effective strategy to isolate potentially spurious densitometric data. However, a decision to accept or reject both low and high resolution components by means of a replication test for relative peak coordinates is necessary in order to provide a reliable outcome.

Because the numerical representation of gliadin electrophoregram composition given by the cultivar signature arrays shown in Figure 14 are not well suited for direct comparisons, a variety of display programs were developed which translate the compact numerical data into a graphic form which is more readily evaluated. Programs GFILTR and GCOMP2 (refer to Figure 9) utilize input data computed at this processing stage to generate electrophoretic pattern graphics which are described below.

Figure 15. Computer graphic display of cv. Talbot electrophoregrams processed from pairwise analysis of replicate densitometric scanning profiles.

Program: GFILTR
Graphic display of replicate gliadin electrophoregrams
encoded in mean signature arrays computed by program - FILTER.

* Processing result for cultivar: TALBOT



---

* Prog. DISTIL peak ID resolution parameters = 5/1 - shoulder detect on.
A. Generated from computer processed densitomegram replicates 1 and 2.
B. Generated from computer processed densitomegram replicates 1 and 3.
C. Generated from computer processed densitomegram replicates 2 and 3.

Execution of program GFILTR (refer to Figure 9) produces the result illustrated in Figure 15. Each electrophoregram graphically depicted corresponds precisely to band mobility and density data coded in MSA's [1], [2] and [3] computed by program FILTER (Figure 14). The display confirms that preceeding stages of densitometric profile processing have successfully computed similar gliadin electrophoregram distributions. Differences between patterns are evident only in terms of relative mobility for homologous bands.

Figure 15 shows that 26 bands are separately encoded in electrophoregrams designated as "A" and "C". Each pattern, representing the mean of two replicates, additionally possesses one unique component [35.8(2) in A; 73.8(2) in C] which is absent in the other two mean electrophoregrams. Thus, a total of 27 different gliadin bands have been identified, 25 which replicate in all three profiles analyzed, and two bands at the fringe of detection which were each found in distinct densitomegram pairings.

The operator can now proceed with two program options: (i) any one pattern of computed gliadin PAGE composition depicted in Figure 15 can be tested against a data base of manually derived data by executing program GCOMP2, or (ii) program MERGE can be invoked to combine complementary patterns together into a single mean composite electrophoregram which can subsequently be used for comparisons executed by programs GCOMP1 and GMERGE.

To accomodate the first option, the computer prompts the operator to enter a number designating the MSA which will be used to graphically evaluate the agreement of the computed electrophoregram with the

manually derived pattern of Rm and band density data (e.g. "MSA [2]" refer to bottom of Figure 14). If no choice is made, the software will select the first FILTER program derived MSA with the highest number of gliadin bands (i.e. MSA [1], Figure 14A).

The result produced by executing program GCOMP2 is presented in Figure 16 which displays the high level of homology which exists between the pattern of Talbot bands automatically acquired by densitometry and the electrophoregram produced from data obtained by manual calculation. The figure also shows the close qualitative and quantitative correspondence between the computer synthesized electrophoretic patterns and the distribution of bands in the PAGE result for cv. Talbot shown in the photographic inset. It can be seen that program analysis was successful in detecting two shoulders [bands 47.5(4) and 57.9(7)] and 23 replicating densitomegram peaks including a band of limited profile definition [27.5(1)]. The latter component, while resolved by densitometry, is only marginally disciminated on a visual basis and was therefore not included in the manually derived reference pattern for cv. Talbot.

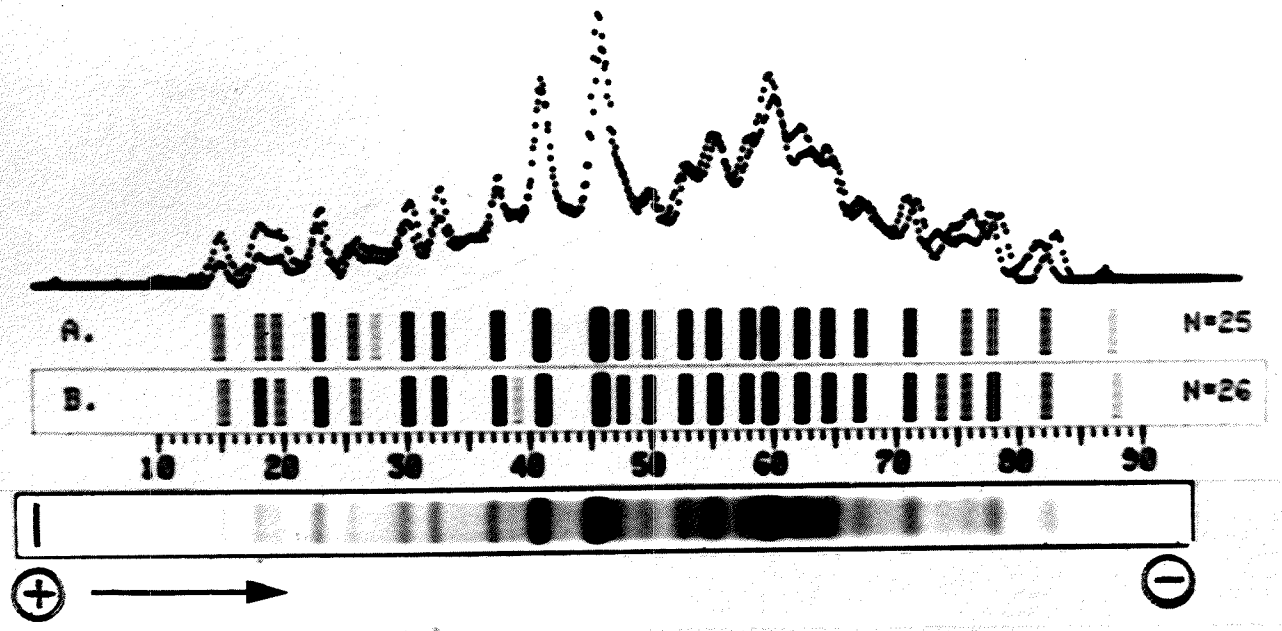
The processing task of program FILTER, to identify homologous bands and then compute their mean mobilities, is especially noticeable in the high mobility region where a lower degree of densitomegram superimposition was manifested. When replication criteria for relative peak coordinates is not satisfied however, and the software cannot identify an homology between pairs of densitometric profiles, a limited number of bands may be passed over as noise. This situation is illustrated in Figure 16 for bands with approximate mobilities of 39

Figure 16. Computer graphic display comparing cv. Talbot gliadin electrophoregrams prepared from data derived manually and automatically computed from densitometric scanning profile replicates R1 and R2.

Program: GCOMP2 >> Comparative display of gliadin electrophoregrams derived from (i) manually determined data and (ii) computer processed replicate densitomegrams via programs - DISTIL* and FILTER.

Processing result for wheat cultivar: TALBOT

Densitomegram for PAGE slab 1 - sample no. 3 Ref. band = 252
Densitomegram for PAGE slab 3 - sample no. 3 Ref. band = 259



Electrophoretic Mobility Relative To The Marquis Reference Band

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* Prog. DISTIL peak ID resolution parameters = 5/1 - shoulder detect on.
A. Pattern generated from computer processed densitomegrams shown.
B. Pattern generated from manually derived electrophoregram data.

and 74 which are visible in the electrophoregram (photographic inset) but were not computed from respective densitometric profiles. An inspection of the data in Figure 14B reveals that bands 38.8(3) and 73.2(2) are in fact isolated, but as unmatched components in densitomegrams R1 and R3 respectively. A homologous counterpart for band 73.2(2) does in fact appear in densitometric profile replicate R2 (refer to Figure 13B, band "745") and the software detects the matching event (Figure 14C) which is graphically displayed by program GFILTR (Figure 15, electrophoregram C).

Complementary Bands and the Program MERGE Processing Step

Because program FILTER handles densitometric profile data in a pairwise fashion, complementary gliadin bands can be detected when more than two replicates are analyzed (refer to Figure 15). These bands however reside in separate data arrays and the task of program MERGE consists of combining them into a single list along with other bands which pass the replication test of program FILTER.

The result is shown in Figure 17 which details the progress of the moving window scanning procedure for relative mobilities (refer to pages 118-119) which is again applied to identify homologous bands in separate replicates. Graphic display of the mean composite signature array generated by program MERGE (e.g. Figure 18A, Electrophoregram D), reveals the minor discrepancies between the computed pattern of 27 bands and its counterpart electrophoregram derived from manually prepared data which contains 26 components. Also, it can be seen that densitometry is sensitive to very low fluctuations in PAGE pattern density. This is

Figure 17. Computer printout of complementary band analysis program. A composite cultivar signature array (bottom of figure) is computed by program MERGE which encodes mean relative mobility and band densities for the set of three replicate electrophoregram signature arrays generated by program FILTER. The list includes gliadin bands in one pair of replicate patterns which complement the data of another (e.g. bands 26 and 27).

Program: MERGE

Computation of a mean composite signature array from (program FILTER)
paired replicate densitomegram data of cultivar: TALBOT.
Scanning window width (Delta) = 0.1 to 1.0 relative mobility units (RMU)
Program DISTIL - peak ID resolution parms. = 5/1 - shoulder detect on

Matching gliadin bands detected in 3 of 3 paired replicates = 25
Complementary bands(***) detected in 2 of 3 paired replicates = 2

Detection sequence	Delta	Densitomegram replicate pair R1/R2	R1/R3	R2/R3	Mean relative mobility	Mean band density
Band 1	0.2	37.6	37.4	37.6	37.6	4
Band 2	0.2	41.2	41.0	41.2	41.2	7
Band 3	0.2	50.0	49.8	49.9	49.9	4
Band 4	0.2	62.5	62.3	62.3	62.4	5
Band 5	0.3	15.2	14.9	15.2	15.1	2
Band 6	0.3	18.5	18.2	18.5	18.4	2
Band 7	0.3	19.8	19.5	19.8	19.7	2
Band 8	0.3	23.2	22.9	23.1	23.1	3
Band 9	0.3	30.4	30.1	30.4	30.3	3
Band 10	0.3	53.1	52.8	53.0	53.0	4
Band 11	0.3	55.4	55.1	55.2	55.2	5
Band 12	0.3	58.2	57.9	57.9	58.0	5
Band 13	0.3	60.0	59.7	59.8	59.9	7
Band 14	0.3	64.7	64.4	64.5	64.5	5
Band 15	0.4	26.1	25.7	25.9	25.9	2
Band 16	0.4	33.0	32.6	33.0	32.9	3
Band 17	0.4	46.1	45.7	46.0	46.0	8
Band 18	0.4	47.9	47.5	47.8	47.8	4
Band 19	0.4	71.5	71.1	71.2	71.3	3
Band 20	0.5	28.0	27.5	27.5	27.7	1
Band 21	0.5	67.6	67.1	67.2	67.3	3
Band 22	0.5	76.2	75.7	75.9	76.0	2
Band 23	0.6	78.4	77.8	77.9	78.1	3
Band 24	0.7	82.8	82.1	82.2	82.4	2
Band 25	0.7	88.2	87.5	87.5	87.7	1
Band 26	***	35.8	0.0	0.0	35.8	2
Band 27	***	0.0	0.0	73.8	73.8	2

[Mean Composite Signature Array] - Number of bands encoded = 27

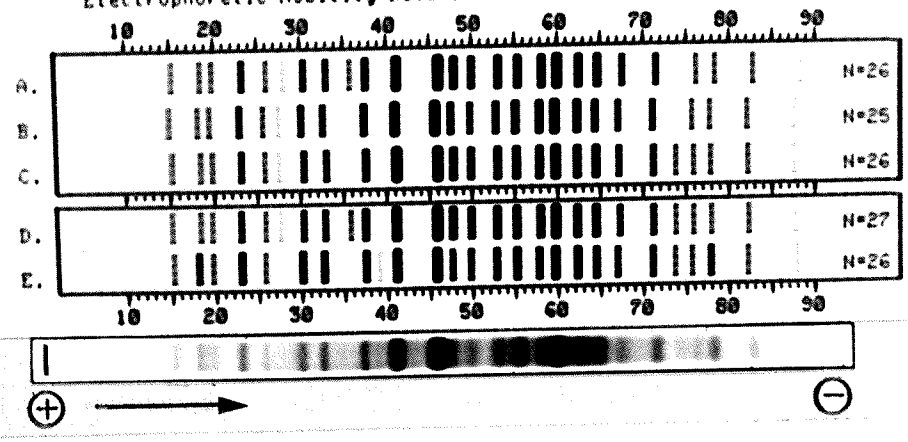
15.1(2)	18.4(2)	19.7(2)	23.1(3)	25.9(2)	27.7(1)	30.3(3)
32.9(3)	35.8(2)	37.6(4)	41.2(7)	46.0(8)	47.8(4)	49.9(4)
53.0(4)	55.2(5)	58.0(5)	59.9(7)	62.4(5)	64.5(5)	67.3(3)
71.3(3)	73.8(2)	76.0(2)	78.1(3)	82.4(2)	87.7(1)	0.0(0)
0.0(0)	0.0(0)	0.0(0)	0.2(*)			

Figure 18. Computer graphic display options for comparison of densitometric scanning profiles and/or electrophoregram data (via program MERGE) generated by programs GCOMP1 and GMERGE.

A

Program: GMERGE
Graphic display of gliadin electrophoregrams encoded in mean signature arrays computed by programs - FILTER & MERGE.

* Processing result for cultivar: TALBOT



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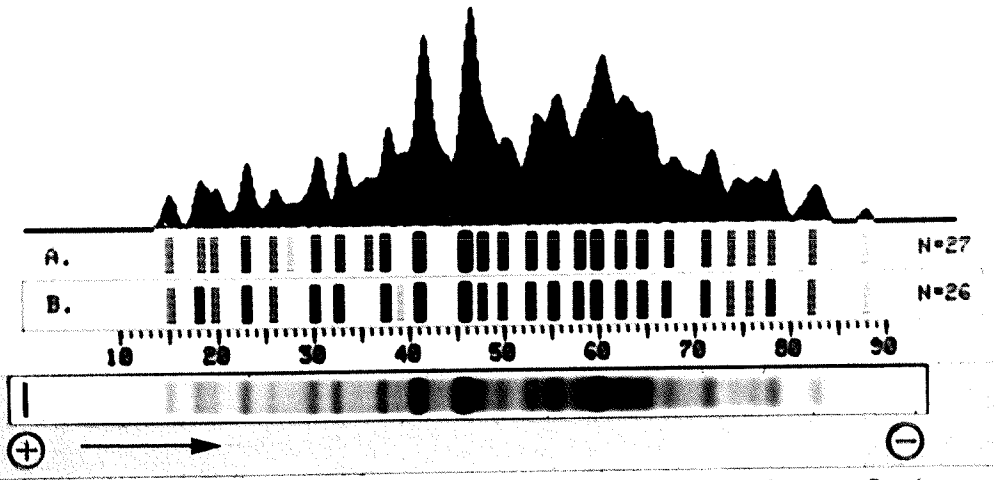
* Prog. DISTIL Peak ID resolution parameters = 5/1 - shoulder detect on.
A. From program FILTER MSA data for densitomegram replicates R1 and R2.
B. From program FILTER MSA data for densitomegram replicates R1 and R3.
C. From program FILTER MSA data for densitomegram replicates R2 and R3.
D. Mean composite electrophoregram computed by program - MERGE.
E. Pattern generated from manually derived electrophoregram data.

B

Program: GCOMP1
Comparative display of gliadin electrophoregrams derived from (i) manually determined data and (ii) replicate densitomegrams processed via programs - DISTIL*, FILTER AND MERGE.

Processing result for wheat cultivar: TALBOT
Densitomegram for PAGE slab 2 - sample no. 3 Ref. band = 253



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* Prog. DISTIL peak ID resolution parameters = 5/1 - shoulder detect on
A. Pattern generated from computer processed densitomegrams.
B. Pattern generated from manually derived electrophoregram data.

particularly indicated by the two computed bands [27.6(1) and 35.8(2)] which are visually ambiguous in the electrophoregram depicted in the photographic inset, but whose presence is confirmed in the accompanying densitometric profile (Figure 18B).

Conversely, a limited number of bands may be visible in the electrophoregram but go completely undetected by the peak finding procedure, or are found in only one replicate densitometric profile and hence are deleted by the program as noise (e.g. band Rm 39, in Figure 18B). This situation may result as indicated, when a faint band migrates between two major contiguous components. Although this particular pattern of bands occurs only infrequently, a possible remedy may lie with a peak detection algorithm based upon the second derivative of the optical density curve. It is a common observation with chromatography spectra, that while the use of second rather than first differentials may be more sensitive to finding shoulders and peaks, the choice depends on noise which is magnified with each successive differentiation (Littlewood et al., 1968). Westerberg (1969) devised a criterion to differentiate second derivative minima, for Gaussian peaks, from those caused by noise, but warned that "very small peaks are hard to separate from noise by any test".

Precision of Computed Electrophoregram Data

The advantage of a computer-based method for the automatic acquisition of gliadin electrophoregram data from optical density curves derives partly from the speed with which relative mobility and band

density values are obtained[1], and the facility to compare normalized PAGE patterns particularly when using graphical formats. The merit of this system for the purpose of cultivar identification, apart from the above mentioned attributes, is dependent upon the precision of the acquired data and their accord with values, mobilities in particular, determined from manual measurements. This is especially relevant as many existing catalogs of cultivar formula data for gliadin electrophoregrams are based on manual measurement reference procedures (Autran and Bourdet, 1975; Ellis and Beminster, 1977; Zillman and Bushuk, 1979; Dal Belin Peruffo et al., 1981; Jones et al., 1982).

A comparison of results of electrophoregram data for cv. Talbot derived by the manual reference procedure (Zillman and Bushuk, 1979) and automatically computed from densitometric profiles is presented in Table 9. As reflected in the graphic display results shown in Figures 16 and 18, it is clear that mean mobility values for both acquisition methods are in excellent agreement. The largest difference in relative mobility for corresponding gliadin components is 0.3 units which is comparable to the experimental error for either method. The average standard deviation was about ± 0.4 distance units for the computer-based approach. The rise in variability for computed bands with high mobilities was traced to a failure of the densitometer to maintain exact linearity for the full length of the scanning interval. The problem was corrected by replacing the entire complement of step-motor circuit resistors possessing ±5% accuracy with high precision (±1%) components.

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[1]The complete analysis of two or three replicate densitometric profiles, to obtain mean electrophoregram mobility and band density parameter values, was handled by the computer in less than 20 seconds.

TABLE 9

Comparison of manually derived and computed electrophoregram data for cv. Talbot

Data Acquisition Method		
Manual	Computer-based	
relative mobility	relative mobility	relative band density
15.2 ± 0.3	15.1 ± 0.3	1.2 ± 0.5
18.1 ± 0.3	18.4 ± 0.3	1.5 ± 0.6
19.7 ± 0.2	19.7 ± 0.4	1.3 ± 0.5
23.0 ± 0.3	23.1 ± 0.3	2.2 ± 0.4
25.8 ± 0.2	25.9 ± 0.4	1.4 ± 0.2
30.1 ± 0.4	30.3 ± 0.3	2.4 ± 0.5
32.6 ± 0.3	32.9 ± 0.4	2.6 ± 0.7
37.5 ± 0.3	37.6 ± 0.3	3.4 ± 0.5
41.1 ± 0.3	41.2 ± 0.3	6.6 ± 0.5
45.8 ± 0.3	46.0 ± 0.4	8.0 ± 0.0
47.6 ± 0.2	47.8 ± 0.4	3.8 ± 0.2
49.8 ± 0.2	49.9 ± 0.2	3.0 ± 0.2
52.8 ± 0.1	53.0 ± 0.4	3.9 ± 0.3
55.2 ± 0.2	55.2 ± 0.3	4.7 ± 0.3
57.8 ± 0.2	58.0 ± 0.4	4.4 ± 0.3
59.7 ± 0.2	59.9 ± 0.4	6.2 ± 0.1
62.3 ± 0.1	62.4 ± 0.3	4.6 ± 0.1
64.5 ± 0.2	64.5 ± 0.3	4.1 ± 0.1
67.0 ± 0.2	67.3 ± 0.5	2.5 ± 0.1
71.1 ± 0.2	71.3 ± 0.4	2.6 ± 0.1
73.7 ± 0.4	73.8 ± 0.9	1.5 ± 0.1
75.7 ± 0.3	76.0 ± 0.5	1.7 ± 0.5
77.9 ± 0.4	78.1 ± 0.6	2.0 ± 0.2
82.2 ± 0.4	82.4 ± 0.8	1.3 ± 0.2
87.9 ± 0.3	87.7 ± 0.8	0.3 ± 0.2

Data reported as mean ± standard deviation for three determinations on replicate electrophoregrams run on separate gel slabs.

These hardware considerations notwithstanding, the average standard error of the mean deviation in relative mobility between manual and computed data was less than 0.1 Rm units.

The decision to use peak heights as the feature parameter for band densities is partly related to the size of the accompanying deviations (Table 9) which indicate that good overall reproducibility can be achieved by normalizing peak maxima relative to an internal profile component. These optimized relative density values yield a reasonable approximation to band protein concentration and can be considered at least as good as calculations based on area determinations, given the variable nature of background staining in PAGE, and the degree of peak overlap.

In the context of the wheat cultivar identification problem, more rigorous quantification of densitometric profile peaks, i.e. by integration of peak areas, is likely not of any practical value. This suggestion is in agreement with published gliadin electrophoregram methodology which have treated all estimates of band density with a cautiously low precision. Some workers having found the relationship between the concentration of gliadin PAGE components and peak areas obtained by densitometry unreliable, used simple visual assessments to make band density assignments on a scale from 1 to 5 (Jones et al., 1982). Others, in the extreme, have chosen to disregard band intensities entirely because of concerns of modifying effects caused by variations in protein content of grain samples (Dal Belin Peruffo et al., 1981). Autran and Bourdet (1975) applied a more compromising strategy by assigning electrophoregram band densities to four discrete levels (trace,+,++,+++) based on relative area percentage values for densitometric profile peaks; however a difference between two components was only confirmed if bands differed by at least two steps in density.

For comparative electrophoregram analysis, Autran and Bourdet's protocol is limited to three classes of quantitative differentiation between bands of similar mobility, i.e. trace/++, trace/+++, or +/+++. By contrast, the procedure described in this study using eight levels of band density based on the optimized relative peak height signature, and applying a conservative difference threshold of three band density units, can recognize 15 classes of band density differences (i.e. 1/4, 1/5, 1/6, 1/7, 1/8, 2/5, etc.). This level of discrimination is more than adequate for cultivar identification as band density may be used only to weight the number of matching and non-matching components for the evaluation of pattern similarities where relative mobility is the critical measurement parameter.

COMPUTER-BASED WHEAT CULTIVAR IDENTIFICATION SYSTEM

This section examines methodology and results of the computer-based system developed for wheat cultivar identification by gliadin electrophoregrams. The computer used to establish the extensive gliadin cultivar formula data base and develop and test programs of the cultivar identification system was the University of Manitoba's Amdahl 580. It was found that core memory size of the minicomputer used previously, and the inflexible nature of its operating system were limiting factors to achieve a satisfactory result for this phase of the research project.

A catalog of PAGE patterns and their respective cultivar formulas for 116 wheat cultivars in the data base is initially presented. Direct computer acquisition of gliadin band migration distances from photographic prints by means of a digitizer is described. A multiple reference band procedure for the determination of relative mobilities is introduced and precision effects are examined compared to the one reference band technique. The fundamental pattern homology analysis formula of the cultivar identification ranking program is described. The identification system is tested using gliadin electrophoregram formulas covering a broad range of genotypes, and numerous examples of program output are given.

The research was also extended to develop a strategy to quantitate the heterogeneity in gliadin composition that is commonly observed in electrophoregram data. To this end, the frequency distribution of gliadin bands as a function of relative mobility is determined. The resulting multi-cultivar profile of gliadin electrophoregram composition

is examined and its potential discussed as a reference spectrum for inter-laboratory comparisons and to provide automatic classification of gliadin bands for the purpose of multivariate analysis.

Gliadin Electrophoregram Results

A comparison of electrophoregrams obtained by the two flatbed PAGE methods used in this study is shown in Figure 19 for a number of common spring wheat cultivars. It is clear that results obtained with the 6 mm horizontal Bushuk and Zillman (1978) apparatus are inferior in resolution to corresponding gliadin patterns acquired using a modified apparatus which incorporates a vertical design with a 50% reduction in gel slab thickness. The effect is in part related to the shorter duration of electrophoresis obtained with the thinner gel slab apparatus. This contributes to improved resolution by minimizing the effect of band spreading, which is related to the square root of migration time (Richards and Lecanidou, 1971; Lunney et al., 1971).

The source of aluminum lactate, which differs in the two electrophoresis systems, and is known to influence the resolution of gliadin electrophoregrams (Lookhart et al., 1982) may also contribute to the better separations obtained with the vertical PAGE system. The overall effect is to increase the number of bands visible in stained electrophoregrams and thereby potentially improve the discrimination power of the electrophoresis test in the computerized cultivar identification process.

Gliadin electrophoregrams, derived by the vertical PAGE system, for bulk wheatmeal samples of cultivars listed in Tables 4 to 6 are shown in

Figure 19. Gliadin electrophoregrams of common spring wheat cultivars Marquis, Neepawa, Pembina, Glenlea and Lemhi 62 obtained by 6 mm horizontal and 3 mm vertical PAGE systems.

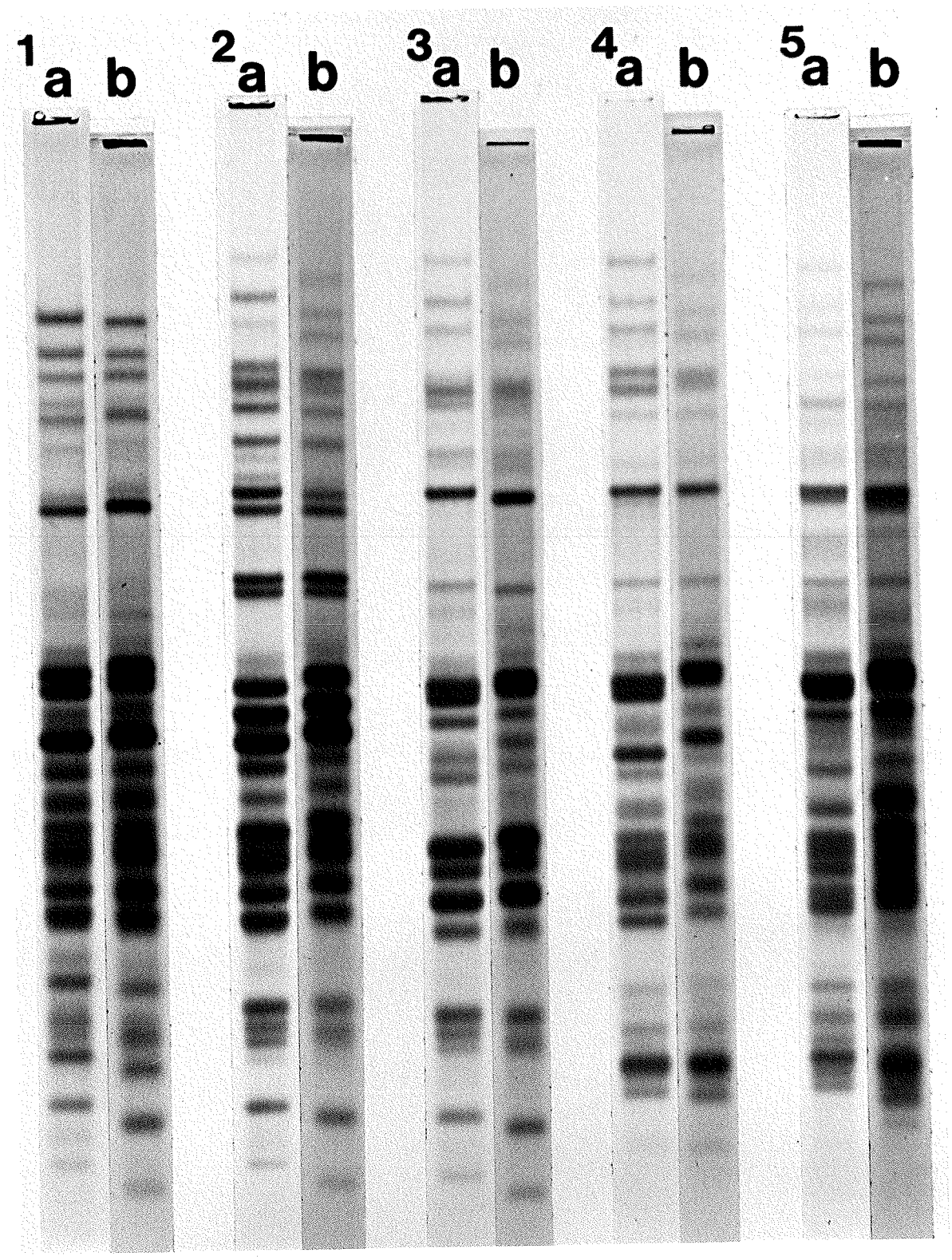
Pattern	Cultivar
1	Marquis
2	Neepawa
3	Pembina
4	Glenlea
5	Lemhi 62

Conditions (a)

Apparatus	: Vertical (3 mm gel bed thickness)
Polyacrylamide gel	: 6%
Buffer	: Aluminum lactate (pH 3.1), source - Fluka Chemical Corp.
Electrophoresis time	: 4 hr.
Protein stain	: Coomassie Blue

Conditions (b)

Apparatus	: Horizontal (6 mm gel bed thickness)
Polyacrylamide gel	: 6%
Buffer	: Aluminum lactate (pH 3.1), source - ROIC Chemical Corp.
Electrophoresis time	: 6 hr.
Protein stain	: Coomassie Blue


1a b
2a b
3a b
4a b
5a b

Figures 20 to 34. In each figure, the two unnumbered flanking electrophoretic patterns are for the reference cultivar Marquis, while the unnumbered center pattern is for the electrophoregram of reference cultivar Neepawa. These PAGE results represent one of an average of three replicate sets of gliadin electrophoregrams used to establish the cultivar identification data base.

This data base, represented as cultivar formula arrays (Zillman and Bushuk, 1979), is presented in computer-generated Tables 10 to 12. Calculated gliadin band mobilities, plotted as shown, are correct to 0.1 Rm units which represents a significant improvement in precision compared to previously reported catalogs of gliadin electrophoregram data (Zillman and Bushuk, 1979; Jones et al., 1982). The use of this highly resolved scale is warranted by the low level of uncertainty in determining relative mobilities by the multiple reference band technique to be described in a following section.

Apart from aspects related to resolution and precision, differences in band relative mobility were observed in both low and high mobility zones compared to the above cited catalogs of gliadin electrophoregram data. The effect can be seen in Figure 19 as electrophoregrams obtained using two different apparatuses, but similar polyacrylamide gel and buffer recipes, fail to align along their entire lengths. This result is not unexpected as Autran et al. (1979) have observed that apparatus design was a factor in explaining varying relative mobilities in different electrophoresis systems.

The slightly different range of relative mobilities associated with the present extensive set of electrophoregram data has no influence upon

the performance of the wheat cultivar identification system, as each laboratory must apply its own set of reference cultivar PAGE patterns as the relevant data base in accordance with its needs. The problem of inter-laboratory comparison and/or utilization of gliadin electrophoregrams is dealt with in greater detail in Appendix D where a calibration formula, derived by regression analysis, is presented as the means to accurately cross-reference between computerized catalogs of electrophoregram data.

Figure 20. Gliadin electrophoregrams of common spring wheat cultivars (nos. 1 to 8 in Table 4) represented in the data base.

Pattern	Cultivar
1	Early Red Fife
2	Garnet
3	Pioneer
4	Prelude
5	Preston
6	Red Fife
7	Ruby
8	Acadia

Conditions:

Polyacrylamide gel	6%
Buffer	Aluminum lactate (pH 3.1)
Protein stain	Coomassie Blue

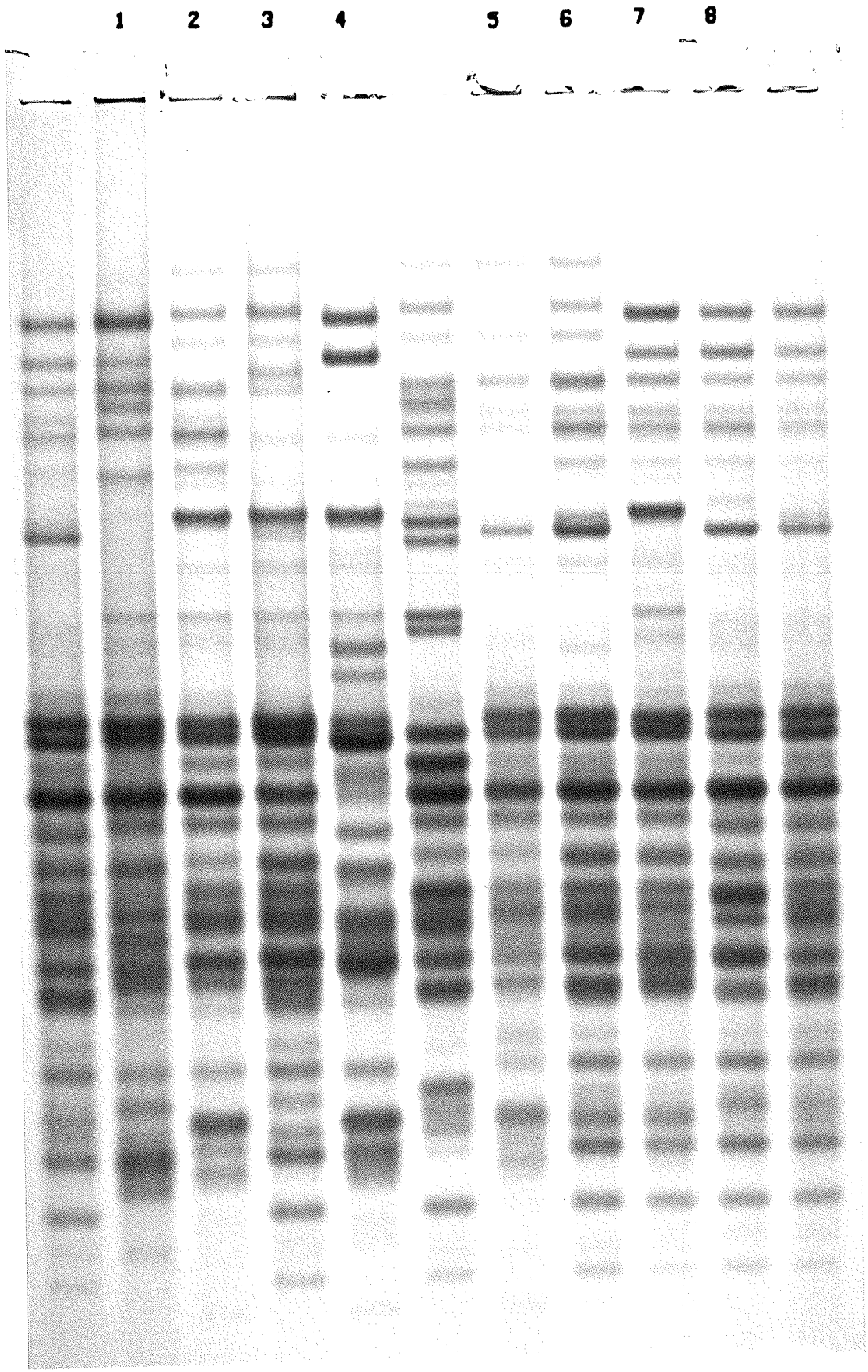

1
2
3
4
5
6
7
8

Figure 21. Gliadin electrophoregrams of common spring wheat cultivars (nos. 9 to 17 in Table 4) represented in the data base.

Pattern	Cultivar
9	Apex
10	Canus
11	Ceres
12	Coronation II
13	Lake
14	Lee
16	Redman
17	Regent

Conditions:

Polyacrylamide gel	6%
Buffer	Aluminum lactate (pH 3.1)
Protein stain	Coomassie Blue

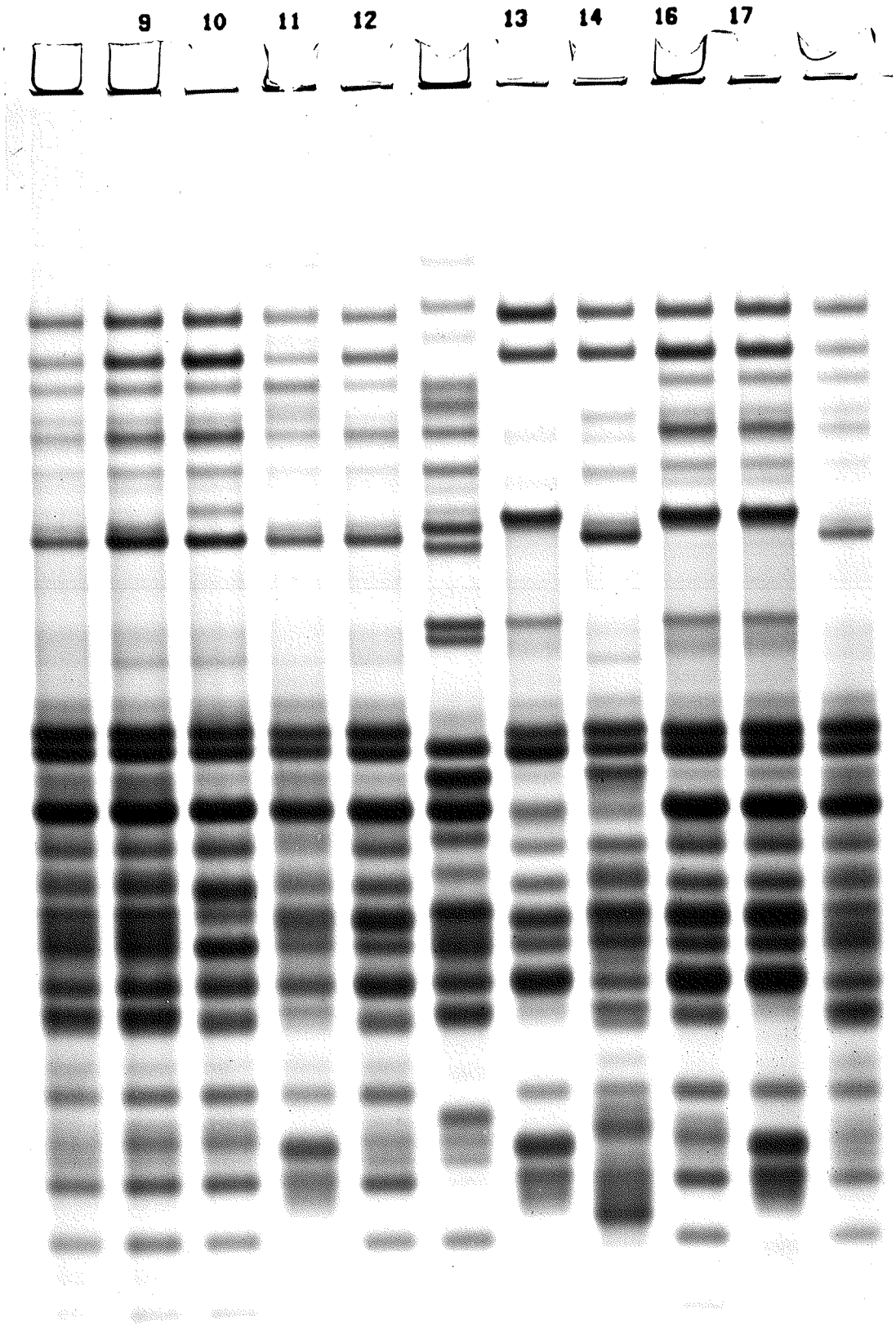

9
10
11
12
13
14
16
17

Figure 22. Gliadin electrophoregrams of common spring wheat cultivars (nos. 18 to 26 in Table 4) represented in the data base.

Pattern	Cultivar
18	Reliance
19	Renfrew
20	Renown
21	Reward
22	Selkirk
23	Benito
24	Canthatch
25	Columbus (BW37)
26	Columbus (BW55)

Conditions:

Polyacrylamide gel	6%
Buffer	Aluminum lactate (pH 3.1)
Protein stain	Coomassie Blue

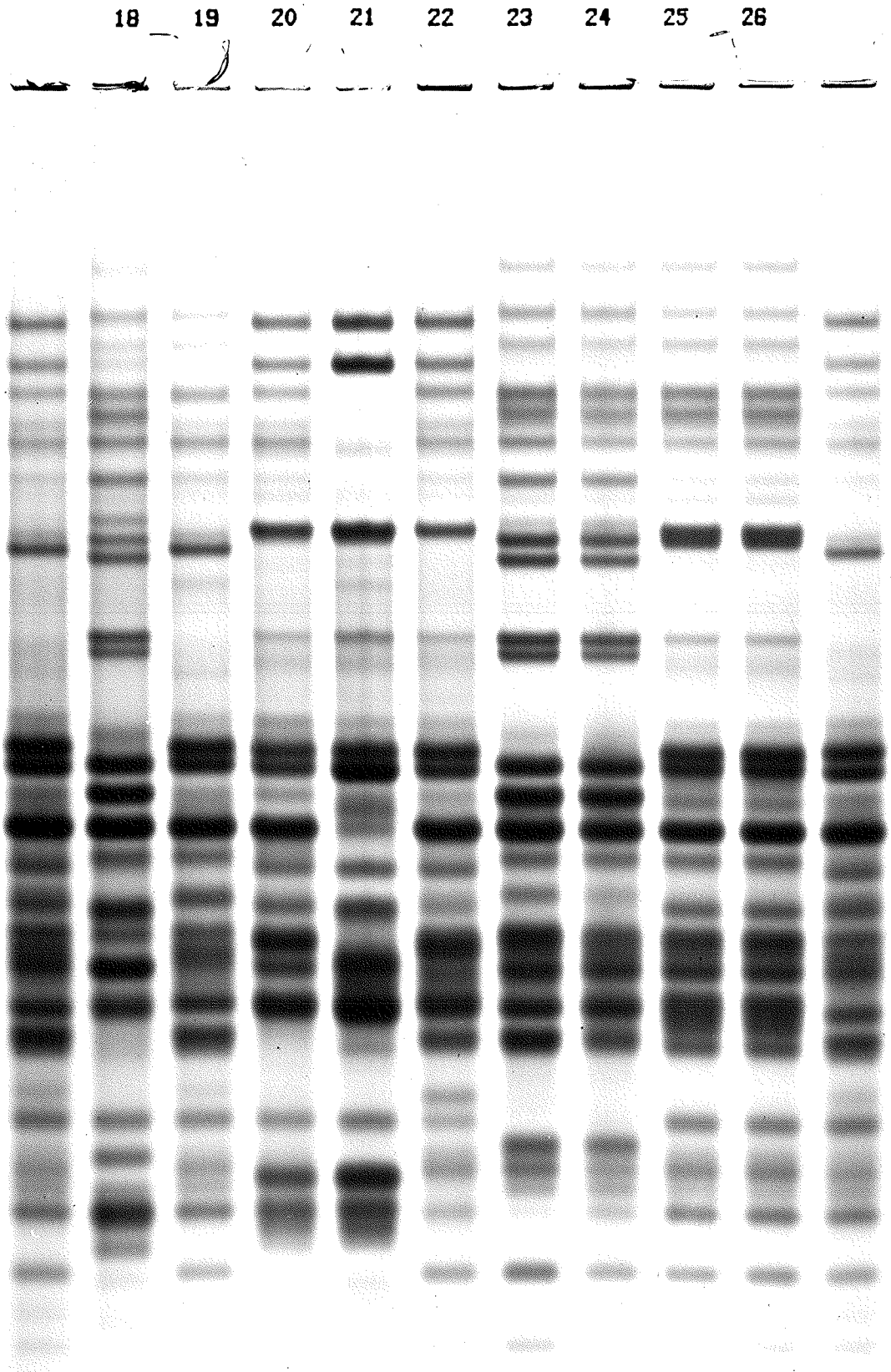

18
19
20
21
22
23
24
25
26

Figure 23. Gliadin electrophoregrams of common spring wheat cultivars (nos. 27 to 35 in Table 4) represented in the data base.

Pattern	Cultivar
27	Katepwa
28	Manitou
29	Napayo
31	Park
32	Pembina
33	Saunders
34	Sinton
35	Thatcher

Conditions:

Polyacrylamide gel	6%
Buffer	Aluminum lactate (pH 3.1)
Protein stain	Coomassie Blue

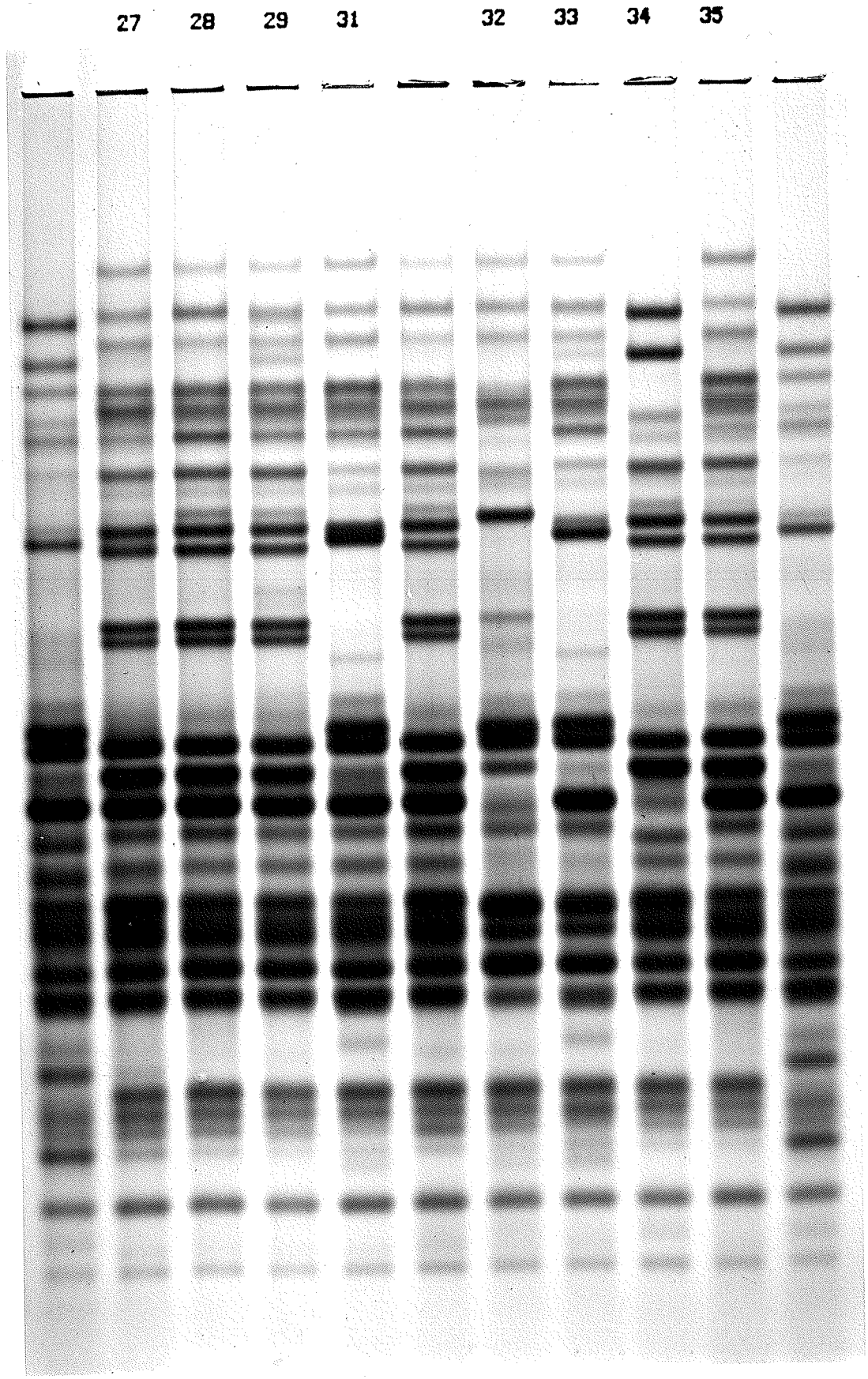

27
28
29
31
32
33
34
35

Figure 24 Gliadin electrophoregrams of common spring wheat cultivars (nos. 36 to 42 in Table 4) represented in the data base.

Pattern	Cultivar
36	Canuck
37	Chester
38	Chinook
39	Cypress
40	Leader
41	Rescue
42	Alex

Conditions:

Polyacrylamide gel: 6%
Buffer : Aluminum lactate (pH 3.1)
Protein stain : Coomassie Blue

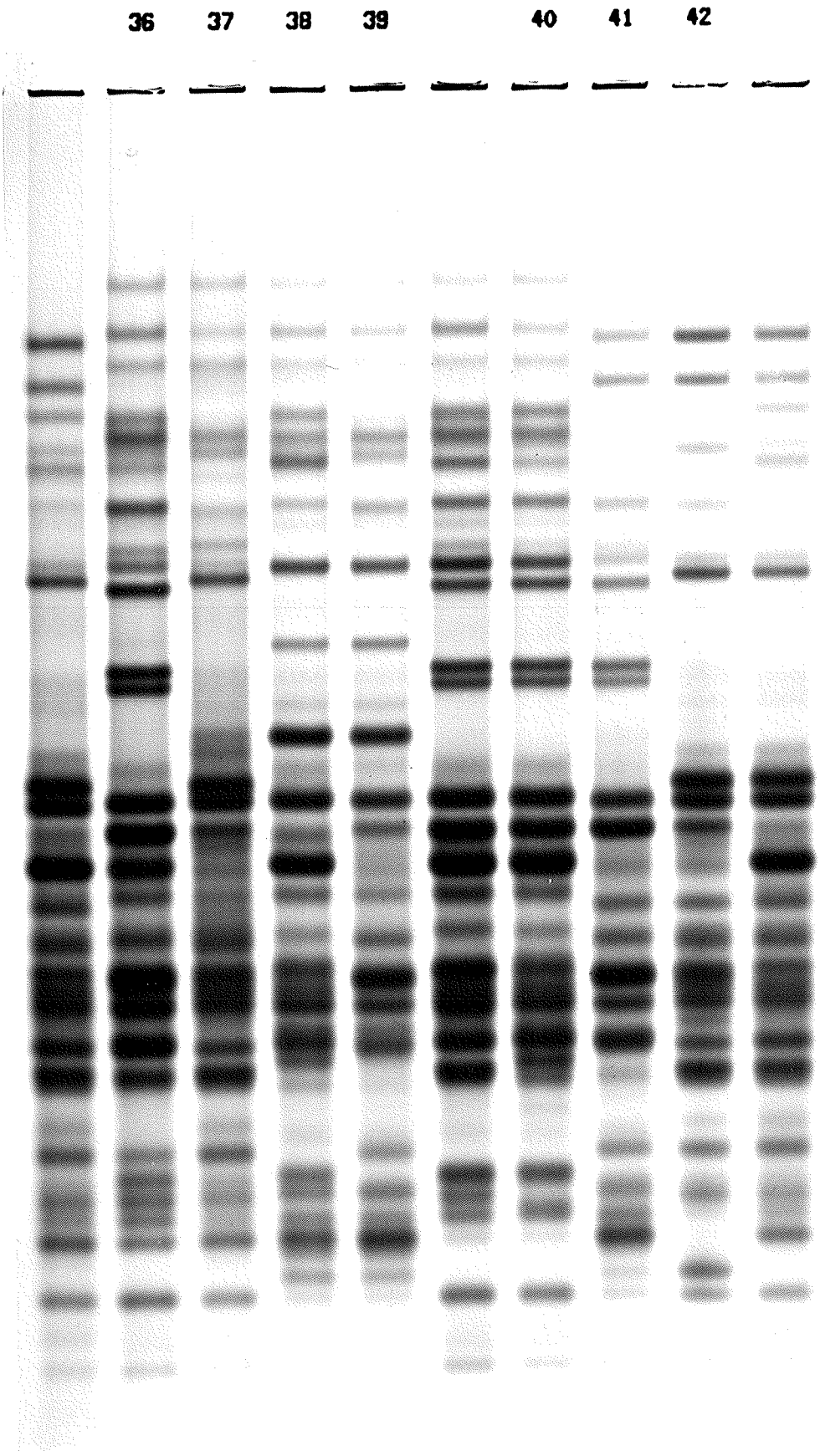

36
37
38
39
40
41
42

Figure 25. Gliadin electrophoregrams of common spring wheat cultivars (nos. 43 to 50 in Table 4) represented in the data base.

Pattern	Cultivar
43	Butte
44	Chris
45	Coteau
46	Era
47	Len
48	Olaf
49	Polk
50	Waldron

Conditions:

Polyacrylamide gel: 6%
Buffer : Aluminum lactate (pH 3.1)
Protein stain : Coomassie Blue

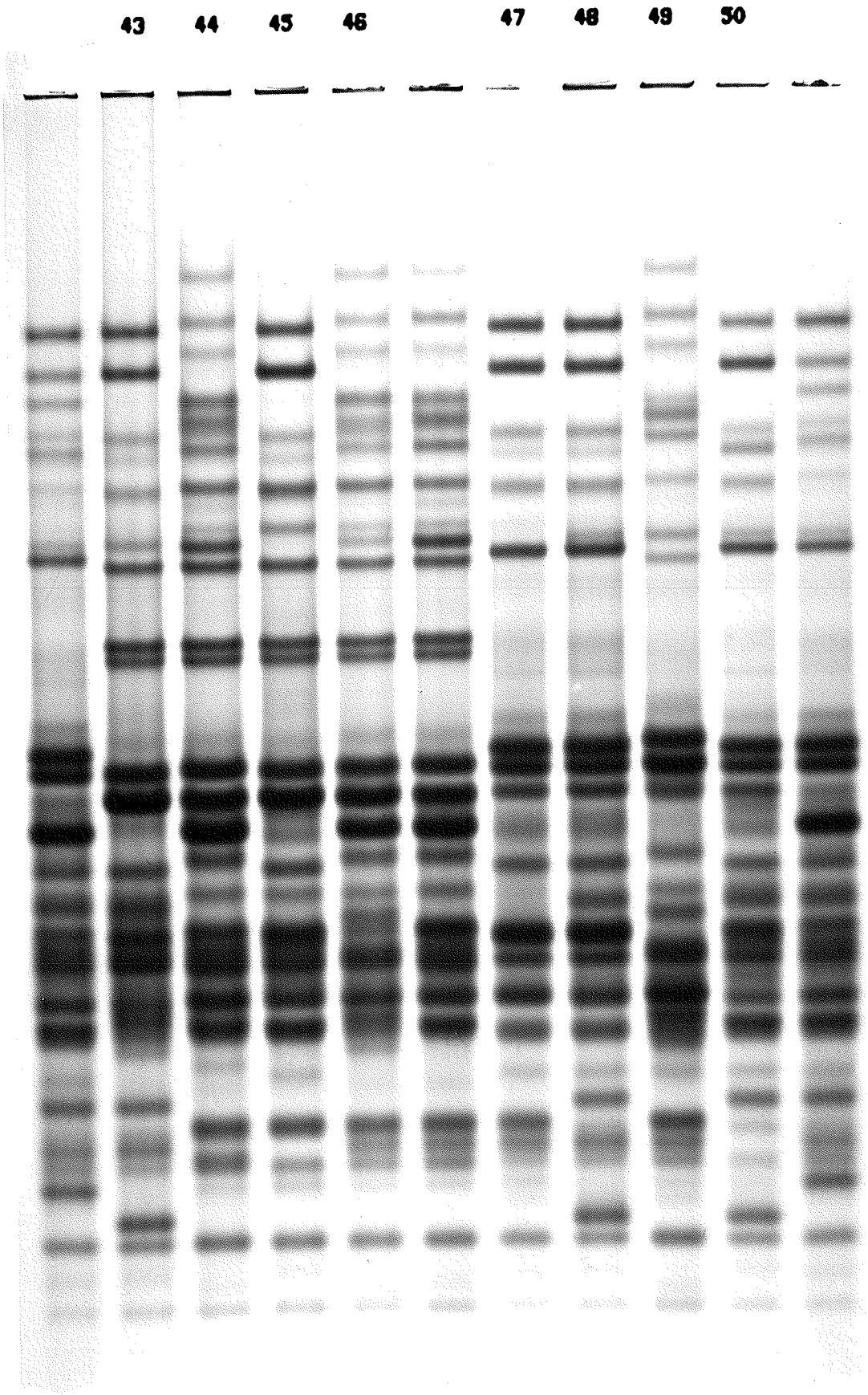

43
44
45
46
47
48
49
50

Figure 26. Gliadin electrophoregrams of common spring wheat cultivars (nos. 51 to 58 in Table 4) represented in the data base.

Pattern	Cultivar
51	Bishop
52	Concorde
53	Dundas
54	Glenlea
55	Huron
56	Kota
57	Laval 19
58	Milton

Conditions:

Polyacrylamide gel: 6%
Buffer : Aluminum lactate (pH 3.1)
Protein stain : Coomassie Blue

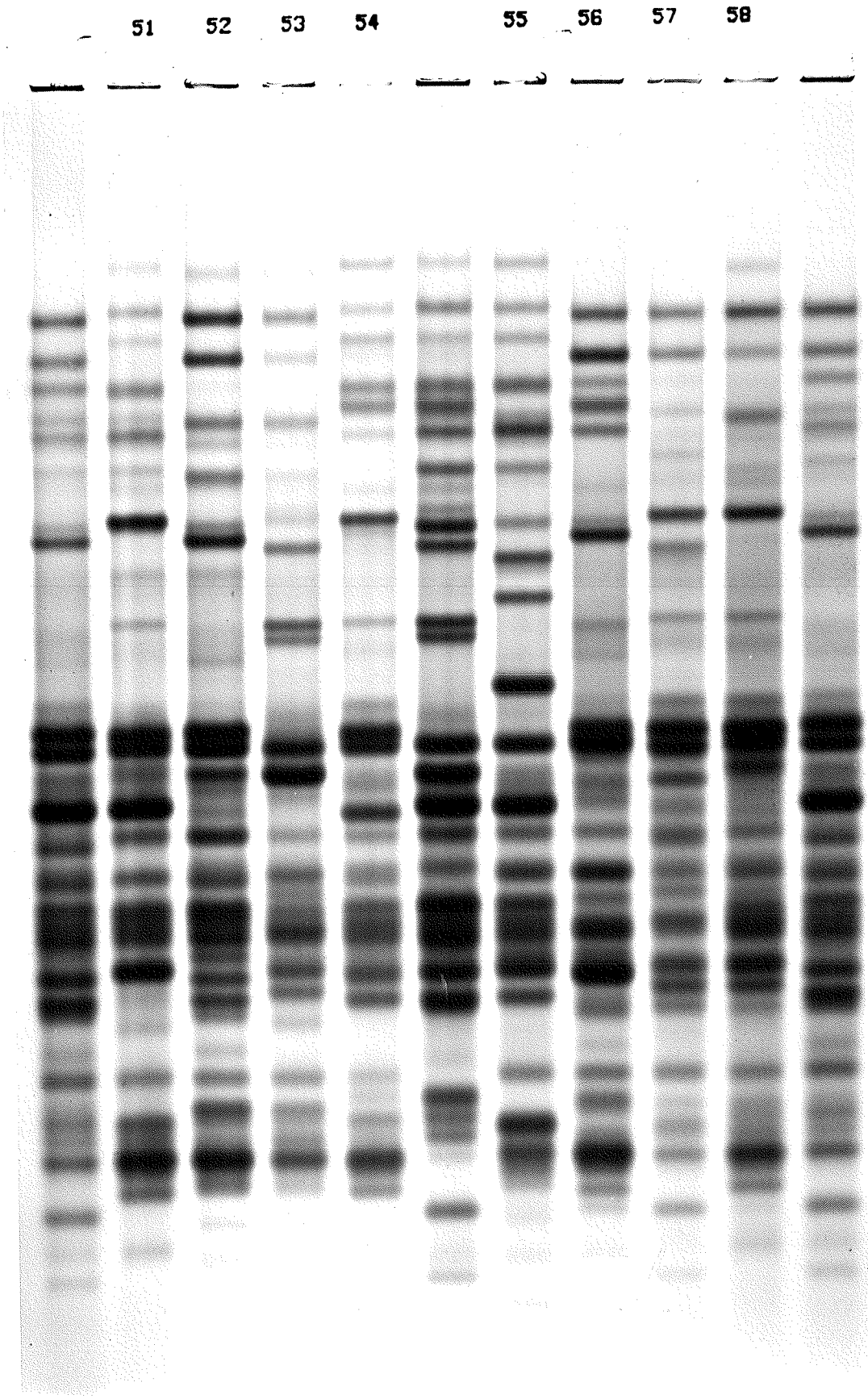

51
52
53
54
55
56
57
58

Figure 27. Gliadin electrophoregrams of common spring wheat cultivars (nos. 59 to 65 in Table 4) represented in the data base.

Pattern	Cultivar
59	Norquay
60	Opal
61	Pitic 62
62	Red Bobs 222
63	UM 632-P
64	UM 684-Q
65	Vernon

Conditions:

Polyacrylamide gel: 6%
Buffer : Aluminum lactate (pH 3.1)
Protein stain : Coomassie Blue

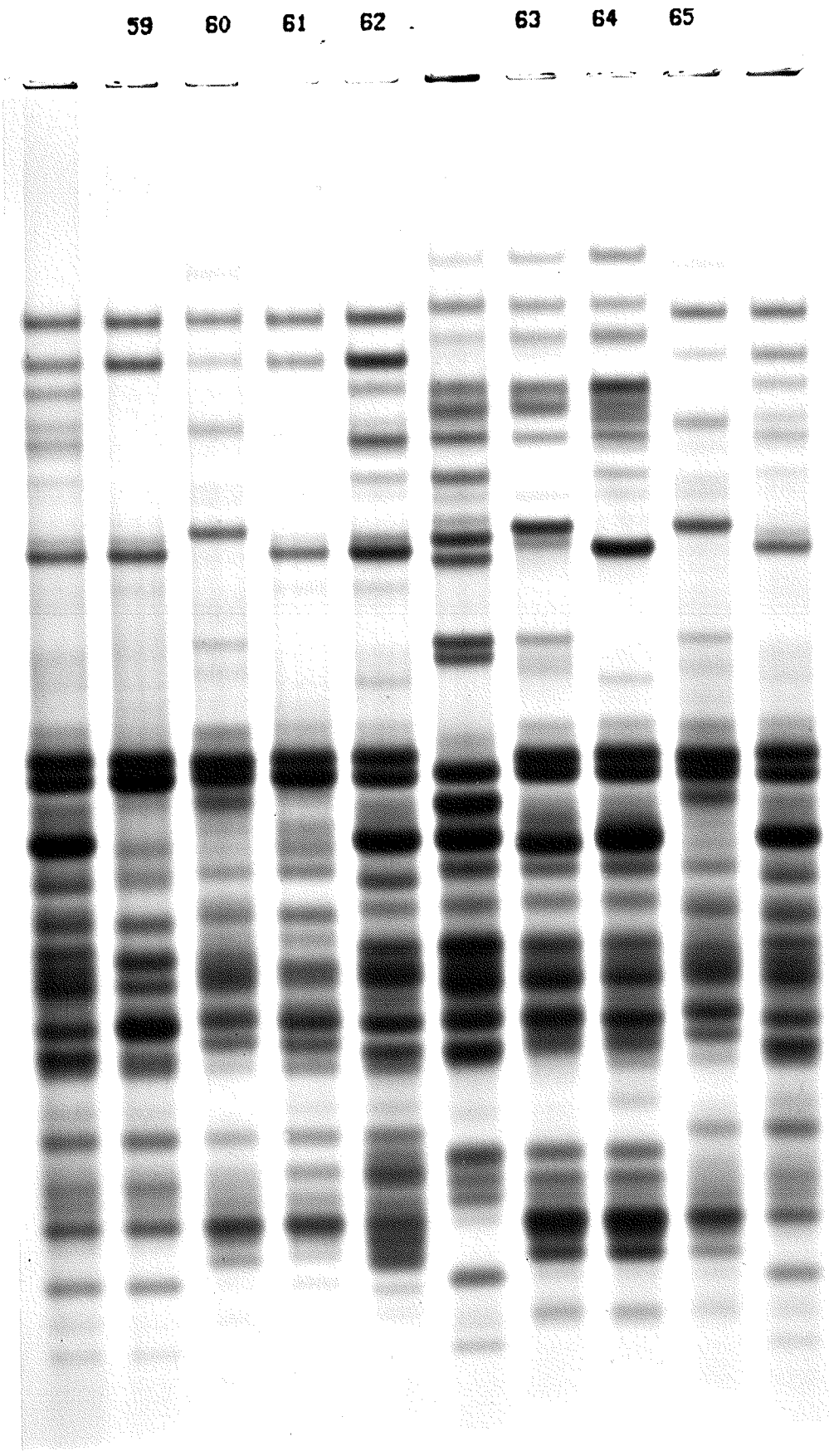

59
60
61
62
63
64
65

Figure 28. Gliadin electrophoregrams of common spring wheat cultivars (nos. 66 to 72 in Table 4) represented in the data base.

Pattern	Cultivar
66	Cascade
67	Fielder
68	Kenhi
69	Lemhi 53
70	Lemhi 62
71	Quality A
72	Springfield

Conditions:

Polyacrylamide gel: 6%
Buffer : Aluminum lactate (pH 3.1)
Protein stain : Coomassie Blue

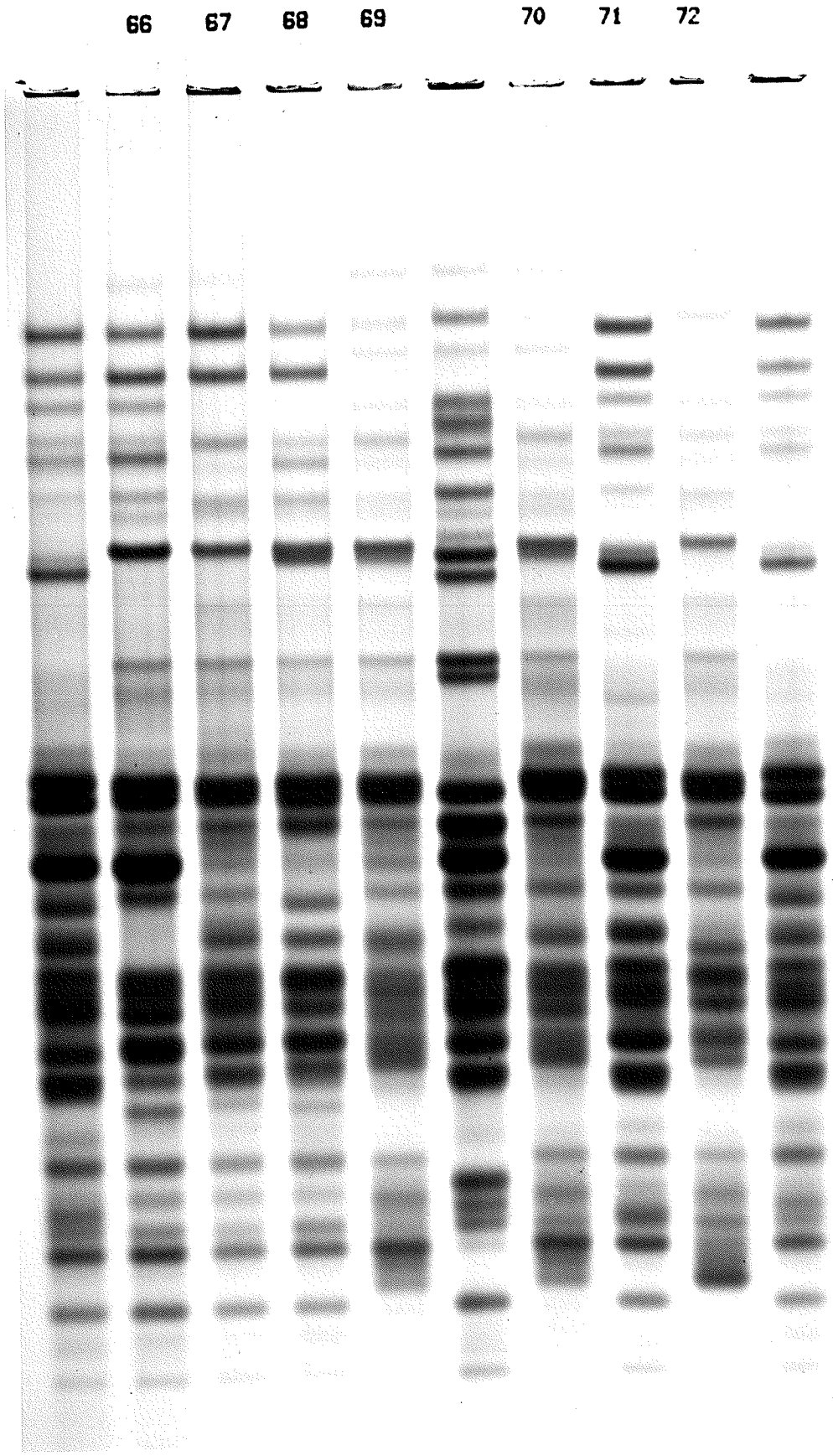

66
67
68
69
70
71
72

Figure 29. Gliadin electrophoregrams of common winter wheat cultivars (nos. 73 to 80 in Table 5) represented in the data base.

Pattern	Cultivar
73	Kharkov 22 M.C.
74	Lennox
75	Monopol
76	Norstar
77	Ridit
78	Sundance
79	Valor
80	Vuka

Conditions:

Polyacrylamide gel	6%
Buffer	Aluminum lactate (pH 3.1)
Protein stain	Coomassie Blue

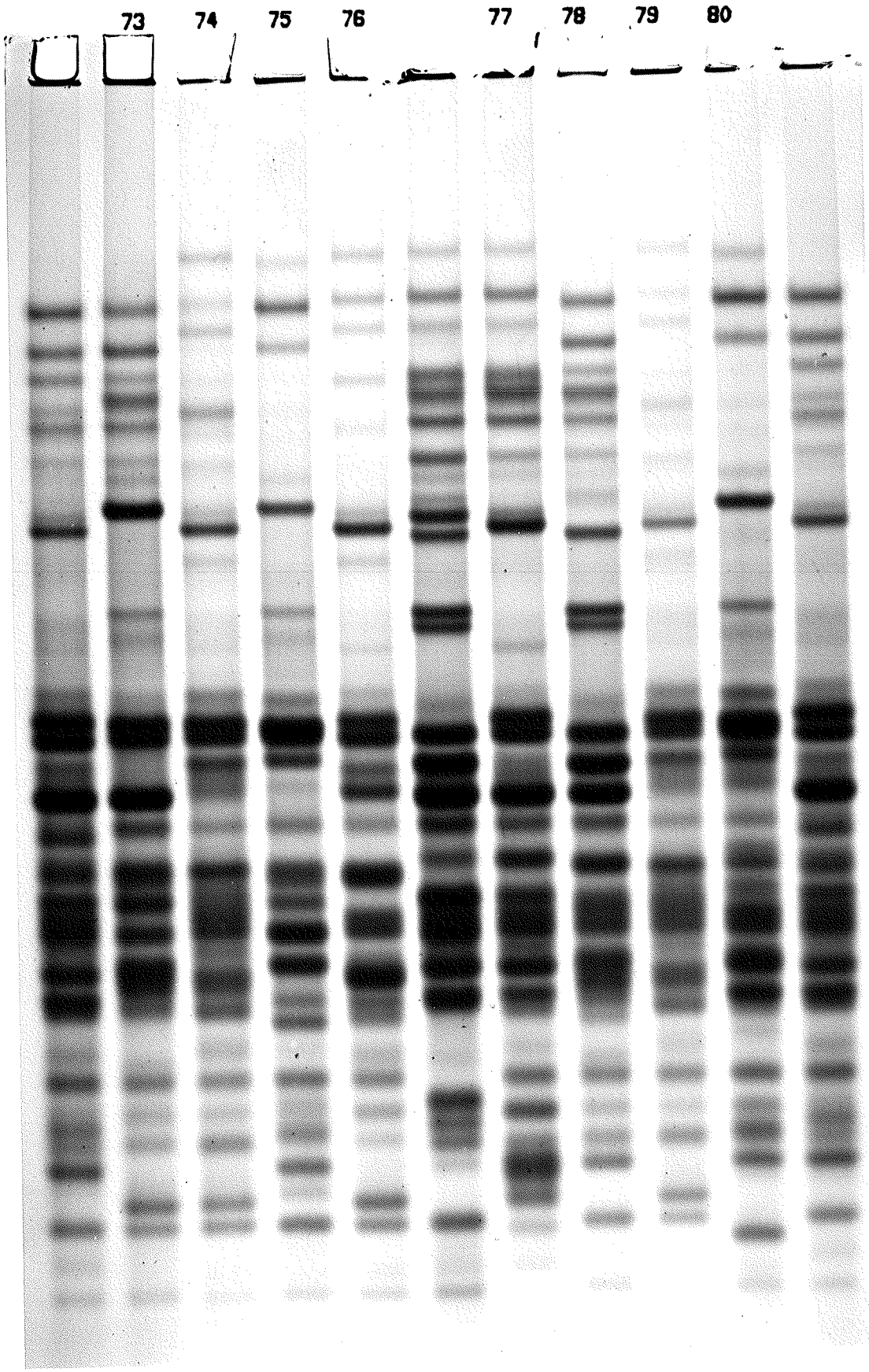

73
74
75
76
77
78
79
80

Figure 30. Gliadin electrophoregrams of common winter wheat cultivars (nos. 81 to 88 in Table 5) represented in the data base.

Pattern	Cultivar
81	Wasatch
82	Westmont
83	Winalta
84	Yogo
85	Cornell 595
86	Dawbul
87	Dawson's Golden Chaff
88	Favor

Conditions:

Polyacrylamide gel	6%
Buffer	Aluminum lactate (pH 3.1)
Protein stain	Coomassie Blue

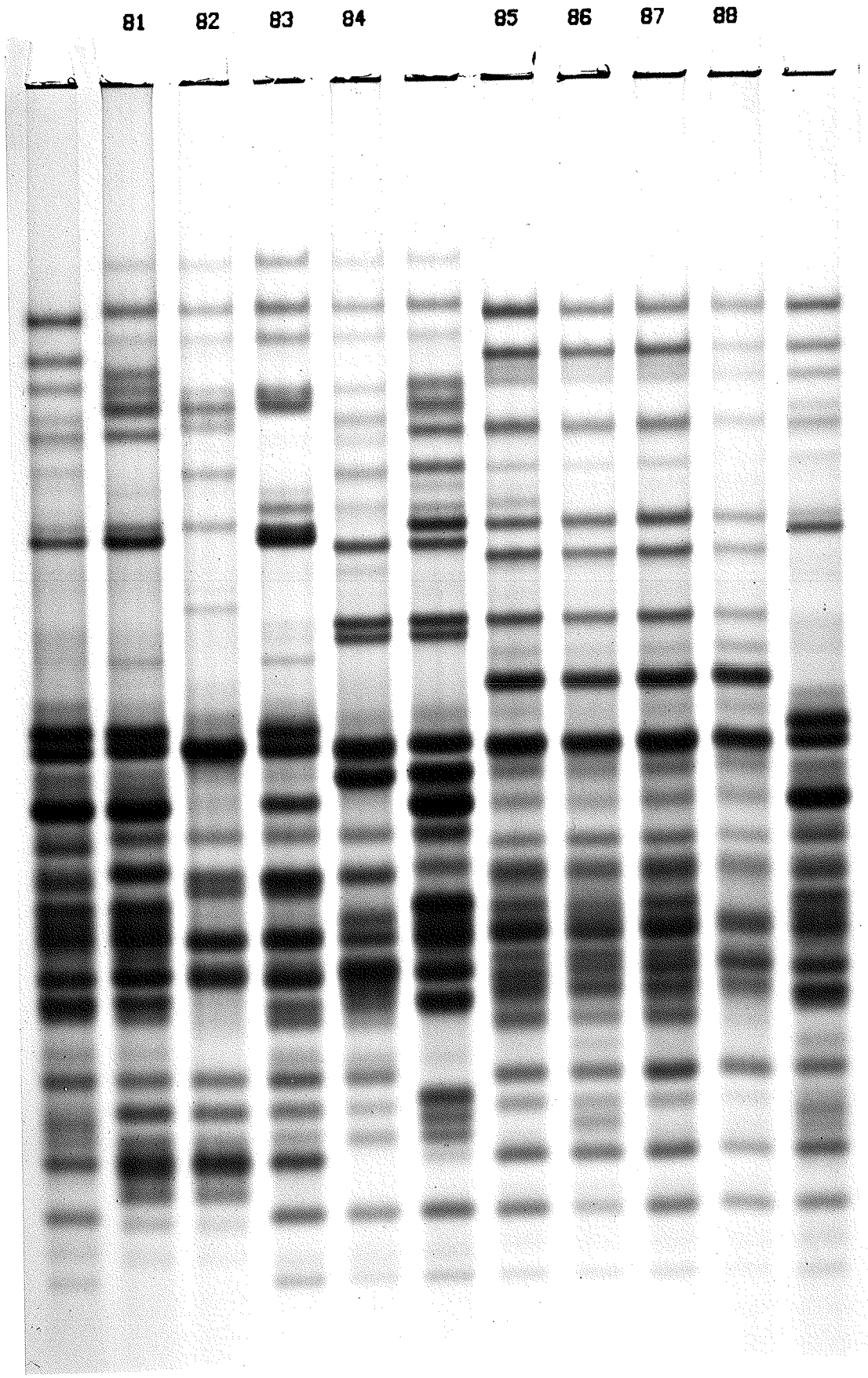

81
82
83
84
85
86
87
88

Figure 31. Gliadin electrophoregrams of common winter wheat cultivars (nos. 89 to 96 in Table 5) represented in the data base.

Pattern	Cultivar
89	Fredrick
90	Gaines
91	Genessee
92	Gordon
93	Houser
94	Jr. No. 6
95	Nugaines
96	O.A.C. 104

Conditions:

Polyacrylamide gel	6%
Buffer	Aluminum lactate (pH 3.1)
Protein stain	Coomassie Blue

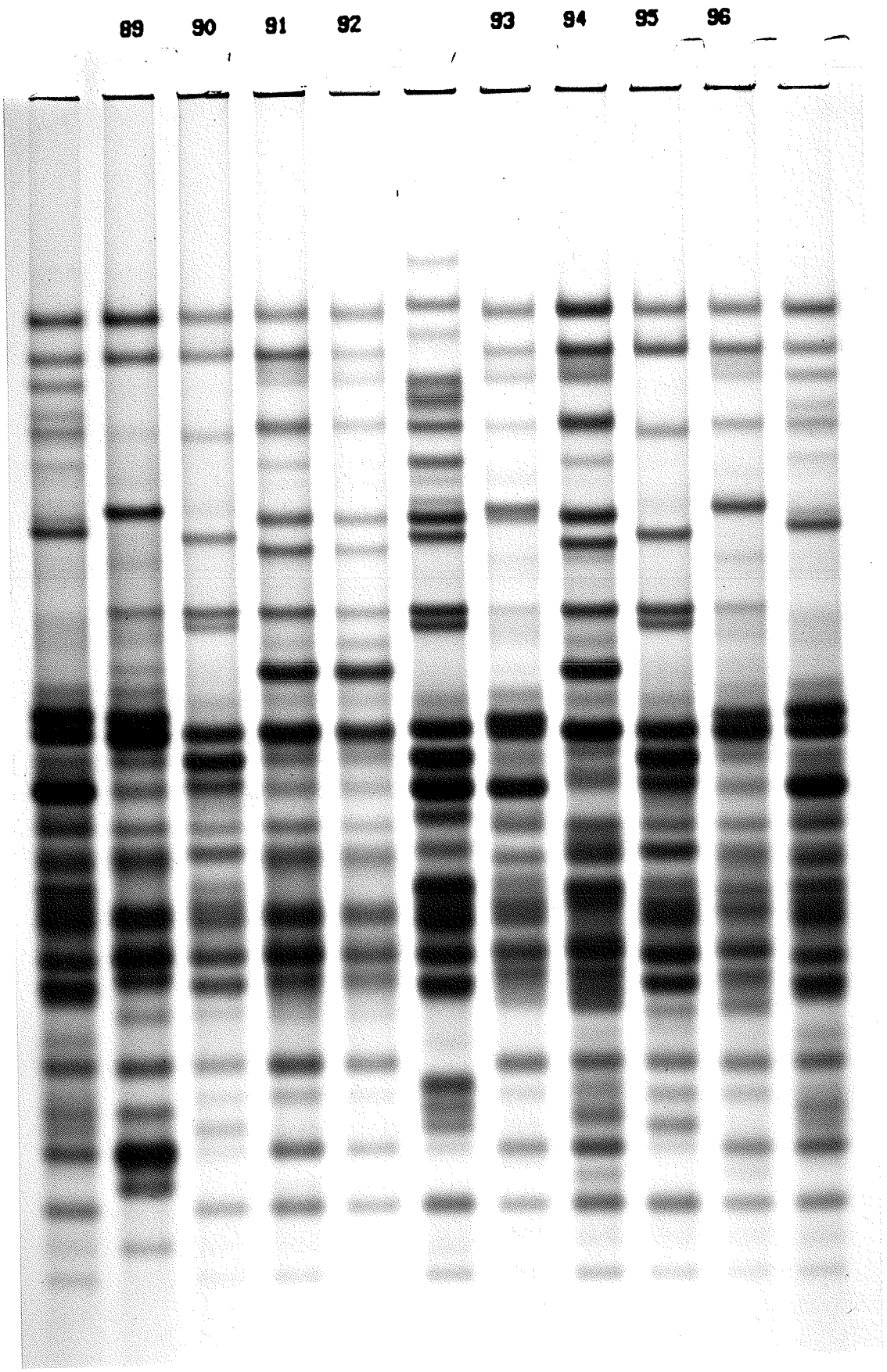

89
90
91
92
93
94
95
96

Figure 32. Gliadin electrophoregrams of common winter wheat cultivars (nos. 97 to 104 in Table 5) represented in the data base.

Pattern	Cultivar
97	Richmond
98	Rideau
99	Talbot
100	Yorkstar
101	Egyptian Amber
102	Fairfield
103	Jones Fife
104	Kent

Conditions:

Polyacrylamide gel: 6%
Buffer : Aluminum lactate (pH 3.1)
Protein stain : Coomassie Blue

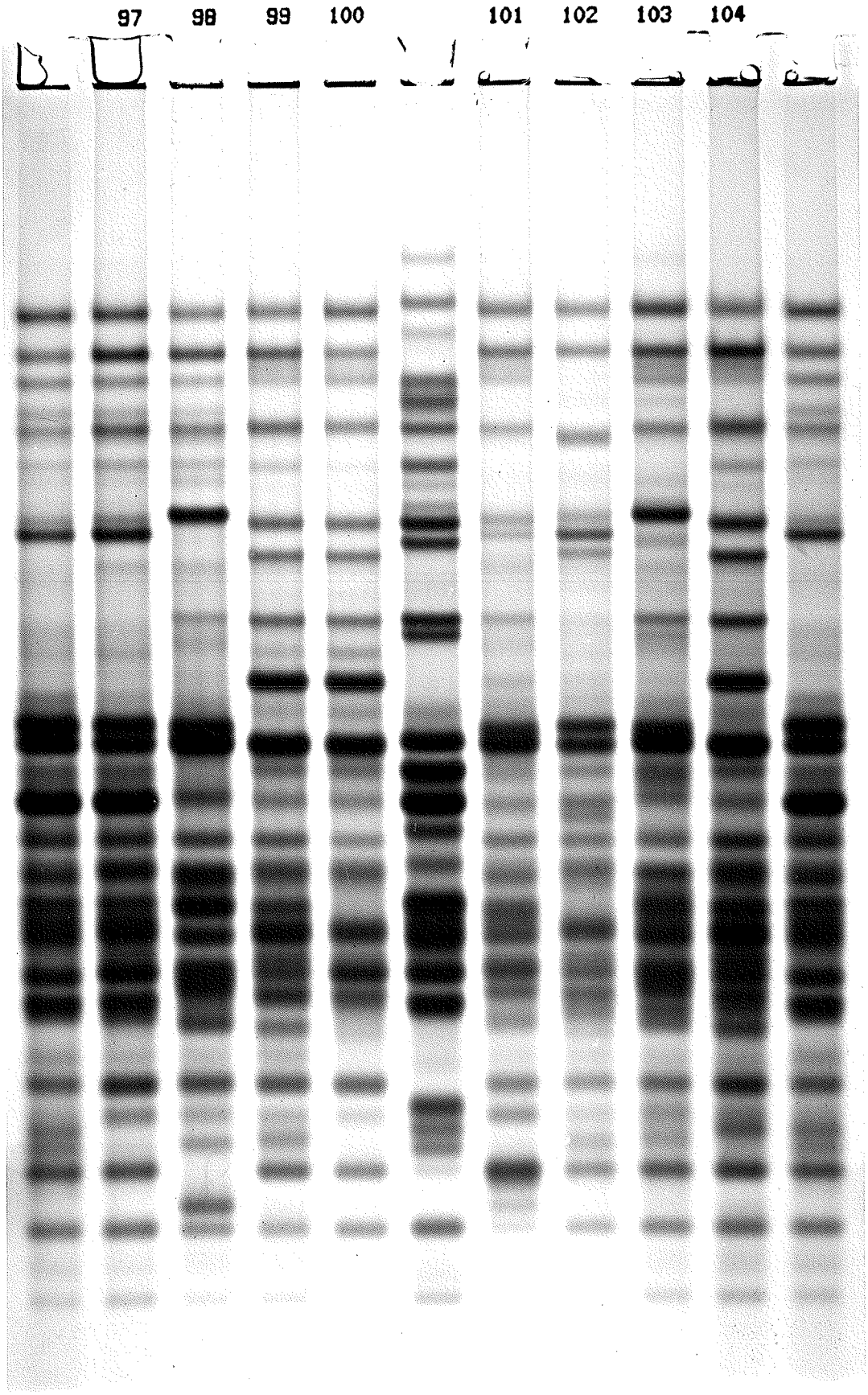

97
98
99
100
101
102
103
104

Figure 33. Gliadin electrophoregrams of common winter and durum wheat cultivars (nos. 105 to 106 in Table 5, nos. 107 to 112 in Table 6) represented in the data base.

Pattern	Cultivar
105	Sun
106	Thorne
107	Carleton
108	Coulter
109	Goldenball
110	Hercules
111	Macoun
112	Medora

Conditions:

Polyacrylamide gel: 6%
Buffer : Aluminum lactate (pH 3.1)
Protein stain : Coomassie Blue

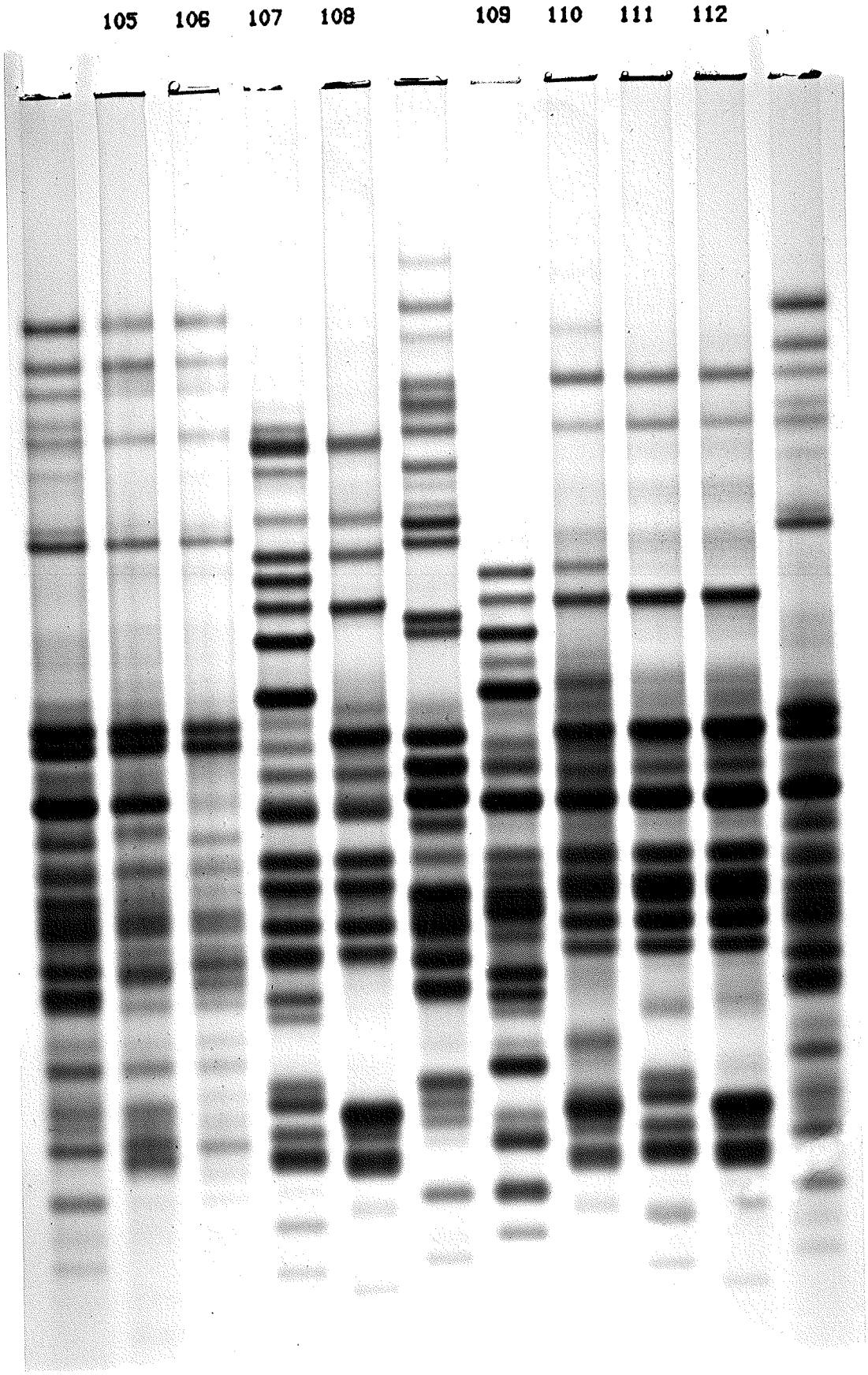

105
106
107
108
109
110
111
112

Figure 34. Gliadin electrophoregrams of durum wheat cultivars (nos. 113 to 120 in Table 6) represented in the data base.

Pattern	Cultivar
113	Mindum
114	Nugget
115	Pelissier
116	Ramsey
117	unknown
118	Stewart 63
119	Wakooma
120	Wascana

Conditions:

Polyacrylamide gel: 6%
Buffer : Aluminum lactate (pH 3.1)
Protein stain : Coomassie Blue

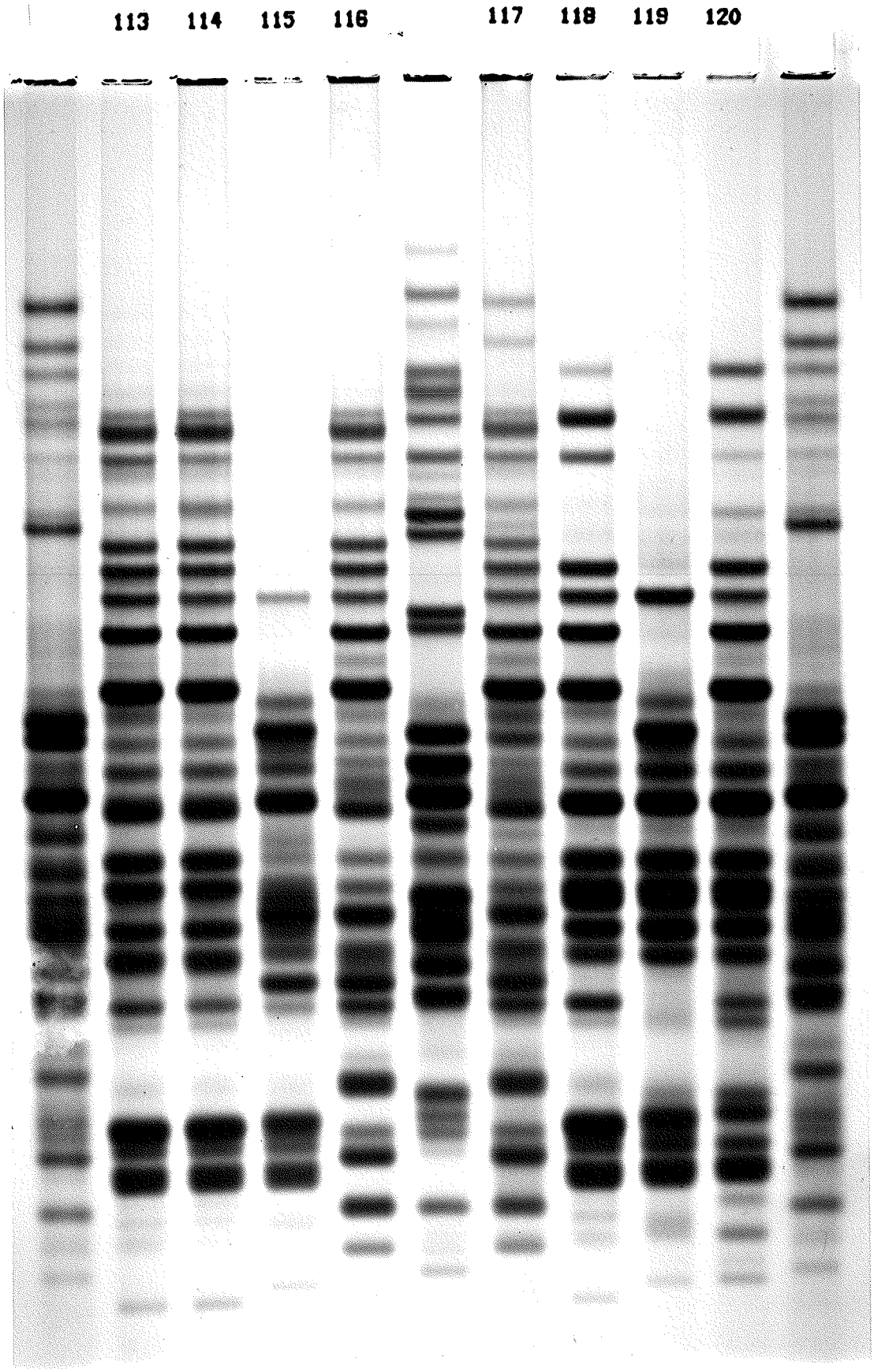

113
114
115
116
117
118
119
120

TABLE 10. CULTIVAR FORMULAS OF COMMON SPRING WHEATS BASED ON GLIADIN ELECTROPHOREGRAMS

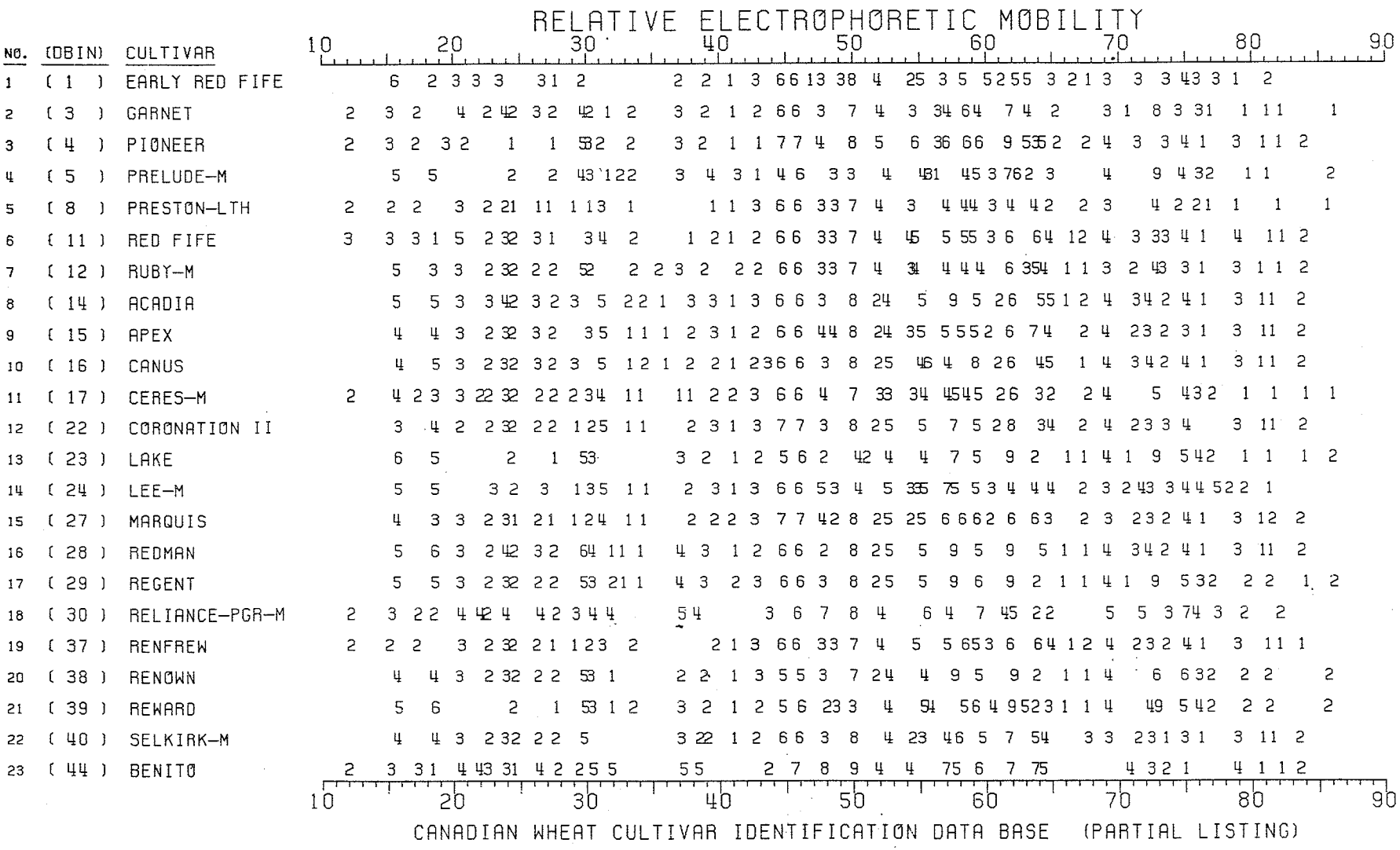


TABLE 10. CULTIVAR FORMULAS OF COMMON SPRING WHEATS BASED ON GLIADIN ELECTROPHOREGRAMS

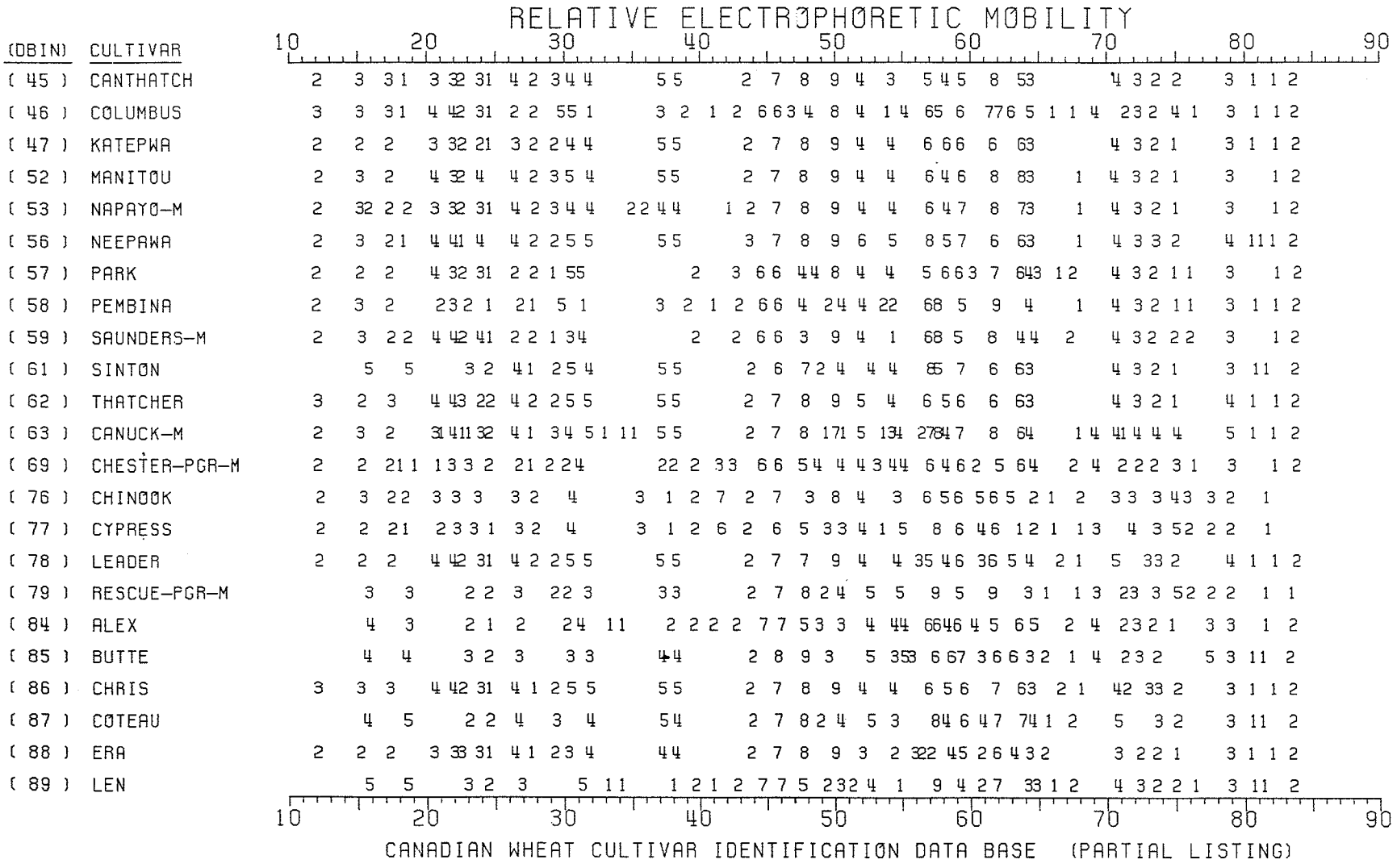
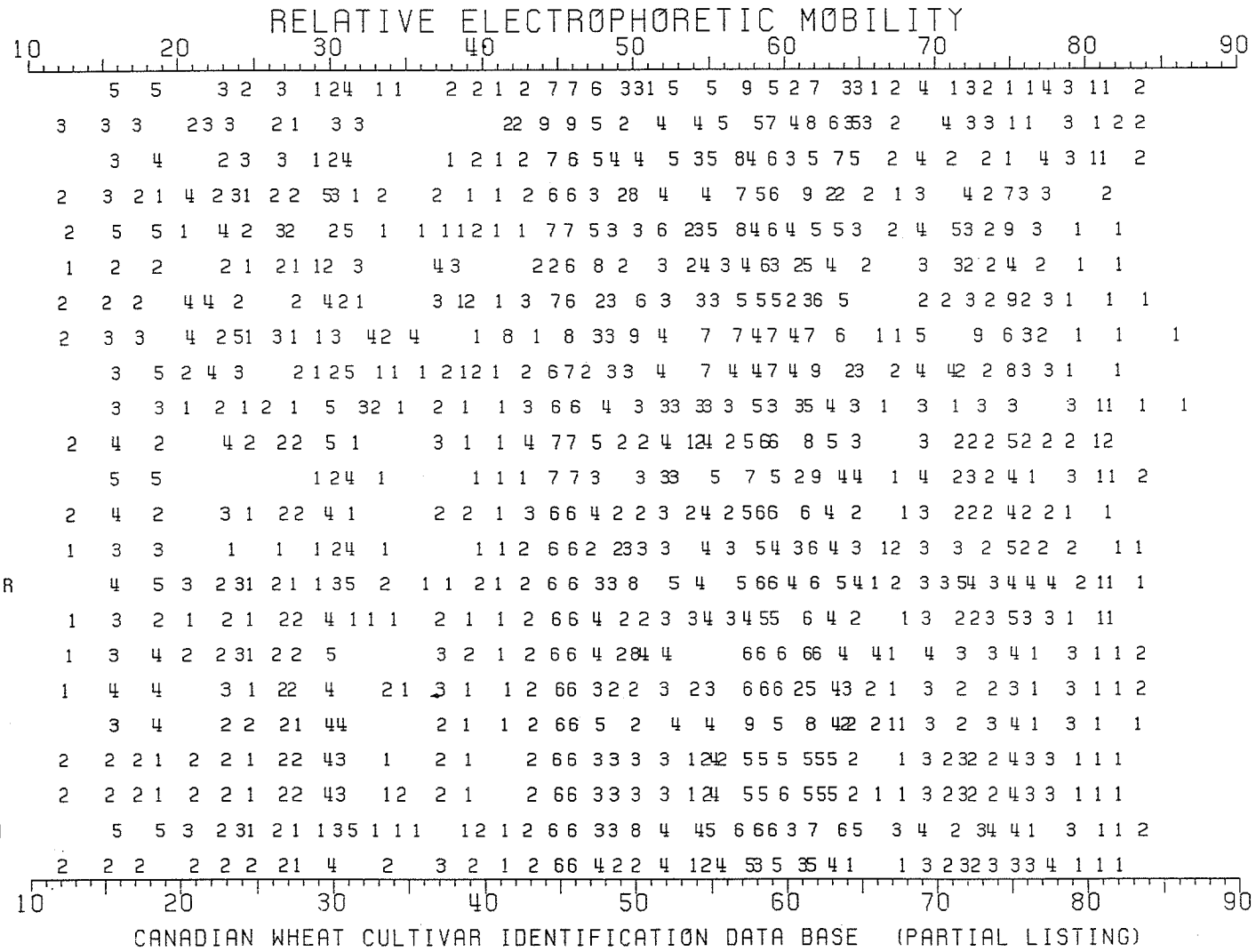


TABLE 10. CULTIVAR FORMULAS OF COMMON SPRING WHEATS BASED ON GLIADIN ELECTROPHOREGRAMS



CANADIAN WHEAT CULTIVAR IDENTIFICATION DATA BASE (PARTIAL LISTING)

TABLE 11. CULTIVAR FORMULAS OF COMMON WINTER WHEATS BASED ON GLIADIN ELECTROPHOREGRAMS

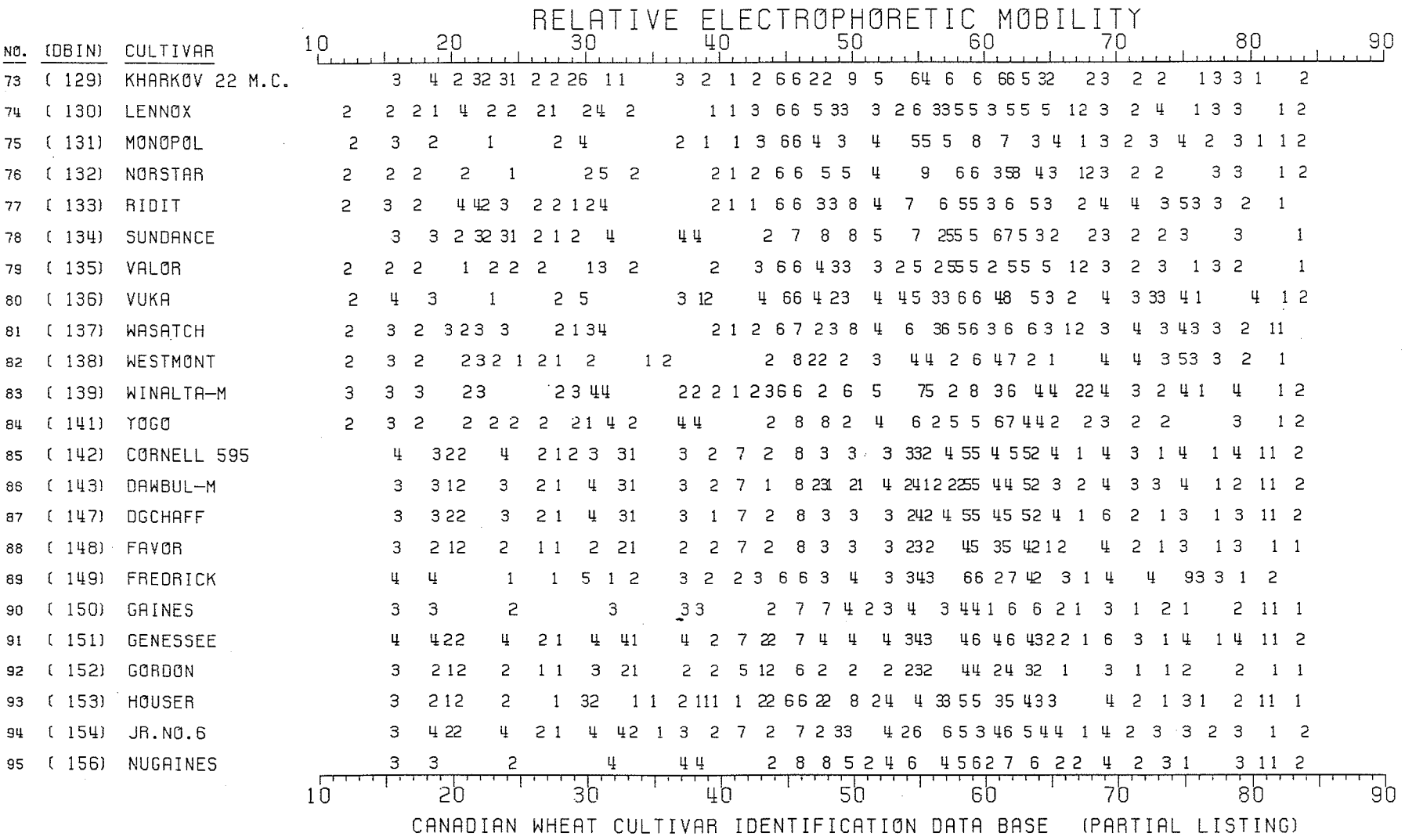


TABLE 11. CULTIVAR FORMULAS OF COMMON WINTER WHEATS BASED ON GLIADIN ELECTROPHOREGRAMS

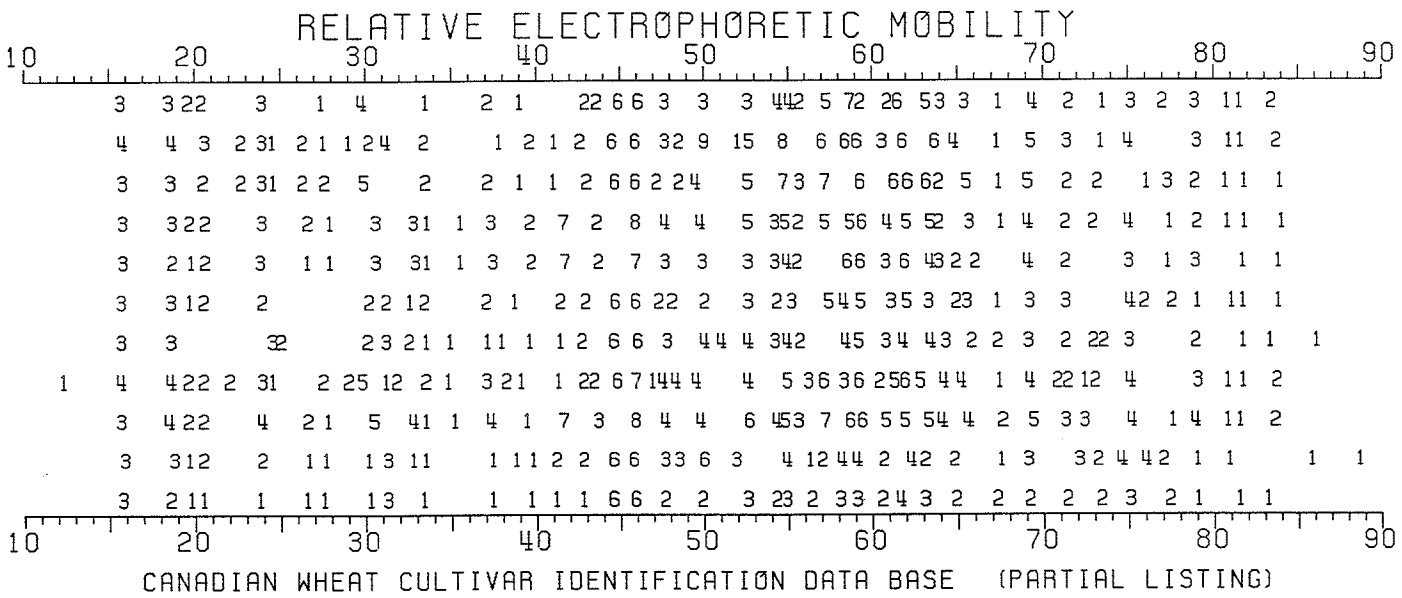
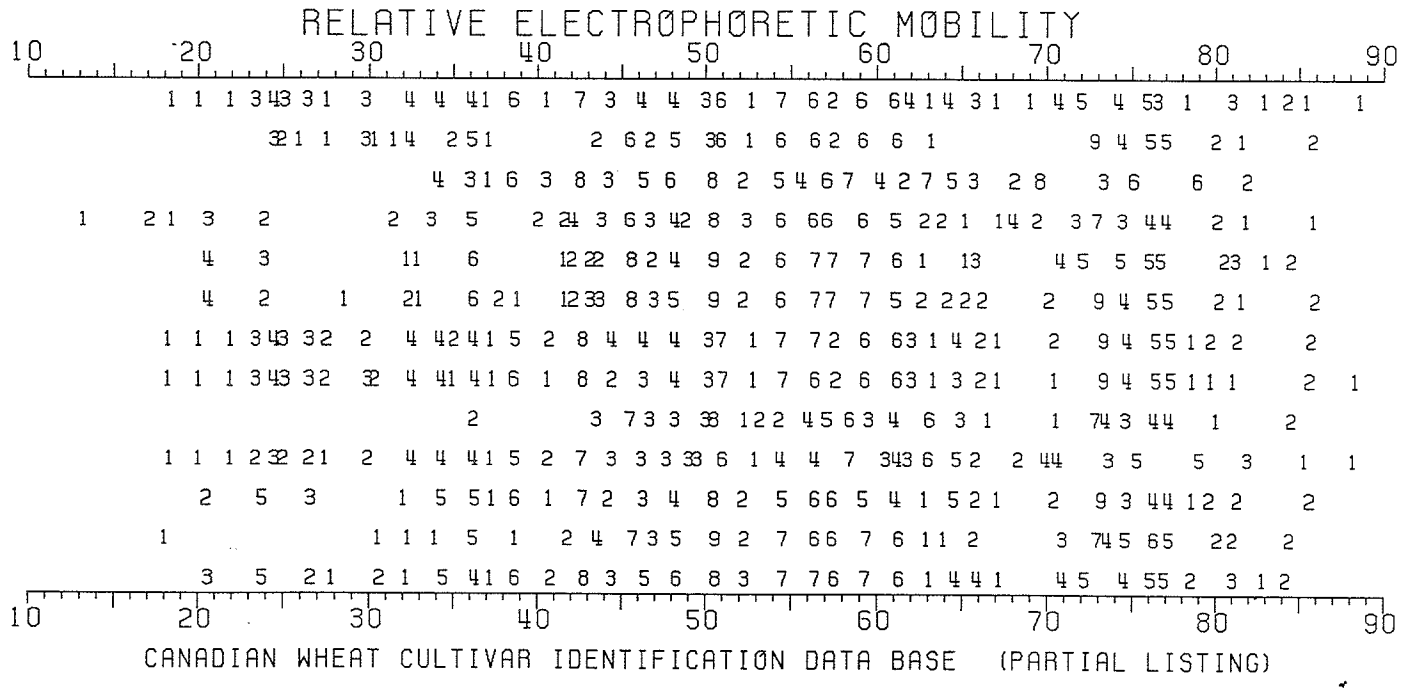


TABLE 12. CULTIVAR FORMULAS OF DURUM WHEATS BASED ON GLIADIN ELECTROPHOREGRAMS



Computer Acquisition of Gliadin Band Migration Distances

Electrophoretic pattern migration distance data from 8 x 10 inch format photographic prints (e.g. Figures 20-34) were acquired in a semi-automatic fashion by means of a digitizing tablet[1] which computed and fed the values directly into a computer file thus obviating the need to manually transcribe the data. Each positive print was taped securely to the surface of the tablet and three parallel lines were drawn across the print in order to join homologous reference bands in the flanking (cv. Marquis) and centre (cv. Neepawa) standard electrophoretic patterns (refer to following section). These lines were used as guides to mark the equivalent positions of reference band migration distances in sample electrophoregrams.

The first step in the digitization process for a given electrophoregram involved the definition of position coordinates for the origin. An instrument protocol was invoked to compute distances (as opposed to area measurements for example). A hand held cursor, partly comprised of electronically wired crosshairs enclosed between two circular glass lenses, was placed over the leading edge of the centre of the slot and a button on the cursor was depressed to digitize the point. Subsequently, each band in the electrophoregram was visually identified and digitized in rapid succession. The list was finished by digitizing the equivalent positions for gliadin reference bands in the pattern. For each cultivar in Tables 4-6, the acquisition of band migration

[1]The digitizing tablet was a Talos model 648 with a resolution of 1000 lines per inch, and accuracy of ±0.005 inches (approximately 39 lines/mm, ±0.013 mm).

distances was considered complete when values for all replicate electrophoregrams (typically 2-5) were collected into the cultivar's data file (refer to Appendix B for example).

Application of the digitizer to quantify band migration distances (and hence relative mobilities) substantially minimizes the tedium and transcription errors that would otherwise be involved with the manual measurement of the data by means of a ruler or microcomparator. Still problematic however, are decisions concerning the inclusion of very faint bands in cultivar formulas (i.e. density=1, in Tables 10-12) and the interpretation of overlapping bands as one or two closely contiguous components. The capability of surveying an average of three replicate PAGE patterns per cultivar sample was an advantage in minimizing the subjectivity of identifying marginal bands. In the program logic for cultivar identification (to be discussed), the uncertainty of very faint bands is taken into account to prevent their having a disproportionate influence on cultivar rankings.

Application of Multiple Reference Bands to Compute Relative Mobilities

In order to improve the day to day reproducibility of relative mobilities used for comparative analysis of gliadin electrophoregrams, as well as to increase overall data base accuracy, the number of reference proteins was increased to three from the single reference band approach proposed by Bushuk and Zillman (1978). The two additional or secondary reference bands are designated as "R24" and "R79" based upon their respective mobilities relative to the primary reference, Marquis band "R50" (see below). These PAGE reference components are indicated

in the electrophoregrams of cvs. Marquis and Neepawa (Figure 35), which were employed as standard patterns for every PAGE run (e.g. Figures 20-34). As with gliadin R50, bands R24 and R79 were found to be common gliadin components among the population of more than 120 wheat cultivars that was studied.

Before these secondary reference bands could be incorporated into the algorithm used to normalize migration distance data, an accurate determination of their relative mobilities was required. Relative mobilities for gliadins R24 and R79 in cvs. Marquis and Neepawa were established relative to band R50 following the method of Bushuk and Zillman (1978). Results of these calculations are presented in Table 13, and show no significant difference in relative mobility for corresponding bands in electrophoregrams of Marquis and Neepawa. On this basis, gliadin reference bands R24 and R79 were assigned mean relative mobility (Rm) values of 23.88 and 78.95 respectively.

The relationships used to compute relative mobilities by multiple reference bands are given in Table 14. The expression which is invoked to make the calculation (equation 1,3,6 or 7) is contingent upon the position of a gliadin band in the electrophoregram field. Thus the influence of a reference protein in determining the relative mobility of a gliadin band depends on the proximity of the former to the latter.

The process begins by dividing each electrophoregram into four mobility zones partitioned along the boundaries formed by the three internal reference proteins (refer to bottom of Table 14). An initial estimate of relative mobility is obtained by applying Bushuk and Zillman's formula (equation 2). If the value returned by Ri(x) is less

Figure 35. Standard gliadin reference bands: R24, R50 and R79 in electrophoregrams of cultivars Marquis (a) and Neepawa (b), used to compute relative mobilities for the cultivar identification data base.

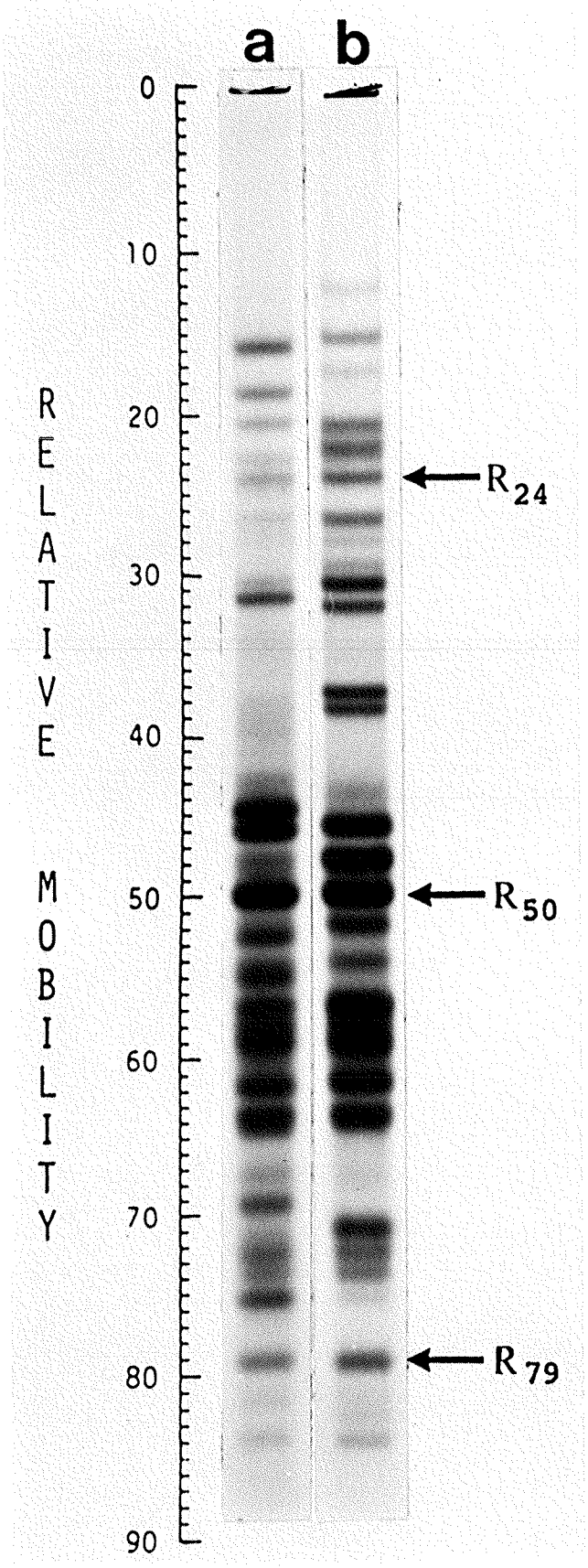

a
b
0
10
20
30
40
50
60
70
80
90
RELATIVE MOBILITY
R_{24}
R_{50}
R_{79}

TABLE 13

Comparison of relative mobilities in cultivars Marquis and Neepawa for reference gliadin bands R24 and R79 calculated relative to Marquis band 50

Reference band	Number of observations	Mean relative Mobility	Standard deviation
Marquis R24	74	23.90	0.21
Neepawa R24	36	23.85	0.22
Marquis R79	74	78.96	0.87
Neepawa R79	36	78.94	0.80

than 23.88 then the computed relative mobility becomes solely a function of the position of the low mobility reference protein according to equation 1. Similarly if Ri(x) returns a value greater than 78.95, then band mobility is computed relatively to the position of reference protein R79 according to equation 3.

If a gliadin component migrates within the range of the reference bands, e.g. in the zone between R24 and R50, then relative mobility is calculated by summing the weighted contributions of equations 1 and 2 using equation 6. The weighting function li(x) for the latter is given by equation 4. Likewise, if a gliadin band is bounded by reference proteins R50 and R79, then equation 7 is applied to compute relative mobility using the weighting function hi(x) defined by equation 5.

The weighting functions li(x) and hi(x), within their respective ranges, can vary between 0 and 1, and are equal to 0 or 1 at the reference protein boundaries. Thus, as the mobility of a gliadin

TABLE 14

Relationships used to compute relative mobilities by multiple reference bands

Mobility relative to reference band R24	$Qi(x) = \frac{xi}{xR24} * 23.88$[1]	(1)
Mobility relative to reference band R50	$Ri(x) = \frac{xi}{xR50} * 50.00$	(2)
Mobility relative to reference band R79	$Si(x) = \frac{xi}{xR79} * 78.95$	(3)
Weighting function for gliadin bands between R24 and R50	$li(x) = \frac{Ri(x)-23.88}{50.00-23.88}$	(4)
Weighting function for gliadin bands between R50 and R79	$hi(x) = \frac{Ri(x)-50.00}{78.95-50.00}$	(5)

Evaluate [Mi=Ri(x)]	Computed relative mobility	
if $Mi < 23.88$	$Mi = Qi(x)$	
$23.88 < Mi < 50.00$	$Mi = [1-li(x)]Qi(x)+li(x)Ri(x)$	(6)
$50.00 < Mi < 78.95$	$Mi = [1-hi(x)]Ri(x)+hi(x)Si(x)$	(7)
$Mi > 78.95$	$Mi = Si(x)$	

[1]xi = migration distance of ith gliadin band.

component nears that of a reference protein, the contribution of the more distant counterpart reference band to the computed relative mobility declines. For example, if a gliadin band approaches the mobility of reference band R50 from a higher or lower mobility, equations 6 and 7 will gradually assume the same identity; at Ri(x)=50.0, the contributions of Qi(x) and Si(x) disappear. Similarly,

the pair of equations 1 and 6 and equations 3 and 7 converge in identity for gliadin bands which migrate with velocities approaching respectively, the low and high mobility reference proteins.

Due to the moderate complexity of the relationships described above, the task of normalizing migration distance data to relative mobilities was implemented by means of a computer program (STATWT3). Arithmetic mean and standard deviations are generated as program output for each gliadin band along with the mean cultivar signature array (refer to Appendix B for example).

Precision of Determination of Relative Mobilities

A comparison of the single (REF1) and multiple reference band (REF3) techniques on the precision of relative mobility results for cultivar Neepawa is given in Table 15. Corresponding plots of standard deviations (SD) and coefficient of variability (CV) as a function of mobility are shown in Figure 36. Statistical analysis of the two Rm measurement procedures was based on a common set of 13 replicates of raw migration distance data including Marquis reference band 50 positions used in the respective formulas of the REF1 and REF3 methods.

The plotted data in Figure 36 shows that the uncertainty (standard deviation) in the calculation of relative mobility using either approach is not constant, but varies with the position of a gliadin band in the electrophoregram field, reaching minima in the vicinity of reference band positions. Not surprisingly the size of relative errors (CV's) is proportional to the distance of a gliadin band from its closest reference protein. This is indicated most clearly for the single

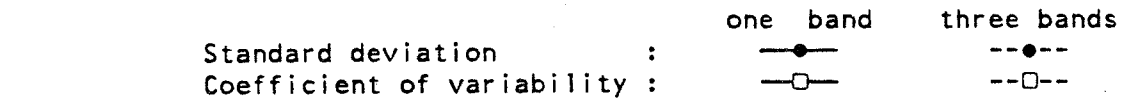
TABLE 15

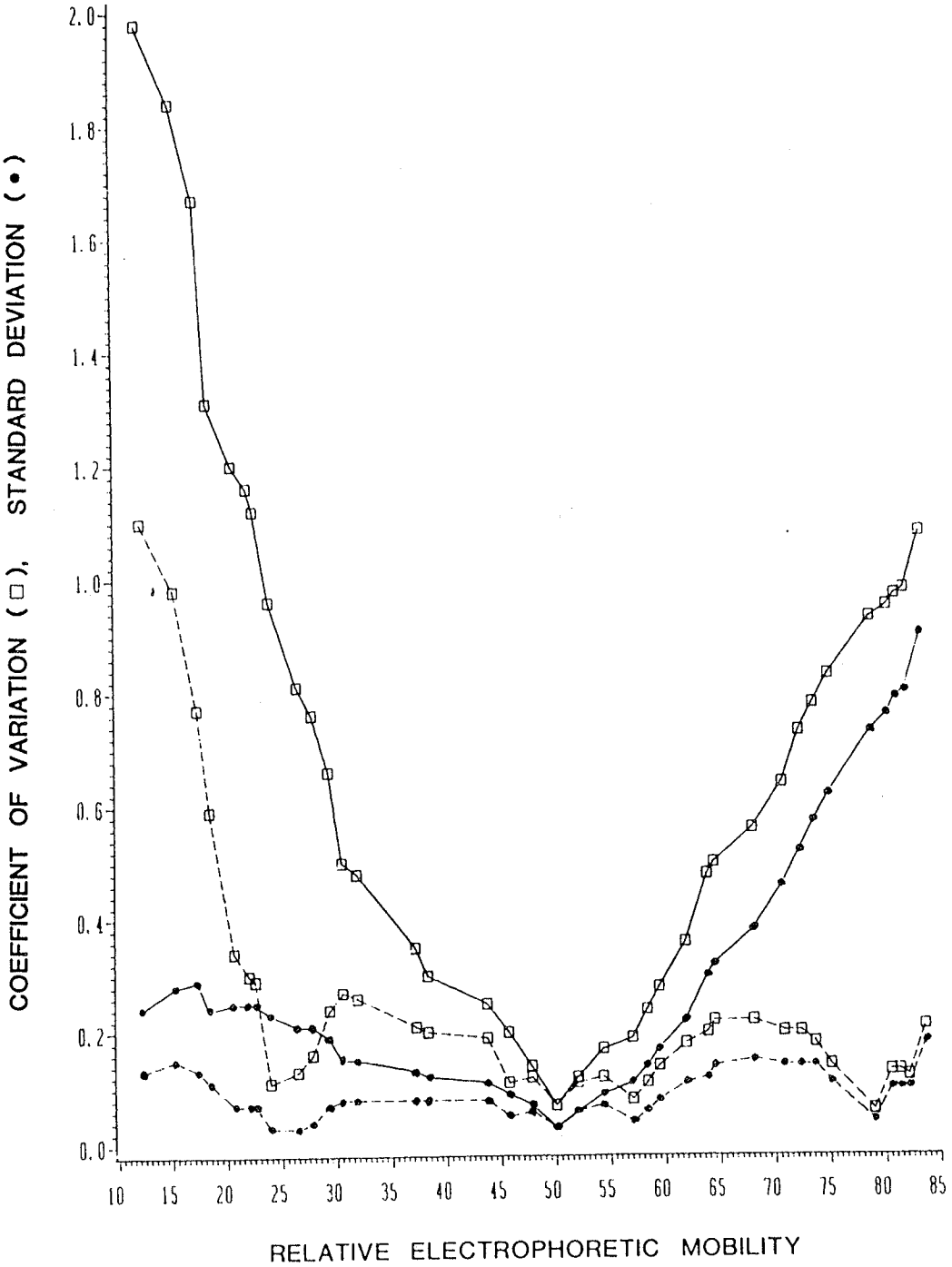
Relative mobilities, standard deviations, coefficient of variabilities and F-ratios for gliadin bands of cv. Neepawa based on single and multiple reference band methods

REF1 procedure			REF3 procedure			
Rm	SD	CV	Rm	SD	CV	F-ratio
12.03	0.24	1.98	12.07	0.13	1.10	3.20*
15.11	0.28	1.84	15.16	0.15	0.98	3.53*
17.21	0.29	1.67	17.26	0.13	0.77	4.67**
18.27	0.24	1.31	18.34	0.11	0.59	4.85**
20.53	0.25	1.20	20.60	0.07	0.34	11.96**
21.90	0.25	1.16	21.98	0.07	0.30	15.16**
22.45	0.25	1.12	22.53	0.07	0.29	15.01**
23.80	0.23	0.96	23.89	0.03	0.11	79.34**
26.36	0.21	0.81	26.44	0.03	0.13	39.79**
27.71	0.21	0.76	27.79	0.04	0.16	22.92**
29.19	0.19	0.66	29.27	0.07	0.24	7.29**
30.41	0.15	0.50	30.49	0.08	0.27	3.38*
31.76	0.15	0.48	31.84	0.08	0.26	3.49*
37.10	0.13	0.35	37.17	0.08	0.21	2.59
38.22	0.12	0.30	38.29	0.08	0.20	2.25
43.66	0.11	0.25	43.70	0.08	0.19	1.72
45.63	0.09	0.20	45.66	0.05	0.11	3.15*
47.75	0.07	0.14	47.77	0.06	0.12	1.36
49.99	0.03	0.07	49.99	0.03	0.07	1.03
51.97	0.06	0.12	51.98	0.06	0.11	1.37
54.27	0.09	0.17	54.30	0.07	0.12	1.93
56.95	0.11	0.19	56.99	0.04	0.08	6.10**
58.29	0.14	0.24	58.34	0.06	0.11	4.82**
59.36	0.17	0.28	59.42	0.08	0.14	3.91*
61.75	0.22	0.36	61.82	0.11	0.18	4.20**
63.70	0.30	0.48	63.78	0.12	0.20	5.94**
64.30	0.32	0.50	64.39	0.14	0.22	5.43**
67.88	0.38	0.56	67.99	0.15	0.22	6.33**
70.62	0.46	0.64	70.76	0.14	0.20	10.75**
72.18	0.52	0.73	72.33	0.14	0.20	13.41**
73.44	0.57	0.78	73.61	0.14	0.18	17.60**
74.87	0.62	0.83	75.04	0.11	0.14	32.75**
78.72	0.73	0.93	78.94	0.04	0.06	271.99**
80.24	0.76	0.95	80.47	0.10	0.13	55.69**
81.01	0.79	0.97	81.24	0.10	0.13	58.18**
81.83	0.80	0.98	82.07	0.10	0.12	65.29**
83.36	0.90	1.08	83.61	0.18	0.21	26.07**

* Significance at the 5% level.
** Significance at the 1% level.

Figure 36. Standard deviation and coefficient of variability as a function of gliadin band relative electrophoretic mobility calculated for cv. Neepawa data using one and three reference band methods.




COEFFICIENT OF VARIATION (□), STANDARD DEVIATION (•)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
RELATIVE ELECTROPHORETIC MOBILITY

reference band CV curve which varies in a symmetrical fashion about Rm=50. The likely source of this error relates to localized variations in gel buffer composition, pH, voltage and temperature which arise during the course of the electrophoresis period. The only time in fact when the gel is truly a homogeneous medium is before the run begins. Clearly then, the greater the number of reference bands applied across the gel in determining relative mobilities, the more the effects of these sources of error will be minimized.

As previously discussed, low and high mobility reference bands R24 and R79 have a decreasing influence on the calculation of relative mobilities for gliadin components with migration velocities approaching the intermediate reference band R50. The effect is reflected in the SD curves shown in Figure 36 which converge in the neighborhood of Rm=50, but become increasingly disparate as the distance between a gliadin band and reference band R50 increases. Differences in precision are most marked in the region of high mobility components where the uncertainty in Rm measurements by the single reference band procedure is at its maximum (SD=0.9). This value is similar to those reported elsewhere (Caldwell and Kasarda, 1978; Lookhart et al., 1983) for gliadin PAGE components with high relative mobilities.

The F-ratios given in Table 15 support what is graphically apparent in Figure 36 that a highly significant difference exists in the precision between the two sets of data (with the exception of gliadin bands possessing intermediate mobilities). It can be therefore concluded that variability in Rm values is stabilized at a significantly lower level using the REF3 procedure, with mean CV's for the REF3 and

REF1 method at 0.2 and 0.7 respectively. This reflects the fact that the average uncertainty in the measurement of relative mobilities by the REF1 procedure is more than three times greater than by multiple reference bands (mean SD's: 0.31 versus 0.09 respectively).

Significant differences notwithstanding, these low values suggest that both procedures are relatively efficient in generating reproducible Rm values. The question may be asked, what level of precision is required of the cultivar identification process. As will be described in the following section, the latter involves matching an unknown or sample electrophoregram with all reference PAGE patterns encoded in the data base taken one at a time. For each pairwise comparison, assessments of gliadin band identity are inferred by comparing recorded relative mobilities ± threshold. This threshold must be set wide enough to accept "truly" homologous protein components, but not so wide that mismatches result between different protein species.

The problem is a classical one of minimizing both so-called type I and type II errors[1] when the null hypothesis is that no significant difference exists between Rm's of compared bands. Minimizing type I error can be achieved with relative ease by selecting a threshold value not less than the 95% confidence limit (95% CL), which for "n" determinations of mean Rm is given as

$$95\%\ CL = \pm\ t_{.05}\sqrt{SD^2/n}$$

---

[1]Type I error refers to the rejection of true gliadin band identity. Type II error is the acceptance of false gliadin band identity.

where $t_{.05}$ is the critical value of Student's t on (n-1) degrees of freedom. Unfortunately the size of this threshold may conflict with the ability to detect the alternative hypothesis when it is true (i.e. minimizing type II error). Some workers have avoided dealing with this intractable problem by not including contributions from non-matching bands in their cultivar identification strategy (Lookhart et al., 1983). Ironically only differences between proteins may be detected with certainty by electrophoresis.

In this regard, the important parameter is the distance between contiguous different gliadin components in compared PAGE patterns. For electrophoregrams run on the same gel slab, direct evidence suggests that this distance can be as small as one-half a mobility unit. By applying the above formula using three replicates as the basis, it can be determined that in order to detect a difference of 0.5 Rm units, in addition to having a 95% confidence that mean mobilities for identical bands in compared patterns lie within this interval, requires that standard errors (SE) be less than 0.12 Rm units.

The data in Table 16 indicates that this level of precision is well within the limits of error for relative mobilities calculated by multiple reference bands. By the single reference band approach however, only for bands possessing mobilities less than 50, does the average uncertainty and therefore 95% CL (0.47) approach a value similar to counterpart data of the REF3 method where 0.45 represents a maximum. The latter was used as the guideline in setting the difference threshold (LSD) of 0.5 Rm units in numerous examples of program output for the cultivar identification system to be described later.

TABLE 16

Standard errors and 95% Confidence limits for Rm measurements by single and multiple reference band methods based on three determinations

	REF1 procedure		REF3 procedure	
SE	SE	95% CL	SE	95% CL
maximum	0.52	2.24	0.10	0.45
average	0.18	0.77	0.05	0.22
Rm < 50	0.11	0.47	0.05	0.22
Rm > 50	0.25	1.09	0.06	0.25

Thus high precision becomes the critical factor if reliable results are to be expected when using relative mobilities to infer the identity of gliadin bands run on different gels. While an increase in sample size by replication will result in a narrowing of confidence limits,[1] this is generally not a practical solution when due consideration is given to the time, labor and cost associated with performing the electrophoresis test on a large number of samples. Accordingly, catalogs of gliadin PAGE data for cultivar identification incorporating few replicates are typical (e.g. N=2, Jones et al., 1982; N=1, Zillman and Bushuk, 1979). As will be shown later, when the average uncertainty in relative mobilities for an entire population of cultivars is at a minimum (e.g. 0.10 or less), improved estimates of gliadin heterogeneity

[1]Based upon average uncertainties in the determination of relative mobilities by the REF3 and REF1 methods, the latter requires about 10 replications to obtain the equivalent level of precision achieved by the REF3 method using three replicates.

can be realized from one-dimensional electrophoretic data.

Calculation of Electrophoretic Pattern Homology

The procedure of cultivar identification developed in this study compares an unknown or sample electrophoregram with all reference patterns encoded in the data base taken one at a time, to obtain a measure used to assess the degree of matching. As such, the process is similar in concept to the identification by matching strategy described by Pankhurst (1975), but does not depend upon an a priori classification of gliadin bands into an attribute list or character set, as this can involve some simplification and interpretation (Wrigley, 1980) and is otherwise time consuming. The problem of classification will be dealt with in a following section.

Rm and density values for each protein band in a gliadin electrophoregram are treated as continuous variables, where Rm represents the primary feature parameter in the assessment of overall similarity in protein composition for two electrophoregrams. The latter is expressed by a score termed percent pattern homology (%PH) and cultivars in the data base are ultimately ranked on this basis. For each comparison involving the unknown and a reference cultivar, the pair of electrophoregrams are scanned from low to high mobility to assess the extent of pattern homology. Matching gliadin bands are tallied if the numerical differences in both their Rm and density parameter values fall within prescribed threshold levels. The least significant difference in Rm is programmable in increments of 0.1 relative mobility units (RMU) and can be set in accord with the uncertainty in Rm measurements. For

the present study, this threshold was fixed at 0.5 RMU which corresponds to the 95% confidence limit (df=2) when comparing mean relative mobilities with a standard error of ±0.10 RMU[1]. For band densities, a difference limit of three units was predefined subjectively as the minimum criterion to reject matching status for paired PAGE components which were not significantly different in Rm. Thus for gliadin bands sharing only a positional homology, the event was scored as a band difference (see below).

Accordingly, the comparative analysis of two electrophoregrams (denoted below as "A" for an unknown and "B" for a reference cultivar) yields four possible pattern homology conditions defined by one matching and three types of non-matching events:

1. m - pairs of matching bands with respect to both Rm and band density parameter values.
2. j - bands present in cv. "A" but absent from cv. "B", i.e. the forward component of non-matching bands.
3. k - bands present in cv. "B" but absent from cv. "A", i.e. the reverse component of non-matching bands.
4. l - pairs of bands with matching Rm values but possessing significantly different levels in density.

Percent pattern homology was then defined as

$$\%PH = \frac{m \times 100}{m + (j + k + l)} \qquad (8)$$

and is equivalent to the expression:

---

[1]The standard error in mean Rm value averaged for over 7,000 gliadin bands from approximately 180 data base reference PAGE patterns was 0.073 RMU; each Rm value represented the mean of approximately three replicate patterns run on separate gels.

$$\%PH = \frac{\text{(no. of pairs of matching bands)} \times 100}{\text{no. of pairs of matching bands + no. of different bands}}$$

Equation 8 is similar in form to the "simple matching coefficient" described by Sneath and Sokal (1973) and is a common means to assess the variation in banding patterns of two gels (Ladizinsky and Hymowitz, 1979).

The above relationship is qualitative in the sense that for each matching or non-matching event between two electrophoregrams, an equivalent score of unity is incremented to the appropriate terms in the expression. While band density is taken into consideration for gliadin bands which match on a mobility basis (i.e. terms m and l in equation 8), terms j and k are not sensitive to the protein concentration of non-matching bands which they enumerate. Very faint bands (density=1) for example, which tend to be non-reproducible, would carry the same weight in the equation as very dense non-matching bands. This lack of sensitivity was found to limit the discrimination power of the gliadin electrophoregram to differentiate between cultivars. The expression in equation 8 was therefore modified to yield a measure of pattern homology as a function of the number of gliadin components weighted by their band density (WBD) values. The general form of this relationship, which is outwardly similar to equation 8 is

$$\%PH = \frac{Ma,b \times 100}{Ma,b + (Fa + Rb + Da,b)} \qquad (9)$$

The individual terms of equation 9 are summation expressions which are defined as follows:

Matching band component: $$Ma,b = \sum_{i=1}^{m} (da + db)\, i/2Wt$$

Forward non-matching band component: $$Fa = \sum_{i=1}^{j} (da)\, i/Wt$$

Reverse non-matching band component: $$Rb = \sum_{i=1}^{k} (db)\, i/Wt$$

Density non-matching band component: $$Da,b = \sum_{i=1}^{l} |(da-db)|\, i/Wt$$

where da and db represent band densities for the ith band or pair of bands in cultivars "a" and "b" respectively, and Wt is a constant equal to the average density assignment for gliadin bands encoded in the data base.

Cultivar Identification System Program Outline

The identification system is comprised of a set of three programmed procedures, dedicated to different aspects of the comparative analysis problem. The scope of each is outlined below as follows:

1. Program CVID produces a ranking of possible cultivars in a short list arranged in order of declining PAGE pattern homology with the unknown electrophoregram. A threshold value for %PH controls entry into the list. Printed output includes the cultivar name, pedigree, attribute summary and tabulation by number of matching and non-matching bands for each ranked pairwise comparison. Also included is the distribution of forward and reverse components of non-matching bands, as well as the number of bands that differ significantly on a density basis alone.

2. Programs IDHOM and IDPLOT combine to produce a graphic analysis of electrophoregram composition for cultivars specified in the list generated by program CVID. For each pairwise comparison of cultivar PAGE data between the unknown and data base member, IDHOM identifies gliadin bands by mobility and density which contribute to matching or non-matching states of pattern homology. The printout gives a detailed summary of results on standard forms. Program IDPLOT uses as input the numerical data

derived from the IDHOM routine. The cultivar formula cataloguing format of Zillman and Bushuk (1979) was adapted to visualize the gliadin electrophoregram composition of matching and non-matching bands which are respectively isolated in separate plots for the list of ranked cultivars.

3. Program CVMAP computes the minimum number of gliadin bands that must be deleted from the unknown and each reference electrophoregram in the data base, in order to yield patterns of identical composition. The printout is a frequency distribution which plots each cultivar's data base identification number and homology score against the value of the continuous variable i.e. total number of composition differences with the unknown electrophoregram. The result assists in evaluating the uniqueness of the latter and identifies cultivars lying at the margins of the distribution which are of diverse genotype.

In addition to these programs of the cultivar identification system, the comparative analysis process can be focused on selected pairs of electrophoregrams to produce numerical hard data (HOMOLOGY2) and graphics (HOMPLOT2) for two cultivars of special interest. The above cited software, with the exclusion of plotting programs IDPLOT and HOMPLOT2, were written in FORTRAN IV language using standard data items with the exception that character type variables and arrays were included in the source. As such, these programs must be compiled under WATFIV or equivalent compilers which can translate the character data type. The plotting programs IDPLOT and HOMPLOT2 were developed in FORTRAN but also incorporate several subroutines of CALCOMP[1] Basic Software to produce results on a Versatec D1200A matrix plotter. These programs were compiled under FORTX. All programs were tested on an IBM 470, and Amdahl 470/580 computers and as is common with all installations, the only major machine dependencies are in the input and output routines.

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[1]California Computer Products, 2411 West La Palma, Anaheim, California, 92801.

Cultivar Identification Printouts and Pattern Homology Analysis Plots for Cv. Neepawa

To illustrate the performance of the cultivar identification system, the signature array encoding the gliadin electrophoregram for the Canadian hard red spring wheat cv. Neepawa (refer to Figure 8) was selected to represent an unknown sample. The full complement of computer printouts and graphic analysis plots are shown in Figures 37 to 39.

The summary report produced by program CVID (Figure 37) represents a short list of cultivars ranked in order of decreasing weighted percent pattern homology (%PH) according to the formula given previously (equation 9, p. 181). At the head of the output are several lines which specify the various free parameters chosen for the program run. As indicated, 55%PH was selected as the cutoff value for cultivar entry into the short list ranking. This limit in combination with specified difference thresholds for gliadin band identity (i.e. <0.5 Rm units, <3 density units), typically resulted in the ranking of 10% to 20% of the primary population (excluding biotypes) of 122 common spring, winter and durum wheat reference cultivars in the data base.

The top ranked cultivar in Figure 37 shows that the identification program has been successful in precisely matching the input PAGE pattern for cv. Neepawa with its data base counterpart. Succeeding %PH values indicate further that the Neepawa electrophoregram is very similar in composition to band patterns of a group of seven cultivars which have been isolated with high levels of pattern homology (>90%). The influence of common genetic background has largely contributed to this result as all eight cultivars are dominated by cv. Thatcher or related genotypes

Figure 37. Cultivar identification ranking program result cv. Neepawa.

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - I. RANKING BY PATTERN HOMOLOGY

* 122 DATA BASE CULTIVAR PATTERNS ANALYZED
* DATA BASE SEARCH CUTOFF AT 55% PATTERN HOMOLOGY (WEIGHTED BY BAND DENSITY).
* LSD(RELATIVE MOBILITY) = 0.5, MOBILITY RANGE: 10.0 - 90.0. LSD(BAND DENSITY) = 3, DENSITY RANGE: 1 - 9.
* UNKNOWN (OR TEST) CULTIVAR ELECTROPHOREGRAM CONTAINS 37 GLIADIN BANDS; TOTAL, WEIGHTED BY BAND DENSITY (WBD) = 44.6

	CULTIVAR	WEIGHTED % PATTERN HOMOLOGY	GLIADIN BANDS IN PATTERN NO.	GLIADIN BANDS IN PATTERN WBD	MATCHING BANDS NO.	MATCHING BANDS WBD	DISTRIBUTION OF NON-MATCHING BAND DATA: TOTAL NO.	TOTAL WBD	MOBILITY BASIS-R NO.	MOBILITY BASIS-R WBD	DENSITY BASIS NO.	DENSITY BASIS WBD	MOBILITY BASIS-U NO.	MOBILITY BASIS-U WBD	CLASS/TYPE	REGION
1	NEEPAWA	100	37	(44.6)	37	(44.6)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	HRS-SMQ	W.CAN
2	MANITOU	98	34	(41.5)	34	(42.6)	3	(0.9)	0	(0.0)	0	(0.0)	3	(0.9)	HRS-SMQ	W.CAN
3	KATEPWA	97	35	(39.0)	34	(41.2)	4	(1.2)	1	(0.3)	0	(0.0)	3	(0.9)	HRS-SMQ	W.CAN
4	CANTHATCH	96	36	(39.9)	34	(39.8)	4	(1.9)	1	(0.3)	1	(0.9)	2	(0.6)	HRS-SMQ	W.CAN
5	THATCHER	96	35	(42.1)	34	(42.6)	4	(1.5)	1	(0.6)	0	(0.0)	3	(0.9)	HRS-SMQ	W.CAN
6	NAPAYO_M	93	40	(43.0)	35	(42.3)	7	(3.1)	5	(2.5)	0	(0.0)	2	(0.6)	HRS-SMQ	W.CAN
7	CHRIS	91	38	(43.7)	34	(42.1)	7	(4.0)	4	(2.5)	0	(0.0)	3	(1.5)	HRS-SMQ	USA
8	BENITO	91	36	(43.7)	34	(42.1)	5	(4.0)	2	(1.9)	0	(0.0)	3	(2.2)	HRS-SMQ	W.CAN
9	CANUCK_M	82	49	(52.0)	35	(43.5)	16	(9.6)	14	(9.0)	0	(0.0)	2	(0.6)	HRS-EMQ	SAWFLY
10	LEADER	80	39	(43.7)	32	(37.8)	11	(9.6)	6	(5.3)	1	(0.9)	4	(3.4)	HRS-SMQ	SAWFLY
11	ERA	75	39	(37.2)	30	(33.3)	13	(10.8)	6	(4.3)	3	(3.7)	4	(2.8)	HRS-NEMQ	USA
12	PARK	63	40	(41.8)	27	(31.1)	21	(18.6)	11	(9.6)	2	(2.2)	8	(6.8)	HRS-SMQ	W.CAN
13	SAUNDERS_M	62	37	(38.7)	27	(30.8)	18	(19.2)	8	(8.4)	2	(2.8)	8	(8.0)	HRS-EMQ	W.CAN
14	SINTON	61	32	(37.8)	24	(29.9)	19	(19.5)	6	(6.5)	2	(2.8)	11	(10.2)	HRS-SMQ	W.CAN
15	RELIANCE_PGR_M	59	35	(42.7)	25	(30.5)	19	(21.4)	7	(9.3)	3	(4.0)	9	(8.0)	HRS-NEMQ	W.CAN
16	CHINOOK	59	37	(39.3)	26	(30.3)	21	(21.4)	10	(10.2)	1	(1.5)	10	(9.6)	HRS-EMQ	SAWFLY
17	COTEAU	58	32	(38.4)	22	(29.3)	23	(21.4)	8	(6.8)	2	(2.8)	13	(11.8)	HRS-SMQ	USA
18	SUNDANCE	56	35	(39.3)	24	(29.1)	22	(22.6)	9	(10.5)	2	(2.8)	11	(9.3)	HRW-BW	W.CAN
	MEAN VALUE:	78	37	(41.6)	30	(36.8)	12	(10.6)	5	(4.9)	1	(1.4)	5	(4.4)		

	CULTIVAR	DATA BASE INDEX NO.	PEDIGREE DATA
1	NEEPAWA	56	THATCHER*7/FRONTANA//THATCHER*6/KENYA FARMER/3/THATCHER*2//FRONTANA/THATCHER, CANADA
2	MANITOU	52	THATCHER*7/FRONTANA//CANTHATCH/3/PI 170925/6*THATCHER, CANADA
3	KATEPWA	47	NEEPAWA*6/RL2938/3/NEEPAWA*6//C.I.8154/2*FROCOR, CANADA(RL2938 = LEE*2/KENYA FARMER).
4	CANTHATCH	45	THATCHER*6/KENYA FARMER, CANADA
5	THATCHER	62	MARQUIS/IUMILLIO/MARQUIS/KANRED, CANADA
6	NAPAYO_M	53	MANITOU*2/4/THATCHER*5/LEE/3/THATCHER*7/FRONTANA//THATCHER*6/KENYA FARMER, CANADA
7	CHRIS	86	FRONTANA/3*THATCHER/3/KENYA 58/NEWTHATCH/2*THATCHER, USA
8	BENITO	44	NEEPAWA/3/RL4255*4//MANITOU/CI7090, CANADA
9	CANUCK_M	63	CANTHATCH/3/MIDA/CADET//RESCUE, CANADA
10	LEADER	78	FORTUNA/CHRIS, CANADA
11	ERA	88	II-50-10/4/PEMBINA/II-52-329/3/II-53-38/III-58-4//II-53-546, USA
12	PARK	57	MIDA/CADET//THATCHER, CANADA
13	SAUNDERS_M	59	HOPE/REWARD//THATCHER, CANADA
14	SINTON	61	MANITOU/3/THATCHER*6/KENYA FARMER//LEE*6/KENYA FARMER, CANADA
15	RELIANCE_PGR_M	30	KANRED/MARQUIS, USA
16	CHINOOK	76	THATCHER/S-615-11, CANADA
17	COTEAU	87	ND496 SIB//ND487/FLETCHER, USA (ND496=WALDRON/ND269; ND487=ND259/CONLEY//CONLEY/ND122/3/JUSTIN/ND142)
18	SUNDANCE	134	CHEYENNE/KHARKOV 22 M.C., CANADA

WBD VALUES IN PARENTHESES GIVE THE PAIRED NUMBER COUNT WEIGHTED BY BAND DENSITY. THE WEIGHTING INCREMENT = (X/3.23)
WHERE 3.23 = POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND IN THE ELECTROPHOREGRAM.

in their pedigrees. Not surprisingly these cultivars all share common class attributes as hard red spring bread wheats of excellent milling and baking quality (refer to Table 8 for definitions of "CLASS/TYPE" quality codes).

Apart from providing cultivar names, %PH scores, class attributes and pedigrees, the cultivar identification printout includes an extensive tabulation of the distribution of matching and non-matching bands for compared electrophoregrams. This data shows that as cultivars become further removed in identity with the Neepawa gliadin PAGE pattern, band differences (weighted or not) accumulate at about twice the rate at which the number of matching bands fall. This result is explained by the fact that for any pairwise comparison of PAGE data, positional differences accrue from two sources, i.e. gliadin bands in one pattern which fail to match with its counterpart, and vice versa. Clearly then, differences as opposed to similarities, represent a much more sensitive scale for discrimination. When both parameters are combined in the form given by equation 9 (refer to page 181) a sensitive relative measure of electrophoretic pattern resemblance is obtained which fully quantifies the heterogeneity of gliadin band patterns for the purposes of comparative analysis.

As discussed previously, the "unweighted" version of this relationship (equation 8, page 180) is qualitative in the sense that for each matching or non-matching event between two compared electrophoregrams a unit value is incremented to appropriate terms of the formula. Implicit in this accounting is the assumption that band densities are evenly distributed among matching and non-matching bands.

There appears to be no apriori reason why this should be so, especially considering that very faint bands (density=1) represent a common but most uncertain attribute for electrophoregrams in the present or any other data base (Jones et al., 1982). Left uncorrected, faint band disagreements would have a disproportionate influence on cultivar rankings as measures of cultivar resemblance would most probably be underestimated.

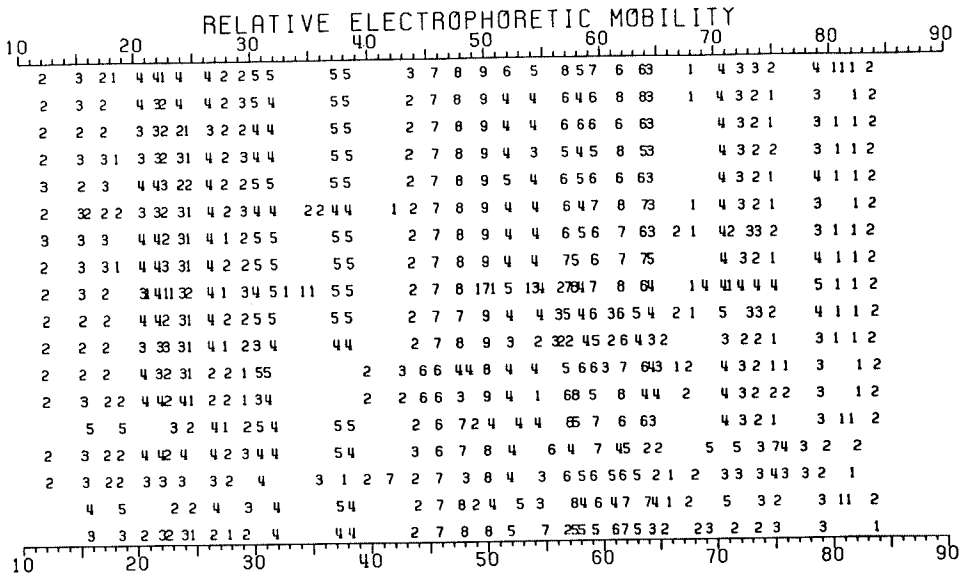
The number and weighting by band density (WBD) counts shown in Figure 37 (and other cultivar ranking printouts) provide strong evidence of an existing bias in the densities of non-matching bands as well as matching components in the electrophoregrams of ranked cultivars. This bias is manifested by states of higher than average densities for matching bands and/or lower than average values for non-matching components. The second of these is plainly shown in the plot of non-matching gliadin bands (Figure 38D) and suggests that some uncertainty exists in differentiating Neepawa from cultivars with pattern homology scores greater than 95%. Fortunately, the need to discriminate amongst these closely related genotypes has no present commercial relevance as they all possess similar functional quality characteristics.

The electrophoregram cultivar formula plots shown in Figures 38(A-D) were produced by program IDPLOT, and provide the necessary graphics to evaluate the computed cultivar identification end result tabulated in Figure 37. The performance level of this comparative analysis process is illustrated with striking detail in the plot of cultivar formulas for isolated matching bands (Figure 38B) which clearly

Figure 38. Cultivar identification pattern homology analysis plots for cv. Neepawa.

A

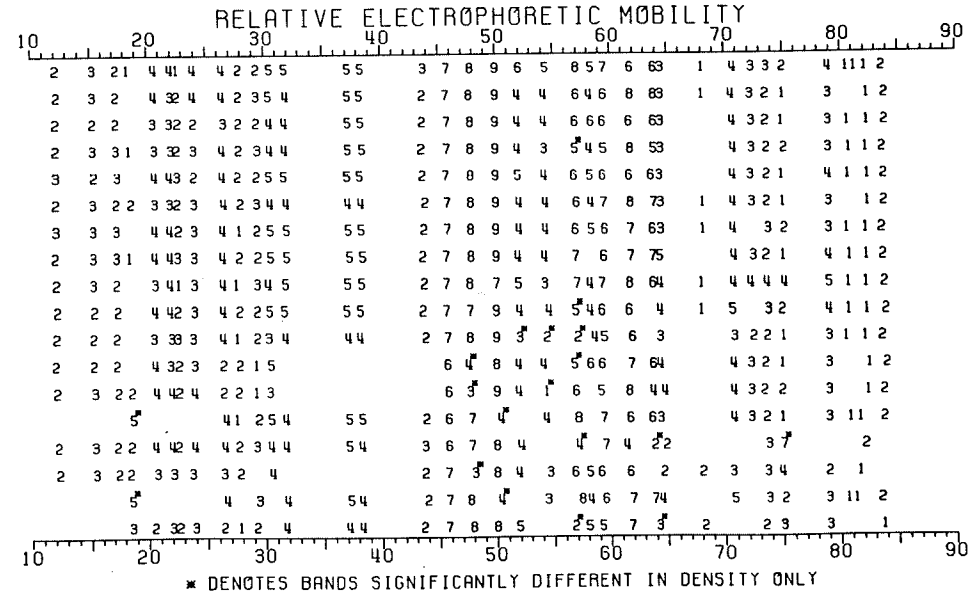
WHEAT CULTIVAR IDENTIFICATION - III. PATTERN HOMOLOGY ANALYSIS
COMPLETE FORMULAS FOR RANKED CULTIVARS



XPH	(DBIN)	CULTIVAR
100	(56)	NEEPAWA
98	(52)	MANITOU
97	(47)	KATEPWA
96	(45)	CANTHATCH
96	(62)	THATCHER
93	(53)	NAPAYO-M
91	(86)	CHRIS
91	(44)	BENITO
82	(63)	CANUCK-M
80	(78)	LEADER
75	(88)	ERA
63	(57)	PARK
62	(59)	SAUNDERS-M
61	(61)	SINTON
59	(30)	RELIANCE-PGR-M
59	(76)	CHINOOK
58	(87)	COTEAU
56	(134)	SUNDANCE

B

WHEAT CULTIVAR IDENTIFICATION - III. PATTERN HOMOLOGY ANALYSIS
MATCHING GLIADIN BANDS - LSD (MOBILITY) = 0.5; LSD (DENSITY) = 3

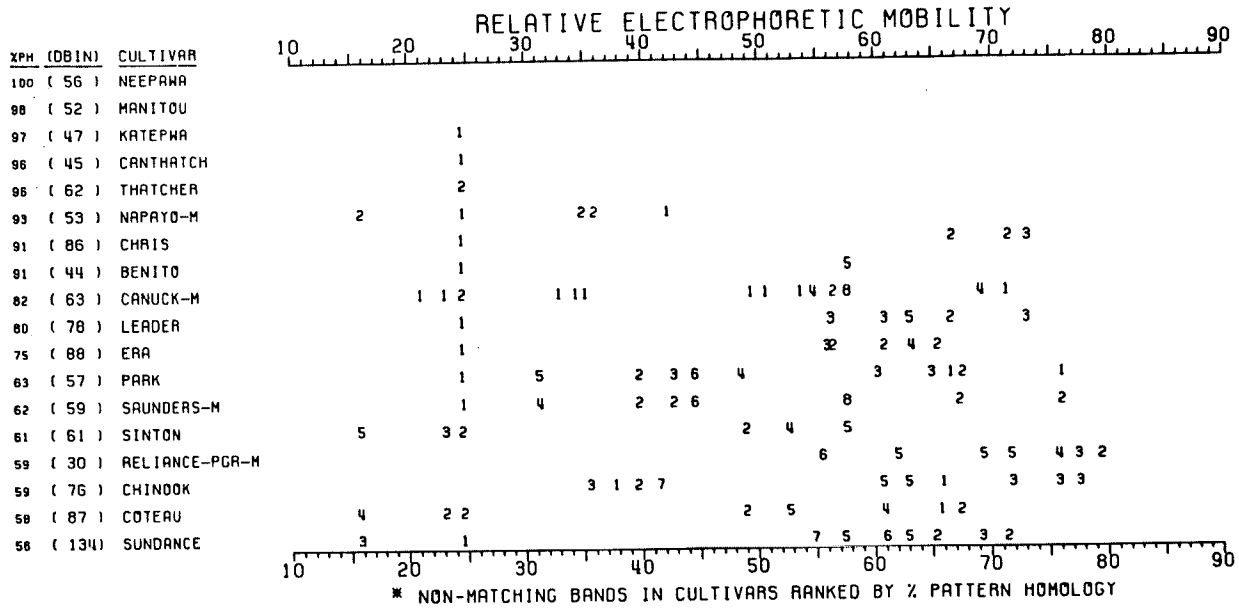


XPH	(DBIN)	CULTIVAR
100	(56)	NEEPAWA
98	(52)	MANITOU
97	(47)	KATEPWA
96	(45)	CANTHATCH
96	(62)	THATCHER
93	(53)	NAPAYO-M
91	(86)	CHRIS
91	(44)	BENITO
82	(63)	CANUCK-M
80	(78)	LEADER
75	(88)	ERA
63	(57)	PARK
62	(59)	SAUNDERS-M
61	(61)	SINTON
59	(30)	RELIANCE-PGR-M
59	(76)	CHINOOK
58	(87)	COTEAU
56	(134)	SUNDANCE

* DENOTES BANDS SIGNIFICANTLY DIFFERENT IN DENSITY ONLY

C

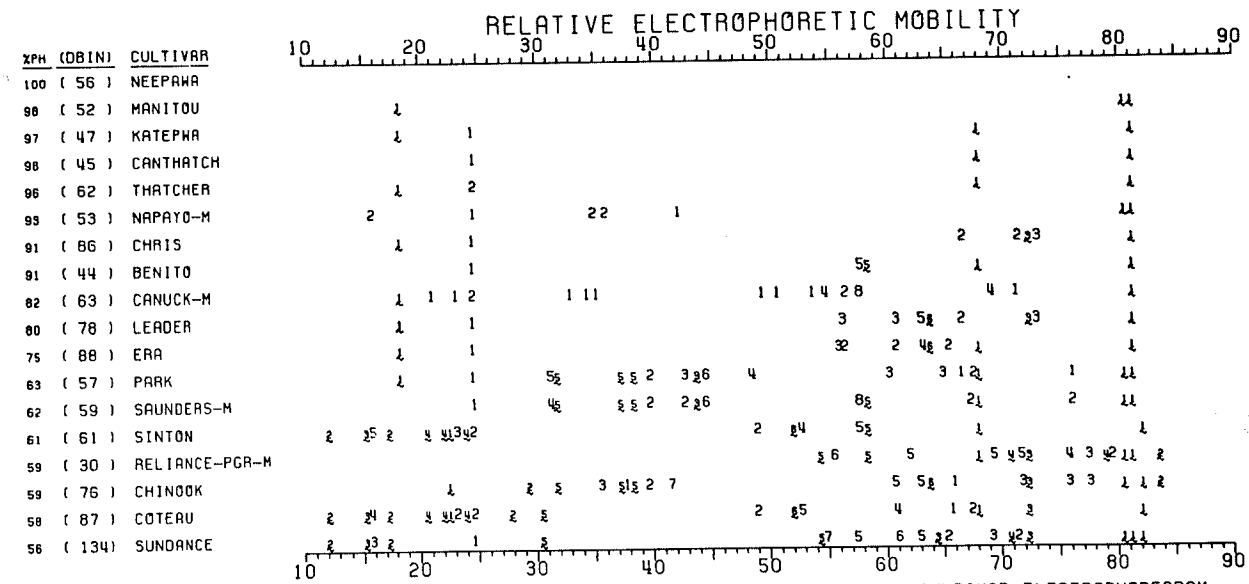
WHEAT CULTIVAR IDENTIFICATION - III. PATTERN HOMOLOGY ANALYSIS
NON-MATCHING GLIADIN BANDS* - LSD (MOBILITY) = 0.5; LSD (DENSITY) = 3



* NON-MATCHING BANDS IN CULTIVARS RANKED BY % PATTERN HOMOLOGY

D

WHEAT CULTIVAR IDENTIFICATION - III. PATTERN HOMOLOGY ANALYSIS
NON-MATCHING GLIADIN BANDS - LSD (MOBILITY) = 0.5; LSD (DENSITY) = 3



~ DENOTES NON-MATCHING BANDS IN UNKNOWN (OR TEST) CULTIVAR ELECTROPHOREGRAM
LARGER DIGITS INDICATE NON-MATCHING BANDS IN CVS. RANKED BY % HOMOLOGY

identifies common bands similar in density or otherwise, among cv. Neepawa and the list of ranked cultivars prepared by program CVID.

The third and final program element of the cultivar identification system developed in this study computes the frequency distribution of positional differences between the gliadin electrophoregram of an input cultivar and counterpart patterns in the data base. For each pairwise comparison, the independent positional difference variable includes both forward and reverse components of non-matching bands as well as bands significantly different on a density basis alone. The removal of these differences will accordingly yield electrophoregrams of identical composition. This strategy implemented by program CVMAP effectively complements analysis results shown previously by using a different criterion for ranking, as well as providing an output representation which is extended to include the entire cultivar population of common and durum wheats, in which each member is explicitly identified.

Typical results are illustrated in Figures 39A and 39B which show frequency distributions for weighted and unweighted positional differences respectively between the Neepawa electrophoregram and 122 reference cultivars designated by their data base identification numbers (DBIN's; refer to Tables 10 to 12 for a complete listing) and %PH scores in parentheses.

Each difference distribution termed a "cultivar distance map" (CVMAP) shows a wide gap or genotypic distance separating cv. Neepawa (DBIN=56) from the bulk of the data base population. Cultivars which are relatively distinct in electrophoregram composition by their similarity with the Neepawa pattern are dispersed in the upper part of

Figure 39. Cultivar identification distance maps for cv. Neepawa.

A. Positional differences weighted by band density.
B. Positional differences not weighted by band density.

A

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - II. DISTRIBUTION BY DIFFERENCES

* INPUT TEST CULTIVAR = NEEPAWA
* 122 DATA BASE CULTIVAR PATTERNS ANALYZED ; LSD(RELATIVE MOBILITY) = 0.5, LSD(BAND DENSITY) = 3.

WEIGHTED POSITIONAL DIFFERENCES

```
 0   56(**)
 1   47(97) 52(98)
 2   45(96) 62(96)
 3   53(93)
 4   44(91) 86(91)
 5
 6
 7
 8
 9
10   63(82) 78(80)
11   88(75)
12
13
14
15
16
17
18
19   57(63) 59(62)
20   61(61)
21   30(59) 76(59) 87(58)
22
23  134(56)
24   97(52)156(51)
25   69(51)150(43)
26   11(52)133(51)
27   46(52) 79(44) 91(50)
28    4(49) 40(46) 58(43) 66(45)124(52)137(51)
29   12(46) 15(47)112(47)
30   27(46) 95(43)110(44)
31   97(38)101(47)141(43)158(44)
32    8(36) 85(45)118(37)139(41)
33   99(37)122(37)163(39)
34    3(37) 16(41) 17(39)109(42)151(40)170(43)
35  142(37)147(35)152(27)153(34)164(36)
36   32(35)113(32)129(40)148(29)
37   84(35)128(34)138(29)
38   22(34) 28(36) 77(33) 92(33)154(35)157(28)
39
40  167(36)
41    1(28)102(29)103(24)115(26)132(29)
42    9(26) 14(34) 96(30)105(22)107(24)111(23)130(28)165(21)166(26)
43  104(28)131(24)143(27)171(18)172(16)
44  178(28)
45  135(23)162(28)
46   29(24) 38(21)108(20)136(27)180(25)186(24)
47   89(19)
48   90(23)
49   24(26)127(20)149(20)155(21)184(12)
50  179(20)187(20)
51
52
53   39(18)181(19)
54    5(19)174(20)185(19)189(21)
55   23(11)177(12)
56  183(15)
57
58  182(16)
```

WEIGHTED POSITIONAL DIFFERENCE GIVES THE TOTAL NUMBER OF BAND DIFFERENCES WEIGHTED BY DENSITY BETWEEN THE INPUT PATTERN AND DATA BASE CULTIVAR ELECTROPHOREGRAMS. THE WEIGHTING INCREMENT = 1-(X/3.23) WHERE 3.23 = CULTIVAR POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND. TABULATED DATA IS LISTED IN PAIRS, AND REPRESENTS RESPECTIVELY FOR EACH CULTIVAR ANALYZED, ITS DATA BASE INDEX NUMBER AND % PATTERN HOMOLOGY IN PARENTHESES.

B

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - II. DISTRIBUTION BY DIFFERENCES

* INPUT TEST CULTIVAR = NEEPAWA
* 122 DATA BASE CULTIVAR PATTERNS ANALYZED ; LSD(RELATIVE MOBILITY) = 0.5, LSD(BAND DENSITY) = 3.

POSITIONAL DIFFERENCES

```
 0   56(**)
 1
 2
 3   52(92)
 4   45(89) 47(89) 62(89)
 5   44(87)
 6
 7   53(83) 86(83)
 8
 9
10
11   78(74)
12
13   88(70)
14
15
16   63(69)
17
18   59(60)
19   30(57) 61(56)
20
21   57(55) 76(55)
22  134(52)
23   87(49)
24   91(50)
25   46(52)
26   37(49)156(42)
27   58(45)
28   11(47) 40(43) 69(46)133(44)
29   79(38)150(37)
30    4(42)124(44)137(43)141(40)
31   85(39)112(40)139(39)
32   12(41) 15(41) 66(40) 99(36)101(40)151(40)158(38)170(40)
33   95(37)110(40)118(39)
34   16(38) 27(38) 28(36)122(38)
35   22(34) 77(35)109(39)128(36)129(36)142(35)147(34)
36   97(32)154(33)163(35)
37    3(31) 17(36) 32(33) 92(30)148(29)164(31)178(23)
38    8(30) 14(33)132(28)138(27)152(27)
39    1(28) 84(30)115(26)153(30)157(28)
40  105(23)131(25)
41    9(25)113(27)167(34)
42  102(25)103(25)104(26)136(25)162(28)186(21)187(19)
43   38(20)107(23)180(20)
44   29(21)130(25)143(27)149(19)189(21)
45   96(22)111(22)
46   89(19)135(21)155(18)165(19)166(22)172(16)184(10)
47   90(22)179(19)
48   24(23) 39(17)108(19)
49   23(13)185(20)
50    5(17)127(17)171(17)181(15)
51  177(11)
52  182(16)
53
54  174(17)183(14)
```

POSITIONAL DIFFERENCE GIVES THE TOTAL NUMBER OF BAND DIFFERENCES BETWEEN INPUT AND DATA BASE CULTIVAR ELECTROPHOREGRAMS. TABULATED DATA IS LISTED IN PAIRS, AND REPRESENTS RESPECTIVELY FOR EACH CULTIVAR ANALYZED, ITS DATA BASE INDEX NUMBER AND % PATTERN HOMOLOGY IN PARENTHESES.

the CVMAP distribution. Not unexpectedly, this group is exclusively comprised of cultivars (framed) listed in the CVID program ranking result given in Figure 37.

The high level of pattern homology which exists between Neepawa and seven of these cultivars is shown to be more clearly reflected in the weighted CVMAP result (Figure 39A) where they are better isolated and are clustered within a tighter band difference range than in the counterpart distribution shown in Figure 39B. Moreover, the weighted analysis makes a further distinction among the 10 remaining cultivars in the ranked sub-population by isolating cvs. Canuck, Leader and Era (DBIN's 63, 78 and 88, respectively). Both Canuck and Leader are sawfly resistant Canadian hard red spring bread wheats of good quality. Cultivar Era on the other hand, is a U.S. licensed hard red spring semi-dwarf wheat of poor breadmaking quality and low protein content (R. Zillman, personal communication). These three cultivars while of the same class of wheat, are logically different genotypes from those cultivars immediately above and below them in the CVMAP result and the ranked list provided in Figure 37; and it is noteworthy that their gliadin electrophoregrams reflect these differences.

In contrast to the general pedigree and/or class similarities between cultivars with high pattern homologies scores (low positional differences) i.e. close to Neepawa in the CVMAP distribution, cultivars at the far end of the distribution (e.g. Figure 39A) are of diverse genotypes. These are in the main durum (DBIN>172) or common wheat cultivars whose electrophoregrams are different in the extreme from that of cv. Neepawa. For example, at the weighted positional difference

level of 55, the durum wheat cv. Coulter (DBIN=177) shares only a 12% pattern homology with cv. Neepawa. Direct comparison of their electrophoregrams (Figure 33) shows that the correspondence between these two cultivars with different species affiliation is extremely low. Likewise cv. Lake (DBIN=23) has a very low level of pattern homology (11%) with the Neepawa electrophoregram. Cultivar Lake, unlike cv. Coulter, is a hard red spring bread wheat of good milling and baking quality. This result indicates that gliadin composition within hard red spring wheats of similar functional quality can be as heterogeneous as that between common and durum wheats; and by implication suggests that much inherent variability remains to be exploited among Canadian bread wheat cultivars.

An explicit comparative analysis of gliadin PAGE patterns for cvs. Neepawa and Lake is shown in Figure 40. The corresponding pattern homology plot (Figure 41) indicates only seven common gliadin components which match within specified program thresholds for relative mobility and band density. Visual inspection of electrophoregrams for cvs. Neepawa and Lake, which are adjacent in Figure 21, confirms the marked dissimilarity in their gliadin compositions.

This form of comparative electrophoregram analysis at the two cultivar level is identical to the process implemented by programs of the cultivar identification system which compare an unknown electrophoregram with each reference entry in the data base. By using the relevant data provided in Figure 40, the measure of pattern homology between cvs. Neepawa and Lake, indicated as 13%, can be confirmed in accordance with equation 8 (page 180) as:

Figure 40. Pattern homology analysis printout for cultivars Neepawa and Lake.

GLIADIN ELECTROPHORETIC PATTERN HOMOLOGY ANALYSIS
CVS. ANALYZED: LAKE NEEPAWA

* LSD (RELATIVE MOBILITY) = 5; LSD (BAND DENSITY) = 3
* NOTE: RELATIVE MOBILITY DATA IS IN INTEGER FORMAT

SIGNATURE ARRAY FOR CULTIVAR LAKE BANDS=33

158(6)	186(5)	243(2)	276(1)	298(5)	304(3)	371(3)	390(2)	412(1)	429(2)
448(5)	462(6)	478(2)	504(4)	510(2)	527(4)	553(4)	576(7)	593(5)	618(9)
637(2)	662(1)	674(1)	691(4)	707(1)	728(9)	749(5)	760(4)	769(2)	795(1)
813(1)	839(1)	860(2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
0(0)	33(3)	2(2)	3(2)	23(0)					

SIGNATURE ARRAY FOR CULTIVAR NEEPAWA BANDS=37

121(2)	152(3)	173(2)	183(1)	206(4)	220(4)	225(1)	239(4)	264(4)	278(2)
293(2)	305(5)	318(5)	372(5)	383(5)	437(3)	457(7)	478(8)	500(9)	520(6)
543(5)	570(8)	583(5)	594(7)	618(6)	638(6)	644(3)	680(1)	708(4)	723(3)
736(3)	750(2)	789(4)	805(1)	812(1)	821(1)	836(2)	0(0)	0(0)	0(0)
0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
0(0)	37(*)	3(2)	4(2)	56(0)					

DISTRIBUTION OF ELECTROPHOREGRAM HOMOLOGY DATA

CULTIVARS:	LAKE		NEEPAWA	
NO. OF BANDS IN PATTERN	33 (34.7)		37 (44.6)	
TOTAL NO. OF UNIQUE BANDS		56 (61.6)		
TOTAL NO. OF MATCHING BANDS		7 (6.5)		
TOTAL NO. OF NON-MATCHING BANDS		49 (55.1)		
* RELATIVE MOBILITY BASIS	19 (21.1)		23 (25.4)	
** DENSITY BASIS		7 (8.7)		

TOTAL PATTERN HOMOLOGY *** 13% (10.6%) ***

NON-MATCHING BANDS OF CV. LAKE

158(6)	186(5)*	298(5)	390(2)	412(1)	429(2)	448(5)	462(6)	478(2)*	504(4)*
510(2)	527(4)	553(4)	576(7)	618(9)*	637(2)*	662(1)	674(1)	691(4)	707(1)*
728(9)	749(5)*	760(4)	769(2)	795(1)	860(2)				

NON-MATCHING BANDS OF CV. NEEPAWA

121(2)	152(3)	173(2)	183(1)*	206(4)	220(4)	225(1)	264(4)	293(2)	318(5)
383(5)	437(3)	457(7)	478(8)*	500(9)*	520(6)	543(5)	570(8)	583(5)	618(6)*
638(6)*	644(3)	680(1)	708(4)*	723(3)	736(3)	750(2)*	789(4)	805(1)	821(1)

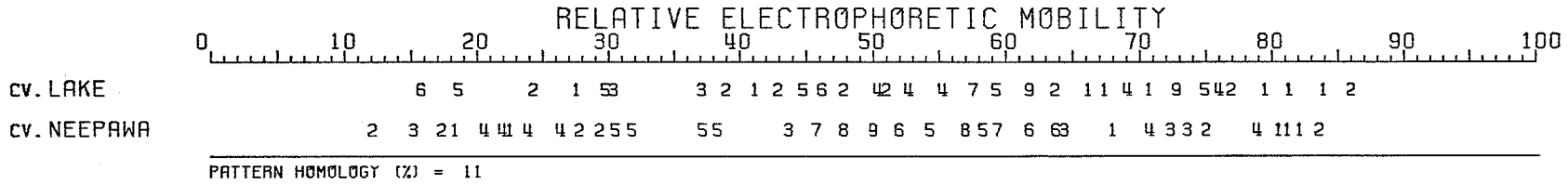
MATCHING ELECTROPHOREGRAM COMPONENTS BY MOBILITY

CV.	LAKE	NEEPAWA
1.	243(2)	239(4)
2.	276(1)	278(2)
3.	304(3)	305(5)
4.	371(3)	372(5)
5.	593(5)	594(7)
6.	813(1)	812(1)
7.	839(1)	836(2)

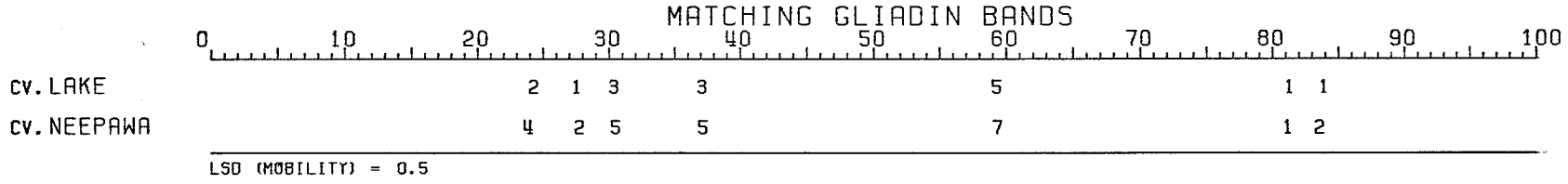
PATTERN HOMOLOGY DISTRIBUTION DATA IN PARENTHESES GIVES THE NUMBER TALLY WEIGHTED BY BAND DENSITY. THE WEIGHTING INCREMENT = (X/3.23) WHERE 3.23 = CULTIVAR POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND IN THE ELECTROPHOREGRAM.

Figure 41. Cultivar formula plot of pattern homology analysis for cultivars Neepawa and Lake.

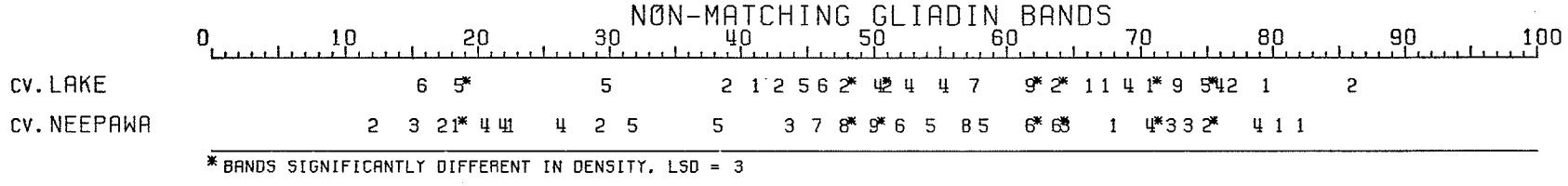
GLIADIN ELECTRØPHØREGRAM HØMØLØGY ANALYSIS



PATTERN HØMØLØGY (%) = 11



LSD (MØBILITY) = 0.5



* BANDS SIGNIFICANTLY DIFFERENT IN DENSITY, LSD = 3

$$\text{Unweighted } \%PH = \frac{7 \times 100}{7 + (23+19+7)} = 12.5\%$$

The denominator in the above relationship is of particular interest as the sum of its terms [56 = (matching bands + non-matching bands)] represents the total number of different or unique gliadin components shared between these two cultivars. This result compared to previously reported estimates of gliadin heterogeneity by starch gel electrophoresis, e.g. 43 bands in 73 French spring and winter wheats (Autran and Bourdet, 1975) or 34 bands in 78 Australian wheat cultivars (du Cros et al., 1980), clearly demonstrates the superiority of the polyacrylamide gel system in resolving gliadins, and underscores as well the effectiveness of the presented computerized technique to detect similarities and differences for compared cultivars within this complex protein fraction.

These results also suggest that the methodology would be especially relevant to studies involved with the inheritance of gliadin protein composition where typically large numbers of lines are evaluated in terms of discrete electrophoretic pattern similarities, differences and recombinants. (e.g. Doekes, 1973; Mecham et al, 1978; Baker and Bushuk, 1978; Sozinov and Poperelya, 1980; Branlard, 1983). The utility of protein electrophoresis to areas of wheat genetics and quality has been discussed by Bietz (1976). The speed and detail offered by a computerized methodology is observed to be ideally suited for these applications.

Cultivar Identification Printouts for Cvs. Sinton, Opal, Springfield, Sundance, Yorkstar and Wascana

To further evaluate the performance of the cultivar identification system, gliadin electrophoregram signature arrays for the following cultivars were selected as test input data:

1. Sinton - hard red spring bread wheat
2. Opal - hard red spring feed wheat
3. Springfield - soft white spring wheat
4. Sundance - hard red winter bread wheat
5. Yorkstar - soft white winter wheat
6. Wascana - durum wheat

These cultivars possess a wide variety of gliadin distribution patterns for wheats of various classes as indicated. Their respective short list rankings and cultivar distance maps are shown in Figures 42 to 47. With the exception of the analysis result for cv. Yorkstar (Figure 46) and Neepawa (Figure 37) discussed earlier, no more than two cultivars (2% of the data base population) in any given list possess pattern homology scores greater than 80% with the test electrophoregram. This level of discrimination was typical of cultivar identification program runs in general for which the average number of isolated cultivars in both 90% (i.e. 90-100%) and 80% (80-89%) pattern homology classes was approximately one (of 121 cultivars) in each case.

These numbers reflect the facility with which differences can be distinguished between cultivars by gliadin electrophoregrams. However, unequivocal differentiation is not possible in every instance mainly due to close genetic relationships. A list of nine cultivar groupings in the data base which are affected in this way is given in Table 17. This list can be sub-divided into 16 pairs of cultivars with similar gliadin PAGE patterns; a total which is relatively insignificant when compared

TABLE 17

Cultivar groups in the data base for which discrimination by gliadin electrophoregrams is uncertain

	Cultivars	Class	%PH[1]
1.	Apex-Marquis	HRS	99
2.	Regent-Renown	HRS	95
3.	Manitou-Neepawa	HRS	98
	Canthatch-Katepwa	HRS	99
4.	Milton-Opal-Vernon	HRS	>95
5.	Lemhi 53-Lemhi 62	SWS	96
6.	Lennox-Valor[2]	HRW	94
7.	Yorkstar-Favor[2]-Genessee-Gordon	SWW	>92
8.	Gaines-Nugaines	SWW	100
9.	Mindum-Nugget	DURUM	98

[1]Computed percent pattern homology score.
[2]Electrophoregram not consistent with pedigree for indicated cultivar.

with more than 7,380 possible pairwise combinations among 122 cultivar electrophoregrams in the data base which can be differentiated.

Of greater importance are values for percent pattern homology which were computed among cultivar groups in Table 17. This data indicates that the comparative analysis of gliadin electrophoregrams characterized by pattern homology scores greater than about 94% must be interpreted with caution, as implied band differences may not be significant. In practice this situation will occur only infrequently. As for the few cultivars which may be ranked at these very high levels of pattern homology, direct visual inspection of their electrophoregrams is recommended to bring the analysis to a satisfactory conclusion.

Two instances are noted in Table 17 where similarities in gliadin composition were inconsistent with published pedigree data. The hard red winter wheat cvs. Lennox and Valor[1] for example, (patterns 74 and 79 respectively in Figure 29) cannot be differentiated in spite of their very different pedigrees (refer to Table 5). The anomaly was traced to the Valor electrophoregram, as a gliadin pattern for cv. Lennox similar to its counterpart in Figure 29 has been reported elsewhere (Tkachuk and Mellish, 1980). While the reference sample for cv. Valor obtained for the present study is logically suspect, a comparison of its varietal description report with that of cv. Lennox reveals similar plant, spike, kernel, and agronomic characteristics for feed wheats that are both specifically adapted to the Atlantic Maritime region[2]. The present ambiguity argues in favor of documenting gliadin electrophoregrams as part of the process of varietal licensing so that questions regarding authenticity of seed samples can be avoided.

Compared to the often small number of differences in gliadin composition between cultivars of the same class of wheat, cultivars with different class affiliations invariably possessed relatively low levels of pattern homology. Accordingly, the computed alignment of cultivars relative to test electrophoregrams for wheats of different utilization type (Figures 42 to 47) was found to cluster in general, mutually exclusive sets of wheat genotypes.

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[1]Cultivars Lennox and Valor were licensed in 1975 and 1981 respectively.
[2]Source: Description of Variety reports, Agriculture Canada, Production and Marketing Branch, Plant Products Division, Ottawa, Ontario.

Most striking is the result for the durum wheat cultivar Wascana (Figure 47B). The main body of the data base population (all common wheats) lies from 10 to 20 difference units further removed (at 49 difference units approximately) from the input cultivar, when compared with any other distance map result involving a common wheat as the reference test cultivar (Figures 39, 42B-46B). The distinction of durum wheats by gliadin electrophoregrams, which features a general absence of bands with relative mobilities less than 20, is consistent with their genetic composition as all lack the D genome.

A further interesting distinction of cultivars by class and hence by quality, can be observed in the result for cv. Yorkstar (Figure 46). Of the 22 soft white or red winter wheats in the data base a total of 17 cultivars (77%) have been isolated in the ranking result. This proportion increases to 100% if those cultivars not adapted to the Ontario region are excluded[1]. The ranking is made more significant by the fact that pedigree does not appear to be the common factor. The cluster of seven cultivars with high pattern homology scores (>85%) and few positional differences (<7) with the Yorkstar electrophoregram (Figure 46B) are relatively dissimilar in pedigree, in contrast to the identification result discussed earlier for the Neepawa electrophoregram (Figure 37). The ancestry of the 17 wheat cultivars in the Yorkstar ranking includes contributions from more than 36 different parents from

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[1]The soft white winter wheat cvs. Gaines, Nugaines and Sun are adapted to the Pacific Northwest; cv. Jones Fife which is an obscure soft to semi-hard white winter wheat which was grown to a limited extent in Alberta; cv. Rideau has kernel characteristics similar to Jones Fife and possesses only fair quality for pastry flour, presumably as a result of inheriting relatively strong gluten characteristics from one of its parents, cv. Kharkov 22 M.C., a HRW bread wheat.

Figure 42. Cultivar identification ranking (A) and distance map (B) results for cv. Sinton. Framed cultivars in (B) correspond to cultivars ranked in (A).

A

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - I. RANKING BY PATTERN HOMOLOGY

- 122 DATA BASE CULTIVAR PATTERNS ANALYZED
- DATA BASE SEARCH CUTOFF AT 55% PATTERN HOMOLOGY (WEIGHTED BY BAND DENSITY).
- LSD(RELATIVE MOBILITY) = 0.5, MOBILITY RANGE: 10.0 - 90.0. LSD(BAND DENSITY) = 3, DENSITY RANGE: 1 - 9.
- UNKNOWN (OR TEST) CULTIVAR ELECTROPHOREGRAM CONTAINS 32 GLIADIN BANDS; TOTAL, WEIGHTED BY BAND DENSITY (WBD) = 37.8

	CULTIVAR	WEIGHTED % PATTERN HOMOLOGY	GLIADIN BANDS IN PATTERN NO.	GLIADIN BANDS IN PATTERN WBD	MATCHING BANDS NO.	MATCHING BANDS WBD	DISTRIBUTION OF NON-MATCHING BAND DATA: TOTAL NO.	TOTAL WBD	MOBILITY BASIS-R NO.	MOBILITY BASIS-R WBD	DENSITY BASIS NO.	DENSITY BASIS WBD	MOBILITY BASIS-U NO.	MOBILITY BASIS-U WBD	CLASS/TYPE	REGION
1	SINTON	100	32	(37.8)	32	(37.8)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	HRS-SMQ	W.CAN
2	COTEAU	82	32	(38.4)	28	(34.2)	8	(7.7)	4	(3.4)	0	(0.0)	4	(4.3)	HRS-SMQ	USA
3	BENITO	66	36	(43.7)	25	(31.3)	16	(15.8)	9	(8.4)	2	(2.8)	5	(4.6)	HRS-SMQ	W.CAN
4	RESCUE_PGR_M	64	33	(34.4)	23	(26.0)	16	(14.6)	7	(5.0)	3	(3.1)	6	(6.5)	HRS-EMQ	SAWFLY
5	WALDRON	63	37	(39.3)	25	(28.9)	17	(17.3)	10	(10.6)	2	(2.2)	5	(4.6)	HRS-SMQ	USA
6	KATEPWA	62	35	(39.0)	24	(28.3)	18	(17.6)	10	(8.4)	1	(1.6)	7	(7.7)	HRS-SMQ	W.CAN
7	NEEPAWA	61	37	(44.6)	24	(29.9)	19	(19.6)	11	(10.2)	2	(2.8)	6	(6.5)	HRS-SMQ	W.CAN
8	NAPAYO_M	61	40	(43.0)	23	(28.6)	23	(18.6)	14	(10.2)	3	(3.4)	6	(5.0)	HRS-SMQ	W.CAN
9	THATCHER	60	35	(42.1)	24	(29.1)	18	(19.2)	10	(9.9)	1	(1.6)	7	(7.7)	HRS-SMQ	W.CAN
10	MANITOU	59	34	(41.6)	22	(28.6)	21	(19.5)	11	(9.3)	1	(1.5)	9	(8.7)	HRS-SMQ	W.CAN
11	CANUCK_M	59	49	(52.0)	25	(31.0)	28	(21.7)	21	(14.2)	3	(2.8)	4	(4.6)	HRS-EMQ	SAWFLY
12	CANTHATCH	59	36	(39.9)	23	(26.5)	19	(18.6)	10	(8.7)	3	(3.7)	6	(6.2)	HRS-SMQ	W.CAN
13	BUTTE	59	35	(40.6)	24	(29.1)	19	(20.1)	11	(12.4)	0	(0.0)	8	(7.7)	HRS-SMQ	USA
14	KENHI	56	35	(33.1)	21	(24.9)	23	(19.2)	12	(8.0)	2	(1.9)	9	(9.3)	SWS-PASTRY	S.ALTA
15	NUGAINES	55	29	(32.8)	20	(24.1)	20	(19.8)	8	(7.4)	1	(1.2)	11	(11.1)	SWW-PASTRY	BC
16	ALEX	55	39	(40.2)	23	(27.4)	24	(22.0)	15	(13.6)	1	(0.9)	8	(7.4)	HRS-SMQ	USA
17	CHRIS	55	38	(43.7)	23	(28.2)	23	(22.6)	14	(12.4)	1	(1.6)	8	(8.7)	HRS-SMQ	USA
	MEAN VALUE:	63	36	(40.4)	24	(29.1)	18	(17.3)	10	(8.9)	1	(1.8)	6	(6.5)		

	CULTIVAR	DATA BASE INDEX NO.	PEDIGREE DATA
1	SINTON	61	MANITOU/3/THATCHER*6/KENYA FARMER//LEE*6/KENYA FARMER, CANADA
2	COTEAU	87	ND496 SIB//ND487/FLETCHER, USA (ND496=WALDRON/ND269; ND487=ND259/CONLEY//CONLEY/ND122/3/JUSTIN/ND142)
3	BENITO	44	NEEPAWA/3/RL4255*4//MANITOU/C17090, CANADA
4	RESCUE_PGR_M	79	APEX/S-615, CANADA
5	WALDRON	92	JUSTIN/4/LEE/3/KENYA 338//LEE/MIDA(ND81), USA
6	KATEPWA	47	NEEPAWA*6/RL2938/3/NEEPAWA*6,/C.I.8154/2*FROCOR, CANADA(RL2938 = LEE*2/KENYA FARMER).
7	NEEPAWA	56	THATCHER*7/FRONTANA//THATCHER*6/KENYA FARMER/3/THATCHER*2//FRONTANA/THATCHER, CANADA
8	NAPAYO_M	53	MANITOU*2/4/THATCHER*5/LEE/3/THATCHER*7/FRONTANA//THATCHER*6/KENYA FARMER, CANADA
9	THATCHER	62	MARQUIS/10MILLIO/MARQUIS/KANRED, CANADA
10	MANITOU	52	MARQUIS/10MILLIO//MARQUIS/KANRED, CANADA
11	CANUCK_M	63	THATCHER*7/FRONTANA//CANTHATCH/3/PI 170925/6*THATCHER, CANADA
12	CANTHATCH	45	CANTHATCH/3/MIDA/CADET//RESCUE, CANADA
13	BUTTE	85	THATCHER*6/KENYA FARMER, CANADA
14	KENHI	116	ND480/POLK//WISCONSIN 261, USA
15	NUGAINES	156	KENYA 338 AC2E3/2*LEMHI, CANADA
16	ALEX	84	SIB. OF GAINES, USA
17	CHRIS	86	ND507/ND496, USA (ND507 = WALDRON/RL4205)
			FRONTANA/3*THATCHER/3/KENYA 58:NEWTHATCH/2*THATCHER, USA

WBD VALUES IN PARENTHESES GIVE THE PAIRED NUMBER COUNT WEIGHTED BY BAND DENSITY. THE WEIGHTING INCREMENT = (X/3.23) WHERE 3.23 = POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND IN THE ELECTROPHOREGRAM.

B

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - II. DISTRIBUTION BY DIFFERENCES

- INPUT TEST CULTIVAR = SINTON
- 122 DATA BASE CULTIVAR PATTERNS ANALYZED ; LSD(RELATIVE MOBILITY) = 0.5, LSD(BAND DENSITY) = 3.

WEIGHTED POSITIONAL DIFFERENCES

```
  0   61(**)
  1
  2
  3
  4
  5
  6
  7
  8   87(82)
  9
 10
 11
 12
 13
 14
 15   79(64)
 16   44(66)
 17   92(63)
 18   47(62)
 19   45(59) 53(61) 62(60)115(56)150(51)
 20   52(59) 56(61) 85(59)156(55)
 21   40(51) 88(54)
 22   63(59) 84(55)
 23   86(55)
 24   89(49)113(48)
 25  141(49)
 26   15(47) 58(41) 90(48)
 27   24(50) 28(44) 69(48) 78(48)
 28   12(40) 27(45) 97(37)110(42)134(45)
 29   69(40)107(36)
 30   22(41)105(36)124(44)147(39)152(28)154(43)157(37)158(40)
 31   77(39)104(40)163(39)170(45)
 32   14(42) 96(43)162(39)164(35)166(35)
 33   23(32) 57(37)111(32)112(37)127(35)128(34)143(37)148(30)151(39)153(32)
 34   76(36) 91(37)103(31)109(39)118(33)142(35)149(36)165(27)
 35   29(33) 66(34)108(29)
 36   16(35)129(35)131(27)
 37   17(31) 32(27) 37(29) 38(23) 99(30)138(26)155(34)167(36)172(20)
 38    9(29) 30(31) 46(33)122(28)
 39    4(28)
 40    8(19) 11(29) 95(23)
 41    3(22)  5(27)130(26)135(22)174(28)187(24)
 42   39(26)137(28)171(18)180(24)186(23)189(26)
 43    1(22)136(25)177(20)
 44  133(22)
 45  101(25)102(21)139(23)
 46  179(20)184(12)
 47  178(18)182(20)183(18)185(20)
 48  132(19)
 49
 50  181(19)
```

WEIGHTED POSITIONAL DIFFERENCE GIVES THE TOTAL NUMBER OF BAND DIFFERENCES WEIGHTED BY DENSITY BETWEEN THE INPUT PATTERN AND DATA BASE CULTIVAR ELECTROPHOREGRAMS. THE WEIGHTING INCREMENT = (X/3.23) WHERE 3.23 = CULTIVAR POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND. TABULATED DATA IS LISTED IN PAIRS, AND REPRESENTS RESPECTIVELY FOR EACH CULTIVAR ANALYZED, ITS DATA BASE INDEX NUMBER AND % PATTERN HOMOLOGY IN PARENTHESES.

Figure 43. Cultivar identification ranking (A) and distance map (B) results for cv. Opal. Framed cultivars in (B) correspond to cultivars ranked in (A).

A

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - I. RANKING BY PATTERN HOMOLOGY

* 122 DATA BASE CULTIVAR PATTERNS ANALYZED
* DATA BASE SEARCH CUTOFF AT 65% PATTERN HOMOLOGY (WEIGHTED BY BAND DENSITY).
* DATA BASE MOBILITY RANGE: 10.0 - 90.0. LSD(BAND DENSITY) = 3. DENSITY RANGE: 1 - 9.
* LSD(RELATIVE MOBILITY) = 0.6. MOBILITY RANGE: 10.0 - 90.0. LSD(BAND DENSITY) = 3. DENSITY RANGE: 1 - 9.
* UNKNOWN (OR TEST) CULTIVAR ELECTROPHOREGRAM CONTAINS 38 GLIADIN BANDS; TOTAL, WEIGHTED BY BAND DENSITY (WBD) = 33.7

	CULTIVAR	WEIGHTED % PATTERN HOMOLOGY	GLIADIN BANDS IN PATTERN NO.	GLIADIN BANDS IN PATTERN WBD	MATCHING BANDS NO.	MATCHING BANDS WBD	DISTRIBUTION OF NON-MATCHING BAND DATA: TOTAL NO.	TOTAL WBD	MOBILITY BASIS-R NO.	MOBILITY BASIS-R WBD	DENSITY BASIS NO.	DENSITY BASIS WBD	MOBILITY BASIS-U NO.	MOBILITY BASIS-U WBD	CLASS/TYPE	REGION
1	OPAL	100	38	(33.7)	38	(33.7)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	HRS-FEED	R/ W.CAN
2	VERNON	95	42	(34.4)	38	(33.4)	4	(1.2)	4	(1.2)	0	(0.0)	0	(0.0)	SRS-FEED	R/ W.CAN
3	MILTON	95	39	(38.7)	37	(35.3)	3	(1.9)	2	(1.5)	0	(0.0)	1	(0.3)	HRS-FEED	R/ W.CAN
4	FIELDER	70	40	(34.4)	27	(28.0)	24	(12.1)	13	(6.6)	0	(0.0)	11	(5.6)	SWS-PASTRY	S.ALTA
5	VUKA	69	36	(39.0)	28	(29.7)	18	(13.3)	8	(8.4)	0	(0.0)	10	(5.0)	HRW-FEED	ATLANTIC
6	LEMHI 53_M	64	44	(37.2)	30	(27.6)	22	(15.8)	14	(10.2)	0	(0.0)	8	(5.6)	SWS-PASTRY	S.ALTA
7	KENHI	64	35	(33.1)	26	(24.9)	20	(13.9)	8	(5.0)	1	(1.2)	11	(7.7)	SWS-PASTRY	S.ALTA
8	SPRINGFIELD	64	42	(35.0)	28	(25.4)	22	(14.2)	12	(7.7)	2	(1.9)	8	(4.6)	SWS-PASTRY	S.ALTA
9	LEMHI 62_M	63	45	(37.8)	30	(27.6)	23	(16.4)	15	(10.8)	0	(0.0)	8	(5.6)	SWS-PASTRY	S.ALTA
10	LENNOX	62	41	(37.6)	28	(26.5)	21	(16.4)	11	(8.0)	2	(1.9)	8	(6.5)	HRW-FEED	ATLANTIC
11	MONOPOL	61	32	(31.9)	24	(24.9)	22	(15.8)	8	(6.2)	0	(0.0)	14	(9.6)	HRW-BW	ATLANTIC
12	SUN	61	42	(31.3)	27	(23.5)	23	(14.9)	12	(6.9)	3	(3.1)	8	(5.9)	SRW-PASTRY	BC
13	BISHOP	60	40	(40.2)	27	(24.9)	21	(16.7)	10	(7.7)	3	(3.7)	8	(5.3)	HWS-EMQ	W.CAN
14	PITIC 62	60	38	(31.6)	26	(24.1)	23	(16.4)	11	(6.8)	1	(0.9)	11	(8.7)	SRS-FEED	UTILITY
15	KHARKOV 22 M.C.	60	41	(41.2)	28	(27.6)	22	(18.6)	12	(9.3)	1	(2.2)	9	(7.1)	HRW-BW	W.CAN
16	PIONEER	58	40	(43.0)	25	(26.0)	25	(18.8)	12	(8.0)	3	(3.7)	10	(6.8)	HRS-EMQ	W.CAN
17	HOUSER	58	43	(34.7)	26	(24.0)	27	(17.3)	15	(9.0)	2	(2.8)	10	(5.6)	SWW-PASTRY	ONTARIO
18	EGYPTIAN AMBER	58	39	(31.0)	25	(23.1)	26	(16.7)	13	(8.4)	1	(0.9)	12	(7.4)	SRW-PASTRY	ONTARIO
19	SELKIRK_M	57	38	(38.1)	27	(25.7)	21	(19.2)	10	(9.3)	1	(1.8)	10	(8.0)	HRS-EMQ	W.CAN
20	DUNDAS_M	56	36	(31.0)	23	(20.7)	24	(16.4)	9	(6.2)	4	(4.6)	11	(5.6)	SRS-FEED	R/ W.CAN
21	ALEX	56	39	(40.2)	25	(25.7)	24	(20.1)	11	(10.5)	3	(2.8)	10	(6.8)	HRS-SMQ	USA
22	RUBY_M	55	43	(41.2)	27	(26.0)	26	(21.7)	15	(12.4)	1	(1.5)	10	(7.7)	HRS-EMQ	W.CAN
23	LAVAL 19	55	40	(32.8)	25	(22.3)	26	(18.3)	13	(8.7)	2	(1.9)	11	(7.7)	SHPS-FEED	E.CAN
24	VALOR	55	37	(33.4)	24	(23.5)	26	(18.9)	12	(7.4)	1	(0.9)	13	(10.5)	SHRW-FEED	ATLANTIC
25	EARLY RED FIFE	55	38	(37.8)	27	(24.6)	20	(19.8)	9	(9.3)	2	(3.1)	9	(7.4)	HRS-NEMQ	W.CAN
	MEAN VALUE:	64	39	(36.0)	27	(26.4)	20	(15.0)	10	(7.4)	1	(1.6)	8	(6.0)		

	CULTIVAR	DATA BASE INDEX NO.	PEDIGREE DATA
1	OPAL	107	TRIESDORF STAMM 21/40 x VON ROMKE ERLI; PEDIGREE INCLUDES GARNET, GERMANY
2	VERNON	111	OPAL*4/POMPE, CANADA
3	MILTON	104	KENTVILLE SELECTION*6/POMPE, CANADA; (KENTVILLE SELECTION = AWNED PLANT SEL. FROM OPAL).
4	FIELDER	113	YAKTANA 54A*4//NORIN 10/BREVOR/3/2*YAQUI 50/4/NORIN 10/BREVOR//BAART/ONAS, USA
5	VUKA	136	TOEERING 2/MERLIN//CARSTEN 8, FRG
6	LEMHI 53_M	118	CALIFORNIA 3098/5*LEMHI, USA
7	KENHI	115	KENYA 338 AC2E3/2*LEMHI, CANADA
8	SPRINGFIELD	128	NORIN 10/BREVOR//3*LEMHI 53/3/LEMHI 62, USA
9	LEMHI 62_M	122	LEMHI 53*5/3/LEE*7//CHINESE/AE. UMBELLATA, USA
10	LENNOX	130	SELECTION, MIRONOVSKAJA, USA
11	MONOPOL	131	PANTHUS/ADMIRAL, WEST GERMANY
12	SUN	171	AS SOL, SELECTION OF LOCAL VARIETY/ENGLISH STANDUP, SWEDEN
13	BISHOP	95	LADOGA/GEHUN, CANADA
14	PITIC 62	108	YAKTANA 54//NORIN 10/BREVOR 26-IC, MEXICO
15	KHARKOV 22 M.C.	129	SELECTION OF KHARKOV, CANADA
16	PIONEER	4	RIGA/PRESTON, CANADA
17	HOUSER	153	BREVOR/NORIN 10//NY WHEAT RYE SEL./3/HOPE HUSSAR/YORKWIN/4/GENESSEE//CT12658/ALASKAN/3/AVON, USA
18	EGYPTIAN AMBER	165	FULTZ/LANCASTER, USA
19	SELKIRK_M	40	MCMURACHY/EXCHANGE/3*REDMAN, CANADA
20	DUNDAS_M	97	OPAL/INIA 66, CANADA
21	ALEX	84	ND507/ND496, USA (ND507 = WALDRON/RL4205)
22	RUBY_M	12	DOWNY RIGA/RED FIFE, CANADA
23	LAVAL 19	103	F.W.606-A/OPAL//OPAL, CANADA
24	VALOR	135	KENT/SANGASTE (RYE), CANADA
25	EARLY RED FIFE	1	SELECTION OF RED FIFE, CANADA

WBD VALUES IN PARENTHESES GIVE THE PAIRED NUMBER COUNT WEIGHTED BY BAND DENSITY. THE WEIGHTING INCREMENT = (X/3.23)
WHERE 3.23 = POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND IN THE ELECTROPHOREGRAM.

B

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - II. DISTRIBUTION BY DIFFERENCES

* INPUT TEST CULTIVAR = OPAL
* 122 DATA BASE CULTIVAR PATTERNS ANALYZED ; LSD(RELATIVE MOBILITY) = 0.6, LSD(BAND DENSITY) = 3.

WEIGHTED POSITIONAL DIFFERENCES

```
      0   107(**)
      1   111(96)
      2   104(95)
      3
      4
      5
      6
      7
      8
      9
     10
     11
     12   113(70)
     13   136(69)
     14   115(64)128(64)
     15   171(61)
     16    97(56)108(60)118(64)122(63)130(62)131(61)
     17    95(60)153(58)166(58)172(52)
     18   103(55)
     19     4(58)  40(57)129(60)135(55)
     20     1(55)  84(56)
     21    66(54)  79(47)149(52)162(53)
     22    12(55)  37(53)138(48)
     23   105(47)112(52)157(49)
     24    58(45)  69(52)  96(48)167(52)
     25    17(48)  99(46)137(49)158(48)166(45)
     26     8(43)  77(44)127(43)148(40)152(37)184(38)
     27     3(44)  92(43)
     28     5(43)   9(40) 11(46) 15(46) 23(41) 27(45) 28(43) 32(42) 38(40) 89(38)110(45)124(45)134(43)
     29    24(44)  61(36) 87(38)102(40)141(40)155(41)
     30    22(41)  39(43) 46(45) 76(41) 86(41) 90(39)147(38)
     31    59(37)109(43)
     32   143(36)185(34)
     33    16(37)  29(36)142(36)164(39)163(38)
     34    14(35)132(31)133(35)151(35)156(32)164(32)
     35   150(26)
     36    67(34)  63(38)139(30)
     37    88(28)170(36)186(25)
     38    53(31)  78(30)177(23)182(26)187(24)
     39    30(27)  47(26)183(23)
     40    91(26)101(30)
     41   179(26)180(22)181(24)
     42    45(24)  68(24)174(21)178(18)
     43    62(23)189(20)
     44    52(23)  86(25)
     45
     46
     47
     48     44(19)
```

WEIGHTED POSITIONAL DIFFERENCE GIVES THE TOTAL NUMBER OF BAND DIFFERENCES WEIGHTED BY DENSITY
BETWEEN THE INPUT PATTERN AND DATA BASE CULTIVAR ELECTROPHOREGRAMS. THE WEIGHTING INCREMENT
= (X/3.23) WHERE 3.23 = CULTIVAR POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY
FOR EACH GLIADIN BAND. TABULATED DATA IS LISTED IN PAIRS, AND REPRESENTS RESPECTIVELY FOR
EACH CULTIVAR ANALYZED, ITS DATA BASE INDEX NUMBER AND % PATTERN HOMOLOGY IN PARENTHESES.

Figure 44. Cultivar identification ranking (A) and distance map (B) results for cv. Springfield. Framed cultivars in (B) correspond to cultivars ranked in (A).

A

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - I. RANKING BY PATTERN HOMOLOGY

- 122 DATA BASE CULTIVAR PATTERNS ANALYZED
- DATA BASE SEARCH CUTOFF AT 55% PATTERN HOMOLOGY (WEIGHTED BY BAND DENSITY).
- LSD(RELATIVE MOBILITY) = 0.5, MOBILITY RANGE: 10.0 - 90.0. LSD(BAND DENSITY) = 3, DENSITY RANGE: 1 - 9.
- UNKNOWN (OR TEST) CULTIVAR ELECTROPHOREGRAM CONTAINS 42 GLIADIN BANDS; TOTAL, WEIGHTED BY BAND DENSITY (WBD) = 35.0

	CULTIVAR	WEIGHTED % PATTERN HOMOLOGY	GLIADIN BANDS IN PATTERN NO.	GLIADIN BANDS IN PATTERN WBD	MATCHING BANDS NO.	MATCHING BANDS WBD	DISTRIBUTION OF NON-MATCHING BAND DATA: TOTAL NO.	TOTAL WBD	MOBILITY BASIS-R NO.	MOBILITY BASIS-R WBD	DENSITY BASIS NO.	DENSITY BASIS WBD	MOBILITY BASIS-U NO.	MOBILITY BASIS-U WBD	CLASS/TYPE	REGION
1	SPRINGFIELD	100	42	(35.0)	42	(35.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	SWS-PASTRY	S.ALTA
2	LEMHI 53_M	86	44	(37.2)	40	(33.4)	6	(5.3)	4	(3.4)	0	(0.0)	2	(1.9)	SWS-PASTRY	S.ALTA
3	LEMHI 62_M	78	45	(37.8)	39	(31.9)	9	(9.0)	6	(5.6)	0	(0.0)	3	(3.4)	SWS-PASTRY	S.ALTA
4	FIELDER	70	40	(34.4)	33	(28.5)	16	(12.4)	7	(5.9)	0	(0.0)	9	(6.5)	SWS-PASTRY	S.ALTA
5	LENNOX	67	41	(37.5)	32	(28.5)	18	(14.2)	8	(6.8)	1	(1.2)	9	(6.2)	HRW-FEED	ATLANTIC
6	BISHOP	65	40	(40.2)	29	(27.1)	21	(14.9)	8	(5.0)	3	(4.3)	10	(5.6)	HWS-EMQ	W.CAN
7	OPAL	64	38	(33.7)	28	(25.4)	22	(14.2)	8	(4.6)	2	(1.9)	12	(7.7)	HRS-FEED	R/ W.CAN
8	VALOR	61	37	(33.4)	28	(25.2)	21	(16.1)	7	(6.2)	2	(1.9)	12	(8.0)	SHRW-FEED	ATLANTIC
9	VERNON	60	42	(34.4)	29	(25.4)	25	(16.7)	12	(7.1)	1	(0.9)	12	(8.7)	SRS-FEED	R/ W.CAN
10	GLENLEA_M	58	40	(38.7)	27	(26.0)	26	(19.2)	11	(8.4)	2	(3.1)	13	(7.7)	SHRS-FEED	UTILITY
11	MILTON	58	39	(38.7)	27	(25.9)	26	(18.9)	11	(9.3)	1	(0.9)	14	(8.7)	HRS-FEED	R/ W.CAN
12	CASCADE	58	38	(39.6)	27	(25.2)	22	(18.6)	7	(5.9)	4	(4.6)	11	(8.0)	SHWS-GHP	E.CAN
13	GARNET	57	40	(38.1)	29	(25.7)	22	(19.2)	9	(7.1)	2	(3.4)	11	(8.7)	HRS-NEMQ	W.CAN
14	KHARKOV 22 M.C.	56	41	(41.2)	27	(25.9)	26	(20.1)	11	(7.7)	3	(4.3)	12	(8.0)	HRW-BW	W.CAN
15	RELIANCE_LTH_M	56	43	(39.3)	28	(25.9)	26	(20.1)	12	(9.9)	3	(3.7)	11	(6.6)	HRS-NEMQ	W.CAN
16	EGYPTIAN AMBER	56	39	(31.0)	26	(23.7)	29	(18.6)	13	(8.4)	0	(0.0)	16	(10.2)	SRW-PASTRY	ONTARIO
17	CHESTER_BRDR	56	42	(37.2)	30	(25.7)	23	(20.1)	11	(9.3)	1	(1.5)	11	(9.3)	HRS-SMQ	SAWFLY
18	WESTMONT	55	34	(30.0)	24	(22.8)	27	(18.3)	9	(5.6)	1	(0.9)	17	(11.8)	HRW-BW	W.CAN
	MEAN VALUE:	64	40	(36.5)	30	(27.1)	20	(15.3)	8	(6.4)	1	(1.8)	10	(7.1)		

	CULTIVAR	DATA BASE INDEX NO.	PEDIGREE DATA
1	SPRINGFIELD	128	NORIN 10/BREVOR//3*LEMHI 53/3/LEMHI 62, USA
2	LEMHI 53_M	118	CALIFORNIA 3098/5*LEMHI, USA
3	LEMHI 62_M	122	LEMHI 53*5/3/LEE*7//CHINESE/AE. UMBELLATA, USA
4	FIELDER	113	YAKTANA 54A*4//NORIN 10/BREVOR/3/2*YAQUI 50/4/NORIN 10/BREVOR//BAART/ONAS, USA
5	LENNOX	130	SELECTION, MIRONOVSKAJA, USA
6	BISHOP	95	LADOGA/GEHUN, CANADA
7	OPAL	107	TRIESDORF STAMM 21/40 X VON ROMKE ERLI; PEDIGREE INCLUDES GARNET, GERMANY
8	VALOR	135	KENT/SANGASTE (RYE), CANADA
9	VERNON	111	OPAL*4/POMPE, CANADA
10	GLENLEA_M	99	PEMBINA*2/BAGE//CB 100, CANADA
11	MILTON	104	KENTVILLE SELECTION*6/POMPE, CANADA; (KENTVILLE SELECTION = AWNED PLANT SEL. FROM OPAL).
12	CASCADE	112	QUALITY A/PACIFIC BLUE STEM//C26-89-2D/3/ONAS, CANADA
13	GARNET	3	PRESTON A/RIGA M, CANADA
14	KHARKOV 22 M.C.	129	SELECTION OF KHARKOV, CANADA
15	RELIANCE_LTH_M	32	KANRED/MARQUIS, USA
16	EGYPTIAN AMBER	165	FULTZ/LANCASTER, USA
17	CHESTER_BRDR	66	RENOWN/S-615//RESCUE/3/KENDEE/4/MIDA/CADET, CANADA
18	WESTMONT	138	RIO/REX//NEBRED, USA

WBD VALUES IN PARENTHESES GIVE THE PAIRED NUMBER COUNT WEIGHTED BY BAND DENSITY. THE WEIGHTING INCREMENT = (X/3.23) WHERE 3.23 = POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND IN THE ELECTROPHOREGRAM.

B

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - II. DISTRIBUTION BY DIFFERENCES

- INPUT TEST CULTIVAR = SPRINGFIELD
- 122 DATA BASE CULTIVAR PATTERNS ANALYZED ; LSD(RELATIVE MOBILITY) = 0.5, LSD(BAND DENSITY) = 3.

WEIGHTED POSITIONAL DIFFERENCES

```
 0   128(**)
 1
 2
 3
 4
 5   118(86)
 6
 7
 8
 9   122(78)
10
11
12   113(70)
13
14   107(64) 130(67)
15    95(65)
16   135(61)
17   111(60)
18   138(55)
19     3(57)  58(53)  99(58) 104(58) 112(58) 131(54) 165(56)
20    32(56)  66(56)  97(52) 116(52) 129(56) 172(49)
21
22     8(50) 105(50) 149(52)
23    46(54)  77(49) 133(52) 152(44) 153(50) 162(51) 166(50)
24    12(51)  17(51)  88(47) 110(52)
25     4(49)  30(48)  69(50)  90(48) 103(46) 108(46) 141(43) 157(46) 171(44)
26    11(49)  22(46)  37(45)  89(43) 132(43) 136(43) 148(42)
27     1(44)  23(42)  24(49)  28(44)  29(45)  76(45)  91(43) 163(45) 167(49)
28    40(44)  79(36)  92(45)  96(46) 127(41) 134(42) 137(44) 151(45)
29    38(38)  39(43) 109(45) 139(39)
30    16(42)  59(38)  78(42) 101(42) 102(40) 143(39) 147(39) 184(33)
31     5(39)  84(42) 188(39)
32    14(41)  45(35)  57(39)  62(39)  85(39) 150(29) 154(39) 164(36)
33    15(40)  27(39)  47(34)  61(34)  86(38) 124(39)
34     9(31)  44(36)  63(38)  87(32) 142(36)
35    52(32) 156(28) 170(38)
36    53(32)
37    66(34) 155(31)
38
39
40   187(23)
41   179(26)
42   180(23)
43   177(17)
44   178(15) 185(24)
45   174(23)
46   181(22)
47
48   186(18)
49   183(19)
50   182(18) 189(18)
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WEIGHTED POSITIONAL DIFFERENCE GIVES THE TOTAL NUMBER OF BAND DIFFERENCES WEIGHTED BY DENSITY BETWEEN THE INPUT PATTERN AND DATA BASE CULTIVAR ELECTROPHOREGRAMS. THE WEIGHTING INCREMENT = (X/3.23) WHERE 3.23 = CULTIVAR POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND. TABULATED DATA IS LISTED IN PAIRS, AND REPRESENTS RESPECTIVELY FOR EACH CULTIVAR ANALYZED, ITS DATA BASE INDEX NUMBER AND % PATTERN HOMOLOGY IN PARENTHESES.

Figure 45. Cultivar identification ranking (A) and distance map (B) results for cv. Sundance. Framed cultivars in (B) correspond to cultivars ranked in (A).

A

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - I. RANKING BY PATTERN HOMOLOGY

- 122 DATA BASE CULTIVAR PATTERNS ANALYZED
- DATA BASE SEARCH CUTOFF AT 55% PATTERN HOMOLOGY (WEIGHTED BY BAND DENSITY).
- LSD(RELATIVE MOBILITY) = 0.5, MOBILITY RANGE: 10.0 - 90.0. LSD(BAND DENSITY) = 3, DENSITY RANGE: 1 - 9.
- UNKNOWN (OR TEST) CULTIVAR ELECTROPHOREGRAM CONTAINS 35 GLIADIN BANDS; TOTAL, WEIGHTED BY BAND DENSITY (WBD) = 39.3

	CULTIVAR	WEIGHTED % PATTERN HOMOLOGY	GLIADIN BANDS IN PATTERN NO.	GLIADIN BANDS IN PATTERN WBD	MATCHING BANDS NO.	MATCHING BANDS WBD	DISTRIBUTION OF NON-MATCHING BAND DATA: TOTAL NO.	TOTAL WBD	MOBILITY BASIS-R NO.	MOBILITY BASIS-R WBD	DENSITY BASIS NO.	DENSITY BASIS WBD	MOBILITY BASIS-U NO.	MOBILITY BASIS-U WBD	CLASS/TYPE	REGION
1	SUNDANCE	100	35	(39.3)	35	(39.3)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	HRW-BW	W.CAN
2	YOGO	67	34	(34.7)	25	(29.6)	19	(14.9)	9	(5.0)	0	(0.0)	10	(9.9)	HRW-BW	W.CAN
3	LEADER	65	39	(43.7)	26	(30.5)	19	(16.4)	10	(8.0)	3	(2.8)	6	(5.6)	HRS-SMQ	SAWFLY
4	KHARKOV 22 M.C.	65	41	(41.2)	28	(31.0)	18	(16.7)	11	(9.0)	2	(2.8)	5	(5.0)	HRW-BW	W.CAN
5	ERA	63	39	(37.2)	26	(28.2)	19	(16.4)	10	(7.1)	3	(3.7)	6	(5.6)	HRS-NEMQ	USA
6	CANUCK_M	61	49	(52.0)	28	(32.5)	26	(20.7)	19	(13.0)	2	(1.9)	5	(5.9)	HRS-EMQ	SAWFLY
7	CANTHATCH	59	36	(39.9)	25	(28.9)	20	(20.1)	10	(8.4)	1	(0.9)	9	(10.8)	HRS-SMQ	W.CAN
8	RELIANCE_PGR_M	59	35	(42.7)	24	(29.3)	20	(20.4)	9	(8.7)	2	(2.2)	9	(9.6)	HRS-NEMQ	W.CAN
9	NAPAYO_M	58	40	(43.0)	26	(28.9)	21	(21.4)	12	(9.6)	2	(2.5)	7	(9.3)	HRS-SMQ	W.CAN
10	CHRIS	57	38	(43.7)	26	(29.6)	20	(22.6)	11	(10.8)	1	(1.2)	8	(10.5)	HRS-SMQ	USA
11	BENITO	57	36	(43.7)	25	(29.6)	20	(22.6)	10	(10.2)	1	(1.5)	9	(10.8)	HRS-SMQ	W.CAN
12	RESCUE_PGR_M	57	33	(34.4)	23	(24.6)	20	(18.9)	8	(5.9)	2	(2.5)	10	(10.5)	HRS-EMQ	SAWFLY
13	THATCHER	56	35	(42.1)	24	(28.6)	21	(22.9)	10	(9.9)	1	(1.2)	10	(11.8)	HRS-SMQ	W.CAN
14	NEEPAWA	56	37	(44.6)	24	(29.1)	22	(22.6)	11	(9.3)	2	(2.8)	9	(10.5)	HRS-SMQ	W.CAN
15	NUGAINES	56	29	(32.8)	21	(24.9)	21	(19.2)	7	(5.6)	1	(0.9)	13	(12.7)	SWW-PASTRY	BC
16	KATEPWA	55	35	(39.0)	23	(26.6)	22	(22.0)	10	(8.0)	2	(2.2)	10	(11.8)	HRS-SMQ	W.CAN
17	MANITOU	55	34	(41.5)	23	(27.6)	21	(22.6)	9	(8.4)	2	(2.8)	10	(11.5)	HRS-SMQ	W.CAN
18	HOUSER	55	43	(34.7)	23	(23.7)	27	(19.2)	15	(8.7)	5	(5.6)	7	(6.0)	SWW-PASTRY	ONTARIO
	MEAN VALUE:	61	37	(40.6)	25	(29.0)	19	(18.9)	10	(8.1)	1	(2.1)	7	(8.7)		

	CULTIVAR	DATA BASE INDEX NO.	PEDIGREE DATA
1	SUNDANCE	134	CHEYENNE/KHARKOV 22 M.C., CANADA
2	YOGO	141	MINTURKI/BELOGLINA//BUFFUM, USA
3	LEADER	78	FORTUNA/CHRIS, CANADA
4	KHARKOV 22 M.C.	129	SELECTION OF KHARKOV, CANADA
5	ERA	88	II-50-10/4/PEMBINA/II-52-329/3/II-53-38/III-58-4//II-53-546, USA
6	CANUCK_M	63	CANTHATCH/3/MIDA/CADET//RESCUE, CANADA
7	CANTHATCH	45	THATCHER*6/KENYA FARMER, CANADA
8	RELIANCE_PGR_M	30	KANRED/MARQUIS, USA
9	NAPAYO_M	53	MANITOU*2/4/THATCHER*5/LEE/3/THATCHER*7/FRONTANA//THATCHER*6/KENYA FARMER, CANADA
10	CHRIS	86	FRONTANA/3*THATCHER//3/KENYA 58/NEWTHATCH/2*THATCHER, USA
11	BENITO	44	NEEPAWA/3/RL4255*4//MANITOU/CI7090, CANADA
12	RESCUE_PGR_M	79	APEX/S-615, CANADA
13	THATCHER	62	MARQUIS/IUMILLIO/MARQUIS/KANRED, CANADA
14	NEEPAWA	56	THATCHER*7/FRONTANA//THATCHER*6/KENYA FARMER/3/THATCHER*2//FRONTANA/THATCHER, CANADA
15	NUGAINES	156	S1B. OF GAINES, USA
16	KATEPWA	47	NEEPAWA*6/RL2938//3/NEEPAWA*6//C.I.8154/2*FROCOR, CANADA(RL2938 = LEE*2/KENYA FARMER).
17	MANITOU	52	THATCHER*7/FRONTANA//CANTHATCH/3/PI 170925/6*THATCHER, CANADA
18	HOUSER	153	BREVOR/NORIN 10//NY WHEAT RYE SEL./3/HOPE HUSSAR/YORKWIN/4/GENESSEE//CT12668/ALASKAN/3/AVON, USA

WBD VALUES IN PARENTHESES GIVE THE PAIRED NUMBER COUNT WEIGHTED BY BAND DENSITY. THE WEIGHTING INCREMENT = (X/3.23) WHERE 3.23 = POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND IN THE ELECTROPHOREGRAM.

B

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - II. DISTRIBUTION BY DIFFERENCES

- INPUT TEST CULTIVAR = SUNDANCE
- 122 DATA BASE CULTIVAR PATTERNS ANALYZED ; LSD(RELATIVE MOBILITY) = 0.5, LSD(BAND DENSITY) = 3.

WEIGHTED POSITIONAL DIFFERENCES

```
  0   134(**)
  1
  2
  3
  4
  5
  6
  7
  8
  9
 10
 11
 12
 13
 14
 15   141(67)
 16    78(65) 88(63)
 17   129(65)
 18   150(54)
 19    79(57)153(65)156(56)
 20    30(59) 45(59)
 21    53(58) 63(61) 87(63) 97(52)
 22    47(55)
 23    22(63) 44(57) 52(55) 56(56) 62(56) 76(53) 86(57)103(47)
 24    46(54) 85(61)112(52)139(60)142(60)167(54)
 25    12(50)
 26    14(49)113(45)115(41)148(40)
 27   147(44)152(35)165(40)172(35)
 28    28(45) 32(45) 37(44) 61(45) 77(43)102(46)107(43)128(42)143(41)151(43)157(40)158(46)
 29     4(47) 40(44) 90(43)111(40)136(46)
 30     1(41) 16(41) 17(43)138(36)154(44)163(41)164(36)170(45)
 31    27(43)118(40)124(45)130(42)162(42)171(34)
 32    15(42) 29(41) 69(39)
 33     3(37) 11(40) 38(35)104(37)105(35)108(34)110(39)135(36)
 34    59(36) 66(33) 95(36)131(32)133(38)
 35    57(38)122(35)166(27)
 36     8(29) 58(30) 84(32) 91(36) 92(33)101(38)
 37     9(30) 89(26)132(30)137(37)
 38
 39   127(28)184(23)
 40    99(26)178(25)187(26)
 41   186(25)
 42    24(27) 96(30)109(31)179(26)
 43   181(24)
 44   149(19)177(17)180(23)
 45    23(21)185(24)
 46    39(24)189(26)
 47     5(22)
 48   183(19)
 49   156(21)
 50   174(18)
 51   182(17)
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WEIGHTED POSITIONAL DIFFERENCE GIVES THE TOTAL NUMBER OF BAND DIFFERENCES WEIGHTED BY DENSITY BETWEEN THE INPUT PATTERN AND DATA BASE CULTIVAR ELECTROPHOREGRAMS. THE WEIGHTING INCREMENT = (X/3.23) WHERE 3.23 = CULTIVAR POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND. TABULATED DATA IS LISTED IN PAIRS, AND REPRESENTS RESPECTIVELY FOR EACH CULTIVAR ANALYZED, ITS DATA BASE INDEX NUMBER AND % PATTERN HOMOLOGY IN PARENTHESES.

Figure 46. Cultivar identification ranking (A) and distance map (B) results for cv. Yorkstar. Framed cultivars in (B) correspond to cultivars ranked in (A).

A

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - I. RANKING BY PATTERN HOMOLOGY

- 122 DATA BASE CULTIVAR PATTERNS ANALYZED
- DATA BASE SEARCH CUTOFF AT 55% PATTERN HOMOLOGY (WEIGHTED BY BAND DENSITY).
- LSD(RELATIVE MOBILITY) = 0.5. MOBILITY RANGE: 10.0 - 90.0. LSD(BAND DENSITY) = 3. DENSITY RANGE: 1 - 9.
- UNKNOWN (OR TEST) CULTIVAR ELECTROPHOREGRAM CONTAINS 37 GLIADIN BANDS; TOTAL, WEIGHTED BY BAND DENSITY (WBD) = 33.1

	CULTIVAR	WEIGHTED % PATTERN HOMOLOGY	GLIADIN BANDS IN PATTERN NO.	GLIADIN BANDS IN PATTERN WBD	MATCHING BANDS NO.	MATCHING BANDS WBD	DISTRIBUTION OF NON-MATCHING BAND DATA: TOTAL NO.	TOTAL WBD	MOBILITY BASIS-R NO.	MOBILITY BASIS-R WBD	DENSITY BASIS NO.	DENSITY BASIS WBD	MOBILITY BASIS-U NO.	MOBILITY BASIS-U WBD	CLASS/TYPE	REGION
1	YORKSTAR	100	37	(33.1)	37	(33.1)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	SWW-PASTRY	ONTARIO
2	FAVOR	98	37	(29.7)	36	(31.1)	2	(0.6)	1	(0.3)	0	(0.0)	1	(0.3)	SWW-PASTRY	ONTARIO
3	GENESSEE	95	40	(39.8)	36	(35.6)	5	(1.9)	4	(1.5)	0	(0.0)	1	(0.3)	SWW-PASTRY	ONTARIO
4	GORDON	94	36	(24.8)	34	(28.0)	5	(1.9)	2	(0.6)	0	(0.0)	3	(1.2)	SWW-PASTRY	ONTARIO
5	TALBOT	91	40	(38.1)	35	(33.9)	5	(3.4)	4	(2.8)	0	(0.0)	1	(0.6)	SWW-PASTRY	ONTARIO
6	CORNELL 595	90	40	(37.8)	35	(33.6)	7	(3.7)	5	(2.8)	0	(0.0)	2	(0.9)	SWW-PASTRY	ONTARIO
7	DGCHAFF	87	39	(36.2)	34	(32.2)	8	(5.0)	5	(3.4)	0	(0.0)	3	(1.5)	SWW-PASTRY	ONTARIO
8	KENT	86	40	(45.2)	35	(35.4)	6	(5.6)	4	(4.0)	1	(0.9)	1	(0.6)	SRW-PASTRY	ONTARIO
9	DAWBUL_M	76	44	(37.6)	33	(29.9)	14	(9.6)	10	(6.8)	1	(1.2)	3	(1.5)	SWW-PASTRY	ONTARIO
10	JR.NO.6	75	39	(39.6)	32	(30.3)	11	(10.2)	6	(6.5)	1	(0.9)	4	(2.8)	SWW-PASTRY	ONTARIO
11	EGYPTIAN AMBER	64	39	(31.0)	28	(24.6)	19	(13.6)	10	(7.7)	1	(1.5)	8	(4.3)	SRW-PASTRY	ONTARIO
12	HOUSER	61	43	(34.7)	28	(24.8)	22	(15.8)	13	(8.4)	2	(3.4)	7	(4.0)	SWW-PASTRY	ONTARIO
13	OAC104	61	38	(34.1)	27	(25.1)	20	(15.8)	10	(7.7)	1	(1.2)	9	(6.8)	SWW-PASTRY	ONTARIO
14	THORNE_M	59	37	(24.1)	27	(19.8)	18	(13.9)	8	(5.6)	2	(1.9)	8	(6.5)	SRW-PASTRY	ONTARIO
15	FAIRFIELD	58	40	(32.8)	25	(24.0)	26	(17.3)	14	(9.9)	1	(1.9)	11	(5.6)	SRW-PASTRY	ONTARIO
16	RICHMOND_M	56	40	(41.2)	25	(25.1)	25	(19.8)	13	(9.0)	2	(3.1)	10	(7.7)	SWW-PASTRY	ONTARIO
17	FREDRICK	56	34	(36.2)	23	(23.8)	23	(18.6)	9	(8.0)	2	(3.4)	12	(7.1)	SWW-PASTRY	ONTARIO
	MEAN VALUE:	76	39	(35.1)	31	(28.8)	12	(9.2)	6	(5.0)	0	(1.1)	4	(3.1)		

	CULTIVAR	DATA BASE INDEX NO.	PEDIGREE DATA
1	YORKSTAR	164	GENESEE*5/3/YORKWIN//NORIN 10/BREVOR, USA
2	FAVOR	148	DIGA DIJON//GABO/NEW ZEALAND 496.01, CANADA
3	GENESSEE	151	YORKWIN//HONOR*2/FORWARD, USA
4	GORDON	152	CD7561(RELATED TO ETOILE DE CHOISY)/GENESSEE/2/CD7561/ KENT/3/7453-4-2-4(FREDRICK SIB)/4/2*YORKSTAR, C
5	TALBOT	163	TRUMBULL//HOPE/HUSSAR/3/DAWSON'S GOLDEN CHAFF*2/RIDIT//CORNELL 595, CANADA
6	CORNELL 595	142	HONOR/FORWARD//NURED/3/HONOR, USA
7	DGCHAFF	147	SELECTION OF CLAWSON, CANADA
8	KENT	170	CALDWELL 10/DAWSON'S GOLDEN CHAFF, CANADA
9	DAWBUL_M	143	DAWSON'S GOLDEN CHAFF/BULGARIAN, CANADA
10	JR.NO.6	154	AS GOLDCOIN, SELECTION OF REDCHAFF OR OF REDCHAFF BALD, USA
11	EGYPTIAN AMBER	165	FULTZ/LANCASTER, USA
12	HOUSER	153	BREVOR/NORIN 10//NY WHEAT RYE SEL./3/HOPE HUSSAR/YORKWIN/4/GENESSEE//CT12658/ALASKAN/3/AVON, USA
13	OAC104	157	DAWSON'S GOLDEN CHAFF/BULGARIAN, CANADA
14	THORNE_M	172	PORTAGE/FULCASTER, USA
15	FAIRFIELD	166	PURKOF/FULHIO, USA
16	RICHMOND_M	158	DAWSON'S GOLDEN CHAFF*2/RIDIT, CANADA
17	FREDRICK	149	WASHINGTON 1//GENESEE/CAPELLE, CANADA

WBD VALUES IN PARENTHESES GIVE THE PAIRED NUMBER COUNT WEIGHTED BY BAND DENSITY. THE WEIGHTING INCREMENT = (X/3.23) WHERE 3.23 = POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND IN THE ELECTROPHOREGRAM.

B

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - II. DISTRIBUTION BY DIFFERENCES

- INPUT TEST CULTIVAR = YORKSTAR
- 122 DATA BASE CULTIVAR PATTERNS ANALYZED ; LSD(RELATIVE MOBILITY) = 0.5, LSD(BAND DENSITY) = 3.

WEIGHTED POSITIONAL DIFFERENCES

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 0  164(**)
 1  148(98)
 2  151(95)152(94)
 3  163(91)
 4  142(90)
 5  147(87)
 6  170(86)
 7
 8
 9
10  143(76)154(75)
11
12
13
14  165(64)172(59)
15
16  153(61)157(61)
17  166(58)
18
19  149(56)150(49)
20  158(56)171(51)
21   76(54) 77(53) 85(54)113(52)
22   12(52)108(49)156(47)
23    5(50)
24    9(45) 27(51)103(46)115(47)138(44)
25   15(49) 17(49) 84(48)
26   22(47)105(41)110(48)124(47)
27    1(43) 16(46) 32(43) 97(39)101(46)109(46)132(42)141(41)167(47)
28   28(42) 39(43) 40(43) 79(37) 88(40) 90(42)102(40)104(43)118(42)135(40)137(43)162(43)
29    8(36) 11(43) 14(44) 37(40) 66(40)111(40)112(42)130(41)131(37)136(43)
30   87(37) 91(40) 92(39)134(36)
31   38(34) 69(41) 95(37)129(40)133(38)139(37)
32    4(38) 29(38) 61(35)127(35)128(36)
33   23(30) 24(39) 45(35) 47(36) 57(38) 78(37) 89(34)122(36)
34    3(35) 53(35) 62(36) 63(41) 99(33)107(32)155(35)
35   30(31) 52(32) 56(36) 86(35) 96(36)
36   59(32)184(24)
37   44(31) 46(36)185(29)
38
39
40  179(25)
41   58(22)181(25)
42
43
44
45  177(15)186(19)187(18)
46  178(16)180(17)182(19)183(19)
47
48  174(18)
49
50
51  189(18)
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WEIGHTED POSITIONAL DIFFERENCE GIVES THE TOTAL NUMBER OF BAND DIFFERENCES WEIGHTED BY DENSITY BETWEEN THE INPUT PATTERN AND DATA BASE CULTIVAR ELECTROPHOREGRAMS. THE WEIGHTING INCREMENT = (X/3.23) WHERE 3.23 = CULTIVAR POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND. TABULATED DATA IS LISTED IN PAIRS, AND REPRESENTS RESPECTIVELY FOR EACH CULTIVAR ANALYZED, ITS DATA BASE INDEX NUMBER AND % PATTERN HOMOLOGY IN PARENTHESES.

Figure 47. Cultivar identification ranking (A) and distance map (B) results for cv. Wascana. Framed cultivars in (B) correspond to cultivars ranked in (A).

A

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - I. RANKING BY PATTERN HOMOLOGY

* 122 DATA BASE CULTIVAR PATTERNS ANALYZED
* DATA BASE SEARCH CUTOFF AT 35% PATTERN HOMOLOGY (WEIGHTED BY BAND DENSITY).
* LSD(RELATIVE MOBILITY) = 0.5, MOBILITY RANGE: 10.0 - 90.0. LSD(BAND DENSITY) = 3, DENSITY RANGE: 1 - 9.
* UNKNOWN (OR TEST) CULTIVAR ELECTROPHOREGRAM CONTAINS 35 GLIADIN BANDS; TOTAL, WEIGHTED BY BAND DENSITY (WBD) = 43.0

	CULTIVAR	WEIGHTED % PATTERN HOMOLOGY	GLIADIN BANDS IN PATTERN NO.	GLIADIN BANDS IN PATTERN WBD	MATCHING BANDS NO.	MATCHING BANDS WBD	DISTRIBUTION OF NON-MATCHING BAND DATA: TOTAL NO.	TOTAL WBD	MOBILITY BASIS-R NO.	MOBILITY BASIS-R WBD	DENSITY BASIS NO.	DENSITY BASIS WBD	MOBILITY BASIS-U NO.	MOBILITY BASIS-U WBD	CLASS/TYPE	REGION
1	WASCANA	100	35	(43.0)	35	(43.0)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)	DURM-PASTA	W.CAN
2	STEWART 63	79	33	(36.8)	29	(35.3)	10	(9.3)	4	(4.6)	0	(0.0)	6	(4.6)	DURM-PASTA	W.CAN
3	CARLETON_PGR	77	44	(43.0)	31	(36.7)	15	(10.8)	11	(7.1)	2	(2.2)	2	(1.5)	DURM-PASTA	W.CAN
4	MACOUN	64	31	(36.2)	22	(29.9)	20	(17.0)	7	(5.3)	2	(2.8)	11	(9.0)	DURM-PASTA	W.CAN
5	NUGGET	59	44	(43.0)	26	(30.5)	24	(21.4)	15	(12.1)	3	(3.7)	6	(5.6)	DURM-PASTA	W.CAN
6	MINDUM	58	42	(44.6)	25	(30.8)	24	(22.3)	14	(12.4)	3	(3.7)	7	(6.2)	DURM-PASTA	W.CAN
7	WAKOOMA_M	49	30	(35.3)	19	(25.7)	26	(26.3)	10	(9.9)	1	(1.5)	15	(14.9)	DURM-PASTA	W.CAN
8	MEDORA	43	34	(38.7)	15	(23.5)	36	(31.0)	16	(13.3)	3	(4.0)	17	(13.6)	DURM-PASTA	W.CAN
9	HERCULES	40	37	(37.8)	16	(22.8)	39	(34.1)	20	(15.8)	1	(0.9)	18	(17.3)	DURM-PASTA	W.CAN
10	GOLDENBALL	39	26	(36.8)	15	(21.7)	28	(33.4)	8	(11.1)	3	(4.3)	17	(18.0)	DURM-PASTA	W.CAN
	MEAN VALUE:	60	35	(39.5)	23	(30.0)	22	(20.6)	10	(9.2)	1	(2.3)	9	(9.1)		

	CULTIVAR	DATA BASE INDEX NO.	PEDIGREE DATA
1	WASCANA	189	LAKOTA*2/PELISSIER, CANADA
2	STEWART 63	186	ST 464/B*STEWART, CANADA
3	CARLETON_PGR	174	VERNAL EMMER/MINDUM, USA
4	MACOUN	180	RL 3607/ DT 182, CANADA
5	NUGGET	183	MINDUM/CARLETON//HEITI/STEWART, USA
6	MINDUM	182	? FOUND IN BREAD WHEAT FIELD, USA
7	WAKOOMA_M	187	LAKOTA*2/PELISSIER, CANADA
8	MEDORA	181	WARD/MACOUN, CANADA
9	HERCULES	179	RL 3097/RL 3304//STEWART/RL 3380, CANADA
10	GOLDENBALL	178	? FROM S. AFRICA

WBD VALUES IN PARENTHESES GIVE THE PAIRED NUMBER COUNT WEIGHTED BY BAND DENSITY. THE WEIGHTING INCREMENT = (X/3.23) WHERE 3.23 = POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND IN THE ELECTROPHOREGRAM.

B

WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - II. DISTRIBUTION BY DIFFERENCES

* INPUT TEST CULTIVAR = WASCANA
* 122 DATA BASE CULTIVAR PATTERNS ANALYZED ; LSD(RELATIVE MOBILITY) = 0.5, LSD(BAND DENSITY) = 3.

WEIGHTED POSITIONAL DIFFERENCES

```
     0  189(**)
     1
     2
     3
     4
     5
     6
     7
     8
     9  186(79)
    10
    11  174(77)
    12
    13
    14
    15
    16
    17  180(64)
    18
    19
    20
    21  183(59)
    22  182(58)
    23
    24
    25
    26  187(49)
    27
    28
    29
    30
    31  181(43)
    32
    33  178(39)
    34  179(40)
    35  184(29)
    36  177(33)
    37
    38
    39  162(32)
    40  127(24)165(23)185(31)
    41   95(27)111(23)171(20)
    42   23(25) 58(24) 59(30) 61(26) 76(30)
    43   38(26) 40(27) 99(24)107(20)131(25)
    44   79(20) 84(26)103(16)141(26)
    45   22(27) 32(24) 57(26)115(20)172(14)
    46    9(17) 29(24) 46(29) 47(25) 89(16)118(22)129(25)134(26)147(23)154(26)
    47    3(19)  5(24) 28(23) 66(20) 87(23)104(20)148(16)
    48   16(24) 37(23) 92(22)102(21)105(19)124(24)135(18)153(16)167(26)
    49    8(11) 14(22) 39(23) 90(18)112(21)138(17)150(12)156(21)166(16)
    50   27(22) 44(24) 62(23) 91(22)101(25)113(17)122(18)128(18)142(21)143(20)163(22)
    51    4(19) 12(17) 24(21) 77(20) 78(22)108( 7)137(20)149(16)155(19)157(15)164(18)
    52   15(21) 45(21) 63(26) 96(17)130(14)152( 7)
    53    1(14) 17(18) 53(21) 85(21) 97( 8)109(21)
    54   30(22) 56(21) 69(15) 88(16)
    55   11(18)110(17)133(18)151(18)
    56   52(18)136(15)170(18)
    57  132(12)158(16)
    58
    59   86(16)
    60
    61
    62
    63
    64  139( 9)
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WEIGHTED POSITIONAL DIFFERENCE GIVES THE TOTAL NUMBER OF BAND DIFFERENCES WEIGHTED BY DENSITY BETWEEN THE INPUT PATTERN AND DATA BASE CULTIVAR ELECTROPHOREGRAMS. THE WEIGHTING INCREMENT = (X/3.23) WHERE 3.23 = CULTIVAR POPULATION MEAN BAND DENSITY AND X = ASSIGNED DENSITY FOR EACH GLIADIN BAND. TABULATED DATA IS LISTED IN PAIRS, AND REPRESENTS RESPECTIVELY FOR EACH CULTIVAR ANALYZED, ITS DATA BASE INDEX NUMBER AND % PATTERN HOMOLOGY IN PARENTHESES.

at least five countries. This result therefore suggests that gliadin composition is a likely factor in the inherited functional quality characteristics of these soft winter wheat cultivars.

The cultivar identification ranking result for cv. Sinton (Figure 42) provides another example where apparent relationships between gliadin composition and functionality lacks a common pedigree component. Cultivar Sinton is a Canadian HRS bread wheat with superior milling and baking qualities, which like cv. Neepawa has Thatcher as the recurrent parent in its pedigree. Surprisingly, the cultivar which appears in the ranking result most similar to cv. Sinton (at 82%PH) is the USA developed cv. Coteau which has excellent functional characteristics as a bread wheat (R. Zillman, personal communication) but with a pedigree unlike its Canadian counterparts.

The fact that Neepawa and other closely related genotypes are isolated from both Sinton and Coteau (Figure 42B) at about the 60%PH level indicates further that a Neepawa or Thatcher type electrophoregram is not a necessary feature or attribute for membership in this quality class of wheat; only that it may be sufficient.

In instances where wheats of mixed class comprise the list of ranked cultivars, the common factor in addition to electrophoregram identity was observed to be end use quality usually in terms of bread or so-called "non-bread" wheat status. The effect is illustrated in the cultivar identification ranking result generated for the Sundance electrophoregram (Figure 45). Cultivar Sundance is a hard red winter wheat with good milling and baking quality. Of the 11 remaining HRW wheats in the cultivar identification data base, only cvs. Yogo (67%PH)

and Kharkov 22 M.C. (65%) possess gliadin patterns of sufficient resemblance to be included in a list dominated by hard red spring wheats of at least equal to Marquis quality.

In an analogous fashion, HRS bread wheats[1] are generally excluded or represent the minority of cultivars which are ranked by gliadin pattern homology to an electrophoregram of a soft white spring (SWS) or soft red spring (SRS) wheat. A typical example of this is shown in the cultivar identification printout for the SWS cv. Springfield (Figure 44). Of the five hard red spring wheats in the list, only one, the seventeenth ranked cultivar (Chester), has functional attributes at least equal to the standard cv. Marquis.

Within the HRS class itself, a clear indication of how great the dissimilarity can be between electrophoregrams of bread and non-bread wheat cultivars is given by the identification program result for cv. Opal (Figure 43). The latter is a hard red spring wheat cultivar of German origin, with a regional license for production in Eastern Canada or areas of British Columbia not designated under the Canadian Wheat Board Act. Opal's licensing status relates to its low protein content, poor baking quality and visual indistinguishability from top grade HRS wheats involved in the grain commerce of Western Canada. The next two cultivars in the list below Opal in Figure 43A, cvs. Vernon and Milton, are of a similar regional type and share a common genetic background with the test cultivar. This factor explains the very high pattern homology scores which were obtained.

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[1]The cultivar identification data base includes a total of 59 HRS wheats, 41 of which are at least equal to Marquis in milling and baking quality.

Monopol, the first bread wheat cultivar ranked in the list generated by the Opal electrophoregram (at 61%PH), is a hard red winter wheat which has a similar German origin. Of the total of five HRS wheats in the ranking result (all below the 60%PH level), the only cultivar with any present commercial significance is Selkirk[1], which is shown to have 21 gliadin band positional differences with the Opal electrophoregram. Moreover, an inspection of the cultivar distance map result (Figure 43B) indicates that Neepawa (DBIN=56, at 42 positional differences) and other Thatcher type wheats which presently dominate the grain commerce in Western Canada, represent the most distinct group of cultivars in the data base with respect to Opal's gliadin composition.

Characterization of the Heterogeneity in Gliadin Electrophoregram Composition

While the aims of the present research are not involved per se with investigating the association between gliadin protein composition and utilization quality, the relationship can be considered an important factor in the successful long term application of the electrophoresis test for wheat cultivar identification. If a definitive association can be established, then grain with undesireable quality attributes can always be expected to be differentiated by PAGE from otherwise visually identical wheat of acceptable quality. At the same time, the information gained would be valuable to breeders in selecting parents for potential crosses and in screening early generation material.

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[1]Selkirk represented 2.4% of the wheat acreage seeded in Western Canada in 1982. (Source: 1982 Prairie Grain Variety Survey, Canadian Co-operative Wheat Producers Limited, Regina, Saskatchewan).

In view of the extensive heterogeneity of the gliadins which parallels the extremely wide range in functional quality possessed by common and durum wheat cultivars, an association between protein composition and quality appears likely. However, the many comparative analysis studies undertaken to identify individual gliadins as determinants of quality have met with little success. A notable exception is the correspondence found between gluten strength in durum wheats and the presence of gliadin band "45" (Damidaux et al., 1978; Kosmolak et al., 1980) named after its relative electrophoretic mobility according to the nomenclature of Bushuk and Zillman (1978).

Only recently has the formidably complex nature of the relationship been exposed with the application of computer-based multivariate analysis methods for the study of French wheats by Branlard and Rousset (1980) and Australian wheat cultivars in a series of reports by Wrigley et al. (1981, 1982b, 1982c). These studies have substantially affirmed the relevance of gliadin composition to utilization quality. However, the inability to relate results of the French and Australian work in terms of identifiable gliadin components, outside the context of their respective data bases, limits their impact considerably.

Branlard and Rousset (1980) for example partitioned their starch gel electrophoregram data into 43 relative mobility classes using the gliadin nomenclature and classification provided earlier by Autran and Bourdet (1975). Of the total number of variables in this scheme, 14 pairs possessed contiguous integer mobility positions (e.g. 21/22, 25/26, etc.) while an additional 22 were for attributes separated by two mobility units. As statistical parameters (standard errors,

replication, confidence limits) were not presented, the repeatability in identifying named classes and allocating gliadin bands to them was open to question. The Australian classification, also for starch gel electrophoregrams, involved a total of 34 gliadin attributes. Problems of nomenclature were avoided by assigning gliadin bands to numbered classes based on visual assessments and experience with their electrophoregrams. It was acknowledged that "conversion of electrophoretic patterns to numerical form involved some simplification and interpretation" (Wrigley, 1980).

Given the multiplicity of gliadin components and their close proximity to one another in electrophoregrams, a subjective approach to formulating a character set structure of gliadin composition cannot be considered a practical solution. Moreover, gains in resolution by PAGE while on the one hand improving the discrimination power of the electrophoresis test for cultivar identification, have made classification problems more acute. What is required is an objective characterization of gliadin electrohoregram heterogeneity to obtain a reference pattern which contains all the occuring components that may be resolved. The reference pattern should additionally be defined using a standardized mobility scale to which cultivar electrophoregrams may be compared in a relatively unambiguous fashion.

A possible approach might be to electrophorese a mixture of gliadin extracts from diverse genotypes and prepare a standard type pattern from the resulting composite electrophoregram. However, the interpretation of electrophoretic patterns from complex mixtures can be extremely difficult (Wrigley and Baxter, 1974). Also, faint bands may be obscured

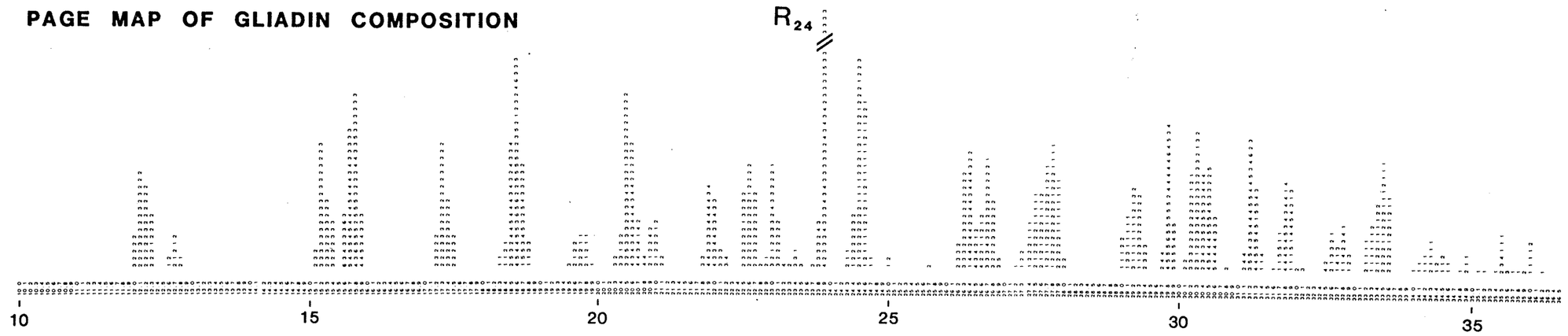
by the abundance of dense components, and uncommon gliadins would likely be under-represented in the sample of genotypes selected for the composite. These limitations notwithstanding, such a process was used by Gubareva et al. (1975) to derive cultivar formulas of gliadin electrophoregrams for Russian cultivars. The basis for their system (refer to Literature Review) was a reference pattern containing 29 bands which they claimed represented all the main gliadin components in common wheat. In light of Autran and Bourdet's classification and what was commonly known at the time from two-dimensional electrophoresis data on single cultivars (Wrigley and Shepherd, 1973), it is evident that Gubareva's accounting of gliadin heterogeneity was considereably underestimated.

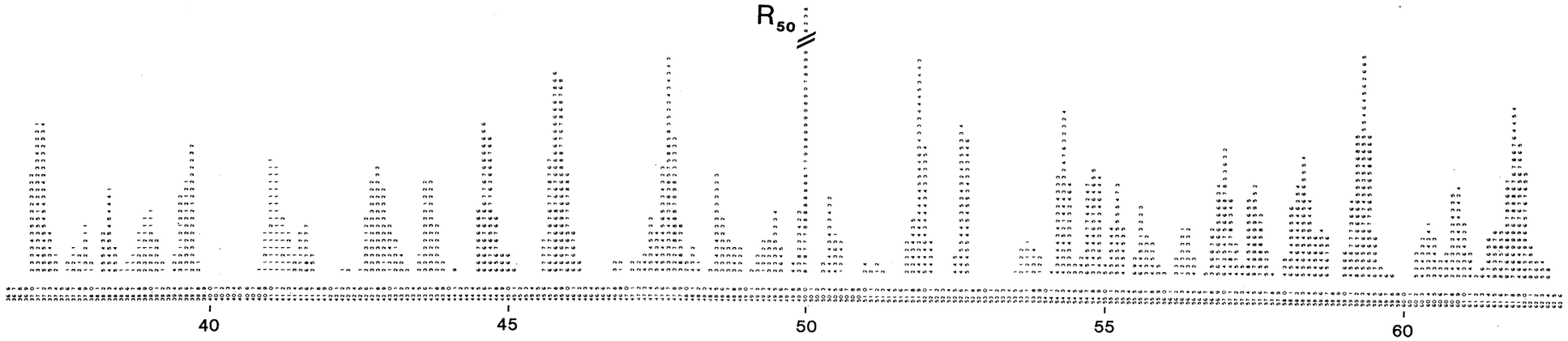
An alternative strategy, which represents the approach taken in the present study, is to build up a composite pattern by plotting out the relative position of every gliadin component detected in a broad-based collection of electrophoregrams. To minimize the variation between bands attributable to experimental error, this method requires the rigorous standardization of gliadin band migration distances to relative mobilities using multiple reference bands as described previously (pp. 165-171). Because the separation between different gliadin components may be of the order of one-half mobility unit or less in certain regions of the electrophoregram field, another requirement is for the data to be recorded to at least one decimal place to minimize the occurrence of ovelapping distributions.

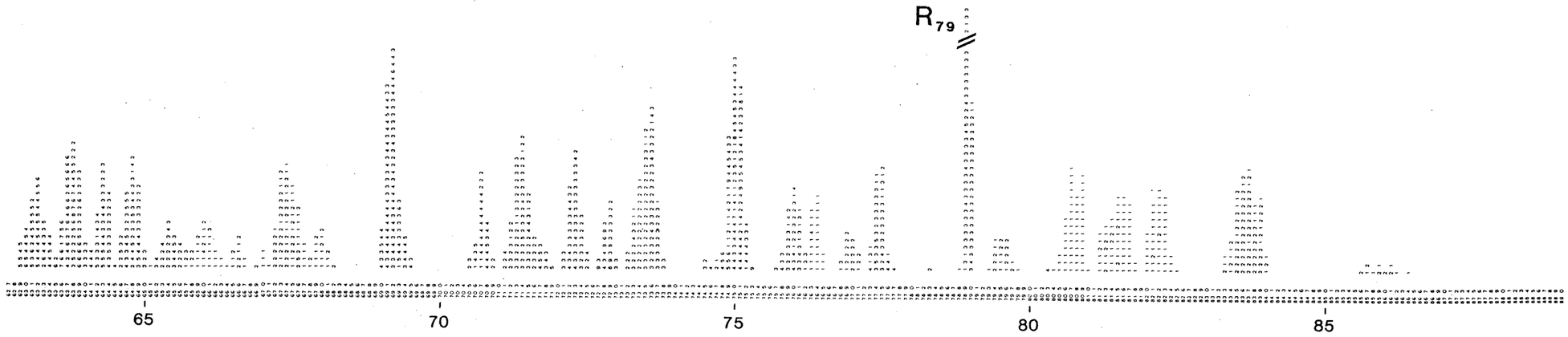
The set of electrophoregrams which were analyzed are for 98 common spring and winter wheats in the cultivar identification data base

Figure 48. PAGE map of gliadin composition for 98 common spring and winter wheat cultivars. The profile represents the frequency distribution of gliadin bands (indicated by their densities) as a function of relative mobility computed by the multiple reference band technique described in the text.

PAGE MAP OF GLIADIN COMPOSITION







RELATIVE ELECTROPHORETIC MOBILITY

(Tables 10-11). Due to the large number (>4,000) of gliadin bands involved, the task of generating the composite pattern was implemented by a FORTRAN program (PAGEMAP). The computed distribution pattern or "PAGE map" of gliadin composition is shown in Figure 48 and represents a frequency histogram with relative mobilities segmented into 800 (0.1 Rm unit) classes from 10.0 to 90.0 corresponding to the approximate range of values encountered. Plotted numbers are for band densities of gliadin components allocated along the relative mobility axis.

The most prominent feature in Figure 48 is that gliadin electrophoregram heterogeneity is sufficiently discontinuous so that common proteins are identifiable as more or less isolated clusters of points across the PAGE composition map. Approximately 90 such clusters may be counted; fewer than eight comprise uncommon components which are present in less than 15% of the spring and winter wheat cultivar electrophoregrams that were studied (e.g., bands in the mobility range: 12.6-12.8, 23.3-23.5, 34.8-36.2, 51.0-51.3, 66.4-66.7); while two are for essentially invariant proteins characterized by mobilities in the range 45.6-46.2 and 59.0-59.8. Also indicated are a limited number of apparently spurious points. All are for confirmed bands with anomalous mobilities (e.g. 25.0, 25.7, 30.8, 44.1, 78.3 and 80.3) which preclude their classification. This atypical variation likely reflects sampling limitations as no doubt some small number of gliadin components are poorly represented in the present collection of cultivars.

The unimodal shape and spread of individual groups of PAGE map components, typically over 0.4-0.5 distance units, suggests that a majority are homogeneous, although numerous instances of incomplete

resolution can be observed. For example, the bimodal distribution between mobilities 36.2 to 36.9 strongly suggests the presence of two distinct gliadin band populations. Slightly overlapping gliadin band populations are also evident within mobilities 40.8-41.7 and 75.7-76.5, especially considering the respective discontinuities in the underlying distribution of band densities.

Severe cases of overlapping band distributions are observed within mobility ranges 58.0-58.7 and 61.3-62.4. The second of these, which contains a continuous cluster of 107 points, is plainly heterogeneous as at least nine members of the base population of 98 cultivars are represented twice. Given the spread of bands over 1.2 relative mobility units, and the distribution's shape with its prominent leading and trailing perturbations, it is likely that three different populations of gliadin components are present.

Clearly for purposes of classificatory analysis, some difficulty exists in establishing discriminant boundaries for variate populations which are strongly overlapped. While they number only a few, a possible remedy might be to place an additional reference band in the problematic PAGE map region. For example, an inspection of gliadin electrophoregrams for standard cultivars Marquis and Neepawa (Figure 35) indicates that both have readily identifiable components positioned precisely in the center of the poorly separated zone noted above (Rm=61.8).

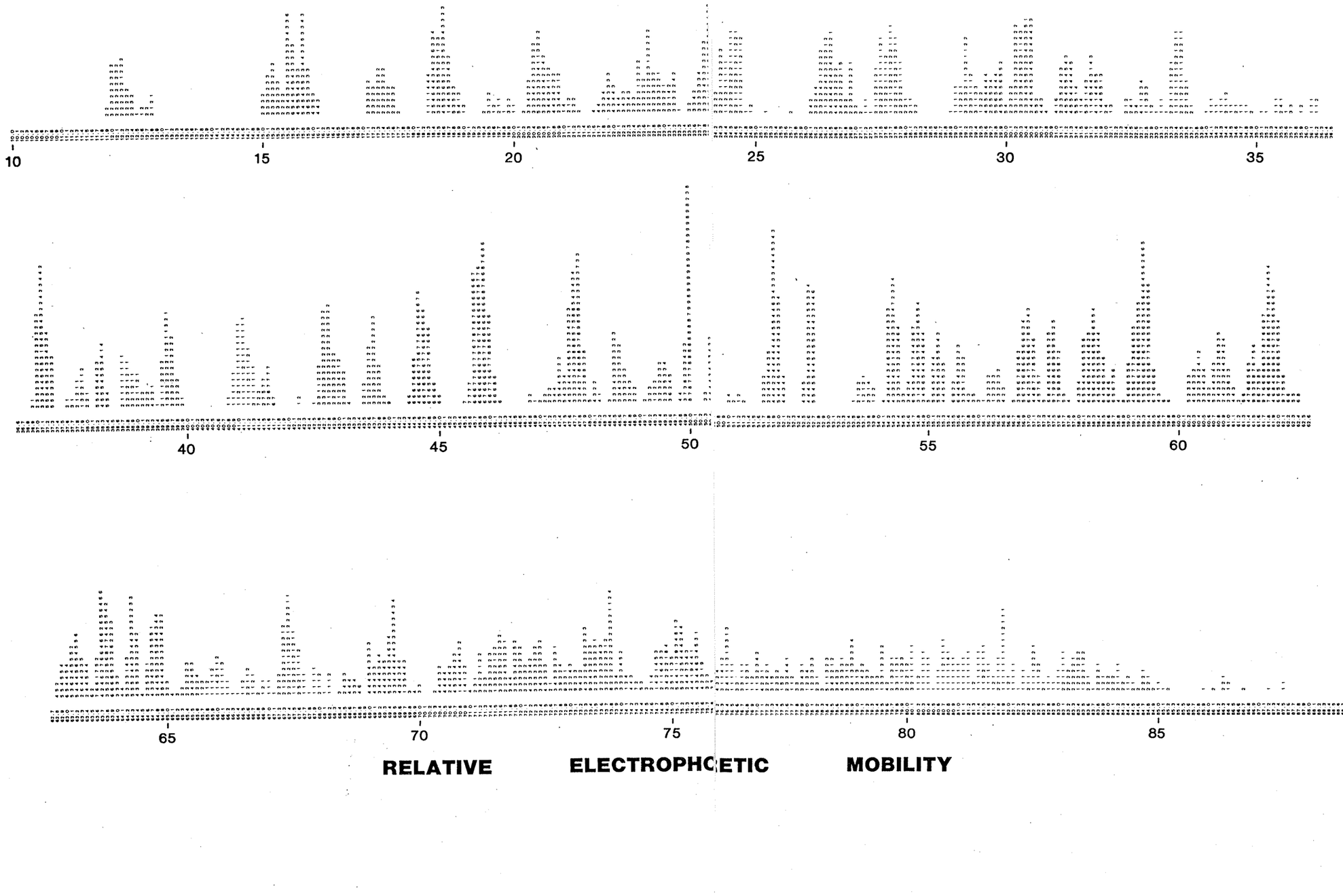
Implementation of a fourth standard protein for electrophoresis would represent only a minor programming task as described previously. The payoff would likely be twofold: (i) a significant narrowing of the

relative mobility distribution for the reference band population to approximately 0.2 distance units as indicated in Figure 48 for bands R24, R50 and R79, and (ii) reduction in the error component for neighboring gliadin components as well, as it was previously shown (Figure 36) that the uncertainty in the calculation of relative mobilities approaches a minimum in the vicinity of reference band positions. The combination would likely result in an improved if not complete separation of the various overlapping gliadin band distributions in this densely populated region of the electrophoregram field.

The practical effect of a multiple reference band protocol on the characterization of gliadin electrophoregram heterogeneity is most easily demonstrated by generating a composite pattern from relative mobility data normalized using a single reference band approach (Bushuk and Zillman, 1978). The resulting PAGE composition map (Figure 49) shows a significant decrease in the number of differentiated variate populations compared with Figure 48. Most striking is the continuous nature of the single reference band composite pattern above a mobility of 70, where all distinctions between different gliadin band populations are obscured. In regards to the cultivar identification method, it is clear that by using relative mobilities derived by the single reference band technique, the ability to minimize both type I and II errors is effectively undermined by the variability in the data.

These results are consistent with statistical data given in Table 15 which indicated that precision in electrophoregram relative mobilities can be significantly improved by increasing the number of

Figure 49. PAGE map of gliadin composition for 98 common spring and winter wheat cultivars. The profile represents the frequency distribution of gliadin bands (indicated by their densities) as a function of relative mobility computed by the single Marquis reference band technique of Bushuk and Zillman (1978).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RELATIVE ELECTROPHORETIC MOBILITY

reference bands in the methodology. Evidence is thus provided that such rigorous standardization is required to prevent significant levels of gliadin heterogeneity from escaping detection for purposes of classification, and to improve the reliability of the pattern homology analysis process for computerized electrophoregram comparisons.

VI

GENERAL DISCUSSION AND CONCLUSIONS

The principal aim of this research project was to use a computer to extend current methods for quantifying and comparing electrophoregrams of wheat gliadin for the purposes of cultivar identification and related applications.

The underlying basis for this study stems from well established observations that the electrophoresis test applied to the gliadin protein fraction can successfully differentiate wheat cultivars independent of common environmental factors (Lee and Ronalds, 1967; Feillet and Bourdet, 1967; Doekes, 1968). Accordingly, the gliadin electrophoregram effectively represents a stable genetic signature of a wheat cultivar. However, due to the experimental variation inherent in the electrophoretic method and the complex heterogeneity of gliadin composition which is resolved, protein pattern results are rarely comparable between different gel slab preparations.

To facilitate the reproducibility of gliadin electrophoregram data for broad-based cultivar comparisons, numerical nomenclatures were introduced for starch (Autran and Bourdet, 1975) and polyacrylamide gels (Bushuk and Zillman, 1978) to specify protein band patterns in terms of a list of paired relative mobility and band density values expressed as cultivar formulas. Still, the location and manual measurement of migration distances and subjective evaluation of band densities for

often confluent electrophoregram components are limiting aspects for cultivar identification. More problematic, is the comparative use of electrophoregram data itself. Whether for genetic or functional considerations, or to identify an unknown sample, assessing the resemblance or composition of band patterns by visual means in continually expanding numerically formatted compendia (e.g. Zillman and Bushuk, 1979; Jones et al., 1982) or photographic records, represents a time consuming and imprecise process. The resolution of these difficulties provided the incentive for this research project.

Because a scanning densitometer can produce an objective and quantitative record of an electrophoregram, the instrument, suitably interfaced to a laboratory-scale computer, supplied the means for an automatic and standardized quantification of electrophoregram band mobilities and density values. Positive film transparencies were found to be more suitable image media for densitometric scanning than the original Coomassie Blue-stained gel slabs. Apart from convenience, the use of film avoided the introduction of errors arising from physical distortion of the polyacrylamide gel slabs upon handling.

One problem in quantitating electrophoregram data relates to inter-gel variation in band densities caused by variability in gel slab staining and destaining. This problem was minimized by calibrating the densitometer light source to 100% transmittance against the background opacity of the film. The region of each electrophoregram which best specified this background was located in the low mobility zone in front of the slot, which is uniformly devoid of gliadin protein bands. This operation invariably yielded a horizontal baseline of zero O.D. across

each densitometric profile, thus referencing all traces to a standard level.

Accuracy and reproducibility are the primary goals of any quantification procedure. In proceeding to satisfy these objectives for the present study, the question arises as to how densitometric scanning profiles of gliadin electrophoregrams may be best characterized for the cultivar identification application. Clearly, the X-coordinate position of all peak maxima, and leading and trailing shoulder inflection points should be determined as these values correspond to band migration distances that ordinarily would be acquired by a visual identification and manual measurement process.[1] This was accomplished by using a peak finding method based upon the first derivative of the densitometric profile. The method also accomodated the acquisition of peak heights as the raw measurement parameter for band densities. Each densitomegram is thus initially reduced to a set of two-value parametized peaks. The sensitivity of this procedure was such that virtually all major and minor electrophoregram components visible on film or photographic prints were detected by the program.

An important requirement for computerized cultivar identification is data comparability, particularly for gliadin band position measurements. Migration distance values acquired by the peak finding algorithm are scaled in essentially arbitrary units whose range depends upon the sampling rate used to convert the analog profile to a digital

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[1]Zillman and Bushuk (1979) for example, used a microcomparator to gather migration distance data from 35 mm negatives of gel slabs. Jones et al. (1982) used a different technique, and manually transcribed peak and shoulder position data from densitometer tracings of gliadin electrophoregrams and subsequently calculated their relative mobilities.

form. In the present work, each gliadin densitomegram was sampled by a total of 512 data points. This was found to be an adequate level of sampling for the 20-30 peaks that were be resolved per scanning profile. Some authors have suggested a somewhat higher level of sampling i.e. from 30-50 samples per peak to ensure an accurate digital representation of the electrophoregram (Yakin et al., 1982). Whatever the level of sampling employed, the need exists to standardize the presentation of the data for purposes of further replicate analysis and/or subsequent cultivar comparisons. This was easily accomplished by using an algorithm of Bushuk and Zillman's (1978) gliadin band nomenclature to transform the the digital scale of peak abscissa values to one of relative mobility.

Like raw peak position coordinates, the absolute range of peak height values, which is a function of the bit resolution of the computer's A/D converter, can vary among installations. Peak height values were normalized by establishing an optimized relative band density scale possessing eight levels to which detected components were automatically assigned by comparison of their respective ordinate values with the largest peak in the profile. This effectively minimizes the variability in band intensities which may arise from variation in the protein content of the grain sample. Good overall reproducibility was achieved using this approach which indicated that relative peak heights can be applied as a useful approximation to quantify band densities. for the cultivar identification application.

Quantification of gliadin densitometric profile peak areas as an alternative feature parameter for band density nevertheless remains a

challenging area for study. Compromises however are inherent in the various analytical strategies which have been developed. Commercially available integrators for example, commonly employ perpendicular drop and triangulation techniques to make peak area estimates. For gas chromatograms, which are not unlike electrophoretic pattern absorbance profiles, Westerberg (1969) showed that these procedures were too inaccurate and poorly reproducible especially when approaching the shoulder limit of two components.

Sophisticated computer programs exist in the field of chromatography to analyze spectra and resolve components by iterative least squares curve fitting to various peak models which include Gaussian (Frazer and Suzuki, 1966; Littlewood et al., 1969), exponential/Gaussian (Anderson et al., 1970a) or several alternatives if a fit for Gaussian components is unsatisfactory (van Rijswick, 1974). One drawback with these procedures is the usual requirement for programs to be run on large mainframe computers involving long execution times with high relative costs. Also, despite the emphasis of accurate models in the curve fitting algorithms, the potential to generate artificial peaks is well known especially for electrophoretic data (Trotman and Greenwell, 1979) where the fitted result on a densitometric profile can always be compared for agreement with the original gel band pattern.

The limits and applicability of the curve-fitting method has been discussed by Anderson et al. (1970b) and Vandeginste and De Galan (1975) who concur that the number of bands or peaks in the system must be reliably identified or known before curve fitting is applied. This condition is especially problematic for the gliadin protein fraction

which separated in a cathodic electrophoretic system yields a spectrum of discrete, overlapped and severely overlapped protein components in which the number of bands apparently resolved in the electrophoregram field may be less than one-half of the total proteins actually present (Wrigley, 1970; Wrigley and Shepherd, 1973). A second source of error relates to the uncertainty in specifying the true position of the densitomegram baseline. The latter is not known due to the variable nature of the background staining in PAGE caused by relatively high MW protein which is extractable along with gliadins from gluten or flour in 70% ethanol and will streak rather than yield discrete bands upon electrophoresis. While this "undesireable" protein can be eliminated by size exclusion chromatography, this step is time consuming and not practical for routine electrophoretic analysis. These observations suggest that the potential payoff in terms of improved accuracy is uncertain in the use of curve-fitting to resolve overlapping components in gliadin electrophoregram densitometric profiles. The technique remains to be explored in the course of future program development.

The main task of the presented data acquisition system is to reduce single densitomegrams to a standardized list of normalized relative mobility and band density parameter values, termed a cultivar signature array. This permits comparisons to be made with band patterns on other gel slabs. A second important feature of this system is replicate analysis, i.e. combining individually computed gliadin band patterns for the same cultivar into a single mean signature array. The task is complicated by three factors: (i) variation in Rm for homologous bands in different replicates, (ii) small separation distances between

contiguous gliadin components in each pattern, and (iii) variation in the number of detected peaks, typically one or two components, in replicate scans. Variability of the third type commonly arises from imperfections in the data which originate from absorbance of precipitated dye or other anomalies on the surface of the PAGE slab. Replicate analysis thus functions not only to improve the precision of Rm and band density data, but also to eliminate spurious information which is detected by the program as unmatched relative peak coordinates.

The program was found to effectively handle variation in band positions of up to 1.5 relative mobility units and still successfully match homologous components in different replicate traces. No mis-matches were observed with the present set of replicate densitometric profiles although the program needs to be tested on a larger set of data to confirm its reliability. Electrophoregram results from the 3 mm PAGE method used in this study should prove useful as gliadin resolution was significantly improved yielding more complex absorbance patterns.

One problem which was encountered relates to visualized bands in the gel which remain undetected by the peak finding algorithm, or are found in only one replicate densitomegram and thus are identified by the program as noise. This situation, although infrequent, may arise when the densitometer fails to adequately resolve a faint or diffuse band migrating adjacent to a major dense component. Designing the data acquisition system to handle up to three replicate profiles in pairwise comparisons was shown to be an effective means to manage bands which lie at the fringe of detection. This permitted the acceptance of a peak if

it were found in only one of three pairwise combinations of replicate traces.

The inherent heterogeneity of gliadin composition confers a high level of discrimination ability. It has been shown that relatively few cultivars among Canadian and United States wheats possess identical PAGE patterns which occurs only for closely related genotypes (Zillman and Bushuk, 1979; Jones et al., 1982). Thus given a low likelihood that any one gliadin band is a critically diagnostic character, a specific band omission resulting from a failure to replicate would have little or no effect on the successful outcome of a computerized cultivar identification procedure.

A more important influence upon results of this process is the precision of the computed relative peak position data and their agreement with relative mobilities determined manually. This is especially relevant as the majority of cataloged gliadin electrophoregram data are based upon manual measurement reference procedures (Autran and Bourdet, 1975; Ellis and Beminster, 1977; Zillman and Bushuk, 1979; Dal Belin Peruffo et al., 1981; Jones et al., 1982). In the present study, the average variation of relative mobilities computed from densitometric profiles was $\pm$ 0.4 distance units which is comparable to values reported by other workers (Zillman and Bushuk, 1979; Jones et al., 1982). When the computed data was compared with Rm values determined manually from photographic prints, only small and insignificant deviations were obtained. These results indicate that the facility of a densitometer and minicomputer can be successfully implemented to provide an objective and precise method to quantitate

gliadin electrophoregrams in a format which is optimal for the cultivar identification application.

A variety of electrophoresis apparatuses were employed in this research project. Several important differences among apparatuses of different type were observed in the electrophoregram results. Not unexpectedly, application of a 3 mm flatbed machine compared to a 6 mm flatbed counterpart resulted in a significant improvement in the resolution of gliadin band patterns. The effect is largely related to the shortened duration of electrophoresis associated with the thinner gel slab (4 hr vs. 6 hr) which contributes to better separations by minimizing the effect of band spreading by diffusion. Accordingly, the 3 mm apparatus, a modified Bushuk and Zillman (1978) type of vertical design, was used to prepare an extensive collection of gliadin electrophoregrams for the computerized cultivar identification system.

As noted by other workers, electrophoretic apparatus design had a significant effect on band relative mobilities. In electrophoregrams derived by vertical PAGE, relative mobilities for bands of lowest and highest migration velocity were respectively increased and decreased by 5% compared to corresponding bands in horizontal PAGE slab results. The differential declined steadily as gliadin bands approached in mobility the position of the central reference protein, i.e. Marquis band 50. This result indicates that potential difficulties exist in the comparison of literature values for relative mobilities of gliadin components notwithstanding the application of a common nomenclature.

Ideally all elements of the electrophoresis system must be uniformly standardized to obtain reproducible data among laboratories,

however in practice this may be difficult to achieve. The finding that the relative mobility scale in one electrophoresis system can be stretched or compressed by curvilinear regression to facilitate accurate registration with the scale of mobilities in a different system has the potential use to mitigate problems associated with inter-laboratory comparison of results.

Several advantages are associated with the gliadin PAGE pattern data base prepared for this study apart from its computerized format which facilitates the comparative analysis process. Compared to the first catalog of electrophoregram formulas for 88 Canadian wheat cultivars (Zillman and Bushuk, 1979), the list of Canadian cultivars documented in this study was updated to 107. The increase accounts for 17 common and two durum wheat cultivars that have been licensed since the previous publication. The use of a thinner gel slab electrophoretic apparatus yielded a significant increase in the average number of digitized bands per electrophoregram which potentially improves the discrimination power of the cultivar identification system. A better resolved electrophoregram also improves the accuracy in determining relative mobilities as migration distance measurements are often ambiguous for overlapping protein bands which are more frequent in a less well resolved system.

The comprehensiveness of the data base was also improved as the entire collection of cultivar samples was surveyed electrophoretically on a bulk wheatmeal and single kernel basis. Approximately 20% were found to possess composite electrophoregrams with varying degrees of admixture. Many offtype patterns were thus incorporated into the

computerized catalog of cultivar formulas. While the identity of heterogeneous cultivar samples is important if single kernels are to be used for cultivar identification, no attempt was made in this study to determine the proportion of genotypes therein. This is not a trivial undertaking as it may require the sampling hundreds of single grains to obtain reliable estimates (Wrigley and Baxter, 1974).

The potential to detect small differences between gliadin bands was also increased as on average three replicate electrophoregrams were analyzed to obtain relative mobility values for a single cultivar formula. The data base was presented in the convenient form of cultivar formula arrays with relative mobilities reported to the nearest 0.1 unit. This represents a significant improvement in apparent precision compared to other catalogs of gliadin electrophoregram data where either half-integer (Zillman and Bushuk, 1979) or simple integer values (Autran and Bourdet, 1975; Jones et al., 1982; Dal Belin Peruffo et al., 1981) have been employed to specify band relative mobilities.

The use of a highly resolved electrophoregram mobility scale was warranted by the low level of uncertainty in the data determined by a novel computational procedure involving multiple reference bands. In this regard, as part of the computer-based system for cultivar identification developed in this study, a new gliadin nomenclature was introduced, substantially modifying the single reference band method of Bushuk and Zillman (1978). Two additional reference proteins were selected in the low and high mobility zones in electrophoregrams of the standard cultivar patterns used for every PAGE run. The three reference bands are then used in a weighted nearest neighbor algorithm which was implemented to compute relative mobilities for cultivar formulas.

The major advantage of this procedure is to significantly improve the precision of results compared to the single reference band approach by stabilizing the variability in Rm measurements at a low level across the entire electrophoregram field. The average uncertainty in the relative position of gliadin bands was reduced more than three-fold with mean standard deviations falling below 0.1 relative mobility units. The overall level of precision displayed by this method is substantially higher than that cited elsewhere in the relevant literature, and is considered to be unobtainable by the single reference band normalization approach.

Rigorous standardization of gliadin band positional data is justified for a number of reasons. For electrophoregrams run on the same gel slab, direct evidence suggests that a mobility separation of 0.5 units is sufficient to distinguish different gliadin bands in adjacent patterns. In practice however, comparative electrophoretic analysis for cultivar identification and related applications involves the comparison of mean relative mobilities for gliadin electrophoregrams run on different gels. If inherently small mobility differences are to be recognized then the critical requirement exists to minimize the variation between bands attributable to experimental error. In program logic for cultivar identification, assessments of gliadin band identity are made by comparing relative mobilities ± threshold. The size of this threshold on the one hand, will set the detection limit for different gliadin components in compared PAGE patterns, but must also be optimized to minimize rejecting true gliadin band identities. To detect a difference of 0.5 Rm units with a 95% confidence that mean mobilities

for identical bands in compared patterns fall inside this interval, requires that standard errors be less than 0.12 Rm units on a three replicate basis. This level of precision is well within the limits of experimental error for relative mobilities calculated by multiple reference bands, indicating that even smaller differences between gliadin bands are likely to be detected.

Strong evidence was also presented that rigorous standardization of relative mobility data is required to prevent significant levels of gliadin heterogeneity from escaping detection. A computer program was developed which assimilates cultivar formula data on a population basis and generates the frequency distribution of gliadin bands as a function of fine scale relative mobility. Visual analysis of the resulting distribution pattern termed a PAGE map, confirmed the existence of over 90 individual gliadin components among a population of 98 common spring and winter wheats in the cultivar identification data base. This number more than doubles previous estimates of gliadin heterogeneity by one-dimensional electrophoresis (Autran and Bourdet, 1975) and is more in line with the number of components revealed in single cultivars by two-dimensional methodology (Wrigley and Shepherd, 1973).

It was observed that gliadin electrophoregram heterogeneity was sufficiently discontinuous so that common distributions of proteins were identifiable as more or less isolated clusters of points across the range of relative mobilities. The significance of this result for inter-laboratory comparisons or classification analysis relates to the ease with which discriminant boundaries may be established across the PAGE map to facilitate the identification of different gliadin variate

populations. For inter-laboratory comparisons, gliadin components would be identified not by a discrete mobility value, but in accord with the specific band population with which it is affiliated. Using the PAGE map as a reference spectrum would also effectively solve the problem of converting electrophoregrams to numbered classes based on subjective and possibly erroneous interpretation of electrophoregrams. Such a conversion is fundamental in research investigating the complex relationships between variation in protein composition and functional quality using multivariate analysis methods. Given the substantial increase in the number of gliadin bands revealed in the PAGE map compared to attribute lists used by other workers, a multivariate analysis study on the present set of data should yield considerable further knowledge and insight on the nature of the association between protein quality and electrophoregram composition.

During the course of this research project, workers affiliated with the U.S. Grain Marketing Research Laboratory at Manhattan Kansas reported a computer-assisted method for wheat cultivar identification by gliadin electrophoregrams (Lookhart et al., 1983). Compared to this recent work, the use of programs of the cultivar identification system developed in the present study offers several advantages:

1. It provides an explicit estimate of errors associated with the determination of band relative mobilities which can be computed by the single or higher precision multiple reference band method.

2. Relative mobility values are calculated and manipulated using decimal accuracy to minimize false band matches and improve discrimination ability.

3. Difference thresholds for band mobilities and densities are user defined and can be established in accordance with experimental error estimates.

4. The equation used to compute pattern homology scores which determine the order of cultivar ranking includes both matching and non-matching band counts in the formula. This fully quantifies the heterogeneity of gliadin band patterns for comparative electrophoregram analysis, and provides a sensitive measure for discrimination.

5. The system is information oriented to provide a comprehensive basis to interpret program output. The short list ranking program, in addition to generating cultivar names and pattern homology scores, includes pedigrees and summary attributes for quality, class and primary growing region. The printout also provides an extensive tabulation of the distribution of matching and non-matching bands for compared electrophoregrams.

6. To evaluate this numerical result a graphic analysis program permits the user to visualize the changing gliadin composition of matching and non-matching bands which are respectively isolated in separate plots for the list of ranked cultivars. An alternate pattern homology analysis program can also be invoked to provide a similar type of output focused on selected pairs of electrophoregrams of special interest. The speed and detail of the analysis is also ideally suited to genetic studies where typically large numbers of lines are evaluated in terms of discrete electrophoretic pattern similarities, differences and recombinants.

7. A third program element of the cultivar identification system complements other analyses by using positional differences in electrophoregrams as the criterion for ranking. It also provides an output result which is extended to include the entire data base population in which each member is explicitly identified in a frequency distribution which comprises the printout. This program assists in evaluating the uniqueness of the unknown or test electrophoretic pattern, and identifies cultivars lying at the margins of the distribution which are of diverse genotype. The analysis yielded evidence which indicated that much inherent variability remains to be exploited among certain Canadian bread wheat cultivars.

In conclusion, the major contribution of this thesis is the elaboration and implementation of numerous computer-based strategies to improve precision and accuracy and reduce the arbitrariness in the quantitation and comparative use of gliadin electrophoregrams. The methodology described offers a fast, reliable and comprehensive system for cultivar identification and has provided new estimates of gliadin

heterogeneity in a standardized format which has much potential to further knowledge on the nature of the association between protein quality and electrophoregram composition.

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Appendix A

THE EFFECT OF GLIADIN EXTRACTION TIME ON PAGE RESOLUTION

Due to the large number of cultivar samples used to establish a data base of gliadin PAGE patterns for cultivar identification, gliadin extraction time was an important factor in determining the total number of electrophoresis samples which could be prepared in a given period. Common extraction times using ground grain or single kernels are from 1 h (e.g. Bushuk and Zillman, 1978; Wrigley and McCausland, 1977) to 2 h (e.g. Autran and Bourdet, 1975). However an optimum or minimum extraction interval with respect to gliadin PAGE composition has not been reported in the literature. Similarly, centrifugation procedures used to clarify the gliadin extract vary considerably, e.g. from 4,550xg, 10 min (Lookhart et al., 1982) to 150,000xg, 10 min (Tkachuk and Mellish, 1980).

To determine an optimum or minimum extraction time to produce a suitable electrophoretic result, the following times were examined:

1. 0 time, i.e. initial mix followed immediately by centrifugation;
2. 10 min;
3. 20 min;
4. 1 h;
5. 2 h;
6. 3 h;

Ground subsamples of grain (50 mg, Udy Cyclone Mill) of cv. Neepawa were placed in 1.5 ml microfuge tubes to which 100 ul of 70% ethanol was added. The mixtures were initially vortexed for 5 sec and again every 20 min for the duration of the standing interval for the respective extraction sample. Mixtures were vortexed briefly prior to centrifugation. Three procedures for clarifying the gliadin extract were also examined:

1. 8,000xg, 5 min with a tabletop microcentrifuge (Beckman Microfuge B);
2. As above, but for 10 min;
3. 20,000xg, 10 min using a Beckman Model J2-21 centrifuge (JA-20 rotor).

After centrifugation, 30 ul of supernatant was diluted with 45 ul of a gliadin extract dilution solution which consisted of electrophoresis tank buffer (refer to Table 7) containing 40% w/v sucrose and 0.6% w/v methyl green dye. Electrophoresis was carried out as described by Bushuk and Zillman (1978) with some modifications.

Essentially the same effects were observed for each of the centrifugation procedures. The PAGE result is shown for the 5 min spin (Figure 50) and indicates virtually no modification in the apparent density of the gliadin bands by increasing the extraction time. Thus a 10 min gliadin extraction time using a highly portable tabletop centifuge to clarify the extract was adequate to obtain suitable electrophoregrams for cultivar identification.

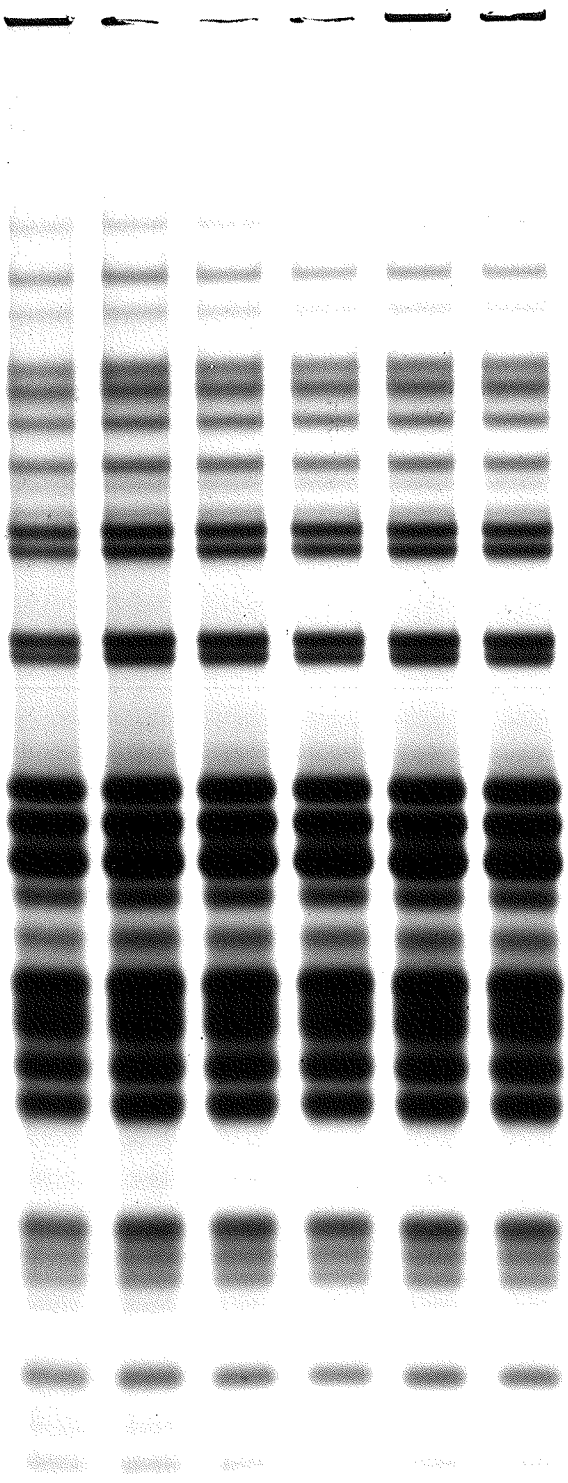
Figure 50. The effect of gliadin extraction time on electrophoregrams of cultivar Neepawa.

Extraction times from left to right:

0 time - 10 sec (meal + extracting solution) mix followed immediately by centrifugation.
10 min
20 min
1 hr
2 hr
3 hr

Conditions:

Extracting solution	: 70% ethanol
Polyacrylamide gel	: 6%
Buffer	: Aluminum lactate (pH 3.1)
Protein stain	: Coomassie Blue



Appendix B

STRUCTURE OF MIGRATION DISTANCE INPUT DATA FILE AND PROGRAM STATWT3 OUTPUT OF RELATIVE MOBILITIES COMPUTED BY MULTIPLE REFERENCE BANDS

Figure 51 illustrates the structure of a typical cultivar data file required to satisfy the input format requirements of program STATWT3. The latter computes relative mobilities and along with band densities, further orders the data into the standardized format of a cultivar signature array (refer to Table 18) which can be used directly by the various programs of the cultivar identification system.

The first row or record of the data file is reserved for the cultivar name. The first column in Figure 51 lists the assigned densities of gliadin bands for cv. Sinton. The remaining columns of data give the individual replicate migration distances in cm for each gliadin electrophoregram. The last three rows in the file following a blank data record contain migration distance values for reference bands R50, R24 and R79 respectively. The program can handle missing migration distances (excluding reference bands) so long as the value for at least one replicate is specified. Missing entries would ordinarily denote a band that could not be reliably identified in the positive print either because it was too faint to be detected or due to some anomaly on the surface of the gel slab or print which made the position of the band ambiguous.

```
SINTON
5    03.830   03.790   03.705
5    04.531   04.480   04.394
3    05.567   05.520   05.453
2    05.910   05.862   05.770
4    06.403   06.376   06.286
1    06.629   06.574   06.545
2    07.066   07.026   06.946
5    07.325   07.292   07.239
4    07.640   07.605   07.546
5    08.920   08.852   08.836
5    09.184   09.119   09.098
2    10.480   10.384   10.447
6    10.988   10.833   10.883
7    11.442   11.305   11.361
2    11.722   11.560   11.635
4    12.080   11.920   11.976
4    12.613   12.438   12.542
4    13.015   12.801   12.936
8    13.680   13.432   13.599
5    13.793   13.567   13.731
7    14.172   13.952   14.142
6    14.742   14.503   14.717
6    15.171   14.949   15.202
3    15.319   15.111   15.387
4    16.784   16.543   16.972
3    17.129   16.914   17.337
2    17.434   17.237   17.637
1    17.749   17.562   17.988
3    18.681   18.420   18.978
1    19.090   18.890   19.441
1    19.278   19.052   19.649
2    19.784   19.560   20.142

     11.968   11.831   11.890
     05.760   05.758   05.618
     18.676   18.463   18.963
```

Figure 51: Computer file of band densities and migration distance values in cm for replicate electrophoregrams of cv. Sinton

Table 18 represents the computer printout of the input data file (Figure 51) processed by program STATWT3. Standard deviations across

TABLE 18

Computer printout of relative mobilities, statistics and cultivar signature array for cv. Sinton generated by program STATWT3

BAND	DENS.	MEAN RM	S.DEV.	REP.1	REP.2	REP.3
1	5	15.78	0.09	15.9	15.7	15.7
2	5	18.68	0.10	18.8	18.6	18.7
3	3	23.05	0.15	23.1	22.9	23.2
4	2	24.45	0.11	24.5	24.3	24.5
5	4	26.59	0.10	26.6	26.5	26.7
6	1	27.54	0.22	27.5	27.3	27.8
7	2	29.36	0.10	29.3	29.3	29.5
8	5	30.50	0.16	30.4	30.4	30.7
9	4	31.82	0.13	31.7	31.7	32.0
10	5	37.18	0.15	37.1	37.1	37.4
11	5	38.30	0.12	38.2	38.2	38.4
12	2	43.81	0.20	43.7	43.7	44.0
13	6	45.78	0.12	45.9	45.6	45.8
14	7	47.76	0.06	47.8	47.7	47.8
15	2	48.91	0.08	49.0	48.8	48.9
16	4	50.41	0.06	50.5	50.4	50.4
17	4	52.69	0.07	52.8	52.6	52.7
18	4	54.33	0.14	54.5	54.2	54.3
19	8	57.10	0.20	57.3	56.9	57.0
20	5	57.63	0.15	57.8	57.5	57.6
21	7	59.30	0.13	59.4	59.2	59.3
22	6	61.70	0.16	61.9	61.6	61.6
23	6	63.62	0.11	63.7	63.5	63.6
24	3	64.32	0.09	64.4	64.2	64.4
25	4	70.68	0.18	70.7	70.5	70.8
26	3	72.21	0.11	72.2	72.1	72.3
27	2	73.53	0.02	73.5	73.5	73.6
28	1	74.94	0.05	74.9	75.0	75.0
29	3	78.90	0.15	78.9	78.7	79.0
30	1	80.81	0.12	80.7	80.8	80.9
31	1	81.59	0.19	81.5	81.5	81.8
32	2	83.71	0.13	83.6	83.6	83.9

SIGNATURE ARRAY FOR CULTIVAR:
SINTON

158 5 187 5 231 3 245 2 266 4 275 1 294 2 305 5 318 4 372 5
383 5 438 2 458 6 478 7 489 2 504 4 527 4 543 4 571 8 576 5
593 7 617 6 636 6 643 3 707 4 722 3 735 2 749 1 789 3 808 1
816 1 837 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
0 0 32 3 0 0 0 0 0 0

the Rm spectrum for cv. Sinton range from 0.02 to 0.22 relative mobility units. This shows the positive effect of the three reference band computing technique (refer to pages 165-179) to stabilize the variability in Rm values at a low level.

Program STATWT3 can also be used in the one reference band mode to compute Rm data relative to Marquis band 50 following the approach of Bushuk and Zillman (1978). This is accomplished by deleting program records tagged with the letter "A" in the source listing which is available from the author.

Appendix C

OFF-TYPE ELECTROPHOREGRAMS IN THE CULTIVAR IDENTIFICATION DATA BASE

Heterogeneity in PAGE composition of a cultivar sample can derive from a number of likely sources which include variation indigenous to the breeder seed from segregants of the original cross(es), mechanical mixture with grain of known or unknown identity and outcrossing. The identity of heterogeneous cultivar samples is important if single kernels are to be used for varietal identification. Also important is the fact that relative mobility position assignments in complex mixtures of gliadin components may become ambiguous for overlapping protein bands. Previously reported biotypes in Canadian wheat cultivars include BW20[1] and Sinton (Kosmolak, 1979), Marquis (Kosmolak and Kerber, 1980), Canuck, Chester and Napayo (Tkachuk and Mellish, 1980).

The strategy to identify heterogeneous cultivar samples is described below. Where variant electrophoregrams were found contributing to the PAGE composition of the bulk, no attempt was made to determine the proportion of genotypes therein. The latter is not a trivial undertaking and requires the sampling of hundreds of single grains to obtain reliable estimates (Wrigley and Baxter, 1974). For each cultivar listed in Tables 4-6, a minimum of two gliadin extracts were prepared for electrophoresis. These were derived from at least one single kernel

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[1]Licensed as Benito.

and one ground sample of grain; for the first 50 numbered cultivars, a minimum of three extracts was used. All replicates were run on separate gel slabs.

If the electrophoregram from the bulk wheatmeal source precisely matched its single kernel counterpart(s), then the cultivar sample was assumed to be homogeneous and mean Rm data was subsequently computed from the replicate electrophoretic patterns. If on the other hand, the two patterns were found not to correspond, usually where the electrophoregram from the ground sample contained bands which complemented the single kernel pattern but not vice versa, then a series of single grains was examined (typically 9-27) to twice account for the dominant type pattern(s) contributing to the composite electrophoregram of the mixture.

To distinguish pure samples in the wheat cultivar identification data base, the name of a cultivar determined to be electrophoretically heterogeneous was appended with the code letter "M", to indicate that the electrophoregram from the ground sample was a composite pattern derived from a mixture. Lines or biotypes within the mixture possessing electrophoregrams consistent with progeny derived from the same parents and contributing to the composition of the bulk pattern were given the name extension "CB" (only one found), or "CB1", "CB2", etc. to denote contributing biotypes.

Cultivars for which the complement of found biotype patterns completely account for the PAGE composition of the ground sample include Prelude (Figure 52C), Ceres (Figure 52E), Lee (Figure 52F), Selkirk (Figure 53B), Napayo (Figure 53D), Canuck (Figure 54A), and Richmond

(Figure 56C). Cultivars for which one or more biotypes remain to be found include Rescue (Figure 55B), Lemhi 53 (Figure 55F) and Jones Fife (Figure 56D). Additional cultivars for which minor disagreements were observed in gliadin PAGE composition of the ground grain sample and single kernels include Ruby, Saunders, Glenlea, Norquay, Lemhi 62, Winalta, Thorne and Wakooma. These eight cultivar are not represented here by figures depicting their gliadin PAGE compositions.

Single kernel PAGE analysis frequently uncovered electrophoregrams which were similar in composition to the bulk sample, except for a few unique gliadin components which could not be identified in the electrophoregram of the mixture. These patterns were given the data base name extension "NCB" to denote a non-contributing biotype. Cultivar samples which were found to possess this kernel type include Early Red Fife (Figure 52A), Katepwa (Figure 53C), Chester (Figure 54C) and Kenhi (Figure 55E).

Electrophoregrams from single kernels which had little homology with the bulk pattern and therefore are not consistent with having been derived from the same cross were given the offtype name extensions "CU" or "NCU". These codes denote kernels of unknown origin which respectively contribute or do not contribute to the PAGE composition of the bulk. Cultivar samples which include patterns of this type are Katepwa (Figure 53,C5), Chester (Figure 54,E6). Waldron (Figure 55,C3,C4), Lemhi 53 (Figure 55,F4), Quality A (Figure 56,A4) and Dawbul (Figure 56,B4).

In some instances the contributing unknown pattern was subsequently identified. For example, the Katepwa sample from the 1980 Central Bread

Figure 52. Gliadin electrophoregrams for off-type cultivar samples.

Pattern	Cultivar Sample	(OFF-TYPE CODE FOR DATA BASE)
A 1	Early Red Fife - Ag.Can. Lethbridge	(LTH)
A 2	Early Red Fife - "	(LTH_CB)
A 3	Early Red Fife - "	(LTH_NCB)
B 1	Early Red Fife - Ag.Can. Ottawa	(PGR_M)
B 2	Early Red Fife - "	(PGR_CU1)
B 3	Early Red Fife - "	(PGR_CU2)
B 4	Early Red Fife - "	(PGR_CU3)
C 1	Prelude -	(M)
C 2	Prelude -	(CB1)
C 3	Prelude -	(CB2)
D 1	Preston - Ag.Can. Lethbridge	(LTH)
D 2	Preston - Ag.Can. Ottawa	(PGR)
E 1	Ceres -	(M)
E 2	Ceres -	(CB1)
E 3	Ceres -	(CB3)
E 4	Ceres -	(CB2)
E 5	Ceres -	(CB4)
F 1	Lee -	(M)
F 2	Lee -	(CB1)
F 3	Lee -	(CB2)

Conditions:

Polyacrylamide gel: 6%
Buffer : Aluminum lactate (pH 3.1)
Protein stain : Coomassie Blue

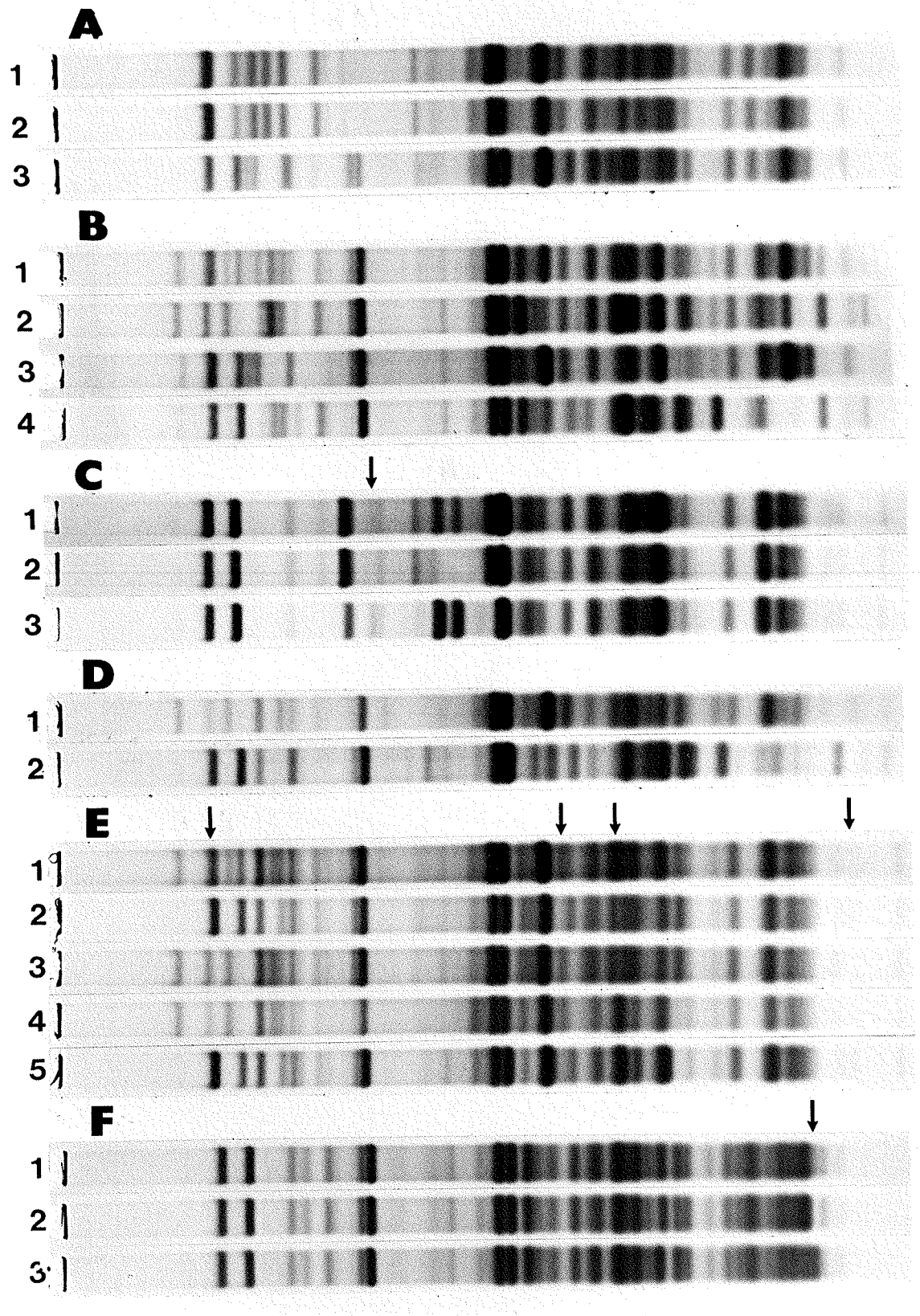
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Figure 53. Gliadin electrophoregrams for off-type cultivar samples.

Pattern	Cultivar Sample	(OFF-TYPE CODE FOR DATA BASE)
A 1	Reliance - Ag.Can. Ottawa	(PGR)
A 2	Reliance - Ag.Can. Lethbridge	(LTH_M)
A 3	Reliance - "	(LTH_CB1)
A 4	Reliance - "	(LTH_CB2)
A 5	Reliance - "	(LTH_CB3)
A 6	Reliance - "	(LTH_CB4)
B 1	Selkirk - (M)	
B 2	Selkirk - (CB1)	
B 3	Selkirk - (CB2)	
B 4	Selkirk - (CB3)	
C 1	Katepwa - Breeder seed	
C 2	Katepwa - "	(NCB)
C 3	Katepwa - 1980 CBWC Test	(CBW_M)
C 4	Katepwa - "	(CBW_CB)
C 5	Katepwa - "	(CBW_CU)
C 6	Katepwa - "	(CBW_NCB)
D 1	Napayo - (M)	
D 2	Napayo - (CB1)	
D 3	Napayo - (CB2)	

Conditions:

Polyacrylamide gel: 6%
Buffer : Aluminum lactate (pH 3.1)
Protein stain : Coomassie Blue

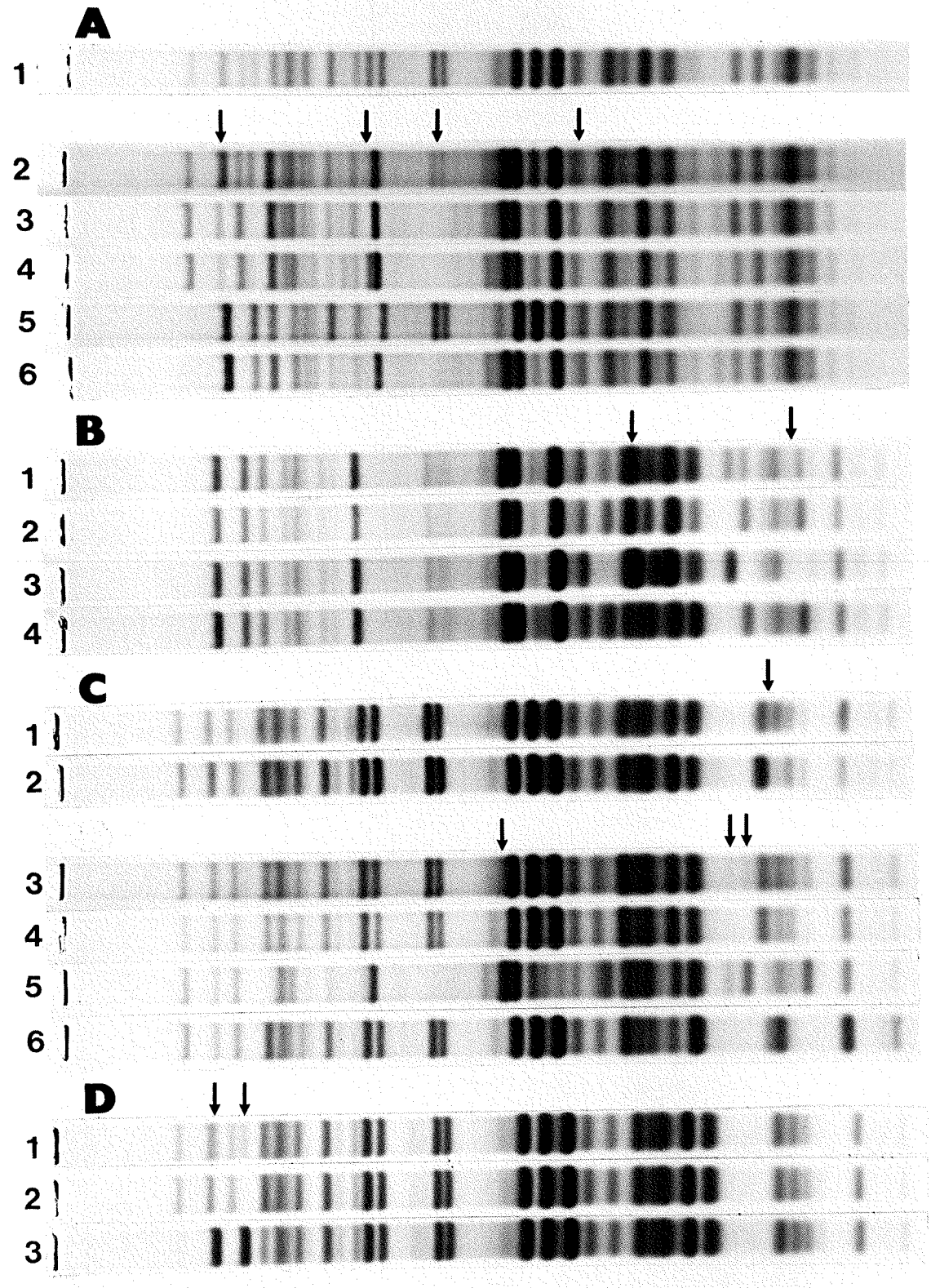
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Figure 54. Gliadin electrophoregrams for off-type cultivar samples.

Pattern	Cultivar Sample (OFF-TYPE CODE FOR DATA BASE)
A 1	Canuck - (M)
A 2	Canuck - (CB1)
A 3	Canuck - (CB2)
B 1	Chester - Breeder seed (M)
B 2	Chester - Ag.Can. Ottawa (PGR_M)
B 3	Chester - 1978 WBWC test (WBW_M)
B 4	Chester - 1980 UQN test (UQN_M)
C 1	Chester - Breeder seed (M)
C 2	Chester - " (CB)
C 3	Chester - " (NCB1)
C 4	Chester - " (NCB2)
D 1	Chester - Ag.Can. Ottawa (PGR_M)
D 2	Chester - " (PGR_CB)
E 1	Chester - 1980 UQN (UQN_M)
E 2	Chester - " (UQN_CB)
E 3	Chester - " (UQN_NCB1)
E 4	Chester - " (UQN_CU1)
E 5	Chester - " (UQN_CU2)
E 6	Chester - " (UQN_NCU)

Conditions:

Polyacrylamide gel: 6%
Buffer : Aluminum Lactate (pH 3.1)
Protein stain : Coomassie Blue

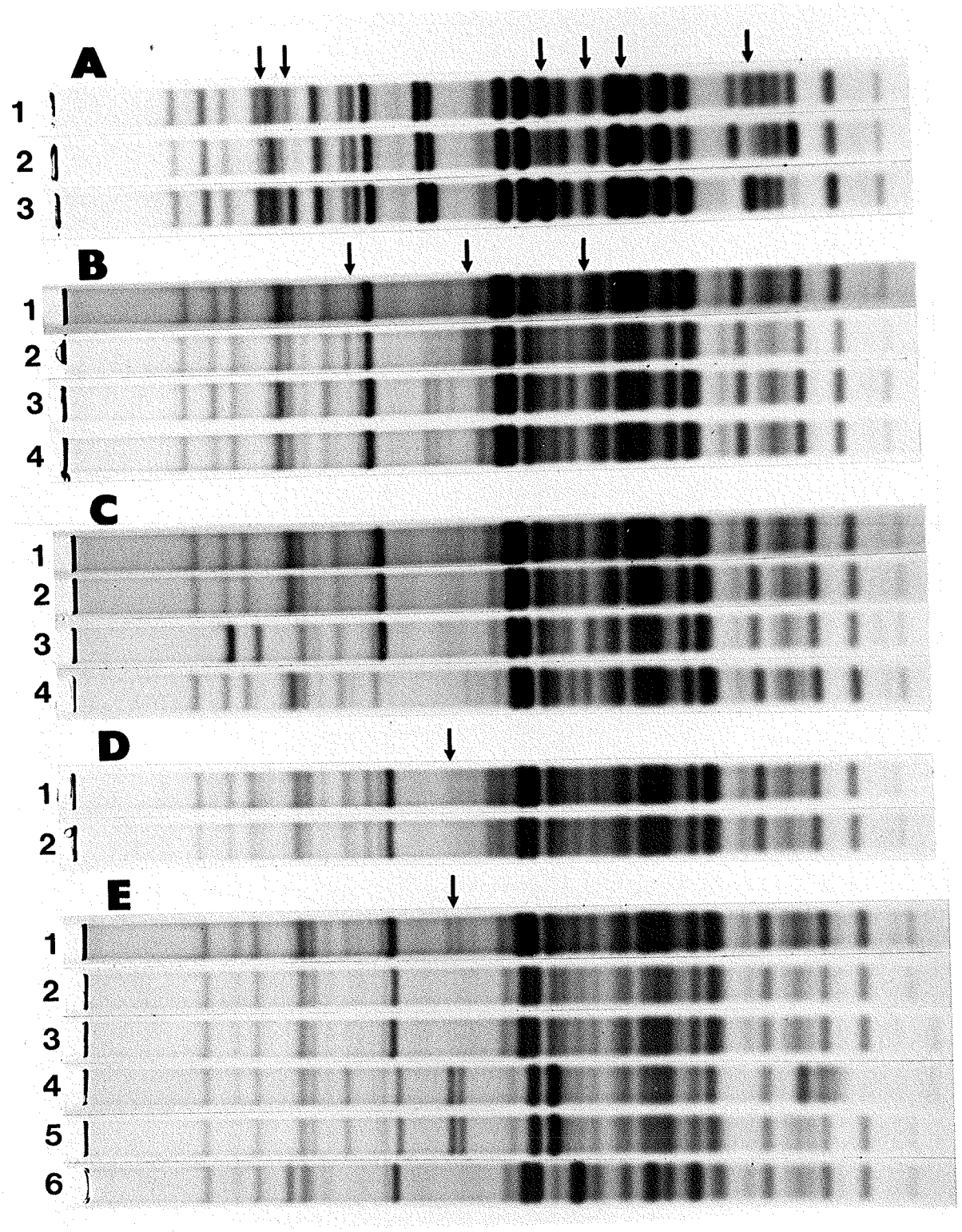
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Figure 55. Gliadin electrophoregrams for off-type cultivar samples.

Pattern	Cultivar Sample (OFF-TYPE CODE FOR DATA BASE)
A 1	Rescue - Ag.Can. Ottawa (M)
A 2	Rescue - " (CB1)
A 3	Rescue - " (CB2)
B 1	Rescue - Ag.Can. Lethbridge (M)
B 2	Rescue - " (CB) high protein
B 3	Rescue - " (CB) low protein
C 1	Waldron - bulk
C 2	Waldron - (CB)
C 3	Waldron - (NCU1)
C 4	Waldron - (NCU2)
D 1	Red Bobs 222 - Ag.Can. Lethbridge (LTH)
D 2	Red Bobs 222 - Ag.Can. Ottawa (PGR)
E 1	Kenhi - bulk
E 2	Kenhi - (CB)
E 3	Kenhi - (NCB)
F 1	Lemhi 53 - (M)
F 2	Lemhi 53 - (CB1)
F 3	Lemhi 53 - (CB2)
F 4	Lemhi 53 - (NCU)

Conditions:

Polyacrylamide gel	6%
Buffer	Aluminum Lactate (pH 3.1)
Protein stain	Coomassie Blue

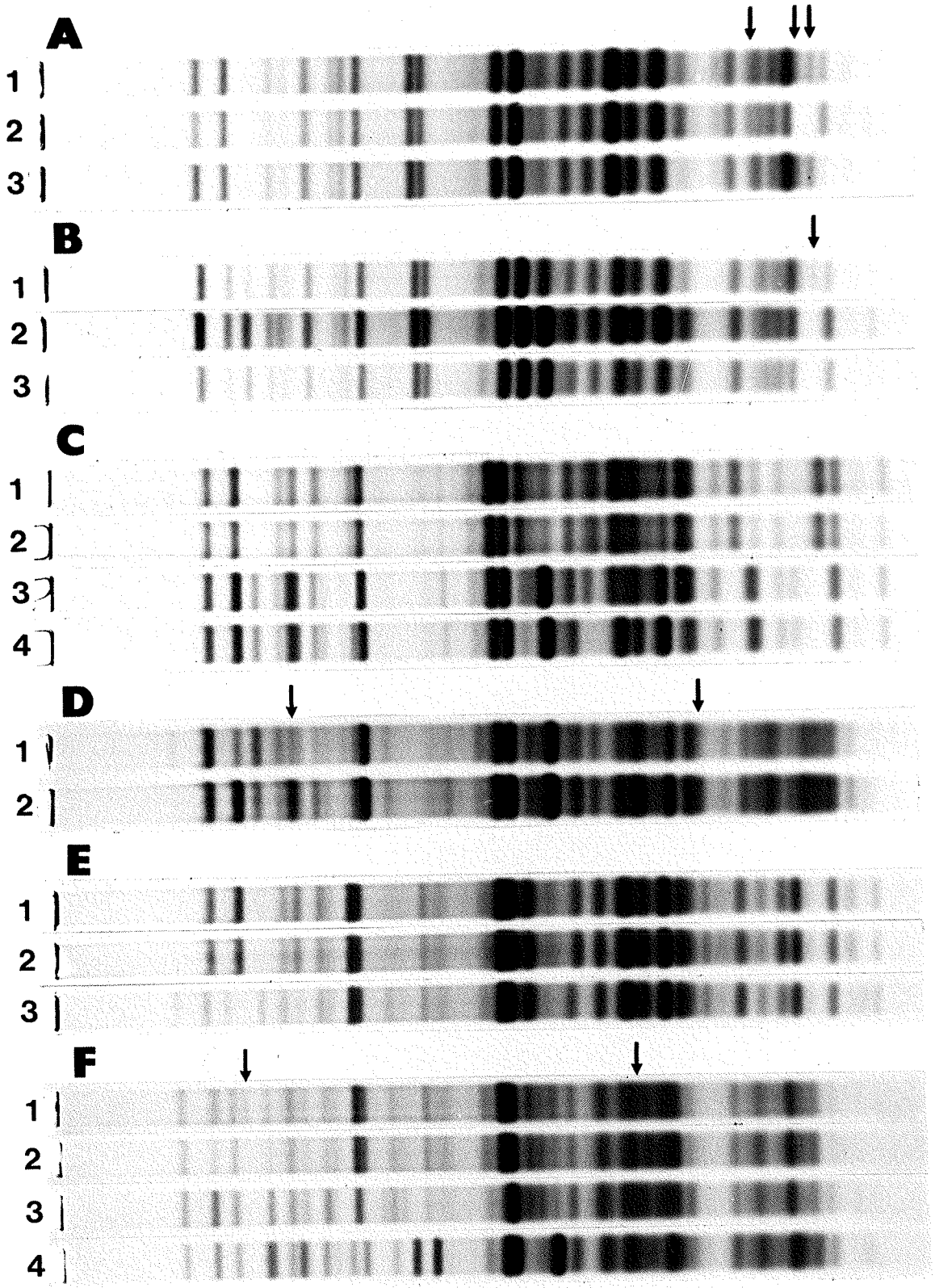
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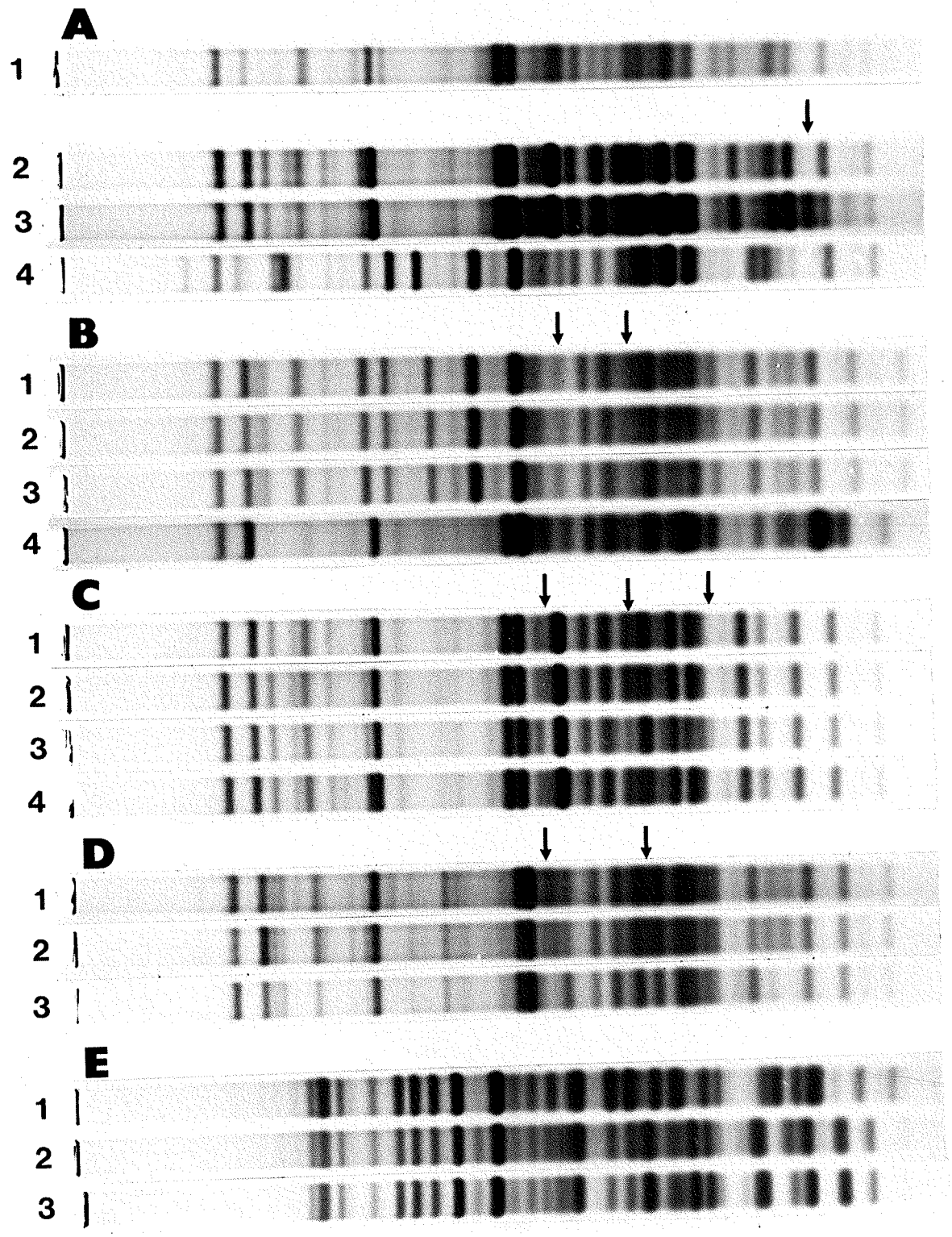
Figure 56. Gliadin electrophoregrams for off-type cultivar samples.

Pattern	Cultivar Sample (OFF-TYPE CODE FOR DATA BASE)
A 1	Quality A - Ag.Can. Lethbridge (LTH)
A 2	Quality A - Ag.Can. Ottawa (PGR)
A 3	Quality A - " (PGR_NCB)
A 4	Quality A - " (PGR_NCU)
B 1	Dawbul - (M)
B 2	Dawbul - (CB1)
B 3	Dawbul - (CB2)
B 4	Dawbul - (NCU)
C 1	Richmond - (M)
C 2	Richmond - (CB2)
C 3	Richmond - (CB3)
C 4	Richmond - (CB1)
D 1	Jones Fife - (M)
D 2	Jones Fife - (CB1)
D 3	Jones Fife - (CB2)
E 1	Carleton - Ag.Can. Ottawa (PGR)
E 2	Carleton - Ag.Can. Winnipeg (WPG)
E 3	Ramsey

Conditions:

Polyacrylamide gel: 6%
Buffer : Aluminum Lactate (pH 3.1)
Protein stain : Coomassie Blue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2
3

Wheat Co-op Test (Figure 53,C3) was found to be contaminated to a significant extent with grain from cv. Chester (Figure 54C) as the latter is identical to the electrophoregram of the "contributing unknown" shown in Figure 53,C5. Not surprisingly cv. Chester was present in the same Co-op test.

The final category of offtype PAGE pattern was characterized by duplicate samples from different sources which gave non-identical electrophoregrams. PAGE pattern discrepancies involving the two major sources of seed samples used in this study [Agriculture Canada Research Station, Lethbridge (given the offtype code "LTH") and The Plant Gene Resources of Canada at The Ottawa Research Station of Agriculture Canada (given the code "PGR")] include the cultivars Early Red Fife, Preston, Reliance, Rescue, Red Bobs 222 and Quality A. All these cultivars represent wheats licensed in Canada prior to 1947, non are in current production and thus the problem of non-matching patterns is relatively innocuous. Authentic patterns for three of these cultivars on the basis of pedigree are as follows:

Early Red Fife - Figure 52A (LTH)
Preston - Figure 52,D1 (LTH)
Rescue - Figure 55B (LTH)

For cvs. Reliance, Red Bobs 222 and Quality A, cultivar samples from at least one additional source needs to be evaluated before a determination of authenticity can be made.

Three types of PAGE composition for cv. Chester is presented in Figure 54 (patterns B1, B2 and B3) derived from a bulk sample of breeder seed (source: Canadian Grain Commission, Grain Research Laboratory), and

cultivar samples from Plant Gene Resources, and the 1978 Western Bread Wheat Co-op Test respectively. The latter is identical to the electrophoregram derived from the 1980 Uniform Quality Nursery (Figure 54,B4).

One final example of offtype electrophoregrams arising from source disagreements is shown by the PAGE patterns of the durum wheat cultivar Carleton obtained from The Plant Gene Resources (Figure 56,E1) and Agriculture Canada Research Station, Winnipeg (ACRSW, Figure 56,E2). The latter is similar to the electrophoregrams represented as Ramsay and Carleton by Zillman (1978). The Carleton sample obtained for this thesis from ACRSW is likewise shown to be identical to the gliadin pattern of cv. Ramsay (Figure 56,E3). Because the pedigrees of cvs. Carleton and Ramsay (refer to Table 6) are sufficiently different to account for significant differences in PAGE composition, the Carleton sample from The Plant Gene Resources of Canada is presumed to be authentic.

Appendix D

CALIBRATION OF ELECTROPHOREGRAM DATA BETWEEN PAGE SYSTEMS

Since 1975, systematic keys or numerical catalogs of gliadin band data by cathodic SGE and PAGE have been separately published for over 500 Australian, Canadian, English, French, Italian, U.S.S.R. and U.S. common and durum wheat cultivars (see literature review). Unfortunately the composite value of this bulk of protein composition data whether for genetic or functional considerations is severely limited as the correspondence between laboratories with respect to the identity of individual gliadin bands is largely unknown. The difficulties in attempting to cross-reference within and among different gel support media were discussed in a collaborative study of major electrophoretic systems by Autran et al. (1979) who observed that "reproducibility of pattern should be possible with strict attention to standardization of reagents, extraction procedure, gel medium, and apparatus".

Progress in recent years towards a consensus on a universal electrophoresis system for the purpose of cultivar identification is evident by the number of workers who have adopted, for example, the basic features of the cathodic PAGE method of Bushuk and Zillman (1978). However modifications with respect to the type of electrophoresis buffer (Khan et al., 1983), buffer concentration and apparatus (Lookhart et al., 1982), polyacrylamide concentration (Dal Belin Peruffo et al., 1981) and type of apparatus alone (Redman et al., 1980) continue to

preclude an ideal basis for direct inter-laboratory comparison of results in the literature. An attempt was therefore made to find a reliable empirical relationship between gliadin band relative mobilities for cultivars run on different electrophoresis systems.

The electrophoregrams used for this investigation were derived from the horizontal and vertical flatbed PAGE methodologies previously described (see Materials and Methods section). As these two systems possess differences with respect to polyacrylamide gel composition, source of aluminum lactate and buffer volumes, in addition to the years in which gliadin electrophoregrams were obtained (horizontal PAGE - 1979, vertical PAGE - 1983), they reasonably reflect experimental conditions that can be associated with separate laboratories.

More significant is the fact that calculated relative mobilities (Bushuk and Zillman, 1978) for electrophoregrams by vertical PAGE were found to be retarded by approximately 5% for bands which move the greatest distance into the gel, and are increased by 5% for bands with low migration velocity. This differential, for outlying bands in the electrophoregram field, reduces to zero as migrating components approach the mobility of the central Marquis reference band. This effect is reflected in the comparison of vertical and horizontal PAGE patterns shown in Figure 19 where, apart from resolution differences, it can be seen that patterns from corresponding cultivars fail to align along the full length of the electrophoregram field.

In order to eliminate confounding experimental factors, duplicate PAGE runs were performed concurrently using common gel (200 ml) and tank buffer (1000 ml) solutions (refer to Table 7) divided equally between

vertical and horizontal 3 mm flatbed electrophoresis apparatuses. Both were modified from the 6 mm design described by Bushuk and Zillman (1978). Electrophoresis of gliadin extracts from cvs. Marquis and Neepawa was carried out at a constant current of 55 ma in both apparatuses, with circulating coolant maintained at a constant temperature of 20°C.

Results of these experiments are shown in Figure 57, and clearly demonstrate that factors related to the physical design of the electrophoresis apparatus can contribute to significantly modify band relative mobilities. Similar observations have only infrequently been referred to in the relevant literature. Autran et al. (1979) made the general observation that apparatus design was a factor that could partly explain different SGE results in terms of band number and distribution. Khan et al. (1983) found that relative mobility values for gliadin components of cv. Marquis varied between a horizontal (Bushuk and Zillman, 1978) and vertical (E-C 470) apparatus.

While further study is required to ascertain the source of the apparatus effect, derived relative mobility data provided the means to evaluate the accuracy of transforming vertical PAGE results to the scale of relative mobilities obtained from horizontal PAGE by least squares regression procedures.

The analysis was based on a set of 167 paired observations[1] of relative mobility (Rm) values for corresponding gliadin bands in electrophoregrams of six cultivars (spring wheats Marquis, Napayo,

[1]Each observation in the set of data submitted to regression analysis represents the mean of approximately three relative mobility determinations for replicate electrophoregrams run on separate PAGE slabs.

Figure 57. Gliadin electrophoregrams of cultivars Neepawa and Marquis derived by vertical and horizontal 3 mm flatbed PAGE

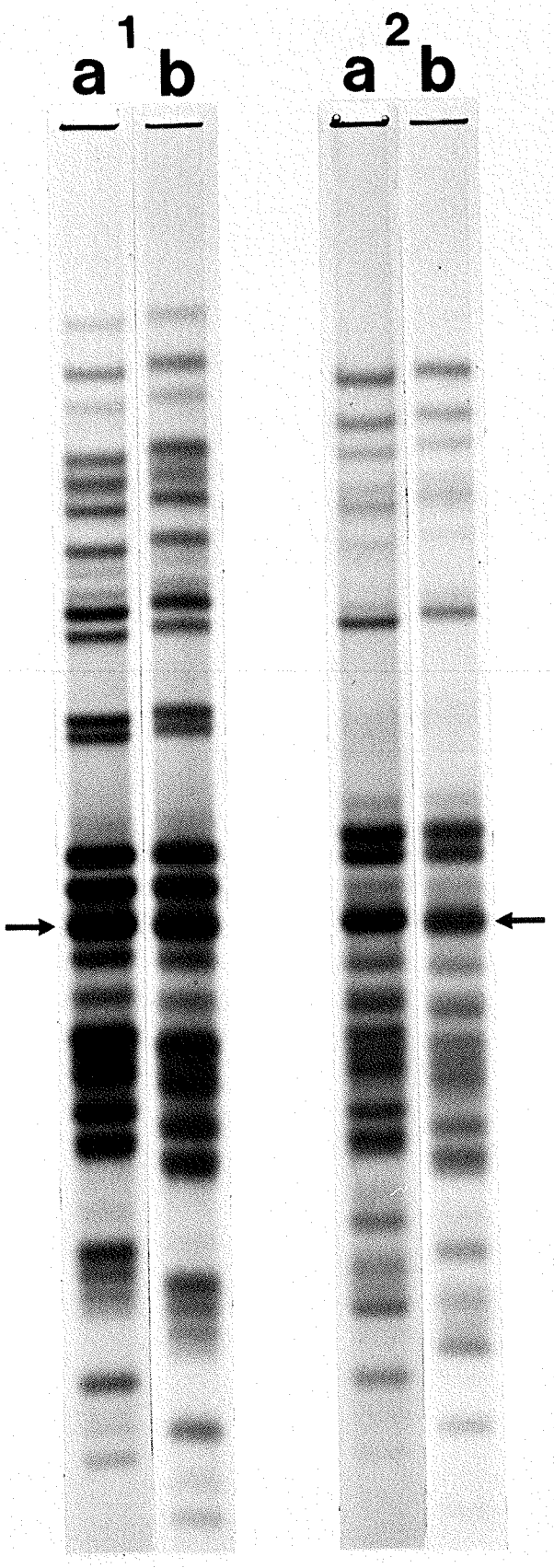
Patterns	Cultivar
1	Neepawa
2	Marquis

Conditions (a)

Apparatus	: Vertical (3 mm gel bed thickness)
Polyacrylamide gel	: 6%
Buffer	: Aluminum lactate (pH 3.1)
Protein stain	: Coomassie Blue

Conditions (b)

Apparatus	: Horizontal (3 mm gel bed thickness)
Polyacrylamide gel	: 6%
Buffer	: Aluminum lactate (pH 3.1)
Protein stain	: Coomassie Blue

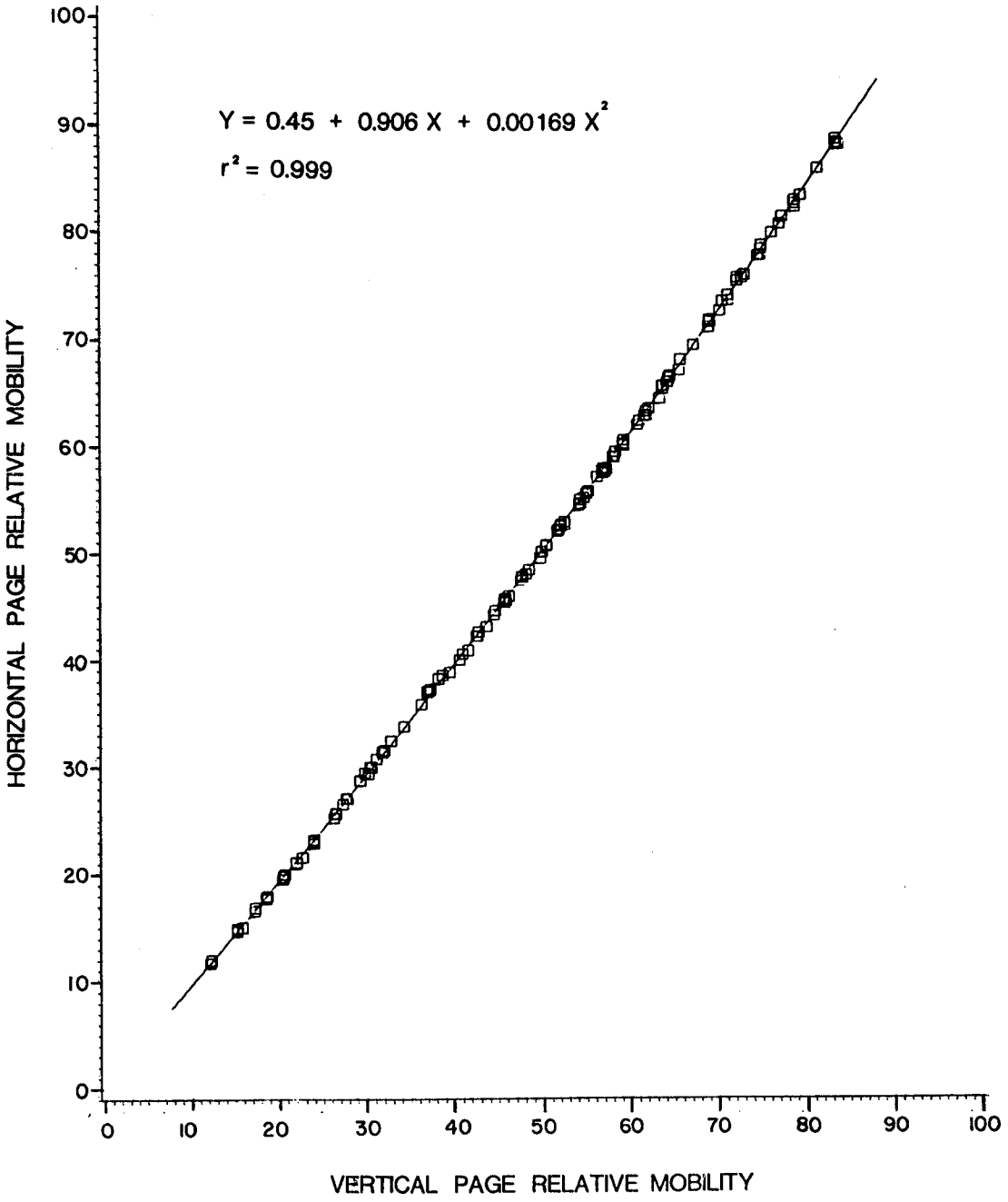

a 1
b
a 2
b

Neepawa and Glenlea; winter wheat cv. Talbot; durum cv. Stewart 63). Cultivars were chosen to provide an adequate distribution of data points across the entire electrophoregram field, as no one cultivar can satisfy this requirement. Rm values for horizontal PAGE results (the dependent variable) were calculated by the method of Bushuk and Zillman (1978). Counterpart data from vertical PAGE patterns were determined by multiple reference bands as described previously (refer to Results and Discussion section) to effectively minimize the experimental error in the independant variable.

Regression analysis was performed using the SAS statistical program package (Ray, 1982) on the Amdahl 580 computer of the University of Manitoba. The regression line shown in Figure 58 for the curvilinear model $Y=a+bX+cX^2$ represents the best fit by least squares to the observed set of data. It is clear that an excellent functional relationship exists between relative mobilities derived from the two flatbed PAGE systems used in this study. Moreover, the mean deviation between observed and predicted horizontal PAGE values is less than 0.20 ± 0.15 Rm units for either the full mobility spectrum or for gliadin bands with Rm's above and below 70 and 30 respectively, where the greatest Rm differential occurs. Subsequent testing of the computed regression equation on numerous electrophoregrams in the cultivar identification data base similarly yielded good fits.

These results provide strong evidence for the level of accuracy that can be expected in calibrating the entire set of vertical PAGE relative mobilities in the cultivar identification data base to the expanded scale of mobilities obtained in this study by horizontal PAGE.

Figure 58. Least-squares curvilinear regression analysis of relative mobility data from vertical and horizontal PAGE systems. Gliadin electrophoregrams from the following cultivars were used to establish the calibration curve: Glenlea, Marquis, Napayo, Neepawa, Stewart 63 and Talbot.


Y = 0.45 + 0.906 X + 0.00169 X²
r² = 0.999
HORIZONTAL PAGE RELATIVE MOBILITY
VERTICAL PAGE RELATIVE MOBILITY
0
10
20
30
40
50
60
70
80
90
100

In practice, the application of simple or curvilinear regression analysis to facilitate inter-laboratory comparison or cross-referencing of gliadin electrophoregram data is bound by few requirements[1]. The value of the result depends of course on comparable resolution and good precision in Rm estimates, especially for cultivars selected to establish the calibration curve. In this regard, the use of multiple reference bands to compute relative mobilities as applied in this study might prove useful.

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[1]Various statistical considerations in regards to using calibration curves on electrophoresis data are discussed by Rodbard (1976).