WHEAT CULTIVAR IDENTIFICATION
BY GLIADIN ELECTROPHOREGRAMS

by

RICHARD REID ZILLMAN

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ABSTRACT

Zillman, Richard Reid. M.Sc., The University of Manitoba, May, 1978. Wheat Cultivar Identification by Gliadin Electrophoregrams. Major Professor: Dr. W. Bushuk.

An electrophoresis test, suitable for identifying wheat cultivars, was developed. The test uses the gliadin pattern (electrophoregram) as the genotypic character.

The test incorporates a continuous aluminum lactate-lactic acid buffer system at pH 3.1 and a flat-bed 6% polyacrylamide gel support medium in a specially constructed apparatus. Seventy percent aqueous ethanol was used for gliadin extraction. The nomenclature, developed in this study, uses a major band in the electrophoregram of the Canadian hard red spring wheat cultivar, Marquis, as the reference. The reference band was assigned an arbitrary mobility of 50; all other bands of that and other cultivars were identified on the basis of mobility relative to 50 for the reference band under identical electrophoresis conditions. A method for subjective quantification of band intensities was developed.

The possible effect of environment on the electrophoregram was examined using five cultivars, each grown at ten different locations in Manitoba and Saskatchewan. The electrophoregram was not affected by environment. This confirmed previous findings that the gliadin

electrophoregram is a genotypic character, and substantiates its use for wheat cultivar identification.

Electrophoregrams were determined for 88 wheats (common and durum) licenced for production in Canada. Based on the nomenclature described in this thesis, electrophoregram formulas for these cultivars were determined and catalogued. Of the 88 cultivars, 85 had distinct formulas. A method for identifying an unknown wheat sample or determining its cultivar purity using the electrophoresis test and the catalogue of cultivar formulas is given. Practical implications of this study in the Canadian wheat industry are discussed.

TABLE OF CONTENTS

Page

I. INTRODUCTION 1

II. LITERATURE REVIEW 3

III. MATERIALS 12

IV. METHODS 17

A. Principle of Electrophoresis Test 17

B. Electrophoresis System 17

C. Electrophoresis Apparatus 18

D. Grain Sample Grinding 18

E. Gliadin Extraction 19

F. Preparation of the Gel 19

G. Sample Application 27

H. Electrophoresis 28

I. Staining 28

J. Photography 29

V. RESULTS AND DISCUSSION 30

A. Effect of Environment on Gliadin Electrophoregram . . 30

B. Gliadin Nomenclature 30

C. Catalogue of Cultivar Formulas 38

1. Determination of Gliadin Band Mobilities 65

2. Precision of Determination of Band Mobilities . 66

Page

3. Determination of Band Intensities 67

4. Use of the Catalogue of Cultivar Formulas . . . 68

5. General Comments on the Catalogue of Cultivar Formulas 68

VI. SUMMARY AND CONCLUSION 70

LITERATURE CITED 73

APPENDIX I: SELECTION OF A GLIADIN EXTRACTION PROCEDURE 77

APPENDIX II: SELECTION OF A STAINING PROCEDURE 82

LIST OF TABLES

Table Page

1. Common spring wheat cultivars analyzed by polyacrylamide gel electrophoresis 13

2. Common winter wheat cultivars analyzed by polyacrylamide gel electrophoresis 15

3. Durum wheat cultivars analyzed by polyacrylamide gel electrophoresis 16

4. Recipes for gel and tank buffers 26

5. Cultivar formulas of common spring wheats based on gliadin electrophoregrams 61

6. Cultivar formulas of common winter wheats based on gliadin electrophoregrams 63

7. Cultivar formulas of durum wheats based on gliadin electrophoregrams 64

LIST OF FIGURES

Figure | Page

1. A detailed drawing of the electrophoresis apparatus 20
2. An isometric drawing of the electrophoresis apparatus 22
3. The set-up of the electrophoresis apparatus, power supply and circulating water bath during electrophoresis 24
4. Gliadin electrophoregrams of hard red spring wheat cv. Marquis grown at ten locations in Manitoba and Saskatchewan 32
5. Gliadin electrophoregrams of hard red spring wheat cv. Neepawa grown at ten locations in Manitoba and Saskatchewan 34
6. Gliadin electrophoregram of hard red spring wheat cv. Marquis with the nomenclature proposed in this study and those of Woychik *et al*. (1961) and Autran and Bourdet (1975) 37
7. Gliadin electrophoregrams of common spring wheat cultivars (nos. 1 to 8 in Table 1) 40
8. Gliadin electrophoregrams of common spring wheat cultivars (nos. 9 to 16 in Table 1) 42
9. Gliadin electrophoregrams of common spring wheat cultivars (nos. 17 to 24 in Table 1) 44
10. Gliadin electrophoregrams of common spring wheat cultivars (nos. 25 to 32 in Table 1) 46
11. Gliadin electrophoregrams of common spring wheat cultivars (nos. 33 to 40 in Table 1) 48

Figure | Page

12. Gliadin electrophoregrams of common spring wheat cultivars (nos. 41 to 48 in Table 1) 50

13. Gliadin electrophoregrams of common spring wheat cultivars (nos. 49 to 50 in Table 1) and common winter wheat cultivars (nos. 51 to 56 in Table 2) 52

14. Gliadin electrophoregrams of common winter wheat cultivars (nos. 57 to 64 in Table 2) 54

15. Gliadin electrophoregrams of common winter wheat cultivars (nos. 65 to 72 in Table 2) 56

16. Gliadin electrophoregrams of common winter wheat cultivars (nos. 73 to 76 in Table 2) and durum wheat cultivars (nos. 77 to 80 in Table 3) 58

17. Gliadin electrophoregrams of durum wheat cultivars (nos. 81 to 88 in Table 3) 60

18. Gliadin electrophoregrams of hard red spring wheat cv. Neepawa for which the gliadin extract was prepared using ten different solvents 80

I. INTRODUCTION

Schedule 1 of the 1971 Canada Grain Act specifies that the cultivars of hard red spring wheat that qualify for grades No. 1 and No. 2 Canada Western Red Spring (CWRS) shall be "Marquis or any variety (cultivar) equal to Marquis". Over the years, the cultivar specification has been both an asset and a liability to Canada's wheat industry. The main implication of this specification is that cultivars that are not equal in quality to Marquis must be identifiable so that they can be excluded from No. 1 and No. 2 grades. Furthermore, the mechanics of Canada's wheat grading practices require that these cultivars must be "visually" distinguishable for rapid grading and segregation. This requirement eliminates the possibility of licencing cultivars that are not visually distinguishable, regardless of their yield potential, disease resistance, milling quality, or end-use quality (e.g. for products other than bread).

Canada's visual identification of wheat cultivars is based entirely on physical characteristics of the grain. The grading regulations do not permit the use of chemical or biochemical tests for identifying cultivars. The main reason for this is the concern that such tests would severely interfere with the throughput of grain in the handling system. On the other hand, Australia has, for a number of years, used the phenol and electrophoresis tests to exclude undesirable cultivars

from their "Prime Hard" and "Hard" grades. The European Economic Community is considering statutory application of an electrophoresis test to identify undesirable "non-bread" cultivars such as Maris Huntsman.

The object of the present study is to present an electrophoresis test that might be suitable for the identification of wheat cultivars in the Canadian wheat grading and handling system. An electrophoretic apparatus and method and a nomenclature for the gliadin components in the electrophoretic patterns (electrophoregrams) are described. The effect of growing location on the gliadin electrophoregram is examined. A catalogue of wheat cultivars licenced in Canada is prepared on the basis of gliadin electrophoregrams using the nomenclature described.

Throughout the text of this study, wheats of the species *Triticum aestivum* L. em Thell. (hexaploid wheats having the genomic formula AABBDD) will be referred to as common wheats, whereas wheats of the species *Triticum turgidum* L., s.sp. *durum* (tetraploid wheats having the genomic formula AABB) will be referred to as durum wheats. Common wheats may have spring or winter habit, and are used for bread making (hard red spring wheats in Canada) or for cake, biscuit and pastry products. Durums are spring wheats used for pasta production.

II. LITERATURE REVIEW

Electrophoretic fractionation of wheat protein has progressed steadily during the past several decades. The earliest experiments were able to differentiate only four or five components of wheat gluten. It is now known that wheat gliadin comprises up to forty or more distinct protein components, and the gliadin electrophoretic pattern can be used to distinguish between cultivars. This review chronologically traces the application of electrophoretic techniques to the study of wheat proteins from those early experiments to the present use of gliadin electrophoregrams for cultivar identification.

The separation of proteins by free or moving boundary electrophoresis was first reported by Tiselius (1937), and applied to wheat protein by Schwert _et al_. (1944). Wheat gluten was first classified by Jones _et al_. (1959) into four groups designated as α, β, γ, and ω on the basis of four distinct peaks in moving boundary electrophoresis. They found several suitable buffer systems for the electrophoretic study of gluten proteins, all at pH 3.1 and nominal ionic strengths of about 0.03. Aluminum lactate - lactic acid (nominal ionic strength of 0.03 to 0.12) was recommended over all others because of the excellent peak symmetry attained with this buffer even at high protein concentrations. With certain buffer solutions α and β peaks were resolved into two components. Thus wheat gluten was shown to consist of at least six distinct

components (α_1, α_2, β_1, β_2, γ, and ω). Cluskey et al. (1961) were able to distinguish between hard and soft wheats on the basis of the electrophoretic profiles of the gluten proteins obtained with the Tiselius apparatus.

Introduction of the starch gel support medium by Smithies (1955) was a major advance in the application of zone electrophoresis to the study of proteins. Elton and Ewart (1960) first applied starch gel electrophoresis, using the aluminum lactate buffer system of Jones et al. (1959), to the study of wheat proteins. Electrophoretic patterns of glutens from four cultivars examined showed the presence of the same eight distinct and separate bands for each.

Woychik et al. (1961) showed that the four groups of gluten proteins established by Jones et al. (1959) using moving boundary electrophoresis could also be distinguished as four groups of bands by starch gel electrophoresis using aluminum lactate buffer at pH 3.1 containing 3M urea. Eight bands were readily evident for the cultivar Ponca. The β peak was resolved into four distinct bands, and the α peak into two. Within each group the resolved bands were identified by Arabic numerals, with the band having the highest mobility being assigned number one. Protein that failed to migrate into the gel (remained at the origin) was characterized as being identical to the α_1 gluten component reported by Jones et al. (1959) and to the major portion of the classical glutenin fraction of wheat gluten. All protein components that migrated in the gel were considered to be gliadins.

Elton and Ewart (1962) compared starch gel electrophoregrams of protein dispersions prepared in aluminum lactate buffer (pH 3.1,

μ = 0.05) from flours of eight wheat cultivars. They observed significant differences in gliadin patterns, while those of the albumins and globulins remained virtually the same for all cultivars tested. This was also found by Bourdet et al. (1963) for two cultivars of common wheat and one of durum. The starch gel electrophoregram of each cultivar showed at least 14 gliadin bands. Graham (1963), in comparing starch gel electrophoregrams of seven common wheat cultivars and one durum wheat cultivar, noted marked differences in the slow-moving gliadin components.

Another important advance in zone electrophoresis was the introduction of the polyacrylamide gel by Raymond and Wang (1960). Polyacrylanide gel electrophoresis (PAGE) was first applied to wheat gluten analysis by Lee (1962) using an alkaline buffer of pH 8.6 containing 2M urea. Because polyacrylamide gels are transparent, he was able to scan the stained gel by transmission densitometry and thereby obtain a quantitative measure of the amount of protein in each band.

Lee and Wrigley (1963) used the PAGE method described by Lee (1962) to study the gluten proteins of 12 wheat cultivars (eight common and four durum wheats). Each cultivar had a characteristic electrophoretic scan profile consisting of eight or nine peaks. Marked differences were noted between the profiles of tetraploid and hexaploid cultivars. They inferred genetic control of the protein patterns since closely related cultivars had similar profiles. Nimmo et al. (1963) found 15 to 17 bands by PAGE at pH 6.1 (sodium cacodylate - sodium chloride buffer) of water soluble flour proteins. They reported reproducible differences among the patterns (both anionic and cationic protein bands) of four cultivars.

Coulson and Sim (1964) examined aqueous extracts of flours of 34 different common wheat cultivars using starch gel electrophoresis. They observed that the pattern in the gliadin region was typical for each cultivar, and was not affected by the area of growth. They concluded their report with the statement:

> These results seem to suggest the possibility of a new method of wheat protein classification as well as the accurate identification of different wheat varieties (which is at present extremely difficult). The evaluation of new varieties and inheritable factors of quality, which would be extremely useful to the wheat breeder, may also be possible.

Feillet and Bourdet (1964) reported that they could identify some common wheat cultivars by starch gel gliadin electrophoregrams. The same workers (1967) were able to separate gliadin into 15 to 19 bands using starch gel electrophoresis in aluminum lactate buffer at pH 3.25. They also pointed out that gliadin composition was not affected by environmental conditions. This conclusion was based on results for two common wheat cultivars grown in four locations and in the greenhouse with the application of nitrogen fertilizer over two years.

Lee and Ronalds (1967), using starch gel electrophoresis and chromatography on carboxymethyl cellulose, made a systematic study of the effect of environment on wheat gliadin. Their study involved four Australian cultivars grown at six widely separated locations in four Australian states. They concluded that location, soil type and season, which can markedly influence the total protein content, have little effect on the gliadin pattern.

Huebner and Rothfus (1968) used sulfoethyl cellulose column chromatography and subsequent starch gel electrophoresis to compare

the gliadins of ten wheat cultivars representing five different classes grown in the United States. Differences in gliadin composition were greatest between cultivars of different classes and least between cultivars of the same class.

Doekes (1968) carried out an extensive electrophoretic study (on starch gel) of the gliadins of 80 wheat cultivars from Western, Central and Eastern Europe, India, and North and South America. He classified the electrophoretic patterns into five main groups. Group I had a compact gliadin pattern and group V a well spread pattern. Groups II, III and IV were intermediate. This arrangement provided a morphological series in that group I comprised soft Western European winter wheats, groups II, III and IV included varieties of diverse origins varying in kernel hardness, and group V comprised hard non-European spring wheats. In addition, Doekes investigated the effects of climate and soil conditions and of nitrogen fertilizer on the gliadin pattern. He concluded that the electrophoretic pattern of the gliadin fraction was not influenced by any of those environmental factors, and that the electrophoregram was a genotypic character of a cultivar.

Wrigley (1970) used a two dimensional technique that combined gel electrofocusing and starch gel electrophoresis to examine the gliadin extracts of a number of wheat cultivars. Protein maps, showing over 40 components, revealed considerable differences in gliadin composition among cultivars. On the other hand, the patterns of samples of the cultivar Spica, in which protein content varied from 9.9 to 26.7%, were qualitatively constant.

Ellis (1971) found, by starch gel electrophoresis, cultivar-specific differences in the slow moving gliadins of 32 commercially grown British wheats (hard and soft, spring and winter). On the basis of the number of dark-staining slow bands, the cultivars were divided into four main groups (0, 1, 2, or 3 dark slow gliadin bands). Differences in band mobility, definition and intensity and the presence of reproducible minor bands allowed differentiation of individual cultivars. However, it was not possible to identify every cultivar by electrophoretic pattern alone. Seasonal and environmental effects did not affect the gliadin patterns, except in the case of sprouted or overheated seed samples which showed a great reduction in band intensities.

Wrigley and Shepherd (1974) described the systematic use of gliadin electrophoresis, based on the starch gel procedure of Graham (1963), to distinguish many of about 50 Australian cultivars (bread wheats and durums). For the purpose of classification, patterns were considered on the basis of four regions, α, β, γ, and ω, identified according to the convention of Jones _et al_. (1959). A table was prepared for the systematic identification of a cultivar according to the appearance of specific distinctive bands in the electrophoregram. Patterns for some cultivars were indistinguishable. Such cultivars were generally closely related genetically. Electrophoretic analyses performed on samples of a number of cultivars grown at different sites in different seasons substantiated previous reports that the gliadin pattern was independent of growth conditions and protein content.

The Greek letter - Arabic numeral system introduced by Woychik _et al_. (1961) was amplified and used for cultivar identification by

the Soviet workers Gubareva _et al_. (1975). They developed a composite formula for common wheats on the basis of number of gliadin bands (7.5% polyacrylamide gel cylinders, 0.013 N acetic acid, pH 3.1) in the electrophoregrams of the putative progenitors of the A (_Triticum boeticum_), B (_Aegilops speltoides_) and D (_Ae. squarrosa_) genomes. The composite gliadin electrophoregram was given the formula α 1 to 7, β 1 to 5, γ 1 to 5, and ω 1 to 12. The numbers representing bands that stained intensely were underlined, those that stained weakly were shown in parenthesis, and doublets were indicated by two dots above the number. A minor deviation in mobility of a β or ω band was indicated by a subscript 1 if the displacement was in the direction of the faster neighboring band, and by a subscript 2 if the deviation was in the direction of the slower neighboring band. An example of the formula that was generated by this nomenclature for the cultivar Kharkovskaya 46 is:

$$\alpha\ 5\ (6)\ 7\quad \beta\ (2)\ 3_2\ 4\ \underline{5}\quad \gamma\ 2\ \underline{5}\quad \omega\ \underline{3}\ \ddot{4}\ \ddot{6}\ 7.$$

In this manner the Soviet workers published the gliadin electrophoregram formulas for 130 cultivars including hard and soft winter and spring wheats grown in the U.S.S.R.

Another nomenclature for identifying gliadin components in the electrophoregram for the purpose of cultivar identification was published by the French workers Autran and Bourdet (1975). This nomenclature was considerably simpler than the one proposed by Woychik _et al_. (1961) and used by the Soviet workers (Gubareva _et al_. 1975). Autran and Bourdet (1975) used starch gel electrophoresis and aluminum lactate buffer at pH 3.2 containing 0.5 M urea. For 60 winter and

13 spring wheat cultivars grown in France, they obtained a total of 43 different bands (a maximum of 25 bands in any one cultivar), each identified by a relative mobility figure relative to 65 for a γ-gliadin band common to all the cultivars analyzed. The range of mobilities for all the gliadin bands was from 21 to 100. Of the 12 electrophoregrmas described in detail, only one had component 100. The nomenclature of Woychik et al. (1961) is related to the nomenclature of Autran and Bourdet (1975) as follows:

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

Band intensities were designated as +++, ++, +, trace, or 0 in order of decreasing intensity by visual examination. Autran and Bourdet (1975) further quantified the dissimilarity of cultivar electrophoregrams using the statistical procedure of Dedio et al. (1969) for the similarity of rye cultivars on the basis of phenolic components. This calculation gives a value of zero for complete identity. Of the 12 French cultivars analyzed in this manner, the highest relative index of dissimilarity (IRD) was 50. These indicies were intended to quantify the similarity of cultivar electrophoregrams and show indirectly a form of genetic relationship between cultivars.

Coles and Wrigley (1976), using the electrophoretic procedure described by Wrigley and Shepherd (1974) and Wrigley and McCausland (1975), showed that positive electrophoretic identification of New

Zealand cultivars was possible. Patterns were grouped according to the position of the gliadin band with highest mobility in the ω region. Other specific bands and the arrangement of bands provided further distinctions among cultivars.

III. MATERIALS

The wheat samples that were used in this study were all cultivars (spring and winter common wheats and durum wheats) that had been licenced in Canada. They are listed along with their pedigree and nationality (Zeven and Zeven-Hissink 1976) in Tables 1 to 3.

The effect of location of growth on the gliadin electrophoregram was investigated further (for Canadian conditions) by examining grain of five hard red spring cultivars (Manitou, Marquis, Napayo, Neepawa, and Sinton) grown in ten locations in Manitoba (Brandon, Dauphin, Glenlea, Morden, and Portage la Prairie) and Saskatchewan (Indian Head, Melfort, Regina, Saskatoon, and Yorkton).

All chemicals used were of laboratory reagent grade. Only deionized distilled water was used in preparing solutions for gliadin extraction and electrophoresis.

TABLE 1. Common spring wheat cultivars analyzed by polyacrylamide gel electrophoresis.

No.	Cultivar	Pedigree and Nationality
1	Acadia	Marquis/Pentad//Canus, Canada
2	Apex	H.44-24/Double Cross//2X Marquis, Canada
3	Bishop	Ladoga/Gehun, Canada
4	Canthatch	Thatcher 6X/Kenya Farmer, Canada
5	Canuck	Canthatch/3/Mida/Cadet//Rescue, Canada
6	Canus	Marquis/Kanred, USA and Canada
7	Cascade	Quality A/Pacific Blue Stem//C26-59-2D/3/Onas, Canada
8	Ceres	Marquis/Kota, USA
9	Chester	Renown/S-615//Rescue/3/Kendee/4/Mida/Cadet, Canada
10	Chinook	Thatcher/S-615-11, Canada
11	Coronation II	Pentad/Marquis, Canada
12	Cypress	Rescue/Chinook, Canada
13	Early Red Fife	selection of Red Fife, Canada
14	Fielder	Yaktana 54A X4//Norin 10/Brevor/3/2X Yaqui 50 /4/ Norin 10/Brevor//Baart/Onas, USA
15	Garnet	Preston A/Riga M, Canada
16	Glenlea	Pembina 2X/Bage//CB 100, Canada
17	Huron	White Fife/Ladoga, Canada
18	Kenhi	Kenya 338 AC2E3/2X Lemhi, Canada
19	Kota	found in USSR durum, USA
20	Lake	Regent/Canus, Canada
21	Lee	Hope//Bobin X2/Gaza, USA
22	Lemhi 53	California 3098/5X Lemhi, USA
23	Lemhi 62	Lemhi 53 X5/3/Lee X7//Chinese/*Ae. umbellulata*, USA
24	Manitou	Thatcher X7/Frontana//Canthatch/3/PI 170925/ 6X Thatcher, Canada
25	Marquis	Hard Red Calcutta/Red Fife, Canada
26	Napayo	Manitou 2X/4/Thatcher 5X/Lee/3/Thatcher 7X/ Frontana//Thatcher 6X/Kenya Farmer, Canada

TABLE 1. Common spring wheat cultivars analyzed by polyacrylamide gel electrophoresis (cont'd).

No.	Cultivar	Pedigree and Nationality
27	Neepawa	Thatcher X7/Frontana//Thatcher X6/Kenya Farmer /3/Thatcher X2//Frontana/Thatcher, Canada
28	Norquay	Lerma Rojo/Sonora 64//Justin, Canada
29	Park	Mida/Cadet//Thatcher, Canada
30	Pembina	Thatcher/3/McMurachy/Exchange//3X Redman, Canada
31	Pioneer	Riga/Preston, Canada
32	Pitic 62	Yaktana 54//Norin 10/Brevor 26-1C, Mexico
33	Prelude	Downy Gehun/Fraser, Canada
34	Preston	Ladoga/Red Fife, Canada
35	Quality A	selection of Florence, Canada
36	Red Bobs 222	selection of Early Triumph, Canada
37	Red Fife	introduced from S. Poland into Canada
38	Redman	Regent/Canus, Canada
39	Regent	H-44/Reward, Canada
40	Reliance	Kanred/Marquis, USA
41	Renfrew	selection of Marquis, Canada
42	Renown	H-44/Reward, Canada
43	Rescue	Apex/S-615, Canada
44	Reward	Marquis/Prelude, Canada
45	Ruby	Downy Riga/Red Fife, Canada
46	Saunders	Hope/Reward//Thatcher, Canada
47	Selkirk	McMurachy/Exchange//3X Redman, Canada
48	Sinton	Manitou/3/Thatcher X6/Kenya Farmer//Lee X6/ Kenya Farmer, Canada
49	Springfield	Norin 10/Brevor//3X Lemhi 53/3/Lemhi 62, USA
50	Thatcher	Marquis/Iumillo//Marquis/Kanred, Canada

TABLE 2. Common winter wheat cultivars analyzed by polyacrylamide gel electrophoresis.

No.	Cultivar	Pedigree and Nationality
51	Cornell 595	Honor/Forward//Nured/3/Honor, USA
52	Dawbul	Dawson's Golden Chaff/Bulgarian, Canada
53	Dawson's Golden Chaff	selection of Clawson, Canada
54	Egyptian Amber	Fultz/Lancaster, USA
55	Fairfield	Purkof/Fulhio, USA
56	Fredrick	Washington 1//Genesee/Cappelle, Canada
57	Gaines	Norin 10/Brevor//Orfed/Brevor sib./3/Burt, USA
58	Genesee	Yorkwin//Honor X2/Forward, USA
59	Jones Fife	Fultz/?/Mediterranean/?/Russian Velvet, USA
60	Junior No. 6	as Goldcoin, selection of Redchaff or of Redchaff Bald, USA
61	Kent	Caldwell 10/Dawson's Golden Chaff, Canada
62	Kharkov 22 M.C.	selection of Kharkov, Canada
63	Nugaines	sib. of Gaines, USA
64	O.A.C. 104	Dawson's Golden Chaff/Bulgarian, Canada
65	Richmond	Dawson's Golden Chaff X2/Ridit, Canada
66	Rideau	Kharkov 22 M.C./Dawson's Golden Chaff, Canada
67	Ridit	Turkey/Florence, USA
68	Sun	as Sol, selection of local variety/English Stand Up, Sweden
69	Sundance	Cheyenne/Kharkov 22 M.C., Canada
70	Talbot	Trumbull//Hope/Hussar/3/Dawson's Golden Chaff X2/Ridit//Cornell 595, Canada
71	Thorne	Portage/Fulcaster, USA
72	Wasatch	Relief/Ridit, USA
73	Westmont	Rio/Rex//Nebred, USA
74	Winalta	Minter/Wichita, USA
75	Yogo	Minturki/Beloglina//Buffum, USA
76	Yorkstar	Genesee X5/3/Yorkwin//Norin 10/Brevor, USA

TABLE 3. Durum wheat cultivars analyzed by polyacrylamide gel electrophoresis.

No.	Cultivar	Pedigree and Nationality
77	Carleton	Vernal Emmer/Mindum, USA
78	Golden Ball	? from S. Africa
79	Hercules	RL 3097/RL 3304//Stewart/RL 3380, Canada
80	Macoun	RL 3607/DT 182, Canada
81	Mindum	found in bread wheat field, USA
82	Nugget	Mindum/Carleton//Heiti/Stewart, USA
83	Pelissier	introduced from Algeria into USA
84	Ramsey	Carleton/PI 94701, USA
85	Stewart	Mindum X2/Vernal Emmer, USA
86	Stewart 63	St.464/8X Stewart, Canada
87	Wakooma	Lakota X2/Pelissier, Canada
88	Wascana	Lakota X2/Pellissier, Canada

IV. METHODS

A. Principle of Electrophoresis Test

The electrophoresis test, as applied to wheat cultivar identification, separates the gliadin components into protein bands of different mobility. It has been reported previously (Lee and Wrigley 1963; Coulson and Sim 1964; Feillet and Bourdet 1964 & 67; Lee and Ronalds 1967; Doekes 1968; and Wrigley 1970), and confirmed for Canadian conditions in this study, that cultivars give band patterns (electrophoregrams) that are characteristic of the genotype and independant of growth conditions.

B. Electrophoresis System

The procedure that was developed uses polyacrylamide gel as the support material. Most previous studies on the use of electrophoresis for cultivar identification (Doekes 1968; Ellis 1971; Wrigley and Shepherd 1974; Autran and Bourdet 1975; and Coles and Wrigley 1976) used starch gel because of its apparent superior resolving power, and because of the difficulty in polymerizing a polyacrylamide gel at low pH. The resolving power of polyacrylamide gel was improved in the present study to a level that is as good as, or perhaps better than, that of starch gel. Furthermore, with the polymerization technique described, it is much easier to prepare reproducible, highly uniform

polyacrylamide gels than starch gels. In addition, polyacrylamide gels are stable, inert and transparent, and have good mechanical stability during handling.

The electrophoretic system devised for this study uses the aluminum lactate - lactic acid buffer described first by Jones et al. (1959) and the acid acrylamide polymerization technique of Jordan and Raymond (1969).

C. Electrophoresis Apparatus

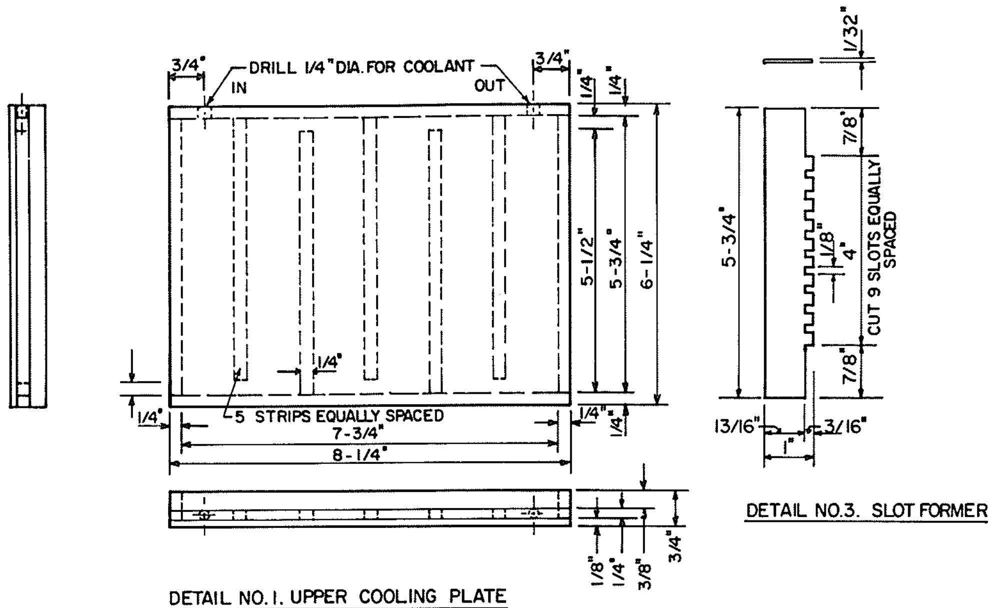
The flat-bed apparatus (Figures 1 to 3) was constructed in the laboratory from acrylic plactic when it was found that the available commercial equipment did not meet requirements, in particular for handling instantaneous polymerization of the gel. The electrodes (removable for cleaning or replacement) were made of B & S Guage 18 platinum wire attached to male "banana" plugs. The apparatus incorporates the following useful features: long gel slab for improved resolution; efficient gel cooling; small electrode buffer wells; and single unit construction. Furthermore, it is easy to construct and is inexpensive.

D. Grain Sample Grinding

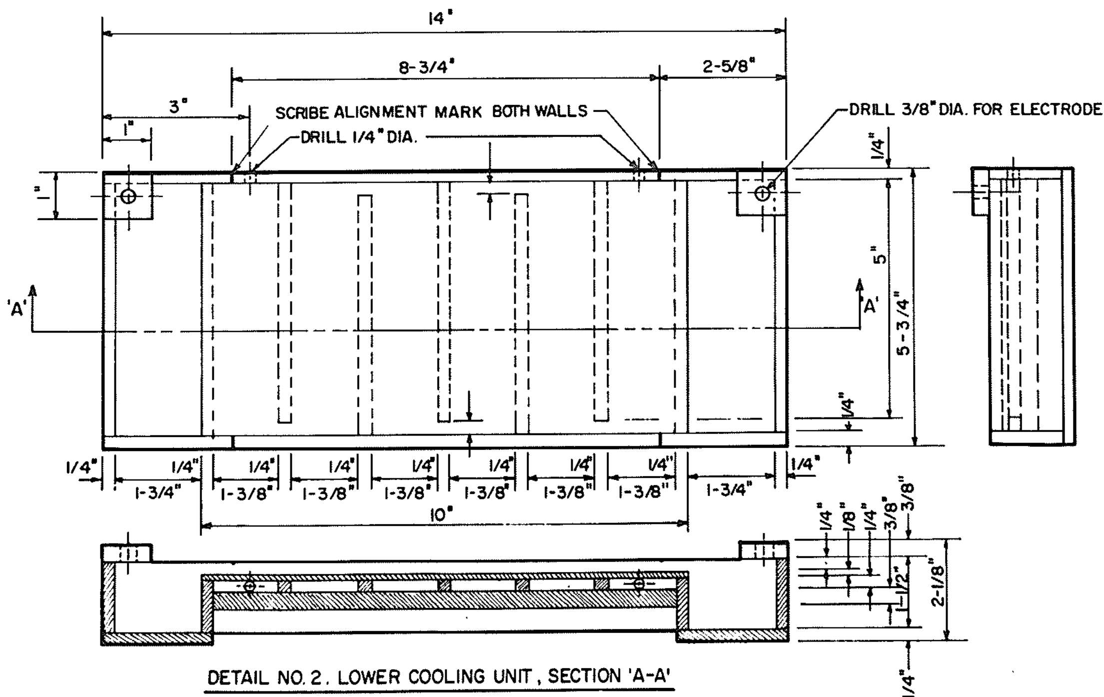
If sufficient grain (e.g. one gram) was available, the entire sample was ground in a Udy Cyclone (or similar) sample mill fitted with a one mm sieve. Single kernels could be ground by hand using a mortar and pestle. There was no attempt to separate the bran or germ from the endosperm (see Appendix I).

Figure 1

A detailed drawing of the electrophoresis apparatus.



DETAIL NO. I. UPPER COOLING PLATE



DETAIL NO. 2. LOWER COOLING UNIT, SECTION 'A-A'

ELECTROPHORESIS APPARATUS

DATE : MAY 10. 1977   SHEET NO. 2 OF 2

NOTE : ALL MATERIAL ACRYLIC. SLOT FORMER TEFLON.
ALL JOINTS TO BE FUSED WITH SOLVENT.
BUFF ALL EXPOSED EDGES.

Figure 2

An isometric drawing of the electrophoresis apparatus.

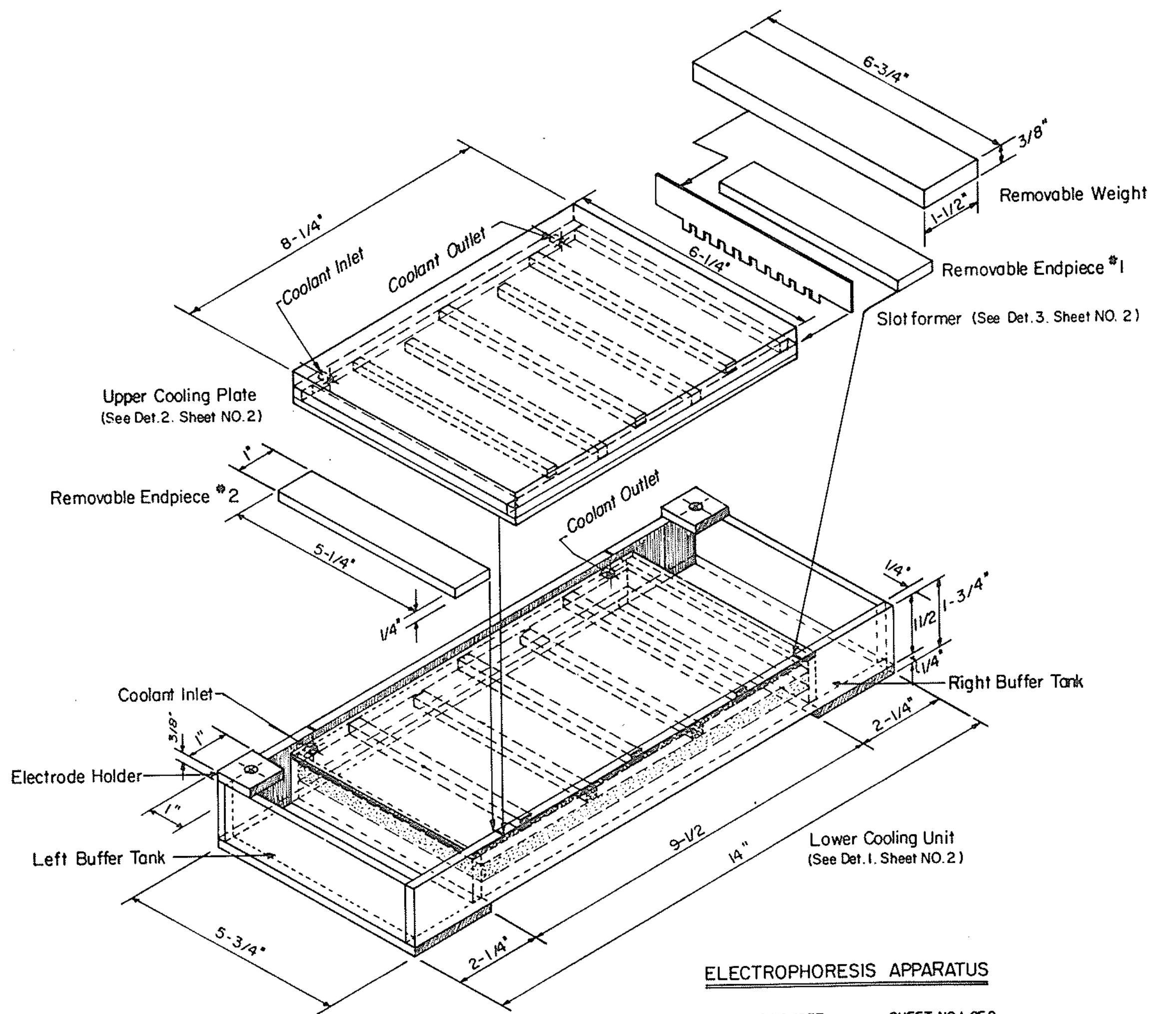
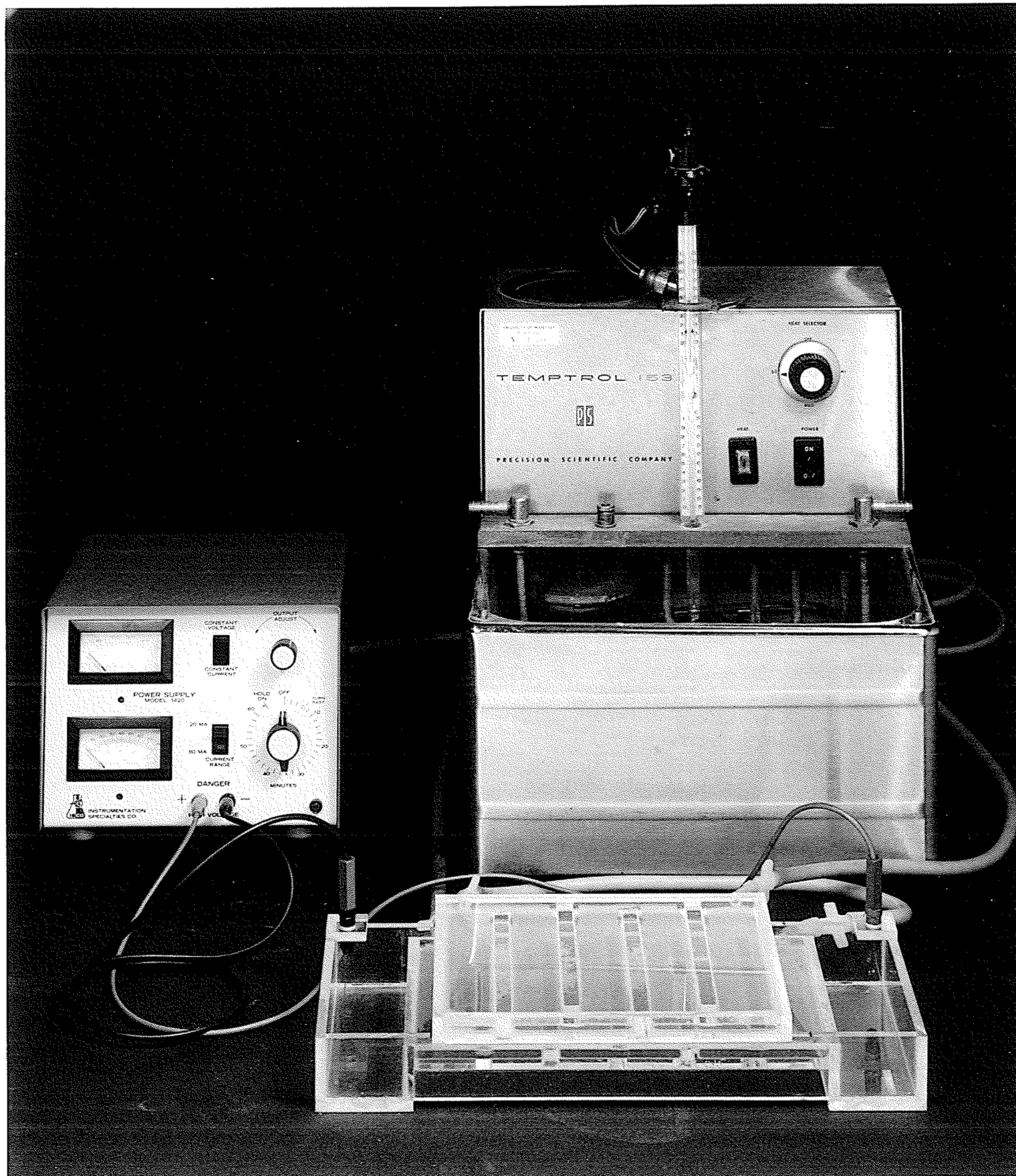

6-3/4"
3/8"
1-1/2"
Removable Weight
Removable Endpiece #1
6-1/4"
Slot former (See Det.3. Sheet NO. 2)
8-1/4"
Coolant Inlet
Coolant Outlet
Upper Cooling Plate
(See Det.2. Sheet NO.2)
1"
Removable Endpiece #2
5-1/4"
1/4"
Coolant Outlet
1/4"
1-3/4"
11/2
1/4"
Right Buffer Tank
2-1/4"
Coolant Inlet
3/8"
1"
Electrode Holder
1"
Left Buffer Tank
9-1/2
14"
Lower Cooling Unit
(See Det.1. Sheet NO.2)
5-3/4"
2-1/4"
ELECTROPHORESIS APPARATUS
DATE: MAY 10. 1977
SHEET NO 1 OF 2

Figure 3

The set-up of the electrophoresis apparatus, power supply and circulating water bath during electrophoresis.


TEMPTROL 153
PS
PRECISION SCIENTIFIC COMPANY
HEAT SELECTOR
HEAT
POWER
ON
CONSTANT VOLTAGE
CONSTANT CURRENT
OUTPUT ADJUST
POWER SUPPLY
20 MA
80 MA
CURRENT RANGE
DANGER
MINUTES
INSTRUMENTATION SPECIALTIES CO.

E. Gliadin Extraction

The ground grain was extracted with three times its weight of 70% aqueous ethanol in a stoppered centrifuge tube by mixing briefly on a vortex mixer and allowing the mixture to stand at room temperature for at least one h. The contents were centrifuged for ten min at 20,000 X g at room temperature. The supernatant was removed and mixed with two times its volume of electrophoresis buffer (Table 4). Sucrose was then added to about 30% to increase the density and thereby facilitate sample application to the sample slot in the gel. Finally, a minute quantity of methyl green dye was added to serve as the marker during electrophoresis, and to facilitate visual observation of sample application into the slot. The original gliadin extracts could be stored in sealed vials in a refrigerator for several weeks without any noticeable change occurring in the electrophoregram. Gliadin extractions using several other solvents were examined in the exploratory part of this study and are described in Appendix I.

F. Preparation of the Gel

The apparatus (Figures 1 to 3) was prepared for gel polymerization by inserting the two removable acrylic end-pieces, thus forming a shallow gel tray, and starting the coolant (water at 21°C) circulation through both upper and lower gel cooling plates. One ml of peroxide catalyst solution was added to 200 ml of gel solution (Table 4), which had been cooled to about 1°C in order to slow down the polymerization reaction. The mixture was swirled briefly and poured quickly into the gel tray. The upper cooling plate was placed immediately over the gel

TABLE 4. Recipes for gel and tank buffers

	Amount required for 200 ml of gel or buffer
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	0.5 g
Lactic acid	to pH 3.1
Catalyst solution	
Hydrogen peroxide, 3%	1.0 ml
Tank buffer solution	
Aluminum lactate	0.5 g
Lactic acid	to pH 3.1

solution in such a way as not to trap any air bubbles underneath, and so that one end of the cooling plate was lined up evenly with one end of the gel solution. The teflon slot-former was then placed against the other end of the upper cooling plate so that the slot "teeth" extended down into the uncovered portion of the gel solution. The slot-former was held in place during polymerization by a heavy strip of removable acrylic which also served to completely cover the gel solution and provide a uniform over-all gel thickness.

Polymerization was essentially instantaneous, although there was adequate time to perform all the operations described above. After approximately five min the three removable pieces of acrylic and the slot-former were carefully removed, exposing the two ends of the gel, the sample slots, and a small portion of the gel surface between the sample slots and the end of the gel.

The electrodes were positioned in the buffer compartments so that they extended the width of the gel parallel to each end. Tank buffer (250 ml) was then added to each electrode compartment to a level even with the upper surface of the gel.

G. Sample Application

A ten-place slot-former was used so that each gel could accomodate eight different samples flanked on each side by the Marquis reference sample. Just prior to the start of electrophoresis, sample solutions (25 μl) were carefully deposited into the slots using a microsyringe. This process was best accomplished if the slots were first filled with buffer so that the sample solution, due to its greater density, would settle uniformly into the slot, displacing the buffer.

H. Electrophoresis

The ends of the lead wires of the power supply were fitted with female "banana" plugs, thus facilitating their connection to the electrodes. The electrode at the slot end of the gel was the anode. As a precautionary measure, the power connections should not be made until after the sample solutions have been added to the slots.

A current of 72 to 74 mA was maintained throughout the electrophoretic run. This corresponded to a voltage drop of approximately 14 V/cm at the beginning which decreased gradually to 13 V/cm at the conclusion of electrophoresis. Since current is sensitive to change in temperature, the coolant was maintained at a constant temperature of 21°C. To maximize the separation of gliadin components which migrate slowly, electrophoresis was continued for 6.5 h. During this time, the albumins and globulins, which were extracted by the alcohol solution with the gliadins, ran off the gel, leaving only the gliadin components spread out over the entire length of the gel. Tank buffer was added from time to time to replace what was lost due to evaporation. At the conclusion of electrophoresis, the positions of the protein bands in the gel could be determined by ultra-violet densitometry or by staining.

I. Staining

On completion of electrophoresis, the upper cooling plate was removed and the gel transferred, by means of a gel remover made from a thin sheet of acrylic, to the staining tray. Polyethylene "Frig-O-Seal" food savers proved to be excellent staining trays. For the gliadins

the best stain was found to be Coomassie brilliant blue dissolved in 12% trichloroacetic acid. Ten ml of a 1% stock solution of the dye in 95% ethanol and 250 ml of freshly prepared 12% trichloroacetic acid were required for each gel. Some gliadin components stained rather slowly, so staining was continued for at least 72 h.

Bands could be detected and their separation observed reasonably well with no destaining other than a water rinse. However, destaining overnight in 12% trichloroacetic acid improved the appearance of the bands, probably because the dye that settled out as a fine precipitate on the surface of the gel during staining was removed. If gels were left in destaining solution too long, stained bands began to fade and, eventually, some disappeared. Other staining-destaining procedures that were tried in the developmental stages of this study are described in Appendix II.

J. Photography

Following destaining, the gel was transferred from the staining tray onto a sheet of clear glass and rinsed with water. A piece of nylon mesh material, somewhat larger in size than the gel, was used for transferring the gel. The gel was illuminated from below with diffused flourescent light and photographed on Kodak 5069 High Contrast Copy film. The film was developed with D19 developer. Printing was done on Kodak Ektamatic SC photographic paper using Kodak Ektamatic A10 activator and Kodak Ektamatic S30 stabilizer.

V. RESULTS AND DISCUSSION

A. Effect of Environment on Gliadin Electrophoregram

Growing location had no qualitative effect (no change in number of bands) on the gliadin electrophoregrams of any of the five Canadian cultivars grown at ten stations in Manitoba and Saskatchewan. Typical results are shown for the cultivars Marquis and Neepawa only (Figures 4 and 5). These results confirm those of previous workers (Coulson and Sim 1964; Feillet and Bourdet 1967; Lee and Ronalds 1967; Doekes 1968; and Wrigley 1970) that the gliadin electrophoregram is independent of environment, and substantiate the suitability of the electrophoregram obtained by the method described for wheat cultivar identification.

B. Gliadin Nomenclature

The use of gliadin electrophoregrams for accurate identification of wheat cultivars requires a systematic nomenclature of the electrophoregram bands. The system used by the Soviet workers (Gubareva *et al*. 1975) could not be successfully replicated. It is subject to the major difficulty of locating the group boundaries inherent in the nomenclature that divides the gliadin proteins into four groups designated as α, β, γ, ω (Jones *et al*. 1959 and Woychik *et al*. 1961). This difficulty is particularly evident in electrophoregrams on slab type polyacrylamide gels. In electrophoregrams of some of the

Figure 4

Gliadin electrophoregrams of hard red spring wheat cv. Marquis grown at ten locations in Manitoba and Saskatchewan.

1. Brandon
2. Dauphin
3. Glenlea
4. Morden
5. Portage la Prairie
6. Indian Head
7. Melfort
8. Regina
9. Saskatoon
10. Yorkton

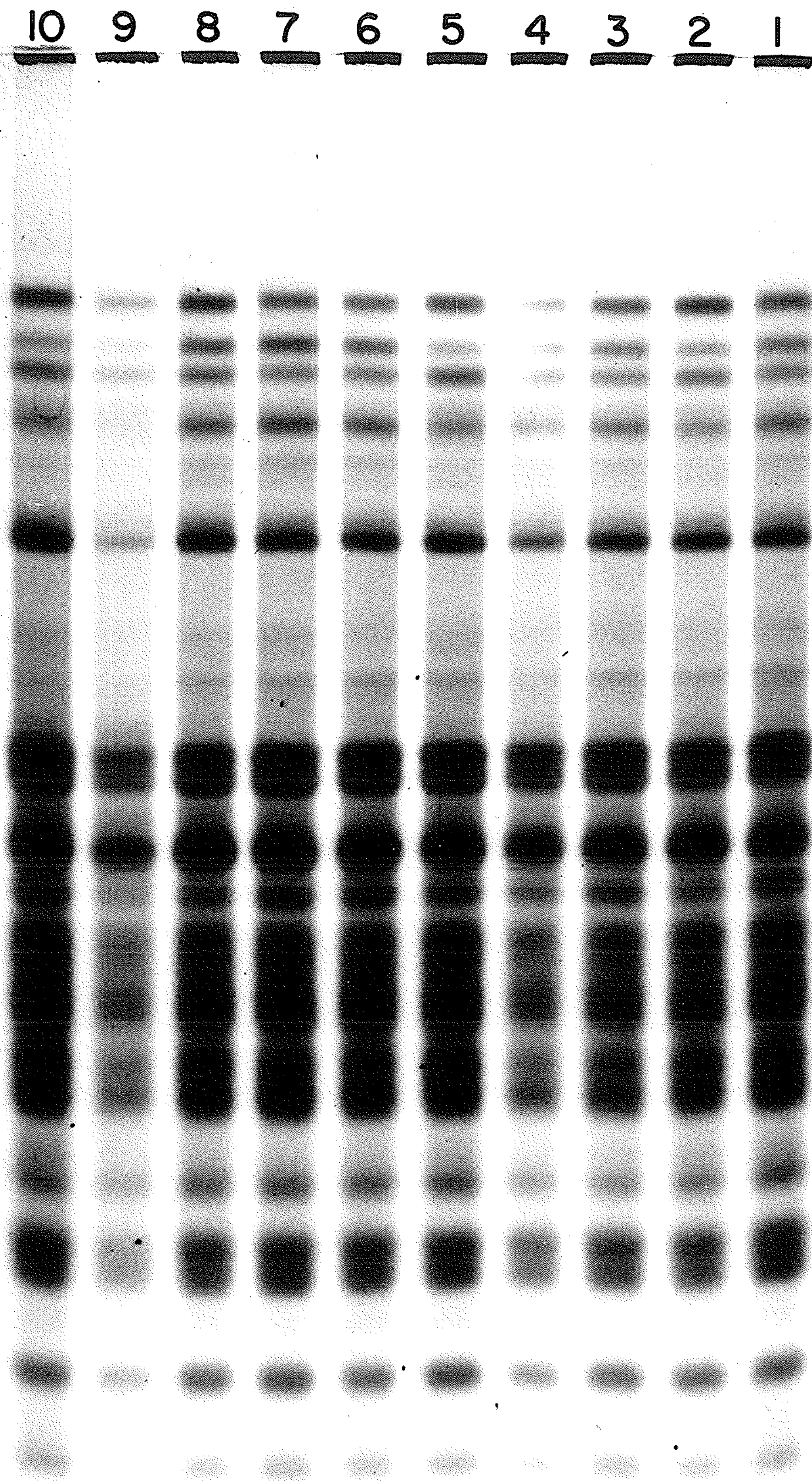
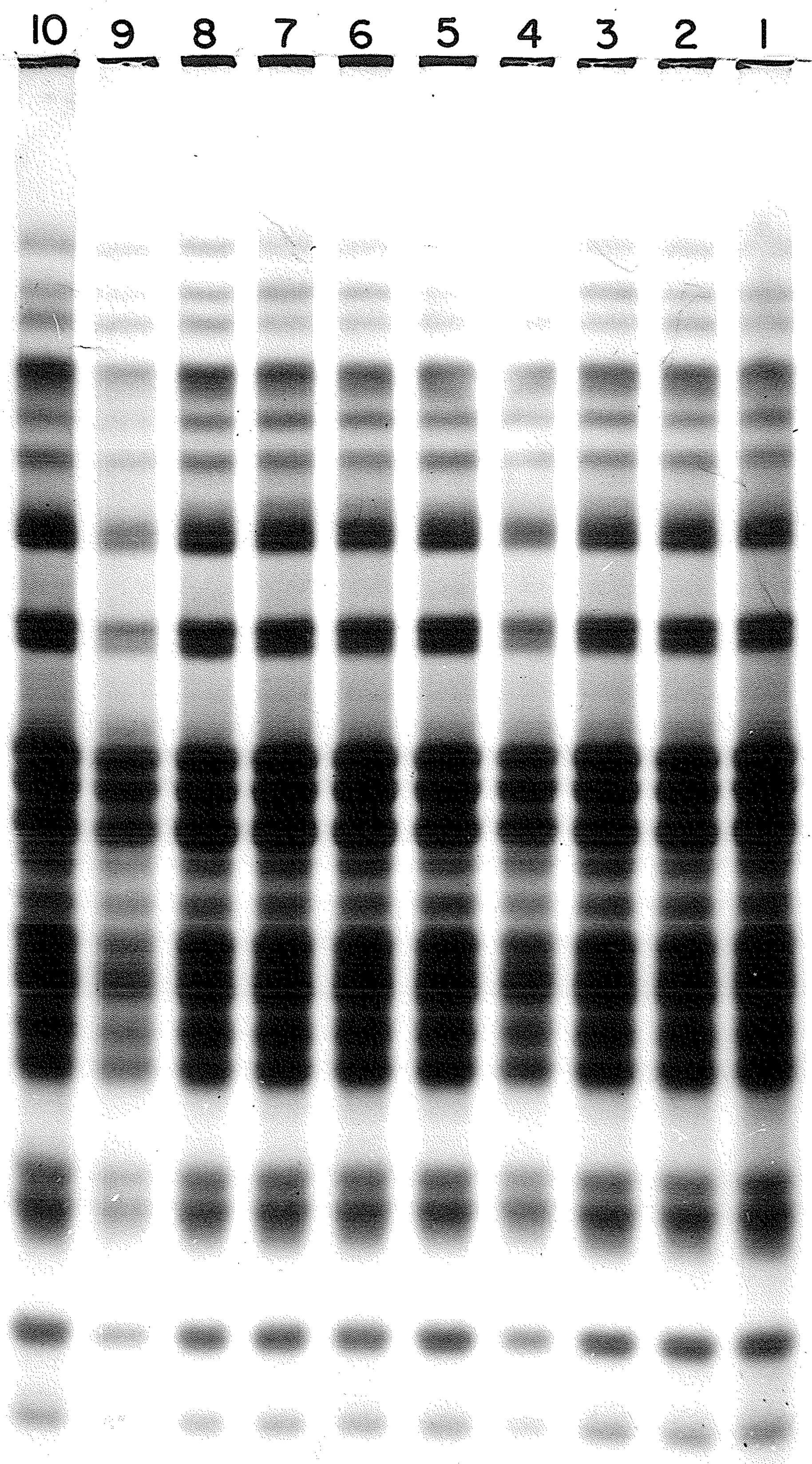

10 9 8 7 6 5 4 3 2 1

Figure 5

Gliadin electrophoregrams of hard red spring wheat cv. Neepawa grown at ten locations in Manitoba and Saskatchewan.

1. Brandon
2. Dauphin
3. Glenlea
4. Morden
5. Portage la Prairie
6. Indian Head
7. Melfort
8. Regina
9. Saskatoon
10. Yorkton


10 9 8 7 6 5 4 3 2 1

cultivars used in this study, it was not possible to unequivocally determine where one group ends and another begins. This was particularly true for the α-β-γ region.

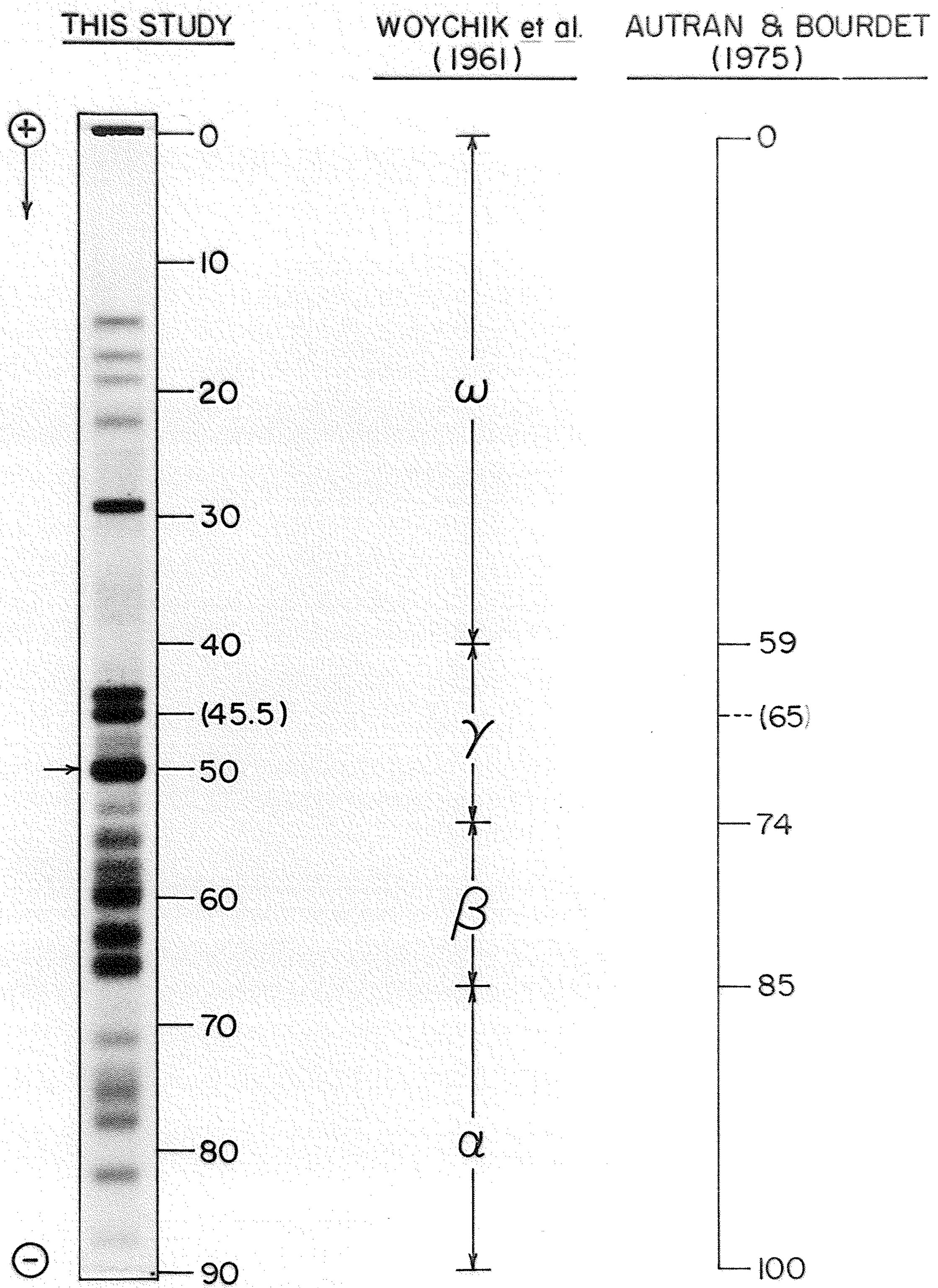
In attempting to apply the French system of gliadin nomenclature (Autran and Bourdet 1975) to the classification (identification) of cultivars in this study, a minor, but extremely critical, difficulty was encountered. With polyacrylamide gel electrophoregrams it was difficult to locate the reference γ-gliadin band to which one would assign a relative mobility value of 65. That band often occurs as the leading band of a prominent doublet and is not always clearly resolved. Furthermore, some durum wheat cultivars do not have band 65.

The nomenclature that is proposed in this study is a modification of that of Autran and Bourdet (1975). Instead of using a γ-gliadin band in the electrophoregram as the reference, it is proposed that one of the major bands (readily identifiable in both polyacrylamide and starch gel electrophoregrams) of the historic Canadian cultivar Marquis be assigned a mobility value of 50 (or 0.50) and used as the reference. All other bands of Marquis and other cultivars can then be identified by a mobility relative to this reference. The proposed reference band is identified by an arrow in Figure 6. This band is readily identifiable in electrophoregrams because it is followed (lower mobility) by a distinctive doublet. An approximate comparison of the proposed nomenclature and that of the French workers is shown in Figure 6.

Adoption of the proposed system of nomenclature would require the use of the Canadian cultivar Marquis as the reference cultivar. This suggestion has been well received by the members of the Wheat Protein

Figure 6

Gliadin electrophoregram of hard red spring wheat cv. Marquis with the nomenclature proposed in this study and those of Woychik *et al*. (1961) and Autran and Bourdet (1975). The published nomenclatures shown are the author's approximations. The reference band of Autran and Bourdet, indicated as (65), is the band indicated as (45.5) in the electrophoregram shown. The proposed reference band is shown by an arrow.


THIS STUDY
WOYCHIK et al. (1961)
AUTRAN & BOURDET (1975)
+
0
10
20
30
40
(45.5)
50
60
70
80
90
−
ω
γ
β
α
0
59
(65)
74
85
100

Nomenclature Study Group of the International Association for Cereal Chemistry. The proposed nomenclature for the bands in the gliadin electrophoregram has a number of distinct advantages:

1) The use of a standard reference cultivar would facilitate inter-laboratory comparison of results. Adjustments in electrophoretic procedure could be made until identical electrophoregrams were obtained.

2) Selection of a reference band that is located near the midpoint of the electrophoregram would ensure maximum accuracy in the mobilities over the entire range.

3) The nomenclature is amenable to the statistical calculation of the relative index of dissimilarity as proposed by Autran and Bourdet (1975).

For more detailed description of the electrophoregram, the intensity of each stained band can be subjectively quantified by a subscript number from 1 to 5 (the darker the band the higher the number). On the basis of the proposed nomenclature the cultivar formula of Marquis is:

16_3 18.5_3 20.5_3 23.5_3 26_1 30.5_4 37_1 38_1 39.5_1 44.5_5 45.5_5
47.5_2 50_5 53_3 55.5_4 58_4 60_4 63_4 65.5_4 71_3 75_3 77_3 82_3 87.5_1.

C. Catalogue of Cultivar Formulas

Gliadin electrophoregrams for all the wheat cultivars listed in Tables 1 to 3, obtained by the procedure described, are shown in Figures 7 to 17. In each figure, the two outer electrophoregrams are of the reference cultivar Marquis. The numbered electrophoregrams correspond to the numbered cultivars listed in Tables 1 to 3. Resolution of the bands was considered to be satisfactory when the

Figure 7

Gliadin electrophoregrams of common spring wheat cultivars (nos. 1 to 8 in Table 1).

1. Acadia
2. Apex
3. Bishop
4. Canthatch
5. Canuck
6. Canus
7. Cascade
8. Ceres

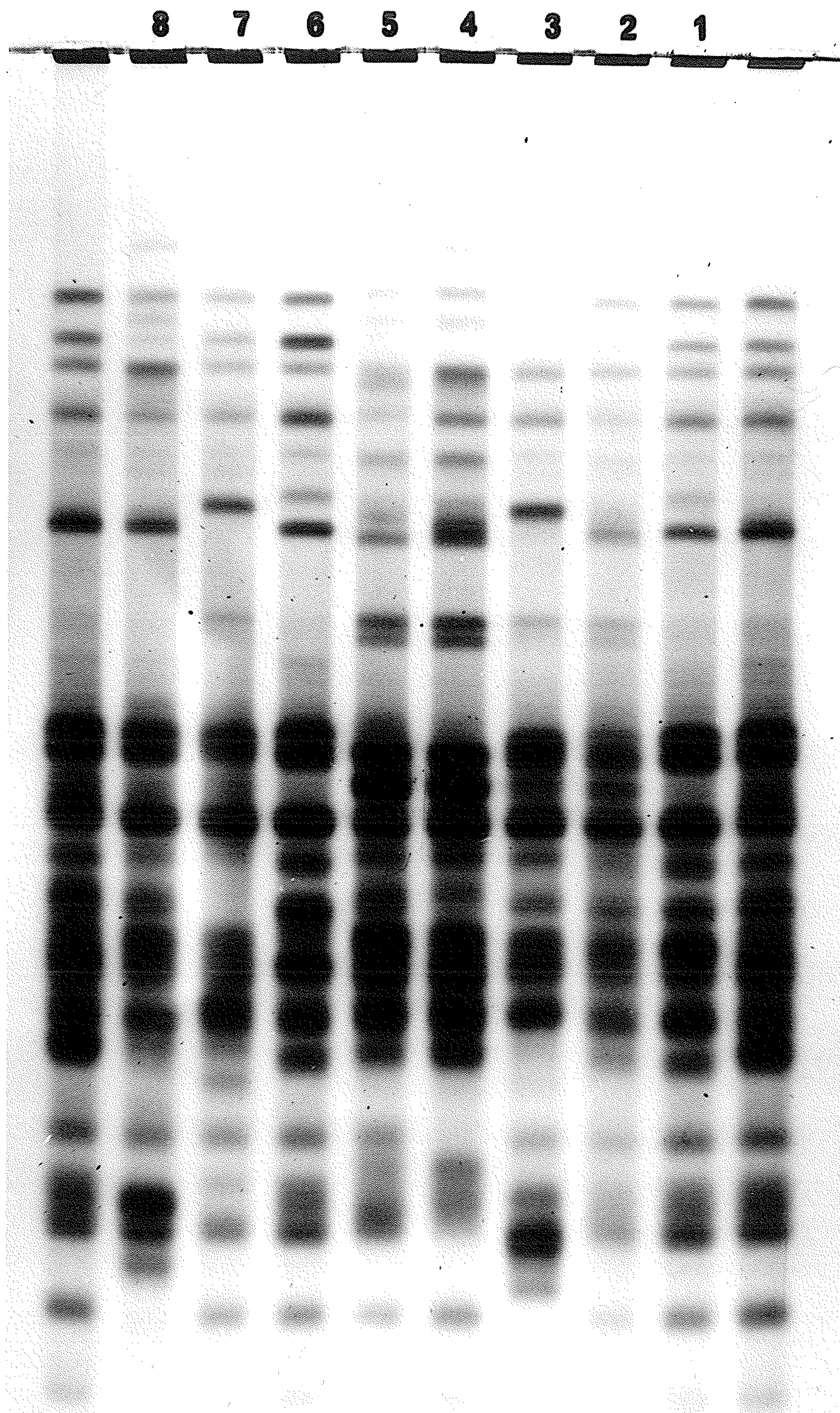

8 7 6 5 4 3 2 1

Figure 8

Gliadin electrophoregrams of common spring wheat cultivars (nos. 9 to 16 in Table 1).

9.	Chester	13.	Early Red Fife
10.	Chinook	14.	Fielder
11.	Coronation II	15.	Garnet
12.	Cypress	16.	Glenlea

16 15 14 13 12 11 10 9

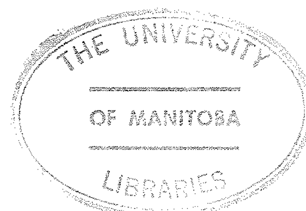


Figure 9

Gliadin electrophoregrams of common spring wheat cultivars (nos. 17 to 24 in Table 1).

17. Huron

18. Kenhi

19. Kota

10. Lake

21. Lee

22. Lemhi 53

23. Lemhi 62

24. Manitou

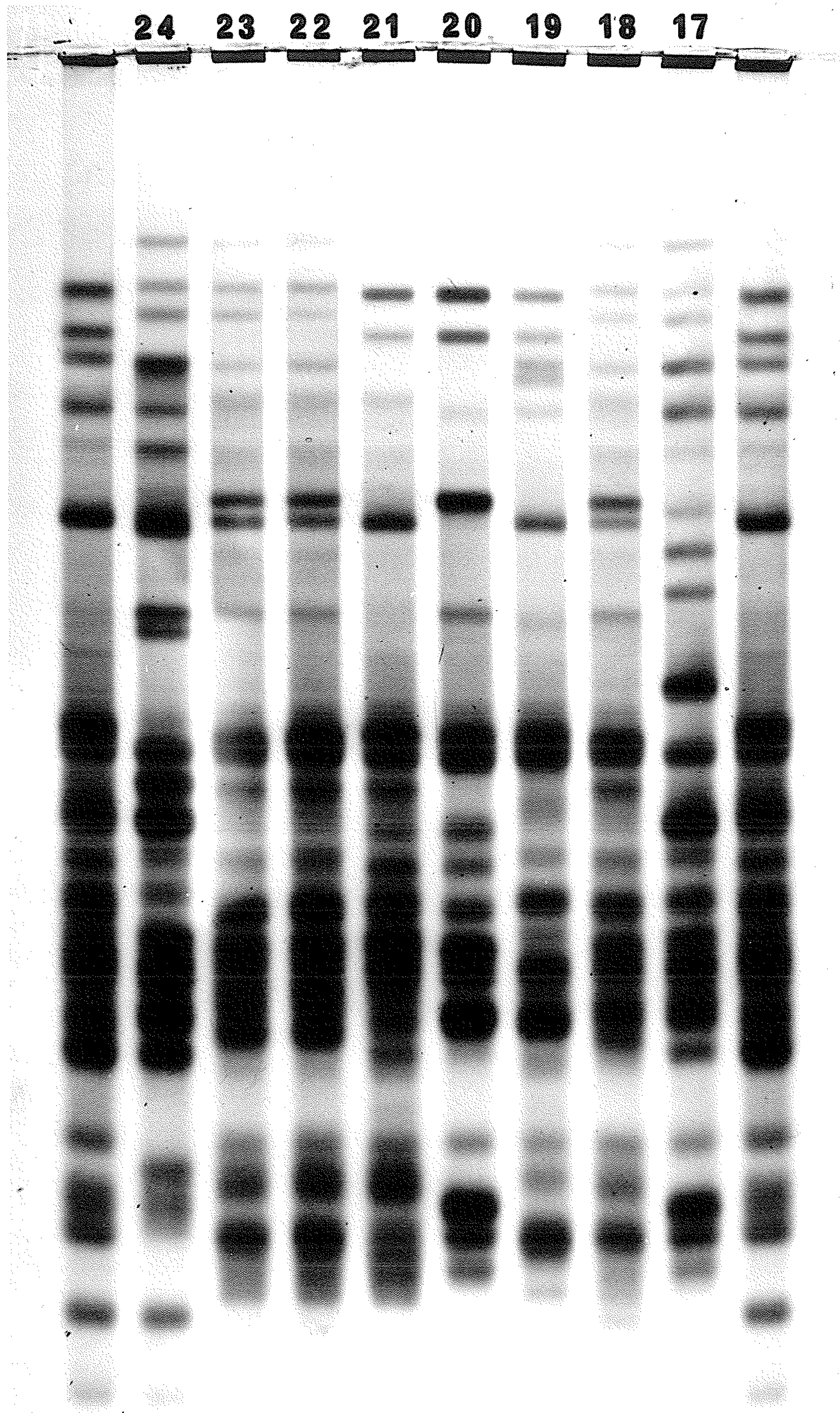

24 23 22 21 20 19 18 17

Figure 10

Gliadin electrophoregrams of common spring wheat cultivars (nos. 25 to 32 in Table 1).

25. Marquis
26. Napayo
27. Neepawa
28. Norquay
29. Park
30. Pembina
31. Pioneer
32. Pitic 62

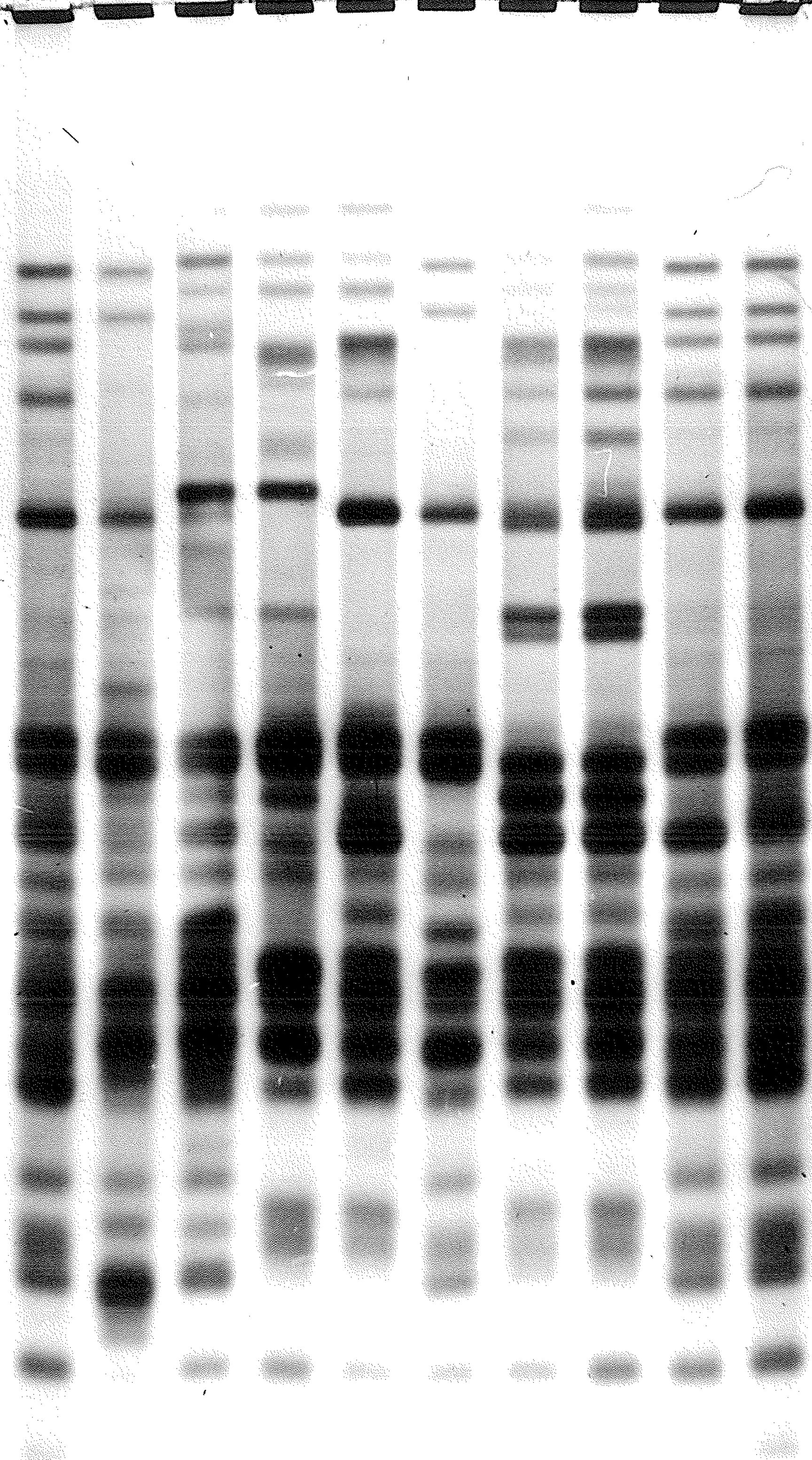

32
31
30
29
28
27
26
25

Figure 11

Gliadin electrophoregrams of common spring wheat cultivars (nos. 33 to 40 in Table 1).

33. Prelude
34. Preston
35. Quality A
36. Red Bobs 222
37. Red Fife
38. Redman
39. Regent
40. Reliance

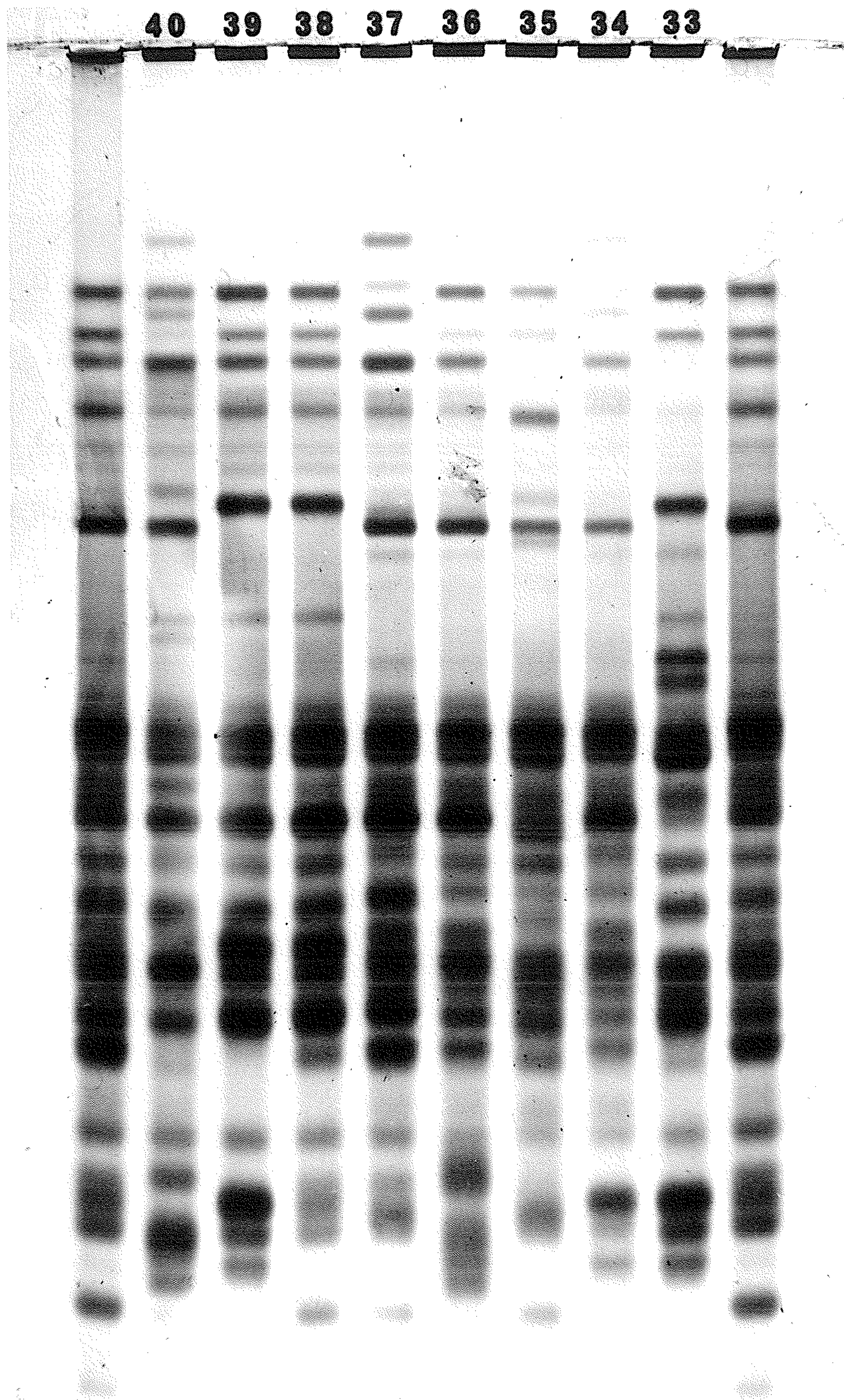

40
39
38
37
36
35
34
33

Figure 12

Gliadin electrophoregrams of common spring wheat cultivars (nos. 41 to 48 in Table 1).

41.	Renfrew	45.	Ruby
42.	Renown	46.	Saunders
43.	Rescue	47.	Selkirk
44.	Reward	48.	Sinton

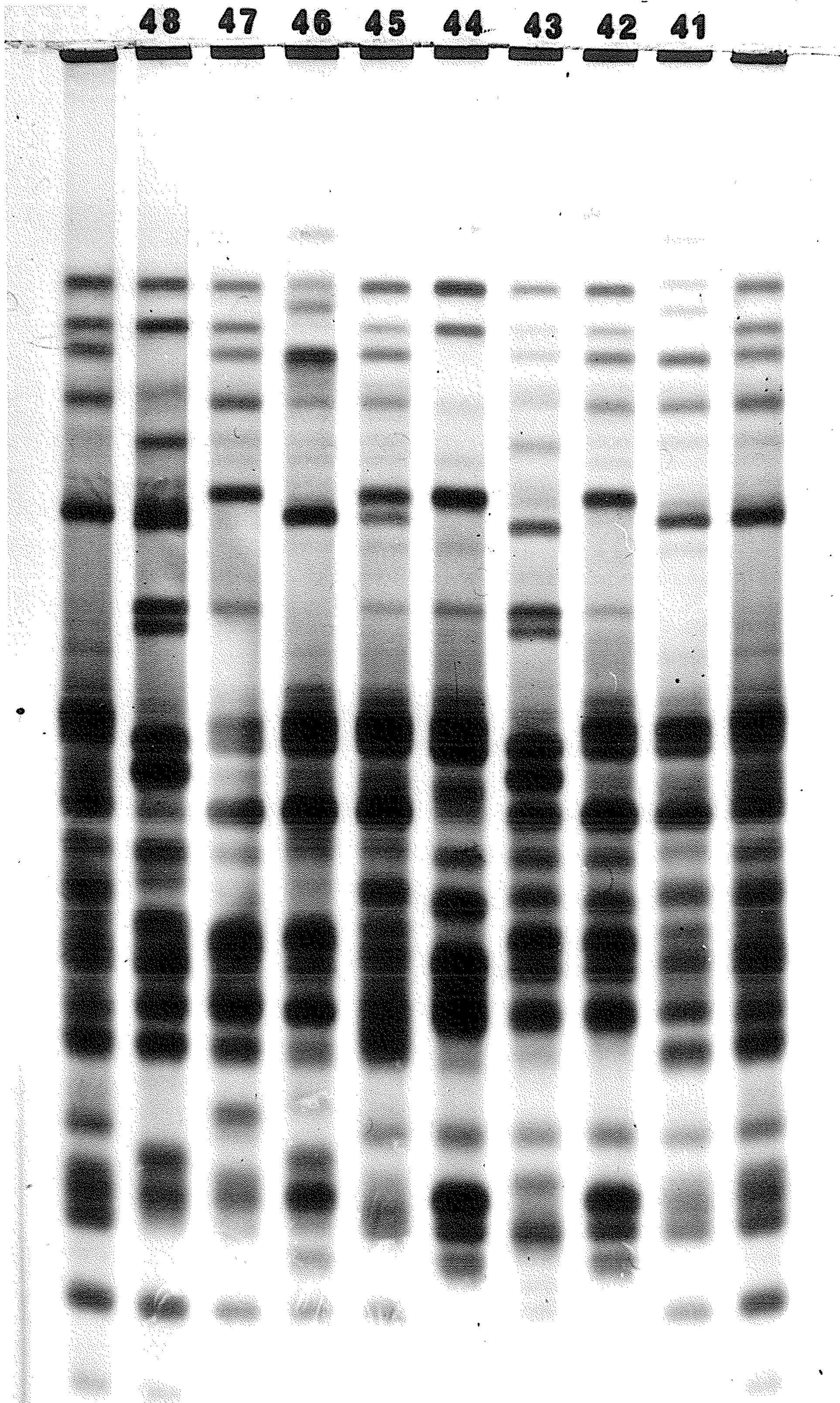

48
47
46
45
44
43
42
41

Figure 13

Gliadin electrophoregrams of common spring wheat cultivars (nos. 49 to 50 in Table 1) and common winter wheat cultivars (nos. 51 to 56 in Table 2).

49. Springfield
50. Thatcher
51. Cornell 595
52. Dawbul
53. Dawson's Golden Chaff
54. Egyptian Amber
55. Fairfield
56. Fredrick

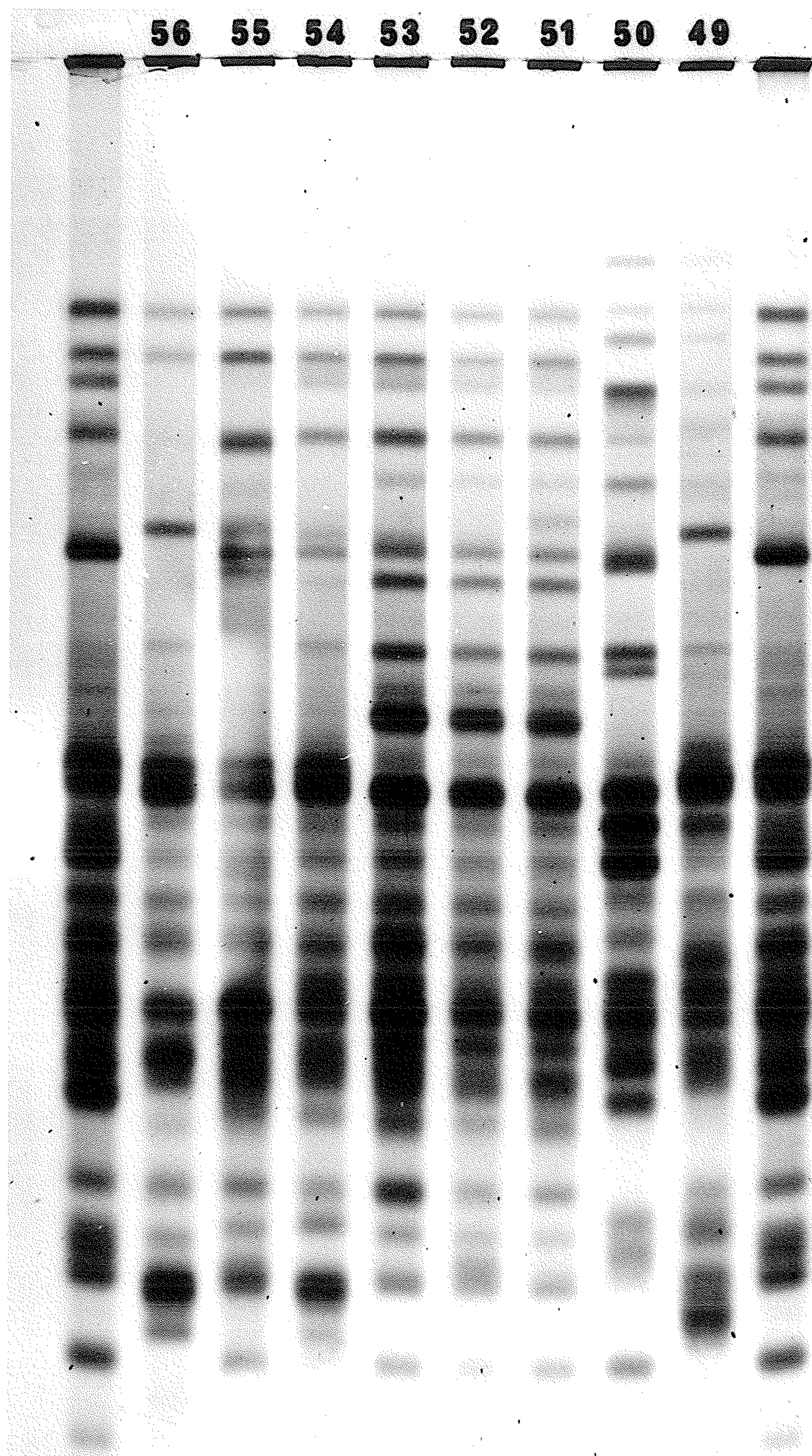

56
55
54
53
52
51
50
49

Figure 14

Gliadin electrophoregrams of common winter wheat cultivars (nos. 57 to 64 in Table 2).

57. Gaines
58. Genesee
59. Jones Fife
60. Junior No. 6
61. Kent
62. Kharkov 22 M.C.
63. Nugaines
64. O.A.C. 104

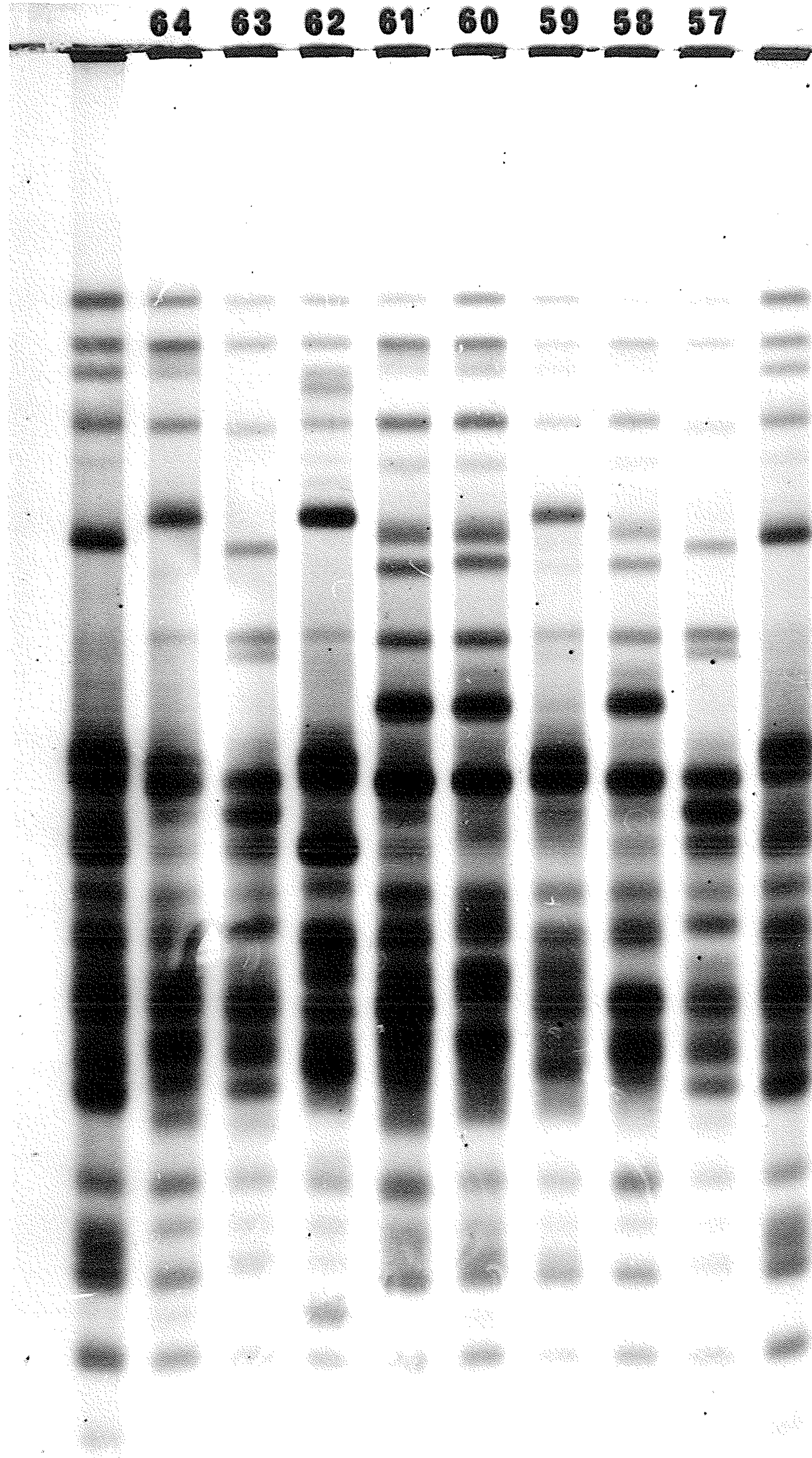

64
63
62
61
60
59
58
57

Figure 15

Gliadin electrophoregrams of common winter wheat cultivars (nos. 65 to 72 in Table 2).

65. Richmond
66. Rideau
67. Ridit
68. Sun
69. Sundance
70. Talbot
71. Thorne
72. Wasatch

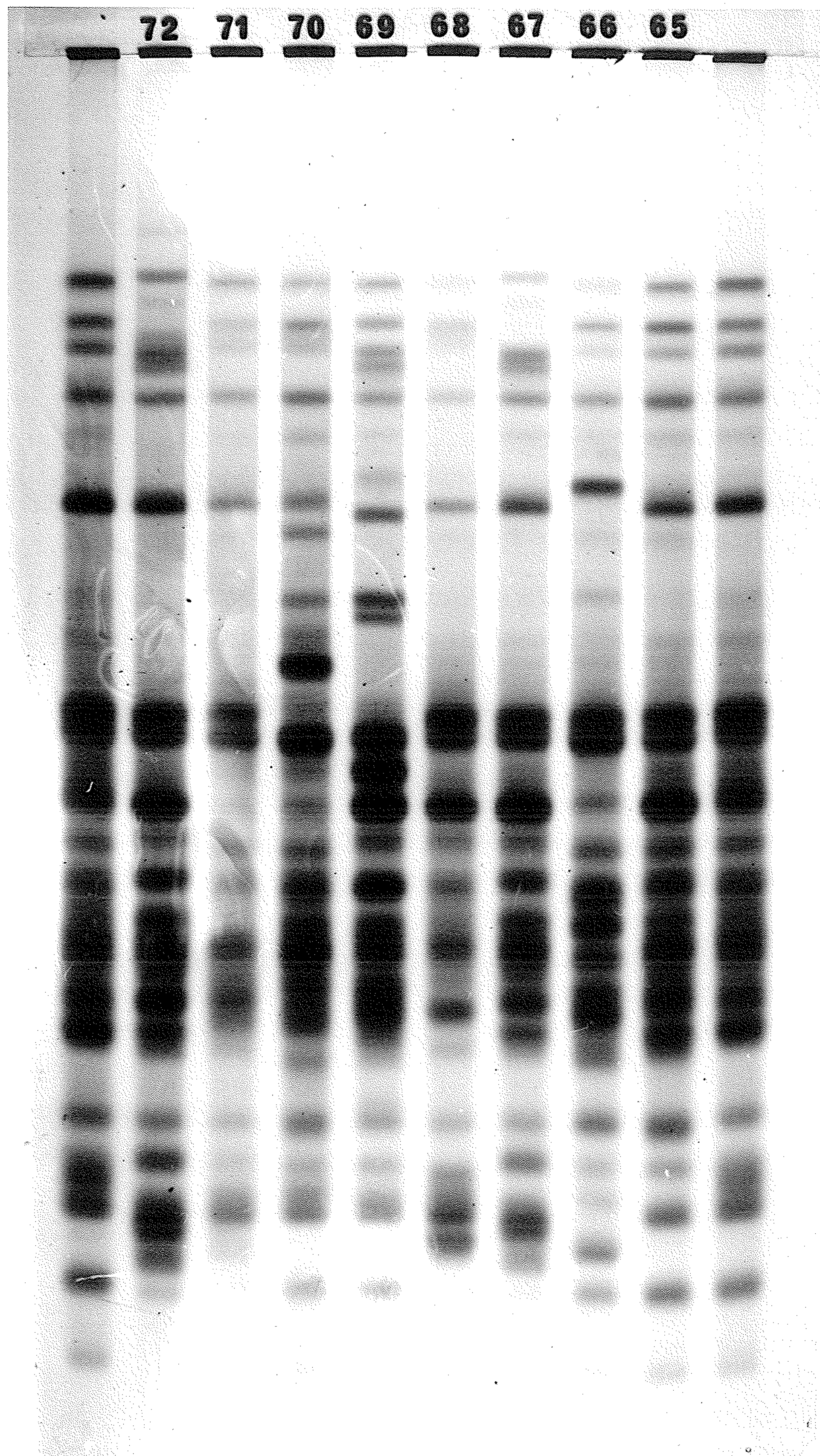

72
71
70
69
68
67
66
65

Figure 16

Gliadin electrophoregrams of common winter wheat cultivars (nos. 73 to 76 in Table 2) and durum wheat cultivars (nos. 77 to 80 in Table 3).

73. Westmont
74. Winalta
75. Yogo
76. Yorkstar
77. Carleton
78. Golden Ball
79. Hercules
80. Macoun

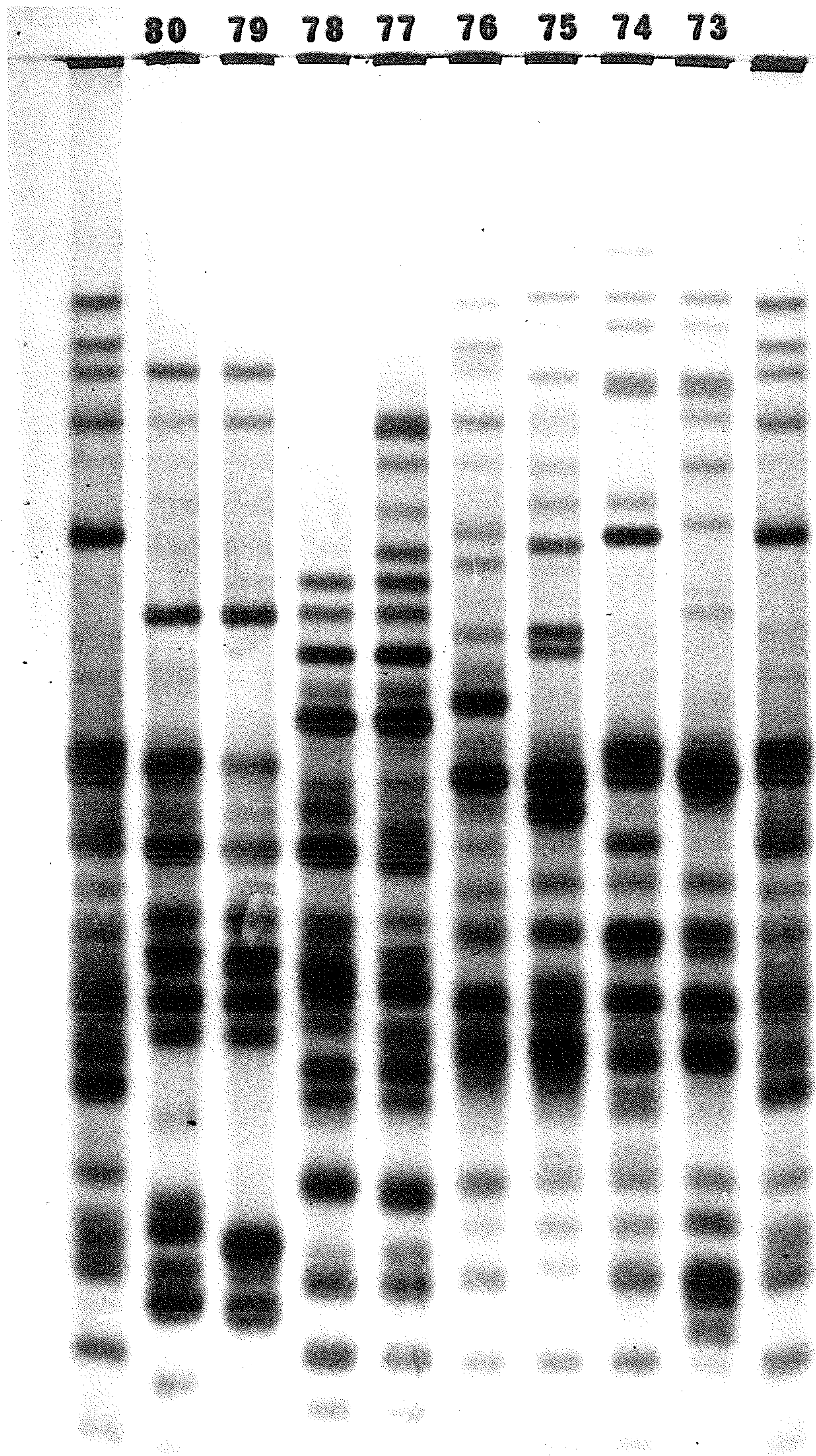

80
79
78
77
76
75
74
73

Figure 17

Gliadin electrophoregrams of durum wheat cultivars (nos. 81 to 88 in Table 3).

81. Mindum
82. Nugget
83. Pelissier
84. Ramsey
85. Stewart
86. Stewart 63
87. Wakooma
88. Wascana

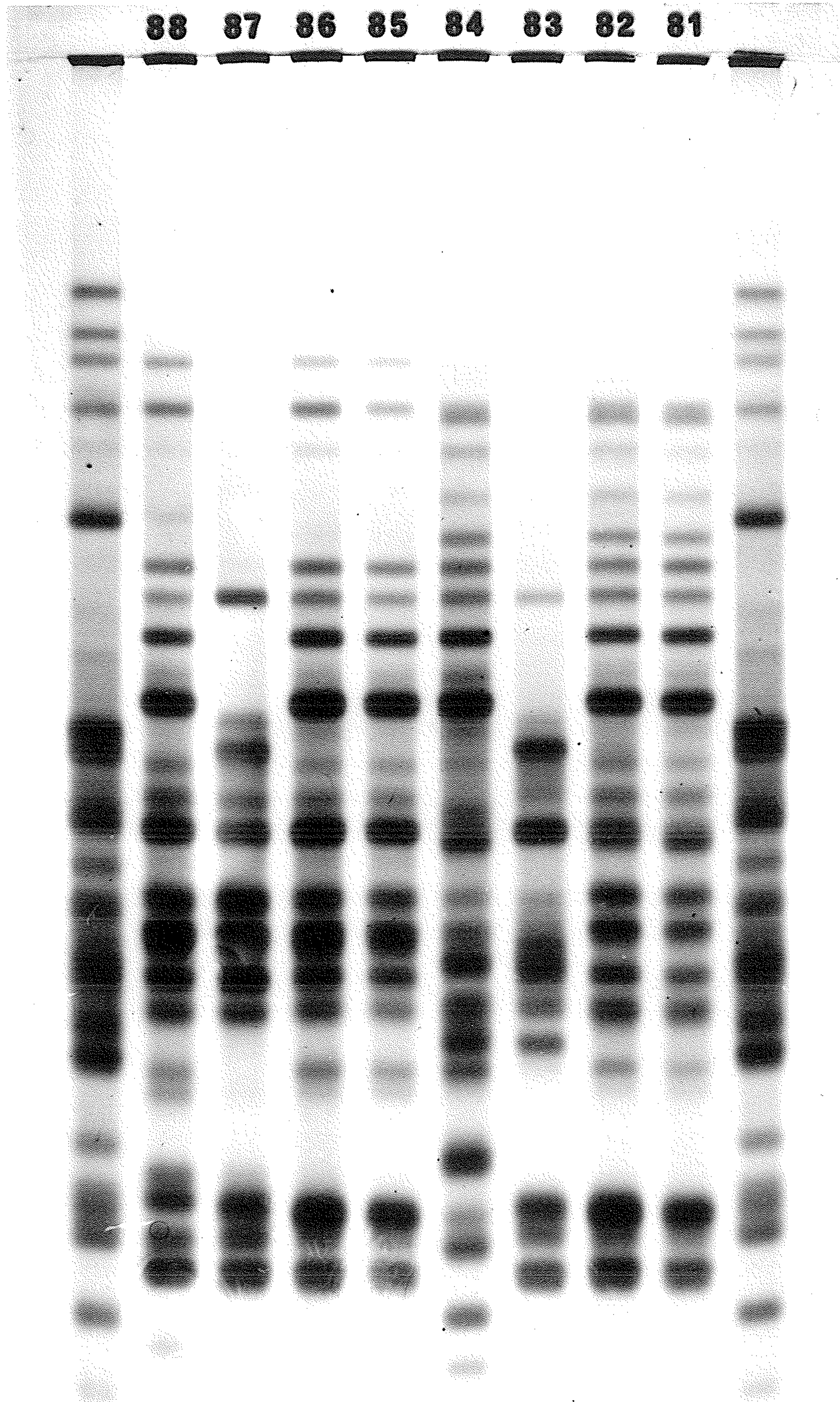

88
87
86
85
84
83
82
81

Table 5. Cultivar formulas of common spring wheats based on gliadin electrophoregrams

Band mobility relative to the Marquis reference band (scale: 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90)

No.	Cultivar	Band values (in order of increasing mobility)
1	Acadia	3* 3 3 3 1 1 4 5 5 1 5 3 3 4 3 4 3 3 2 3 2 1
2	Apex	2 1 2 1 1 2 1 3 4 3 5 2 2 3 2 3 2 1 1 2 1
3	Bishop	1 2 1 3 3 1 4 1 5 5 3 5 3 3 4 4 4 2 3 5 2 1
4	Canthatch	2 2 2 3 3 3 1 4 3 2 5 5 5 3 2 4 4 4 4 3 3 2 1
5	Canuck	1 2 1 2 1 2 1 2 3 2 1 5 5 4 3 3 4 4 4 3 2 1 3 2 1
6	Canus	3 4 3 4 2 2 4 1 5 5 5 3 4 1 4 4 3 3 2 3 2 1
7	Cascade	2 2 2 2 2 1 1 3 1 1 4 4 1 5 1 3 3 4 2 2 2 2 3 2 1
8	Ceres	1 2 1 1 3 2 1 1 3 1 5 5 1 5 2 3 3 3 3 2 2 4 3 2 1 1
9	Chester	2 2 2 3 1 1 4 1 1 5 5 4 3 3 4 5 5 3 4 2 3 3 3 1
10	Chinook	1 2 2 4 4 2 4 3 5 5 2 5 3 2 3 4 3 3 4 2
11	Coronation II	2 2 2 2 1 4 5 5 5 3 3 4 2 4 3 3 1 3 2 1
12	Cypress	1 2 1 2 2 2 4 3 5 5 5 2 3 3 5 4 4 3 3 5 2
13	Early Red Fife	4 1 4 1 3 2 1 1 2 5 4 5 2 3 1 2 3 3 1 2 3 4 2 1
14	Fielder	4 4 1 1 5 1 5 4 3 1 2 3 3 4 3 3 2 1 3 2 1
15	Garnet	2 2 2 3 2 5 1 3 3 1 4 2 2 1 2 4 1 2 5 2 2
16	Glenlea	2 2 2 31 2 4 1 1 5 4 5 2 2 2 3 2 2 1 2 5 3 1
17	Huron	3 2 2 3 3 1 1 3 3 5 5 5 3 3 3 4 4 3 2 5 4 3 1 1
18	Kenhi	1 2 1 1 1 1 4 3 1 5 4 3 1 2 3 3 3 3 2 2 3 4 2 1
19	Kota	3 2 2 2 1 4 5 5 2 2 4 1 4 4 2 2 2 5 2
20	Lake	5 4 1 5 3 5 5 3 3 3 4 3 5 2 5 4 3 1 1
21	Lee	4 2 1 1 4 5 5 3 2 3 3 4 4 3 2 2 4 3 2
22	Lemhi 53	1 2 1 1 1 1 4 3 1 5 5 3 2 3 4 4 2 3 4 1 2 3 4 2
23	Lemhi 62	2 2 2 1 1 1 3 3 1 4 3 2 1 1 4 4 3 3 3 1 2 3 4 2
24	Manitou	3 3 3 4 3 3 1 4 4 4 1 5 5 5 2 2 3 4 4 3 2 2 2 1
25	Marquis	3 3 3 3 1 4 1 1 1 5 5 1 5 3 4 4 4 4 4 3 3 3 3 1
26	Napayo	2 3 2 1 4 1 3 3 1 5 5 4 5 5 5 3 3 5 5 5 5 3 2 3 1

Table 5. Cultivar formulas of common spring wheats based on gliadin electrophoregrams (cont'd)

Band mobility relative to the Marquis reference band

No.	Cultivar	<15	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85–90
27	Neepawa	1	1 1	3 1 1	1	4	4 4		5 5	5 3	3 5	5 5	5	3	2	3	1
28	Norquay		3 3			4			5 5	3 3	3 4	3 5	3	2	2 3	2	1
29	Park	2	2 3	4 2		5	1	2	5 5 2	5 2	3 3	4 4	4 1	3	2	2	1
30	Pembina	2	2 2	3 1 1	2	4	2		5 5 3	3 2	5	4 5	3	3	3	3	1
31	Pioneer	1	3 2	1 2 1		4 2 1	1		5 5 3	4 3	4 3	4 4 3	3 1	2 2	3	2	1
32	Pitic 62		2 2	1		3	1 1 1 1	2	4 5 3	3 3	3 1	4 4 2	1	2 2	5	1	
33	Prelude		4 3	1		5 2	2	5 4 1	4 5 3	1 3	4	5 2 5	2	2	5 4 3	1	1
34	Preston	1	1	3 1 1		4 1		2	5 4 1	5 2	2 3	3 2 3	3 1	2	4 2 3		
35	Quality A		3 1	4	1	2 4 2		2	5 5 2	3 3	2 2	4 2 4	3 1	2	3 1	2	1
36	Red Bobs 222		4 1	4 1 2		5 1		2	5 4 2	5 3	3 3	4 1 3	3	2 5	3 3	3 1	
37	Red Fife	3	2 3	5 1 3	1 1 1	5 2		2 2	5 4 3	5 3	4 3	4 1 5	5	3 2	3 1	2	1
38	Redman		4 3	4 1 3	1 1	5	3	2	5 5 2	5 3	4 5	4 5	3	3 1	3 2	3	1
39	Regent		5 3	4 1 3	1 1	5	2	2	5 5 1	5 3	4 5	4 5	3		5 3 3	1	
40	Reliance	1	3 2 1	4 1 1	1 2	4	2 2	1 2	5 5 3	5 2	3	5 3	1	3 3	5	3 1	
41	Renfrew	2	3 3	4 3	1 1	2 4 1	1	2	5 5 2	5 3	4 3	4 4	4	3	3 3	3	1
42	Renown		4 3	4 3	1 1	5	2	2	5 5 2	5 3	4 5	4 5		3	5 4 3	1	1
43	Rescue		3 2	2 2	2	1 4	5 4	1	5 5	5 4	4 5	4 5	1	3 3	4	1 2	
44	Reward		5 4	1	1	5 1		2	5 5 3	2 3	5	5 5	2	3	5 4 3	1	1
45	Ruby		4 2	3 3	1 1	4 3 1	2	2	5 5 2	5 2	4 3 3	4	4	3	3	3	1
46	Saunders	2	3 3	5 1 2	1 1	5	1	2	5 5 2	5 2	1 5	3 5	3 2	3	5 2 3	3	1
47	Selkirk		3 3	3 3	1 1	4	3	1	4 4 2	5 2	5	1 5	4 3	2	3 2	3	1
48	Sinton		3 4	1	3 1	45	5 4	1	5 5	3 4	3 5	5 5	4 1 1	3	3	4	2
49	Springfield	1	1 1	1 1	1	4	1	2	5 5 3	2 3	4 4	4 4 3		2 3	3 5		
50	Thatcher	3	2 3	5 1	3	5	5 4	1	5 5	5 3	3 4	4 4	4	3	3 1	3	1

*Relative band intensity is indicated by a number (1 to 5; 5 is most intense) in the appropriate position according to the mobility scale at the top of the table.

Table 6. Cultivar formulas of common winter wheats based on gliadin electrophoregrams

Band mobility relative to the Marquis reference band

No.	Cultivar	<15	15–19	20–24	25–29	30–34	35–39	40–44	45–49	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85–90
51	Cornell 595		3* 3	1 4	1 1	3 3	4 2	5 1	5 3	3 3	4 2	5 3 4	3	3 3	3	3	1
52	Dawbul		2 2	1 3	1	3 3	3 2	5 1	5 2	2 3	3 1	4 3 2 3	2	2 2	3 3	2	1
53	Dawson's G. Chaff		3 4	2 5	2	4 4	4 2	5 1	5 3	3 3	4 3	5 4 4 3	3	4 3	3	3	1
54	Egyptian Amber		2 3	1 3	1	2	1	5	5 2	3 3	3 3 4	4 2	2	2 3	5	2	
55	Fairfield		3 4		2	4 2		4	4 3	3 2 3	3	5 3 4	3	3 3	4	3	
56	Fredrick		2 2		4		1	1 1 5	5 3	2 3	3 4	4 3	1	3 2	5	3 1	
57	Gaines		2 2	2		3	3 3	1	5 5	4 3	4 2	4 4	3 1	2 1	2	3	1
58	Genesee		2 3	1 3	1	3 3	3 2	5 1	5 3	3 3	4	5 5	3	4 2		3 3	1
59	Jones Fife		3 2	1 2		4 1 2	2 1	1 5	5 3 2	3	3 4	4 2 4	2	2 2	1 2	2	1
60	Junior No. 6		4 4	2 5	2	4 4	4 2	5 2	5 1 2	3	4 4 3	4 3	3	3 2	3 3	1 3	1
61	Kent		2 4	1 4	2	3 4	4	5 1	5 3	3 4	4 4	5 3 3 2	3	4 3	3	2	1
62	Kharkov 22 M.C.		3 3	2 3 3	1 1 5		2	1 2 5	4 1	5 3	4 3	3 2 4	2	2 2	2 3	2	1
63	Nugaines		2 2	2		3	3 3	1	5 5	3 2	3 2	4 4	3 1	2 2	2	2	1
64	O.A.C. 104		3 4	1 3	4		1	4	5 3	2 3	3 3	4 4 3	3	3 2	3 1	3	1
65	Richmond		3 4	3 4	2	5 1	1	1 5	5 3	5 3	4 4 5	5	5 2	4 3	4	4	1
66	Rideau		2 3	2 3	1 1 5	1	2 1	1 5	5	3 3	4 4	4 4 5	3	3 2	2 3	3	1
67	Ridit	1	3 1	4 3 3	1	5	1	1 5	5	3 3	4 4 5	1 4	3 1	3 4	2 5	3 1	
68	Sun		1 1	2		3		1 5	5 1	5 3	3 1 3	2 4	3	3 3	4 4		
69	Sundance		3 3	3 3 3	1 2	4	4 3	2	5 5	5 3	5 4 4	4 5	3 1 1	2 2	3	3	1
70	Talbot		2 3	2 4	2	3 3	3 2	5 2	5 3	3 3	4 4	5 4 4 4	3	3 2	2 3	3	1
71	Thorne		2 1	1 2		2		1 5	4 1	2 2	2 3	1 3 2	1	2 1	2 3	1 1	
72	Wasatch	1	3 1 1	4 3 3	1	5	1	1 5	5 2	5 3	4 4 5	2 4	3 2	3 3	3 5	3 2	
73	Westmont	1	3 2 1	3 2 3	3	3 1	2	2	5 3	1 4	4 3	5 4		3 4	5	4 2	
74	Winalta	3	3 3	4	3	5	1	1 5	5 1	5 3	5 5	2 3	3 2	3 3	4	4	2
75	Yogo	1	3 2	3 1	2 3	4 1	4 4	2	5 5	1 3	4 4	4 5 3	2 1	3 3	2	3	2
76	Yorkstar		2 2	1 3	1	3 3	3 3	5 2	5 3	3 3	4 5	4 2		4 2	3	3	1

*Relative band intensity is indicated by a number (1 to 5; 5 is most intense) in the appropriate position according to the mobility scale at the top of the table.

Table 7. Cultivar formulas of durum wheats based on gliadin electrophoregrams

Band mobility relative to the Marquis reference band

No.	Cultivar	20.5	23.5	26	28.5	29	30	31	31.5	33.5	35.5	38	40.5	42	43	43.5	44	44.5	45	46	46.5	47.5	48	48.5	49	50	50.5	51	52
77	Carleton		5*	3		3			4	4	4	5	3	5			2			3		1			3			4	
78	Golden Ball									4	4	5	3	5			1			3		4				5			
79	Hercules	4	3	1	1			1		1	5								4				2			4			
80	Macoun	4	2	1	1			1			5				1			5		2			3			5			1
81	Mindum		4	2		3			4	4	4	5	2	5			2			2				3				5	
82	Nugget		4	3		3	1		4	4	4	5	2	5			2			3				3			5		
83	Pelissier										3					2			5		2		2				5		
84	Ramsey		4	3		3			4	4	4	5	2	5			2			2					13			5	
85	Stewart	3	3	1						4	3	5	1	5			1			3				3			5		
86	Stewart 63	3	4	2						4	4	5	1	5			2			3				3			5		
87	Wakooma										5					3			5					3			5		
88	Wascana	3	3	1			1			4	3	5	2	5			1			3				3			5		

No.	Cultivar	54.5	55	56	56.5	57	57.5	59	59.5	60	61	61.5	62	63.5	64	65.5	66	66.5	67	71	71.5	72.5	74	74.5	75	75.5	76.5	77	77.5	78.5	79	82	83	83.5	85	87.5	89.5
77	Carleton	3			4			5				4			5		3				5					3			5			4			3		
78	Golden Ball	3		2			5	5			4			4		3			1	5						2		5				5			3		
79	Hercules	5				5			5			4													5		2				5	1					1
80	Macoun	4			5				5			4						1				3	5				4			5				2		1	
81	Mindum	5				4			4				4			2			1						5		2				5	1					1
82	Nugget	5				5			5				5			3			1						5		3				5	1					1
83	Pelissier		2				4	5				3			4									5			3				5	1				1	
84	Ramsey	3				3		5				4			4		4				5					2		4				3			2		
85	Stewart	4					5			4			3				3		2						5		2				5	1			1		1
86	Stewart 63	4					5			4			4				3		2						5		3				5	1			1		1
87	Wakooma	5					5			5			4									1		5			4				5		2			1	
88	Wascana	4					5			4			4				3		2			3	4				3				5			2		1	

*Relative band intensity is indicated by a number (1 to 5; 5 is most intense) in the appropriate position according to the mobility scale at the top of the table.

doublet of Marquis (bands 44.5 and 45.5 in Figure 6) was resolved. On the basis of the gliadin electrophoregrams shown in Figures 7 to 17, a catalogue of cultivar formulas was prepared (Tables 5 to 7) using the nomenclature described in the previous section.

1. Determination of Gliadin Band Mobilities

Photographs of stained electrophoregrams were compared using three different types of film (High Contrast Copy, Panatomic X, and Plus X). The best results were obtained with High Contrast Copy film. However, in preparing prints, there was an apparent loss of some faint bands and a decrease in the resolution of some closely-spaced intensely-stained bands. In order to obtain accurate mobilities for all bands present in the original stained electrophoregram, measurements were made on the photographic negative rather than on the print.

The negative (representing eight electrophoregrams flanked on each side by the Marquis electrophoregram used as the reference) was held between two strips of thin glass plate and placed in the travelling stage of a Nikon profile projector Model 6C fitted with a 10X projection lens. The negative was aligned by rotating the stage slightly so that the vertical crosshair on the projection screen could be centered on the same band in the Marquis electrophoregram located at the top and bottom of the negative. The horizontal crosshair on the projection screen should be parallel to each electrophoregram on the negative. When the travelling stage (holding the negative) was moved longitudinally the vertical crosshair would then indicate the same position (read off the revolving micrometer scale) on each electrophoregram on the negative.

The distance from the origin to the center of the Marquis reference band was the reference distance that was assigned a mobility of 50. In each electrophoregram, the distance from the origin to the center of a band was the migration distance of that band. The migration distance of a specific band divided by the migration distance of the Marquis reference band and multiplied by 50 equals the relative mobility of the band. In this manner, the relative mobilities of the bands in each of the eight electrophoregrams on each negative were determined.

2. Precision of Determination of Band Mobilities

In all electrophoretic methods, reproducibility of the electrophoregram is extremely difficult. This aspect has, most often, been disregarded in the relevant literature. Lee and Ronalds (1967) are among the few who mentioned the difficulty of controlling experimental factors. Non-uniform gels (particularly starch), inefficient gel cooling during electrophoresis, and ineffective gliadin extraction are probably the three most critical factors that can affect the results. Doekes (1969) estimated the reproducibility of his gliadin densitograms from results of experiments in which the extraction of the flour and the composition of the gel were taken into account.

The precision of the electrophoretic procedure used in this study was determined by comparing the mobilities of three bands (two ω-gliadin bands and one α-gliadin band) in the Marquis reference electrophoregram. It was found that, for any one gel, the relative mobility of each band was exactly the same in both reference electrophoregrams (i.e. top and bottom of the negative). This shows that the rate of protein

migration is uniform across the gel.

The average values of the mobilities of the three bands of the reference electrophoregram (selected to determine the precision) on eleven different gels were 15.98 ± 0.13, 30.64 ± 0.23, and 87.18 ± 0.38 respectively. The mean deviations indicate that band mobilities are highly reproducible from one gel to another. As might be expected, the precision is higher for the lower mobility bands. A precision of ± 0.5 is considered satisfactory for the intended purpose of using the electrophoregram for cultivar identification.

3. Determination of Band Intensities

Intensities of the stained gliadin bands were quantified subjectively by a number from one to five, five representing the most intensely stained bands (see Tables 5 to 7). The precision of this method is considered to be ± 1. Because band intensities depend on the protein content of the grain sample (hence protein in extract used for electrophoresis), they are of minor importance, compared with the total number of bands and band mobilities, in the cultivar formula. A small difference in band intensity (or in the occurrence of a very faint band) alone is not sufficient to distinguish between two cultivar formulas which may otherwise be the same. The most accurate way to quantify band intensities is by scanning the electrophoregram with a transmission densitometer and computing the area under each peak in the densitogram. This was not done in the present study.

4. Use of the Catalogue of Cultivar Formulas

The catalogue (Tables 5 to 7) can be used to determine (or confirm) the cultivar identity of a sample of grain. This is accomplished by first determining the cultivar formula from the electrophoregram by the procedure described above and then identifying the same formula in the catalogue. This identifies the cultivar directly. If the unknown grain sample is suspected to be a mixture of grain of more than one cultivar, then electrophoregrams of single kernel extracts should be examined. Mixtures can be confirmed by examining at least 10 kernels. A much larger sample (e.g. 100 kernels) must be examined to obtain information on the composition of the mixture. The electrophoregram formula of the unknown wheat sample, when written on a strip of paper using the same mobility scale as in the catalogue (see Tables 5 to 7), can simply be compared with each listing in the catalogue until a match is found.

As an extension of the present study, it would be of interest to investigate the use of the computer for comparing the formula of an unknown cultivar with an identical one (hence identification of the cultivar) in the computer memory. The catalogue could then conceivably be expanded to include formulas for a large number of varieties that wheat breeders use in their breeding programs.

5. General Comments on the Catalogue of Cultivar Formulas

With only a few exceptions, every cultivar in the catalogue is identifiable on the basis of its cultivar formula. There are several cultivars which are very closely related genetically to one or more

other cultivars (see Tables 5 to 7). Examples of close relatives are Canus and Reliance, Garnet and Pioneer, Regent and Renown, Marquis and Renfrew, Lake and Redman, Lemhi 53 and Lemhi 62, and Manitou, Napayo, Neepawa, and Thatcher among the common spring wheats; Dawbul and O.A.C. 104 and Gaines and Nugaines among the common winter wheats; and Stewart and Stewart 63 and Wakooma and Wascana among the durum wheats. There are many other examples of two or more cultivars in the catalogue having one or more common parents in their pedigrees. Nevertheless, with the exception of Lemhi 53 and Lemhi 62, Gaines and Nugaines, and Stewart and Stewart 63, each cultivar has at least one band in its cultivar formula which distinguishes it from its closest relative. The cultivars Manitou, Napayo, Neepawa, and Thatcher can be distinguished from one another only by taking into account band intensities and the occurrence of several faint bands.

Durum wheats can immediately be distinguished from common wheats on the basis of the number of the slow-migrating (ω-gliadin) bands (relative mobilities in the range 12 to 20). Not one of the twelve durum wheats listed has a band in this region, whereas every one of the 76 common wheats listed has at least two bands in this region.

VI. SUMMARY AND CONCLUSION

An electrophoresis test (including apparatus, method and nomenclature for bands in the gliadin electrophoregram) was developed for the purpose of identification of wheat cultivars. The apparatus and method were described in considerable detail. The apparatus was designed so that it would accommodate a long gel slab for optimum resolution of gliadin components. It provided efficient gel cooling and required a small volume of electrode buffer. A simple, rapid and highly reproducible polyacrylamide gel polymerization technique was perfected and described.

Several gliadin extraction, staining and photographic procedures were investigated in the process of developing the electrophoresis test. Sixty or 70% aqueous ethanol and 25% aqueous 2-chloroethanol were found to be the best extraction solvents. A staining solution of 0.04% Coomassie brilliant blue in 12% trichloroacetic acid was the most effective. High Contrast Copy film was the best film for photographing stained gels.

The electrophoresis test was used to show that growing location had no qualitative effect on the gliadin electrophoregrams of five Canadian cultivars grown at ten locations in Manitoba and Saskatchewan. This confirms previous reports that the gliadin electrophoregram is a genotypic character and therefore can be used for identifying wheat cultivars.

A new nomenclature for the bands in the gliadin electrophoregram was described. A prominent band near the center of the electrophoregram of the cultivar Marquis was adopted as the reference band and was assigned an arbitrary mobility of 50. All other bands of that and other cultivars were identified by mobility relative to that of the reference band. Three distinct advantages were cited to justify the proposed nomenclature.

A method for determining the relative mobility from the actual mobility measured by microcomparator directly from the photographic negative of the stained gel was developed. It was shown that band mobility was constant across any one gel (i.e. gel polymerization was uniform), and reproducible to within ± 0.5 from one gel to another (i.e. gel polymerization was reproducible). A subjective quantification of band intensities was described.

Using the electrophoresis test, cultivar formulas of 88 wheats (common and durum wheats) licenced for production in Canada were determined and catalogued. There were 85 distinct cultivar formulas and three pairs that were essentially identical; for each pair the cultivars were very closely related genetically. The use of the electrophoresis test and the catalogue of cultivar formulas for identifying unknown grain samples was discussed.

Two recommendations were proposed for further study to expedite the process of practical identification of wheat grain samples.

1) Cultivar formulas could be obtained by transmission densitometry of electrophoregrams in the gel. Scanning with ultraviolet light, if feasible, would eliminate staining and photography as well,

thereby considerably reducing the time required.

2) Cultivar formulas could be stored and analyzed in a computer memory. This would facilitate the storage of an extremely large and constantly expanding catalogue of cultivar formulas. Comparison (by computer) of the formula of an unknown wheat sample with each of the formulas in the memory would provide immediate identification.

In conclusion, a centralized service based on the electrophoresis test described in this study (and including the two proposed recommendations) could conceivably be used in the Canadian wheat grading and handling system for the purpose of cultivar identification.

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APPENDIX I: SELECTION OF A GLIADIN EXTRACTION PROCEDURE

Wheat gliadin proteins cannot be dissolved in aqueous salt solutions. They are soluble in lower aliphatic carbinols (e.g. 50 to 70% ethanol), in dilute alkali and acid solutions (although the original protein configuration is liable to be altered due to pH effects), and in urea. They exhibit slight solubility in water, some polar organic solvents (ketones and ethers), and some buffers (e.g. acetate, salicylate and lactate).

Based on the known solubility properties of gliadin, ground grain samples (one part by weight) were extracted with each of the following solvents by mixing briefly on a vortex mixer and allowing the mixture to stand at room temperature for one h, centrifuging ten min at 20,000 X g, and decanting the supernatant (gliadin extract):

1) six parts deionized distilled water (after two brief washes with five parts deionized distilled water to remove salt ions);

2) seven parts electrophoresis buffer (see Table 4);

3) six parts electrophoresis buffer (after two brief water washes);

4) seven parts 0.05N acetic acid;

5) six parts 0.05N acetic acid (after two brief water washes);

6) seven parts 1M urea;

7) seven parts 2M urea;

8) seven parts 25% aqueous 2-chloroethanol;

9) three parts 60% aqueous ethanol followed by addition of four parts electrophoresis buffer to the supernatant;

and 10) three parts 70% aqueous ethanol followed by addition of four parts electrophoresis buffer to the supernatant (standard procedure).

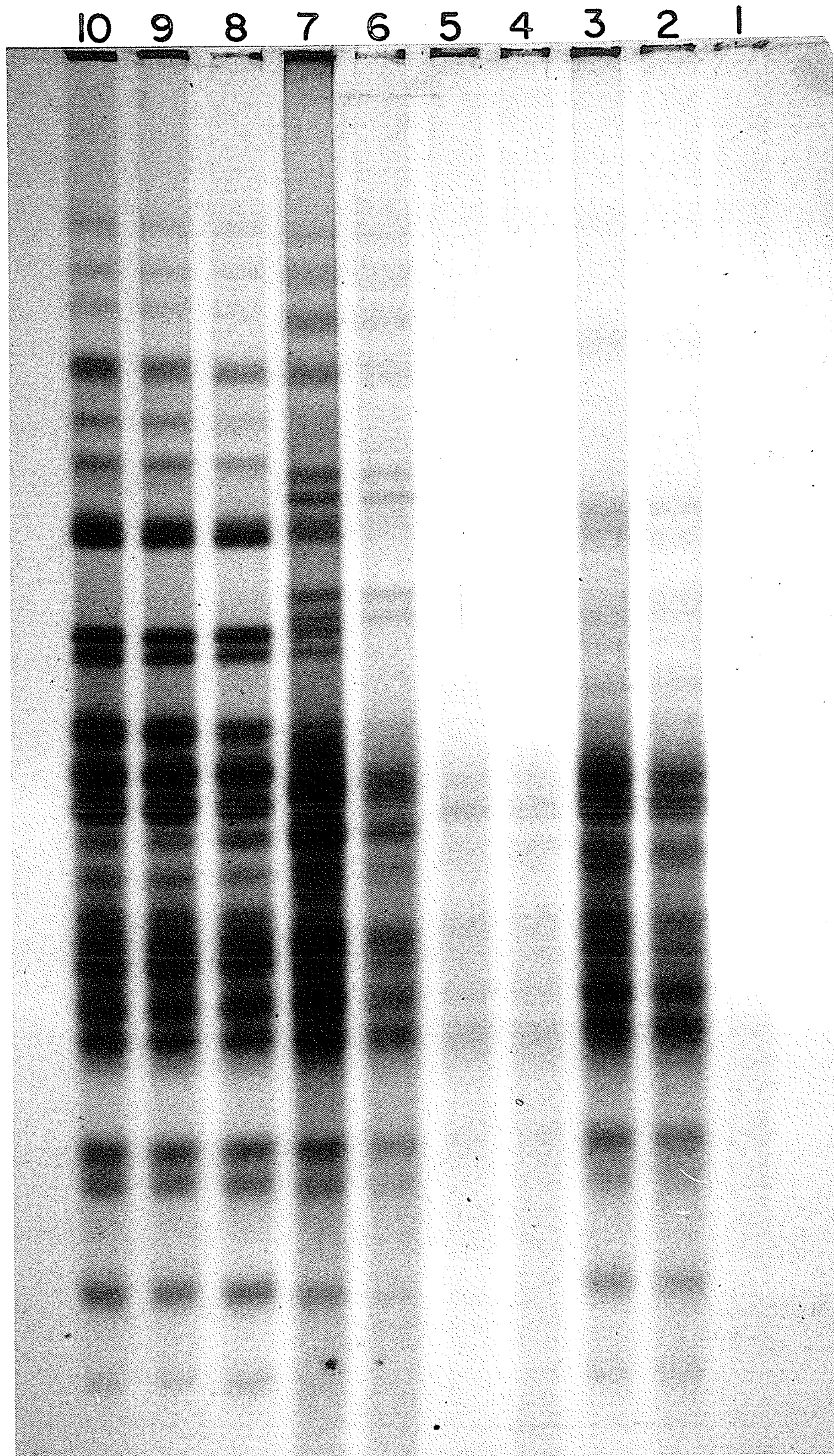
Electrophoregrams of the ten extracts were compared using grain of five different cultivars (bread wheats Marquis, Neepawa and Pembina, utility wheat Glenlea, and durum wheat Wascana). There were obvious quantitative differences (band intensities) and minor qualitative differences (number of bands and band mobilities) in the electrophoregrams of the same cultivar obtained using the nine different extraction procedures and the standard procedure. Essentially the same differences were observed for each of the five cultivars used. Results are shown for the cultivar Neepawa (Figure 18).

Deionized distilled water, electrophoresis buffer and dilute acetic acid did not extract sufficient gliadin, even with prior removal of salt ions by two water washes, to produce the characteristic electrophoregram obtained with the standard procedure (70% ethanol extract). Presumably, inefficient extraction led to a loss of some of the minor bands and a decrease in the intensity of the major bands. The use of 2M urea, although more effective than 1M urea as a gliadin solvent, caused considerable background staining and streaking of bands in the electrophoregram. There were some qualitative differences between electrophoregrams of urea and ethanol extracts. The electrophoregrams of the 2-chloroethanol and the ethanol extracts were the same. Sixty percent ethanol appeared to be equally as effective as 70% ethanol.

Figure 18

Gliadin electrophoregrams of hard red spring wheat cv. Neepawa for which the gliadin extract was prepared using ten different solvents.

1. water wash - water wash - water
2. electrophoresis buffer
3. water wash - water wash - electrophoresis buffer
4. 0.05N acetic acid
5. water wash - water wash - 0.05N acetic acid
6. 1M urea
7. 2M urea
8. 25% aqueous 2-chloroethanol
9. 60% aqueous ethanol
10. 70% aqueous ethanol


10 9 8 7 6 5 4 3 2 1

The latter was chosed for routine use (in the standard procedure) because it is the historically conventional solvent for wheat prolamine (Osborne 1907).

The gliadin electrophoregram (for each of the above five cultivars) was the same for extracts prepared (by the standard extraction procedure) from ground whole grain, from ground whole grain defatted with chloroform-benzene (5:2 v/v), and from flour (bran and germ removed). Similarly, an increase in the extraction time (2-, 5- or 10-fold) of the standard procedure had no effect on the electrophoregram. Therefore, one h at room temperature is adequate time for complete extraction of the gliadin protein.

APPENDIX II: SELECTION OF A STAINING PROCEDURE

To select the most appropriate staining-destaining procedure (i.e. the one that ensured the most distinct electrophoregrams), the following procedures were examined:

1) staining with 0.025% solution of Coomassie brilliant blue in a 7:20:80 (v/v/v) solution of glacial acetic acid, methanol and water containing 6% trichloroacetic acid and destaining with the same solvent without trichloroacetic acid (Koenig *et al*. 1970);

2) staining with a 0.1% solution of aqueous nigrosine in 10% trichloroacetic acid and destaining with 10% trichloroacetic acid (Ellis 1971);

3) staining with a 1% solution of amido black 10B in 7.5% acetic acid and destaining with 7.5% acetic acid (Ellis 1971);

4) staining with a 0.025% solution of aqueous nigrosine in 10% acetic acid and destaining with a 4:1:5 (v/v/v) solution of methanol, glacial acetic acid and water (Wrigley and Shepherd 1974);

5) staining with a 0.05% solution of aqueous nigrosine in 2% trichloroacetic acid and destaining with 40-50% aqueous ethanol (Autran and Bourdet 1975);

and 6) staining with a 0.04% solution of Coomassie brilliant blue in 12% trichloroacetic acid and destaining with 12% trichloroacetic acid (standard procedure).

The standard procedure proved to be most effective. Coomassie brilliant blue appeared to be a more effective dye for staining gliadin proteins in polyacrylamide gel than either amido black 10B or aqueous nigrosine. The latter two dyes did not seem to penetrate the gel, so that they stained the protein only at the gel surface. Of the dye solvents and destaining solutions used in these six procedures, trichloroacetic acid is the one in which gliadin protein is least soluble. Consequently, 12% trichloroacetic acid was used in the standard procedure in order to minimize diffusion of bands during the staining process, and to maximize the length of time that stained gels could be kept after completion of the staining process without the fading of stained bands occurring.