# Computer-Aided Analysis of Gliadin Electrophoregrams. II. Wheat Cultivar Identification and Class Comparisons<sup>1</sup>

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### ABSTRACT

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A computer-based methodology is described that facilitates identification and comparison of wheat cultivars based on gliadin electrophoregram relative mobility and band density data. The basic programmed task compares a numerically encoded unknown or test polyacrylamide gel electrophoretic (PAGE) pattern with all reference PAGE patterns in the data base. For each comparison, four classes of events are scored: matching bands, nonmatching bands in the unknown and reference electrophoregrams, and bands that differ significantly only in staining intensity. These parameters are used in an equation to quantify electrophoretic pattern homology that determines the order of cultivar ranking. A separate computer program evaluates the uniqueness of the unknown electrophoregram and identifies diverse genotypes. Plotting software that provides a graphic analysis of electrophoretic pattern

composition was developed to identify and differentiate matching and nonmatching bands in compared electrophoregrams. User-established parameters include least significant difference thresholds for comparing relative mobility and band density. The user may supply pedigree information and coded wheat class, quality, or region attributes for each reference electrophoregram. These data assist in the interpretation of program output and provide a basis for differentiation of cultivars according to functional type by gliadin composition. Two versions of the cultivar-ranking formula are described, and the performance of the computerized system is illustrated for several test input electrophoregrams against a PAGE pattern data base of 122 common spring, winter, and durum wheat cultivars.

The extensive heterogeneity of gliadin electrophoretic composition can confer a high level of discrimination among wheat cultivars. This attribute, combined with the stability of the gliadin electrophoregram in response to common environmental factors (Feillet and Bourdet 1967, Lee and Ronalds 1967, Wrigley 1970), gives the electrophoresis test its utility for cultivar identification (Autran and Bourdet 1975, Bushuk and Zillman 1978, du Cros and Wrigley 1979). However, experimental variation inherent in the electrophoretic method together with the multiplicity of gliadin components make the task of visually assessing the resemblance or composition of electrophoregrams both time consuming and imprecise.

Different approaches have been proposed to evaluate wheat cultivar identity using gliadin electrophoretic data (Wrigley et al 1982a). Diagnostic keys (Autran and Bourdet 1975, Ellis and Beminster 1977) or catalogs of cultivar formulas (Dal Belin Peruffo et al 1981, Jones et al 1982, Zillman and Bushuk 1979) based on band relative mobility (Rm) and relative staining intensity (density) values can reduce the arbitrariness of the identification process. These methods have limited value in routine applications where accuracy and speed are best achieved by computerized analysis.

An automated approach to expedite wheat cultivar identification was first reported by Bushuk et al (1978), who quantified gliadin electrophoregrams by minicomputer processing of densitometric scanning profiles. Computer programs to

manipulate the derived Rm and band density data encoded as "cultivar signature arrays" were outlined (Sapirstein et al 1980) for wheat cultivar identification and other comparative analyses. Wrigley (1980) used a computerized strategy to identify Australian varieties by implementing a program designed to solve problems in taxonomy. Apart from the need for a large mainframe computer to run this program, this approach depended upon an a priori classification of bands which had limited precision. Lookhart et al (1983) described a computer program for identifying wheat cultivars similar to our earlier approach based on Rm and band density features; however, only integer accuracy was used to encode band relative mobilities. A second factor limiting discrimination was use of a similarity coefficient that left out the contribution from nonmatching bands. Program evaluation was limited to a single computer plot that traced the declining distribution of data base cultivars as a function of computed similarity to the unknown.

This study considers a more rigorous methodology for computer-based wheat cultivar identification. A set of programs designed for generalized comparative analysis of two-value parameterized lists is described which, in the present application, encodes the gliadin electrophoregram.

# MATERIALS AND METHODS

# Wheat Cultivars

Wheat cultivars used to establish a data base of gliadin electrophoregrams are listed along with their data base identification numbers (DBIN) in Table I. Represented are 122 common spring, winter, and durum wheat cultivars licensed in Canada before 1984. The list includes cultivars of commercial and historic importance as well as those possessing regional or restricted licenses. Several U.S.-registered hard red spring (HRS) wheats (DBIN 84-92) were also included in the data base.

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# Gliadin Extraction and Electrophoresis

Gliadin extraction and polyacrylamide gel electrophoresis (PAGE) in 6% gels were performed as described by Sapirstein and Bushuk (1985a). For each cultivar listed in Table I an average of three gliadin extracts was prepared for electrophoresis. These were derived from at least one single kernel and one ground sample of grain. All replicates were run on separate gel slabs. Gaps in the sequence of DBIN (Table I) indicate that offtype electrophoregrams were obtained for the preceding cultivar sample in the list. A characterization of offtype patterns observed is reported elsewhere (Sapirstein 1984).

# **Determination of Gliadin Band Mobilities and Densities**

Mean band Rm values from replicated gliadin electrophoregrams were determined as described by Sapirstein and Bushuk (1985a). First, band migration distances were computed from photographs of gel slabs using a digitizing tablet, and then the absolute positional data were adjusted to the Rm scale using a new three reference band standardization implemented by means of a computer program.

Subjectively determining relative band densities from photographs of electrophoregrams was found to be sufficient, in terms of precision and speed, for cultivar comparisons. Bands were assigned an integer value from 1 (very faint) to 9 (very dense), relative to the band densities in the Neepawa reference electrophoregram run as an internal standard on each gel slab. This procedure adequately minimized run-to-run variation in the absolute level of band densities which may occur from gel staining

and destaining. The precision of this method was  $\pm 1$  density level. However, for computerized determination of electrophoregram homologies, a band density variation of  $\pm 2$  units was routinely used as the margin of experimental error. As only sound and mature kernels were used for gliadin extraction, we generally observed no significant difference in the distribution of relative band densities in electrophoregrams from single kernels, or between single kernels and ground samples of grain.

# Data Base Encoding of Gliadin Electrophoregrams

To encode Rm and band density data for computer analysis, each reference PAGE pattern entry in the data base was represented by three character and six numerical records in a set structure (Fig. 1). Character records specify the cultivar name and pedigree. The numerical portion comprises a 110-element, onedimension cultivar signature array (row vector) of integer data. The first 100 positions contain paired Rm 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 information on the cultivar and PAGE result. Included are the total number of gliadin bands in the pattern, the number of replicates averaged in computing Rm values, the 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 (refer to following section).

TABLE I
Wheat Cultivars Analyzed by Polyacrylamide Gel Electrophoresis for Cultivar Identification Data Base

DBIN <sup>a</sup>	Hard Red Spring Wheats	DBIN		DBIN	
1	Early Red Fife	86	Chris	139	Winalta
3	Garnet	87	Coteau	141	Yogo
4	Pioneer	88	Era		
5	Prelude	89	Len	1.40	Soft white winter wheats
8,9 <sup>b</sup>	Preston	90	Olaf	142	Cornell 595
11	Red Fife	91	Polk	143	Dawbul
12	Ruby	92	Waldron	147	Dawson's Golden Chaf
14	Acadia		**.**	148	Favor
15	Apex	0.5	Utility or miscellaneous class/type wheats	149	Fredrick
16	Canus	95	Bishop	150	Gaines
17	Ceres	96	Concorde	151	Genessee
22	Coronation II	97	Dundas	152	Gordon
23	Lake	99	Glenlea	153	Houser
24	Lee	101	Huron	154,155	Jr. No. 6
27	Marquis	102	Kota	156	Nugaines
28	Redman	103	Laval 19	157	O.A.C. 104
29	Regent	104	Milton	158	Richmond
30,32	Reliance	105	Norquay	162	Rideau
37	Renfrew	107	Opal	163	Talbot
38	Renown	108	Pitic 62	164	Yorkstar
39	Reward	109,110	Red Bobs 222		
40	Selkirk	111	Vernon		Soft red winter wheats
44	Benito			165	Egyptian Amber
45	Canthatch		Soft white spring wheats	166	Fairfield
46	Columbus	112	Cascade	167	Jones Fife
47	Katepwa	113	Fielder	170	Kent
52	Manitou	115	Kenhi	171	Sun
53	Napayo	118	Lemhi 53	172	Thorne
56	Neepawa	122	Lemhi 62		
57	Park	124,127	Quality A		Durum wheats
58	Pembina	128	Springfield	174	Carleton
59	Saunders		TT- ad and and and and and and and and and	177	Coulter
61		100	Hard red winter wheats	178	Goldenball
	Sinton	129	Kharkov 22 M.C.	179	Hercules
62	Thatcher	130	Lennox	180	Macoun
63	Canuck	131	Monopol	181	Medora
66,69	Chester	132	Norstar	182	Mindum
76	Chinook	133	Ridit	183	Nugget
77	Cypress	134	Sundance	184	Pelissier
78 78	Leader	135	Valor	185	Ramsey
79	Rescue	136	Vuka	186	Stewart 63
84	Alex	137	Wasatch	187	Wakooma
85	Butte	138	Westmont	189	Wascana

<sup>&</sup>lt;sup>a</sup>DBIN = Data base identification number.

<sup>&</sup>lt;sup>b</sup>Two DBINs indicate different electrophoregrams for cultivar samples obtained from different sources.

The standard format of a data base reference PAGE pattern entry for Neepawa is shown in Figure 1. Because the cultivar name appears without an extension, a homogeneous cultivar sample is implied. To distinguish homogeneous from heterogeneous cultivar samples, the name of a cultivar appended with the code letter "M" indicates that the electrophoregram from the ground sample was a composite pattern derived from a mixture. The additional numbers specified by the signature array (element positions 103-109) indicate that the electrophoregram encodes 37 gliadin bands with Rm values averaged using 13 replicate PAGE patterns, and that the cultivar is a HRS wheat, superior to Marquis quality, grown in western Canada, and represents entry number 056 in the data base. A listing and definition of attribute summary codes (signature array element positions 106-108) is presented in Table II.

#### RESULTS AND DISCUSSION

# Calculation of Electrophoretic Pattern Homology

Rm and band density values for each protein band in an electrophoregram were treated as continuous variables, where Rm represented the primary feature to assess the overall similarity in protein composition for two PAGE patterns. As such, the process did not require a classification of gliadin bands into a character set structure, as this can involve some simplification and interpretation (Wrigley 1980) and is otherwise time consuming. This problem is discussed in another article (Sapirstein and Bushuk 1985b).

For each comparison of an unknown and a reference cultivar, the pair of electrophoregrams were scanned from low to high mobility to quantify the extent of pattern homology. Compared bands were scored as matching if the differences in respective Rm and density values were within prescribed threshold levels. The least significant difference in Rm was programmable in increments of 0.1 distance units and was set in accord with the uncertainty in Rm measurements; in this study it was fixed at 0.5 Rm units, which corresponds to a significant difference (P = 0.05, two degrees of freedom) when comparing mean relative mobilities with a standard error of  $\pm 0.08$  Rm units. The precision in determining Rm for the data base was previously found to be at least equal to this level, for all band positions in the electrophoregram field (Sapirstein and Bushuk 1985a). For band densities, which were quantified on an integer scale from one to nine, a difference of three units was arbitrarily used as the threshold to reject a match for compared bands not significantly different in mobility. Thus, for bands sharing only a positional homology, this event was scored as a band difference.

```
NEEPAWA 3
THATCHER*7/FRONTANA//THATCHER*6/KENYA FARMER/3/THATCHER
*2//FRONTANA/THATCHER, CANADA2
4 2 56 0
 0 0 3713
        0 2
```

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

Fig. 1. Standard data base coding format for the gliadin electrophoregram of cultivar Neepawa.

In the algorithm which analyzes the composition of two electrophoregrams (denoted below as "A" for an unknown and "B" for a reference cultivar), four classes of events were differentiated: 1) m, pairs of bands with matching relative mobility and density values; 2) j, bands present in the unknown "A" but absent from the reference "B"; 3) k, bands present in "B" but absent from "A"; 4) l, pairs of bands that share a positional homology but possess significantly different levels in density. Percent pattern homology (% PH), which determined the basis for cultivar ranking in several programs of the cultivar identification system, was expressed as the

$$100 \times (No. of pairs of matching bands)$$
  
No. of pairs of matching bands + No. of different bands

This definition is similar to the simple matching coefficient described by Sneath and Sokal (1973) and is a common form used to assess the variation in seed protein banding patterns of two electrophoregrams (Ladizinsky and Hymowitz 1979). In the present application, the above ratio was specified as follows

$$\% \text{ PH} = \frac{100 \times m}{m + (j + k + l)}.$$
 (1)

No weights are attached to the terms in equation 1. This means. for example, that extremely faint bands (density = 1), which are commonly observed in electrophoregrams but tend to be nonreproducible as they are difficult to visualize, carry the same weight in the equation as the most intensely stained components. Because a gliadin band represents a multistate character with a relative intensity that is largely an expression of genotype, the problem can be resolved by weighting band number counts as a function of individual band densities. In this way, the protein composition of an electrophoregram can be quantified in addition to the presence or absence of bands.

TABLE II Cultivar Signature Array Attribute<sup>a</sup> Summary Index Codes and Definitions

	Signature Array Element Position											
Attribute Index No.	106 Class Code	Definition	107 Quality Code	108 Region Code								
1	Blank		Blank	Blank								
2	HRS	Hard red spring	$NEMQ^b$	W.CAN								
3	SHRS	Semi-hard red spring	EMQ <sup>b</sup>	SAWFLY								
4	HWS	Hard white spring	$SMQ^b$	S.ALTA								
5	SHWS	Semi-hard white spring	FEED	BC								
6	SWS	Soft white spring	<b>PASTRY</b>	ONTARIO								
7	SRS	Soft red spring	$BW^c$	QUEBEC								
8	SHPS	Semi-hard purple spring	PASTA	ATLANTIC								
9	HRW	Hard red winter	$GHP^d$	ATL/BC								
10	SHRW	Semi-hard red winter	nd <sup>e</sup>	R/W.Can <sup>f</sup>								
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								

<sup>a</sup>Source of attribute data: Handbook of Canadian Varieties of Barley, Field Beans, Field Peas, Flax, Oats, Rye, and Spring, Durum, and 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.

 $^{b}NEMQ = HRS$  wheat not equal to Marquis, EMQ = HRS wheat equal to Marquis, and SMQ = HRS wheat superior to Marquis in milling and baking quality.

BW = non-HRS bread wheat.

<sup>d</sup>GHP = general household purpose.

end = not defined.

f R/W.CAN = restricted from West Canadian region by kernel characteristics.

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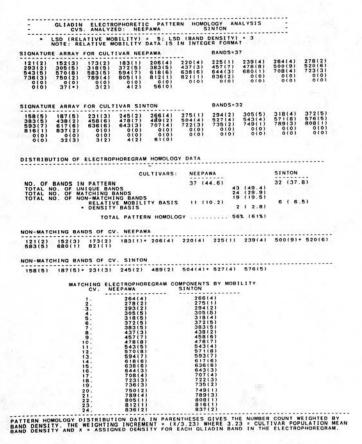


Fig. 2. Pattern homology analysis printout (program HOMOLOGY2) for cultivars Neepawa and Sinton.

In the case of a single electrophoregram with an average distribution of band densities, it can be determined that

$$\sum_{i=1}^{n} (d)_i / \text{Dav} = n, \tag{2}$$

where  $(d)_i$  represents the density assignment for the ith band in the electrophoregram, and Dav is a constant equal to the average density value for all bands in the data base cultivar population. The term on the left-hand side of equation 2 gives the weighted-by-band density (WBD) score for the electrophoregram. A WBD value significantly lower or higher than the number (n) of bands in the pattern indicates that the electrophoregram contains bands with a lower or higher than average level of staining intensity or protein concentration.

In an analogous fashion, equation 1 was modified to quantify significant departures from average band density for matching and nonmatching bands in compared electrophoregrams:

Weighted % PH = 
$$\frac{100 \times \sum_{i=1}^{m} (dA + dB)_{i}/2Dav}{\sum_{i=1}^{m} (dA + dB)_{i}/2Dav + \sum_{i=1}^{j} (dA)_{i}/Dav + \sum_{i=1}^{j} (dA-dB)_{i}/Dav},$$
 (3)

where dA and dB represent band densities for the ith band or pair of bands in cultivars "A" and "B." For cultivar identification, an algorithm implementing equation 3 was found to provide better discrimination than simple matching and nonmatching band count ratios (Sapirstein 1984).

**Outline of Computer Programs** 

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

1. The program designated "CVID" produces a short list ranking of data base reference cultivars in order of declining pattern

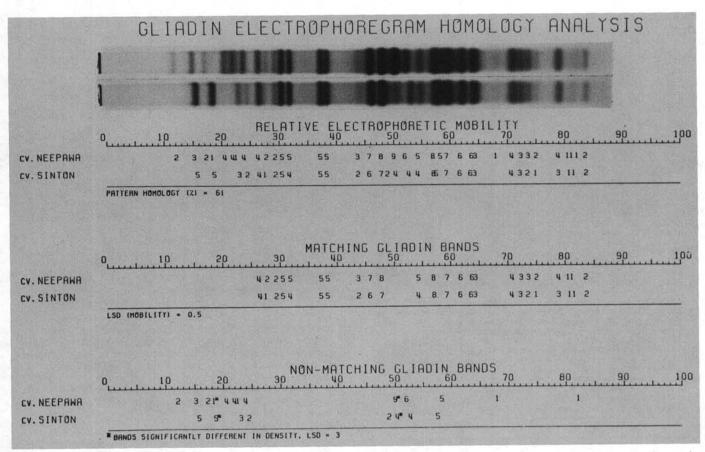


Fig. 3. Cultivar formula plots of pattern homology analysis (program HOMPLOT2) for cultivars Neepawa and Sinton, along with photographs of respective electrophoregrams.

homology with the unknown electrophoregram. A threshold value for % PH, which may be set by the user, controls entry into the list. Printed output includes the cultivar name, pedigree, class/quality attributes, and tabulation of matching and nonmatching bands for each paired comparison.

- 2. Programs "IDHOM" and "IDPLOT" combine to produce a graphic analysis of electrophoregram composition for cultivars specified in the list generated by program CVID. IDHOM identifies matching and nonmatching bands for each comparison of cultivar PAGE data between the unknown and data base member. The printout gives a detailed summary of results on standard forms. Program IDPLOT uses as input the data derived from the IDHOM routine to graphically visualize the composition of matching and nonmatching bands, which are isolated in separate plots for the list of ranked cultivars.
- 3. Program "CVMAP" computes the minimum number of bands that must be deleted from the unknown and each reference electrophoregram to yield patterns of identical composition. The printout is a frequency distribution that plots each cultivar's data base identification number and pattern homology score against the value of the independent variable, i.e., the total number of differences with the unknown electrophoregram. The result assists in evaluating the uniqueness of the unknown and identifies cultivars lying at the margins of the distribution which are of diverse genotype.

In addition to these ranking programs of the cultivar identification system, the comparative analysis could be focused on selected pairs of electrophoregrams to produce numerical data ("HOMOLOGY2") and graphic output ("HOMPLOT2") results for two cultivars of special interest. All software, with the exclusion of plotting programs IDPLOT and HOMPLOT2, was written in FORTRAN using standard data items, with the exception that character expressions were used in the source code. As such, these programs must be compiled under compilers that can translate the

character data type (e.g., WATFIV, FORTRAN 77). The plotting programs IDPLOT and HOMPLOT2 were developed in FORTRAN but also incorporate several subroutines of CALCOMP basic software (California Computer Products, Anaheim, CA) to produce results on a Versatec D1200A matrix plotter or a Xerox 8700 laser printer. All programs were tested on IBM 470 and Amdahl 470/580 mainframe computers. Program implementation on a laboratory-scale mini/micro computer (256K memory) should be readily feasible, as the only major machine dependencies are in the input and output routines. Commented program source listings can be obtained from the first author on request.

# Analysis of Pattern Homology for Two Cultivars

The various programs of the cultivar identification system implement a common procedure for paired comparison of electrophoregram data. The basic processing characteristics of the larger system can be well demonstrated with an analysis of two cultivars. Figures 2 and 3 show typical printout and graphic analysis results generated by HOMOLOGY2 and HOMPLOT2, respectively. In this example, electrophoregram data were analyzed for the HRS wheat cultivars Neepawa and Sinton.

An important feature of the comparative analysis printout is the comprehensive tabulation of the distribution and identity of matching and nonmatching bands in compared patterns. All pattern homology parameter values are given in their weighted and unweighted form. In the absence of computer resources for plotting data, program HOMOLOGY2 or its counterpart for cultivar identification (program IDHOM, results not shown), can be run to provide a satisfactory substitute for the graphic display described in this paper.

The measure of pattern homology between cultivars Neepawa and Sinton was determined to be 56% (unweighted), derived from

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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
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       DISTRIBUTION OF NON-MATCHING BAND DATA
                                                                                                                                                                                                                                                            GLIADIN
BANDS IN
PATTERN
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COLLIVAR

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MANITOU
KATEPWA
CANTHATCH
THATCHER
NAPAYO_M
CHRIS
BENITO
CANUCK_M
LEADER
ERA
SAUNDERS_M
SINTON
CHIONOK
CHIONOK
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CHIONOK
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     COTEAU
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   W. CAN
                                                  MEAN VALUE:
                                                                                                                                                                                                                                                            37 (41.6)
                                                                                                                                                                                                                                                       PEDIGREE DATA

THATCHER*7/FRONTANA//THATCHER*6/KENYA FARMER/3/THATCHER*2//FRONTANA/THATCHER, CANADA
THATCHER*7/FRONTANA//CANTHATCHER*6/KENYA FARMER/3/THATCHER, CANADA
NEEPAWA*6/RL293B/3/NEEPAWA*6/C.1.8154/2*FROCOR, CANADA(RL293B = LEE*2/KENYA FARMER).
THATCHER*6/KENYA FARMER, CANADA
MARQUIS/10/MILLIO/MARQUIS/KANRED, CANADA
MARQUIS/10/MILLIO/MARQUIS/KANRED, CANADA
MANITOU*2/4/THATCHER*5/KENYA 58/NEWTHATCHER*7/FRONTANA//THATCHER*6/KENYA FARMER, CANADA
FRONTANA/3*THATCHER*3/KENYA 58/NEWTHATCH/2*THATCHER* USA
NEEPAWA/3/RL4255*4/MANITOU/C17090, CANADA
CANTHATCH/3/MIDA/CADET//RESCUE, CANADA
FORTUNA/CHRIS, CANADA
11-50-10/4/FEMBINA/11-52-329/3/11-53-38/111-58-4//11-53-546, USA
MIDA/CADET//THATCHER, CANADA
MANITOU/3/THATCHER, CANADA
MANITOU/3/THATCHER, CANADA
MANITOU/3/THATCHER, CANADA
MANITOU/3/THATCHER, CANADA
MANITOU/3/THATCHER*6/KENYA FARMER//LEE*6/KENYA FARMER, CANADA
THATCHER/S-615-11, CANADA
THATCHER/S-615-11, CANADA
ND496 S1B//ND487/FLETCHER, USA (ND496*WALDRON/ND269; ND487*ND259/CONLEY//CONLEY/ND122/3/
JUSTIN/ND142) CHEYENNE/KHARKOV 22 M.C., CANADA

GIVE THE PAIRED NUMBER COUNTY WEIGHTED BY BAND DENSITY. THE WEIGHTING INCREMENT = (X/3).23
                                                                                                                                                                                NO.
                                                                                                                                                                                                                                                            PEDIGREE DATA
   CHRIS
   CANUCK
   SAUNDERS_M
   SINTON
RELIANCE_PGR_M
CHINOOK
   COTE AU
SUNDANCE
   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.
```

Fig. 4. Cultivar identification short list ranking (program CVID) printout for hard red spring wheat Neepawa. The "Mobility Basis-R" data column scores the number of bands in ranked cultivar electrophoregrams lacking positional homology with the unknown pattern. The "Mobility Basis-U" data column scores the converse band number count for the unknown. See text for additional details.

equation 1 as follows:

Unweighted % PH = 
$$\frac{24 \times 100}{24 + (11 + 6 + 2)} = 56\%$$
.

The weighted % PH score (61%) is somewhat higher, owing to the higher WBD count for the 24 bands that matched.

The performance of the process is illustrated with clear detail in the computer plot of electrophoregram composition (Fig. 3), which isolated matching and nonmatching bands from the total patterns. The computed result is largely confirmed by visual inspection of the Neepawa and Sinton electrophoregrams, which lack homology in the regions of low and intermediate mobility.

# Cultivar Identification System Program Output

To evaluate the performance of the identification system, representative gliadin electrophoregrams for wheats of various class types were selected as test input data to represent unknown samples, namely Neepawa (HRS bread wheat), Wascana (durum wheat), Yorkstar (soft white winter [SWW] wheat), Springfield (soft white spring [SWS] wheat), Sundance (hard red winter [HRW] bread wheat), and Opal (HRS feed wheat).

The typical complement of computer printouts and plots for a complete cultivar identification analysis is shown in Figures 4 through 6. The summary report produced by program CVID (Fig. 4) represents a short list of data base cultivars ranked in order of decreasing weighted % PH compared to the unknown



HHEAT CULTIVAR IDENTIFICATION - III. PATTERN HOMOLOGY ANALYSIS

<u>COMPLETE FORMULAS FOR RANKED CULTIVARS</u>

						RELATIV		ECTF	ROPHO	BRE	TICMOBI		
ZPH	(DBIN)	CULTIVAR	10	<u>.</u> سىيار	20	30	40	ىلىب	50	1	60	70	80 90
100	(56)	NEEPAWA	2	3 21	4 41 4	42255	5 5	3 7	8 9	6 5	8 5 7 6 63	1 4332	4 111 2
98	( 52 )	MANITOU	2	3 2	4324	4 2 35 4	5 5	2 7	8 9	4 4	646 8 83	1 4321	3 12
97	( 47 )	KATEPHA	2	5 5	3 32 21	3 2 2 4 4	5 5	2 7	8 9 1	44	6 6 6 6 63	4 3 2 1	3 1 1 2
96	( 45 )	CANTHATCH	2	3 3 1	3 32 31	42344	5 5	2 7	8 9	4 3	5 4 5 8 53	4322	3 1 1 2
96	( 62 )	THATCHER	3	2 3	4 43 22	4 2 25 5	5 5	2 7	8 9	5 4	6 5 6 6 63	4321	4 1 1 2
93	( 53 )	NAPAYO-M	2	32 2 2	3 32 31	42344 2	244	1 2 7	8 9	4 4	647 8 73	1 4321	3 12
91	( 86 )	CHRIS	3	3 3	4 42 31	41255	5 5	2 7	8 9	44	6 5 6 7 63	2 1 42 33 2	3 1 1 2
91	(44)	BENITO	2	3 31	4 43 31	4 2 25 5	5 5	2 7	8 9	44	75 6 7 <b>7</b> 5	4 32 1	4 1 1 2
82	(63)	CANUCK-M	2	3 2	31 411 32	41 34 51 11	1 5 5	2 7	8 171	5 134	27647 8 64	1441444	5 1 1 2
80	( 78 )	LEADER	2	5 5	4 42 31	4 2 25 5	5 5	2 7	7 9	4 4	35 4 6 3 6 5 4	2 1 5 33 2	4 1 1 2
75	( 88 )	ERA	2	2 2	3 33 31	41 23 4	ųų	2 7	8 9	3 2	322 45 26 4 3 2	3 2 2 1	3 1 1 2
63	( 57 )	PARK	2	5 5	4 32 31	2 2 1 55	2	3 6 6	44 8	44	5 6 6 3 7 643	12 43211	3 12
62	( 59 )	SAUNDERS-M	2	3 2 2	4 42 41	2 2 1 34	2	2 6 6	3 9	4 1	68 5 8 44	2 43222	3 12
61	(61)	SINTON		5 5	3 2	41 25 4	5 5	2 6	724	44	85 7 6 63	4321	3 11 2
59	( 30 )	RELIANCE-PGR-M	2	3 2 2	4,424	42344	5 4	3 6	78	4 6	3 4 7 45 22	5 5 3 74 3	322
59	(76)	CHINOOK	2	3 2 2	3 3 3	324	3 1 2 7	2 7	3 8	4 3	6 5 6 5 6 5 2 1	2 33 343	3 2 1
58	( 87 )	COTEAU		4 5	2 2	4 3 4	54	2 7	824	5 3	84647 741	2 5 32	3 11 2
56	( 134)	SUNDANCE		3 3	2 32 31	212 4	44	2 7	8 8	5 7	255 5 67 5 3 2	23 2 2 3	3 1
			10	.1,	50	30	40		50	1	60	70	80 90

B

WHERT CULTIVAR IDENTIFICATION - III. PATTERN HOMOLOGY ANALYSIS

MATCHING GLIADIN BANDS - LSD (MOBILITY) = 0.5; LSD (DENSITY) = 3

						LAT		C T	R			RE			B I I	_ I	ΤΥ					
ZPH (DBIN) CU	JLTIVAR	10	. ـ ـ ـ ـ ـ ـ ـ ـ ـ ـ ـ ـ ـ ـ ـ ـ ـ ـ ـ	20 		30	40		بيا	5	50	لب	60	)	بيلي	7	Έ	لب	8	0	بلب	90
100 ( 56 ) NE	EEPAWA	2	3 21	4 41 4	4 2	255	5 5	3	7	8	9 6	5	857	6	63	1	4 3	3 2	4	11	1 2	
98 (52) MA	UOTINE	2	3 2	4,32,4	4 2	35 4	5 5	2	7	8	9 4	4	646	8	83	1	4 3	2 1	3		1 2	
97 (47 J KA	ATEPWA	2	2 2	3 32 2	3 2	244	5 5	2	7	8	9 4	ų	6 6 6	6	ස		4 3	2 1	3	1	1 2	
96 (45 ) CA	ANTHATCH	2	3 3 1	3 32 3	4 2	344	5 5	2	7	8	9 4	3	5 4 5	8	23		4 3	2 2	3	1	1 2	
96 (62) TH	HATCHER	3	2 3	4 43 2	4 2	255	5 5	2	7	8	9 5	ų	6 5 6	6	63		4 3	2 1	ų	1	1 2	
93 (53 ) NA	APAYO-M	2	3 2 2	3 32 3	4 2	344	44	2	7	8	9 4	4	647	8	73	1	4 3	2 1	3		1 2	
91 ( 86 ) CH	HRIS	3	3 3	4 42 3	4 1	255	5 5	5	7	8	9 4	ų	6 5 6	7	63	1	4	3 2	3	1	1 2	
91 (44) BE	ENITO	2	3 3 1	4 43 3	4 2	255	5 5	2	7	8	9 4	ų	7 6	7	75		4 3	321	ų	ı	1 2	
82 ( 63 ) CF	ANUCK-M	2	3 2	3 41 3	4.1	34 5	5 5	5	7	8	7 5	3	747	8	64	1	4 4	44	5	1	1 2	
80 (78 ) LE	EADER	2	2 2	4 42 3	4 2	255	5 5	2	7	7	9 4	4	5 4 6	6	ų	1	5	3 2	4	1	1 2	
75 (88 ) ER	RA	2	5 5	3 33 3	4.1	234	44	5	7	8	9 3	, S,	2 45	6	3		3 8	2 2 1	3	1	1 2	
63 (57) PA	ARK	2	2 2	4 32 3	2 2	1 5			6	ų.	8 4	4	5 66	7	64		4 3	2 1	3		1 2	
62 ( 59 ) SA	AUNDERS-M	2	3 2 2	4 42 4	5 5	1 3			6	3	9 4	1	6 5	8	44		4 3	5 5	3		1 2	
61 (61) SI	INTON		5	•	4.1	254	5 5	5	6	7	ų	ų	8 7	6	63		4 3	2 1	3	1 !	1 2	
59 ( 30 ) RE	EL IANCE-PGR-M	2	3 2 2	4 42 4	4 2	3 4 4	5 4	3	6	7	8 4	1	ų 7	4	5,5			3 7			2	
59 ( 76 ) CH	HINOOK	2	3 22	3 3 3	3 2	4		5	7	3	8 4	3	6 5 6	6	2	2	3	3 4	2	1	l	
se ( 87 ) CO	TEAU		5	•	ų	3 4	54	2	7	8	ď	3	84 6	7	74		5	3 2	3	11	2	
56 ( 134) SU	JNDANCE		3	2 32 3	2 1	2 4	44	2	7	8	8 5		255	7	3	5		2 3	3	_	1	
		10	1,,,,	20		30	40	* *	Γ''		50	, 17	6	ָ כ	[ .	7	70		8	0		90
				<b>*</b> (	DENO	TES I	BANDS SIGN	ΙF	I C	AN1	ΓLΥ	DIF	FEREN	١T	IN D	ENS	IT1	( ON	ΙLΥ			

Fig. 5. Cultivar identification pattern homology analysis plots (program IDPLOT) for cultivar Neepawa.

electrophoregram. At the head of the output are several lines specifying 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 the selected difference thresholds for gliadin band identity (i.e., 0.5 Rm units, 3 density units), generally resulted in ranking 10 to 20% of the primary population (excluding biotypes) of 122 common spring, winter, and durum wheat reference cultivars in the data base. The number of ranked cultivars varies depending on the uniqueness of the input electrophoregram. For example, the ranking by pattern homology to the Marquis electrophoregram (result not shown) lists 43 cultivars above the 55% PH threshold, evidence of the

significant contribution of this historic Canadian variety to the genetic composition of other wheat cultivars in the data base.

The top-ranked cultivar in Figure 4 shows that the identification program was successful in precisely matching the input PAGE pattern for Neepawa with its data base counterpart. Successive % PH values indicate further that the Neepawa electrophoregram is very similar in composition to band patterns of a group of seven cultivars that have been isolated with very high levels of pattern homology (>90%). The influence of common genetic background has contributed largely to this result, as all eight cultivars are dominated by the cultivar Thatcher or a related genotype as the recurrent parent in respective pedigrees. Not surprisingly, these

# C

WHEAT CULTIVAR IDENTIFICATION - [1]. PATTERN HOMOLOGY ANALYSIS

NON-MATCHING GLIADIN BANDS\*\* - LSD (MOBILITY) = 0.5; LSD (DENSITY) = 3

		F	RELATIVE	ELECT	ROPHORE 1	TIC MOBIL	ITY
XPH (DBIN) CULTIVAR	10	. 20	30	40	50	60	70 80 90
100 ( 56 ) NEEPAWA							· · · · · · · · · · · · · · · · · · ·
98 (52) MANITOU							
97 ( 47 ) KATEPWA		1					
96 ( 45 ) CANTHATCH		1					
96 (62) THATCHER		2					
93 ( 53 ) NAPAYO-M	2	1	22	1			
91 (86 ) CHRIS		1				2	2 3
91 (44) BENITO		1				5	
82 ( 63 ) CANUCK-M		1 1 2	1 11		11 14	2 8	4 1
80 (78 ) LEADER		1				3 3 5 2	3
75 ( 88 ) ERA		1			3	2 2 4 2	•
63 ( 57 ) PARK		1	5	2 3 6	ų	3 3 1 2	1
62 (59) SAUNDERS-M		1	ų	2 2 6		8 2	2
61 (61) SINTON	5	3 2			2 4	5	•
59 ( 30 ) RELIANCE-PGR-M					6	5	5 5 4 3 2
59 ( 76 ) CHINOOK			3	1 2 7		5 5 1	3 3 3
58 (87) COTEAU	ų	5 5			2 5	4 12	
56 ( 134) SUNDANCE	3	1			7	5 6 5 2	3 2
	10	50	30	40	77.50	00	70 80 90
		* NON-MAT	CHING BAND	S IN CULT			TERN HOMOLOGY

# D

WHEAT CULTIVAR IDENTIFICATION - III. PATTERN HOMOLOGY ANALYSIS

NON-MATCHING GLIADIN BANDS - LSD (MOBILITY) - 0.5; LSD (DENSITY) - 3

	RELATIVE ELECTROPHORETIC MOBILITY
ZPH (DBIN) CULTIVAR	$\frac{10}{10}$ $\frac{20}{10}$ $\frac{30}{10}$ $\frac{40}{10}$ $\frac{50}{10}$ $\frac{60}{10}$ $\frac{70}{10}$ $\frac{80}{10}$ $\frac{90}{10}$
100 ( 56 ) NEEPAWA	
98 (52) MANITOU	i ii
97 ( 47 ) KATEPWA	į i
98 ( 45 ) CANTHATCH	1
96 (62) THATCHER	1 2
95 ( 53 ) NAPAYO-M	2 1 22 1
91 ( 86 ) CHRIS	1 1 2 223 1
91 (44) BENITO	1 S <sub>5</sub> 1
82 ( 63 ) CANUCK-M	1 1 1 2 1 11 11 14 28 4 1 1
80 (78 ) LEADER	1 3 3 51 2 23 1
75 (88 ) ERA	į 1 32 2 4 5 2 1 1
63 (57) PARK	1 5 5 5 5 2 3 2 6 4 3 3 1 2 1 11
62 ( 59 ) SAUNDERS-M	1 Mg 5 5 2 2 26 85 21 2 11
61 (61) SINTON	\$ 72 \$ 4 A73A5 5 BA 25 T
59 ( 30 ) RELIANCE-PGR-M	
59 ( 76 ) CHINOOK	1
58 (87) COTEAU	\$ \$4 \$ \$ \$75.55 \$ 5 \$2 4 151 \$ T
56 ( 134) SUNDANCE	\$ \frac{1}{2} \fra
	10 20 30 40 50 60 70 80 gb
	~ DENOTES NON-MATCHING BANDS IN UNKNOWN (OR TEST) CULTIVAR ELECTROPHOREGRAM
	LARGER DIGITS INDICATE NON-MATCHING BANDS IN CVS. RANKED BY % HOMOLOGY

cultivars all share common class attributes for HRS bread wheats with excellent milling and baking quality characteristics.

Apart from providing cultivar names, % PH scores, class attributes, and pedigrees, the CVID printout includes an extensive tabulation of the distribution of matching and nonmatching bands for compared electrophoregrams. The total nonmatching band data column shows that WBD scores (in parentheses) for the six cultivars ranked below Neepawa are considerably less than the paired number count. This provides evidence that cultivar discrimination was derived primarily from differences involving very faint bands.

The cultivar formula plots shown in Figure 5 were produced by program IDPLOT and provide the means to evaluate the CVID printout (Fig. 4) by visualizing the pattern homology analysis process for the entire set of ranked wheats. The detail and precision of the computerized methodology is especially well demonstrated in the plot of cultivar formulas for isolated matching bands (Fig. 5B), which clearly identifies common groups of gliadins similar in density or otherwise. The plots for nonmatching bands (Fig. 5C and D) confirm that only extremely faint bands are the basis for discrimination. Considerable uncertainty therefore exists in differentiating Neepawa from cultivars with pattern homology scores greater than 95%. Fortunately, the need to discriminate among these closely related genotypes has no present commercial relevance, as they all possess similar end-use quality characteristics.

These computer-generated plots also illustrate the potential of the method for studying genetic relationships, where large numbers

of progeny are to be evaluated in terms of discrete electrophoregram similarities, differences, and recombinants compared to parental type protein patterns. In this application, the electrophoregram (or portion thereof) of a parent genotype would replace the unknown as the input pattern, and electrophoregrams for the F2 generation or other derived material would constitute the data base

The third and final element (program CVMAP) of the cultivar identification system, illustrated in Figure 6, computes the frequency distribution of band position differences between the gliadin electrophoregram of an input cultivar and counterpart patterns in the data base. For each pair compared, the positional difference variable scores the total nonmatching band count, including bands that significantly differ by density alone. Removal of these differences will yield electrophoregrams of identical composition. The strategy implemented by program CVMAP complements results shown previously by using a different criterion for ranking. It also provides an output extended to include the entire reference population of common and durum wheats, in which each entry is explicitly identified.

A typical result is illustrated in Figure 6, which depicts the frequency distribution for weighted positional differences between the Neepawa electrophoregram and 122 reference cultivars. The difference distribution, which we termed a "cultivar distance map" (CVMAP), shows a wide gap or genotypic distance separating the cultivar Neepawa (DBIN 56) from the bulk of the data base population. Cultivars that are relatively distinct in electrophoregram

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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
                                                             56(++)
47(97) 52(98)
45(96) 62(96)
53(93)
44(91) 86(91)
                      01234567890123456789012345678901234567890123456789012345678901234567
                                                                                                                                                                                                                                                                                                                                                                                CULTIVARS MOST SIMILAR TO CV.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           NEEPAWA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    NEEPPWA
KATEPWA
MANITOTCH
CANTITATER
NAPAYO
BENITO
CHNISCK
M
CHNISCK
M
LERA
PARK
PARK
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           56
47
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 63(82) 78(80)
88(75)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        SAUNDERS_M
                                                                                                        59(62)
                                                                                                                                                                                                                                                                                                                                                                                CULTIVARS MOST DIFFERENT FROM CV
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  NEEPAWA
                                                                                                        76(59) 87(58)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     MINDUM
NUGGET
LAKE
COULTER
PRELUDE M
CARLETON PGR
RAMSEY
WASCANA
REWARD
MEDORA
HERCULES
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | 137 | 152 | 156 | (51) | 69 | (51) | 150 | (43) | 11 | (52) | 133 | (51) | 46 | (52) | 79 | (44) | 91 | (50) | 44 | (49) | 40 | (46) | 58 | (43) | 66 | (45) | 124 | (52) | 137 | (51) | 124 | (51) | 124 | (51) | 124 | (51) | 124 | (51) | 124 | (51) | 124 | (51) | 124 | (51) | 124 | (51) | 124 | (51) | 124 | (51) | 124 | (51) | 137 | (51) | 124 | (51) | 137 | (51) | 137 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 144 | (51) | 
                                                           167(36)

1(28)102(29)103(24)115(26)132(29)

9(26) 14(34) 96(30)105(22)107(24)111(23)130(28)165(21)166(26)

104(28)131(24)143(27)171(18)172(16)

178(28)

135(23)162(28)

29(24) 38(21)108(20)136(27)180(25)186(24)

89(19)

90(23)

24(26)127(20)149(20)155(21)184(12)

179(20)187(20)
                      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 PARENTHESSES.
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Fig. 6. Cultivar identification distance map (program CVMAP) printout for Neepawa. Framed data in the frequency distribution corresponds to cultivars ranked in Figures 4 and 5. Data in inset represents a separate segment of program CVMAP output which identifies diverse genotypes relative to the input electrophoretic pattern. See text for details.

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 DISTRIBUTION OF NON-MATCHING BAND DATA GLIADIN BANDS IN PATTERN DENSITY MOBILITY BASIS-R WEIGHTED % PATTERN HOMOLOGY TOTAL BASIS-R
NO. WBD
0 (0.0)
4 (4.6)
11 (7.1)
7 (5.3)
15 (12.1)
14 (12.4)
10 (9.9)
16 (13.3)
20 (15.8)
8 (11.1) CULTIVAR NO. WBD NO. WBD JMOLO( 79 77 64 59 58 49 43 40 39 NO. WBD NO. WBD CLASS/TYPE REGION WASCANA STEWART 63 CARLETON\_PGR MACCUN NUGGET MINDUM WAKOOMA\_M MEDORA HERCULES GOLDENBALL 35 (43.0) 33 (36.8) 44 (43.0) 31 (36.2) 44 (43.0) 42 (44.6) 30 (35.3) 34 (38.7) 37 (37.8) 26 (36.8) 35 (43.0) 29 (35.3) 31 (36.7) 22 (29.9) 26 (30.9) 25 (30.8) 19 (25.7) 15 (23.7) 16 (22.8) 15 (21.7) 0 (0.0) 0 (0.0) 2 (2.2) 2 (2.8) 3 (3.7) 3 (3.7) 1 (1.5) 3 (4.0) 1 (0.9) 3 (4.3) DURM-PASTA 0 ( 0.0) 6 ( 4.6) 2 ( 1.5) 11 ( 9.0) 6 ( 5.6) 7 ( 6.2) 15 (14.9) 17 (13.6) 18 (17.3) 17 (18.0) W.CAN W.CAN W.CAN W.CAN W.CAN W.CAN W.CAN W.CAN MEAN VALUE: 23 (30.0) 22 (20.6) 1 (2.3) 10 ( 9.2) 9 ( 9.1) PEDIGREE DATA NO. PEDIGREE DATA

WASCANA 189 LAKOTA-2/PELISSIER, CANADA
SIEWART 63 186 ST 464/8\*STEWART, CANADA
SIEWART 63 186 ST 464/8\*STEWART, CANADA
MACOUN 174 VERNAL EMMER/MINDUM, USA
MACOUN 180 RL 3607/ DT 182. CANADA
NUGGET 183 MINDUM/CARLETON//HEITI/STEWART, USA
MINDUM 183 MINDUM/CARLETON//HEITI/STEWART, USA
MINDUM 182 TOUND IN BREAD WHEAT FIELD, USA
MAKOOMA, 187 TOUND IN BREAD WHEAT FIELD, USA
MEDORA 181 KARTA\*2/PELISSIER, CANADA
MEDORA 181 KARTA\*2/PELISSIER, CANADA
MEDORA 179 R. ASSIGNOMA/STEWART/RL 3380, CANADA
GOLDEMBALL 178 7 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. 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 189(\*\*) 890123456789012345678901234567890123456789012345678901 186(79) 174(77) 180(64) 187 (49) 181(43) 178(39) 179(40) 184(29) 177(33) 162(32)
127(24)165(23)185(31)
95(27)111(23)171(20)
23(25) 58(24) 59(30) 61(26) 76(30)
38(26) 40(27) 99(24)107(20)131(25)
79(20) 84(26)103(16)14(26)
22(27) 32(24) 57(26)115(20)172(14)
9(177) 29(24) 46(29) 47(25) 89(16)118(22)129(25)134(26)147(23)154(26)
3(19) 5(24) 28(23) 66(20) 87(23)104(20)148(16)
16(24) 37(23) 39(23) 90(18)112(21)138(17)156(18)153(16)167(26)
8(11) 14(22) 39(23) 90(18)112(21)138(17)150(12)156(21)156(16)
27(22) 44(24) 62(23) 91(22)101(25)113(17)122(18)128(18)14(21)143(20)163(22)
4(19) 12(17) 24(21) 77(20) 78(22)108(7)137(20)149(16)155(19)157(15)164(18)
15(21) 45(21) 63(26) 96(17)130(14)152(7)
1(14) 17(18) 53(21) 85(21) 97(8)109(21)
30(22) 56(21) 69(15) 88(16)
11(18)110(17)133(18)151(18)
52(18)136(16)170(18) 86(16) 139(9) 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 = (4/3.23) 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.

Fig. 7. Cultivar identification ranking (A) and distance map (B) printouts for durum wheat Wascana. Framed cultivars in (B) correspond to cultivars ranked in (A).

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WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - I. RANKING BY PATTERN HOMOLOGY
 122 DATA BASE CULTIVAR PATTERNS ANALYZED
DATA BASE SEARCH CUTOFF AT 58% 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
                                                                                                                                                                                                                                                                 DISTRIBUTION OF NON-MATCHING BAND DATA
                                                                                                                                   GLIADIN
BANDS IN
PATTERN
                                                                                                                                                                                                                                                                                                                        MOBILITY
BASIS-R
                                                                                                                                                                                                                                                                                                                                                                                  DENSITY
                                                                                                                                                                                           MATCHING
BANDS
NO. WBD
                                                                                                                                                                                                                                                                         TOTAL
                                                                             WEIGHTED
% PATTERN
HOMOLOGY
                                                                                                                                                                                                                                                              TOTAL

NO. WBD

0 ( 0.6)
6 ( 1.9)
5 ( 1.9)
5 ( 3.4)
7 ( 3.7)
8 ( 5.0)
6 ( 5.6)
114 ( 10.5)
129 ( 15.8)
120 ( 15.8)
20 ( 15.8)
20 ( 15.8)
21 ( 17.3)
22 ( 18.6)
23 ( 18.6)
                                                                                                                                                                                                                                                                                                                                                                                                                                       BASIS-U
NO. WBD
0 (0.3)
1 (0.3)
3 (1.2)
1 (0.6)
2 (0.9)
3 (1.5)
1 (0.6)
4 (2.3)
9 (6.8)
8 (4.3)
9 (6.8)
11 (5.6)
11 (5.6)
                                                                                                                                                WBD
                                                                                                                                                                                                                WBD
                                                                                                                                                                                                                                                                                                                          NO. WBD
                                                                                                                                                                                                                                                                                                                                                                                     NO. WBD
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       REGION
  CULTIVAR
                                                                                                                                   NO
                                                                                                                                                                                           NO. WBD

37 (33.1)
36 (31.1)
36 (31.1)
34 (28.0)
34 (28.0)
35 (33.6)
35 (33.5)
36 (35.4)
33 (29.9)
32 (30.3)
28 (24.6)
28 (24.6)
27 (19.8)
27 (25.1)
27 (19.8)
                                                                                                                                                                                                                                                                                                                          NO. WBD

O ( 0.0)
1 ( 0.3)
4 ( 1.5)
2 ( 0.6)
4 ( 2.8)
5 ( 2.8)
5 ( 3.4)
4 ( 4.0)
10 ( 6.8)
10 ( 7.7)
113 ( 8.7)
12 ( 9.9)
14 ( 9.9)
13 ( 8.9)
                                                                                                                                                                                                                                                                                                                                                                                        NO. WBD
0 (0.0)
0 (0.0)
0 (0.0)
0 (0.0)
0 (0.0)
1 (0.0)
1 (1.2)
1 (1.2)
1 (1.9)
2 (3.4)
1 (1.9)
2 (3.4)
CULTIVAR
YORKSTAR
FAVOR
GENESSEE
GENESOT
CORNELL 595
OGCHAFF
KENT
DAWBUL M
JR.NO.5
EGYPTIAN AMBER
HOUSER
HOUSER
THORNE M
FAIRFIELD
RICHMOND M
FREDRICK
                                                                                                                                 NO WBD

37 (29.7)
40 (39.9)
36 (24.8)
40 (38.1)
40 (37.8)
39 (36.2)
44 (37.5)
39 (39.6)
39 (31.0)
38 (34.1)
38 (34.1)
40 (41.2)
41 (36.2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       CLASS/ITP
SWW - PASTRY
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 ONTARIO
                                                                                            100
95
95
97
97
87
87
87
66
11
55
55
55
                                                                                                                                                                                                                                                                  12 ( 9.2)
                                                                                                                                                                                                                                                                                                                               6 ( 5.0)
                                                                                                                                   PEDIGREE DATA
                                                                                                                                 GENESEE-5/3/YORKWIN//NORIN 10/BREVOR, USA
DIGA DIJON//GABO/NEW ZEALAND 496.01, CANADA
YORKWIN//HONDR-2/FORWARD, USA
OTSEL (RELATED TO ETOILE DE CHOISY)/GENESSEE/2/CD7561/ KENT/3/7453-4-2-4(FREDRICK SIB)/4/2*YORKSTAR, C
TRUMBULL//HOPE/HUSSAR/3/DAWSON'S GOLDEN CHAFF*2/RIDIT//CORNELL 595, CANADA
HONOR/FORWARD//NURED/3/HONOR, USA
SELECTION OF CLAWSON, CANADA
CALDWELL 10/DAWSON'S GOLDEN CHAFF, CANADA
DAWSON'S GOLDEN CHAFF BULGRIAN, CANADA
DAWSON'S GOLDEN CHAFF BULGRIAN, CANADA
CHUSTAL CANADA
DESCRIPTION OF REDCHAFF OR OF REDCHAFF BALD, USA
BREVOR/HORIN 10//NY WHEAT RYE SEL./3/HOPE HUSSAR/YORKWIN/4/GENESSEE//CT12658/ALASKAN/3/AVON. USA
DAWSON'S GOLDEN CHAFF BULGARIAN, CANADA
PORTAGE/FULCASTER, USA
DAWSON'S GOLDEN CHAFF SULGARIAN, CANADA
PORTAGE/FULCASTER, USA
DAWSON'S GOLDEN CHAFF SULGARIAN, CANADA
WASHINGTON 1//GENESSEE/CAPELLE, CANADA
MASHINGTON 1/GENESSEE/CAPELLE, CANADA
MASHINGTON 1/GENESSEE/CAPELLE, CANADA
GIVE THE PAIRED NUMBER COUNT WEIGHTED BY BAND DENSITY. THE WEIGHTING INCREMENT * (X/3.23)
YORKSTAR
FAVOR
GENESSEE
GORDON
TALBOT
CORNELL 595
DOCHAFF
KENT
DAWBUL M
JR.NO. 5
ECYPTIAN AMBER
ROCTO 4
HORNE M
FAIRFIELD
RICHMOND M
FREDRICK
                                                                                             1648
151153
1470
1470
1470
1553
1470
1553
1572
166
  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.
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- WHEAT CULTIVAR IDENTIFICATION BASED ON GLIADIN ELECTROPHOREGRAMS - 11. DISTRIBUTION BY DIFFERENCES

- INTO DATA BASE CULTIVAR PATERNS ANALYZED; LSD(RELATIVE MOBILITY) = 0.5, LSD(BAND DENSITY) = 3.

WEIGHTED POSITIONAL DIFFERENCES

- INTO DATA BASE CULTIVAR PATERNS ANALYZED; LSD(RELATIVE MOBILITY) = 0.5, LSD(BAND DENSITY) = 3.

WEIGHTED POSITIONAL DIFFERENCES

- INTO DATA BASE CULTIVAR PATERN ANALYZED; LSD(RELATIVE MOBILITY) = 0.5, LSD(BAND DENSITY) = 3.

WEIGHTED POSITIONAL DIFFERENCES

- INTO DATA BASE CULTIVAR PATERN ANALYZED; LSD(RELATIVE MOBILITY) = 0.5, LSD(BAND DENSITY) = 3.

WEIGHTED POSITIONAL DIFFERENCE SHEET OF ALL NUMBER OF BAND DIFFERENCES WEIGHTED BY DENSITY BETWEEN THE MOBILITY AND DESCRIPTION AND DESCRIPTION OF SECTIVELY PAGE AND DIFFERENCES WEIGHTED BY DENSITY BETWEEN THE MOBILITY AND DESCRIPTION OF SECTIVELY PAGE AND DIFFERENCES WEIGHTED BY DENSITY BETWEEN THE MOBILITY BETWEEN THE WEIGHTED BY DENSITY BETWEEN THE MOBILITY BY
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Fig. 8. Cultivar identification ranking (A) and distance map (B) printouts for soft white winter wheat Yorkstar. Framed cultivars in (B) correspond to cultivars ranked in (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 DISTRIBUTION OF NON-MATCHING BAND DATA GLIADIN BANDS IN PATTERN MATCHING BANDS NO. WBD DENSITY BASIS WEIGHTED % PATTERN HOMOLOGY MOBILITY BASIS-U TOTAL TOTAL

NO. WBD

0 ( 0.0)
19 (14.9)
19 (16.4)
26 (20.7)
20 (20.1)
20 (20.1)
20 (20.1)
20 (22.6)
20 (12.9)
21 (22.6)
22 (22.6)
22 (22.6)
21 (19.2)
21 (22.6)
22 (22.6)
27 (19.2) BASIS
NO. WBD
0 (0.0)
3 (2.8)
3 (3.7)
1 (0.2)
1 (2.2)
2 (2.6)
2 (2.6)
2 (2.8)
1 (0.2)
2 (2.8)
1 (1.2)
2 (2.8)
5 (5.6) NO. WBD CULTIVAR NO. WBD NO. WBD NO. WBD

35 (39.3)
39 (34.7)
39 (43.7)
39 (52.0)
49 (52.0)
36 (39.9)
35 (42.7)
38 (43.7)
36 (43.7)
37 (44.6)
37 (44.6)
38 (34.7)
37 (44.6)
43 (34.7)
43 (34.4) NO. WBD

0 ( 0.0)
10 ( 8.0)
10 ( 8.0)
11 ( 9.0)
19 ( 13.4)
19 ( 8.7)
12 ( 9.6)
11 ( 10.8)
10 ( 10.2)
8 ( 5.9)
11 ( 9.3)
11 ( 9.3)
11 ( 9.3)
12 ( 9.6)
13 ( 10.8)
14 ( 10.8)
15 ( 8.7) NO. WBD

0 ( 0.0)
6 ( 5.6)
6 ( 5.6)
9 (10.8)
9 (10.8)
10 (10.5)
10 (11.8)
9 (10.1)
13 (12.7)
13 (12.7)
10 (11.8)
7 ( 5.0) CLASS/TYPE REGION CLASS/TYPE
HRW-BW
HRW-BW
HRW-BW
HRW-SWMQ
HRRS-SWMQ
HRRS-SWMQ SUNDANCE 100 67 65 SUMDANCE
YOGO
LEADER
KHARKOV 22 M.C.
ERA
KHARKOV 22 M.C.
CANUCK M
CANTHATCH
RELIANCE—PGR\_M
NAPAYO\_M
CHRIS
BENITO
BENITO
RESCUE PGR\_M
THATCHER
NEEPAWA
NUGAINES
KATEPWA
MANITOU
HOUSER W. CAN W. CAN SAWFLA USAW FAN USAW FAN W. CAN ON TARIO MEAN VALUE: 61 37 (40.6) 19 (18.9) 10 ( 8.1) 7 (8.7) 1 (2.1) SUNDANCE 134 CHEYENNE (MARROY 22 M.C., CANADA VOGO 141 MINTURK / BELGOGLINA / BUFFUM, USA LEADER 78 FORTUNA / CHRIS, CANADA USA KHARKOY 22 M.C. 129 SELECTION OF KHARKOY, CANADA CANUCK M 63 IL-80-10/4/PEMBINA/II-52-329/3/II-53-38/III-58-4//II-53-546, USA CANTHATCH 45 THATCHER\*6/KENYA FARMER, CANADA CANTHATCH 45 THATCHER\*6/KENYA FARMER, CANADA CANTHATCH 30 MANITOU\*2/4/THATCHER\*5/LEE/3/THATCHER\*7/FRONTANA//THATCHER\*6/KENYA FARMER, CANADA GENITO 96 FRONTANA/3\*THATCHER\*5/KENYA 58/NEWTHATCH/2\*THATCHER. USA NAGAYOM 53 MANITOU\*2/4/THATCHER\*5/KENYA 58/NEWTHATCH/2\*THATCHER. USA RESCUE PGR M 79 KEPAWA/3/RL 4255\*4//MANITOU/C17090, CANADA RESCUE PGR M 79 KEPAWA/3/RL 4255\*4//MANITOU/C17090, CANADA NIGATURE 62 MAROUIS/KANED LISKANDA GENITO 65 MAROUIS/KANED CANADA NIGATURE 7/FRONTANA/THATCHER\*5/KENYA FARMER/3/THATCHER\*2/FRONTANA/THATCHER\*CANADA NIGATURE 62 MAROUIS/KANED CANADA RESCUE PGR M 79 KEPAWA/3/RL 4255\*4//MANITOU/C17090, CANADA RESCUE PGR M 79 KEPAWA/S/RL 4255\*4//MANITOU/C17090, PEDIGREE DATA 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 134(\*\*) 0 ,89011234567890123456789012333333444444667 141(67)
78(65) 88(63)
129(65)
150(75)
150(75)
150(75)
150(75)
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Fig. 9. Cultivar identification ranking (A) and distance map (B) printouts for hard red winter wheat Sundance. Framed cultivars in (B) correspond to cultivars ranked in (A).

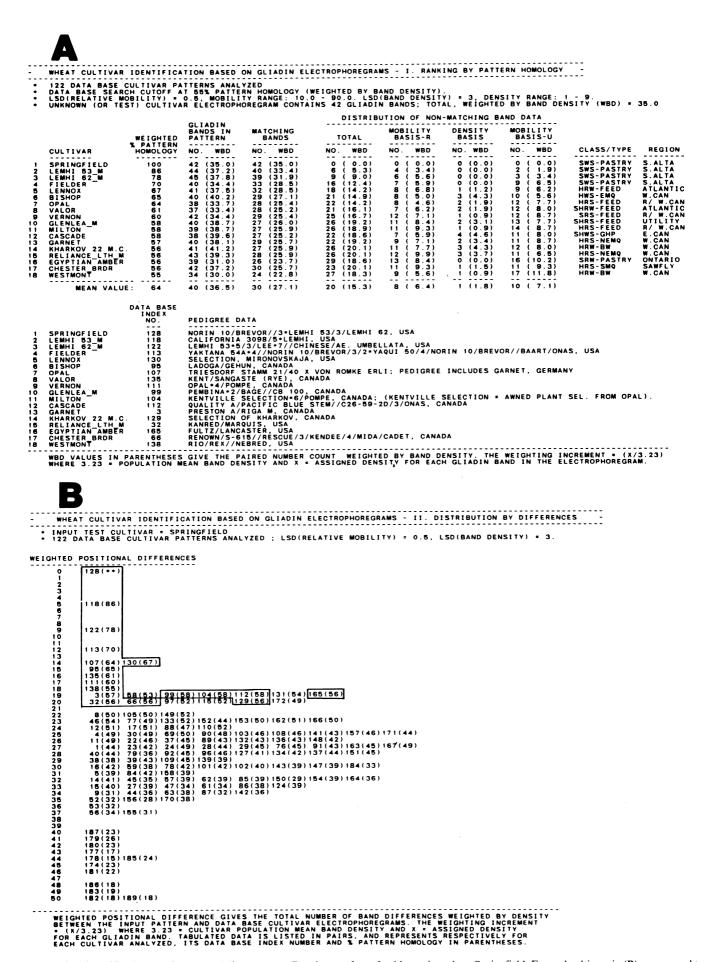


Fig. 10. Cultivar identification ranking (A) and distance map (B) printouts for soft white spring wheat Springfield. Framed cultivars in (B) correspond to cultivars ranked in (A).

B

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.

Fig. 11. Cultivar identification ranking (A) and distance map (B) printouts for hard red spring wheat Opal. Framed cultivars in (B) correspond to cultivars ranked in (A).

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composition because of their similarity to the Neepawa pattern are tightly clustered in the upper part of the CVMAP distribution. As expected, this group was exclusively comprised of cultivars listed in the CVID program ranking result shown in Figure 4.

A further distinction among the 10 remaining cultivars in the ranked subpopulation relates to the isolation of cultivars Canuck, Leader, and Era (DBINs 63, 78, and 88, respectively). Both Canuck and Leader are sawfly-resistant Canadian HRS bread wheats of good quality. Cultivar Era is a U.S.-registered HRS semi-dwarf wheat of poor breadmaking quality and relatively low protein content. These three cultivars of the same wheat class are logically different genotypes from those cultivars immediately above and below them in the CVMAP result and the ranked list provided in Figure 4. Their gliadin electrophoregrams reflect these differences. It is noteworthy particularly for Era, which cannot be visually differentiated by kernel characteristics from commercial HRS wheat cultivars of good breadmaking quality.

These observations indicate that the CVMAP analysis can stand alone as a satisfactory "summary" version of the cultivar identification procedure, requiring no input of pedigree, functional quality, or adaptation information to set up the data base. Clearly, however, the CVMAP program presents information complementary to the CVID short-list ranking. In this regard, a most useful feature is the identification of diverse genotypes with respect to the input cultivar. These are located at the margins of the CVMAP distribution and can be listed separately (illustrated in inset, Fig. 6) by the user. Cultivars in the lower tail of the distribution are in the main durum (DBIN > 172) or common wheat cultivars with electrophoregrams differing in the extreme from that of Neepawa. For example, at the weighted positional difference level of 55, the durum wheat Coulter (DBIN 177) shares only a 12% PH with Neepawa. Lake (DBIN 23) also has a very low level of pattern homology (11%) with the Neepawa electrophoregram. Cultivar Lake, unlike Coulter, is an HRS bread wheat of good milling and baking quality. The CVMAP process can therefore be used to quickly and comprehensively identify inherent variability in large populations of material that could be exploited through plant breeding.

The CVMAP distribution also yields information on overall discriminative ability of the procedure using the present data base. For cultivars at the bottom end of the ranking list, pattern homology scores around 10-20% are common. In other words, the data base cultivar population is resolved over 80-90% of the pattern homology measurement scale, which indicates a significant advantage in cultivar discrimination compared to the 60-65% range of "relative percent similarity" obtained by Lookhart et al (1983).

# Wheat Class Comparisons

In terms of class or quality type discrimination, the most striking results were obtained using a durum wheat cultivar as the test unknown. An example shown in Figure 7 was derived from a program run for the cultivar Wascana. The computer clearly identifies a durum wheat cultivar as the "unknown" (Fig. 7A) by exclusively ranking other cultivars of like class below the topranked Wascana. Note that the cutoff threshold for entry into the ranking list was reduced to 35% PH from the 55% level usually used for common wheats.

The CVMAP result (Fig. 7B) also shows how easily durum cultivars can be discriminated by gliadin electrophoregrams. The main body of the CVMAP distribution is comprised entirely of common wheats. With a mode value of 50 band differences for the Wascana electrophoregram, this level of discrimination between durum and common wheats is about 50% higher than that within each class alone. The distinction of durum wheats by gliadin electrophoregrams features a substantially different pattern distribution; and a general absence of bands with Rm <20 is consistent with their genetic composition, as all lack the D genome.

The identification of cultivars by class or quality characteristics was also easily determined for soft winter wheats. Electrophoregram data for Yorkstar (a SWW wheat) gave a ranking that included only soft white or red winter pastry wheats (Fig. 8A) with common

adaptation. The remaining soft winter wheat cultivars in the data base that were excluded from the list were five genotypes with different attributes. The excluded set comprised cultivars Gaines, Nugaines, and Sun, which are all SWW types adapted to the Pacific Northwest; Jones Fife, an obscure soft to semi-hard white winter wheat grown to a limited extent in Alberta; and Rideau, with kernel characteristics similar to Jones Fife, possessing only fair quality for pastry flour, presumably as a result of inheriting relatively strong gluten characteristics from one of its parents, Kharkov 22 M.C., an HRW bread wheat.

Another feature of the soft winter wheat ranking (Fig. 8A) is the apparent unimportance of common pedigree in deriving the result. The cluster of seven cultivars with high pattern homology scores (>85%) and few positional differences (<7) with the Yorkstar electrophoregram (Fig. 8B) are relatively dissimilar in pedigree. In total, the ancestry of the 17 wheat cultivars in the Yorkstar ranking includes contributions from more than 36 different parents from at least five countries. This indicates that gliadin composition may be the predominant factor in clustering genotypes by functional type.

Compared to the soft winter wheats, HRW wheats were a less strongly associated cultivar class. A typical result is illustrated in Figure 9, which shows the ranking derived by the Sundance electrophoregram as the test unknown. Cultivar Sundance is an HRW wheat with good milling and baking quality. Of the 11 remaining HRW wheats in the cultivar identification data base, only cultivars Yogo and Kharkov 22 M.C. possess gliadin patterns with sufficient resemblance to be included in a list dominated by Thatcher-type HRS bread wheats. The ranking may be at least partly explained by the limited number of HRW bread quality wheats in the data base, as well as by the contribution of Kanred, an HRW wheat, in the pedigree of Thatcher. Nevertheless, when wheats of different classes comprised the list of ranked cultivars, as in this example, we routinely found that the common factor in the ranking could generally be reduced to end-use quality in terms of bread or so-called "nonbread" wheat status.

A similar trend was observed when the data base was ranked by gliadin pattern homology to an electrophoregram from either a soft (white or red) spring, or an HRS feed wheat cultivar. Results are shown in Figures 10 and 11 for the SWS cultivar Springfield and the HRS wheat Opal. Both cultivars may be characterized as relatively low-protein wheats, unsuitable for breadmaking. These quality attributes, and Opal's visual indistinguishability from topgrade HRS bread wheats, also account for its restricted license for production in eastern Canada or areas of British Columbia not designated under the Canadian Wheat Board Act.

The computer outputs for Springfield and Opal (Figs. 10 and 11) have several common features. The majority of wheats with high % PH in respective short rankings, correctly reflect the class of the input cultivar. Both lists are also comparable in the proportion of pastry and feed type cultivars which were isolated, as well as the high proportion of HRS bread wheat cultivars which were excluded. (The cultivar identification data base includes 59 HRS wheats, 41 of which are at least equal to Marquis in milling and baking quality.) The CVMAP distributions (Figs. 10B and 11B) also reveal that Neepawa (DBIN 56) and other Thatcher-related wheats, which presently dominate the grain commerce in western Canada, are the most distant common wheat cultivars in electrophoregram identity with Springfield and Opal type wheats.

These results point to substantial differences in gliadin composition between bread and nonbread wheat genotypes, notwithstanding grain class affiliation or distinguishability by kernel characteristics. In order to explain the similarity in the ranking lists for Springfield and Opal, it should be noted that both cultivars, despite having very different pedigrees, share a 64% electrophoretic pattern homology. This was sufficient to place each cultivar in the upper tail of the other's CVMAP distribution (Fig. 10B, DBIN 107; Fig. 11B, DBIN 128).

The foregoing provides firm evidence of the influence of gliadin composition in differentiating wheats of different functional type. Comparable results were routinely observed in extensive testing of programs of the cultivar identification system. As the ranking results were generated by the computer based solely on gliadin composition, the latter must be considered an important factor relating to inherent wheat quality characteristics. Indeed, the relevance of gliadin composition to wheat quality parameters has been well documented for French (Branlard and Rousset 1980) and Australian (Wrigley et al 1981, 1982b) varieties, in studies where complete electrophoretic pattern data was submitted to the computer for analysis. It is not surprising, therefore, that ranking Canadian cultivars by gliadin electrophoregram homologies also exposes a similar underlying relationship.

The aims of the present study were not involved with investigating the association between gliadin protein composition and utilization quality. The relationship is nonetheless important to the successful long-term application of the electrophoresis test for wheat cultivar identification, as it suggests that grain with undesirable or different quality attributes can always be expected to be differentiated by PAGE from (otherwise visually identical) wheat of acceptable or contrasting quality.

#### **General Considerations**

With the exception of the short-list ranking results for Neepawa and Yorkstar (Figs. 4 and 8, respectively), no more than two cultivars (or  $<\!2\%$  of the data base population) in a given ranking achieved 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.

While these numbers reflect the facility with which differences could be distinguished between cultivars by gliadin electrophoregrams, unequivocal differentiation was not possible in every instance, mainly when genetic relationship was very close. A list of nine cultivar groupings in the data base that were affected in this way is given in Table III. This list can be subdivided into 16 pairs of cultivars with similar gliadin PAGE patterns, a relatively insignificant total when compared with more than 7,380 possible pairs among 122 cultivar electrophoregrams in the data base that can be differentiated.

Of greater importance are values for % PH that were computed among cultivar groups in Table III. The data indicate 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.

It should be emphasized that the reliability of results in general will depend on good precision in relative mobility determination and the establishment of a data base comprising a broad-based collection of reference electrophoregrams derived from authentic seed samples of known pedigree. Information concerning the presence of offtype patterns is also important if single kernels are used as the basis for cultivar comparisons.

# **CONCLUSIONS**

The complex heterogeneity of gliadin proteins demands the utility of a computerized strategy to evaluate the resemblance of electrophoregrams. Even on a small scale, the task of identifying matching and nonmatching bands in compared patterns is impractical by visual means alone. The elaborate system of programs described in this article has several practical and research applications, not the least of which is to quantify these types of assessments for cultivar identification. The power of these methods clearly relates to the comparative analysis of gliadin electrophoregram composition, especially where a large sample of patterns comprises the data base. The speed and detail of this process should therefore be well suited for determining genetic relationships, or to characterize the diversity or identify unique forms in a population of genotypes. Where satisfactory resolution and reproducibility of banding patterns exist, data on other protein fractions, notably sodium dodecyl sulfate PAGE patterns of high molecular weight glutenin subunits, should lend themselves well to

TABLE III
Cultivar Groups in the Data Base for Which Discrimination
by Gliadin Electrophoregrams is Uncertain

Attribute Index No.	Cultivars	Class	Computed % Pattern Homology Score
1	Apex-Marquis	Hard Red Spring	99
2	Regent-Renown	. •	95
3	Manitou-Neepawa		98
	Canthatch-Katepwa		99
4	Milton-Opal-Vernon		>95
5	Lemhi 53-Lemhi 62	Soft White Spring	96
6	Lennox-Valor	Hard Red Winter	94
7	Yorkstar-Favor-		
	Genessee-Gordon	Soft White Winter	>92
	Gaines-Nugaines		100
9	Mindum-Nugget	Durum	98

similar analyses. The potential also exists to successfully apply the computer-based methodology for the comparative analysis of high-performance liquid chromatography separations of cereal proteins as described by Bietz et al (1984). In this rapidly developing field, cultivar identification based on chromatographic data can also be reduced to a process of comparing lists of two-value parameters (relative elution time and absorbance) that characterize the cultivar.

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