VALUE ADDED WHEAT CRC PROJECT REPORT

Rapid Electrophoretic Verification of Varietal Identity:

Application to 30 Current Australian Wheats

R.E. Tonkin[1,2], G.B. Cornish[1,2] and C.W. Wrigley[2,3]

[1] SARDI Grain Quality Research Laboratory, Adelaide, S.A.
[2] Value Added Wheat CRC Ltd, North Ryde, NSW
[3] Food Science Australia, North Ryde, NSW

Date: October 2002

VAWCRC Report No: 9
Copy No: 31

Rapid Electrophoretic Verification of Varietal Identity: Application to 30 Current Australian Wheats

R.Tonkin[1, 2], G.B.Cornish[1, 2] and C.W.Wrigley[2, 3]

[1]SARDI Grain Quality Research Laboratory, Adelaide, South Australia,
[2]Value-Added Wheat CRC, North Ryde, NSW, and
[3]Food Science Australia, North Ryde, NSW

SUMMARY

This report describes a procedure for performing electrophoretic verification of wheat variety faster than the conventional method (acidic electrophoresis of gliadin proteins). The increased speed is achieved by the use of short-length gels (25 mm 'MicroGels') that have recently become commercially available. This rapid method retains good ability to provide distinction between varieties, as detailed in a table showing which of 30 current varieties are distinguishable by visual examination of the electrophoretic patterns. The method is initially recommended for 'verification' of identity, that is, checking the patterns of authentic and declared samples side-by-side.

INTRODUCTION

Various situations demand that the varietal identity of a wheat consignment must be verified quickly. These situations include the harvest scene, when grain is delivered to the silo, and the export terminal, where grain being loaded into the ship's hold must be checked. When large numbers of grain samples are being analysed for identity, even without strict time constraints, the use of a rapid method increases the general efficiency of analysis.

The electrophoretic method described in this report is based on the routine procedure that has been in use for many years, namely, acidic polyacrylamide gel electrophoresis (A-PAGE), to determine the composition of the readily-extractable gliadin proteins of the grain. Under the acidic conditions of pH-3 electrophoresis, the gliadins move cathodically (to the negative electrode).

A standard method for performing A-PAGE in a gradient of gel concentration (to sharpen protein bands) was approved in 1988 as Method No. 08-01 by the Cereal Chemistry Division of the Royal Australian Chemical Institute. This method, as revised in 1995, is described in the latest version of these Methods (see reference to Westcott and Ross, 1995 at the end of this report).

The speed of this standard method has been increased by shortening the length of the standard pre-cast gel from about 70 mm to 25 mm, thus reducing the time taken for the electrophoresis step from a few hours to about 25 minutes. In addition, the gel-staining step has been shortened. The MicroGels still contain a gradient of acrylamide concentration (3–20%T), but of course, this gradient is greatly compressed.

The rapid method was published early in the 1990s, but it has only been possible to implement the concept as a routine practice following the recent manufacture of the MicroGels by Gradipore (Sydney). Originally, the MicroGels were developed to monitor the progress of preparative electrophoresis in real time – as the preparative fractionation was taking place (Wrigley and Margolis, 1992). Its suitability for wheat identification was immediately evident, as described in several publications of the time (Bekes *et al.*, 1991; Gore *et al.*, 1991a, 1991b; Wrigley *et al.*, 1991, 1992).

EQUIPMENT NEEDED

The MicroGels are compatible with many gel tanks designed for minigels, because their cross-section has not been altered, only the length of the gel. Compatible gel tanks include:-

- Gradipore Mini 4-cell or MicroGel unit
- Bio-Rad Mini-Protean
- Novex XCell I and II

The MicroGels are available from Gradipore (info@gradipore.com). The gradient range used in this method is 3 – 20%T. The gels measure 80 X 25 X 1 mm, contained in a cassette that is 100 mm wide, 50 mm deep (measured top to bottom in the direction of migration), and 4.5 mm thick. They are provided with ten sample wells, each holding up to 50 μL of sample. Routinely, store at 4^{o}C; do not freeze! Shelf life is about five months from the date of manufacture. The gels are manufactured with Tris-borate buffer (pH 8.6) in the gel, so a pre-electrophoresis step is necessary to introduce the sodium lactate buffer (pH 3) into the gel. Note the instructions provided with the gel, especially guidance concerning trouble-shooting.

A power supply is needed, capable of delivering at least 300 volts (direct current).

CHOICE OF SAMPLE

Authentic samples must be available to cover the range of varieties of interest. Ideally these are foundation seed or original breeders' stock obtained from the Australian Winter Cereals Collection, RMB 944, Tamworth. NSW 2340.

Samples must be crushed or milled before extracting the gliadin proteins, but it is not critical that the grinding should be to a fine particle size. For initial analyses, wholemeal or flour samples are recommended, as the grinding step has the effect of 'averaging out' the composite varieties if the sample is a mixture. On the other hand, the analysis of a few single grains individually must be interpreted in terms of the statistical significance of how their identities relate to the composition of the whole consignment (Wrigley and Batey, 1995).

For breeding studies, it is valuable to be able to cut off the brush-end of the grain and crush it, leaving the germ end to be grown on after analysis of protein composition. In this case, crush the grain (or brush end of half grain) using pliers or with a hammer and metal plate.

PROCEDURE

The method below has been developed as a routine laboratory procedure; it is not necessarily optimised to achieve the fastest results.

1. Sample extraction

1.1 Weigh the crushed sample (15–100 mg) into an Eppendorf tube.

1.2 Add extracting solution at about ten times the weight of the dry sample, using ethylene glycol (Reagent 1.1) or 6% urea solution (Reagent 1.2). For example, if there is 20 mg of grain, add 200 μL of ethylene glycol. **N.B.** Ethylene glycol is viscous; use a pipette with a large tip. A small amount of crystal violet (Reagent 1.3) or other suitable acid marker dye (such as methyl green) may be added to indicate the progress of electrophoresis, and to ensure that the electrodes are connected with the correct polarity.

1.3 Vortex the sample. Allow it to stand for some minutes, but preferably for an hour or even overnight.

1.4 Centrifuge for 10 minutes at about 4,000 rpm.

2. Electrophoresis

2.1 Fit the MicroGel cassette into the gel-holder tank, so that the loading wells can be seen above the gasket and within reach of the sample-loading syringe. Remove air bubbles from wells and from spaces above the gel using sodium lactate buffer. Follow the instructions provided with the MicroGels.

2.2 Fill the apparatus with sodium lactate buffer (Reagent 2.1)**,** with the gel and upper electrode compartment in place. Use the cooling unit if available, set to an initial temperature of 25–30^{o}C, mainly to ensure reproducibility of run conditions. Connect the tank to the power supply so that the lower electrode is negative (reversed polarity to pH-8 electrophoresis) and set to 300 volts. The current should be about 40 mA per gel. Run for about ten minutes or longer to start the process of equilibrating the gel with the sodium lactate buffer.

2.3 Load 5 µl of sample into each well using a micro-syringe, after the gel is equilibrated and the power is switched off. Rinse the syringe with buffer between each sample. Each well may be loaded with 1 µl of crystal violet dye, if a marker dye has not been included in the extracting solution.

2.4 Switch on the power and continue electrophoresis for about five minutes after the marker dye has run off the bottom of the gel (about 25 minutes). The dye moves ahead of the proteins. The best run time must be determined experimentally for the apparatus in use.

2.5 Remove gel cassette after power is switched off and electrodes are disconnected. Gently prise the cassette open. Mark the gel corner corresponding to lane #1.

3. Staining, interpretation and drying

3.1 Immerse the gel in dye solution. Use Gradipure stain (Reagent 3.1) or Reagent 3.2 at room temperature. To accelerate staining, heat the dye to 50°C and immerse for only about 15 minutes.

3.2 Destain the gel by rinsing in Reagent 3.3 (6 % v/v acetic acid) if Gradipure stain was used, or in Reagent 3.4 (methanol-acetic acid) after the standard Coomassie stain. This is recommended to enhance contrast. Destaining after Gradipure stain takes about twice the stain time.

3.3 Examine gel patterns, to see if there is a match between the authentic sample and the test samples declared to be that variety. If these patterns are seen as being similar by visual inspection, the sample is likely to be correct as declared, with the rider that there may be other varieties that give the same or similar pattern. Table 1 indicates varieties with patterns that are indistinguishable by this method. If a test sample does not show the pattern as declared, it may be another variety or it may be a mixture of varieties with different patterns. In such cases, identification is much more difficult than verification of identity. Initial steps towards identification involve a repeat of this method including authentic samples of varieties likely to be involved, plus the analysis of individual grains if a mixture of varieties is suspected.

3.4 Dry gel by soaking in GradiDry II solution (Reagent 3.5, ~20 mL per gel, plus cellophane sheets) for up to 20 minutes (no longer), if the gel is to be stored dry. The cellophane sheets can alternatively be soaked in Reagent 3.6. Place a cellophane sheet on frame and apply about 1 mL GradiDry II or in soaking solution. Place the gel on the sheet and remove any bubbles. Cover with the second cellophane sheet and remove any more bubbles. Clamp the drying frame holder down and stand the gels upright to dry overnight, or place in drier for at least 2 hours at about 45°C.

REAGENTS

1. Reagents for sample extraction

1.1 Ethylene glycol

This reagent (ethanediol, glycol, ethylene alcohol) is recommended for the extraction of gliadin proteins, using it undiluted (< 4 % water content).

CAUTION: This reagent is hazardous; see the MSDS for it.

1.2 6% Urea solution

Urea (reagent grade)	0.6 g
Deionised water	9.5 mL

This reagent is an alternative to ethylene glycol for gliadin extraction, as specified in RACI Standard Method 08.01.

1.3 Crystal Violet 1%

Dissolve 1 g crystal violet in about 90 mL of 50 % sucrose solution and make up to 100 mL with sucrose solution.

2. Reagents for electrophoresis

2.1 Sodium lactate electrophoresis buffer, pH 3.1

NaOH (sodium hydroxide)	170 mg
Concentrated lactic acid	~ 1.7 mL
Deionised water	to 1 L

Dissolve 170 mg NaOH in ~ 900 ml water. Add concentrated lactic acid (~1.7 mL) until the pH is 3.1, and dilute to 1 L. The buffer may be made up at ten times concentration and diluted as needed. Store in refrigerator (4°C).

CAUTION: These reagents are hazardous. Wear gloves, cover clothing, eye protection, and use fume hood. See MSDS information.

3. Reagents for staining and drying

3.1 Gradipure stain

Obtainable from Gradipore, Sydney.

3.2 Coomassie stain

Methanol	100 mL
Acetic Acid (glacial)	100 mL
Coomassie Blue R-250	1 g
Deionised water	to 1 L

Dissolve Coomassie Blue R-250 in 100 mL water. Dissolve methanol and acetic acid in about 500 mL water, stirring well. Add Coomassie solution, make up solution to 1 L, store in a dark bottle.

3.3 Destaining solution (for use with Reagent 3.1, Gradipure stain)

Acetic acid (glacial)	60 mL
Deionised water	to 1 L

Add acetic acid to ~ 900 ml water and make up to 1 L, stirring well.

3.4 Destaining solution (for use with Reagent 3.2, Coomassie stain)

Acetic acid (glacial)	100 mL
Methanol	100 mL
Deionised water	to 1 L

Add acetic acid and methanol to about 750 mL water, make up to 1 L, stirring well.

CAUTION: These reagents are hazardous. Wear gloves, cover clothing, eye protection, and use fume hood. See MSDS information.

3.5 GradiDry II Gel Drying Solution
Obtainable from Gradipore, Sydney.

3.6 Gel-Drying Solution

Ethylene glycol	40 mL
Ethanol	350 mL
Deionised water	610 mL

As described in the Gradipore instruction sheet:- Add the water, then ethanol, then ethylene glycol to a container and mix for 10 minutes. Store in a tightly sealed container.

3.7 Acid Wash solution

Hydrochloric acid (33%)	100 mL
Deionised water	900 mL

This reagent is for cleaning equipment. Add the acid to the water. Store the reagent in a glass bottle. This gives a 3% HCl solution; if the concentrated HCl is of a different grade, adjust the formula accordingly.
CAUTION: HAZARDOUS CHEMICALS! Use heavy gloves, acid-resistant apron, face shield and fume cupboard. Beware of hot solutions. Take all safety precautions!

RESULTS

Thirty varieties were chosen for evaluation by the MicroGel method. They are listed in Table 1, together with the alleles for their high-molecular-weight glutenin subunits (HMW-GS) (as *Glu-A1*, *Glu-B1*, and *Glu-D1*) and their low-molecular-weight glutenin subunits (LMW-GS) (as *Glu-A3*, *Glu-B3* and *Glu-D3)*. Distinction according to gliadin composition should be similar to that based on the composition of the low-molecular-weight subunits, because the latter are closely linked to the gliadin genes (Cornish *et al*., 1993). No *Glu-D1* data are shown for the four durum wheats (27-30) as they lack the D genome. The actual discrimination ability of the method is shown in Table 2, with a tick or a cross indicating distinction or similarity, respectively.

Table 1. The 30 varieties evaluated for distinction by the MicroGel method, showing their alleles for glutenin subunits (HMW-GS and LMW-GS).

NO.	VARIETY	*Glu-A1*	*Glu-B1*	*Glu-D1*	*Glu-A3*	*Glu-B3*	*Glu-D3*	*Notes*
1	Anlace SP-1	1	7+9	2+12	c	g	c	
2	Arrino SP-1	2*	17+18	2+12	c	b	c	May vary at D3
3	Bowie SP-1	1	7+9	2+12	b	b	c	May vary at A3
4	Brookton SP-1	2*	17+18	2+12	c	h	a	
5	Cadoux SP-1	2*	17+18	2+12	b	b	a	
6	Camm SP-1	1	7+9	5+10	b	h	c	
7	Corrigin SP-1	2*	7+8	2+12	f	g	a	
8	Datatine SP-1	2*	7+8	2+12	f	b	a	
9	Diamondbird SP-1	1	17+18	5+10	b	h	b	
10	Frame SP-1	1	7+8	5+10	c	h	c	
11	Grebe SP-1 (1B/1R)	N	7+9	5+10	c	j	b	
12	Kalannie SP-1	2*	7+9	5+10	b	b	c	May vary at D1, A3 & D3
13	Krichauff SP-1	1	7+9	5+10	c	b	a	
14	Kukri SP-1	1	7+8	5+10	d	h	b	
15	Lorikeet SP-1	2*	7+8	2+12	b	b	c	
16	Perenjori SP-1	2*	17+18	2+12	d	b	a	May vary at D1
17	Rosella SP-1	2*	7+8	2+12	b	b	b	
18	Snipe SP-1	N	7+8	2+12	c	b	b	
19	Sunsoft SP-2	2*	7+8	2+12	b	b	b	
20	Tammin SP-2	2*	17+18	2+12	c	b	b	
21	Thornbill SP-1	N	7+8	2+12	c	b	a	May vary at A3
22	Triller SP-1	N	7+8	2+12	b	j	b	May vary at A1
23	Westonia SP-1	2*	17+18	2+12	c	h	c	
24	Wyuna SP-2	N	17+18	2+12	c	h	b	
25	Yanac SP-2	2*	20a+b	2+12	b	b	b	
26	Yitpi SP-1	1	7+8	5+10	c	h	c	
27	Gundaroi SP-2	N	7+8					
28	Kamilaroi SP-1	N	20a+b					
29	Wollaroi SP-1	N	7+8					
30	Yallaroi SP-1	N	13+16					

Table 2. Discrimination ability of the MicroGel method for 30 wheat varieties currently grown in Australia. A tick indicates that the two varieties give patterns that are visually distinguishable. Similarity of pattern is indicated by a cross.

	Anlace	Arrino	Bowie	Brookton	Cadoux	Camm	Corrigin	Data Tine	Diamondbird	Frame	Grebe	Kalannie	Krichauff	Kukri	Lorikeet
Anlace	-	✓	×	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Arrino	✓	-	✓	✓	×	✓	×	×	✓	✓	✓	✓	✓	✓	✓
Bowie	×	✓	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Brookton	✓	✓	✓	-	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓
Cadoux	✓	×	✓	✓	-	✓	×	×	✓	✓	✓	✓	✓	✓	✓
Camm	✓	✓	✓	✓	✓	-	✓	✓	✓	×	✓	✓	✓	✓	✓
Corrigin	✓	×	✓	✓	×	✓	-	×	✓	✓	✓	✓	✓	✓	✓
Datatine	✓	×	✓	✓	×	✓	×	-	✓	✓	✓	✓	✓	✓	✓
Diamond Bird	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	✓	×	✓
Frame	✓	✓	✓	✓	✓	×	✓	✓	✓	-	✓	✓	✓	✓	✓
Grebe	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	✓
Kalannie	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	✓	✓
Krichauff	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	✓
Kukri	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	-	✓
Lorikeet	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-
Perenjori	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rosella	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×
Snipe	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×
Sunsoft	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×
Tammin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Thornbill	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Triller	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓	✓	✓
Westonia	✓	✓	✓	×	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓
Wyuna	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	×	✓
Yanac	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Yitpi	✓	✓	✓	✓	✓	×	✓	✓	✓	×	✓	✓	✓	✓	✓
Gundaroi	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Kamilaroi	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Wollaroi	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Yallaroi	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 2. (continued)

	Perenjori	Rosella	Snipe	Sunsoft	Tammin	Thornbill	Triller	Westonia	Wyuna	Yanac	Yitpi	Gundaroi	Kamilaroi	Wollaroi	Yallaroi
Anlace	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Arrino	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Bowie	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Brookton	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓	✓
Cadoux	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Camm	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	×	✓	✓	✓	✓
Corrigin	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Datatine	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Diamond Bird	✓	✓	✓	✓	✓	✓	✓	×	×	✓	✓	✓	✓	✓	✓
Frame	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓
Grebe	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Kalannie	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Krichauff	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Kukri	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	✓	✓
Lorikeet	✓	×	×	×	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Perenjori	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rosella	✓	-	×	×	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Snipe	✓	×	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sunsoft	✓	×	✓	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tammin	✓	✓	✓	✓	-	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Thornbill	✓	✓	✓	✓	✓	-	✓	✓	✓	✓	✓	✓	✓	✓	✓
Triller	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	✓	✓	✓	✓	✓
Westonia	✓	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	✓	✓	✓	✓
Wyuna	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	✓	✓	✓
Yanac	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	✓	✓
Yitpi	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	✓	✓	✓
Gundaroi	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	✓	✓	✓
Kamilaroi	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	×	×
Wollaroi	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	-	×
Yallaroi	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	×	-

REFERENCES

Bekes, F., Batey, I.L., Wrigley, C.W., and Gore, P.J. (1991). Rapid electrophoresis of gliadin proteins: integration of lab tests to efficiently identify wheat varieties. Pages 467-475 in: *Gluten Proteins 1990.* W.Bushuk and R.Tkachuk, eds. Amer. Assoc. Cereal Chemists: St Paul, MN, USA.

Cornish G.B., Burridge, P.M., Palmer, G.A. and Wrigley, C.W. (1993). Mapping the origins of some HMW & LMW glutenin subunit alleles in Australian wheat germplasm. Proc. 43rd Aust. Cereal Chem. Conf., Royal Aust. Chem. Inst., Melbourne. Pp 255-260.

Gore, P.J., Bekes, F., Batey, I.L., and Wrigley., C.W. (1991a). Making variety identification easier: Rapid electrophoresis and software packages. In: Proc. 40th Aust. Cereal Chem. Conference Pp. 222-224. Eds. Westcott, T., and Williams, Y. Roy. Aust. Chem. Inst., Melbourne

Gore, P.J., Bekes, F., Batey, I.L, and Wrigley, C.W. (1991b). Rapid electrophoresis and new software to facilitate genotype identification in wheat. In: Proc. 6th Assembly Wheat Breeding Soc. Aust. Pp. 403-406. Eds. L. O'Brien *et al.*

Westcott, T., and Ross, D. (1995) 'Official Testing Methods of the Cereal Chemistry Division of the Royal Australian Chemical Institute.' Royal Australian Chemical Institute, Melbourne, Vic.

Wrigley, C.W. (Editor) (1995). 'Identification of Food-Grain Varieties' (Book of 283 pages). Amer. Assoc. Cereal Chemists, St Paul MN, USA.

Wrigley, C.W., and Margolis, J. (1992). Rapid pore-gradient electrophoresis of proteins and peptides in Micrograd gels. Appl. Theor. Electrophoresis 3: 13-16.

Wrigley, C.W., and Batey, I.L. (1995). Efficient strategies for variety identification. In: Identification of Food-Grain Varieties. Ed., Wrigley, C.W. Amer. Assoc. Cereal Chemists, St Paul MN, USA. Pp. 19-33.

Wrigley, C.W., Gore, P.J. and Manusu, H.P. (1991). Rapid (<10 min.) electrophoresis for identification of wheat varieties. Electrophoresis 12: 384-385

Wrigley, C.W., Batey, I.L., Bekes, F., Gore, P.J., and Margolis, J. (1992). Rapid and automated characterisation of seed genotype using Micrograd electrophoresis and pattern-matching software. Appl. Theor. Electrophoresis 3: 69-72.