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Wheat Cultivar Identification by Gliadin Electrophoregrams. IV. Comparison of International Methods[1]

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ABSTRACT

Gliadin electrophoregrams were obtained for two Canadian hard red spring wheats using several different procedures. Comparison of results from laboratories in Australia, Canada, and France indicated that reproducibility of pattern should be possible with strict attention to standardization of reagents, extraction procedure, gel medium, and apparatus. We compared the relative merits for cultivar identification of cathodic electrophoresis in flat gels consisting of 6% polyacrylamide, 10 or 12% starch, and 3–27% and 3–15% gradients of polyacrylamide.

Electrophoretic separation of wheat proteins has progressed steadily over the past few decades. The earliest experiments differentiated only four or five components of wheat gluten (1). Gliadin alone is now known to have more than 40 distinct protein components (2), and the gliadin electrophoretic pattern (electrophoregram) can be used to identify cultivars. Various methods of gliadin electrophoresis that have been applied to wheat cultivar identification have been described. (3–11).

We compared gliadin electrophoregrams of two Canadian wheat cultivars obtained by several different methods in three separate laboratories. The methods are currently used in Australia, Canada and the European Economic Community. The main purpose of this article is to record some important advantages and disadvantages of the methods and to identify experimental details that contribute to the reproducibility of the electrophoregram. This is preliminary work to a current, collaborative attempt of an ICC study group to produce a standardized procedure for electrophoretic identification of cereal varieties.

MATERIALS AND METHODS

Two Canadian hard red spring wheat cultivars, Marquis and Neepawa, were used. Table I shows the laboratory where the experiments were done and the gliadin solvents and electrophoresis procedures used. All procedures were done with a flat-gel support medium and a continuous lactate-lactic acid buffer system at pH 3.1–3.2 with aluminum or sodium as the cation.

RESULTS AND DISCUSSION

Extracting solvent

Figures 1 and 2 show the electrophoregrams of gliadins extracted with the three most commonly used solvents (70% ethanol, 25% 2-chloroethanol, and 6% urea) on each of several gel media. The pattern differences relate mainly to the staining intensities of equivalent bands, but differences also occur in the number of bands and in band positions (compare pattern 2 with 3, 5 with 6, and 8 with 9). The differences are significant and standardization of extraction procedure (solvent, gliadin concentration, and sample volume) is important for optimum reproducibility.

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Uniform 6% polyacrylamide gel

Polyacrylamide gel (PAG) electrophoregrams (ie, the number and relative mobilities of bands) were qualitatively similar for analyses performed in the three laboratories (Figs. 1 and 2, patterns 1–4). The bands in pattern 4 probably blurred because the samples deteriorate with age.

Reproducibility of the pattern depended to some extent on the size and design of the electrophoretic apparatus. Standardization of the aluminum concentration is also important to obtaining reproducible electrophoregrams as the aluminum ions apparently form an association with the proteins. Variation of the concentration of polyacrylamide and thus of gel pore size alter the relative mobilities of the bands. Pore size apparently is also affected by the method of polymerization. We concluded that satisfactory reproducibility with PAG electrophoresis can be achieved between laboratories with strict attention to experimental details.

Starch Gel

Starch gel (SG) electrophoregrams 5 and 6 (Figs. 1 and 2), obtained with the same apparatus as 6% PAG (patterns 2 and 3), closely resembled the 6% PAG patterns in the low-mobility region. Although poorer resolution of the faster-moving bands makes comparisons between SG and PAG electrophoregrams difficult, some general similarities are evident. However, differences in band number and distribution are sufficient to illustrate clearly the qualitative effects of gel support medium on the gliadin electrophoregrams.

Further differences are evident from comparisons of starch gel patterns (5 and 6 with 7) obtained with different apparatuses and gel formulas. Previous experience suggested that apparatus design may partly explain the different results. Pattern 7 was obtained using a simplified, inexpensive electrophoresis unit (12) designed for routine identification of cultivars in Australia where fewer genotypes need be considered than in Europe.

TABLE I. Summary of Electrophoretic Methods Used in this Study

No.	Laboratory	Gliadin Extraction Solvent	Apparatus	Gel Support Medium
1	Winnipeg, Canada	70% ethanol (11)	Bushuk and Zillman (11)	6% polyacrylamide (11)
2	Montpellier, France	70% ethanol (11)	Autran and Bourdet (8)	6% polyacrylamide (11)
3	Montpellier, France	25% 2-chloroethanol (8)	Autran and Bourdet (8)	6% polyacrylamide (11)
4	North Ryde, Australia	70% ethanol (11)	Wrigley and McCausland (12)	6% polyacrylamide (11)
5	Montpellier, France	70% ethanol (11)	Autran and Bourdet (8)	10% starch; 0.5% urea (8)
6	Montpellier, France	25% 2-chloroethanol (8)	Autran and Bourdet (8)	10% starch; 0.5% urea (8)
7	North Ryde, Australia	6% urea (12)	Wrigley and McCausland (12)	12% starch; 12% urea (12)
8	North Ryde, Australia	70% ethanol (11)	Wrigley and McCausland (12)	3–27% polyacrylamide gradient (12)[a]
9	North Ryde, Australia	6% urea (12)	Wrigley and McCausland (12)	3–27% polyacrylamide gradient (12)
10	North Ryde, Australia	6% urea (12)	Wrigley and McCausland (12)	3–15% polyacrylamide gradient (Wrigley unpublished)

[a] Preformed gel from Gradient Pty. Ltd., Lane Cove, NSW, Australia.

Although simplification of the method somewhat compromised resolution of protein bands, the patterns obtained were satisfactory for making the required distinctions between different Australian cultivars. In fact, working with the simpler electrophoretic patterns facilitated the practical recognition of electrophoregrams for specific cultivars.

Polyacrylamide-gradient gel

Electrophoregrams 8 and 9 (Figs. 1 and 2) were obtained using a Gradipore precast gel with an acrylamide concentration gradient from 3% at the top (sample origin) to 27% at the bottom. Zone sharpening, attainable with these gels, results in distinct narrow bands, but advancing bands become stacked together. A general correlation can be seen between the number and general grouping of bands (particularly the slower moving gliadins) on the gradient gel, and the uniform polyacrylamide gel. However, relative mobilities are very different in the gradient gel since the mobility of a particular component depends on its position in the gradient.

In the gradient system, sodium was preferred to aluminum in the buffer system as sodium gave better protein resolution and was readily available in good purity. Patterns were reproducible for the same electrophoresis time using different gel batches from the same manufacturer. However, slightly different electrophoregrams were obtained for gels from two sources (Pharmacia AB, Uppsala, Sweden; and Gradient Pty. Ltd., Lane Cove, Australia). Gels from Gradient Pty. Ltd. had higher cross-linking in the lower part of the gel (13).

Electrophoregram 10 (Figs. 1 and 2) shows that it is possible to moderate the "stacking up" of the faster moving gliadins in patterns 8 and 9 by using a more shallow acrylamide concentration gradient. Although the gel for pattern 10 was made in the laboratory, several precast gradient gels with concentration ranges of 3 to 15% are commercially available.

General Discussion

This interlaboratory comparison showed that reproducibility of the electrophoregram for grain of the same genotype should be possible with strict attention to standardizing procedures, apparatus, and reagents.

The collaboration compared the relative merits of several systems currently used to identify cultivars by electrophoresis. All methods provided clear distinction between the two cultivars used in this study; however, the two sets of patterns and our general experience indicate that PAG provides better potential than SG for protein resolution and for differentiation between cultivars. In addition, PAG is easier to handle than SG; slicing is unnecessary before staining; and the need, with starch, for rigid control over gelatinization conditions is avoided.

The toxicity of acrylamide monomer should be considered in relation to the routine casting of PAG in quality control laboratories. Furthermore, when requirements such as rapid identification of large numbers of samples are considered, the currently used 6% PAG is cumbersome to handle and store, is slow to stain, and there is a gradual loss of the stained patterns. The precast gradient PAG overcomes the toxicity problem and eliminates gel preparation time. Its small size (70 × 70 × 3 mm) makes it convenient to handle and shortens the time for electrophoresis and staining, but it is not as well suited to densitometric scanning, and the "stacking up" of the fast

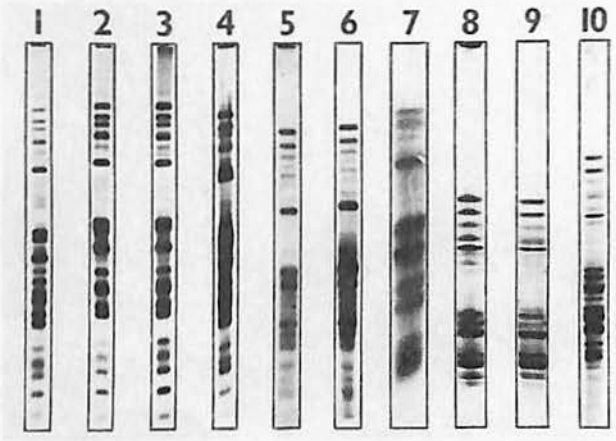

Fig. 1. Gliadin electrophoregrams of the cultivar Marquis (electrophoregrams 1–10 as in Table I).

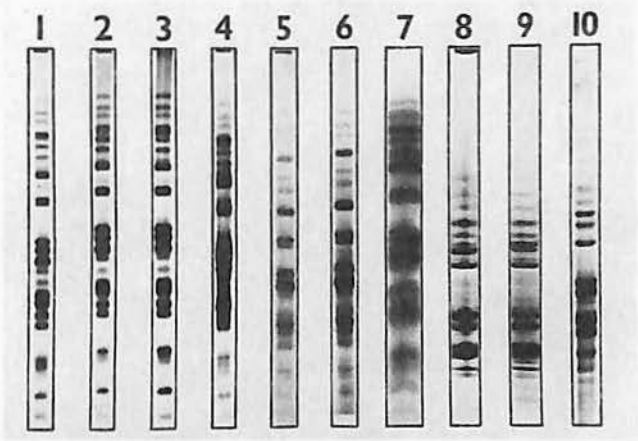

Fig. 2. Gliadin electrophoregrams of the cultivar Neepawa (electrophoregrams 1–10 as in Table I).

moving gliadins detracts from its discriminating ability (especially for the 3–27% gradient gel).

The use of polyacrylmide offers the possibility of keeping the dried, stained gel as a record, thereby avoiding the need for routine photography and perhaps for scanning. Whereas stained bands gradually fade in wet gel, they remain constant in dried gradient and uniform PAG if the gel is polymerized with persulphate.

The most suitable electrophoretic method depends on the purpose. The two main possibilities, routine screening of many samples to verify identity or to eliminate "rogue" wheats and thorough identification of a smaller number of samples from a larger selection of possible genotypes, may seem to conflict. The solution is to choose a working method based on convenience to suit a local need and to standardize, for the second type of application, a universal and more comprehensive procedure that may form the basis of an internationally accepted catalog of electrophoregrams. Our results suggest that such standardization should be possible and provide a basis for choosing a method to best suit local needs. The method that appears to be the most suitable as an international reference procedure is that of Bushuk and Zillman (11), which uses the 6% PAG.

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