NOTE

A Labor-Saving Technique for Polyacrylamide Gel Electrophoresis of Gliadins from Large Numbers of Single Wheat Kernels

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ABSTRACT

Gel capacity was increased by use of a 40-place well-former, and labor was reduced by steeping crushed kernels in ethylene glycol and applying extracts to gels without centrifugation. About 30 min of labor was required to process 80 kernels for simultaneous polyacrylamide gel electrophoresis on two gels. Large numbers of single kernels were extracted with minimal effort, permitting rapid screening for establishing homogeneity in grain samples and for application to inheritance studies.

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Polyacrylamide gel electrophoresis (PAGE) of gliadins is an established technique for identifying wheat genotypes and for determining degree of homogeneity. The latter application usually requires extraction of single kernels or portions of kernels. Several reviews have been concerned with use of PAGE for these purposes (Cooke 1984, Khan 1982, Wrigley et al 1984). Techniques for electrophoresis of extracts of single kernels usually involve crushing or grinding kernels, addition of 70% aqueous ethanol, and centrifugation to obtain clear extracts for application to gels. These procedures require several operations and are time-consuming in terms of labor and personal attention. In addition, microcentrifuges and associated accessories are required. When several hundred individual kernels must be extracted and analyzed, the technique may be too demanding to be practical (particularly if technical assistance is not available).

Recently the author became involved in efforts to determine degree of homogeneity in individual field plots of several old cultivars of soft red winter wheats. Because this project required extraction and electrophoresis of more than 600 individual kernels, studies were undertaken to reduce to a minimum time spent on operations. This report describes techniques that evolved from the study and presents examples of results obtained in the homogeneity study. Innovations include construction and use of a 40-place well-former and an extraction procedure that requires minimum kernel treatment and no centrifugation.

MATERIALS AND METHODS

Construction of the Well-Former ("Comb")

The acrylic strip (depth stop) was removed from a 3-mm thick, 15-well Teflon comb (Hoefer Scientific Instruments, San Francisco, CA), and the "teeth" were shortened to 0.5 cm by being cut off off squarely with a single-edge razor blade. The back (flat edge) of the 13-cm (5 1/8-in.) long comb was first marked off in 1/16-in. intervals with a pen. (A thin plastic 6-in. rule graduated in 1/16-in. and taped to the comb provided a convenient template for establishing the intervals.) The edge was then marked off in lines at the intervals and at right angles to the sides of the comb, and alternate spaces (starting with the second space) were inked in. With a single-edge razor blade, V-shaped notches were cut to a depth of about 3 mm (1/8 in.) in the inked-in spaces (i.e., the inked-in spaces were removed). Every fifth notch was cut to a depth of about 5 mm (3/16 in.) to produce higher partitions at 5-well intervals to serve as markers and facilitate identification of wells. The resulting well-former produced 40 wells, each about 1.5 mm (1/16 in.) wide (Fig. 1, top).

Gel Casting

The electrophoretic apparatus was a Hoefer model SE 600 (Hoefer Scientific Instruments, San Francisco, CA), using 14 × 16 cm plates separated by 3-mm thick spacers. Sandwiches were assembled as usual, but the well-former was inserted before the assembly was clamped in the casting stand so that the well-former was inverted and at the bottom of the assembly (Fig. 1, bottom). Gel solutions containing 12% total acrylamide (T) cross-linked with 3% bisacrylamide (12% T, 3% bisacrylamide concentration as a percentage of T) and buffered with acetic acid were prepared and deaerated as described previously (Clements 1987b). Before

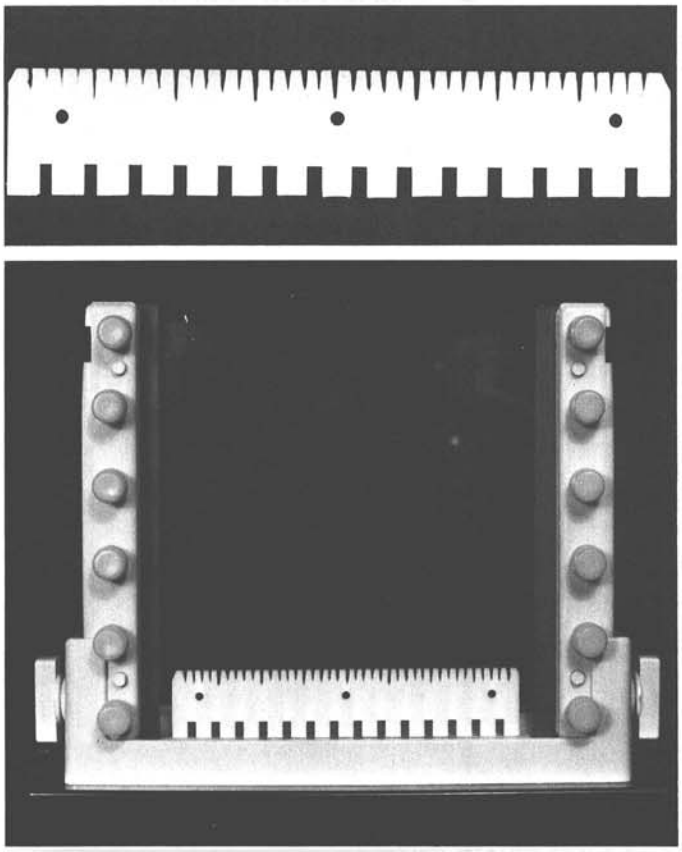

Fig. 1. Top, Details of 40-place well-former ("comb"). **Bottom,** Well-former in place for gel casting.

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cooling the solution and adding catalyst, however, a portion of the solution was pipetted into the cavities at the ends of the well-former by means of a hypodermic syringe equipped with an 8-cm long 19-gauge needle to which an 8-cm long 2-mm o.d. Teflon tube was attached. Solution was added slowly to just fill the end cavities, and additional solution was added as it seeped down and along the comb to fill the notches (partition-formers) from the bottom. As the notches of the comb filled, solution was added slowly to layer over the comb to a depth of about 5 mm. Only small amounts of solution penetrated into the large cavities remaining from the 15-well edge of the comb. The remaining gel solution was then cooled (Clements 1987a), hydrogen peroxide catalyst was mixed in, and the solution was added rapidly to fill the sandwich. The hypodermic needle, with tubing attached, was dragged through the gel quickly to mix the solution and to dislodge air bubbles, and additional solution was added as necessary to fill the sandwich. When gelling had occurred (1–2 min), the assembly was removed from the stand and cleaned of adhering gel, and the comb was extracted carefully with long-nosed pliers (by grasping the central "tooth" remaining from the 15-well comb). Gels were cast 24 hr before use and were stored at 4° C in sealed plastic bags.

Sample Extraction

Initially, spot plates were used to contain kernels during extraction. However, 5-ml flat-bottomed plastic vials (Sarstedt, Inc., Princeton, NJ) contained in 40-place racks were found to be more convenient. Single kernels were crushed between the smooth faces of a pair of pliers (with force sufficient to compress the kernels into wafers) and were allowed to fall into the vials. The kernels, usually breaking up to some degree, were not further comminuted in any way. Ethylene glycol (150 μl), containing crystal violet to provide visibility, was pipetted into the vials, and the vials were lightly covered with sheets of aluminum foil and allowed to stand overnight at room temperature. Suspensions were not mixed or agitated at any time. (It was helpful to maintain the rack in a tilted position after jarring the kernels into the low sides of the vials. During loading, the rack was tilted gently in the opposite direction to allow the extract to drain from the kernel.)

Electrophoresis

Gels were inverted from the casting position to place the wells at the top, and the cavity above the wells was filled with upper reservoir buffer (1.0 ml of glacial acetic acid in 700 ml of water; Clements 1987b). Extracts (3 μl) were pipetted directly from vials into the wells without centrifugation or other treatment. Lower reservoir buffer was acetic acid (10 ml of glacial acetic acid in 4,100 ml of water).

RESULTS AND DISCUSSION

Patterns

The methodology was developed primarily for screening large numbers of individual kernels for the purpose of establishing homogeneity. For such applications, differences among patterns (rather than the patterns themselves) are important. The two electrophoregrams shown (Fig. 2) illustrate extremes encountered in the study of old cultivars. The Todd and Trumbull specimens (left) were relatively pure; the Fultz specimen (right) was extremely heterogeneous. Continuities and discontinuities between adjacent patterns are immediately apparent. Although intensities of patterns vary because of differences in kernel size and protein content (and also because of some variation in well width), the variations do not obscure qualitative differences.

The 40-Place Well-Former Comb

The comb was designed to situate wells sufficiently below the tops of the glass plates (2.5 cm) to avoid turbulence from addition of upper reservoir buffer. The notches (3 mm deep) produce partitions high enough to isolate samples (1 mm deep) from lateral turbulence. The triangular notches provide a rigidity that avoids deformation that can occur in combs with narrow, square teeth. The remains of the teeth from the original 15-well comb provide a means for grasping the comb to extract it after polymerization.

The unusual casting procedure, i.e., with the well-former at the bottom, avoids air entrapment that results from conventional insertion of the comb from the top. The two-stage addition of gel solution, carried out as described, completely eliminated bubble entrapment in the comb.

Extraction Procedure

In an earlier study, Clements (1987a) showed that ethylene glycol extracts of meals give gliadin patterns that are essentially

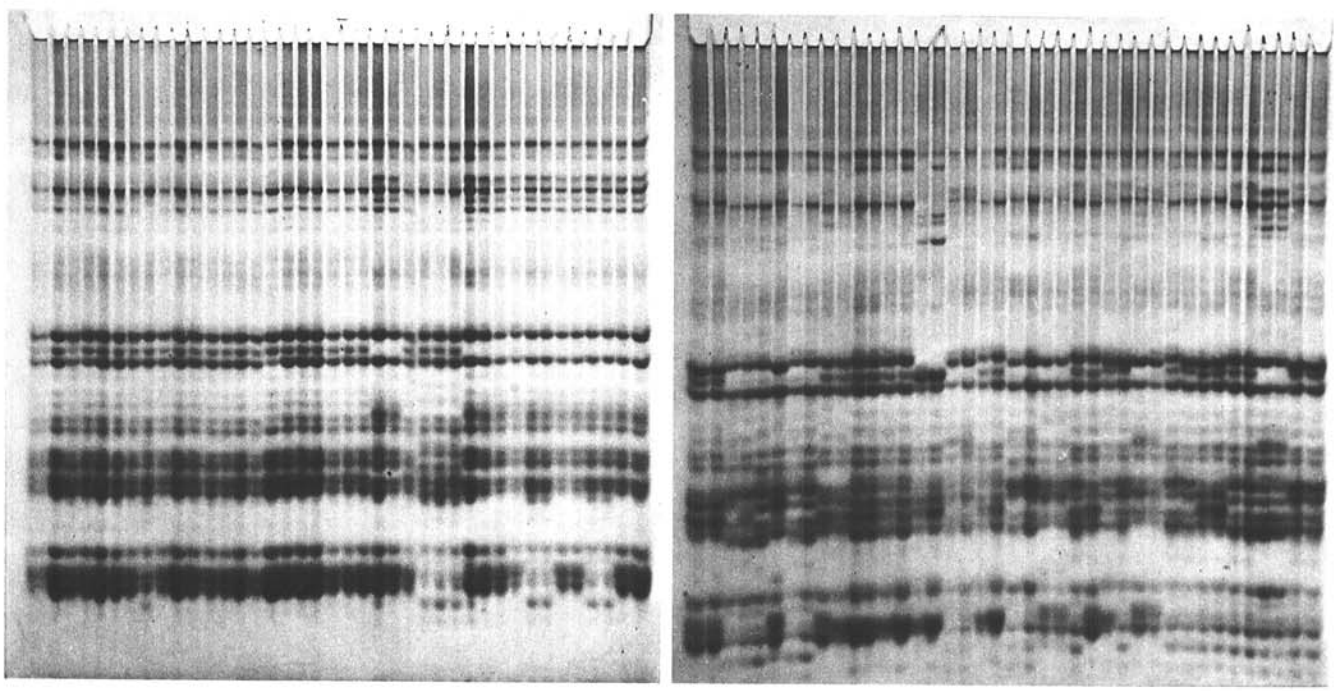

Fig. 2. Gliadin patterns from ethylene glycol extracts (uncentrifuged) of single wheat kernels applied to 40-well polyacrylamide gel electrophoresis gels (12% total acrylamide, 3% bisacrylamide concentration as a percentage of total acrylamide) acetic acid buffered. Load: 3 μl per well. **Left,** wells 1–22, Todd; wells 23–40, Trumbull. **Right,** wells 1–40, Fultz.

identical to patterns obtained with conventional 70% ethanol extracts. Further experiments showed that single kernels extracted with ethylene glycol without centrifugation produce gliadin patterns that are identical to patterns obtained by techniques involving centrifugation. The high density of ethylene glycol permits direct application to gels without the addition of sucrose or other additives. Also, because of the very low volatility of ethylene glycol, only minimum measures are required to prevent evaporation. Usually kernels are allowed to steep for about 16 hr; steeping for longer times (3–4 days) gives the same results. A 2-hr extraction gives discernible (but weak) patterns. Increased load or agitation probably would improve results from such extractions.

The procedures described were developed to minimize requirements for researchers' time and attention, and may not be appropriate to situations in which results must be obtained without delay. However, in this laboratory, determinations of homogeneity typically have not been of an urgent nature, and economy of labor has been of much greater importance. Although steeping is relatively prolonged (as compared with conventional techniques), no attention is required during this time. Likewise, since kernels need not be reduced to meals, very little time is required for processing, and centrifugation is eliminated. Selection and crushing of 80 kernels and addition of solvent required about 30 min. The extracts could be pipetted onto gels in less than 30 min. This approach permits high-volume single-kernel screening on a continuous basis with minimum expenditure of labor.

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