NOTE

Rapid Electrophoresis of Oat (*Avena sativa* L.) Prolamins from Single Seeds for Cultivar Identification

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ABSTRACT

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A quick method for the extraction of alcohol-soluble proteins (prolamins) of oats from single seeds and flour samples was developed. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was carried out using a programmable, microprocessor-controlled electrophoresis unit and ultrathin prefabricated polyacrylamide gels. This system allowed gels to be run, stained, and destained in 1 hr. The prolamin patterns from seven different oat cultivars were sufficiently unique for cultivar identification. With this equipment, most variables that hindered reproducibility in the past were removed. With minimal experience it was possible to obtain standardized, repeatable electrophoregram prolamin patterns quickly.

The electrophoresis of grain proteins and use of the subsequent banding patterns for cultivar identification is well established (Cooke 1984). The alcohol-soluble prolamin fraction is considered to give the greatest variability between cultivars of a given species (Bietz et al 1984, Konarev et al 1979). Extraction methods are uncomplicated, requiring pulverized single seeds or flour samples to be extracted with an alcohol-based solvent. The alcohol-soluble proteins are then prepared for electrophoresis.

Traditional methods of polyacrylamide gel electrophoresis (PAGE) have prevented the widespread routine application of this technique for commercial or industrial use. Batch-to-batch variation in gel casting and changes in electrophoretic parameters, i.e., buffer strength, temperature, or electrical settings, can adversely affect reproducibility. Controlling these parameters is an involved process that increases the amount of time needed to complete the test (Autran et al 1979, Bietz et al 1984, Sapirstein and Bushuk 1985).

The recent availability of precast, ultrathin-layer polyacrylamide gels and an automated PAGE system has led to a rapid and reproducible method of electrophoresis.

MATERIALS AND METHODS

Plant Material

Oat (*Avena sativa* L.) cultivars were supplied by V. Burrows, Agriculture Canada (Hinoat, Ogle, Donald), and F. Webster, Quaker Oats Company, Barrington, IL (1984 U.S. Co-op tests: Centennial, Dal, Marathon, Preston, Wright). Flour samples were obtained by manually dehulling oats and milling them in the presence of dry ice in a small domestic electric coffee grinder (Melita).

Prolamin Extraction

Each of the groats was placed between two flat steel plates and individually crushed with a hammer blow. The resulting flour was put into 1.5-ml conical polypropylene centrifuge tubes. The flour from each grain was vortexed in 52% ETOH (24 μl/mg flour) for 1 min, then centrifuged 10 min at 22° C (Robert et al 1983). Thirty microliters of the supernatant was decanted to a 0.5-ml polypropylene centrifuge tube and centrifuged in vacuo to dryness. The prolamin extracts were stored at −20° C.

Sample Preparation

Sample buffer contained 2.5% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 10 m*M* Tris HCl (pH 8.0) and 1 m*M* ethylenediaminetetraacetic acid. Dried prolamin fractions were resuspended in 13 μl of sample buffer and boiled for 3 min. Quantities required for electrophoresis were taken from this 13 μl of solution, and the remaining stock solution was saved. Samples (1 μl) were simultaneously applied to the gel using a disposable plastic comb (Pharmacia Canada Inc., Dorval, Quebec).

Polyacrylamide Gels

Prepackaged 43 × 50 × 0.45 mm PhastGel media (Pharmacia) were used. The 10–15% gradient PhastGel media contained 2% cross-linker in the resolving gel and had stacking gels composed of 4.5% acrylamide and 3% cross-linker. The gels were buffered with 0.112*M* acetate, and 0.112*M* Tris (pH 6.4). Gel buffer strips consisted of 2% Agarose IEF, 0.2*M* Tricine, and 0.2*M* Tris pH 8.0 (Pharmacia 1986).

Rapid Electrophoresis

Prolamin samples were resolved by high-resolution ultra-thin layer sodium dodecyl sulfate-PAGE using the PhastSystem (Pharmacia Canada Inc.). The standard electrophoretic parameters were up to 250 V, 10 mA, 3 W for 67 Vhr for 10–15% gels. The gels were electronically cooled to 15° C for the duration of electrophoresis.

Staining

Gels were stained for 8 min using the microprocessor-controlled development unit of the PhastSystem at 50° C in a solution containing 10% acetic acid, 30% methanol, and 0.1% Coomassie R350 dye (Pharmacia). Gels were destained at 50° C in acetic acid, methanol, water (1:3:6) for a total of 23 min. Gels were then washed in a preserving solution of 5% glycerol and 10% acetic acid for 5 min. From the development unit, moist gels were dried on petri plates at room temperature.

RESULTS AND DISCUSSION

Prolamins extracted from flour samples and single seeds of several varieties of oats were analyzed using this rapid method of electrophoresis. Cultivar purity and the absence of environmental effects on prolamin banding patterns was established (data not shown).

The fingerprints obtained from individual oat cultivars were sufficiently unique to provide the basis for discrimination between the different cultivars (Fig. 1). A high degree of repeatability not previously possible was obtained using this system. When the same samples were run on successive gels, the patterns were virtually identical. The electrophoretic parameters were monitored and

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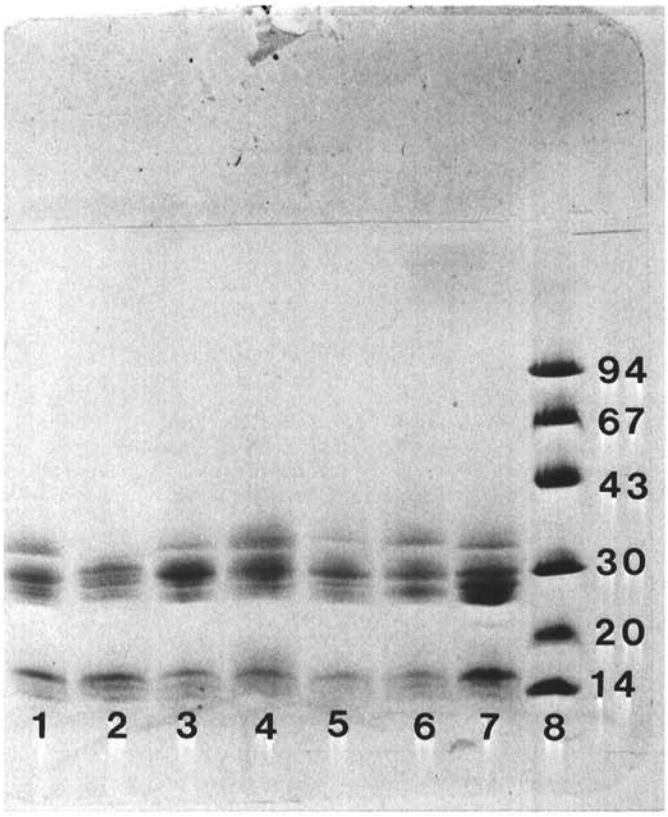

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (on a 10–15% gradient PhastGel) of oat prolamins extracted with 52% ethanol (v/v) from single seeds of seven different cultivars. **1** = Wright, **2** = Preston, **3** = Marathon, **4** = Dal, **5** = Centennial, **6** = Ogle, **7** = Hinoat, **8** = low molecular weight markers (Pharmacia).

controlled by a microprocessor. Once programmed, all gels were subjected to identical conditions during successive electrophoretic runs. Consequently, any variability is limited to sample preparation as the gels and buffers are prefabricated.

We found that minimal training was required for competent operation of this apparatus. Two major advantages experienced with this system of electrophoresis were the rapid acquisition of data and reproducibility of results. In the past it has taken from 24 to 72 hr with conventional electrophoresis equipment to obtain satisfactory results, i.e., clear banding patterns. Now results can be obtained in 1 hr. In addition to improving one's own repeatability, this apparatus also allows different laboratories to compare results with greater confidence, uniformity, and improved accuracy. The speed and quality of data from such an integrated approach will interest and benefit cereal chemists, breeders, and seed analysts.

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