Electrophoresis and electrofocusing identify wheat varieties

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Many wheat-producing countries are turning to electrophoresis for speedy variety identifications.

Fig. 1. A stained starch gel, showing the pattern of protein bands, separated by electrophoresis, for seven wheat varieties. The patterns are characteristic of each grain protein and therefore can be used for identifying varieties.

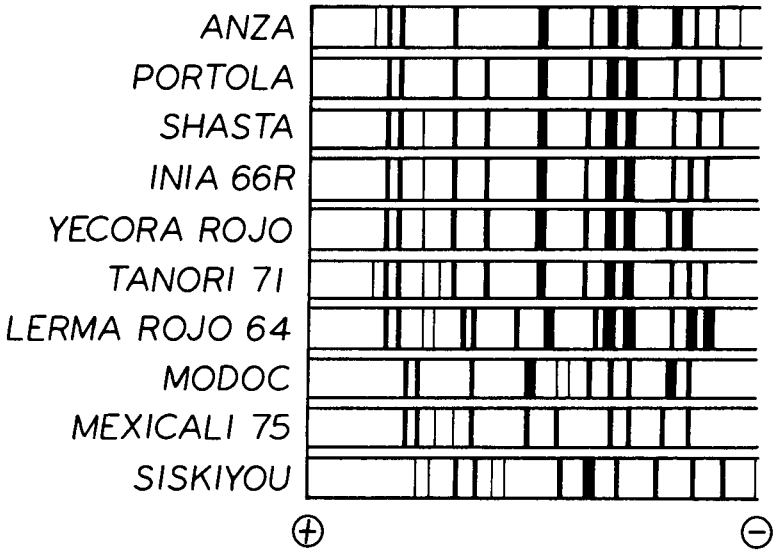


Fig. 2. Diagrams of starch-gel patterns for bread wheat (Anza, Portola, Shasta, Inia 66R, Yecora Rojo, Tanori 71, and Lerma Rojo 64), durum (Modoc and Mexicali 75), and triticale (Siskiyou) varieties.

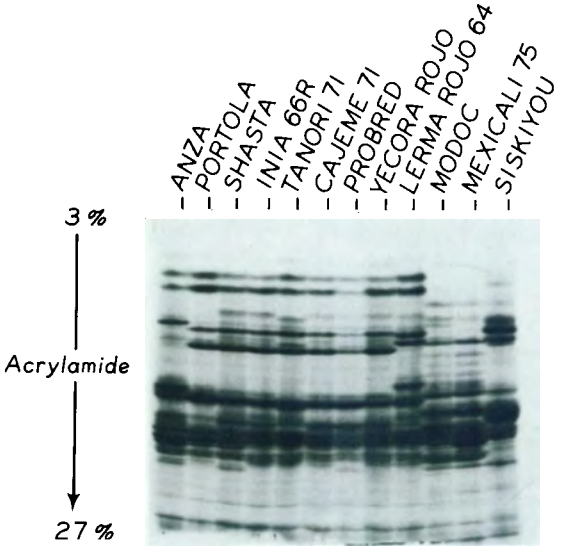


Fig. 3. Electrophoretic patterns obtained for grain proteins separated on a polyacrylamide gradient gel. All varieties except Cajeme 71 and Yecora Rojo can be distinguished by this method.

The development of a new wheat variety is a long and complicated procedure in which the breeder painstakingly tailors the new variety to perform well in a certain region, to have particular disease resistances, and to be suitable for specific uses, such as for bread, pastry, or pasta production. These and many more characteristics are "built in" by the breeder, but the results of these efforts are not fully realized if growers do not obtain properly labeled seed or if the harvested grain of one quality type is mixed with grain of another type, thus making the mixture poorly suited to any end use.

It is important that methods be available for quickly and positively identifying wheat varieties—either as seed for sowing or as harvested grain. "Grow-out" tests from seed for morphological and other characteristics take too long for most farmers' purposes and fail to identify all varie-

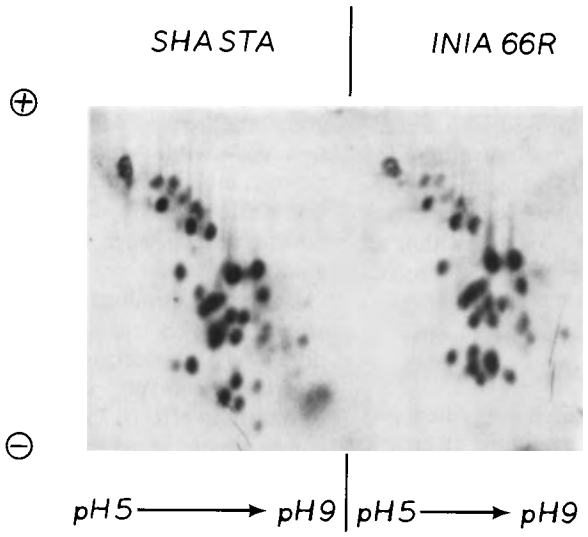
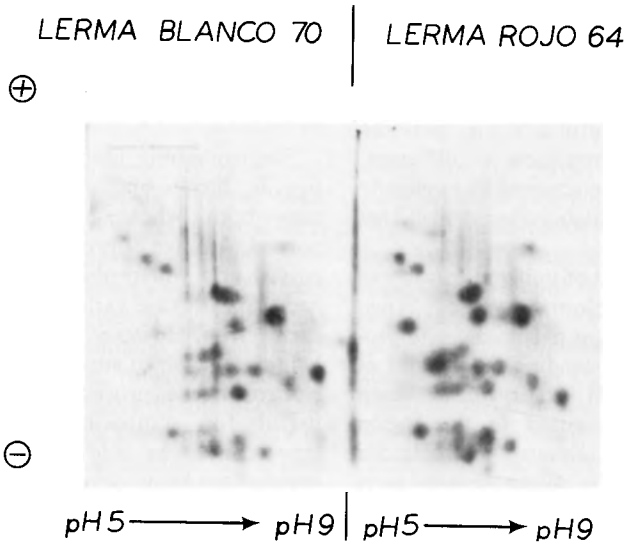


Fig. 4. Two-dimensional separations of proteins. First, gel isoelectric focusing is used to separate proteins horizontally as the pattern is shown; then the proteins are further separated vertically in the second dimension by starch-gel electrophoresis. The combination of the two methods reveals differences that are not evident in the patterns for either one used singly. (A) Shasta (left) and Inia 66R. (B) Lerma Blanco 70 (left) and Lerma Rojo 64.



ties. An examination of grain shape and color is also often inconclusive.

Electrophoresis of grain proteins

A method which is proving very reliable for variety identification in many wheat-producing countries is electrophoresis of the grain proteins. There are a few variations of the method, but all produce a pattern of protein "bands," like those illustrated in figure 1. The pattern is analogous to a fingerprint—distinctive and characteristic for the variety, and the same irrespective of the conditions under which the variety was grown.

The many different proteins of wheat grain have been grouped into a few classes according to their function and solubility. The group of proteins most useful in variety identification are the gliadin proteins. Gliadin accounts for about 40 percent of the protein of wheat endosperm. It is the part of gluten that is particularly responsible for the extensibility of dough. Gliadin is poorly soluble in water, but can readily be dissolved (extracted) in a solvent, such as a mixture of ethanol and water, or in 6 percent urea solution. The extraction procedure involves crushing the grain (as little as one-half of a kernel), mixing it with six times its weight of solvent, and allowing it to stand for about one hour. The mixture is centrifuged so that a clear solution can be used for electrophoresis.

In the acidic (pH 3) solution used in this electrophoresis system, the gliadin proteins are all positively charged, moving toward the negative electrode in the electric field. However, their speeds of migration differ, depending on the strength of their respective positive charges and their molecular size.

Thus, if the proteins can be stabilized (as in a gel medium which permits electrophoretic migration but prevents mixing), staining can reveal the positions of the proteins after they have moved in the electric current for a few hours. The result is a series of parallel bands lined up in sequence from the starting point to the fastest-moving component. Typical electrophoresis patterns for some California wheat varieties are illustrated in figure 1.

The speed of movement of each protein band is characteristic of the protein, indicating its charge and also its molecular size if the pores of the gel are small. The combined migrations of all the gliadin proteins reflect not only genetic composition and thus variety of the grain, but they can also provide additional information, such as pedigree. The pattern is constant for a particular genotype: the proportions and positions of the zones are not altered by growing conditions or even by the protein content of the grain.

Many varieties have unique electrophoretic patterns. The extent to which the varieties can be distinguished depends on the conditions of the electrophoresis system used—the pH, buffer composition, and gel medium. In general, greater protein-resolving power is required of the electrophoresis system if it is to distinguish between closely related varieties than to distinguish distantly-related varieties or different market types, such as durum and bread wheats. We have examined several electrophoretic systems to determine the extent to which each can be used to distinguish between wheats currently grown in California.

Partly hydrolysed starch was one of the first materials used for gliadin electrophoresis in a stabilizing medium, and starch gel has remained popular. Traditionally, the buffer used is aluminum lactate at about pH 3.

A thin slab of gel, about six inches square and 1/4 inch thick, is prepared by heating a starch slurry until the starch gelatinizes. This custard-like mixture is poured into the tray of the electrophoresis unit. A cover is applied together with a slot-forming device which leaves a series of depressions (slots) in the gel when it sets after cooling. Each slot is filled with a solution made with the protein extract from one variety.

After the extracts have been applied, a direct current (about 80 milliamps and 200 volts) is placed across the ends of the gel. After about four hours, the current is turned off, the gel is sliced in half to make two sheets about 1/8 inch thick, and the halves are placed in a staining solution which dyes the proteins and not the gel material.

Figure 1 shows a starch gel after stain-

ing, with eight bread-wheat varieties now or formerly grown in California. Of these, only Cajeme 71 and Yecora Rojo could not be distinguished by this method. These two varieties were obtained from the same hybrid population so this result was not unexpected. The electrophoretic patterns can be shown schematically, as in figure 2 where several electrophoresis runs highlight the easily distinguished band differences. Note that the two durum wheats Modoc and Mexicali 75 and the triticale Siskiyou differ in many bands from the bread wheats, especially in the regions of lower mobility (the left side of the diagram).

Gradient gel electrophoresis

Because of its high resolving power, polyacrylamide has become the preferred medium for electrophoresis. The gels are clear and easier to handle than starch, and the molecular sieving ability of the gel can be adjusted extensively by altering the acrylamide concentration.

A disadvantage of polyacrylamide is that the acrylamide monomer is toxic; however, with preformed gradient gels there is no danger to the user. Furthermore, preformed gels eliminate the step of gel preparation. The gel, obtained in cassette form between glass plates, is about three inches square and 1/10 inch thick. It is held vertically in the electrophoresis unit. A sample holder, inserted above the gel, provides for up to 14 samples in each gel slab. Before samples are applied, the gel is equilibrated with the buffer to be used (sodium lactate, pH 3) by preelectrophoresis for an hour.

The concentration of acrylamide in the gradient gel increases from the top where it is very weak (3 percent) to about 27 percent at the bottom. Electrophoresis in a gel of increasing acrylamide concentration offers the advantage that optimal gel porosity is provided to sharpen zones of all proteins in a mixture. As the proteins move down into a gradient gel, their mobilities decrease progressively as the increasing acrylamide concentration restricts migration. Electrophoresis can be varied from one to six hours to emphasize the bands most useful for variety identification. The protein zones are sharpened in this way, and resolution is enhanced. Thus, more gliadin components are separated and better distinctions between varieties can be obtained.

These characteristics can be seen in the gradient gel, illustrated in figure 3, which was obtained after electrophoresis for 3 3/4 hours. This method has provided obvious pattern differences for all but the most closely-related varieties. For example, the varieties Cajeme 71 and Yecora Rojo give very similar patterns, but Probred, a closely-related variety, can be distinguished from the other two.

Separation between the faster proteins (α-gliadins) can be improved with a shallower gradient of gel concentration, for example, from 2 to 16 percent. Electrophoresis is also quicker: the actual electrophoretic step can be completed within an hour with a 2 to 16 percent gel at 400 volts.

Electrophoresis combined with isoelectric focusing

Electrophoresis in starch or gradient gels does not extract the maximum of genetic information from the grain proteins. These techniques were designed as compromises between reasonable resolving power and fast, convenient analysis. Additional information may be obtained by combining gel electrophoresis with gel isoelectric focusing to produce a two-dimensional array of protein components.

The application of gel isoelectric focusing is similar to that of electrophoresis. However, the information it provides about protein composition is different, since separation is determined by isoelectric properties of the protein rather than charge and size.

An acrylamide gel of uniform concentration is used for isoelectric focusing. When voltage is supplied, a pH gradient is produced in the gel, extending from pH 3 at one end to about pH 10 at the other. Each protein becomes focused to the region where the gel pH equals the isoelectric point of the protein. When separation is complete, the positions of the proteins are revealed with a protein-staining dye.

Alternatively, this strip of gel can be used as the starting material—placed across the end of a gel slab—for electrophoretic separation in a second dimension. The resulting two-dimensional array of spots shows aspects of the protein composition and clear distinctions between closely-related wheats that are not revealed by either of the two techniques used alone in a one-dimensional separation. For example, Shasta and Inia 66R are quite similar with one-dimensional electrophoresis (see figure 1), but are clearly different in the two-dimensional array of separated proteins (figure 4A). Lerma Rojo 64 and its white-grained mutant Lerma Blanco 70 cannot be distinguished by one-dimensional electrophoresis, while minor but significant differences are seen in the combined procedure (figure 4B).

Other applications

These electrophoretic methods are applicable, in principle, to varietal identification in other cereal grains, such as barley, rye, oats, rice, and maize, although the extracting solvent must be altered for some grains to suit the solubility properties of the grain proteins. In addition, as the information provided by the proteins is carried through milling and much of food processing, electrophoretic analysis is finding application in a range of aspects of food analysis.

Pure seed production is another area where there is the need for a procedure—such as electrophoresis—that characterizes genotype, independent of environmental effects. Primarily, the method can be applied to aid in identifying certified seed; secondarily, it is proving useful for characterizing off-types or rogue plants which appear in a crop. Rogues may be identified as another known variety, as segregants of the cross that produced the variety being propagated, or as the product of natural outcrossing with another variety.

Worldwide implications

Electrophoretic identification of varieties is being applied in many countries—both wheat producers and importers. A range of procedures is being employed and will probably continue to be necessary because varieties are different in each region. However, efforts were recently initiated toward adopting a standardized electrophoretic procedure that might be useful for comparing results between laboratories. In addition, computerized systems are being developed for recording and storing electrophoretic patterns to facilitate identification of samples, and for comparing samples so that interrelationships can be examined.

Such developments may lead to the eventual compilation of electrophoretic data on a worldwide basis to permit identification of cereal varieties from many sources. This sophistication may not be applicable for the individual grower, but it would be valuable in international trade, in some forensic and legal applications, and for regulation of breeders' rights.

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