PROTEIN ELECTROPHORESIS AIDS CEREAL VARIETY IDENTIFICATION

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Enzyme proteins obtained from seedling plants show distinct patterns (isozymes) after migration in an electric field. Crop varieties differ in isozymes, and these differences open new possibilities for "fingerprinting" varieties for identification purposes.

IT HAS BECOME increasingly important to develop highly discriminatory methods for identification of varieties of crop plants. Plant breeders and seedsmen need positive identification for the protection of their proprietary rights on varieties. The grower needs assurance that his seedlot is the variety he intended to use. Processors must be assured of varietal identity and freedom from mixtures because different varieties of many crops have widely different quality and use characteristics. Variety identification can often only be guaranteed by the certification of identity back to the original seed lot, because of inadequate discriminatory characteristics especially in seed samples but also in plant characters.

Several techniques have been used successfully to identify varieties based on biochemical differences: for example, the fluorescence and phenol tests. More recently, however, developments in biochemistry and genetics have pointed to the use of molecular differences in enzyme proteins. These differences have been studied in many plant and animal species and it appears that they can be applied to problems of variety identification.

Electrophoretic method

Crude juices are obtained by crushing fresh tissue of young seedling leaves or stems. A sample of the juice is absorbed by a filter paper wick after which the wick is inserted in a slot in a buffered horizontal starch gel. An electric current is passed through the gel. The proteins in the plant juice then migrate through the starch gel. The rate and distance of migration depends upon the electrical charge and size of the protein molecules. When the migration is completed, the starch gel is sliced horizontally and treated with a stain specific for a particular enzyme.

Eleven common wheat varieties (*Triticum aestivum* L.) one durum wheat (*T. durum* Desf.), and one triticale variety (*Triticale hexaploide* Lart.) were used in these studies. The seeds were treated with a fungicide and germinated at 29°C in the dark using the Cobb-Jones slant-board technique. A 1% solution of potassium nitrate was used in the water reservoir of the germination trays.

Seedlings were harvested when 8 days old by excising the shoot directly above the seed. All of the shoot tissue from a single plant was crushed thoroughly and the crude extract was absorbed without filtration by a 6 × 6 mm Beckman paper wick. The saturated wicks were chilled until preparations from all plants to be analyzed in one starch gel were ready for insertion in the gel. Up to 16 plants were analyzed on one gel. The starch gel was prepared using standard techniques with citric acid and Sigma 7–9 buffer. The wicks were inserted in slots in the gel. The wicks were left in the gels for 15 minutes with electric current at 150 volts passing through the gel. The wicks were then removed and the voltage increased to 350 V until the brown borate front had moved 8 cm from the point of wick insertion. The gels were cooled during electrophoresis with Sno-Gel packs.

Upon completion of electrophoresis the gels were sliced horizontally into three parts and stained separately for esterase and two peroxidase enzymes using standard staining procedures for these enzymes. The staining period was about 30 minutes or until the bands were distinct. The gels were then rinsed, fixed in

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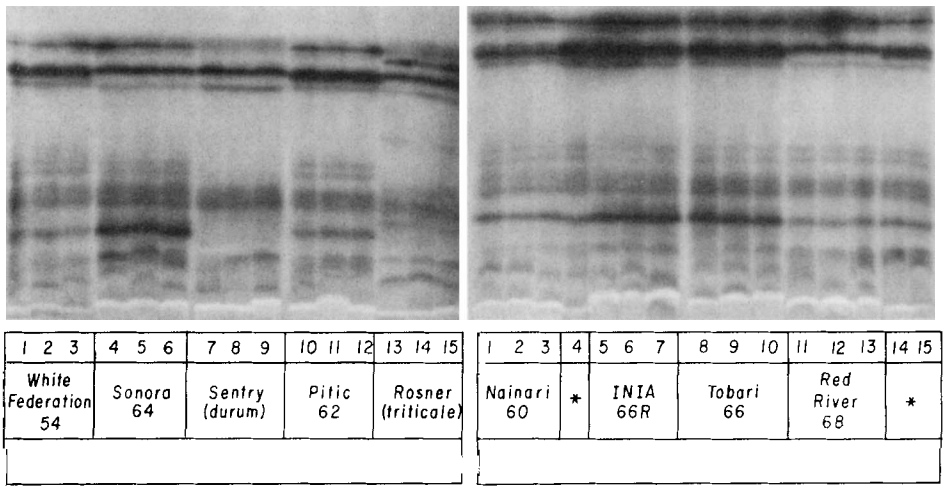
SMALL-SCALE BEEF RAISING: REWARD OR RISK? Leaflet 211. This leaflet is for the inexperienced person who either owns or is considering buying land on which he hopes to start up a small beef-raising business. While such a project is not completely impossible, there are a great many factors involved that might not occur to a beginner. The aim of this publication is to point out these factors so they may be considered by the prospective cattle rancher before he risks too much capital.

THE SMALL FARM HOME. Leaflet 210. This leaflet is directed to city families who are thinking of moving to the country and starting a small farm.

50% methanol, and wrapped for storage until evaluated.

After staining, bands appear on the starch gel. These bands are enzyme proteins which are variants, called isozymes, of the stained enzyme system. The bands vary in position and staining intensity. The molecular structure, and hence the relative position on a starch gel of an isozyme, is genetically determined. This position and staining intensity is relatively constant when electrophoresis and staining conditions are standardized. Since the starch gel is sliced horizontally into three parts before staining, the simultaneous assay of three or more different enzyme systems, is possible.



Fingerprinting varieties

Identifying varieties by electrophoretic techniques is possible because each variety has a unique pattern of isozymes (stained bands). Since several enzyme systems can be assayed and several bands (in some cases 15 or more) can be differentiated for each enzyme, the number of possible combinations of bands present and their positions is virtually limitless. Thus, a pureline variety, such as most small-grain varieties, can be "fingerprinted" by its combinations of isozymes of one or more enzymes.

The inheritance of isozymes has been examined for many species of wild and cultivated plants. The isozymes are generally inherited as major genes segregating in Mendelian fashion.

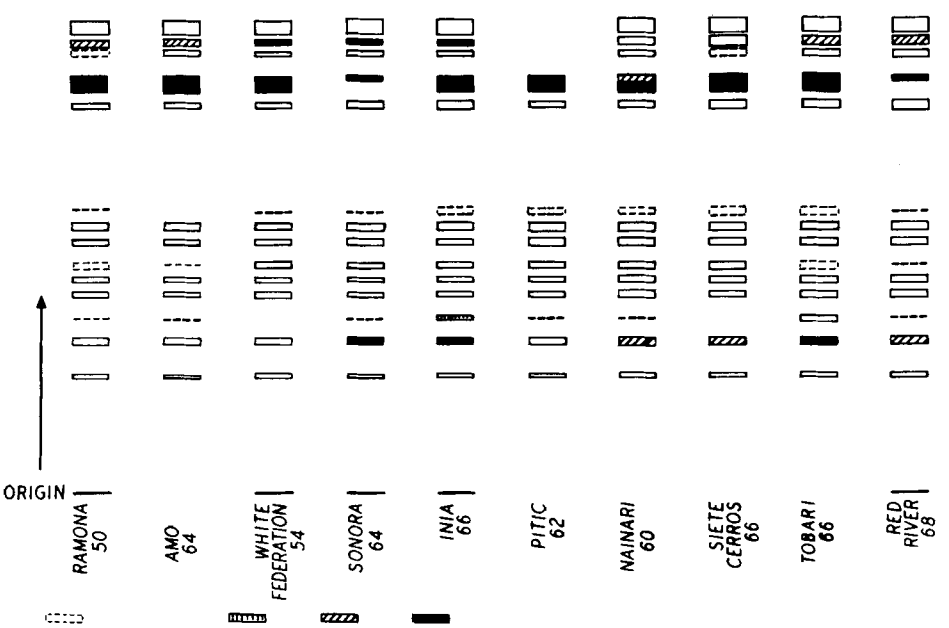
This survey of wheat and triticale varieties was made to examine the possibility of adapting protein electrophoresis for variety identification. The varieties used were chosen because of importance in California agriculture, morphological similarities, or their usefulness in plant improvement programs.

Zymograms

The first phase in the evaluation of this technique for variety identification required reliable establishment of the isozyme patterns from a group of varieties. This was done by making eight or nine separate runs using various combinations of varieties on a gel. On each gel three or four plants from each variety were evaluated—a total of more than 30 plants for each variety in all runs. Photo 1 shows typical results for esterase for two gels. In this figure, and others presented here, the staining pattern for each plant on a gel is identified by a number. For example, numbers 1 to 3 in photo 1 (left) are from three plants of White Federation 54. The figure shows banding patterns for 10 varieties. Within each variety the patterns were very consistent, but among varieties there were large differences in the number, position, and staining intensity of the bands.

After examination of gels from several independent runs, the banding patterns were established and sketched as in the diagram. The pattern for each variety is called a zymogram. Groups of varieties can be identified from the diagram that are similar, for example Ramona 50 and Ramona 64 or Sonora 64, Tobari 66, and INIA 66. Sonora 64 can be distinguished from INIA 66 and Tobari 66 by one isozyme band. Tobari 66 could not be distinguished from Sonora 64 and INIA 66 with esterase isozymes, but could be separated based on peroxidase isozymes (not shown).

With the three enzymes examined, Ramona 50 and Ramona 64 could not be distinguished. Ramona 64 is an unreleased, stem-rust-resistant, backcross derivative of Ramona 50 and it is not surprising that the two varieties were so similar. Similarly, the banding patterns of INIA 66 and INIA 66R were identical; the latter variety is a selection from the former, and the two are expected to be very similar. Some varieties shown in photo above are easily distinguished from all other varieties (e.g., Nainari 60, Sentry durum, and Rosner triticale).



Interpretative diagram of esterase zymograms for 10 wheat varieties. The intensity of staining of bands is indicated by the scale at the bottom of diagram.

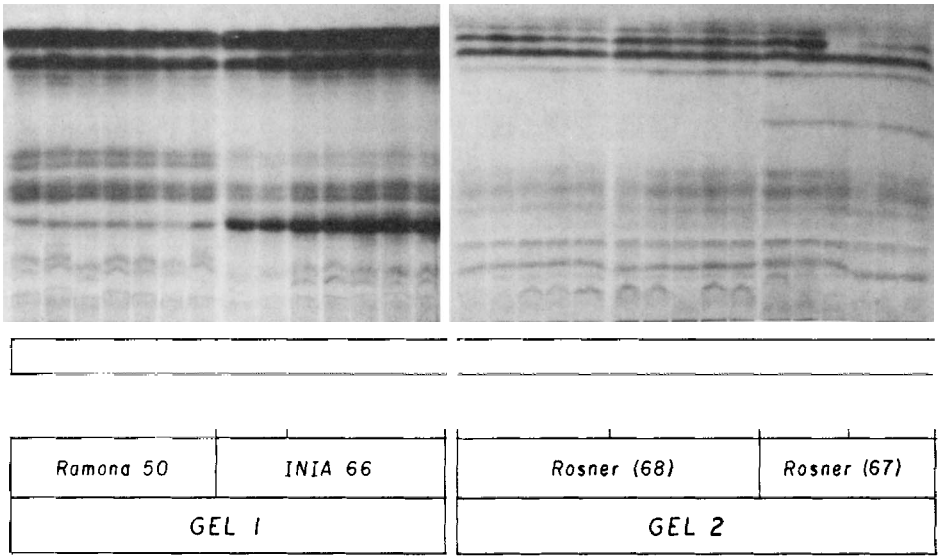


Photo 2. esterase zymograms for Ramona 50 and INIA 66 wheat (Gel 1) and Rosner triticale (Gel 2) grown at Davis and Tulelake in 1969 or at Davis in 1968 and 1969.

Environments compared

Differences among varieties were obvious but these differences must be expressed consistently from location-to-location and year-to-year if isozymes are to be used for variety identification. This was examined in a limited way. Ramona 50 and INIA 66, both produced at Davis and Tulelake in 1969, were compared (gel 1 in photo 2), and plants from both varieties from the two locations were indistinguishable.

Rather different results were obtained for two samples of Rosner triticale (gel 2, photo 2). Rosner (67) and Rosner (68) were obtained from the University of Manitoba in 1967 and 1968, respectively. Rosner (67) was grown at Davis in 1968 and 1969 and Rosner (68) was grown at Davis and Tulelake in 1969. Variation was found among plants of Rosner (67), but the differences were not consistent from year to year. Rosner (67) is therefore a heterogeneous variety for esterase isozymes.

In contrast, the zymograms of Rosner (68) were very uniform among plants within and between locations. A striking difference in one band was noted for the two Rosner introductions. Rosner (68) resulted from a reselection and purification of the variety at Manitoba and the two selections were indistinguishable under field conditions in California. This illustrates that subtle changes in a variety may be easily detected if the appropriate enzymes are evaluated.

Results from the comparisons of three varieties grown at two locations or two years did not show significant differences that could be attributed to environmental effects. Many more varieties and environments should be examined, but one of the critical requisites of a method for variety identification—environmental stability—seems to be satisfied.

Commercial seedlots

Two lots of uncertified seed of Pitic 62 and INIA 66 were submitted by a seedsman so their identity and purity could be confirmed. These varieties were easily distinguished by esterase isozymes (see photo 1 and diagram). Known standards and one of the commercial seedlots were included on a single gel. The commercial sources were readily identified by variety in zymograms. Commercial INIA 66 showed very little variation among plants, but Pitic 62 showed variation at one of the bands. Minor variation of this type has also been observed in standard sources of Pitic 62.

Conclusions

A system for variety identification must rely on rapid and inexpensive methods, yield the same results for samples produced in a wide range of environmental conditions, and it must unambiguously discriminate among varieties. The isozyme system uses rather simple equipment and only a few chemical supplies. The results can be obtained in about the same period of time as a standard seed germination test. A high level of training is not required to perform the tests. Seed technologists could readily learn the techniques in a short time. It seems practical from a technical point of view to consider isozyme analysis for variety identification.

Additional work is needed to establish the consistency of expression of isozymes over environments, although the results presented here suggest that this will not be a problem. Different species and enzyme systems would of course require proper standardization of certain procedural details.

Seedling enzyme proteins

To establish the uniqueness of varieties by isozymes requires that varieties which may differ in many morphological or physiological characters also differ in seedling enzyme proteins. This may not be the case at all. For example, INIA 66 and INIA 66R differ in grain yielding ability, but are indistinguishable during the growth period or as seed samples. Similarly, Ramona 50 and Ramona 64 are indistinguishable except for resistance to stem rust. With the enzyme systems used here the isozyme system failed to distinguish these varieties. Many more enzyme systems remain to be examined, however. It seems obvious that isozyme analysis will not replace all other methods of variety identification, but it does offer a vast number of highly discriminatory traits (bands) to increase the probability of correct identification.

If this technique is to be useful for many crops, additional research is needed (1) to characterize existing varieties, (2) to evaluate which enzyme systems will be useful to identify particular varieties, (3) to standardize techniques so that results from various laboratories will be comparable, (4) to determine the significance of environmental effects, and (5) to improve the procedure so that more rapid analyses can be made on seed or vegetative plant tissues.

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