

Iso-electric Focusing for Reliable Genetic Analyses



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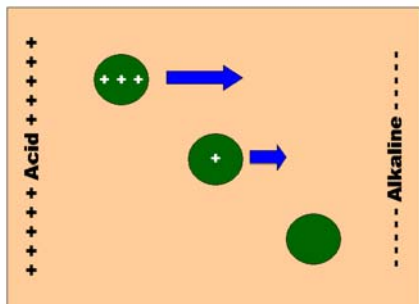


Effective testing for hybrid-purity and varietal identification of vegetable seeds can be accomplished using a number of laboratory techniques. Although DNA-based methods are becoming commonplace in agricultural settings, high development costs have made these assays impractical for routine testing. While STA Laboratories has developed a number of cost-effective DNA methods it is primarily because of cost and simplicity that isozymes have emerged as the preferred tool for evaluation of seed genetics.

Isozymes are structurally different molecular forms of enzymes which have identical catalytic functions. Substitutions of amino acids account for the origin of various isozymes. The result is a slight variation in the net charge of the protein (becoming more positively or negatively charged) causing it to move at a different rate in the presence of an electric field. Historically, the mobility rates of isozymes were measured in a starch-gel matrix using a technique called *Starch Gel Electrophoresis*. Isozymes were first separated and then visualized by allowing the enzymes to catalyze a chemical reaction in the starch gel which would pro-

duce a color change wherever the enzymes were present. Since the different enzymes are encoded by unique genes, the presence, absence, or overall profile of isozymes may be used as an indicator of a plant's genotype.

Isozymes can also be evaluated using an extremely high-resolution method known as *Iso-electric Focusing or IEF*. This technique relies not on the rates of mobility but on the protein's net charge. Isozymes move

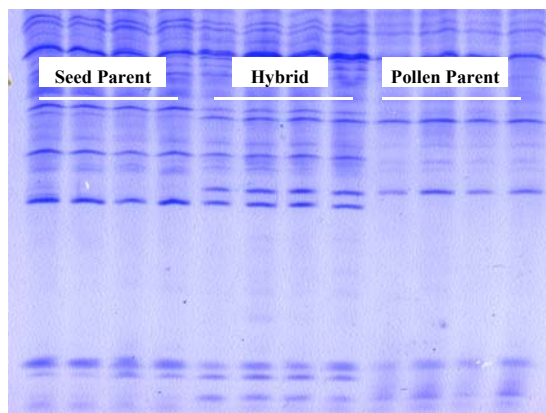


Proteins migrating through a pH gradient will continue to move until their net charge becomes zero.

through a pH gradient under the influence of an electric field. As the enzymes move through acidic regions of the gel and into areas of higher alkalinity the net charge on the protein changes, until eventually, it reaches a pH region where the net charge equals zero. At this point the protein will not migrate any further and is said to be "focused". IEF can be used to differentiate proteins with very subtle changes in amino acid composition. Using STA's proprietary gel systems, the pH gradients may be prepared either narrow or broad and can be tailored specifically to the isozymes and proteins being evaluated. Because of this flexibility and the rapid run times, iso-electric focusing is rapidly replacing starch gel electrophoresis as the method of choice in agricultural testing.

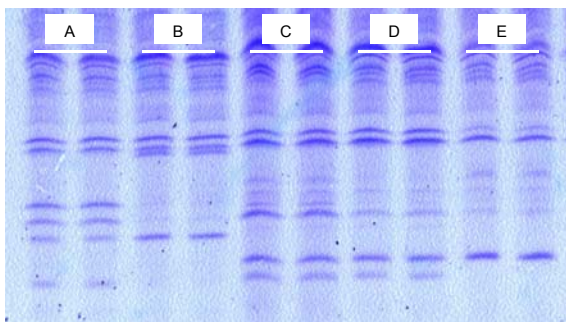
As mentioned above, isozymes catalyze chemical reactions and by using color-producing chemical analogs, it is possible to visualize the location of the enzymes within a starch or IEF. This, however, is not the only way to observe the pattern of proteins from a seed or plant. There are a number of stains which can be used to visualize proteins. Silver Staining is one commonly used technique which is useful for all crop species and is quite sensitive. Unfortunately, silver staining requires several time-consuming steps and is notoriously sensitive to glassware cleanliness and water quality. While silver staining is still used extensively at STA Laboratories, we have developed some new methods which allow us to respond more quickly to our clients' genetic testing needs.

Some of our newer techniques utilize a dye known as Coomassie[®] Brilliant Blue which is used as a general protein stain. First, seeds are crushed into a coarse powder and an extraction solution is added to solubilize the seed proteins. The protein extracts are applied to an IEF gel and the proteins are separated. Staining with Coomassie[®] Brilliant Blue causes the proteins to appear as blue "bands". As many as 50 proteins can be visualized simultaneously to produce a pattern resembling a barcode. Unlike the simple patterns produced by starch gel electrophoresis, iso-electric focusing gels can be used to simultaneously evaluate hybridity and varietal



High-resolution IEF allows rapid and reliable identification of inbreds in your hybrid seed lot.

trueness. These assays can be performed quickly and reliably so that you can make timely decisions for your seed production program.



Using IEF, corn varieties can be evaluated for trueness-to-type simultaneously with hybridity testing.



Call us at 1-800-426-9124 to find out how STA Laboratories, Inc. can be your partner in quality seed production.