

A Method of Varietal Differentiation Using Vertical IEF Electrophoresis with Esterase and Coomassie Stains

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Accurate varietal labeling is essential for seed marketing throughout the world. Seed lot contamination can greatly impede seed trade and result in tremendous losses to both seed companies and growers. Protein gel electrophoresis is a powerful tool that utilizes a vast diversity of seed and seedling proteins to differentiate between varieties within a species and can be used to identify seed lot contaminants. Although SDS PAGE is a more traditional method used for seed varietal testing, it is not suitable for all species. This study focuses on the use of vertical IEF gel electrophoresis in combination with esterase and Coomassie stains for variety differentiation of wheat and sunflower. Pure seeds were ground, extracted in an extraction buffer (75 mM Tris pH 7.5 and 0.1% β -mercaptoethanol), centrifuged, and the supernatant was electrophoresed on pH 5-7 and pH 3-10 IEF gels. After completion of the IEF gel run, the gels were stained at 30 °C for 30 minutes with aryl esterase to reveal esterase activity (resulting in brown-stained bands). After the esterase staining was completed, the gels were subsequently stained with Coomassie blue, a total protein stain resulting in blue-stained bands. Upon completion of staining, each sample (lane) contained two differently colored banding patterns, which provided additional information for distinguishing between varieties wheat and sunflower. Esterase and Coomassie blue stains on the ampholyte pH 5-7 range IEF gel were complementary in wheat and sunflower and provided the greatest diversity in banding patterns. This method promises to be a valuable tool for differentiating varieties of wheat and sunflower. Additional research has been initiated to evaluate the usefulness of IEF gels in combination with esterase and Coomassie blue stains for the differentiation of tall fescue and wheatgrass varieties. Preliminary results indicate that two week old seedlings provide better banding patterns than seeds. Additionally, greater separation of esterase proteins is obtained with the pH 5-7 IEF gel for wheatgrass and the pH 3-10 IEF gel for tall fescue. Working with tall fescue and wheatgrass has revealed an importance for understanding the species genetics. Since wheatgrass and tall fescue are crossing-pollination, evaluation of single seeds or seedlings can yield ambiguous results. A subsample of several seeds or seedlings should be combined and homogenized prior to extraction in order provide meaningful results. In conclusion, the use of two staining systems to stain a single electrophoresed gel increases the power of the test to differentiate varieties. This procedure is quick, simple, relatively lower in cost, and generates highly resolved banding patterns.