ELECTROPHORETIC IDENTIFICATION OF AGROSTIS PALUSTRIS AND POA PRATENSIS CULTIVARS

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Numerous creeping bentgrass and Kentucky bluegrass cultivars have recently been introduced. Cultivar identification is becoming a major problem in that distinguishable morphological differences are lacking for positive identification. The recently enacted Plant Protection Act has made it possible to patent a cultivar. Some means of positive identification is necessary if cultivars are to be properly distinguished.

Electrophoresis has been useful in the cultivar identification of soybeans, alfalfa, barley, and oats. The possibility of using acrylamide gel disc electrophoresis in the identification of turfgrass cultivars was studied. Gel electrophoresis is a technique used in the separation and isolation of proteins. It is based on the differential movement of protein bands through a gel when under an electric current. Cultivar identification is then based on different protein banding patterns produced by the different cultivars.

Six cultivars of creeping bentgrass (Toronto, Cohansey, C-52, Penncross, Pennlu, and Evansville), and ten cultivars of Kentucky bluegrass (Merion, Fylking, Cougar, Prato, Windsor, Belturf, Nugget, Galaxy, Park, and Kenblue) were studied. The bluegrass cultivars were chosen to represent a diverse genetic base. All plant material was placed in flats in a greenhouse under fluorescent lights which extended the normal photoperiod to 14 hours per day with the temperature held as close as possible to 20 C. Bluegrasses were maintained at a 3.75 cm cutting height, and bentgrasses at 2.5 cm. All grasses were watered as required, and received a complete nutrient solution weekly. A fungicide was applied biweekly for powdery mildew control. A six week acclimation period was allowed.

The extraction of proteins from leaf tissue was found to be extremely critical. The entire extraction was carried out in a cold chamber at 4 C to prevent denaturation of proteins. All equipment had to be chilled prior to use. Three grams fresh leaf tissue, a small amount of acid washed sand, and 1.0 ml phosphate buffer were ground in a mortar. The extraction was made with a 0.2 M phosphate buffer, pH 7.0, containing several anti-oxidants and chelating agents. The anti-oxidants were included to prevent the oxidation and subsequent destruction of leaf proteins by phenolic compounds. Chelating agents were included to remove metal ions. This extraction buffer was selected after preliminary studies with Tris and unmodified phosphate buffers. Only a few, lightly stained bands could be obtained with these two extraction buffers. Bentgrasses were particularly sensitive to the extraction buffer used. Bentgrass extracts made with the Tris and unmodified phosphate buffers turned brown, indicating protein precipitation, most likely due to the high phenolic content of the bentgrasses.

After being ground, the tissue was removed, and the mortar washed with an additional 2.0 ml phosphate extraction buffer. The protein extract was squeezed from the tissue through two layers of cheesecloth, and centrifuged at 10,000 XG for 30 minutes at 4 C. The supernatent was used for electrophoresis.

Seven per cent acrylamide gels were established in 9.5 cm tubes. A spacer gel was used as an anti-convectional medium. Water was layered on top of each gel during polymerization to prevent formation of a maniscus. Equal amounts of extract and unpolymerized gel were mixed, and a 0.1 ml aliquot was used in electrophoresis. This was placed on top of the gel after the water was removed.

A Tris-glycine electrode buffer, pH 8.3, was used. Bromo phenol blue dye traced the protein front. Electrophoresis was run at 1 mA per tube for 15 minutes, and then at 2 mA per tube until the tracing dye front was approximately 1 cm from the bottom of the tube. This generally required about two hours.

Gels were removed from the tubes and stained 15 minutes at room temperature in a 0.125% Coomassie Blue solution (45% water, 45% methanol, and 10% acetic acid by volume). Gels were destained over night at 50 C using a solution of 5% methanol, and 7% acetic acid, and then stored in 7% acetic acid for observations. Six replications of both the extraction and electrophoresis were made for each cultivar.

All six bentgrass cultivars examined could be individually distinguished on the basis of different protein banding patterns. A number of unknown samples, brought into the laboratory by a technician, could be identified 100 per cent of the time. Compared to the bluegrasses, the bentgrasses produced more distinct and consistent banding patterns.

The ten Kentucky bluegrass cultivars exhibited between 14 and 24 individual bands. However, banding in some regions was inconsistent or too light for positive identification. Banding in two regions only was reliable enough for cultivar identification. Six cultivars could be placed into three groups of two (Merion and Fylking, Cougar and Prato, and Windsor and Belturf), while Nugget and Galaxy could be identified singly. Park and Kenblue Kentucky bluegrasses exhibited no characteristic banding pattern over the six replications. Bluegrass unknowns, excluding the Park and Kenblue, could always be placed into the proper group, and 75 per cent of the time could be individually distinguished using banding patterns in the less consistent regions.

Background (light staining between darker stained bands) presented a problem in locating individual bands. This was especially true with the Kentucky bluegrasses, where heavy background made it difficult to distinguish lighter bands.

Acrylamide gel disc electrophoresis has potential use in the cultivar identification of creeping bentgrass and Kentucky bluegrass. However, caution must be exercised in several areas. (a) Plant material must be grown under exacting light, temperature, moisture, and nutritional conditions prior to leaf sampling. Additional standardization of these factors may aid in Kentucky bluegrass cultivar identification. Also, supraoptimal temperatures have been shown to cause drastic shifts in the protein banding patterns of bentgrass.

Another approach might be to change the standard growing temperature to where more distinct protein banding patterns possibly could be achieved. (b) The temperature conditions during staining were found to be extremely critical. Fluctuations in room temperature presented difficulty in obtaining uniform staining. Staining temperature should be standardized. (c) Plant material variability must be minimized. The use of clonal material may be necessary. All bentgrass cultivars examined, except Penncross, are vegetatively propagated and thus genetically uniform. Bentgrasses exhibited more consistent and distinct banding patterns than did the bluegrasses. The lower genetic uniformity of the bluegrasses probably contributed to the less characteristic protein banding patterns. This was especially true of Park and Kenblue bluegrasses, which did not show consistent banding patterns over the six replications. Both of these cultivars represent a mixture of several bluegrass lines, all of which differ genetically.