Use of Cellulose Acetate Electrophoresis as an Alternative to Starch Gel Electrophoresis for Detecting Root-Knot Nematode Resistance in Tomato

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ABSTRACT

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Detection of the acid phosphatase-1 (*Aps-1*) isozyme from tomato genotypes susceptible, homozygous resistant, and heterozygous resistant to root-knot nematode was compared by cellulose acetate and starch gel electrophoresis. In contrast to starch gel electrophoresis, cellulose acetate electrophoresis made discrete separations of *Aps-1* bands and gave better resolution. In all tests, cellulose acetate electrophoresis was quicker and more economical than starch gel electrophoresis in assaying for the *Aps-1* isozyme.

Tomato breeding for root-knot nematode resistance was started about 45 yr ago at the Tennessee Agricultural Experimental Station (1). Early studies demonstrated that resistance derived from *Lycopersicon peruvianum* (L.) Mill. is controlled by a single dominant gene (*Mi*), which confers resistance to three important root-knot nematode species, *Meloidogyne incognita*, *M. javanica*, and *M. arenaria* (2,3). In 1974, Rick and Fobes (7) showed that the *Mi* gene is tightly linked to the acid phosphatase-1 (*Aps-1*) locus. This allele, designated *Aps-1*^{1}, was known to occur only in *L. peruvianum*. All other cultivars of *L. esculentum* Mill. carried the allele *Aps-1*$^+$ (4). Because of the dimeric nature of *Aps-1*, zymograms of heterozygous plants show three bands: one each in the parental position (+/+, 1/1) and a dimeric band in the intermediate position (+/1). Since then, detection of the *Aps-1* isozyme in tomatoes has served as a basis for identifying nematode-resistant germ plasm. The electrophoretic procedure currently employed to identify *Aps-1* in tomatoes is the starch gel electrophoresis as outlined by Medina-Filho and Stevens (4). During the past several years, starch gel electrophoresis has gained considerable acceptance and is used as a diagnostic tool for identifying nematode-resistant tomato genotypes (5). The procedure, however, is laborious and time consuming and requires skill and experience.

Recently, Paul and Williamson (6) assayed acid phosphatase isozymes and purified *Aps-1* from tomato cell cultures by cellulose acetate electrophoresis. The simplicity of cellulose acetate electrophoresis, coupled with the relatively short time required to assay for the *Aps-1* isozyme, make this procedure an ideal diagnostic tool for researchers involved in evaluation of root-knot resistance in tomato genotypes. This study, therefore, was undertaken to determine if cellulose acetate electrophoresis could be used as a routine screening method to identify tomato genotypes that are susceptible, homozygous resistant, and heterozygous resistant to root-knot nematode and to compare this electrophoretic procedure with starch gel electrophoresis.

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MATERIALS AND METHODS

Plant material. Seedlings of the cultivars VFN8, Early Pak 7, and hybrid X3717 were used because of their homozygous resistant (7), susceptible, and heterozygous resistant (H. A. Bolkan, *unpublished*) reactions, respectively, to root-knot nematode. Seedlings for all tests were grown in Styrofoam Speedling trays (33 × 66 cm) with 200 growing cells (10 × 10 mm) containing a steam-sterilized mixture of peat, sand, and perlite (6:5:1, v/v). Trays with seedlings were kept in a greenhouse where temperatures varied from 16 C at night to 26 C during the day. At the second-true-leaf stage and 72–96 hr before the *Aps-1* assay, all seedlings were watered with a nutrient solution (1 L/Styrofoam tray) prepared by dissolving 2 g of KH_2PO_4 per liter of Growmore fertilizer solution (Nacco Agricultural Chemicals, Cardena, CA) previously mixed at the rate of 2.5 g/L of water.

Starch gel electrophoresis procedure. Slab gels (183 × 157 mm) 5 mm thick and containing 11% starch (Sigma) were used. With the exception of the gel and the electrophoresis tank buffer pH, the procedure for the extraction, electrophoresis, and staining of the gels was as described by Medina-Filho and Tanksley (5). To improve resolution, the pH of the gel and electrode buffer (0.3 M boric

acid) was adjusted to 8.0 instead of the recommended 8.4.

Cellulose acetate electrophoresis procedure. Cellulose acetate electrophoresis was performed using the Sartophor system for electrophoresis (Sartorius GmbH, Gottingen, West Germany). The electrophoresis tank buffer was 0.3 M boric acid, pH 8.2, adjusted with 4 N NaOH. Seedling sap samples were obtained by grinding the youngest fully expanded true leaves (one leaf per plant per sample) in freshly prepared grinding buffer (1:1.5, w/v) made of 750 mg of reduced glutathione per 20 ml of distilled water, pH 8.2, adjusted with 1 M tris (hydroxymethyl) aminomathane. The grinding buffer was kept on ice throughout the extraction process. After extraction and without prior centrifugation, 10-μl samples from each test plant were transferred separately to the sample holder of the Sartophor system and refrigerated until needed. Samples were transferred to 120-μm-thick cellulose acetate membrane strips (70 × 145 mm) with the multiple-sample applicator of the Sartophor system. Electrophoresis was performed at 200V constant voltage (current 2 mA) at room temperature (25 ± 1 C) for 17 or 20 min, depending on the number of samples assayed. If 10 samples were placed on the cellulose acetate membrane in one application, electrophoresis was run for 20 min; on the other hand, if 20 samples were placed on the cellulose acetate membrane, the running time was reduced to 17 min to avoid sample contamination.

After electrophoresis, *Aps-1* bands were visualized by placing the cellulose acetate membranes in staining solution and incubating in the dark until the bands developed (usually 4–6 hr). The staining solution was prepared fresh each day by mixing 20 mg of Fast Black K Salt, 10 ml of 0.1 M sodium acetate, pH 4.6, 15 μl of 1 M MgCl, 0.5 ml of 1% β-napthylacid phosphate in acetone:water (1:1, v/v), and 10 ml distilled water. The staining solution was poured into plastic petri dishes (100 × 15 mm) and stored in the dark until use. The position of isozyme bands in each starch gel and cellulose acetate membrane was measured, and the relative migration [Rf = band migration (mm)/borate front migration (mm) ×100] values were calculated. The number of samples from each electrophoretic procedure with no *Aps-1* band separation was compared by the *t* test.

RESULTS AND DISCUSSION

Under the conditions that the cellulose acetate electrophoresis was conducted, Rf values for *Aps-1* bands for resistant (1/1) and susceptible (+/+) genotypes were 65.2 and 76.1, respectively (Fig. 1A). Rf values for *Aps-1* bands from heterozygous resistant (+/1) plants were 76.1, 70.7, and 65.2 (Fig. 1A). The Rf for the *Aps-2* band (5) was 43.5. A third band resolved in starch gel electrophoresis below the *Aps-2* band (Fig. 1B) was not detected with cellulose acetate electrophoresis; however, a long unresolved area indicating enzyme activity was present in all plants assayed (Fig. 1A). With starch gel electrophoresis at pH 8, the *Aps-1* Rf values for resistant and susceptible plants were 77.6 and 85.1, respectively (Fig. 1B). The *Aps-2* band had an Rf value of 70.1, and the Rf of the band located below *Aps-2* was 49.2 (Fig. 1B).

Specificity and reliability are primary factors determining the accuracy of all diagnostic procedures including electrophoresis. Both starch gel and cellulose acetate electrophoresis differentiated the *Aps-1* isozyme and allowed classification of tomato genotypes for root-knot nematode resistance. In all our studies, however, cellulose acetate electrophoresis gave better resolution than starch gel electrophoresis. This was especially true for heterozygous resistant plants; the *Aps-1* bands were focused and distinct (Fig. 1A), whereas with starch gel electrophoresis, the bands were diffuse and incompletely separated from each other (Fig. 1B). Furthermore, when the number of unreadable samples that required retesting was determined, the percentage of unreadable samples was significantly less ($P = 0.01$) with cellulose acetate electrophoresis than with starch gel electrophoresis. Of the 4,400 samples assayed with cellulose acetate electrophoresis, only 0.02% were unreadable, whereas of the 798 samples assayed with starch gel electrophoresis, 2.2% needed retesting.

Compared with starch gel electrophoresis, cellulose acetate electrophoresis performed with the Sartophor apparatus appears to be an excellent system for assaying the *Aps-1* isozyme. The procedure is simple and quick; separation of *Aps-1* bands can be accurately done for up to 20 individual plants simultaneously within 17 min. One person can perform the necessary operations (i.e., extraction and running

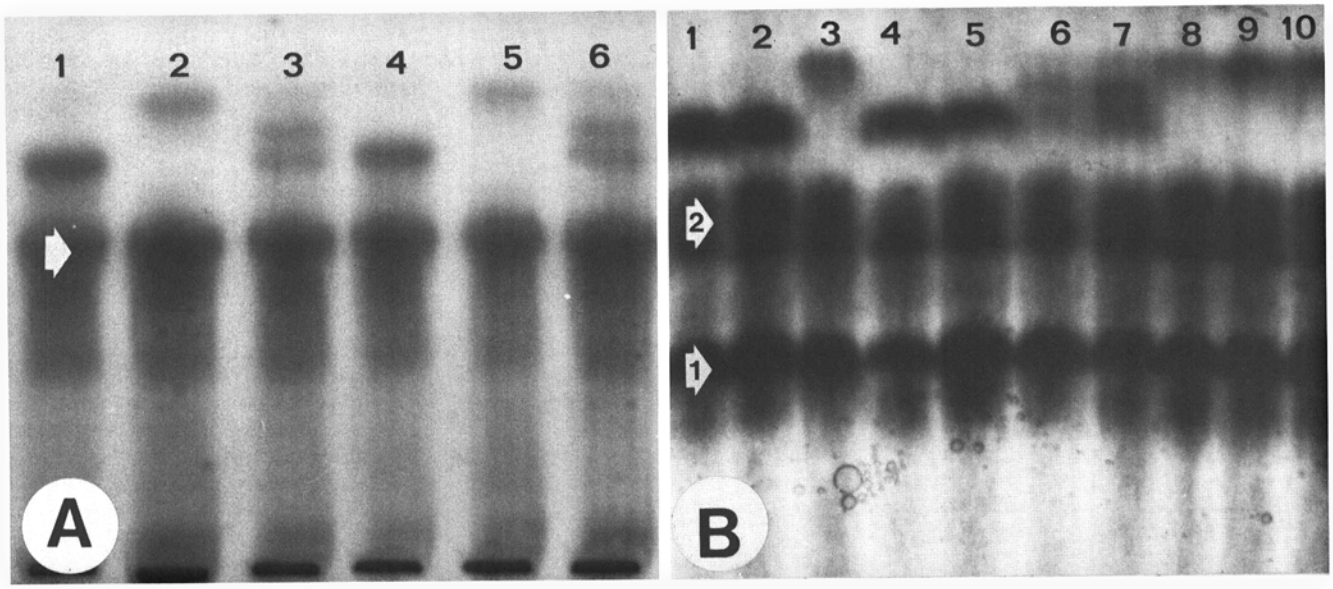

Fig. 1. Typical acid phosphatase (*Aps*) zymograms obtained with **(A)** cellulose acetate and **(B)** starch gel electrophoresis. **(A)** Arrow: *Aps-2* band (Rf = 43.5). Lanes 1 and 4: *Aps-1*$^{1/1}$ (Rf = 65.2, homozygous resistant genotype). Lanes 2 and 5: *Aps-1*$^{+/+}$ (Rf = 76.1, homozygous susceptible genotype). Lanes 3 and 6: *Aps-1*$^{1/1}$ (Rf = 65.2), and *Aps-1*$^{+/1}$ (Rf = 70.7), and *Aps-1*$^{+/+}$ (Rf = 76.1), heterozygous resistant genotype. **(B)** Arrow 1: undesignated *Aps* band (Rf = 49.2). Arrow 2: *Aps-2* band (Rf = 70.1). Lanes 1, 2, 4, and 5: *Aps-1*$^{1/1}$ (Rf = 77.6). Lanes 3 and 8–10: *Aps-1*$^{+/+}$ (Rf = 85.1). Lanes 6 and 7: *Aps-1*$^{1/1}$ (Rf = 77.6), *Aps-1*$^{+/1}$ (Rf = 81.4), and *Aps-1*$^{+/+}$ (Rf = 85.1). Plant material for both electrophoretic procedures was grown under the same conditions, and the *Aps-1* bands were visualized with Fast Black K Salt and β-napthyl acid phosphate.

the electrophoresis) and classify up to 400 individual plants within 8 hr with cellulose acetate electrophoresis. Two systems can be operated at the same time, permitting classification of up to 800 samples within 8 hr. With starch gel electrophoresis, within a comparable time period, using a four-gel apparatus, one individual can only run 140 samples (4.5, H. A. Bolkan and C. M. Waters, *unpublished*). Additionally, the starch gel electrophoresis procedure for assaying *Aps-1* involves a number of variables that affect the reliability and quality of the results obtained (4). The procedure is labor intensive and time consuming. Cellulose acetate electrophoresis can be performed on a laboratory bench without refrigeration. The initial cost of equipment is about $1,400 (U.S.), but the cost of operation is very economical; 500 ml of tank buffer is sufficient to operate the system during an 8-hr period.

This study demonstrates that cellulose acetate electrophoresis is superior to the commonly used starch gel electrophoresis in distinguishing between homozygous and heterozygous nematode resistant tomato genotypes. The new method represents a considerable savings of time, effort, and resources. Since our early experiments with cellulose acetate electrophoresis, we have been routinely screening a wide array of tomato cultivars for their *Aps-1* isozyme with the cellulose acetate electrophoresis procedure. Regardless of the origin of the cultivar tested, cellulose acetate electrophoresis accurately differentiated the *Aps-1* bands. Although our primary concern here was with the *Aps-1* isozyme, cellulose acetate electrophoresis might be useful in studying other isozymes.

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