Identification of Sweetpotato Cultivars Using Isozyme Analysis

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Abstract. The enzymes alcohol dehydrogenase, diaphorase, esterase, glutamate dehydrogenase, glucosephosphate isomerase, isocitrate dehydrogenase, malate dehydrogenase, malic enzyme, 6-phosphogluconate dehydrogenase, phosphoglucomutase, shikimate dehydrogenase, and xanthine dehydrogenase were analyzed by starch gel electrophoresis of leaf tissue from nine sweetpotato [*Ipomoea batatas* (L.) Lam.] cultivars. Bands of most enzymes were well-defined. Polymorphisms were found in nine enzymes, and cultivars were identified by comparing polymorphisms.

Isozyme application was outlined by Pierce and Brewbaker (1973) and included cultivar identification. Isozyme analysis has subsequently been reported for cultivar identification for several clonally propagated species including apple. (Weeden and Lamb, 1985), *Camellia japonica* (Wendel and Parks, 1983), pineapple (Dewaid et al., 1988), raspberry (Cousineau and Donnelly, 1989), and strawberry (Bringhurst et al., 1981). Biochemical markers offer a more precise method for distinguishing cultivars than morphological characteristics and promise to be valuable supplements for patent identifications (Moore and Collins, 1983). In addition, isozymes can be used to study within-population genetic variability and relationships among clones.

Isozyme studies on sweetpotato have been limited and few enzymes have been examined. Variation in esterase (Kokubu and Hirai, 1978) and peroxidase (Kokubu and Maeda, 1978; Kokubu and Nakakawaji, 1982) isozymes was found among sweetpotato cultivars and related wild species using horizontal thin layer agar gel electrophoresis. Xue et al. (1988) reported interspecific diversity between sweetpotato and related wild species in peroxidase isozymes determined by polyacrylamide gel electrophoresis. Our purpose was to determine if starch gel electrophoresis could be used effectively to observe isozyme variability of 12 enzyme systems in sweetpotato for cultivar identification and indication of common ancestry among cultivars. Since we found no previous work with starch gel electrophoresis on sweetpotato, electrophoretic procedures and techniques used on other species (Tanksley and Orton, 1983) were adapted for use on sweetpotato.

'Centennial', 'Jasper', 'Jewel', 'MD708', 'Regal', 'Southern Delite', 'Travis', 'Vardaman', and 'W216' sweetpotatoes were compared for isozyme banding patterns. Plants

used were produced from storage roots originally obtained from the following sources: 'Centennial', 'Jewel', 'Travis', and 'Vardaman'—foundation roots, Mississippi State Univ.; 'MD708'—breeder's roots, Univ. of Maryland; 'Regal', 'Southern Delite', 'W216'—breeder's roots, USDA-ARS Vegetable Laboratory. Genotypic authenticity of cultivars was maintained by producing storage roots under controlled conditions with careful attention to freedom from mechanical mixing and visible mutations.

Enzymes evaluated were alcohol dehydrogenase (ADH; EC 1.1.1.1), diaphorase (DIA; EC 1.6.4.3), esterase (EST; EC 3.1.1.2), glutamate dehydrogenase (GDH; EC 1.4.1.2), glucosephosphate isomerase (GPI; EC 5.3.1.9), isocitrate dehydrogenase (IDH; EC 1.1.1.42), malate dehydrogenase (MDH; EC 1.1.1.37), malic enzyme (ME; EC 1.1.1.38), 6-phosphogluconate dehydrogenase (6-PGD; EC 1.1.1.44), phosphoglucomutase (PGM; EC 2.7.5.1), shikimate dehydrogenase (SKDH; EC 1.1.1.25), and xanthine dehydrogenase (XDH; EC 1.2.1.37). Basic electrophoretic techniques (Arulsekaran and Parfitt, 1986; Brewer and Sing, 1970; Cardy et al., 1983; Shields et al., 1983; Vallejos, 1983)

were used in preparing, electrophoresing, and staining the gels.

Plant materials consisted of two 25-mm plants of each cultivar transplanted to pots and grown in a greenhouse. Analyses were started 4 weeks after transplanting and continued for 3 months. No variation in banding patterns for any enzyme was observed due to differences in plant age. Immature leaves, 5 to 10 mm long, from each of the two plants of each cultivar were collected in early morning as source material for enzyme extraction. About 300 mg of leaf tissue was homogenized in 2 ml of an extraction buffer solution consisting of 0.1 M Tris, 32.5 mM reduced glutathione, 14 mM beta-mercaptoethanol and 200 mg of polyvinylpyrrolidone. The macerated samples were then centrifuged at 4°C for 15 min at 10,000× g. The supernatant was frozen quickly at -20°C for future use. Banding resolution of frozen extracts after 1 week was compared with fresh extracts and no difference was observed. Since activity of some enzymes is known to diminish with freezing time (Wendel and Weeden, 1989), samples older than 1 week were discarded.

Two buffer systems were effective in separating isozymes of sweetpotato. Buffer system A (Cordy et al., 1983) was a discontinuous system that clearly defined DIA, EST, GDH, GPI, ME, PGM, and XDH, and system B (Shaw and Prasad, 1970) was a continuous system that best elucidated banding of ADH, IDH, MDH, 6-PGD, and SKDH (Table 1). A 13.5% (w/v) starch gel was used for system A and a 13% (w/v) starch gel was most effective for system B. A constant current of 40 mA was applied to system A for 5 h and 30 mA to system B for 3 h. The staining solutions were described by Kennedy (1989). Electrophoretic analyses were repeated a minimum of three times for each enzyme after the optimum gel/staining system was determined.

Among the sweetpotato cultivars examined, the enzymes GDH, IDH, and XDH were not useful for cultivar identification be-

Fig. 1. Sweetpotato leaf isozyme phenotypes separated by starch gel electrophoresis. EST had three regions of dense banding with several bands each that appeared monomorphic.

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Table 1. Gel and electrode buffers used in starch gel electrophoresis for isozyme analysis of sweet-potato.

Buffer system	Buffer type	pH	Chemical ingredient
A	Electrode	8.3	0.19 M Boric acid 0.04 M Lithium hydroxide
	Gel (9 parts gel buffer : 1 part electrode buffer)	8.3	0.05 M Tris 0.007 M Citric acid (monohydrate)
B	Electrode	7.0	0.135 M Tris 0.043 M Citric acid (monohydrate)
	Gel (1:14 dilution of electrode buffer)	7.0	

Table 2. Enzyme phenotypes of sweetpotato cultivars.

Cultivar	Enzyme*								
	ADH	DIA	EST	GPI	MDH	ME	6-PGD	PGM	SKDH
Centennial	A	B	A	A	B	B	B	B	B
Jasper	B	B	B	B	B	C	B	B	B
Jewel	A	A	A	A	B	C	C	C	B
MD 708	A	B	B	B	A	A	A	C	A
Regal	A	A	B	B	A	C	A	A	B
Southern Delite	A	B	B	C	B	C	C	B	B
Travis	B	A	B	B	B	C	C	B	B
Vardaman	A	B	C	B	B	B	B	B	B
W216	A	B	B	B	A	C	B	B	A

*See Fig. 1 for phenotypes A, B, and C of different enzymes.

Table 3. Origin of sweetpotato cultivars characterized by isozyme analysis.

Cultivar	Parent(s)* and its origin
Centennial	L5-130, polycross seedling out of Unit 1 Porto Rico x Pelican Processor
Jaspar and Travis	L3-217, polycross seedling out of LO-99, polycross seedling out of [(Mameyita x L4-6) x (Pelican Processor x Triumph)]
Jewel	Centennial x Nugget
MD-708	Maryland polycross seedling
Regal and Southern Delite	W-99, polycross seedling out of SC-1166
Vardaman	M97-4 x HM145
W-216	Resisto, polycross seedling out of W-56

*Polycross = open pollination, male parent unknown.

cause each enzyme showed only one band of equal mobility for all nine cultivars. However, each of the nine remaining enzyme systems had at least one polymorphic region (Fig. 1).

Two regions of activity were exhibited by ADH. One was a fast-migrating, monomorphic zone and the other was a slow-migrating, polymorphic zone that included a single fast-banded and three-banded phenotypes. DIA had five regions of activity, and the fastest migrating one was polymorphic. The fastest migrating activity zone of EST was polymorphic, and the three phenotypes observed were fast, slow, and double-banded. Three additional groups of closely spaced bands identified for EST appeared monomorphic. GPI, MDH, ME, and SKDH produced five, eight, four and six bands, respectively, and each had a polymorphic region near the midrange of speed of migration. Each of three PGM phenotypes included three well-resolved bands, but regions of activity were difficult to interpret. The three phenotypes were distinguishable by differences in rate of migration

of at least one band. The four fastest-migrating isozymes of 6-PGD constituted a region of activity for the separation of three phenotypes.

Systematic identification of the cultivars included in this study was accomplished by a comparison of enzyme phenotypes (Table 2). Determination of the phenotypes of certain enzymes requiring both buffer systems was necessary to identify all cultivars; however, all of the enzymes were not needed. Identification of the nine cultivars was possible by comparing phenotypes for MDH, ADH, and 6-PGD from buffer system B with either a) PGM and GPI, b) EST and ME, c) EST and DIA, or d) GPI and DIA from buffer system A.

To determine if isozyme phenotypes indicate common ancestry, origins of clones used are shown in Table 3 ('Centennial': Miller et al., 1960; 'Jasper': Hernandez et al., 1974; 'Travis': Hernandez et al., 1981; 'Jewel': Pope et al., 1971; 'Regal': Jones et al., 1985; 'Southern Delite': Jones et al., 1987; 'Vardaman': Allison et al., 1981; 'Resisto': Jones et al., 1983). Known re-

lationships are: 'Centennial' is a parent of 'Jewel'; 'Jasper' and 'Travis' are half-sibs or full-sibs; and 'Regal' and 'Southern Delite' are half-sibs or full-sibs. The number of duplicate phenotypes for nine isozymes among related clones was similar to that among unrelated clones for 'Centennial'/'Jewel' and 'Regal'/'Southern Delite' (duplicate phenotypes: 'Centennial'/'Jewel' = 5, average of 'Centennial' and 'Jewel' with all other cultivars = 4; 'Regal'/'Southern Delite' = 4, average 'Regal' and 'Southern Delite' with all others = 4), 'Jaspar' and 'Travis' had more duplicate phenotypes than the average of those cultivars with all others, seven vs. four, respectively.

Conclusions are not possible regarding isozymic determination of common ancestry since exact relationships among clones are not known.

In summary, the nine cultivars used in this study were separated by comparing banding patterns from five of nine polymorphic enzymes, indicating sufficient variability for separation of additional cultivars. The observed variability will, therefore, provide a method for precisely identifying cultivars for patent purposes.

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