Use of Isoenzyme Analysis to Characterize Raspberry Cultivars and Detect Cultivar Mislabeling

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Abstract. Isoenzyme staining was used to characterize 55 of 78 raspberry cultivars (*Rubus idaeus* L., *R. × neglectus* Peck, and *R. occidentalis* L.). Six enzymes were needed to achieve this characterization: isocitrate dehydrogenase, malate dehydrogenase, phosphoglucosomerase, phosphoglucosomutase, shikimic acid dehydrogenase, and arid triose phosphate isomerase. The 23 cultivars that were not uniquely characterized were grouped into eight groups of two and two groups of three and four. Two of these groups comprised black raspberry cultivars, all of which were similar isozymically. Isoenzymes could not distinguish between the cultivar Willamette and a spine-free mutant of the cultivar. Analysis of cultivars obtained from several sources revealed that raspberry cultivar mislabeling exists but is not very prevalent. Regular isoenzyme analysis of raspberry cultivars held by germplasm repositories, certified and other propagators, and breeders is both feasible and advisable for early detection of cultivar mislabeling.

The identification of raspberry cultivars based on morphological markers can be difficult, especially during vegetative stages. DNA minisatellite analysis was found to be useful for the characterization of blackberry and raspberry cultivars (Nybom and Hall, 1991; Nybom et al., 1989). However, this analysis is expensive to perform, requires specialized laboratory equipment, and is time-consuming. Isoenzyme staining of starch gels is a relatively simple, inexpensive technique that requires minimal equipment and time to perform. It was shown to be effective in the characterization of a few red and purple raspberry cultivars (Cousineau and Donnelly, 1989a, 1989b).

The objectives of this study were to determine if isoenzyme staining could be useful to distinguish many and diverse red, purple, and black raspberry cultivars and if staining with six isoenzymes rather than five, as originally reported (Cousineau and Donnelly, 1989a, 1989b), might improve the resolution of cultivars. Also, an indication of the extent that mislabeling occurs in raspberry cultivars was obtained by sampling

plants from various sources.

Sixty-eight red and yellow (*Rubus idaeus*), four purple (*R. × neglectus*), and six black (*R. occidentalis*) raspberry cultivars from various sources (Table 1) were maintained in the greenhouse at ambient temperatures and natural light. The cultivars Festival and Newburgh obtained from source 7 and Titan obtained from source 9 were maintained in the field.

Enzymes were extracted by grinding fresh, mature, fully expanded leaves in 350- μ l of extraction buffer (Cousineau and Donnelly, 1989a). Filter paper wicks (3 $\times$ 12 mm Whatman #1) were dipped into the leaf homogenates and used immediately, stored at -20C for 24 h, or stored at -70C for up to 1 month before use. Electrophoresis was performed using horizontal starch gels, prepared as described by Cousineau and Donnelly (1989b). Two gel and electrode buffer systems were used to separate the isoenzymes. The electrode buffer for system 1 was 0.065 M L-histidine and 0.015 to 0.016 M citric acid, pH 5.7 (Cardy et al., 1981).

The gel buffer was made by adding 42 ml of electrode buffer to 258 ml of water. This system was used to run malate dehydrogenase (MDH, EC 1.1.1.37), phosphoglucosomerase (PGI, EC 5.3.1.9), and shikimic acid dehydrogenase (SKDH, EC 1.1.1.25). The electrode buffer for system 2 consisted of 0.135 M Tris, 0.043 M citric acid, and 0.0012 M Na₂EDTA, pH 7.1 (modified from Ayala et al., 1973). The gel buffer was 0.009 M Tris, 0.003 M citric acid, and 0.0012 M Na₂EDTA, pH 7.1. This system was used to run isocitrate dehydrogenase (IDH, EC 1.1.1.42), phosphoglucosomutase (PGM, EC 2.7.5.1), and triose phosphate isomerase (TPI, EC 5.3.1.1). The gels were electrophoresed in the cold (4C) at 25 and 40 mA for systems 1 and 2, respectively, until the tracking dye had migrated 10 to 12 cm. The longer migration distance was needed to get an adequate separation of the bands for PGM.

After electrophoresis, the gels were sliced horizontally and stained for the appropriate enzyme as described in Cousineau and Donnelly (1989b) with the following modifications. For IDH, 115 mg sodium isocitrate and 8 mg NADP were used instead of 55 and 5 mg, respectively. For MDH, 120 mg dl-malic acid was used instead of 100 mg. For both PGI and PGM, the NADP was replaced with 8 mg NAD. The amount of shikimic acid used to stain for SKDH was increased from 40 to 90 mg per staining reaction.

Fifty-five of 78 cultivars were separated using all six isoenzymes (Table 1, Figs. 1-3). The three most useful enzymes were MDH, PGI, and PGM. By combining data obtained for these enzymes, 30 cultivars could be characterized. TPI, IDH, and SKDH resulted in the separation of 12, nine, and four additional cultivars, respectively, as the results for each enzyme were added sequentially to those of the other isoenzymes. The remaining cultivars were placed into eight groups of two and two groups of three and four inseparable cultivars. Contained within this last group were 'Willamette' and a spineless mutant of this cultivar (S-F Willamette) (Jennings and Brydon, 1990). Cultivars derived by mutation are not expected to vary in their isoenzyme patterns unless the mutation directly affects enzyme mobility (Wendel and Parks, 1983). The PGM pattern

Fig. 1. Interpretative drawing of malate dehydrogenase (MDH), phosphoglucosomerase (PGI), and shikimic acid dehydrogenase (SKDH) isoenzyme patterns run on gel and electrode buffer system 1 (histidine-citric acid, pH 5.7). The letters correspond to those listed in Table 1.

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E, not B as previously published (Cousineau and Donnelly, 1989a), was obtained for 'Boyne'. Better resolution of the band that differentiates patterns B and E (Rf 0.44) was obtained by running the gel overnight.

The level of isoenzyme variation in the six black raspberry cultivars tested was low, which suggests that isoenzymes may not be sufficiently sensitive for the characterization of these cultivars. Nybom and Schaal (1990) found a high level of variation in natural populations of *R. occidentalis* using mini-satellite DNA analysis. This technique may be useful for characterizing black raspberry cultivars.

The following three cultivars exhibited unique bands absent in other plants tested: 'Autumn Bliss' with a band at Rf 0.16 for MDH; 'Prestige' with a band at Rf 0.70 for PGM; and 'Pathfinder' (source 5) with bands at Rf 0.20, 0.46, and 0.50 for MDH. These unique bands may be due to the presence of *R. arcticus* L. and *R. pungens oldhamii* Mig. in the derivation of 'Autumn Bliss' and 'Prestige', respectively, and to the use of a third-generation seedling derived from wild *R. strigosus* plants as one of the parents of 'Pathfinder'.

Designated cultivars from several sources were assayed, whenever possible, to determine the extent of cultivar mixing. Six cultivars were apparently not true-to-type (refer to Table 1 for source codes): 'Carnival' (10), 'Newburgh' (10), 'Nova' (1), 'Pathfinder' (1; two accessions designated #111 and #392), 'Royalty' (10), and 'Southland' (1). Erroneous 'Carnival', 'Newburgh', and 'Royalty' isoenzyme patterns were originally published in Cousineau and Donnelly (1989a, 1989b) because of mislabeling. The source of these erroneously labeled cultivars is unknown; because representative plants have since been assayed from verified sources, the corrected enzyme patterns are those shown in Table 1. When both parental genotypes of a cultivar were available, the isoenzyme patterns were converted to genotypes by assigning an allelic designation to each homodimeric band. The 12 pedigrees tested proved to be consistent (Table 2) and confirmed that 'Nova' and 'Southland', obtained from the National Clonal Germplasm Repository, Corvallis, Ore., were actually mislabeled plants. The Repository has since eliminated these incorrectly labeled plants and is replacing them with plants from verified sources (Humme, personal communication, 1992). Three distinct isoenzyme patterns were demonstrated for the 'Pathfinder' plants obtained from three sources. Unique MDH bands at Rf 0.46 and 0.50, present in all three plants (patterns L, N, and M for plants from sources 5, 1 #111, and 1 #392, respectively), suggested that they were seedlings of one another. Based on isoenzyme patterns, the only 'Pathfinder' plant that could have given rise to the other two was from source 5. For this reason, the patterns for source 5 'Pathfinder' were tentatively placed in Table 1 and the identity of the plants from the other two sources was considered uncertain.

Isoenzyme analysis has proven to be use-

Table 1. Identification of raspberry cultivars from various sources using six isoenzymes. The letters represent the cultivar banding patterns for each enzyme.

Cultivar ^a	Fruit color ^b	Source ^c	Isoenzyme and pattern					
			MDH	PGM	PGI	TPI	IDH	SKDH
Scepter ^A	R	1, 4	A	A	A	A	A	B
Sentry ^A	R	1	A	A	A	A	A	B
Madawaska	R	1, 2, 3	A	A	B	B	A	A
Carnival ^B	R	1, 2	A	A	C	C	A	A
Chilliwick ^B	R	2, 3	A	A	C	C	A	A
Malling Exploit	R	1	A	C	A	B	A	A
Titan	R	4, 9	A	C	A	B	A	B
Comox ^C	R	2, 3	A	C	B	C	A	A
Gatineau ^C	R	2	A	C	B	C	A	A
Skeena	R	3	A	C	B	D	2	A
Algonquin	R	2, 3	A	C	C	A	A	A
Cuthbert	R	1	A	D	2	A	A	A
Festival	R	1, 2, 7	A	D	A	C	2	A
Viking	R	1	A	D	A	C	C	A
Puyallup	R	1	A	D	B	A	A	A
Colossus	R	1	A	E	B	A	A	A
Citadel	R	1, 4	A	E	E	A	A	A
Marcy	R	1, 2, 3	B	C	B	A	A	A
Canby	R	1	B	C	B	C	A	A
Nootka	R	2, 3	B	C	E	A	A	A
Goldenwest	Y	1	B	D	A	A	A	A
Indian Summer	R	1	B	D	D	A	C	A
Boyne	R	1, 2, 6	B	E	A	A	A	A
Augustred	R	1	B	E	A	A	C	A
Killarney	R	1, 2	B	E	A	A	D	A
Malling Leo	R	1	B	G	B	A	A	A
Tahoma	R	1	C	A	A	A	A	C
Chief	R	1		A	A	A	C	A
Latham	R	1, 2, 4		A	A	A	C	C
Summit	R	1, 3	C	B	A	C	A	B
Sunrise	R	1	C	B	D	A	A	D
Avonu	R	2	C	C	A	B	A	A
Meeker ^D	R	3	C	C	A	B	A	A
S-F Willamette ^D	R	4	C	C	A	B	A	A
Willamette ^D	R	2, 3	C	C	A	B	A	A
Trent	R	1	C	C	B	A	A	A
Creston ^E	R	1, 3	C	C	B	C	A	A
Rideau ^E	R	1	C	C	B	C	A	A
Chilcotin	R	2, 3	C	C	D	B	A	A
Sumner	R	3	C	D	A	A	A	A
Fairview	R	1	C	D	A	C	A	A
Milton ^F	R	1	C	D	B	A	A	A
Washington ^F	R	1	C	D	B	A	A	A
Southland	R	4	C	E	D	A	A	A
Norna	R	1	C	G	B	C	A	A
Comet	R	2, 3	D	A	A	B	A	A
Redwing ^G	R	8	D	C	A	A	A	A
Tulameen ^G	R	2, 3	D	C	A	A	A	A
Malling Delight	R	1	D	C	B	A	A	A
Norfolk Giant	R	1	D	C	B	C	A	C
Glen Garry	R	4	D	C	C	A	A	A
Glen Prosen	R	1, 2, 4	D	C	C	C	A	A
Glen Moy	R	2	D	C	E	A	A	A
Heritage	R	1, 2	D	D	A	A	C	A
Thames	R	1	D	G	B	C	A	A
Newman	R	1	E	A	C	C	C	A
Matsqui	R	2, 3	E	A	E	C	A	A
Anelma ^H	R	2	E	D	A	E	A	A
Newburgh ^H	R	1, 2, 7	E	D	A	E	A	A
Reveille	R	1	E	D	C	A	A	A
Haida	R	2, 3	E	D	C	C	A	A
Bonanza	R	2, 5	F	A	A	A	A	C
Liberty	R	1	F	A	A	A	C	B
Nova	R	2, 5, 6		B	D	A	A	A
Brandywine	P	1, 10	E	D	C	A	B	A
Amethyst	P	1	G	E	E	A	B	A
Royalty	P	1, 4	G	F	A	B	A	
Success	P	1	H	B	C	A	B	2
Dundee	B	4	H	B	F	A	B	A
Allen ^I	B	1	H	B	F	A	E	A
Blackhawk ^I	B	1	H	B	F	A	E	A
Munger ^I	B	1, 10	H	B	F	A	E	A
Huron ^I	B	1	H	H	F	A	E	A

Continued on next page.

Table 1. Continued.

Cultivar ^c	Fruit color ^c	Source ^a	Isoenzyme and pattern					
			MDH	PGM	PGI	TPI	IDH	SKDH
Jewel ^b	B	1	H	H	F	A	E	A
Phoenix	R	1	I	B	D	A	A	A
Prestige	R	1	J	J	A	A	A	A
Autumn Bliss	R	1	K	A	C	A	A	A
Pathfinder ^e	R	5	L	I	D	A	A	A

^aCultivars with identical letters cannot be separated using the above isoenzymes.

^bB = black, P = purple, R = red, Y = yellow.

^c1 = National Clonal Germplasm Repository, 33447 Peoria Rd., Corvallis, OR 97333. 2 = Plant Gene Resources of Canada, Agriculture Canada, Smithfield Experimental Farm, P.O. Box 340, Trenton, Ontario K8V 5R5.3 = Hugh A. Daubeney, Agriculture Canada Research Station, 6660 N.W. Marine Dr., Vancouver, B.C. V6T 1X2. 4 = Harry J. Swartz, Dept. of Horticulture, Univ. of Maryland, College Park, MD 20742. 5 = Helene Rousseau, Service de Phytotechnie du Québec, Complexe Scientifique, 2700 Rue Einstein, Ste-Foy, Quebec G1P 3W8, Canada. 6 = Gilbert Allen Nurseries, R.R. #2, Centreville, N.S. B0P 1J0. 7 = Roger Labrecque, 123 Rte. 218 est, Saint-Charles, Comté Bellechasse, G0R 2T0, Canada. 8 = Adam Dale, Horticulture Research Inst. of Ontario, Box 587, Simcoe, Ontario N3Y 4N5, Canada. 9 = Nourse Farms, Box 485 RFD, South Deerfield, MA 01373. 10 = Source unknown.

^eIdentity is tentative.

Fig. 2. Interpretative drawing of isocitrate dehydrogenase (IDH), phosphoglucumutase (PGM), and triose phosphate isomerase (TPI) isoenzyme patterns run on gel and electrode buffer system 2 (Tris-citric acid, pH 7.1). The letters correspond to those listed in Table 1.

Fig. 3. Phosphoglucumutase starch gel of raspberry cultivars (left to right) Latham, Phoenix, Glen Moy, Newburgh, Killarney, Royalty, Jewel, Thames, Pathfinder, and Prestige. Origin at the bottom, anode at the top.

ful for raspberry cultivar identification. Seventy percent of the cultivars analyzed were uniquely characterized using six isoen-

zymes. More difficult and expensive techniques, such as minisatellite DNA analysis (Nyblom et al., 1989), may be needed to sep-

Table 2. Cultivars for which the isoenzyme patterns of both parents have been determined.

Cultivar	Parentage
Algonquin	Haida × Canby
Bonanza	Sunrise × Washington
Boyne	Chief × Indian Summer
Chief	Latham × Latham
Chilcotin	Summer × Newburgh
Killarney	Chief × Indian summer
Matsqui	Summer × Carnival
Meeker	Willamette × Cuthbert
Nootka	Carnival × Willamette
Nova	Southland × Boyne
Sumner	Washington × Tahoma
Tulameen	Nootka × Glen Prosen

arate cultivars that are similar isozymically. Although raspberry cultivar mislabeling was not very prevalent in this study, it does exist. Propagators and breeders should be careful in choosing only plants obtained from verified sources. Regular monitoring of raspberry cultivars using isoenzymes is feasible for germplasm repositories, certified and other propagators, and breeders and is recommended to detect mislabeling at an early stage. In addition, isoenzyme patterns for new cultivars should be described in the original release publication and in patent applications. This step would greatly assist in characterizing the original genotype released and promote its future identification.

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