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J. AMER. Soc. HORT. SCI. 110(4):509–515. 1985. Identification of Apple Cultivars by Isozyme Phenotypes

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Abstract. Fifty-four apple (*Malus domestica* Borkh.) cultivars were characterized electrophoretically using 6 isozyme systems. Intracultivar variation in isozyme phenotype was not observed, whereas intercultivar polymorphism was sufficient to permit reliable and unambiguous identification of nearly every cultivar. The most useful isozyme systems for distinguishing among the cultivars were 6-phosphogluconate dehydrogenase and aspartate aminotransferase. Sports could not be distinguished from the original cultivar. The genetic basic of several polymorphisms was known, enabling the comparison of the isozyme genotype observed in a hybrid with that predicted on the basis of parental genotypes. The 6-phosphogluconate dehydrogenase genotype of 'Spartan' indicated that 'Yellow Newtown' may not have been the paternal parent.

An important aspect of the apple industry, particularly to nurserymen and growers, is reliable verification of the cultivar being grown. The sale of fruit trees and planting of an orchard represent major investments of time and money; yet the identity of the cultivar being planted usually is not verified until fruit is produced several years later. Although many cultivars can be distinguished as budlings on the basis of morphological characters, the necessary expertise is not widely available, nor will it become so in the foreseeable future. Isozyme analysis offers a possible alternative method for cultivar identification which has been successfully applied to several crops (1, 2, 3, 10, 14, 16). Should such techniques prove applicable to the apple, they would provide a reliable method for the verification of cultivar identity which could be performed on a routine basis by laboratory technicians using relatively simple and inexpensive procedures.

In order to use isozyme techniques successfully for cultivar identification, a particular crop must exhibit significant levels of isozyme polymorphism among the cultivars; yet each cultivar should be predominantly monomorphic. In addition, well-resolved isozyme phenotypes must be obtainable from a convenient tissue such as leaf or stem, and such phenotypes should exhibit little or no variability during development or under diverse environmental conditions. A priori apple would appear to be a crop amenable to analysis using isozyme techniques. The clonal character of apple cultivars indicates that intracultivar genetic variation should be minimal. The wide crosses that have been made as part of apple breeding programs, and the outcrossing nature of the species, may be reflected by a high level of isozyme polymorphism among the extant cultivars. Previous studies on several isozyme systems (5, 9, 13) have demonstrated that such polymorphism does exist in apple. These favorable preliminary findings encouraged us to proceed with a detailed investigation of isozyme systems which have been genetically and biochemically characterized in other plant species. Isozyme systems were selected which generally exhibit little or no environmentally induced variation, show good activity in leaf ex-

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tracts, and can be used as genetic tools for studying pedigrees and marking other genes of interest.

Materials and Methods

The cultivars used in this study are part of the extensive apple germplasm collection at the NYSAES in Geneva, N.Y. Most leaf samples were collected shortly after bud break in the spring; however, to test the stability of the isozyme phenotype over time, many cultivars also were sampled by 1) collecting twigs in late winter and forcing these in the greenhouse, and 2) collecting leaves in early summer after most of the leaves had expanded. In addition, pollen and petal tissue samples were taken from several cultivars, and ripe fruit of 'Golden Delicious' also was tested. For most of the analyses, about 1 cm² of young leaf tissue was crushed throughly in 0.3 ml of 50 mM Tris maleate pH 8.5 containing 20% glycerol (w/v), 10% soluble polyvinylprolidone (PVP-40), 0.5% Triton X-100, and 14 mM 2-mercaptoethanol. Starch gels were prepared and electrophoresis conducted as described by Weeden (16). Two buffer systems were used for electrophoresis: a tris citrate/lithium borate system at pH 8.1 (11) and a histidine buffer at pH 6.5 (4). Slices from the tris citrate/lithium borate gel were assayed for aspartate aminotransferase (AAT; EC 2.6.1.1), glucose phosphate isomerase (GPI; EC 5.3.1.9), and triose phosphate isomerase (TPI, EC 5.3.1.1). Diaphorase (DIAP; EC 1.6.4.3) 6phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44) and NADP specific isocitrate dehydrogenase (IDH; EC 1.1.1.42) were assayed on slices from the histidine gel. The assay conditions have been described previously (5).

Results

Considerable polymorphism in isozyme phenotype was observed among the cultivars examined. The genetic basis of the 6PGD, AAT, GPI, and IDH phenotypes has been discussed previously (5) and was confirmed further by progeny tests (Weeden and Lamb, in preparation). Nine cases of apparent allozymic polymorphism were identified among the cultivars, including 2 each in the isozyme systems 6PGD, AAT, and IDH, and one each in the systems GPI, TPI, and DIAP (Table 1). The structures of the isozymes of 6PGD, AAT, GPI, TPI, and IDH have been shown to be dimeric in the considerable number of other plant species examined (see reference 6 for review). The respective apple isozymes exhibited phenotypes characteristic of this dimeric structure (Fig. 1).

Especially diagnostic were the triple-banded phenotypes observed in many cultivars. The 3 bands are produced because monomeric subunits ("a" and "b") can combine to form active enzymes in 3 ways (aa, ab, and bb). The "ab" heterodimeric form nearly always exhibits a mobility equal to the average of the mobilities of the "aa" and "bb" bands. Thus, for descriptive purposes, it is only necessary to give the positions of the bands in homozygous phenotypes, heterozygous individuals always being assumed to exhibit both homodimeric bands plus an intermediate heterodimeric form. The relative positions of the homodimeric forms in the isozyme systems investigated are presented in Fig. 2.

Isozyme phenotypes

6-Phosphogluconate dehydrogenase. Two sets of 6PDG isozymes, designated 6PGD-1 and 6PGD-2 (5), were present in apple leaf extracts (Fig. 1). Four homodimeric 6PGD-1 bands were observed, and these bands were labelled aa, bb, cc, and dd in order of decreasing relative mobility (Fig 2). When any 2 of the homodimeric bands were present in an extract, a third band with an intermediate mobility also was present. In contrast, no additional bands were seen when only 1 homodimer was present. We interpreted this result to indicate that heterodimeric enzymes would form between any 2 6PGD-1 subunits.

The aa, bb, and dd homodimers of 6PGD-1 were relatively common among the cultivars examined and are easily distinguishable on the basis of their mobilities. The cc homodimer had a much more limited distribution, being restricted to 'Delicious', 'Empire', and 'Fuji', the latter 2 cultivars each having 'Delicious' as one parent. The cc homodimer exhibits a mobility only slightly slower than the bb homodimer and could be mistaken for the latter if standards were not run on the same gel.

Three 6PGD-2 phenotypes were easily distinguished. The most common phenotype consisted of a wide band of activity intermediate between the 6PGD-1 bands and the origin. Because of its high frequency, we have designated this phenotype the "normal" (n) one in apples. Some heterogeneity may be present within this phenotype; however, the variability was not reproducible in our hands and, therefore, was not useful for cultivar identification purposes. The other 2 phenotypes which were distinguishable from "normal" were called "slow" and "fast". (Fig. 1). Both phenotypes reflected heterozygosity at 6PGD-2, the "slow" exhibiting 2 additional bands of activity with slower mobilities than the "n" band whereas the fast phenotype displays the "n" plus 2 additional bands with faster mobilities. In the slow phenotype, the least anodal band produces a very faint activity stain so that this phenotype often appears to be a doublet. The fast phenotype was observed only in the cultivar, 'Wagener'.

Aspartate aminotransferase. The 2 loci specifying easily observable AAT isozymes were both highly polymorphic in apple. The terminology used to identify each allele and activity band parallels that described for the 6PGD system and is presented in Fig. 2. A 3rd area of AAT activity occasionally could be observed near the origin, but the bands were very faint and appeared to be similar in most cultivars examined. In contrast to the 6PGD isozymes, the mobilities of the AAT-1 homodimers overlapped those of the AAT-2 set (Fig. 2) making interpretation of some phenotypes difficult. However, most cultivars exhibited clearly resolved and reproducible AAT phenotypes which proved to be exceedingly useful for distinguishing among the cultivars.

Four alleles were responsible for the polymorphism observed in AAT-1 bands. The 3 more anodal homodimers, aa, bb, and cc, were easily distinguishable from each other and from the AAT-2 homodimers (Fig. 1). However, the most slowly migrating AAT-1 homodimer, dd, possessed a mobility similar to the AAT-2 bb homodimer so that AAT-1 dd and also the heterodimer, cd, occasionally would obscure or be obscured by AAT-2 bands. The AAT-1 dd homodimer was the least common of the 4, but helped distinguish 'McIntosh' and several of its derivatives.

The most common AAT-2 homodimers were aa and cc, the bb homodimer being limited to 'Jonathan' and a few other cultivars. All 3 homodimers were easily distinguishable from each other the bc phenotype could be confused with that of cc because the bb homodimer in heterozygous cultivars was faint, and the cc band in homozygous plants often showed an anodal shadow. The aa, ab, and bb bands but were subject to interference from the AAT-1 dd homodimer. Often this interference could be reduced by using petal extracts in which the AAT-1 forms exhibit reduced levels of expression.

Table 1. Isozyme phenotype for 9 polymorphic isozymes in 54 apple cultivars.

Cultivar $6PGD-1$ $6PGD-2$ $AAT-1$ $AAT-2$ $GPL-2$ $DPL-2$ $DIAP-2$ $DIAP-2$ $DIAP-3$ </th <th></th> <th></th> <th></th> <th></th> <th>1</th> <th>sozyme</th> <th colspan="4">yme</th>					1	sozyme	yme			
Ben Davis ad nn cc ac cc ab ab bb ab Calville Blanc bd nn cc ac cc aa ab bb ab Carland bd nn bc cc ac aa ab aa Early Corland bb nn cc ac aa ab bb< aa Early McInosh ab nn cd cc ac aa ab bb< aa Freedom cd nn bc ac ac aa ab bb aa Goltser 67 ab nn ab cc aa ab bb bb aa Goltser 67 ab nn ac cc aa ab bb aa Goltser 67 ab nn ac	Cultivar	6PGD-1	6PGD-2	AAT-1	AAT-2	GPI-2	TPI-2	DIAP-2	IDH-1	IDH-2
Calville BlancbdmmaacccccababababCrimson Beautybdnnbcccccaabbabaa(Red) DeliciousacnnbcccccaaabbbaaEarly CortlandbbnnccacacaaabbbaaEarly McIntoshabnnccacacaaabbbaaFiresideddnnacacccaaabbbaaFiresideddnnacacccaaabbbaaFiresideddnnbbaaccaaabbbaaGalia BeauryddnsbbaaacccaaabbbaaGolenva EarlyadnnacccccababbbaaGolenva EarlyadnnacccccababbbaaGolenva EarlyadnnacccccababbbaaGrimes GoldenadnnacccccababbbaaGrimes GoldenadnnacccccababbbaaGrimes GoldenadnnacccccaaababaaJarey Mithadnnaccccc <td>Ben Davis</td> <td>ad</td> <td>nn</td> <td>cc</td> <td>ac</td> <td>cc</td> <td>ab</td> <td>ab</td> <td>bb</td> <td>ab</td>	Ben Davis	ad	nn	cc	ac	cc	ab	ab	bb	ab
CordiandbdnnccacccacababababCrimson BeautybdnnbcccccaaababaaRed) DeliciousacnnbcncccacaaabbbaaEarly McInoshabnnccacacaaabbbaaEmpirebcnnccccacaaabbbaaFreedomadnnacccccaaabbbaaFreidoddnnbbacccaaabbbaaGalia BeautyddnnabccacaaabbbabGolser 69abnnccacccaaabbbaaGolden DeliciousddnnaaccccababbbaaGranv SmithaannacccccabbbbaaaGranv SmithaannacccccabbbbaaaHoncygoldbdnnacacccaaabbbaaJerseymacadnnacacccaaabbbaaGranv SmithaannacacccaaabbbaaJonzdolbdnnacacccaaabbb </td <td>Calville Blanc</td> <td>bd</td> <td>nn</td> <td>aa</td> <td>сс</td> <td>сс</td> <td>aa</td> <td>ab</td> <td>bb</td> <td>aa</td>	Calville Blanc	bd	nn	aa	сс	сс	aa	ab	bb	aa
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$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	Crimson Beauty	bd	nn	bc	сс	cc	aa	bb	ab	aa
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Early McIntosh ab nn ccl cc ac aa ab bb aa a Freedom bc nn cc cc cc aa aa ab bb aa a Freside dd nn ac ac cc aa ab bb aa a Freside dd nn bc cc ac aa ab bb aa bf ad a bred ad nn bc cc ac aa ab bb aa bf ad ab	Early Cortland	bb	nn	cc	ac	ac	aa	ab	bb	aa
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	Jerseymac	ad	nn	bd	cc	cc	aa	aa	bb	aa
Jonagoldbddnnaacbcccccaabbbb ab^2 aaaJonamacbbnncdacccaaabbbaaJonathanbbnncdacccaaabbbaaJulyredaannbcacacacaaabaaLibertyaannccaaabbbaaaaLodiabnnccccaaabbbaaMacfreebdnsbdbbccaaabbbaaMacounadnnccccaaabbbaaMelnoshabnnbcacccaaabbbaaMelnoseabnnbcacccaaabbbaaMonroebdnnbcacccaaabbbaaMotgomerybdnnccccaaababaaNovamacaannbcccccaabbaaNovamacadnnacacccaabbabNovamacadnnacacccaabbabNovamacadnnacacccaabbabNovamacadnnacacccaaabbbaa <td< td=""><td>Jonadel</td><td>ab</td><td>nn</td><td>bc</td><td>bc</td><td>cc</td><td>aa</td><td>bb</td><td>ab</td><td>aa</td></td<>	Jonadel	ab	nn	bc	bc	cc	aa	bb	ab	aa
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$\begin{array}{ccccccc} Montgomery & bd & nn & cc & cc & ad & ab & aa & bb & aa \\ Mutsu & ddd & nn & aac & ccc & ccc & aab & ab^2 & bbb & aa \\ Nova Easygro & ad & nn & cc & ac & cc & aa & bb & bb & ab \\ Novamac & aa & nn & bc & cc & cc & aa & bb & bb & aa \\ Prima & dd & nn & ac & ac & cc & aa & ab & bb & aa \\ Priscilla & ab & nn & ac & ac & cc & aa & ab & bb & aa \\ Quinte & ad & nn & bb & cc & cc & aa & ab & bb & aa \\ Red Spy & bb & nn & aa & cc & cc & ab & ab & bb & aa \\ Rhode Island Greening & ddd & nn & aa^y & bbb & acc & aab & ab^z & bbb & aa \\ Sir Prize & ddd & ns^z & ac^z & ccc & cc & ab & ab & bb & aa \\ Sir Prize & ddd & ns^z & ac^z & ccc & cc & ab & ab & bb & aa \\ Sig Prize & ddd & ns & ab & ab & ab & ab & ab & ab \\ Sir Prize & ddd & ns & ab & ac & ccc & ab & ab & ab & ab \\ Sir Prize & ddd & ns & ac^z & ccc & ccc & ab & ab & ab & ab \\ Sir Prize & ddd & ns & ac^z & ccc & ccc & ab & ab & ab & ab \\ Sir Prize & ddd & ns & ac^z & ccc & ccc & ab & ab & ab & ab \\ Sir Prize & ddd & ns & ac^z & ccc & ccc & ab & ab & ab & ab \\ Sir Prize & ddd & ns & ac^z & ccc & ccc & ab & ab & ab & ab \\ Sir Prize & ddd & ns & ccc & ccc & ab & ab & ab & ab \\ Sir Prize & ddd & ns & ac^z & ccc & ccc & ab & ab & ab & ab \\ Sir Prize & aa & nn & bc & cc & ccc & aa & ab & ab & ab \\ Sir Prize & aa & nn & cc & ccc & ccc & aa & ab & bbb & aa \\ Spartan & aa & nn & ccc & ccc & ab & ab & bbb & aa \\ Spigold & bbd & nn & aa & ccc & ccc & ab & ab & bbb & aa \\ Wagener & ad & fn & ad & cc & cc & cc & aa & ab & bb & aa \\ Yellow Newton & dd & nn & cc & cc & cc & aa & ab & bb & aa \\ Yellow Transparent & aa & nn & cc & cc & cc & aa & ab & bb & aa \\ Yellow Transparent & aa & nn & cc & cc & cc & aa & ab & bb & aa \\ Yellow Transparent & aa & nn & cc & cc & cc & aa & ab & bb & aa \\ Yellow Transparent & aa & nn & cc & cc & cc & aa & ab & bb & aa \\ Yellow Transparent & aa & nn & cc & cc & cc & aa & ab & bb & aa \\ Yellow Transparent & aa & nn & cc & cc & cc & aa & ab & bb & aa \\ Yellow Transparent & ab & bb & aa & ab & bb & aa \\ Yellow Transparent & dd & nn & cc & cc $	Monroe	bd	nn	bc	aa	cc	aa	ab	bb	aa
Mutsudddnnaacccccccaabab²bbbaaaNova EasygroadnnccacccaabbbbabNovamacaannbcccccaabbbbaaPrimaddnnacacccaaabbbaaPriscillaabnnacacccaaabbbaaQuinteadnnaaccccaaabbbaaRed SpybbnnaaccccababbbaaRhode Island Greeningdddnnaa ^y bbbaccaabab ^z bbbaa ^z Rome BeautydddnsbbaaaccacabababaaSir Prizedddns ^z ac ^z cccccaaabbbaaSigratanaannaaccccaaababaaWageneradfnadccccaaababaaWageneradfnadccccaaaaabaaYellow NewtonddnnccccccaaaaabaaYellow Transparentaannaa ^y ccccaaabbbaaYork Imperialddnnaa ^y ccccac </td <td>Montgomery</td> <td>bd</td> <td>nn</td> <td>cc</td> <td>cc</td> <td>ad</td> <td>ab</td> <td>aa</td> <td>bb</td> <td>aa</td>	Montgomery	bd	nn	cc	cc	ad	ab	aa	bb	aa
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Red Spy Rhode Island Greening Rome Beautybb ddnn nn aayaa abb bbcc acc aab aacc abb abab bbb aa acab abbb aa aaSir Prize Spartan Spigolddddns adns bbacc aa aaab aa aaaa abbb aa aaaa abbb aa aaSir Prize Spartan Spigolddddns acacc accccc cc cc ccaaa abbbb aa abaaa ab abbbb aa aa abWagener Wealthyad bdfn nn abad accc cc cc cc aa aaab abbb aa abYellow Newton York Imperialdd dd nn aa aa aa ddnn aa aa cccc cc cc cc aa aa aa aaaa ab ab aa aa aa aa aa aa acab ab aa <b< td=""><td>Quinte</td><td>ad</td><td>nn</td><td>bb</td><td>сс</td><td>сс</td><td>aa</td><td>aa</td><td>bb</td><td>aa</td></b<>	Quinte	ad	nn	bb	сс	сс	aa	aa	bb	aa
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SharthicaddnoaddccccaddaddaddSpartanaannbcccccaaabababSpigoldbbdnnaaaccccccaababbbbbaaaWageneradfnadccccababbbaaWealthybdnnabacccaaaaababYellow NewtonddnnccccaaaaabaaYellow TransparentaannccccacababbbaaYork Imperialddnnaa ^y ccccaaabbbaa	Sir Prize	ddd	nsz	acz	ccc	ccc	abb	aaa	bbb	aaa
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Yellow Transparent aa nn cc cc ac ab ab bb aa York Imperial dd nn aa ^y cc cc aa ab bb aa	Yellow Newton	dd	nn	CC.	CC	cc	ลล	аа	bb	aa
York Imperial dd nn aa ^y cc cc aa ab bb aa	Yellow Transparent	20 22	nn	cc	cc	ac	ab	ab	bb	aa
	York Imperial	dd	nn	aa ^y	сс	сс	aa	ab	bb	aa

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^zTriploid genotype uncertain.

^yMay have d allele, see discussion in text.

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Fig. 1. Variation in (A) 6PGD, (B) AAT, and (C) GPI isozymes in apple leaf extracts. The sequence of cultivars for 6 PGD is 1) 'Melba', 2) 'Cortland', 3) 'Wagener', 4) 'Yellow Transparent', 5) 'Sir Prize', 6) 'Monroe', 7) 'Melrose', and 8) 'Crimson Beauty'. The sequence for the AAT gel is 1) 'Mutsu', 2) 'Jersey Black', 3) 'Wealthy', 4) 'Haralson', 5) 'Jonathan', 6) 'Rhode Island Greening', 7) 'Fireside', and 8) 'Rhode Island Greening'. The sequence for the GPI gel is 1) 'Fireside', 2) 'Rhode Island Greening', 3) 'Macfree', 4) 'McIntosh', 5) 'Ben Davis', 6) 'Macoun', 7) 'Lodi', and 8) 'Red Spy'. Anode is towards the top of the figure.

Glucose phosphate isomerase. Two areas of activity were identified on gels assayed for GPI activity (Fig. 1). The more anodal zone, GPI-1, showed slight differences in band position. However, this variability was not consistent within a cultivar and may be an artifact of our extraction procedure. The polymorphism in GPI-2 was useful for cultivar identification with at least 3 homodimer forms being present. The most common phenotype contained only the cc homodimer. The ac heterozygous 3 banded phenotype was observed in several cultivars whereas the bc phenotype was limited to 'Freedom'. Several cultivars possessed an additional band of activity cathodal to





the cc homodimer. The genetic basis of this slow band has not been clarified. For the purpose of cultivar identification, we have labelled the slower band as the "dd" homodimer of GPI-2, necessitating that some cultivars be designated by 3 alleles at the GPI-2 locus (Table 1).

Triose phosphate isomerase. Two zones of enzyme activity also were observed on gel slices stained for TPI activity (Fig. 3). The more anodal band, TPI-1, was monomorphic among all cultivars tested. Two phenotypes were observed for TPI-2, the more common phenotype possessing only one slow migrating band labelled "bb". About one-fourth of the diploid cultivars exhibited a 3-banded TPI-2 phenotype, consisting of the aa and bb homodimers as well as the intermediate ab heterodimer (Fig. 3).

Diaphorase. Several zones of activity were observed when gel slices were assayed for DIAP activity. Most of these regions did not show variability within our sample of cultivars; however, the 2nd most anodal set of bands, labelled DIAP-2, was polymorphic (Fig. 3). Three DIAP-2 phenotypes were observed, a single-banded pattern and two 3-banded patterns. The triplebanded patterns could be distinguished by their relative intensities of the more anodal 2 bands. In phenotype aa, these 2 bands were of approximately equal intensities, while in the ab phenotype the most anodal band was very faint (Fig. 3). Phenotype bb consisted only of the most slowly migrating band of the triplet previously described.

Isocitrate dehydrogenase. The most common IDH phenotype among the cultivars was a triple-banded pattern containing the bb band of IDH-1, the aa band of IDH-2, and their intergenetic heterodimer 1b2a (Fig. 3). Three variations of this phenotype were present among the diploid cultivars sampled (Fig. 3). Several cultivars exhibited a triple-banded IDH-1 phenotype as well as a blurring of the 1b2a band. The most anodal band was designated the IDH-1 aa homodimer. The middle band of the triplet represented the ab heterodimer, and the blurring of the 1b2a band apparently was caused by the presence of an adjacent 1a2a heterodimer. Cultivars possessing the IDH-2 aa and bb homodimers produced a triplet of bands in the IDH-2 region and, again, a blurring of the 1b2a band. Those few cultivars ('Delicious', 'Fuji', 'Melrose', and 'Spartan') possessing all 4 homodimers gave 2 faint triplets, one in the IDH-1 region and



Fig. 3. Variation observed in the IDH, DIAP, and TPI isozyme systems. Sequence on IDH gel is 1) 'Cortland', 2) 'Wagener', 3) 'Yellow Transparent', 4) 'Sir Prize', 5) 'Monroe', 6) 'Melrose', and 7) 'Crimson Beauty'. Sequence on DIAP gel is 1) 'Freedom', 2) 'Honeygold', 3) 'Rome Beauty', and 4) 'McIntosh'. The TPI phenotypes are for 1) 'Jonathan' and 2) 'Prima'. Anode is towards the top of figure.

another in the IDH-2 region as well as a faint blur between the 2 triplets (Fig. 3).

Tissue distribution

In order to determine which apple tissues would be appropriate for isozyme analysis and if any of the isozymes exhibited tissue specific differences, we examined extracts from young and mature leaves from plants growing or forced into bud break in the greenhouse or from older trees in the orchards. Young leaves were superior to older leaves for clarity of the banding pattern, but no changes in band positions were apparent. Both petal and pollen tissues gave clear results for some isozymes, but others were faint or lacking. Fruit extracts generally gave low activity in all systems. However, the positions of the bands in all systems in petal and fruit extracts were identical to those observed in leaf extracts. No differences were observed between samples taken from nonbearing vs. mature plants, samples from greenhouse plants vs. field plants, or samples from spur leaves vs. other leaves.

Within-cultivar variability

At least 15 different trees of 'Freedom', 'Liberty', 'Mutsu', and 'Jonathan', as well as several trees of most other cultivars, were examined for their phenotype in all isozyme systems mentioned. Intracultivar polymorphisms were absent in all cases. Electrophoresis of extracts of chlorotic leaf spot virus-infected and virus-free trees of 'Liberty' gave identical results, indicating that the presence of this virus did not affect the isozyme pattern observed after electrophoresis. Identical isozyme phenotypes also were observed when the same cultivar was grown on different rootstocks. Thus, the normal 'Jonathan' isozyme phenotype was expressed in leaf tissue from trees growing on seeding, Malling (M) 7, M 26, and East Malling Long Ashton (EMLA) 9 rootstocks. Similarly, 'Mutsu' on seedling, Malling Merton (MM) 106 and interstem trees MM 111/M 9 showed identical isozymes.

Distinguishing among the cultivars

Of the isozyme systems examined, 6PDG and AAT were particularly useful in discriminating among cultivars. The separation of cultivars into 15 sets on the basis of their 6PGD-1 and -2 phenotypes is presented in Table 2. This grouping uniquely distinguishes the cultivars 'Wagener', 'Liberty', 'Delicious', 'Empire', 'Quinte', 'Spigold', and 'Jonagold'. The last 2 cultivars are triploid, and their unique 6PGD-1 phenotypes were discussed in a previous publication (5). Most of the remaining sets may be separated into their individual cultivars on the basis of AAT phenotypes. For instance, 'Honeygold' and 'Macfree' in set 3 may be differentiated because the former cultivar possesses the aa and cc homodimers of AAT-1 whereas the latter contains the bb and dd homodimers (Table 1). In set 4, 'Sir Prize' may be distinguished from 'Rome Beauty' and 'Gallia Beauty' again on the basis of AAT-1 phenotype, but the 2 latter cultivars could not be distinguished from each other by any of the isozyme systems.

Three of the cultivars of set 5 possess the bc phenotype at AAT-1 with the 4th cultivar, 'Yellow Transparent' having the

Table 2. Differentiation of apple cultivars on the basis of 6PGD phenotype.

Set	6PGD-2	6PGD-1	Cultiver
no.	Pnenotype	Pnenotype	Cultivar
1	fast	ad	Wagener
2	slow	aa	Liberty
3		bd	Honeygold, Macfree
4		dd	Gallia Beauty, Rome Beauty, Sir Prize
5	normal	aa	Gravenstein, Novamac, Spartan, Yellow
			Transparent
6		ab	Early McIntosh, Gloster 69, Jonadel,
			Julyred, Lodi, McIntosh, Melba,
			Melrose, Priscilla
7		ac	Delicious
8		ad	Ben Davis, Freedom, Geneva Early,
			Granny Smith, Grimes Golden,
			Jerseymac, Macoun, Nova Easygro,
			Quinte
9		bb	Early Cortland, Jonathan, Jonamac, Red
			Spy
10		bc	Empire
11		bd	Calville Blanc, Cortland, Crimson
			Beauty, Holiday, Idared, Jersey
			Black, Monroe, Montgomery, Wealthy
12		cd	Fuji
13		dd	Fireside, Golden Delicious, Mutsu,
			Prima, Rhode Island Greening, Yellow
			Newtown, York Imperial
14		bbd	Spigold
15		bdd	Jonagold

cc phenotype. Comparison of the AAT-2 phenotype separates 'Gravenstein' (ac) from 'Spartan' and 'Novamac'. The latter 2 cultivars differ in both their DIAP-2 and IDH-1 zymograms.

Set 6 is one of the larger groups of cultivars, but nearly all can be distinguished on the basis of AAT phenotypes. Only 2 pairs of cultivars ('Early McIntosh' and 'McIntosh'; 'Jonadel' and 'Melrose') possess identical AAT zymograms. However, the former pair differ in their GPI phenotypes, whereas the latter pair differ in DIAP-2 and IDH-2 patterns. Set 8 may be separated similarly when subjected to analysis of AAT phenotype, because the only 2 cultivars exhibiting the same AAT zymogram ('Ben Davis' and 'Nova Easygro') differ in their TPI and DIAP patterns.

All cultivars within set 9 and within the large set 11 possess different AAT phenotypes, leaving only set 13 to be considered. Again, most of cultivars in set 13 differ in their AAT phenotypes; however, in 2 cases, 'Rhode Island Greening' and 'York Imperial', the actual AAT-1 genotype is uncertain because of the overlap between the AAT-1 dd band and AAT-2 bands. The aa band was the major AAT-1 allozyme in extracts of 'Rhode Island Greening', but faint, more slowly migrating bands indicate that ad and dd allozymes also may be present but are obscured partially by the intense bb band of AAT-2. This complication does not interfere with the identification of 'Rhode Island Greening', for its AAT phenotype is still unique within the set. However, a similar problem with 'York Imperial' leaves open the possibility that it may be confused with 'Golden Delicious' if only 6PGD and AAT phenotypes are considered. Fortunately, these 2 cultivars differ in their TPI and DIAP patterns and can be distinguished easily using these additional systems. Finally, 'Prima' and 'Fireside' were not distinguishable using any of the isozyme systems reported here.

Attempts to differentiate sports using isozyme analysis were unsuccessful. The cultivar 'Vance Delicious' exhibited isozyme phenotypes identical to those in 'Delicious'. Similarly 'Boller McIntosh' could not be distinguished from 'McIntosh'. In a more thorough sampling, 7 sports of Jonathan (Freeborn Jonathan, Jonared, Smith Jonathan, Anderson Jonathan, Welday Jonathan, Conkle Jonathan, and Blackjon) were not separable by isozyme phenotype nor were 11 sports of Rome Beauty (Baillie Rome, Ohio Rome, Penrome, Carlson Rome, Ruby Rome, Australian Rome, Cox Rome, Conical Rome, Dalzell Rome, Hotle Rome, and Loop Striped Rome). Thus, it would appear that these genetically well-defined isozyme systems will not be useful for distinguishing sports of apple cultivars.

The list of cultivars tested for isozymes included several instances in which a cultivar and both its parental lines were characterized (Table 3). Such tests provided a useful internal check on our results, for the genotype of the derived cultivar should be extractable from the alleles exhibited in the parents. The phenotypes determined for 6PGD-1, AAT-1, and 2, TPI-2 and IDH were tentatively converted to genotypes by exchanging an allelic designation for each different homodimeric band. A cultivar possessing only one homodimer was presumed to be homozygous for that allele, whereas those showing 2 homodimers were categorized as heterozygous. In 16 of the 17 pedigrees available, the isozyme genotypes of the progeny were entirely consistent with the parental genotypes. The only pedigree showing an inconsistancy was that giving rise to 'Spartan'. 'Yellow Newtown', the male parent of 'Spartan', was homozygous for the d allele at 6PGD-1; yet this allele was not found in 'Spartan'.

Table 3. Cultivars examined for which the isozyme genotype of both parents has been determined.

Cultivar	Parentage
Cortland	Ben Davis x McIntosh
Empire	McIntosh x Delicious
Early McIntosh	Yellow Transparent x McIntosh
Early Cortland	Cortland x Lodi
Geneva Early	Quinte x Julyred
Holiday	Macoun x Jonathan
Idared	Jonathan x Wagener
Jonagold	Golden Delicious x Jonathan
Jonamac	McIntosh x Jonathan
Jonadel	Jonathan x Delicious
Lodi	Montgomery x Yellow Transparent
Melrose	Jonathan x Delicious
Monroe	Jonathan x Rome Beauty
Macoun	McIntosh x Jersey Black
Ouinte	Crimson Beauty x Red Melba
Spartan	McIntosh x Yellow Newtown
Spigold	Red Spy x Golden Delicious

Discussion

The results of this investigation of the isozyme phenotypes of apple cultivars demonstrate that enough genetic polymorphism exists within the 6 isozyme systems of 6PGD, AAT, GPI, TPI, DIAP, and IDH to identify conveniently and unambiguously most cultivars. The first 2 isozyme systems mentioned are especially useful for differentiating among cultivars because of the high level of genetic polymorphism in these systems. It is not obvious why these 2 systems should exhibit such considerable variability, for although polymorphism in 6PGD and AAT has been observed in many other species (3, 8, 12, 15), in most cases the amount of polymorphism has been quite modest. It may be that the loci specifying the isozymes are in regions of the genome that have particularly diverse origins within *Malus*. It would be of interest to trace the pedigrees of several cultivars in order to determine where each of the allozymes arose.

The finding that all sports of a cultivar which we examined possessed isozyme phenotypes identical to the original parent has significance not only for the limitations on applicability of the technique but also for the resolution of a long standing question regarding the origin of 'Gallia Beauty.' Hartman and Howlett (7) believed that this cultivar arose as a chance seedling, whereas others (French and Klein, personal correspondence) have expressed the opinion that 'Gallia' is a color sport of 'Rome Beauty'. Our studies show that 'Rome Beauty' and 'Gallia Beauty' exhibit identical phenotypes, not only in the isozyme systems reported here, but also in esterase, peroxidase, acid phosphatase, leucine aminopeptidase and malic enzyme, all of which are polymorphic among the apple cultivars tested (Weeden, unpublished data). Furthermore, the genotype of 'Rome Beauty' is heterozygous at a minimum of 3 isozyme loci (6PGD-2, GPI-2, and TPI-2). It is unlikely that this heterozygosity would have been maintained even if fertilized by 'Rome Beauty' pollen (probability of maintaining heterozygosity at 3 loci in such a cross = 0.135). We, therefore, believe that the evidence strongly favors the hypothesis that 'Gallia Beauty' is a sport of 'Rome Beauty'.

We were also unable to distinguish between 'Fireside' and 'Prima' using the seven isozyme systems. Preliminary data indicate that these cultivars can be separated on the basis of esterase and acid phosphatase phenotypes (as well as by fruit morphology); however, both of these systems are prone to environmental or developmental variation, and we are hesitant to use such systems for cultivar identification until their genetic basis and consistency of expression have been firmly established.

Our data confirmed previous results regarding the formation of triploid cultivars. Chyi and Weeden (5) demonstrated that intensity differences in the 6PGD-1, AAT-1, AAT-2 and TPI phenotypes indicated that the female parent contributed the unreduced gamete in the crosses forming 'Jonagold' and 'Spigold.' The DIAP phenotypes presented here are consistent with this conclusion. In addition, the results on the triploid cultivar 'Mutsu' indicate that 'Golden Delicious', the female parent, contributed the 2N gamete. Three other triploid cultivars, 'Gravenstein', 'Rhode Island Greening', and 'Sir Prize' also were characterized in this study. Obvious differences in the intensity of the homodimeric allozymes were evident in several systems (Table 1), indicating the presence of 2 doses of one allele and one dose of the 2nd in the genome. However, such skewing of the banding pattern was not observed in other cases where skewing would have been predicted based on the triploid nature of the cultivar. At present we have not determined the reason for these exceptions and are unable to define their triploid genotype.

Our data also are consistent with published pedigrees in 16 of 17 cases. The single exception, involving the cultivar 'Spartan', may be explained by postulating that a different cultivar was the actual paternal parent of 'Spartan'.

The occurrence of inconsistencies in pedigree relationships does not affect the practicality of using isozyme analysis to distinguish apple cultivars. We have found that within-cultivar polymorphism is lacking in the isozyme systems investigated, but considerable variation exists between cultivars. The isozyme genotype of a plant can be determined using a small quantity of young leaf tissue. Most cultivars may be distinguished on the basis of 2 isozyme systems, making the analysis economical and applicable in small laboratories as well as in large scale operations. The constraints imposed by loss of activity in some isozyme systems in older leaves, pollen and fruit, and the inability of our approach to distinguish sports do not appear to be serious limitations. Other isozyme systems such as acid phosphatase and esterase also exhibit polymorphism among the apple cultivars and may be useful for extending the technique beyond the present limitations.

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