IDENTIFICATION OF CRABAPPLE CULTIVARS BY ISOZYMES

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ABSTRACT-- Forty-five crabapple (*Malus spp.*) cultivars were evaluated for 16 isozyme systems by starch gel electrophoresis. Of the 16 systems evaluated, six were useful in separating cultivars. Enzyme systems used to distinguish among the cultivars included alcohol dehydrogenase, aspartate aminotransferase, malate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphoglucoisomerase, and shikimate dehydrogenase. Each enzyme system produced one well resolved polymorphic region except 6-phosphogluconate dehydrogenase which had two. Crabapple selections could be uniquely identified when all six enzymes were evaluated. Alcohol dehydrogenase had the greatest number of banding patterns diagnostic for cultivar separation.

INTRODUCTION

Crabapples are a popular group of ornamental trees that are morphologically very diverse. Nearly 700 crabapple types have been named (see Dirr, 1990) which constitute a gene pool derived from 20-30 species. Young nursery stock can be difficult to identify, parentage can often be unknown or confusing, and synonymy may exist among named crabapple cultivars. Starch gel electrophoresis is a procedure that has long been used in horticultural crops (Peirce and Brewbaker, 1973). Direct and objective chemical analyses are available to help identify cultivars including isozyme analysis. In that isozymes are largely unaffected by the environment, simply inherited, and relatively inexpensive to analyze, they have been used widely in cultivar verification (Tanksley and Orton, 1982; Arulsekar and Parfitt, 1986; Greer, et. al., 1993). While domestic apple has been evaluated for isozyme differences (Menendez, et. al., 1986; Samimy and Cummins, 1992; Weeden and Lamb, 1985), little if any work has been conducted with crabapples.

Given the polyploid origin of Malus spp. as postulated by Chevreau and Laurens (1985, 1987) and the multispecies complex from which they are derived, crabapples should possess sufficient diversity to be exploited by isozyme analyses. The objectives of this study were to determine if crabapple cultivars can be readily delineated by differences in isozyme patterns and to evaluate the suitability of bud and leaf tissue for analyses.

MATERIALS AND METHODS

Dormant bud tissue was collected from 45 crabapple selections from the collections at The Holden Arboretum during the Fall, 1992 and 1993. Samples were kept chilled (4° C) until the analyses could be completed and tissue was stored for no more than one week. Approximately 200mg of tissue was macerated using a glass pestle with ground glass in a chilled spot plate with about 650ul of extraction buffer (Wendel and Parks, 1982). Crude protein extracts were absorbed onto filter paper prepared wicks and loaded into 11.5 percent starch gels using standard procedures (Vallejos, 1983).

Initially, 16 enzymes were stained for after electrophoresis to identify enzymes that would be well resolved and polymorphic. The evaluated enzymes included: aconitase, aminoaspartate transferase (AAT), acid phosphatase, alcohol dehydrogenase (ADH), diaphorase, colorimetric esterase, glucose-6-phosphate dehydrogenase, isocitric dehydrogenase, leucine aminopeptidase, malate dehydrogenase (MDH), malic enzyme, peroxidase (PER), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), 6-phosphogluconate dehydrogenase (6-PGD), and shikimate dehydrogenase (SKDH).

Crude protein extracts of individual samples were loaded into each of three gels in an attempt to maximize the resolution of isozymes. The three buffer systems used included: a morpholine-citric acid system, pH 6.1 (Conkle, et. al., 1982); a lithium hydroxide-boric acid system, pH 8.1 (Cheliak and Pitel, 1984); and a histidine-Tris system, pH 7.0 (Cheliak and Pitel, 1984). These systems are referred to as MC, Cheliak-B, and Cheliak-H, respectively.

For electrophoresis, gels were placed in a refrigerator at 4° C and initially powered at 100-150 volts. Sample wicks were removed after 20 minutes and electrophoresis continued for 3-6 hrs at 300 volts. Gels were horizontally sliced and the top slice was discarded. Standard stain recipes were used to elucidate the isozymes (Wendel and Weeden, 1989; Cheliak and Pitel, 1984). While dormant buds were the predominant tissue analyzed, leaf tissue was also evaluated during the spring, 1994, to verify consistency between bud and leaf tissue. Enzymes from leaf tissue were extracted in a similar manner as for bud tissue.

After the initial screening, further work included six enzyme systems: AAT, ADH, MDH, 6-PGD, PGI, and SKDH. Best results were obtained when MDH and 6-PGD were stained for on the MC buffer system, AAT and PGI were stained for on the Cheliak-B system, and ADH and SKDH were stained for on the Cheliak-H system. All samples were run a minimum of three times to verify reproducability and the selection 'Hargozam' Harvest Gold™ was included on all gels as an internal reference.

RESULTS AND DISCUSSION

After the initial screening of 16 enzymes, eight were

sufficiently polymorphic. These eight polymorphic systems exhibited fair to good resolution and included AAT, ADH, MDH, PER, 6-PGD, PGI, PGM, and SRDH. However, PER and PGM were not included in this report because of unreliable or excessively complex banding patterns. PGM was very complex producing at least 10 different banding patterns which were difficult to interpret because of the closeness and complexity of the bands. In addition, banding expression for PGM was more intense and reliable from bud tissue when compared with leaf tissue.

Of the remaining enzymes, AAT, ADH, MDH, PGI, and SKDH each had one polymorphic region that was scorable, whereas 6-PGD had two polymorphic regions. Staining for AAT produced one darkly staining region that was scored and four banding patterns were observed (Figure 1). ADH produced a fast monomorphic region and a slower polymorphic region with seven different banding expressions (Figure 1). MDH produced three regions of activity including one polymorphic region with four observed phenotypes (Figure 1).

Staining for PGI exhibited two banding regions. The slower region was scored whereas the faster region could not be scored due to poor resolution. Crabapples were classed into one of five phenotypic types for PGI and banding patterns included single, triple and five band types (Figure 1). Occasionally, two closely migrating bands were observed at a relative mobility of 22 percent [(R_f) = 0.221. That is, "twin" bands were sometimes observed for phenotypes A, B, and C. For simplicity, we have grouped cultivars that produced these "twin" bands with cultivars that yielded a single at R_f 0.22. This should improve the ability to score PGI for other researchers.

Staining for 6-PGD produced two polymorphic regions. Cultivars were scored for both regions. The faster [anodal] region for 6-PGD (designated 6-PGD-1) and slower region (6-PGD-2) produced single and triple banding patterns consistent with a dimeric enzyme. Each polymorphic region produced four banding patterns (Figure 1). SKDH produced a single region of activity with excellent resolution and a single or double banding expression was observed which is consistent with a monomeric enzyme (Figure 1).

While enzyme banding patterns were assigned a letter designation corresponding to their phenotypic expression; ADH, 6-PGD-1, 6-PGD-2, and SKDH were well resolved and the patterns of banding could be explained by a simple genetic model. That is, ADH appears to be controlled by two loci of which one is polymorphic and controlled by at least three alleles. Two polymorphic loci apparently control the banding pattern of 6-PGD. Region 6-PGD-1 appears to be controlled by at least four alleles and 6-PGD-2 by at least three alleles. SKDH appears to be controlled by one polymorphic loci and at least three alleles. The other enzyme systems were not so straightforward as to imply a genetic model without progeny testing. For the enzymes reported here, banding patterns were similar in quality and quantity regardless of the tissue that was utilized.

The 45 crabapple selections evaluated in this study were sufficiently different at the isozyme level that nearly all could

be uniquely distinguished (Table 1). Three cultivar pairs had similar banding patterns: purpurea 'Eleyi' & 'Hopa', 'Pink Dawn' & 'Canary', and 'Silver Drift' & 'Donald Wyman'. All six enzymes were required to delineate among most of the cultivars. ADH had the greatest number of banding patterns (7) whereas the other enzymes had either three to five patterns. Some banding patterns were more diagnostic to separate among cultivars. That is, banding patterns observed at a frequency of less than ten percent were arbitrarily considered diagnostic (Table 2). ADH had four diagnostic banding patterns whereas AAT, MDH, and SKDH each had two diagnostic patterns and 6PGD-2 and PGI had one. Cultivars that possessed diagnostic banding patterns included 'Branzam', 'Burgandy', 'Camzam', x robusta 'Dolgo', 'Doubloons', 'Hamzam', 'Lanzam', 'Mazam', 'Mollie Ann', 'Pink Satin', 'Pond Red', 'Red Barron', 'Royal Ruby', *x adstringens* 'Selkirk', 'Sinai Fire', 'Spring Song', and 'Zumarang'.

Chemical analyses can be added to the growing list of characteristics that can be used to distinguish among crabapple cultivars. Unlike morphological characteristics, isozymes are objective traits that are largely unaffected by environment. While inheritance patterns have not been documented here, the inheritance of four loci can be inferred which could be validated by progeny testing. Using these four tentative loci, alleles could be assigned, and the integrity of crabapple pedigrees (if available) could be determined.

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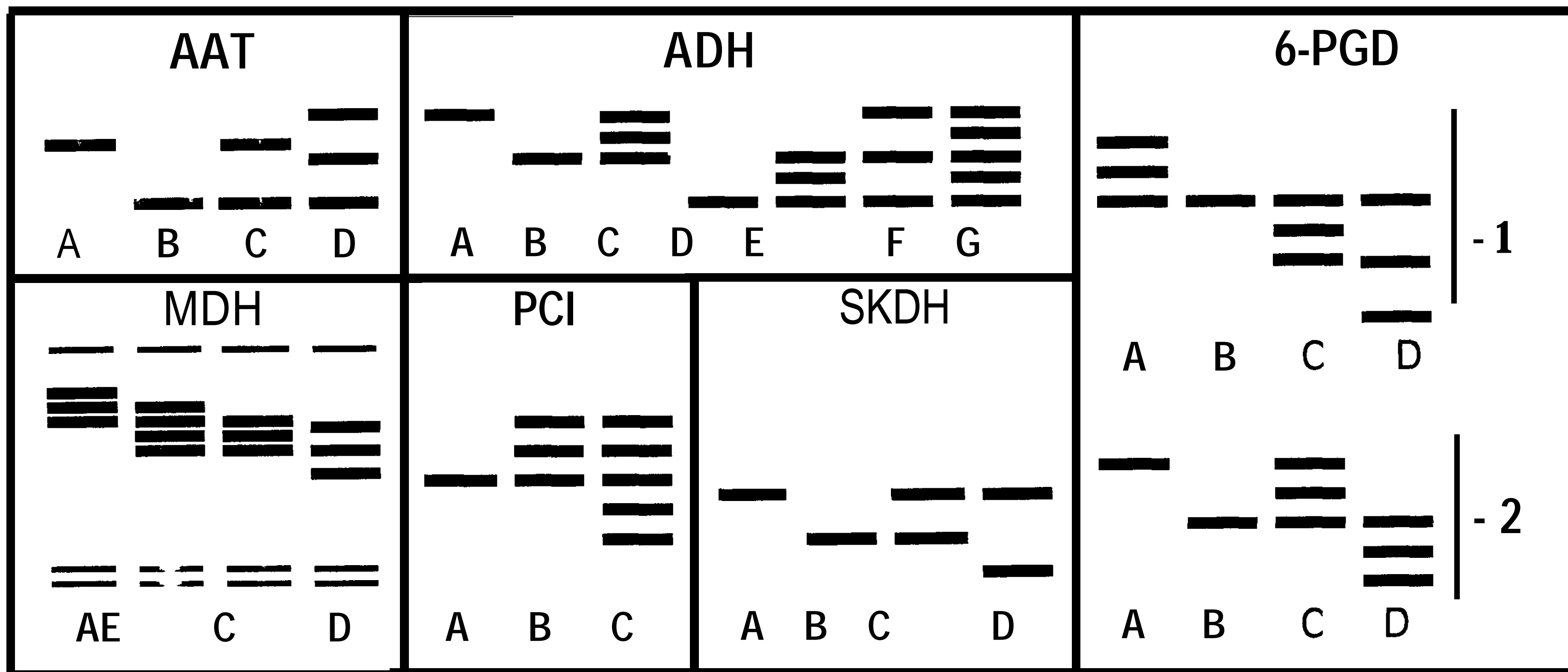
Table 1. Cultivar names and (Trademarks) of selected crabapple taxa evaluated by isozyme analysis along with the corresponding phenotype for the six enzyme systems (and seven polymorphic regions) that were evaluated. Phenotypes reported correspond to banding patterns shown in Figure 1.

ENZYME SYSTEM **Cultivar (Trademarks)**	**AAT**	**ADH**	**MDH**	**6-PGD-1**	**6-PGD-2**	**PGI**	**SKDH**
'Adams'	B	B	**B**	C	B	B	B
'Branzam' (Brandywine)	B	**B**	D	**C**	C	A	A
'Burgandy'	**B**	F	B	**B**	B	A	C
'CamZam' (Camelot)	**C**	A	**C**	**B**	B	B	B
'Canary'	B	B	**B**	**B**	B	A	C
'David'	B	B	C	**B**	B	B	B
x robusta 'Dolgo'	B	E	A	**D**	A	A	B
'Donald Wyman'	B	C	C	**A**	B	B	C
'Doubloons*	B	**D**	C	**B**	A	A	B
'Edna Mullins'	B	**B**	C	**A**	B	B	C
'Excazam' (Excaliber)	C	B	C	**B**	**C**	B	B
prunifolium 'Cheal's Golden Gem'	C	B	C	**C**	**B**	B	C
'Golden Hornet'	B	**C**	**B**	**D**	D	B	B
'Schmidt Cutleaf'(Golden Raindrops)	B	**C**	**C**	**B**	C	A	B
'Hamzam' (Hamlet)	C	G	C	**B**	B	C	B
'Hargozam' (Harvest Gold)	B	B	B	**B**	B	A	B
x adstringens 'Hopa'	B	E	B	**C**	B	A	B
'Jewelberry'	B	**E**	C	**B**	D	B	B
'Lanzam' (Lancelot)	**C**	**A**	C	**A**	C	B	C
'Louisa'	**B**	B	C	**B**	B	**B**	C
'Mazam' (Madonna)	D	**B**	B	**B**	B	**A**	A
'Mollie Ann'	B	**A**	C	**B**	C	A	B
'Ormiston Roy'	B	B	**B**	**B**	**B**	**B**	C
'Pink Cascade'	B	B	**B**	**D**	**B**	**A**	C
'Pink Dawn'	**B**	B	B	**B**	B	A	C
'Pink Satin'	**B**	B	C	**D**	**B**	C	B
'Pond Red'	C	E	C	**B**	**B**	C	C
'Prairie Maid'	C	C	B	**B**	D	A	B
'Prairiefire'	C	**B**	**B**	**B**	**B**	**B**	B
x zumi 'Professor Sprenger'	C	**B**	**B**	**C**	**D**	**B**	C
'Profusion'	**B**	**B**	C	**C**	D	A	B
'Purple Prince'	**B**	**C**	C	**B**	C	B	B
purpurea 'Eleyi'	B	B	B	**C**	**B**	B	B
'Red Barron'	B	B	C	**B**	**B**	B	A
'Jewelcole' (Red Jewel)	C	**B**	**C**	**B**	**B**	A	B
'Royal Ruby'	B	**F**	**C**	**B**	**B**	C	D
sargentii 'Candymint'	B	**C**	C	**C**	C	B	B
x adstrinaens 'Selkirk'	B	**E**	B	**C**	B	A	A
'Sentinel;	C	C	C	**A**	B	B	C
'Silver Drift'	B	C	C	**A**	B	B	C
'Sinai Fire'	B	**B**	**C**	**D**	A	A	B
'Snow Magic'	B	**B**	**C**	**A**	B	B	B
'Spring Song'	B	**F**	**C**	**B**	**B**	A	C
'Weepcanzam'(Weeping Candied Apple)	C	**B**	**C**	**B**	**B**	B	B
'Zumarang'	A	A	C	**A**	A	B	C

Table 2. Frequency of phenotypes observed among 45 crabapple cultivars characterized by isozyme analysis.

Phenotype	AAT	ADH	MDH	6-PGD-1	6-PGD-2	PGI	SKDH
A	1	4	1	7	4	18	4
B	31	23	15	24	29	23	24
C	12	8	28	9	7	4	16
D	1	1	1	5	5	0	1
E	0	5	0	0	0	0	0
F	0	3	0	0	0	0	0
G	0	1	0	0	0	0	0

Figure 1. Diagrammatic representation of the isozyme banding patterns and designated phenotypes of aminoaspartate transferase (AAT), alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), 6-phosphogluconate dehydrogenase (6-PGD), and shikimate dehydrogenase (SKDH) from crude leaf extracts from crabapples. 6-PGD produced two polymorphic regions of activity that were scored (-1 and -2).


AAT
A B C D
ADH
A B C D E F G
6-PGD
- 1
A B C D
- 2
A B C D
MDH
AE C D
PCI
A B C
SKDH
A B C D