Inheritance and Linkage Relationships of Isozymes in Easter Cactus

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ABSTRACT. Polyacrylamide gel electrophoresis was used to study inheritance and linkage of isozymes in Easter cactus (*Hatiora* species and interspecific hybrids). Five isozyme systems were analyzed: aspartate aminotransferase (AAT), glucose-6-phosphate isomerase (GPI), malate dehydrogenase (MDH), phosphoglucosmutase (PGM), and triosephosphate isomerase (TPI). F₁, F₂, BC₁, and S₁ progeny were used for inheritance studies. Six polymorphic loci (*Aat-1*, *Gpi-1*, *Mdh-1*, *Pgm-1*, *Pgm-2*, and *Tpi-2*) were identified. *Aat-1* and *Pgm-1* were linked (recombination frequency = 26% ± 7%), but the other isozyme loci assorted independently. Aberrant segregation ratios were observed in at least one segregating family for all six isozyme loci. We hypothesize that segregation distortion was due to linkage between isozyme loci and other genes subject to pre- or postzygotic selection. The existence of five additional isozyme loci (*Aat-2*, *Gpi-2*, *Mdh-2*, *Mdh-3*, and *Tpi-1*) was inferred from segregation patterns and by comparison of isozyme profiles from phylloclades and pollen. These isozyme loci may prove useful for confirming hybridity in intra- and interspecific crosses, determining parentage of cultivars, and assessing genetic diversity in germplasm collections.

Easter cactus is a succulent, epiphytic shrub that is grown as a flowering potted plant. Plants cultivated under the common name Easter cactus include clonally propagated selections of *Hatiora gaertneri* (Regel) Barthlott, *H. rosea* (Lagerheim) Barthlott, and their interspecific hybrids (*H. × graeseri* Barthlott ex. D. Hunt) (Barthlott and Taylor, 1995; Meier, 1995). More than 100 cultivars of Easter cactus have been named, of which most are complex *H. × graeseri* hybrids (Boyle, 1995; Meier, 1995). As far as is known, *H. gaertneri*, *H. rosea*, and *H. × graeseri* are diploid taxa with a chromosome number of $2n = 2x = 22$ (Barthlott, 1976).

Interest in Easter cactus has increased within the past decade due to its potential as a specialty potted crop for spring sales. Currently, only ≈10 cultivars are grown commercially as potted plants. These 10 cultivars exhibit the combination of traits that are required for commercial production, i.e., an upright habit, extensive branching, rapid rate of growth, and satisfactory postproduction longevity. Many of the remaining 90+ cultivars are inferior in one or more of these traits (T.H. Boyle, unpublished data), thus restricting their commercial potential. Hence, demand for this crop would be likely to increase if more commercial cultivars were available.

Easter cactus breeding was initiated at the Univ. of Massachusetts in 1987, and is directed toward the development of novel and superior clones for the commercial trade. It was therefore highly desirable to have a set of genetic markers for confirming hybridity in intra- and interspecific crosses, determining parentage of cultivars, and assessing genetic diversity in germplasm collections. Crossing studies have revealed that few simply inherited morphological markers exist in Easter cactus (T.H. Boyle, unpublished data).

Isozymes are widely used as genetic markers in horticultural crops (Weeden, 1989). Typically, isozymes exhibit simple inheritance, codominance, complete penetrance, and consistency of expression under a wide range of environmental conditions, and

these traits make them particularly useful as genetic markers (Weeden and Wendel, 1989). The objectives of this research were to 1) document and interpret the banding patterns for five isozyme systems, and 2) determine the genetics and linkage relationships of several isozyme loci.

Materials and Methods

PLANT MATERIAL. Twelve clones were used as parents for this study (Table 1). F₁, F₂, BC₁, and S₁ populations were generated for inheritance studies. S₁ seed of 'Crimson Giant', a highly self-incompatible cultivar, was produced by incubating flowering plants for 12 h in a 40 °C growth chamber and selfing immediately after incubation (Boyle et al., 1994). Henceforth, all crosses will be presented as pistillate parent × pollen parent unless specified otherwise. Pollination, seed germination, and plant culture were conducted as described in Boyle et al. (1994).

ISOZYME EXTRACTION, SEPARATION, AND STAINING. Isozymes were extracted from both mature stem segments (phylloclades) and pollen. Tissue was removed from phylloclades with a no. 1 cork borer. Soluble proteins were extracted by homogenizing 8 mg phylloclade tissue in 100 µL cold extraction buffer [0.050 M Tris-

Table 1. Easter cactus clones used for study, their parentage, and source.

Clone	Parentage ^a	Source ^b
Andre	Unknown	RG
Crimson Giant	Unknown	MD
Evita	Unknown	JV
MD861	Unknown	MD
Red Pride	Unknown	JV
Shocking Pink	Unknown	RG
R891-2	Crimson Giant × MD861 (F ₁)	MA
R891-3	Crimson Giant × MD861 (F ₁)	MA
R891-6	Crimson Giant × MD861 (F ₁)	MA
R891-8	Crimson Giant × MD861 (F ₁)	MA
R891-9	Crimson Giant × MD861 (F ₁)	MA
R892-1	MD861 × Crimson Giant (F ₁)	MA

^aPistillate parent × pollen parent.

^bJV = J. de Vries Potplantencultures, Aalsmeer, The Netherlands; MA = Univ. of Massachusetts, Amherst; MD = Univ. of Maryland, College Park; RG = Rainbow Gardens, Vista, Calif.

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HCl, pH 7.5; 0.014 M mercaptoethanol; 5% (w/v) polyvinylpyrrolidone (PVP-40); and 5% (w/v) sucrose (Wendel and Weeden, 1989)]. Crude homogenates were centrifuged at 10,060 *g*_n for 10 min at 4 °C. Supernatant (60 µL) was collected and mixed with 10 µL cold (4 °C) sample mix [50% (v/v) glycerol and 0.1% (w/v) bromphenol blue (Shields et al., 1983)]. Flowers were harvested on the day of anthesis, and pollen was removed from anthers with a vortexer and collected in scintillation vials. Soluble proteins were extracted by soaking 5 mg pollen in 500 µL pollen extraction buffer (0.050 M Tris-HCl, pH 7.5; 0.014 M 2-mercaptoethanol; and 1 mM EDTA) for 4 h at 4 °C (Weeden and Gottlieb, 1980). Pollen extracts were centrifuged at 10,060 *g*_n for 10 min at 4 °C, and the resulting supernatant was treated as described earlier for phylloclade samples.

Native proteins were separated by polyacrylamide gel electrophoresis (PAGE) using a Mini-Protean II cell (Bio-Rad Laboratories, Hercules, Calif.). Single-percentage polyacrylamide gels were prepared using 0.375 M Tris-HCl, pH 8.8 (Hames, 1981). The electrode buffer was 0.025 M Tris and 0.192 M glycine, pH 8.3 (Hames, 1981). Electrophoresis was conducted at 4 °C under constant voltage (200 v). Running times varied but typically required ≈45 min for completion.

Five enzyme systems were examined: aspartate aminotransferase (AAT, E.C. 2.6.1.1), glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9), malate dehydrogenase (MDH, E.C. 1.1.1.37), phosphoglucumutase (PGM, E.C. 5.4.2.2), and triosephosphate isomerase (TPI, E.C. 5.3.1.1). Polyacrylamide gel concentrations and sample loading volumes (µL) for the five enzyme systems were as follows: 5% gels and 10 µL for AAT; 7.5% gels and 5 µL for GPI; 7.5% gels and 10 µL for MDH and PGM; and 10% gels and 5 µL for TPI. The recipes of Wendel and Weeden (1989) were used for staining gels. Enzyme stains were prepared immediately before use. Gels were fixed in 7% (v/v) acetic acid after suitable banding intensity was attained (Hames, 1981).

DATA COLLECTION AND ANALYSIS. Data were collected immediately after gels were fixed. *R_f* values were determined by dividing the band's migration distance by the tracking dye's migration distance (Hames, 1981). The reported *R_f* values are the means from three or more gels.

Chi-square tests were used to determine the goodness-of-fit between observed segregation ratios and expected Mendelian ratios (1:1, 1:2:1, or 1:1:1:1). Yates' correction for continuity was used for chi-square tests with one degree of freedom (Yates, 1934). Reciprocal crosses were pooled if their observed segregation ratios did not differ significantly (*P* > 0.05) in heterogeneity chi-square tests. The computer program LINKAGE-1 (Suiter et al., 1983) was used to discern linkage relationships among loci.

ISOZYME NOMENCLATURE. Zones of enzyme activity are identified by Arabic numerals and are numbered sequentially from the anode. For each enzyme, loci are numbered sequentially, with 1 denoting the most anodally migrating locus. Alleles at individual loci are designated by letters assigned sequentially from the anode.

Results and Discussion

ASPARTATE AMINOTRANSFERASE. Gels exhibited two polymorphic zones of AAT activity. The more anodal zone (AAT-1) consisted of five bands with *R_f* values ranging from 0.36 to 0.50. Bands c and d (with *R_f* values of 0.45 and 0.38, respectively) were used for genetic studies. Phylloclade extracts yielded either single- or triple-banded phenotypes (Fig. 1). Triple-banded phenotypes exhibited a band of intermediate mobility (*R_f* 0.41), which was absent in gels of soaked-pollen extracts. These results are consis-

tent with a dimeric structure for AAT. Crosses between a single-banded individual and a triple-banded individual produced, in most cases, progenies which segregated in a 1:1 (single band : triple band) ratio (Table 2). However, one BC₁ family ('Crimson Giant' x R891-2) deviated significantly from the expected 1:1 ratio (Table 2). Three families obtained from crossing two triple-banded individuals yielded all three expected phenotypes, but their segregation ratios deviated significantly from the expected 1:2:1 ratio (Table 2). In two of these families ('Crimson Giant' x R891-6 and 'Crimson Giant' S₁ progeny), one of the single-banded phenotypes was deficient whereas the other single-banded phenotype was in excess. Interestingly, the ratio of single-banded to triple-banded individuals did not deviate from the expected 1:1 ratio in either family [$\chi^2 = 0.09$ (*P* > 0.05) for 'Crimson Giant' x R891-6; $\chi^2 = 0.00$ (*P* > 0.05) for 'Crimson Giant' S₁ progeny]. We propose that AAT-1 isozymes are encoded by a single locus (*Aat-1*) with multiple alleles. Bands a through e are tentatively designated *Aat-1* alleles *a* through *e*, respectively.

The more cathodal zone (AAT-2) consisted of bands at *R_f* 0.34 (a), 0.32 (b), and 0.29 (c). Segregation in AAT-1 banding patterns was not accompanied by likewise segregation in AAT-2 banding patterns, suggesting AAT-2 bands are products of a second AAT locus, designated *Aat-2*. Intergenic heterodimers were not observed in phylloclade extracts, and therefore it is likely that *Aat-1* and *Aat-2* encode AAT isozymes with different subcellular locations.

GLUCOSE-6-PHOSPHATE ISOMERASE. Two polymorphic zones of activity were observed on gels. GPI-1 contained three bands with *R_f* values of 0.43 (a), 0.39 (b), and 0.36 (c). Phylloclade extracts displayed either single- or triple-banded phenotypes at GPI-1 (Fig. 1). Triple-banded phenotypes exhibited a band of intermediate mobility that was lacking in soaked-pollen extracts, results that indicate that GPI is a dimer. When a triple-banded individual was crossed with a single-banded individual, progenies were obtained in the expected 1:1 segregation ratio, with the exception of one F₁ family ('Crimson Giant' x 'Evita') (Table 2). Progenies from ab x ab crosses did not significantly differ from the expected 1 aa : 2 ab : 1 bb segregation ratio (Table 2). When plants with different triple-banded phenotypes were crossed (ab x bc), progeny were obtained in the expected 1:1:1:1 ratio (Table 2). These results indicate that GPI-1 isozymes are encoded by a single locus with three codominant alleles. This locus is designated *Gpi-1*, with bands a, b, and c corresponding (respectively) to alleles *a*, *b*, and *c*.

GPI-2 contained five different bands with *R_f* values between 0.23 and 0.32. Phylloclade extracts displayed only single-banded phenotypes at GPI-2. GPI-2 bands are tentatively identified as products of a second locus (*Gpi-2*).

Fig. 1. Diagrammatic representation of phenotypes for aspartate aminotransferase (AAT-1), glucose-6-phosphate isomerase (GPI-1), and triosephosphate isomerase (TPI-2) in Easter cactus.

MALATE DEHYDROGENASE. Four zones of MDH activity were observed. MDH-1 and MDH-4 were polymorphic whereas MDH-2 and MDH-3 were monomorphic. Three different bands have been identified at MDH-1, with R_f values between 0.53 and 0.63. Bands a (R_f 0.63) and c (R_f 0.53) were analyzed genetically. Phylloclade extracts displayed either a single-banded or triple-

banded phenotype (Fig. 2). Triple-banded individuals produced a band of intermediate mobility at R_f 0.58 that was not present in gels of soaked-pollen extracts. These results are consistent with a dimeric enzyme. Crosses between a single-banded individual and a triple-banded individual yielded four families that segregated in the expected 1:1 ratio, but four other families that deviated signifi-

Table 2. Parental and progeny phenotypes for aspartate aminotransferase (AAT), glucose-6-phosphate isomerase (GPI), malate dehydrogenase (MDH), phosphoglucumutase (PGM), and triosephosphate isomerase (TPI) isozymes in Easter cactus and chi-square analysis of segregating progenies.

Isozyme zone	Cross or self ^z	Parental phenotypes	Segregation ratios expected (observed)	χ^2
AAT-1	CG x MD	cd x cc	1 cc:1 cd (18:23)	0.39
	CG x RP ^y	cd x cc	1 cc:1 cd (31:32)	0.00
	CG x EV ^y	cd x cc	1 cc:1 cd (34:36)	0.01
	EV x RP ^y	cc x cc	(61 cc)	---
	12 x 13 ^y	cc x cd	1 cc:1 cd (13:19)	0.78
	12 x 19	cc x cd	1 cc:1 cd (51:44)	0.38
	18 x 16	cc x cd	1 cc:1 cd (6:3)	0.44
	CG x 12 ^y	cd x cc	1 cc:1 cd (18:36)	5.35*
	CG x 13 ^y	cd x cd	1 cc:2 cd:1 dd (1:17:9)	6.56*
	CG x 16 ^y	cd x cd	1 cc:2 cd:1 dd (21:21:3)	14.60*
	CG x 21 ^y	cd x cc	1 cc:1 cd (25:24)	0.00
	12 x MD	cc x cc	(12 cc)	---
	18 x CG	cc x cd	1 cc:1 cd (5:5)	0.10
	CG ⊗	cd x cd	1 cc:2 cd:1 dd (5:53:49)	36.20*
GPI-1	AN x EV ^y	bc x ab	1 ab:1 ac:1 bb:1 bc (25:12:17:14)	5.76
	AN x MD	bc x ab	1 ab:1 ac:1 bb:1 bc (21:22:25:27)	0.96
	CG x MD	bb x ab	1 ab:1 bb (19:21)	0.03
	CG x RP ^y	bb x ab	1 ab:1 bb (25:37)	1.95
	CG x EV ^y	bb x ab	1 ab:1 bb (26:44)	4.13*
	EV x RP ^y	ab x ab	1 aa:2 ab:1 bb (15:23:23)	5.79
	RP x MD	ab x ab	1 aa:2 ab:1 bb (25:50:25)	0.00
	12 x 13 ^y	bb x bb	(32 bb)	---
	18 x 16	bb x ab	1 ab:1 bb (4:5)	0.00
	CG x 12 ^y	bb x bb	(56 bb)	---
	CG x 13 ^y	bb x bb	(26 bb)	---
	CG x 16 ^y	bb x ab	1 ab:1 bb (20:25)	0.36
	CG x 21 ^y	bb x ab	1 ab:1 bb (29:20)	1.31
	12 x MD	bb x ab	1 ab:1 bb (8:4)	0.75
	18 x CG	bb x bb	(10 bb)	---
	CG ⊗	bb x bb	(107 bb)	---
MDH-1	CG x MD	ac x aa	1 aa:1 ac (18:22)	0.23
	CG x RP ^y	ac x aa	1 aa:1 ac (30:33)	0.06
	CG x EV ^y	ac x aa	1 aa:1 ac (42:25)	3.82
	EV x RP ^y	aa x aa	(61 aa)	---
	12 x 13 ^y	aa x ac	1 aa:1 ac (9:22)	4.65*
	18 x 12	ac x aa	1 aa:1 ac (6:9)	0.27
	18 x 16	ac x aa	1 aa:1 ac (6:3)	0.44
	CG x 12 ^y	ac x aa	1 aa:1 ac (15:41)	11.16*
	CG x 13	ac x ac	1 aa:2 ac:1 cc (5:9:8)	1.55
	CG x 16 ^y	ac x aa	1 aa:1 ac (16:29)	3.20
	CG x 21 ^y	ac x ac	1 aa:2 ac:1 cc (10:23:16)	1.65
	12 x MD	aa x aa	(12 aa)	---
	18 x CG	ac x ac	1 aa:2 ac:1 cc (5:3:2)	3.40
	CG ⊗	ac x ac	1 aa:2 ac:1 cc (30:52:19)	2.49
PGM-1	AN x MD	dd x dd	(96 dd)	---
	CG x MD	ff x dd	(40 df)	---
	CG x RP ^y	ff x dd	(62 df)	---
	CG x EV ^y	ff x dd	(67 df)	---
	EV x RP ^y	dd x dd	(59 dd)	---
	EV x SP	dd x df	1 dd:1 df (15:17)	0.03

cantly from the expected 1:1 ratio (Table 2). Progenies from ac x ac crosses did not significantly differ from the expected 1 aa : 2 ab : 1 bb ratio (Table 2). We propose that MDH-1 isozymes are governed by a single locus (*Mdh-1*) with multiple alleles, with bands a, b, and c corresponding (respectively) to *Mdh-1* alleles *a*, *b*, and *c*.

MDH-2 and MDH-3 contained a single band each, with R_f values of 0.39 and 0.34, respectively. MDH-2 and MDH-3 were identified as separate loci (*Mdh-2* and *Mdh-3*, respectively), both with a single allele (*a*). A band that migrated halfway between MDH-2 and MDH-3 ($R_f \approx 0.36$) was tentatively identified as an intergenic heterodimer of *Mdh-2* and *Mdh-3*. Soaked-pollen ex-

Table 2. Continued.

Isozyme zone	Cross or self ^z	Parental phenotypes	Segregation ratios expected (observed)	χ^2
PGM-1	RP x MD	dd x dd	(100 dd)	---
	12 x 13 ^y	df x df	1 dd:2 df:1 ff (4:13:14)	7.26*
	12 x 19	df x df	1 dd:2 df:1 ff (20:53:22)	1.36
	18 x 12	df x df	1 dd:2 df:1 ff (5:9:1)	2.73
	18 x 16	df x df	1 dd:2 df:1 ff (2:7:0)	3.67
	CG x 12 ^y	ff x df	1 df:1 ff (40:16)	9.45*
	CG x 13 ^y	ff x df	1 df:1 ff (9:18)	2.37
	CG x 16 ^y	ff x df	1 df:1 ff (33:12)	8.89*
	CG x 21 ^y	ff x df	1 df:1 ff (35:14)	8.16*
	12 x MD	df x dd	1 dd:1 df (5:7)	0.08
	18 x CG	df x ff	1 df:1 ff (8:2)	2.50
	CG ⊗	ff x ff	(100 ff)	---
PGM-2	AN x MD	ad x ab	1 aa:1 ab:1 ad:1 bd (29:22:22:23)	1.42
	CG x MD	ad x ab	1 aa:1 ab:1 ad:1 bd (9:12:11:8)	1.00
	CG x RP ^y	ad x ab	1 aa:1 ab:1 ad:1 bd (16:9:21:16)	4.71
	CG x EV ^y	ad x aa	1 aa:1 ad (31:36)	0.24
	EV x RP ^y	aa x ab	1 aa:1 ab (27:34)	0.59
	EV x SP	aa x aa	(32 aa)	---
	RP x MD	ab x ab	1 aa:2 ab:1 bb (33:39:28)	5.34
	12 x 13 ^y	ad x aa	1 aa:1 ad (16:15)	0.00
	12 x 19	ad x ad	1 aa:2 ad:1 dd (28:42:25)	1.46
	18 x 12	aa x ad	1 aa:1 ad (8:7)	0.00
	18 x 16	aa x ad	1 aa:1 ad (5:4)	0.00
	CG x 12 ^y	ad x ad	1 aa:2 ad:1 dd (17:26:13)	0.86
	CG x 13 ^y	ad x aa	1 aa:1 ad (9:17)	1.88
	CG x 16 ^y	ad x ad	1 aa:2 ad:1 dd (9:25:11)	0.73
	CG x 21 ^y	ad x ad	1 aa:2 ad:1 dd (13:25:11)	0.18
	12 x MD	ad x ab	1 aa:1 ab:1 ad:1 bd (4:3:3:2)	0.67
	18 x CG	aa x ad	1 aa:1 ad (6:4)	0.10
	CG ⊗	ad x ad	1 aa:2 ad:1 dd (37:51:12)	12.54*
TPI-2	AN x MD	ab x ab	1 aa:2 ab:1 bb (21:55:20)	2.06
	CG x MD	bb x ab	1 ab:1 bb (14:26)	3.03
	CG x RP	bb x ab	1 ab:1 bb (5:26)	12.90*
	CG x EV ^y	bb x ab	1 ab:1 bb (11:59)	31.56*
	EV x RP ^y	ab x ab	1 aa:2 ab:1 bb (0:33:27)	24.90*
	RP x CG	ab x bb	1 ab:1 bb (14:17)	0.13
	RP x MD	ab x ab	1 aa:2 ab:1 bb (22:56:22)	1.44
	12 x 13 ^y	bb x bb	(31 bb)	---
	12 x 19	bb x ab	1 ab:1 bb (50:45)	0.17
	18 x 12	ab x bb	1 ab:1 bb (7:8)	0.00
	18 x 16	ab x bb	1 ab:1 bb (4:5)	0.00
	CG x 12 ^y	bb x bb	(56 bb)	---
	CG x 13 ^y	bb x bb	(26 bb)	---
	CG x 16 ^y	bb x bb	(45 bb)	---
	CG x 21 ^y	bb x bb	(49 bb)	---
	12 x MD	bb x ab	1 ab:1 bb (4:8)	0.75
	18 x CG	ab x bb	1 ab:1 bb (6:4)	0.10
	CG ⊗	bb x bb	(100 bb)	---

^zAN = Andre, CG = Crimson Giant, EV = Evita, MD = MD861, RP = Red Pride, SP = Shocking Pink, 12 = R891-2, 13 = R891-3, 16 = R891-6, 18 = R891-8, 19 = R891-9, 21 = R892-1; self = ⊗.

^yPooled data from reciprocal crosses.

*Significant deviation from Mendelian inheritance ($P \leq 0.05$). Yates' continuity correction used for χ^2 tests with one degree of freedom.

Rf (x100) +	MDH-1			PGM-1			PGM-2					
70												
60	—	—		—	—							
50		—	—		—	—						
40							—	—	—	—	—	—
-	aa	ac	cc	dd	df	ff	aa	ab	ad	bd	bb	dd

Fig. 2. Diagrammatic representation of phenotypes for malate dehydrogenase (MDH-1) and phosphoglucumutase (PGM-1 and PGM-2) in Easter cactus.

tracts also displayed bands at R_f 0.34, 0.36, and 0.39, results that are consistent with an intergenic heterodimer at R_f 0.36. MDH-4 bands were too faint to interpret consistently.

Ascorbic acid selectively inhibits cytosolic MDHs but does not markedly suppress MDHs compartmentalized in organelles (Goodman et al., 1980). Addition of 8.3% (w/v) ascorbic acid to the phylloclade extraction buffer suppressed activity at MDH-1, but did not affect activity at MDH-2 or MDH-3 (O'Leary, 1996). These results suggest that *Mdh-1* encodes a cytosolic form of MDH whereas *Mdh-2* and *Mdh-3* encode organellar forms of MDH.

PHOSPHOGLUCOMUTASE. PAGE gels contained two polymorphic zones of PGM activity (Fig. 2). PGM-1 consisted of six bands with R_f values between 0.55 and 0.70. Bands d and f (with R_f values of 0.62 and 0.55, respectively) were used for genetic studies. Phylloclade extracts yielded either single- or double-banded phenotypes (Fig. 2), results which indicate that PGM is a monomer in Easter cactus. Crosses between a single-banded individual and a double-banded individual resulted in the expected 1:1 segregation ratio in four families, but significant departures from expectations were observed in three other families (Table 2). Crosses between two double-banded individuals (df x df) yielded three families, which approximated a 1 dd : 2 df : 1 ff segregation ratio (Table 2). In one F_2 family (R891-2 x R891-3), however, dd phenotypes were deficient and ff phenotypes were in excess (Table 2). Most of the inheritance data supports the hypothesis that PGM-1 isozymes are encoded by a single locus with multiple alleles. This locus is designated *Pgm-1*, with bands a through f tentatively designated as alleles *a* through *f*, respectively.

PGM-2 bands migrated between R_f 0.39 and 0.48. Bands a, b, and d (with R_f values of 0.48, 0.46, and 0.42, respectively) were subjected to genetic analysis. When a double-banded individual was crossed with a single-banded individual, progenies were obtained in the expected 1:1 segregation ratio (Table 2). When plants with identical double-banded phenotypes were crossed, the expected 1:2:1 segregation ratio was observed in all families except for the S_1 progeny of 'Crimson Giant' (Table 2). Crosses between clones with different double-banded phenotypes yielded progenies in the expected 1:1:1:1 ratio (Table 2). Our results indicate that PGM-2 isozymes are governed by a single locus. This locus is designated *Pgm-2*, with bands a through e tentatively designated as alleles *a* through *e*, respectively.

TRIOSEPHOSPHATE ISOMERASE. Two zones of activity were observed for TPI. TPI-1 consisted of a single band at R_f 0.52; this band is designated allele *a* of locus *Tpi-1*. TPI-2 contained two bands with R_f values of 0.38 (a) and 0.32 (b). Phylloclade extracts produced either single-banded or triple-banded phenotypes at TPI-2 (Fig. 1). Triple-banded phenotypes contained a band at R_f 0.35,

but this band was not observed on gels of soaked-pollen extracts, indicating that TPI is a dimer. When a triple-banded individual was crossed with a single-banded individual, seven families segregated in a ratio of $\approx$ 1:1, but significant departures from expectations were observed in two other families (Table 2). Crosses between two triple-banded individuals yielded two families which fit the expected 1:2:1 ratio, and another family ('Evita' x 'Red Pride') with no aa homozygotes and a surplus of bb homozygotes (Table 2). Our results suggest that TPI-2 isozymes are governed by a single locus (*Tpi-2*), with bands a and b corresponding to alleles *a* and *b*, respectively.

Bands that were observed midway between TPI-1 and TPI-2 were tentatively identified as intergenic heterodimers. Single-banded individuals (those with one band at either R_f 0.32 or 0.38) exhibited only one band of intermediate mobility, whereas individuals with a triple-banded phenotype (those with bands at R_f 0.32, 0.35, and 0.38) always displayed two bands of intermediate mobility, results that agree with those expected for intergenic heterodimers. The intermediate band(s) were also present in soaked-pollen extracts, providing further evidence that they are intergenic heterodimers. Intergenic heterodimers of *Tpi-1* and *Tpi-2* have also been reported in *Annona cherimola* Mill. (Patty et al., 1988).

LINKAGE ANALYSIS. Linkage between *Aat-1*, *Gpi-1*, *Mdh-1*, *Pgm-1*, *Pgm-2*, and *Tpi-2* was tested for all possible pairs of loci. *Aat-1* and *Pgm-1* were linked (recombination frequency = $26\% \pm 7\%$) whereas the other loci assorted independently.

SEGREGATION DISTORTION. Distorted ratios were observed in at least one segregating family for all six isozyme loci (Table 2). We propose that segregation distortion is due to linkage between the isozyme loci and other genes subject to pre- or postzygotic selection. *Hatiora gaertneri*, *H. rosea*, and *H. x graeseri* are highly self-incompatible (Boyle et al., 1994; Ganders, 1976) and are therefore predominantly or exclusively outcrossed. Inbreeding of a normally-outbred taxon would be likely to expose some semilethal or lethal recessive genes, thus leading to the attrition of some genotypes prior to maturity. As a consequence, isozyme loci that are closely linked to semilethal or lethal recessives would also be subject to selection.

Several cases of disturbed segregation ratios at isozyme loci have been attributed to linkage with genes controlling gametophytic self-incompatibility (Manganaris and Alston, 1987; Wendel and Parks, 1984), and a similar phenomenon may have occurred in Easter cactus. The closely related genus *Schlumbergera* has a gametophytic self-incompatibility system that is controlled by a single multiallelic gene (*S*) (Boyle, 1997). The type of self-incompatibility system is highly conserved within families, and therefore it is reasonable to assume that self-incompatibility in *Hatiora* is also a simply-inherited trait under gametophytic control. Only two of the six isozyme loci that we examined were linked (*Aat-1* and *Pgm-1*), and therefore linkage with the *S* locus could account for aberrant segregation ratios at no more than two isozyme loci.

Isozyme segregation ratios may also have been affected by the use of interspecific hybrids in this study. As judged from their phylloclade and flower morphology, most of the clones listed in Table 1 conform taxonomically to *H. x graeseri*. Zamir and Tadmor (1986) reviewed the literature for intra- and interspecific crosses in three genera (*Capsicum*, *Lens*, and *Lycopersicon*). They found that the proportion of loci deviating from expected monogenic segregation ratios was significantly higher for interspecific crosses (54%) than for intraspecific crosses (13%). The genomes of *H. gaertneri* and *H. rosea* may have evolutionarily diverged to the extent that interspecific hybridization, although possible, yields progeny which segregate in non-Mendelian ratios.

Conclusions

Six polymorphic isozyme loci have been identified in Easter cactus. Additional genetic markers are required before linkage groups can be established for this crop. Future research should examine other isozyme systems and randomly amplified polymorphic DNA (RAPD) to identify more genetic markers of Easter cactus.

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