Cultivar Identification and Genetic Diversity within a *Hatiora* (Cactaceae) Clonal Germplasm Collection Using Isozymes

Maureen C. O'Leary¹ and Thomas H. Boyle²

Department of Plant and Soil Sciences, French Hall, University of Massachusetts, Amherst, MA 01003

ADDITIONAL INDEX WORDS. Easter cactus, parentage verification, polyacrylamide gel electrophoresis

ABSTRACT. Isozyme markers were used to identify cultivars and assess the genetic diversity within a germplasm collection of 49 *Hatiora* Britt. & Rose clones. The collection included accessions of Easter cactus [*H. gaertneri* (Regel) Barthlott, *H. ×graeseri* Barthlott ex D. Hunt, and *H. rosea* (Lagerheim) Barthlott] plus *H. herminiae* (Campos-Porto & Castellanos) Backeberg ex Barthlott and *H. salicornioides* (Haworth) Britton & Rose. Seven enzyme systems were analyzed: aspartate aminotransferase, glucose-6-phosphate isomerase, leucine aminopeptidase, malate dehydrogenase, phosphoglucumutase, shikimate dehydrogenase, and triosephosphate isomerase. Thirteen loci and 42 alleles were identified. Twenty-one clones (43%) displayed unique isozyme profiles, but the remaining 28 clones shared isozyme profiles with one to three other clones. Percent polymorphic loci, mean number of alleles per locus, and mean heterozygosity were 69, 3.23, and 0.30, respectively, for the entire collection. Isozymes also proved useful for verifying that some progeny were genuine F₁ hybrids.

The genus *Hatiora* consists of five species of epiphytic or epilithic shrubs that are native to southeastern Brazil (Barthlott, 1987; Barthlott and Taylor, 1995). As currently conceived, the genus is comprised of two subgenera: *Hatiora* and *Rhipsalidopsis* (Barthlott, 1987). Subgenus *Hatiora* contains two species—*H. salicornioides* (Haworth) Britton & Rose and *H. herminiae* (Campos-Porto & Castellanos) Backeberg ex Barthlott—with terete or cylindric stem segments (phylloclades) and terete pericarpels. Subgenus *Rhipsalidopsis* contains three species—*H. epiphylloides* (Campos-Porto & Werdermann) F. Buxbaum, *H. gaertneri* (Regel) Barthlott, and *H. rosea* (Lagerheim) Barthlott—that have flattened or angular phylloclades and angled pericarpels. *Hatiora gaertneri*, *H. rosea*, and *H. salicornioides* are cultivated as ornamentals. *Hatiora gaertneri* and *H. rosea* were introduced into cultivation nearly a century ago (Lagerheim, 1912; Regel, 1884) and have achieved prominence due to their large and showy flowers. These two species and their interspecific hybrid [= *H. ×graeseri* Barthlott ex D. Hunt (Hunt, 1992)] are commonly referred to as Easter cactus. Most (>100) Easter cactus cultivars are complex *H. ×graeseri* hybrids (Boyle, 1995; Meier, 1995). *Hatiora salicornioides* is grown as a potted plant, the chief aesthetic features being its unique jointed stems and small yellow flowers. *Hatiora epiphylloides* and *H. herminiae* are rare in cultivation and are represented in few living collections. Except for *H. ×graeseri*, no other interspecific hybrids have been reported for *Hatiora* (Barthlott and Taylor, 1995).

Easter cactus exhibits considerable potential as a flowering pot crop due to its showy flowers and excellent postproduction longevity. A *Hatiora* germplasm collection was assembled to serve as a genetic resource for a breeding project at the University of Massachusetts. It became desirable to establish a rapid and unambiguous means of identifying clones. Presently, *Hatiora* cultivars are identified by their phylloclade and flower morphology, flower color, and

growth habit. Identifying cultivars is difficult when plants are vegetative because there are few distinguishing features. Isozymes have been used for identifying cultivars in several crops (Nielsen, 1985; Weeden, 1989). The likelihood of identifying *Hatiora* clones by their isozyme phenotypes depends on the level of isozyme polymorphism within the germplasm. In a recent investigation, we identified six polymorphic isozyme loci in Easter cactus (O'Leary and Boyle, 1998a), and demonstrated that considerable isozyme variability exists in this crop. Isozymes can also be used to verify hybridity and assess the level of genetic variation in germplasm collections (Weeden, 1989; Yndgaard and Hoskuldsson, 1985). Therefore, the objectives of the present study were to 1) survey a diverse collection of *Hatiora* clones for seven enzyme systems and ascertain if isozyme phenotypes could be used to identify clones and 2) estimate the genetic diversity within the germplasm collection.

Materials and Methods

PLANT MATERIAL. The germplasm collection consisted of 49 *Hatiora* clones. Most of the clones were Easter cactus cultivars of unknown parentage, but the collection also included accessions of *H. gaertneri*, *H. herminiae*, *H. rosea*, and *H. salicornioides* (Table 1). All *Hatiora* species except for *H. epiphylloides* were represented in the collection.

ISOZYME EXTRACTION, SEPARATION, AND STAINING. Enzymes were extracted from phylloclade tissue. Procedures used in extraction and separation of isozymes are described elsewhere (O'Leary and Boyle, 1998a, 1998b). Native proteins were separated by polyacrylamide gel electrophoresis using a Mini-Protean II cell (Bio-Rad Laboratories, Hercules, Calif.). Single percentage (5% to 10%) gels were prepared using 0.375 M Tris-HCL (pH 8.8) as the gel buffer (Hames, 1981). The electrode buffer for all enzyme systems 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).

Seven enzyme systems were examined: aspartate aminotransferase (AAT, E.C. 2.6.1.1), glucose-6-phosphate isomerase (GPI, E.C. 5.3.1.9), leucine aminopeptidase (LAP, E.C. 3.4.11.1), malate dehydrogenase (MDH, E.C. 1.1.1.37), phosphoglucumutase (PGM, E.C. 5.4.2.2), shikimate dehydrogenase (SKD, E.C. 1.1.1.25), and triosephosphate isomerase (TPI, E.C. 5.3.1.1). Polyacrylamide gel concentrations and sample loading volumes were as follows: 5%

Received for publication 29 July 1998. Accepted for publication 29 Mar. 1999. Massachusetts Agricultural Experiment Station publication 3225. This study was supported in part by a Faculty Research Grant from the Graduate School of the University of Massachusetts. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Graduate research assistant.

²Associate professor. To whom reprint requests should be addressed; e-mail tboyle@pssci.umass.edu.

Table 1. *Hatiora* accessions used for study and their isozyme phenotypes.

Clone	Source ^z	Isozyme profile	Locus								
			<i>Aat-1</i>	<i>Aat-2</i>	<i>Gpi-1</i>	<i>Gpi-2</i>	<i>Lap-1</i>	<i>Mdh-1</i>	<i>Pgm-1</i>	<i>Pgm-2</i>	<i>Tpi-1</i>
Easter cactus cultivars ^y											
Andre	RG	1	cc	cc	bc	ee	ce	aa	dd	ad	ab
Andromeda	GP	2	cc	cc	ab	ee	de	aa	dd	ab	ab
Annika	BF	3	cc	cc	bb	ee	cd	aa	dd	aa	bb
Auriga	GP	4	cc	cc	bb	ee	ee	aa	dd	aa	ab
Bliss	BF	5	cd	cc	bb	ee	ce	ac	ff	ad	bb
Caprice	RG	6	cc	cc	ab	ee	dd	aa	dd	aa	ab
Cassiopeia	GP	7	cc	cc	bb	ee	cd	aa	df	ab	bb
Cetus	GP	8	cc	cc	bb	ee	ce	aa	df	bb	ab
China Rose	RG	9	cc	cc	ab	ee	ee	aa	dd	ab	ab
Christine	RG	10	cc	cc	ab	ee	ce	aa	df	ab	aa
CL1	CL	11	cc	cc	bc	ee	ce	aa	dd	aa	ab
CL2	CL	12	cd	cc	bb	ee	ce	ac	ef	aa	ab
CL3	CL	13	cc	cc	bb	ee	ce	aa	ef	aa	ab
CL16	CL	14	cc	cc	bb	ee	ce	aa	ef	aa	aa
CL32	CL	15	cc	cc	ac	ee	ce	aa	df	ad	ab
Crimson Giant	MD	5	cd	cc	bb	ee	ce	ac	ff	ad	bb
Evita	JV	6	cc	cc	ab	ee	dd	aa	dd	aa	ab
Flash	RG	16	cc	cc	ab	ee	de	aa	df	ab	aa
France	RG	17	cc	cc	ab	ee	ce	aa	df	ab	bb
Gaertneri Giant	RG	2	cc	cc	ab	ee	de	aa	dd	ab	ab
Hatherton Star	RG	17	cc	cc	ab	ee	ce	aa	df	ab	bb
Laura Ann	RG	9	cc	cc	ab	ee	ee	aa	dd	ab	ab
Leo's Pink	RG	18	cc	cc	ab	ee	ce	aa	dd	ab	ab
Mira	GP	19	cc	cc	ab	ee	ce	aa	df	ab	ab
Monarch	RG	16	cc	cc	ab	ee	de	aa	df	ab	aa
Orion	GP	20	cc	cc	bb	ee	ce	aa	dd	ab	ab
Pink Perfection	RG	21	cc	cc	bb	ee	de	aa	df	ab	aa
Purple Pride	JV	22	cc	cc	bb	ee	de	aa	dd	ab	bb
Rainbow	RG	23	cc	cc	ab	ee	de	aa	ef	aa	aa
Red Pride	JV	2	cc	cc	ab	ee	de	aa	dd	ab	ab
Rood	JV	24	cc	cc	bb	ee	cc	aa	df	bb	bb
Shocking Pink	RG	25	cc	cc	ab	ee	ce	aa	df	aa	ab
Sutter's Gold	RG	14	cc	cc	bb	ee	ce	aa	ef	aa	aa
Thor-Anne	GT	7	cc	cc	bb	ee	cd	aa	df	ab	bb
Thor-Ina	GT	26	cc	cc	bb	ee	cc	aa	dd	bd	ab
Vista Delight	RG	9	cc	cc	ab	ee	ee	aa	dd	ab	ab
Yvonne Pelham	RG	10	cc	cc	ab	ee	ce	aa	df	ab	aa
Species clones											
<i>H. gaertneri</i> 06524	BG	24	cc	cc	bb	ee	cc	aa	df	bb	bb
<i>H. herminiae</i> 36170	BH	27	ee	bb	---	bb	aa	bb	aa	cc	bb
<i>H. rosea</i> AG941	AG	28	cc	cc	aa	ee	ee	aa	dd	bb	aa
<i>H. rosea</i> AG942	AG	28	cc	cc	aa	ee	ee	aa	dd	bb	aa
<i>H. rosea</i> EC500	RG	28	cc	cc	aa	ee	ee	aa	dd	bb	aa
<i>H. rosea</i> MD861	MD	9	cc	cc	ab	ee	ee	aa	dd	ab	ab
<i>H. salicornioides</i> HE931	HE	29	aa	aa	---	bc	aa	bb	ab	cc	bb
<i>H. salicornioides</i> H921-2	MA	30	ae	aa	---	cc	bd	bb	ac	ce	aa
<i>H. salicornioides</i> LG891	LG	31	ce	aa	---	cc	dd	bb	cc	ee	ac
<i>H. salicornioides</i> UM875	MA	32	ae	aa	---	cd	bd	bb	aa	bc	ab
<i>H. salicornioides</i> 01625	BG	33	be	aa	---	ac	ad	bb	aa	be	aa
<i>H. salicornioides</i> 52257	HG	33	be	aa	---	ac	ad	bb	aa	be	aa

^zAG = Abbey Gardens, Carpinteria, Calif.; BF = Bliss Farms, Woodcliff Lake, N.J.; BG = Botanischer Garten, Universität Bonn, Bonn, Germany; BH = Botanischer Garten, Universität Heidelberg, Heidelberg, Germany; CL = Crystal Lake Greenhouses, Carver, Mass.; GP = Gartneriet PKM, Odense, Denmark; GT = Gartneriet Thoruplund, Odense, Denmark; HE = Hermann Englemann Greenhouses, Apopka, Fla.; HG = Huntington Botanical Gardens, San Marino, Calif.; JV = J. de Vries Potplantencultures, Aalsmeer, The Netherlands; LG = Logee's Greenhouses, Danielson, Conn.; MA = Univ. of Massachusetts, Amherst; MD = Univ. of Maryland, College Park; RG = Rainbow Gardens, Vista, Calif.

^yClones of unknown parentage. Most cultivars of Easter cactus are complex *H. ×graeseri* hybrids, but some may be selections of *H. gaertneri* or *H. rosea*.

Table 2. The relative mobility (R_f) of each allele scored in the isozyme survey of 49 *Hatiora* accessions. The R_f values represent the means from three or more gels.

Locus	Allele	Mean R_f	Locus	Allele	Mean R_f
<i>Aat-1</i>	<i>a</i>	0.50	<i>Mdh-1</i>	<i>a</i>	0.63
	<i>b</i>	0.48		<i>b</i>	0.58
	<i>c</i>	0.45		<i>c</i>	0.53
	<i>d</i>	0.38	<i>Mdh-2</i>	<i>a</i>	0.39
	<i>e</i>	0.35	<i>Mdh-3</i>	<i>a</i>	0.20
<i>Aat-2</i>	<i>a</i>	0.36	<i>Pgm-1</i>	<i>a</i>	0.70
	<i>b</i>	0.34		<i>b</i>	0.67
	<i>c</i>	0.32		<i>c</i>	0.65
<i>Gpi-1</i>	<i>a</i>	0.43	<i>Pgm-2</i>	<i>d</i>	0.62
	<i>b</i>	0.39		<i>e</i>	0.57
	<i>c</i>	0.36		<i>f</i>	0.55
<i>Gpi-2</i>	<i>a</i>	0.32		<i>a</i>	0.48
	<i>b</i>	0.30		<i>b</i>	0.46
	<i>c</i>	0.27		<i>c</i>	0.44
	<i>d</i>	0.25		<i>d</i>	0.42
	<i>e</i>	0.23		<i>e</i>	0.39
<i>Lap-1</i>	<i>a</i>	0.72	<i>Skd-1</i>	<i>a</i>	0.46
	<i>b</i>	0.70	<i>Tpi-1</i>	<i>a</i>	0.52
	<i>c</i>	0.68	<i>Tpi-2</i>	<i>a</i>	0.38
	<i>d</i>	0.63		<i>b</i>	0.32
	<i>e</i>	0.59		<i>c</i>	0.29

gels and 10 μ L for AAT; 7.5% gels and 5 μ L for GPI and LAP; 7.5% gels and 10 μ L for MDH, PGM, and SKD; and 10% gels and 5 μ L for TPI. Gels were stained according to the recipes of Wendel and Weeden (1989).

Mendelian inheritance was demonstrated for the monomeric enzyme PGM and dimeric enzymes AAT, GPI, MDH, and TPI in *Hatiora* (O'Leary and Boyle, 1998a). Mendelian inheritance was demonstrated for the monomers LAP and SKD in the closely related genus *Schlumbergera* (O'Leary and Boyle, 1998b).

DATA COLLECTION AND ANALYSIS. Isozyme loci and allele designations are as described in O'Leary and Boyle (1998a, 1998b). The percentage of polymorphic loci, mean number of alleles per locus, and average heterozygosity were calculated separately for the following two groups: 1) clones of Easter cactus (= *H. gaertneri*, *H. ×graeseri*, and *H. rosea*), and 2) all *Hatiora* clones. Mean number of alleles per locus and average heterozygosity were calculated for all isozyme loci (monomorphic as well as polymorphic).

Results and Discussion

In total, 13 loci and 42 alleles were identified among the 49 accessions, and 9 of the 13 loci (69%) were polymorphic (Table 2). Phenotypes for *Gpi-1*, *Mdh-3*, *Skd-1*, and *Tpi-1* were not determined for the six *H. salicornioides* clones and *H. herminiae* 36170 due to poor resolution of bands. *Mdh-2*, *Mdh-3*, *Skd-1*, and *Tpi-1* were monomorphic in all clones that were surveyed (49 clones for *Mdh-2* and 42 clones for *Mdh-3*, *Skd-1*, and *Tpi-1*). Among the polymorphic loci, the number of alleles per locus ranged from three (*Aat-2*, *Gpi-1*, *Mdh-1*, and *Tpi-2*) to six (*Pgm-1*) (Table 2).

Several alleles occurred in low frequency. For example, *Aat-1d* occurred only in 'Bliss' 'CL2', and 'Crimson Giant', *Gpi-1c* was detected only in 'Andre', 'CL1', and 'CL32', and *Tpi-2c* was present only in *H. salicornioides* 'LG891' (Table 1). Some alleles were restricted to a particular taxon. For *Aat-2*, allele *a* occurred only in *H. salicornioides*, allele *b* was restricted to *H. herminiae* 36170,

Table 3. *Hatiora* cultivars with identical isozyme profiles.

Isozyme profile	Clones	Clonal differentiation possible by other phenotypic characters?
2	Andromeda, Gaertneri Giant, Red Pride	Yes
5	Bliss, Crimson Giant	No
6	Caprice, Evita	No
7	Cassiopeia, Thor-Anne	Yes
9	China Rose, Laura Ann, MD861, Vista Delight	Yes
10	Christine, Yvonne Pelham	Yes
14	CL 16, Sutter's Gold	Yes
16	Flash, Monarch	Yes
17	France, Hatherton Star	Yes
24	Rood, 06524	Yes
28	AG941, AG942, EC500	Yes
33	01625, 52257	No

Table 4. Genetic diversity statistics for the Easter cactus clones and for the entire *Hatiora* germplasm collection.

Group	Clones analyzed (no.)	Percent polymorphic loci ²	Mean no. alleles/locus	Mean heterozygosity
Easter cactus clones ^{3,x}	40	54	1.85	0.21
All clones ^x	46	69	3.23	0.30

²Calculations based on 13 loci.³Easter cactus cultivars plus the authentic clones of *H. gaertneri* and *H. rosea* (see Table 1).^xDuplicate accessions excluded from diversity calculations (see Table 3).

and allele *c* occurred only in *H. gaertneri*, *H. ×graeseri*, and *H. rosea* (Table 1). Five loci (*Gpi-2*, *Lap-1*, *Mdh-1*, *Pgm-1*, *Pgm-2*) exhibited a similar pattern of allelic distribution, with one set of alleles present only in *H. salicornioides* and *H. herminiae* (subgenus *Hatiora*) and another set of alleles limited to *H. gaertneri*, *H. ×graeseri*, and *H. rosea* (subgenus *Rhipsalidopsis*) (Table 1). Hence, the distribution of alleles for these five loci corresponded with the subgeneric classification of the genus based on morphological characters (Barthlott, 1987).

A total of 33 isozyme profiles were identified among the 49 accessions that were surveyed (Table 1). Twenty-one accessions (43%) could be distinguished solely on the basis of their isozyme profiles. The remaining 28 accessions shared isozyme profiles with other clones and could be classified into one of 12 groups (Table 3). Cultivars within nine of these groups could be distinguished from one another by other phenotypic characters, such as flower and/or phylloclade morphology. Clones within three groups (profiles 5, 6, and 33) could not be distinguished either by isozymes or morphological characters, and most likely represent identical clones. These results demonstrate that isozyme markers can aid in identifying *Hatiora* clones, especially when used in conjunction with morphological characters.

Percent polymorphic loci, mean number of alleles per locus, and mean heterozygosity were 69, 3.23, and 0.30, respectively, for the entire germplasm collection (Table 4). For the Easter cactus clones that were surveyed, percent polymorphic loci, mean number of alleles per locus, and mean heterozygosity were 54, 1.85, and 0.21, respectively (Table 4). The mean number of alleles per locus in the entire collection is nearly twice that found in the Easter cactus clones alone (= *H. gaertneri*, *H. rosea*, and *H. ×graeseri*). This difference is attributable to the *H. herminiae* and *H. salicornioides* clones, which contain many alleles not present in clones of *H. gaertneri*, *H. rosea*, or *H. ×graeseri* (Table 1). Hence, the clones of *H. herminiae* and *H. salicornioides* comprise only a small portion of the germplasm collection but contribute substantially to its genetic diversity.

Isozyme markers were also useful in providing evidence of parentage for several *Hatiora* clones. For example, we wanted to confirm the parentage of *H. salicornioides* H921-2, a putative F₁ hybrid of the cross LG891 × UM875. The presence of *Aat-1a*, *Lap-1b*, *Pgm-1a*, and *Pgm-2c* provide evidence that H921-2 is an F₁ hybrid (Table 1). *Pgm-1* proved particularly useful for confirming that crosses yielded authentic F₁ hybrids. For instance, we were able to confirm that seedlings of the cross 'Crimson Giant' × 'Red Pride' were F₁ hybrids. 'Crimson Giant' was homozygous for *Pgm-1f* and

'Red Pride' was homozygous for *Pgm-1d* (Table 1), whereas the 62 progeny from the cross exhibited the expected (df) phenotype. These examples demonstrate that isozymes can be a useful tool for confirming hybridity in *Hatiora*.

This study indicates that substantial isozyme polymorphism is present in the *Hatiora* germplasm collection. The high level of polymorphism among the clones permitted identification of some cultivars, quantification of genetic diversity present in germplasm collection, and verification of hybridity for some F₁ progeny. However, more polymorphic isozyme loci will need to be identified before *Hatiora* clones can be distinguished solely by their isozyme profiles.

Literature Cited

- Barthlott, W. 1987. New names in Rhipsalidinae (Cactaceae). *Bradleya* 5:97–100.
- Barthlott, W. and N.P. Taylor. 1995. Notes towards a monograph of Rhipsalideae (Cactaceae). *Bradleya* 13:43–79.
- Boyle, T.H. 1995. Flowering responses of Easter cactus clones at optimal and supraoptimal temperatures. *HortScience* 30:613–616.
- Hames, B.D. 1981. An introduction to polyacrylamide gel electrophoresis, p. 1–91. In: B.D. James and D. Rickwood (eds.). *Gel electrophoresis of proteins: A practical approach*. IRL, Oxford, England.
- Hunt, D. 1992. *Hatiora*, p. 25. In: D. Hunt and N.P. Taylor (eds.). *Notes on the miscellaneous genera of Cactaceae* (2). *Bradleya* 10:17–32.
- Lagerheim, G. 1912. *Rhipsalis rosea* Lagerh. N. Sp. *Svensk Bot. Tidskr.* 6:717–720.
- Meier, E. 1995. Easter cacti (*Rhipsalidopsis*; Cactaceae). *Haseltonia* 3:10–24.
- Nielsen, G. 1985. The use of isozymes as probes to identify and label plant varieties and cultivars, p. 1–32. In: M.C. Rattazzi, J.G. Scandalios, and G.S. Whitt (eds.). *Isozymes: Current topics in biological and medical research*. Liss, New York.
- O'Leary, M.C. and T.H. Boyle. 1998a. Inheritance and linkage relationships of isozymes in Easter cactus. *J. Amer. Soc. Hort. Sci.* 123:98–103.
- O'Leary, M.C. and T.H. Boyle. 1998b. Segregation distortion at isozyme locus *Lap-1* in *Schlumbergera* (Cactaceae) is caused by linkage with the gametophytic self-incompatibility (*S*) locus. *J. Hered.* 89:206–210.
- Regel, E. 1884. *Epiphyllum Russelianum* Hook. var. *Gärtneri*. *Gartenflora* 33:323–324.
- Weeden, N.F. 1989. Applications of isozymes in plant breeding. *Plant Breeding Rev.* 6:11–54.
- Wendel, J.F. and N.F. Weeden. 1989. Visualization and interpretation of plant isozymes, p. 5–45. In: D.E. Soltis and P.S. Soltis (eds.). *Isozymes in plant biology*. Dioscorides, Portland, Ore.
- Yndgaard, F. and A. Hoskuldsson. 1985. Electrophoresis: A tool for genebanks. *Plant Genet. Resources Nwsl.* 63:34–40.