Diversity and Distribution of Isozymes in a *Schlumbergera* (Cactaceae) Clonal Germplasm Collection

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ABSTRACT. A germplasm collection of 59 *Schlumbergera* Lemaire clones was assayed for isozymes of aspartate aminotransferase, glucose-6-phosphate isomerase, leucine aminopeptidase, malate dehydrogenase, phosphoglucosmutase, shikimate dehydrogenase, and triosephosphate isomerase. The collection included cultivars of holiday cactus [*S. truncata* (Haworth) Moran and *S. ×buckleyi* (T. Moore) Tjaden] plus accessions of *S. kautskyi* (Horobin & McMillan) N.P. Taylor, *S. opuntoides* (Löfgren & Dusén) D. Hunt, *S. orssichiana* Barthlott & McMillan, *S. russelliana* (Hooker) Britton & Rose, *S. ×exotica* Barthlott & Rauh, and *S. ×reginae* McMillan & Orssich. Twelve loci with 36 alleles were detected. Percent polymorphic loci, mean number of alleles per locus, and mean heterozygosity were 83, 3.00, and 0.24, respectively, for the entire collection. Forty-one clones (69%) could be distinguished solely on the basis of their isozyme profiles, but the remaining 18 clones shared profiles with one or two other clones. Isozymes proved useful for determining the parentage of some clones and verifying that some progeny were interspecific hybrids. About 28% of the genetic diversity within the entire collection is unique to four *Schlumbergera* species that have scarcely been exploited for breeding holiday cactus cultivars.

The genus *Schlumbergera* is comprised of six species of epiphytic or epilithic shrubs native to southeastern Brazil (Barthlott and Taylor, 1995; Hunt, 1969). The genus can be divided into two groups based on vegetative characters. *Schlumbergera kautskyi* (Horobin & McMillan) N.P. Taylor, *S. orssichiana* Barthlott & McMillan, *S. russelliana* (Hooker) Britton & Rose, and *S. truncata* (Haworth) Moran have flattened, crenate or truncate phylloclades with areoles confined to the margins and apices, whereas *S. microsphaerica* (K. Schumann) Hövel and *S. opuntoides* (Löfgren & Dusén) D. Hunt have cylindric, terete or obovoid phylloclades with areoles distributed over the entire surface (Barthlott and Taylor, 1995). *Schlumbergera truncata*, also known as crab cactus or Thanksgiving cactus, is the most commonly grown species in the genus and was introduced into cultivation around 1817 (Tjaden, 1964). The remaining five *Schlumbergera* species are rare in cultivation. Three interspecific hybrids have been bred: *S. ×exotica* Barthlott & Rauh (= *S. opuntoides* × *S. truncata*); *S. ×reginae* McMillan & Orssich (= *S. orssichiana* × *S. truncata*); and *S. ×buckleyi* (T. Moore) Tjaden (= *S. russelliana* × *S. truncata*). *Schlumbergera ×buckleyi*, better known as Christmas cactus, has been in cultivation since the 1850s (Hunt, 1981; Tjaden, 1964), whereas *S. ×exotica* and *S. ×reginae* are of relatively recent origin (Barthlott and Rauh, 1977; Horobin and McMillan, 1985) and are not cultivated extensively. Most of the 1,000+ *Schlumbergera* cultivars in existence are either *S. truncata* or complex *S. ×buckleyi* hybrids (McMillan and Horobin, 1995). Thanksgiving cactus and Christmas cactus are known collectively as holiday cactus (Boyle, 1997).

Much of the germplasm resources within *Schlumbergera* have not been exploited for breeding commercial cultivars (McMillan

and Horobin, 1995). A *Schlumbergera* clonal germplasm collection was assembled at the University of Massachusetts to serve as a resource for a breeding project. One of the project's initial objectives was to develop a rapid and unambiguous means of identifying clones to minimize duplicate accessions in the collection. Presently, *Schlumbergera* cultivars are distinguished by their phylloclade and flower morphology, flower color, growth habit, and flowering time under natural photoperiods (Barthlott and Rauh, 1977; Boyle, 1997; McMillan and Horobin, 1995). Most of these characters, however, are influenced by environmental conditions, thus limiting their usefulness for distinguishing cultivars. Additionally, fewer traits exist for identifying cultivars when plants are in a vegetative state. Isozymes have been used to identify cultivars in many crops (Nielsen, 1985; Weeden, 1989), including some cacti (O'Leary and Boyle, 1999). Typically, isozymes exhibit simple inheritance, codominance, complete penetrance, and consistent expression under a wide range of environmental conditions (Weeden and Wendel, 1989). Isozymes can also be used in plant breeding programs to verify hybridity and assess the level of genetic variation in germplasm collections (Weeden, 1989; Yndgaard and Hoskuldsson, 1985). Although *Schlumbergera* exhibits considerable phenotypic variation (Barthlott and Rauh, 1977; Hunt, 1969), the extent of genetic variation in cultivated material remains unknown. The objectives of the present study were to: 1) ascertain the diversity and distribution of isozyme alleles in a collection of *Schlumbergera* clones; and 2) determine if isozymes could be used to distinguish clones, identify duplicate accessions, elucidate pedigrees, and verify hybridity.

Materials and Methods

PLANT MATERIAL. Fifty-nine *Schlumbergera* clones were included in this study. Most of the clones were holiday cactus cultivars, but accessions of *S. kautskyi*, *S. opuntoides*, *S. orssichiana*, *S. russelliana*, *S. ×exotica*, and *S. ×reginae* were also examined (Table 1). All *Schlumbergera* species except for *S. microsphaerica* were represented in the study. The holiday cactus clones surveyed included commercial cultivars (both contempo-

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

Clone	Source ^z	Isozyme	Locus									
		profile	<i>Aat-1</i>	<i>Aat-2</i>	<i>Gpi-2</i>	<i>Lap-1</i>	<i>Mdh-1</i>	<i>Mdh-2</i>	<i>Pgm-1</i>	<i>Pgm-2</i>	<i>Skd-1</i>	<i>Tpi-2</i>
Holiday cactus cultivars ^y												
Abendroth No. 2	DK	1	aa	bb	cc	cd	bb	aa	bc	ac	cc	bb
Abendroth No. 6	DK	2	aa	bc	bc	bb	bb	bb	bb	cc	cc	bb
Abendroth No. 13	DK	3	aa	bb	ac	cc	bb	aa	bb	cd	cd	ab
Alexis	GP	4	aa	bc	ab	cc	bb	aa	bb	cc	cd	ab
Apricot	DK	5	aa	ab	bc	cc	bb	aa	bb	cc	cc	aa
Barbara	GP	6	aa	cc	ab	cc	bb	aa	bb	cc	cd	ab
Bridgeport	CO	7	aa	bc	bb	bb	ab	aa	cc	cc	cc	bb
Cambridge	CO	8	aa	cc	bc	bb	ab	aa	bc	cc	cc	bb
Christmas Cactus	MA	5	aa	ab	bc	cc	bb	aa	bb	cc	cc	aa
Christmas Charm	CO	9	aa	bc	ab	bb	ab	aa	bb	cc	cd	bb
Christmas Cheer	RG	10	aa	bc	bb	bb	ab	aa	bc	cc	cd	bb
Christmas Fantasy	CO	11	aa	bc	ab	bb	ab	aa	cc	cc	cc	bb
Claudia	GP	12	aa	ab	ab	cc	bb	aa	bb	cc	cc	ab
Dark Marie	GP	13	aa	bc	bb	cd	bb	aa	bb	cc	cc	bb
Eva	JV	14	aa	bb	bb	dd	ab	aa	bc	cc	cc	bb
Gina	GP	15	aa	bc	bb	bb	bb	aa	bb	cc	cd	bb
Gold Charm	CO	8	aa	cc	bc	bb	ab	aa	bc	cc	cc	bb
Huntington 50447	HG	5	aa	ab	bc	cc	bb	aa	bb	cc	cc	aa
Kris Kringle	CO	16	aa	ab	ab	cc	ab	aa	bb	cc	cd	ab
Kris Kringle II	CO	17	aa	ab	bb	cc	ab	aa	dd	cc	cd	bb
Lavender Doll	CO	18	aa	ac	ab	bc	bb	aa	bb	cc	cc	bb
Lavender Doll II	CO	19	aa	bc	bb	bb	ab	aa	bd	cc	cd	bb
Lilofee	DK	20	aa	bc	ab	bd	bb	aa	bc	cc	cd	bb
Linda	GP	21	aa	ab	bb	bd	ab	aa	bc	cc	cd	bb
Madisto	GP	22	aa	ac	ab	cd	bb	aa	bb	cc	cc	ab
Madonga	GP	23	aa	bc	ab	cc	bb	aa	bc	cc	cc	bb
Majestic	DK	24	aa	bc	ab	cd	bb	aa	bc	cc	cd	ab
Marie	GP	13	aa	bc	bb	cd	bb	aa	bb	cc	cc	bb
Naomi	JV	25	aa	cc	bb	dd	bb	aa	bc	cc	cd	bb
Noris	DK	26	aa	bc	ab	cc	bb	aa	bb	cc	cc	aa
Peach Parfait	CO	27	aa	cc	ab	cc	ab	aa	bc	cc	cc	bb
Pinkie	RG	22	aa	ac	ab	cd	bb	aa	bb	cc	cc	ab
Purple Devil	DK	26	aa	bc	ab	cc	bb	aa	bb	cc	cc	aa
Red Radiance	CO	28	aa	ac	aa	cc	bb	aa	bb	cc	cd	bb
Rocket	RG	29	aa	bb	bb	bd	bb	aa	bc	cc	cd	bb
Salmonium Rubrum	DK	30	aa	ac	bb	cc	bb	aa	bb	cc	cc	aa
Santa Cruz	CO	31	aa	ab	ab	bb	ab	aa	cc	cc	cd	bb
Sonja	JV	32	aa	ac	ab	cd	bb	aa	bd	cc	cd	ab
Starbrite	LN	33	aa	ab	ab	bc	ab	aa	bc	cc	cc	ab
Thor-Britta	GT	34	aa	bb	bb	dd	bb	aa	bb	cc	cc	bb
Thor-Louise	GT	35	aa	bc	ab	dd	bb	aa	dd	cc	cc	ab
Twilight Tangerine	CO	15	aa	bc	bb	bb	bb	aa	bb	cc	cd	bb
White Christmas	CO	36	aa	bb	bb	bb	ab	aa	cc	cc	cc	bb
Yantra	JV	37	aa	bb	bb	bb	bb	aa	bb	cc	cc	bb
Zaraika	JV	37	aa	bb	bb	bb	bb	aa	bb	cc	cc	bb
<i>Schlumbergera</i> species and interspecific hybrids												
<i>S. ×exotica</i> Exotica	DK	38	ab	bb	bc	ac	bb	aa	ab	ce	bc	ab
<i>S. ×exotica</i> Minibelle	RG	39	ab	bb	ab	cc	bb	aa	ad	ce	bc	ab
<i>S. kautskyi</i> clonotype	DK	40	aa	bb	bb	dd	bb	aa	bb	cc	ee	bb
<i>S. opuntoides</i> 01537	BG	41	bb	bb	ab	cc	bb	aa	aa	ce	ab	ab
<i>S. opuntoides</i> RG clone	RG	42	bb	bb	ab	ac	bb	aa	aa	ce	ab	ab
<i>S. orssichiana</i> AG clone	AG	43	aa	bd	bb	cd	bb	aa	bb	bc	de	ab
<i>S. orssichiana</i> CO clone	CO	43	aa	bd	bb	cd	bb	aa	bb	bc	de	ab
<i>S. orssichiana</i> RG clone	RG	43	aa	bd	bb	cd	bb	aa	bb	bc	de	ab
<i>S. ×reginae</i> 05584	BG	44	aa	bd	bb	bc	ab	aa	bb	cc	ce	ab
<i>S. russelliana</i> 02636	BG	45	aa	bb	bb	cc	bb	aa	bb	cc	cc	bb
<i>S. russelliana</i> 33160	BH	46	ab	bb	aa	cc	bb	aa	bb	cc	cc	aa
<i>S. russelliana</i> 34533	HG	47	aa	bb	bb	dd	bb	aa	bb	cc	ab	ab
<i>S. russelliana</i> Hunt 6484	DK	48	aa	bb	bb	cc	bb	aa	bb	cc	cc	aa
<i>S. russelliana</i> JFH Clone 1	DK	49	aa	bb	bb	ce	bb	aa	bb	cc	cc	aa

²AG = Abbey Garden, Carpinteria, Calif.; BG = Botanischer Garten, Universität Bonn, Bonn, Germany; BH = Botanischer Garten, Universität Heidelberg, Heidelberg, Germany; CO = B.L. Cobia, Inc., Winter Garden, Fla.; DK = Dolly Kölli, Mashpee, Mass.; GP = Gartneriet PKM, Odense, Denmark; GT = Gartneriet Thoruplund, Odense, Denmark; HG = Huntington Botanical Gardens, San Marino, Calif.; JV = J. de Vries Potplantencultures, Aalsmeer, The Netherlands; LN = Leavitt's Nursery, Lehighton, Pa.; MA = Univ. of Massachusetts, Amherst; RG = Rainbow Gardens, Vista, Calif.

³Clones of unknown parentage. Holiday cactus cultivars are either complex *S. ×buckleyi* hybrids or *S. truncata* selections.

rary and obsolete) plus older clones of horticultural significance (McMillan and Horobin, 1995).

ISOZYME EXTRACTION, SEPARATION, AND STAINING. Enzymes were extracted from phylloclade tissue. Isozyme extraction and separation procedures were described previously (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 was 0.025 M Tris and 0.192 M glycine (pH 8.3) for all enzyme systems. 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), phosphoglucosmutase (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% gels and 10 µL for AAT; 7.5% gels and 10 µL for GPI, LAP, MDH, PGM, and SKD; and 10% gels and 5 µL for TPI. Gels were stained according to procedures of Wendel and Weeden (1989).

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

DATA COLLECTION AND ANALYSIS. Isozyme loci and allele designations were described in O'Leary and Boyle (1998a, 1998b). Percent polymorphic loci, mean number of alleles per locus, and mean heterozygosity were calculated separately for the following three groups: 1) the holiday cactus gene pool (= *S. × buckleyi* and *S. truncata* clones) representing readily exploitable germplasm for cultivar development; 2) the *Schlumbergera* species gene pool (= *S. kautskyi*, *S. opuntoides*, *S. orssichiana*, and *S. russelliana* clones) comprising germplasm that has scarcely been used for breeding; and 3) all *Schlumbergera* clones. Mean number of alleles per locus and mean heterozygosity were calculated for all isozyme loci, monomorphic as well as polymorphic.

Results and Discussion

Thirty-six alleles were detected among the 12 loci that were surveyed (Table 2). *Gpi-1* and *Mdh-3* were monomorphic but the other 10 loci were polymorphic. Among the polymorphic loci, the number of alleles per locus ranged from two (*Aat-1*, *Mdh-1*, *Mdh-2*, and *Tpi-2*) to five (*Lap-1*, *Pgm-2*, and *Skd-1*).

Several alleles were limited to one or a few clones. For example, alleles *Lap-1e*, *Mdh-2b*, *Pgm-2a*, and *Pgm-2d* were detected only in *S. russelliana* JFH Clone 1, Abendroth No. 6, Abendroth No. 2, and Abendroth No. 13, respectively (Table 1). Some alleles were restricted to specific taxa. *Aat-2d* occurred only in *S. orssichiana* and *S. × reginae* 05584, whereas *Lap-1a*, *Pgm-1a*, and *Pgm-2e* were detected only in *S. opuntoides* and *S. × exotica* (Table 1). Due to the paucity of authentic *Schlumbergera* species in this collection, it was not possible to discern taxonomic relationships within this genus using isozyme profiles. However, results suggest that isozymes may aid in distinguishing *Schlumbergera* species and therefore may be useful for resolving taxonomic questions about the genus.

A total of 49 isozyme profiles were identified among the 59 *Schlumbergera* clones (Table 1). Forty-one clones (69%) could be distinguished solely by their isozyme profiles. Among the 45 holiday cactus accessions, 30 (67%) displayed unique isozyme profiles. Eighteen *Schlumbergera* clones shared isozyme profiles with one or two other clones and could be classified into one of eight groups. Clones within six of these groups (profiles 5, 8, 12, 15, 26, and 37) could be distinguished from one another by other characters, i.e., morphological features, flower color, and/or growth habit. As expected, we were unable to distinguish sports using isozyme profiles. For example, the mutant 'Dark Marie' possessed the same isozyme profile as its parent ('Marie'), but these two cultivars differed in flower and phylloclade pigmentation. Clones within two groups (profiles 22 and 43) were indistinguishable by isozymes or other characters, and probably represent identical clones.

Isozymes were used to address some questions of parentage. 'Christmas Cactus' (Table 1) is the clone that is grown commonly as a winter-flowering houseplant in the United States. 'Christmas Cactus' is intermediate between *S. truncata* and *S. russelliana* with

Table 2. The relative mobility (R_f) of each allele scored in the isozyme survey of 59 *Schlumbergera* 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>	0.52	<i>Mdh-3</i>	<i>a</i>	0.36
	<i>b</i>	0.50			
<i>Aat-2</i>	<i>a</i>	0.41	<i>Pgm-1</i>	<i>a</i>	0.64
	<i>b</i>	0.39		<i>b</i>	0.59
	<i>c</i>	0.31		<i>c</i>	0.56
	<i>d</i>	0.27		<i>d</i>	0.54
<i>Gpi-1</i>	<i>a</i>	0.60	<i>Pgm-2</i>	<i>a</i>	0.47
<i>Gpi-2</i>	<i>a</i>	0.49		<i>b</i>	0.45
	<i>b</i>	0.43		<i>c</i>	0.40
	<i>c</i>	0.39		<i>d</i>	0.37
<i>Lap-1</i>	<i>a</i>	0.60	<i>Skd-1</i>	<i>e</i>	0.35
	<i>b</i>	0.58		<i>a</i>	0.47
	<i>c</i>	0.56		<i>b</i>	0.44
	<i>d</i>	0.51		<i>c</i>	0.39
	<i>e</i>	0.47		<i>d</i>	0.37
<i>Mdh-1</i>	<i>a</i>	0.62	<i>Tpi-2</i>	<i>e</i>	0.35
	<i>b</i>	0.59		<i>a</i>	0.38
<i>Mdh-2</i>	<i>a</i>	0.44		<i>b</i>	0.33
	<i>b</i>	0.42			

Table 3. Distribution of isozyme alleles in four different groups of *Schlumbergera* accessions.

Locus	Holiday cactus cultivars ^c			
	Truncata group ^y	Intermediate group ^x	Buckleyi group ^w	<i>S. russelliana</i> clones ^z
<i>Aat-1</i>	a	a	a	a,b
<i>Aat-2</i>	a,b,c	a,b,c	a,b,c	b
<i>Gpi-2</i>	a,b,c	a,b,c	a,b,c	a,b
<i>Lap-1</i>	b,c,d	b,c,d	c	c,d,e
<i>Mdh-1</i>	a,b	a,b	b	b
<i>Mdh-2</i>	a,b	a	a	a
<i>Pgm-1</i>	b,c	b,c,d	b	b
<i>Pgm-2</i>	a,c	c,d	c	c
<i>Skd-1</i>	c,d	c,d	c	a,b,c
<i>Tpi-2</i>	b	a,b	a	a,b

^zRefer to Table 1.^yTruncata group cultivars (Abendroth No. 2, Abendroth No. 6, Bridgeport, Cambridge, Christmas Cheer, Christmas Fantasy, Dark Marie, Eva, Gold Charm, Marie, Peach Parfait, Rocket, Santa Cruz, Thor-Britta, Twilight Tangerine, and White Christmas) have truncate phylloclades, distinctly zygomorphic flowers that are held at an angle above a horizontal plane, yellow pollen, and terete pericarpels.^xIntermediate group cultivars (Abendroth No. 13, Alexis, Barbara, Christmas Charm, Claudia, Gina, Huntington 50447, Kris Kringle, Kris Kringle II, Lavender Doll, Lavender Doll II, Lilofee, Linda, Madisto, Madonga, Majestic, Naomi, Pinkie, Red Radiance, Sonja, Starbrite, Thor-Louise, Yantra, and Zairaika) exhibit characteristics that are intermediate between the Truncata and Buckleyi groups.^wBuckleyi group cultivars (Apricot, Christmas Cactus, Noris, Purple Devil, and Salmoneum Rubrum) have crenate phylloclades, pendulous flowers that are more or less actinomorphic, pink pollen, and angled pericarpels.

regard to morphological characters, which suggests that it is an interspecific hybrid of these two species (Hunt, 1981; Tjaden, 1964). Its exact parentage, however, is unknown. During the 1840s, Wilbraham Buckley of Tooting, England, produced three hybrid seedlings by pollinating one *S. russelliana* flower with *S. truncata* 'Ruckerianum' (Moore, 1852). It has been surmised that 'Christmas Cactus' is one of Buckley's original hybrids (McMillan and Horobin, 1995; Tjaden, 1964). Moran (1953) suggested that Buckley's pollen parent might have been a hybrid of *S. russelliana* and *S. truncata* rather than pure *S. truncata*. Unfortunately, neither of the clones that Buckley used for hybridization are known to exist today. The isozyme data may shed some light on the origin of 'Christmas Cactus'. Table 3 lists the distribution of isozyme alleles in four groups of *Schlumbergera* accessions: holiday cactus clones with *S. truncata*-like characters (=Truncata group), holiday cactus clones with *S. ×buckleyi*-like characters (=Buckleyi group), holiday cactus clones with characters that are intermediate between the Truncata and Buckleyi groups (=Intermediate group), and *S. russelliana* clones. The last group includes five of the seven known *S. russelliana* clones in cultivation (McMillan and Horobin, 1995). The most informative locus is *Tpi-2*. The five clones in the Buckleyi group and most of the seven *S. russelliana* clones have the *Tpi-2a* allele, whereas the 16 clones in the Truncata group are homozygous for *Tpi-2b* (Tables 1 and

3). These results suggest that *Tpi-1a* and *Tpi-1b* in holiday cactus originated from *S. russelliana* and *S. truncata*, respectively. If this hypothesis is true, then all clones in the Buckleyi group (including 'Christmas Cactus') could not be F₁ hybrids of *S. truncata* and *S. russelliana* because they are homozygous for *Tpi-2a* (Table 1). If 'Christmas Cactus' is in fact one of Buckley's original hybrids, then it could be a BC₁ hybrid (*S. russelliana* × *S. ×buckleyi*) as Moran (1953) had suggested.

Isozymes were used to resolve two apparent cases of misidentification. Documentation provided with 05584 stated that it was the *S. orssichiana* clonotype, i.e., the original clone used to describe the taxon, but 05584's phenotype suggested that it was a hybrid of *S. orssichiana* and *S. truncata* (= *S. ×reginae*). A comparison of isozyme profiles for the authentic *S. orssichiana* clonotype (=AG clone) and 05584 indicates differences for *Lap-1*, *Mdh-1*, *Pgm-2*, and *Skd-1*. Hence, 05584 could not be the *S. orssichiana* clonotype. Also, three alleles (*Lap-1b*, *Mdh-1a*, and *Skd-1c*) that were present in 05584 were also common in holiday cactus, but these three alleles did not occur in AG clone (Table 1). These results support the contention that 05584 is a *S. ×reginae* hybrid. The second case concerned Huntington 50447, a field-collected plant that was identified as *S. truncata* by its collectors. However, its phylloclades and flowers were intermediate between *S. truncata* and *S. russelliana*. The *Tpi-2* phenotype for

Table 4. Genetic diversity statistics for the holiday cactus clones, the *Schlumbergera* species clones, and for the entire *Schlumbergera* germplasm collection.

Group	Clones analyzed (no.)	Percent polymorphic loci ^z	Mean no. alleles/locus	Mean heterozygosity
Holiday cactus ^{y,x}	44	75	2.17	0.24
<i>Schlumbergera</i> species ^{w,x}	9	67	2.25	0.17
All clones ^x	56	83	3.00	0.24

^zCalculations based on 12 loci.^yClones of *S. truncata* and *S. ×buckleyi* (see Table 1).^xDuplicate accessions excluded from diversity calculations (see text).^wClones of *S. kautskyi*, *S. opuntoides*, *S. orssichiana*, and *S. russelliana* (see Table 1).

Huntington 50447 (aa) suggests that its ancestry includes *S. russelliana*, thus corroborating the morphological data.

Isozymes were also useful for verifying that some *Schlumbergera* clones were interspecific F₁ hybrids. For example, 'Exotica' and 'Minibelle' are putative F₁ hybrids of *S. opuntoides* and holiday cactus (*S. truncata* or *S. ×buckleyi*) (McMillan and Horobin, 1995). The presence of *Aat-1b*, *Pgm-1a*, *Pgm-2e*, and *Skd-1b* in 'Exotica', 'Minibelle', and the two *S. opuntoides* clones and their absence in all holiday cactus clones (Table 1) lends support to this claim.

Several cytological studies (Barthlott, 1976; Remski, 1954; Stockwell, 1935) have shown that the genus *Schlumbergera* consists of diploid taxa with a chromosome number of $2n = 2x = 22$. However, four of the holiday cactus cultivars included in this study ('Bridgeport', 'Cambridge', 'Gold Charm', and 'Santa Cruz') are reported to be polyploids (Cobia, 1992; Wade et al., 1985). Isozyme data can provide evidence of polyploidy (Weeden, 1989). A diploid ($2n$) individual has a maximum of two alleles per locus whereas a polyploid (Xn where $X \geq 3$) individual can have up to X alleles per locus. Therefore, polyploids with three or more alleles per locus usually exhibit greater numbers of isozyme bands than diploids. The ploidy level of 'Bridgeport', 'Cambridge', 'Gold Charm', and 'Santa Cruz' could not be deduced from their isozyme phenotypes (Table 1). These four cultivars were heterozygous at several isozyme loci, but at most two bands were detected for the monomers (LAP, PGM, and SKD) and three bands for the dimers (AAT, GPI, MDH, and TPI), as would be expected for diploids. Chyi and Weeden (1984), Murawski et al. (1994), and Nielsen (1980) reported that isozyme genotypes can be deduced in heterozygous polyploids by the relative intensity of the banding patterns. Therefore, it should be possible to distinguish simplex (*abbb*), duplex (*aabb*), and triplex (*aaab*) genotypes in tetraploids. Scoring genotypes by this method assumes that staining intensity is directly proportional to gene dosage. Lack of proportionality between staining intensity and gene dosage can lead to incorrect genotype identification. No attempt was made to deduce ploidy level of the four *Schlumbergera* clones from the relative intensities of isozyme bands.

Percent polymorphic loci, mean number of alleles per locus, and mean heterozygosity were 75, 2.17, and 0.24, respectively, for the 44 holiday cactus clones (Table 4). The genetic diversity statistics for the nine *Schlumbergera* species clones were similar to those for the holiday cactus clones. Percent polymorphic loci, mean number of alleles per locus, and mean heterozygosity were 83, 3.00, and 0.24, respectively, for the entire germplasm collection (Table 4).

Among the 36 alleles that were surveyed, 17 were found in both the holiday cactus clones and the *Schlumbergera* species clones (= *S. kautskyi*, *S. opuntoides*, *S. orssichiana*, or *S. russelliana*) but the remaining 19 alleles were limited to one of these two groups. Nine of the alleles occurred only in the holiday cactus clones whereas the other 10 alleles were restricted to the *Schlumbergera* species clones. Thus, about 28% of the genetic diversity within the entire collection is unique to four *Schlumbergera* species that have scarcely been exploited for breeding holiday cactus cultivars. Incorporating some or all of these *Schlumbergera* species into holiday cactus breeding programs may help broaden the genetic diversity of the crop and enhance efforts to develop novel cultivars for commercial use.

This study demonstrates that substantial isozyme polymorphism is present in the *Schlumbergera* germplasm collection. The high level of isozyme polymorphism enabled us to distinguish most cultivars, identify duplicate accessions, rectify cases of misidentification, and address questions of parentage. We anticipate that isozymes will have practical value for identifying *Schlumbergera* cultivars and protecting breeder's rights.

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