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Isozyme polymorphism and inheritance in Hatiora and Schlumbergera (Cactaceae).

Maureen C. O'Leary
University of Massachusetts Amherst

ISOZYME POLYMORPHISM AND INHERITANCE IN *HATIORA* AND *SCHLUMBERGERA* (CACTACEAE)

A Dissertation Presented

by

MAUREEN C. O'LEARY

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Approved as to style and content by:

Thomas H. Boyle, Chair

Wesley Autio, Member

Robert Bernatzky, Member

David Mulcahy, Member

William Bramlage, Department Head
Plant and Soil Sciences

ABSTRACT

ISOZYME POLYMORPHISM AND INHERITANCE IN *HATIORA* AND *SCHLUMBERGERA* (CACTACEAE)

MAY 1996

MAUREEN C. O'LEARY, B.S., WORCESTER POLYTECHNIC INSTITUTE

M.S., UNIVERSITY OF MASSACHUSETTS AMHERST

Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Professor Thomas H. Boyle

Isozyme analysis was used to identify clones, measure levels of genetic variation within groups of clones, and analyze mating systems in two Cactaceae genera - *Hatiora* and *Schlumbergera*. Isozymes were extracted from phylloclades and pollen and were separated by polyacrylamide gel electrophoresis. The inheritance of aspartate aminotransferase (AAT), glucose-6-phosphate isomerase (GPI), malate dehydrogenase (MDH), phosphoglucomutase (PGM), and triosephosphate isomerase (TPI) was examined in *Hatiora*. Six loci (*Aat-1*, *Gpi-1*, *Mdh-1*, *Pgm-1*, *Pgm-2*, and *Tpi-2*) were analyzed, and results were generally as expected for single loci with codominant alleles. For all six isozyme loci segregation distortion was observed in at least one segregating family. *Aat-1* was linked with *Pgm-1* (26 cM), but the other isozyme loci assorted independently. The inheritance of leucine aminopeptidase (LAP), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKD) was investigated in *Schlumbergera*. Three loci were analyzed (*Lap-1*, *Pgm-1*, and *Skd-1*), and results were generally as expected for single loci with codominant alleles. Significant segregation distortion was observed in at least one segregating family for all three isozyme loci. Disturbed segregation at *Lap-1* was due to tight linkage (7 cM) with the locus controlling gametophytic self-incompatibility (*S*). All three loci assorted independently of each other. In a third study, a *Hatiora* germplasm collection composed of 49 clones was assayed for AAT, GPI, LAP, MDH, PGM, SKD,

and TPI. Thirteen putative loci and 42 putative alleles were identified, and 9 of the 13 loci (69%) were polymorphic. Twenty-two clones (45%) could be distinguished solely on the basis of their isozyme profiles, but the other 27 clones shared isozyme profiles with one to five other clones. Thirteen modern *H.* x *graeseri* cultivars exhibited less genetic diversity than 40 *H. gaertneri*, *H.* x *graeseri*, and *H. rosea* clones representing older and modern cultivars plus field-collected specimens. The difference in genetic diversity was primarily attributed to a loss of alleles during breeding. In a fourth study, a *Schlumbergera* germplasm collection composed of 59 clones was assayed for AAT, GPI, LAP, MDH, PGM, SKD, and TPI. Twelve putative loci and 36 putative alleles were identified, and 10 of the 12 loci (83%) were polymorphic. Forty-one clones (69%) could be distinguished solely on the basis of their isozyme profiles, but the other 18 clones shared isozyme profiles with one or two other clones. Forty-two commercial clones of *S. truncata*, *S.* x *buckleyi*, and *S.* x *exotica* exhibited less genetic diversity than 14 field-collected clones of *S. kautskyi*, *S. opuntioides*, *S. orssichiana*, *S. russelliana*, and *S. truncata*. The difference in genetic diversity was attributed to limited sampling from wild populations and loss of alleles during breeding.

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CHAPTER 1

LITERATURE REVIEW

Introduction

Hunter and Markert (1957), using the combined technologies of starch gel electrophoresis and enzyme activity staining, demonstrated that enzymes exist in multiple forms. The multiple forms of an enzyme may be separated by electrophoresis and then visualized by staining. A zymogram is the term used to describe the configuration of bands in the electrophoretic analysis of an enzyme (Richards, 1990). The term allozyme refers to different forms of an enzyme specified by alternative alleles at one gene locus, whereas the term isozyme refers to different forms of an enzyme specified by genes at different loci (Wendel and Weeden, 1989).

Isozymes have been recognized as a more convenient and reliable source of genetic markers than morphological characters which have been traditionally used in plant breeding (More and Collins, 1983). Isozymes also have several attributes to make them especially suited to genetic studies: Mendelian inheritance, codominant expression, complete penetrance, and lack epistatic or pleiotropic interactions among loci (Weeden and Wendel, 1989). The most important attribute of isozymes is their simple genetic basis. A diploid organism contains two copies (alleles) of each gene, and, in most cases, both alleles are transcribed and translated. This codominant expression and complete penetrance allows for the calculation of allele frequency, and identification of heterozygous and homozygous individuals (Weeden, 1989). Other attributes of isozymes include selective neutrality, and consistency of expression of most isozymes regardless of environmental conditions, developmental stage, and tissue sampled. Generally, isozyme analysis requires only a small amount of plant tissue, so that the plant is not destroyed by tissue sampling (Wendel and Weeden, 1989). In addition, the number of loci and subcellular location of the isozymes is highly

conserved in plant evolution (Gottlieb, 1982). It has been found that, in most diploid plant species, many enzymes have the same number of loci, a conserved subunit structure, and specific subcellular locations (Weeden and Wendel, 1989; Gottlieb, 1982).

There are several areas in plant breeding where isozymes can be utilized. Isozymes can be employed to: distinguish individual cultivars; develop markers for commercially important traits; estimate genetic diversity within taxa; confirm existence of intra- or interspecific hybrids; analyze quantitative traits; and increase knowledge about genome structure by the development of linkage maps (Wendel and Weeden, 1989).

Cultivar Identification. Cultivar identification is important for commercial production of agronomic and horticultural crops and in plant patent applications and patent infringement cases. Brewbaker (1966) first proposed the use of isozymes for cultivar identification. Isozyme polymorphism has been successfully used for identifying cultivars in many crops (Nielsen, 1985).

Plant breeders rights (PBR) legislation was first enacted in 1930 (Bailey, 1983). This legislation provides licenses to breeders allowing them to control the marketing and propagation of plant cultivars which they have created. In order to obtain a patent, it must first be established that there is varietal distinctness. Varietal distinctness may be identified with morphological or biochemical characterizations. In some crop species, morphological distinctness is rare and sometimes not convenient for plant identification, i.e., characters that are only distinguishable at plant maturity (Hashemi, et al., 1991). Techniques used in varietal identification must exhibit environmental stability, ability to distinguish intervarietal variation (which may be minimal), and repeatability of results. Isozymes not only conform to the necessary criteria for use in varietal identification, but may potentially be one of the most practical and economic techniques available in many cases.

There are many examples of using isozymes to aid in identifying cultivars (Nielsen, 1985). Isozyme analysis has been conducted on guayule (*Parthenium argentatum*) (Hashemi et al., 1991). Previously, only one morphological marker was available. Isozyme polymorphism was found to be prevalent in guayule, and is currently employed to identify cultivars. Other crops where isozymes have been used successfully for genetic characterization include rice (*Oryza sativa* L.) (Glaszmann, 1987), carnation (*Dianthus caryophyllus* L.) (Messeguer and Arus, 1985), white poplar (*Populus alba* L.) (Rajora and Dancik, 1992) sugarbeet (*Beta vulgaris* L.) (Nagamine et al., 1989), pine (*Pinus*) (Shrunkal et al., 1992), *Azolla lam*, an aquatic nitrogen-fixing fern (Zimmerman, 1989), pecan (*Carya illinensis*) (Marquard et al., 1995) and Olive (*Olea europaea*) (Trujillo et al., 1995).

There are, however, limitations to the usefulness of isozymes. Polymorphism must be present within taxa in order to distinguish genotypes. The number of bands is a function of the number of loci, number of alleles per locus and the quaternary structure of the specific enzyme. The larger the sample of loci, the greater the number of alleles detected and the greater the probability of finding a unique phenotype (Cheliak and Pitel, 1984). In certain crops such as tomato (*Lycopersicon esculentum*) (Rick, 1983), pepper (*Capsicum annuum*) (McLeod, 1983), and safflower (*Carthamus tinctorius*) (Estilai et al., 1994) limited enzyme polymorphism has been detected, making isozyme analysis a poor technique for genetic characterization. Another problem that may be encountered in cultivar identification is distinguishing between sports of a cultivar. Many important horticultural varieties have developed from bud sports. Sports are almost identical to the parent plant with only minor genetic changes. Thus far, detection of isozyme polymorphism in cultivars that have arisen as sports has been unsuccessful (Weeden and Lamb, 1985; Mendenez, 1986).

Estimating Genetic Diversity. Isozyme analysis between and among different taxa can also provide an accurate estimation of genetic diversity and may aid breeding

programs in the evaluation of samples for potential sources of new traits, in the conservation of genetic resources, and in determining the phylogeny of a species (Mowrey and Werner, 1990; Tanksley, 1983). Genetic diversity estimations are useful in both cultivated and wild populations. Eighteen isozyme loci were examined to determine the genetic diversity in a germplasm collection of cultivated *Cucumis sativus* L. (Knerr et al., 1989). The genetic diversity of the collection was determined to be low. However, the information obtained in this study provided a basis to facilitate future additions to the germplasm collection. In a wild population of *Lophocereus schottii* (columnar cactus), the genetic diversity within a population that reproduced primarily by asexual means was found to have a level of genetic diversity similar to sexually reproducing species (Parker and Hamrick, 1992). The authors suggest that this relatively high level of genetic diversity was achieved through the occasional sexual reproduction and immigration through pollen movement and the long distance dispersal of stems by water. Genetic diversity was also studied in two wild populations of *Tillandsia* (Soltis et al., 1987). The species *T. ionantha* and *T. recurvata* have considerably different flower morphology, suggesting different reproduction strategies. Isozyme analysis of the *T. recurvata* population indicated a very low level of genetic diversity, suggesting a high level of inbreeding. The population of *T. ionantha* was found to have a high level of genetic diversity, suggesting outbreeding. The results of this study confirmed reproduction strategies that were consistent with the flower morphology of the two of *Tillandsia* species.

Confirmation of Hybridity. The use of isozymes has been successful in determining if progeny are of zygotic or asexual origin (Soltis, 1989). Confirmation of this can be determined easily if the two parents possess distinct allozyme banding patterns. In polyploid guayule, reproduction is by facultative apomixis, thus producing progenies derived by either maternal tissue or fertilized eggs (Hashemi et al., 1991). The identification of zygotic and asexual progeny can be established at the seedling

stage by isozyme analysis, so that unwanted asexual seedlings can be discarded. There are many examples using isozyme segregation data to distinguish asexual progeny from zygotic progeny, especially in long-term crops. For instance, in citrus, a breeder can avoid 5-10 year costs of growing out and maintaining unwanted asexual seedlings if isozyme analysis is conducted at an early stage (Torres, 1982).

Another application of isozymes in genetic studies is in determining the parentage of a particular cultivar. Torres and Bergh (1984) were able to distinguish the paternal parent that contributed root rot resistance in avocado (*Persea americana* Mill.). In banana (*Musa*), it was determined by isozyme analysis that three cultivars originated from an interspecific cross between *M. acuminata* and *M. balbisiana* (Jarret and Litz, 1986). Also isozyme analysis was used to test the hypothesis that *Paulownia taiwaniana*, a native of Taiwan, originated from a cross between *P. fortunei* and *P. kauakamii* (Finkeldey, 1992).

Marking Monogenic Traits. Isozymes are also useful for marking monogenic traits. Marking these traits would be extremely beneficial in breeding programs where time space and money are usually limited. There are several examples where isozymes have been involved in marking genes conferring disease resistance (Weeden, 1989). Traits such as nematode resistance in tomato (*Lycopersicon esculentum*) (Rick and Fobes, 1974) and more recently apple scab resistance in apple (*Malus pumila* Mill) (Manganaris et al., 1994) have been linked to an isozyme locus. In addition, the gene *Adh-1* in *Pisum sativum* was found to be closely linked to the gene or genes expressing resistance to pea enation mosaic virus (Weeden and Provvidenti, 1988). The benefits of this isozyme marker are enormous due to the ability to test expression for *Adh-1* allele in the seed prior to planting. In the pea, one-fifth of the cotyledon can be removed from the seed and assayed for the expression of this allele without affecting germination or seedling growth.

Other important traits such as sex determination in dioecious asparagus (*Asparagus officinalis* L.) have also been marked by isozymes (Maestri et al., 1991). In apple (*Malus pumila* Mill.), the self-incompatibility (*S*) locus was marked by the isozymes *GOT-1* and *IDH-1* (Manganaris and Alston, 1987; Batlle et al., 1995). Having the self-incompatibility locus tagged, there now exists a sound base for genotyping apple varieties for the *S*-alleles. Analysis for these isozymes is very simple and rapid in comparison to time-consuming pollination studies previously conducted with apple cultivars.

Quantitative Trait Analysis. Isozyme loci are well suited to mapping polygenic traits, for they are found to be well distributed over the plant genome (Powell et al., 1990). The marking of quantitative traits such as yield and cold tolerance has been accomplished in several crops such as corn (*Zea mays*) (Stuber, 1982; Stuber and Moll, 1980) and tomato (*Lycopersicon esculentum*) (Vallejos and Tanksley, 1983). More recently, polygenic traits have been tagged by isozyme markers in soybean (*Glycine soja*) (Graef, 1989), lentil (*Lens culinaris* Medik.) (Havey and Muehlbauer, 1989), barley (*Hordeum spontaneum*) (Powell et al., 1990) and almond (*Prunus amygdalus*) (Asins et al., 1994).

Linkage Maps. Detailed genetic maps using isozymes and morphological characters have received considerable attention for use as a breeding tool and in basic genetic research. A linkage map is a map of the linkage groups in a genome. A linkage group is defined by genes that occur on the same chromosome (Richards 1990). The more linkage groups with markers available, the better the chance of finding associations between these markers and economically-desirable traits. Linkage between pairs of isozyme loci has been reported in a number of horticultural genera (Vezvaei et al., 1995; Huang et al., 1994; Heemstra et al., 1991). Linkage maps are generally a combination of markers that can include morphological traits, isozymes, restriction

fragment length polymorphisms (RFLPs) and/ or random amplified polymorphic DNA (RAPDs).

Electrophoresis. Polyacrylamide was chosen as the support medium for this study over the more commonly used starch medium. Polyacrylamide offers several advantages over starch. Most importantly, polyacrylamide offers greater resolving power that can be achieved by altering the concentrations of acrylamide, thereby increasing or decreasing the molecular sieving effect for enhancing band separation and/or sharpness. Other advantages of polyacrylamide include more rapid running times, uniformity, repeatability, and gel transparency that allows for densitometer readings and lighter colored dye precipitates to be visible that may not be detected with the opaque starch medium (Weeden, 1989). Using the starch gel matrix can potentially result in time savings since the starch gel can be sliced into many gels after electrophoresis so that several enzymes may be assayed at one time. However, projects requiring maximum resolving power generally use polyacrylamide due to the uniform and greater flexibility in sieving properties for the best resolution of enzyme bands possible.

A Review of Cactaceae Literature

Taxonomy. The family Cactaceae is comprised of about 2,200 species (Barthlott, 1983). The epiphytic species, which represent approximately 10% of all cacti are in the subfamily Cactoideae. The epiphytic species are divided into two tribes, Rhipsalideae and Hylocereinae, which are separated based on the geographic location of their evolutionary centers (Barthlott,1983). The evolutionary center of the tribe Rhipsalideae is southern South America, whereas the evolutionary center of Hylocereinae is southern Mexico and Central America. The tribe Rhipsalideae comprises four genera: *Schlumbergera, Hatiora, Rhipsalis,* and *Lepismium*

(Barthlott,1983). The two genera that will be genetically analyzed by isozymes are *Schlumbergera* and *Hatiora.*

Hatiora. The genus *Hatiora* is compised of five species of epiphytic or epilithic shrubs that are endemic to southeastern Brazil (Barthlott, 1987; Hunt, 1992). Plants have a determinate growth pattern and produce a series of leafless stem segments (phylloclades) with a composite areole (Moran, 1953). According to Barthlott (1987), the genus is divided into two subgenera. The subgenus *Hatiora* includes two species with terete phylloclades: *H. salcornioides* (Haw.) Britton & Rose and *H. herminiae* (Campos-Porto & Castellanos) Backeberg ex Barthlott. The subgenus *Rhipsalidopsis* contains three species with flattened or angular phylloclades: *H. epiphylloides* (Campos-Porto & Werdermann) F. Buxbaum, *H. gaertneri* (Regel) Barthlott, and *H. rosea* (Lagerheim) Barthlott. *Hatiora gaertneri* and *H. rosea* were brought into cultivation nearly a century ago (Lagerheim, 1912; Regel, 1884), and have been valued as ornamentals due to their showy flowers. Hybrids between *H. gaertneri* and *H. rosea*, first described by Werdermann (1939), have been used extensively to breed commercial cultivars (Meier, 1992). The collective name for hybrids of *H. gaertneri* x *H. rosea* parentage is *H.* x *graeseri* (Werdermann) Barthlott (Barthlott, 1987). The two species and their interspecific hybrids are commonly known as Easter cactus, and refers to the fact that plants grown in the northern hemisphere flower primarily in spring (near Easter).

Hatiora rosea was discovered by Per Dusen in 1909 growing at 1100-2300 meters in the forests near Caiguava in Parana, Brazil. *Hatiora rosea* was originally named *Rhipsalis rosea,* but in 1920 was transferred to the genus *Rhipsalidopsis* by Britton and Rose (McMillan, 1981). *Hatiora gaertneri* was originally discovered in southern Brazil in 1881 by Gaertner. *Hatiora gaertneri* was first named *Epiphyllum russellianum* var. *gaertneri* by Regel, and was transferred to *Schlumbergera* by Britton and Rose in 1920. The species was transferred several more times until finally in 1953

it was placed into the genus *Rhipsalidopsis* by Moran (1953). Although the genus *Rhipsalidopsis* was accepted by most taxonomists, Barthlott (1987) transfered the genus *Rhipsalidopsis* to the genus *Hatiora*. The transfer of *Rhipsalidopsis* to the genus *Hatiora* has been accepted by most taxonomists (Boyle et al.,1994; Huxley et al., 1992; Ganders, 1976; Taylor, 1976).

Both *H. gaertneri* and *H. rosea* exhibit self-incompatibility but the two species are interfertile (McMillan, 1981). As with most cacti, *Hatiora* has a chromosome number of $2n=22$ (Boyle, unpublished data; Love, 1976).

Easter cactus is a popular crop in Europe and is now gaining in importance in the United States. There are approximately 125 cultivars of Easter cactus in cultivation (Meier, 1992). Many of the commercial cultivars of Easter cactus were developed in Europe and are not adapted to environmental conditions found in North America. There is a great demand for the development of new cultivars of Easter cactus for the U.S. market. However, no information is available about the extent of genetic diversity within *Hatiora* germplasm. Information on genetic diversity on this crop would greatly enhance the breeding programs for future cultivar development.

Schlumbergera. The genus *Schlumbergera* consists of six species: *S. truncata, S. russelliana, S. orssichiana, S. kautskyi, S.obtusangula,* and *S. opuntioides* (Huxley et al.,1992). The six species are native to the states of Sao Paulo, Rio de Janiero, Espirito Santo, and Minas Gerias in southeastern Brazil (Barthlott and Rauh, 1977; Hunt, 1969).

Schlumbergera truncata, the most important *Schlumbergera* species in cultivation, flowers in November in the northern hemisphere and is commonly called Thanksgiving cactus. Hybrids of *S. truncata* and *S. russelliana* (= *S. x buckleyi)* flower in December and are called Christmas cactus. Thanksgiving and Christmas cactus are collectively called holiday cactus (Runger and Poole, 1985).

Although *Schlumbergera* exhibits an enormous amount of phenotypic variation, the amount of genetic variation is unknown. All of the species have the same number of chromosomes ($2n=22$) and some species are able to hybridize (Barthlott and Rauh, 1977). Except for *S. obtusangula* and *S. kautskyi*,all other *Schlumbergera* species are self-incompatible (McMillan, 1991; McMillan and Horobin, 1992).

The genus *Schlumbergera* can also be separated from other closely-related genera by the distinctive flower tube and basal nectar chamber (Barthlott and McMillan, 1978). The genus can be divided into two groups based on vegetative differences: those species with flattened phylloclades (= *S. truncata*, *S. russelliana*, *S. orssichiana*, and *S. kautskyi*), and those species with spiny opuntoid phylloclades (= *S.opuntoides* and *S. obtusangula*) (Barthlott and McMillan, 1978). The taxonomy of *Schlumbergera* species has been a topic of debate since the genus was established by Lemaire in 1858 (Hunt, 1969). The species currently in *Schlumbergera* have passed through a number of different genera before consolidation by Hunt in 1969 (McMillan, 1990).

Today, there are approximately 200 cultivars of holiday cactus in cultivation (McMillan, 1985). Very little is known about the genetics of this crop. Information regarding the genetic diversity would be advantageous for plant breeding programs, and may also elucidate some long-standing taxonomic disputes within this genera.

Isozyme Literature on The Cactaceae. Literature involving isozyme research on the Cactaceae is limited. Wallace and Fairbrothers (1986) isoelectrically focused seed proteins of *Opuntia humifusa* Raf. (prickly pear) to determine the degree of similarity between two north American populations. Similar enzyme profiles were obtained from both populations, but some variability did exist between the two populations. This variability was attributed to small changes in genotypes of isolated populations and genetic fixation. The authors concluded that, despite morphological differences within this phenotypically plastic taxon, that *O. humifusa* should remain a

single species. Parker and Hamrick (1992) analyzed isozymes from several populations of *Lophocereus schottii* (columnar cactus) to determine the amount and distribution of genetic diversity within and among populations, and the relation between plant spacing and genetic diversity. The results of this study showed that *L. schottii*, with occasional sexual reproduction, pollen movement, and dispersal of stems via water, had diversity characteristics similar to species in which reproduction is primarily sexual. The authors also determined that greater numbers of *L. schottii* with identical genotypes occurred closer together than far apart. Murawski et al. (1993) used isozyme analysis to determine the nature of polyploidy (auto vs. alloploidy) of *Pachycereus pringlei*. *Pachycereus pringlei* was confirmed to be an autotetraploid based on isozyme data showing tetrasomic inheritance. Garcia-Carreno (1993) also reported on peroxidase activity in *P. pringlei*. That study focused on the isolation, separation of isozymes, and kinetic characterizations of isoperoxidases isolated from shoots of *P. pringlei*.

CHAPTER 2

MATERIALS AND METHODS

Abstract

Isozyme analysis is a reliable technique that has been used in plant studies to measure levels of genetic variation within populations, analyze mating systems, determine taxonomic relationships among species, identify varieties, and help substantiate parentage in breeding programs. This paper reports on polyacrylamide gel electrophoresis (PAGE), which has proven to be a reliable method for analyzing isozymes in two Cactaceae genera (*Hatiora* and *Schlumbergera*). Information is provided on PAGE equipment and running conditions as well as procedures used for preparing tissue samples, polyacrylamide gels, electrode buffers, and enzyme stains. The techniques described here may be useful for investigations of other Cactaceae taxa.

Introduction

Isozyme markers are widely used in plant research, and are especially suited to genetic studies. Isozymes exhibit Mendelian inheritance, codominant expression, and complete penetrance, but lack epistatic or pleiotropic interactions among loci (Weeden and Wendel, 1989). They also exhibit selective neutrality, and consistency of expression of most isozymes regardless of environmental conditions, developmental stage, and tissue sampled. Isozymes have been successfully used for demonstrating genetic variation, identifying interspecific hybrids, distinguishing individual cultivars, and developing markers for commercially important traits (Wendel and Weeden,1989).

Literature involving isozyme research on the Cactaceae is limited. Murawski et. al (1994) used isozymes to study the breeding behavior of the autotetraploid species *Pachycerus pringlei*. Garcia-Carreno (1993) reported on the isolation, separation of isozymes, and kinetic characterizations of isoperoxidases in *Pachycerus pringlei*.

Parker and Hamerick (1992) analyzed several populations of *Lophocereus schottii* to determine the amount and distribution of genetic diversity within and among populations, and the relation between plant spacing and genetic diversity. Wallace and Fairbrothers (1986) analyzed *Opuntia humifusa* using isozymes to determine the degree of similarity between two North American populations. There have, however, been no isozyme studies on the genera *Hatiora* or *Schlumbergera*. Isozymes may be useful for identifying cultivars on a year-round basis, as a source of genetic markers in breeding programs, and to assess the level of genetic diversity within the species.

Traditionally isozyme studies are based on starch gel eletrophoresis. Polyacrylamide gel electrophoresis (PAGE) offers several advantages over starch and was chosen as the support medium for this study. Most importantly, polyacrylamide offers greater resolving power that can be achieved by altering the concentrations of acrylamide, thereby increasing or decreasing the molecular sieving effect for enhancing band separation and/or sharpness. Also PAGE offers rapid running times,uniformity, repeatability and gel transparency that allows for lighter colored dye precipitates to be visible.

The main objective of this study was to identify enzyme systems that were consistently resolved, intensely stained, and interpretable. Evaluations concerning the best tissue to employ, method of extraction and extraction buffer to use were conducted. Also, the amount of sample loaded, percent polyacrylamide gel, gel and electrode buffers, and enzyme staining protocols, were evaluated to optimize band activity and resolution for *Hatiora* and *Schlumbergera* cultivars.

Materials and Methods

Plant Material. Phylloclades of *Hatiora* and *Schlumbergera* cultivars were collected from greenhouse-grown plants. For most enzyme systems, approximately half of a 5 mm disk (8 mg) of mature phylloclade tissue, immature (2-6 mm) whole

phylloclade segments, or 5-10 mm pieces of young roots were used to determine the best activity for a given enzyme system. Plants were pinched intermittently to enhance the production of immature phylloclades. Root tissue was obtained by placing mature phylloclade segments on wet filter paper in glass petri dishes and kept under lights (≈ 22^{o} C) in the laboratory. Pollen samples were obtained by removing the flowers and using a vortex to collect the pollen into scintillation vials.

Sample preparation. All samples of mature phylloclade, immature phylloclade and root tissue were prepared in the same manner. Approximately 25-100 ul of extraction buffer (50 mM Tris - pH 7.5, 5% sucrose, 14 mM B-mercaptoethanol, and 5% PVP-40) (Wendel and Weeden, 1989) was pipetted into a microcentrifuge tube, and the tube was placed on ice. Plant tissue then was added to the ice-cold extraction buffer in approximately 1:12, 1:10 and a 1:5 tissue to extraction buffer ratio for mature, immature and root samples respectively. The tissue was ground with an art supply pestle until most of the solid tissue was macerated. Samples were then microcentrifuged at 10,000 rpm in a 4^{o}C refrigerator for ten minutes. The supernatant (10-60 ul) was removed and placed into a fresh microcentrifuge tube containing 5-10 ul ice-cold sample mix (50% glycerol and 0.1% of bromphenol blue, with a final gycerol concentration of ≈ 4-7% in samples) (Shields et al., 1983). For pollen sample preparation, approximately 5 mg of pollen was placed into a microcentrifuge tube containing 500 ul of pollen extraction buffer (50 mM Tris-HCl containing 1 mM EDTA and 14 mM B-mecaptoethanol) for 4 hours at 4°C (Weeden and Gottlieb, 1980). The pollen then was centrifuged and the supernatant was placed in sample mix as described earlier.

The majority of samples were prepared and analyzed on the same day. Storage of samples for up to one month at -20°C was possible for most enzyme systems, with only a minor loss of activity. However, almost complete loss of activity was observed

for some isozymes of aspartate aminotransferase (AAT) and malate dehydrogenase (MDH) within a week when samples were stored at -20°C.

Gel Preparation. A Mini-Protean II multicasting chamber (Bio-Rad, Richmond, CA.) was used to cast 12 single percentage polyacrylamide gels at the same time. The acrylamide solution (30:1 acrylamide to bisacrylamide) was poured into the casting chamber from the top quickly and evenly to avoid polymerization and bubbles. Afterwards, a 15-well comb was placed into each gel in the casting chamber starting from the back. The gels were removed from the chamber approximately 45 minutes after they were poured. Combs were removed under the running tap and the gels were rinsed and shaved of excess polymerized acrylamide with a razor blade. Single percentage gels (5-10%) could be stored upright for up to two weeks at 4°C when held in air tight plastic bags containing approximately 1-3 mls of electrode buffer. The gel buffer used for preparing the polyacrylamide gels was 375 mM tris-HCl pH 8.8 (Hames, 1981).

Electrophoresis. The Mini-Protean II dual slab cell system (Bio-Rad, Richmond, CA.) was used for running gels. The reservoir buffer consisted of 25 mM tris 1.92 mM glycine pH 8.3 (Hames, 1981). Electrophoresis was conducted under a constant 200 voltage at 4^{o}C. Gels were completely immersed in cold (4^{o}C) electrode buffer in the electrophoresis cell at least a half hour prior to sample loading. The electrophoresis cell was removed for approximately 10 minutes from the incubator for sample loading. Running times varied with the enzyme system assayed, but was usually about 45 minutes.

Staining and Gel Preservation. The enzyme stains were prepared immediately prior to use. Stocks of many common solutions were prepared in advance and stored at the appropriate temperature and volume (table 2.1).The enzyme staining procedures were obtained from Wendel and Weeden (1989). All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). After the appropriate staining

intensities were achieved, gels were rinsed with water and fixed in 7% acetic acid (Hames, 1981). Gels were dried using the Bio/Gel Wrap System (Biodesign Inc., Carmel, NY.)

Results and Discussion

Many different enzyme systems were evaluated. Only enzyme systems that had consistently sharp and intense banding patterns were chosen for further studies and presented in this paper. The enzymes AAT, GPI, LAP, MDH, PGM, SKD, and TPI all were found to contain polymorphisms and have consistently resolved and intensely stained bands. Gel and sample preparation conditions for each enzyme are described in Table 2.

The main problem that was encountered in isozyme analysis of *Hatiora* and *Schlumbergera* was the mucilaginous tissue. The mucilage made samples very difficult to prepare and load onto gels, and caused problems in the banding resolution. The mucilage also limited the tissue: extraction buffer ratios that could be used in sample preparation. Attempting to concentrate the sample to obtain more enzyme activity was difficult. This problem was partially overcome in some instances by adding PVP-40 to the sample extraction buffer. Mature phylloclade tissue was the tissue of choice to use in sample preparations, only because it was the most abundant and available at all times. However, in some instances immature phylloclade tissue or root tissue was used because of the inability to concentrate the mature tissue extract enough for the appropriate band staining.

Pollen samples were used mainly to confirm intralocus and interlocus heterodimers. Generally, the pollen samples contained considerable enzymatic activity and, in some cases, pollen specific bands were observed.

Traditionally, it has been thought that using starch gels results in time savings, since the starch gel can be sliced into many gels after electrophoresis for assaying

several enzymes simultaneously. However, in this study, we employed mini-polyacrylamide gels which saved a great amount of time and supplies. With the use of the multi-casting chamber, up to 12 polyacrylamide gels, each containing 15 wells for samples, can be cast in approximately one hour. This, together with the small size of the gels, made it possible to run approximately 20-24 gels per day, assuming that the samples were previously prepared. In addition, less enzyme stain was required to obtain the necessary band intensities on these small gels. Each gel required only 25 mls of enzyme stain, half of what is commonly used to stain starch gels.

In conclusion, this study provided seven enzyme systems with twelve loci in total to be used in future inheritance and genetic diversity studies with *Hatiora* and *Schlumbergera*.

Table 2.1. Solutions, concentrations, storage conditions and life of many commonly used stocks in isozyme analysis.

Stock	Concentration	Storage temperature/duration
Acrylamide	30%	4°C months
AAT substrate solution	200 mls	4°C 1 week
Ammonium persulfate (APS)	10 mg/ml	21°C fresh daily
Arsenic acid	75 mg/ml	21°C months
Bis-acrylamide	1%	4°C months
Bromphenol Blue	5 mg/ml	21°C months
Fast Black K salt	20 mg/ml	4°C months
Fast Blue BB salt	50 mg/ml	4°C months
Glucose-6-phosphate dehydrogenase	20 units/40ul	4°C months
Glyceraldehyde-3-phosphate dehydrogenase	300 units/ml	-20°C months
Tetrazolium thiazolyl blue (MTT)	10 mg/ml	4°C months
$MgCl_2$	50 mg/ml	21°C months
NAD	10 mg/ml	-20°C months
NADP	5 mg/ml	-20°C months
Phenazine methosulfate (PMS)	50 mg/ml	4°C 1-2 weeks
Sample mix (50% glycerol, 0.1% bromphenol blue)	25 mls	21°C months
Tris-HCl	1 M-1.5M	21°C months

Table 2.2 Polyacrylamide gel concentrations and sample loading volumes used for electrophoresis

Enzyme	Gel concentration (%)	Loading volume (µl)
Aspartate aminotransferase (AAT)	5	10
Glucose-6-phosphate isomerase (GPI)	7.5	5
Leucine aminopeptidase (LAP)	7.5	5
Malate dehydrogenase (MDH)	7.5	10
Phosphoglucomutase (PGM)	7.5	10
Shikimate dehydrogenase (SKD)	7.5	10
Triosephosphate isomerase (TPI)	10	5

CHAPTER 3

INHERITANCE OF SIX POLYMORPHIC ISOZYME LOCI IN *HATIORA*

Abstract

Polyacrylamide gel electrophoresis (PAGE) was performed to investigate the inheritance of aspartate aminotransferase (AAT), glucose-6-phosphate isomerase (GPI), malate dehydrogenase (MDH), phosphoglucomutase (PGM), and triosephosphate isomerase (TPI) in the genus *Hatiora* (Cactaceae). F_1, F_2, BC_1, and S_1 populations were examined to determine the mode of inheritance. The results of inheritance studies for six polymorphic loci (*Aat-1*, *Gpi-1*, *Mdh-1*, *Pgm-1*, *Pgm-2*, and *Tpi-2*) were generally as expected for single loci with codominant alleles. However, segregation distortion was observed in at least one segregating family for all six isozyme loci, and was more common in BC_1 and S_1 families. Linkage analysis revealed that *Aat-1* and *Pgm-1* were linked (26 cM), whereas the other isozyme loci assorted independently. The monomeric structure of *Pgm-1* and *Pgm-2* and the dimeric structure of *Aat-1*, *Gpi-1*, *Mdh-1*, and *Tpi-2* were consistent with quaternary structures previously reported for other plant species.

Introduction

Easter cactus [*Hatiora gaertneri* (Regel) Barthlott, *H. rosea* (Lagerheim) Barthlott, and their interspecific hybrids (= *H.* x *graeseri* (Werdermann) Barthlott)] is a popular flowering potted plant in northern Europe and has been increasing in popularity in North America (Boyle, 1991). Plants have showy flowers and produce a series of flattened or angular stem segments (phylloclades) that function as the primary photosynthetic organ (Moran, 1953). *Hatiora gaertneri* and *H. rosea* are endemic to southeastern Brazil (Barthlott, 1987; Hunt, 1992) and were brought into cultivation nearly a century ago (Lagerheim, 1912; Regel, 1884). The first interspecific hybrids

of *H. gaertneri* and *H. rosea* were produced in 1928 by Alfred Gräser of Nürnberg, Germany (Tjaden, 1986; Werdermann, 1939). More than 100 cultivars of Easter cactus are in existence, and the majority of these are complex hybrids of *H.* x *graeseri* (Boyle, 1995; Meier, 1992). The common name for these taxa refers to the fact that plants grown in the northern hemisphere flower primarily in spring, i.e., near Easter. *Hatiora gaertneri*, *H. rosea*, and *H. x graeseri* are diploid taxa with a chromosome number of $2n=2x=22$ (Barthlott, 1976).

Breeding of Easter cactus was initiated at the University of Massachusetts in 1987, and is directed towards the production of novel and superior clones. It was therefore highly desirable to have a set of genetic markers for identifying cultivars and selecting progeny in the seedling stage. Crossing studies have revealed that few simply-inherited morphological markers exist in Easter cactus (T.H. Boyle, unpublished data).

Isozymes are widely used in horticulture for identifying cultivars and as genetic markers for breeding programs (Nielsen, 1985). Several attributes of isozymes make them particularly useful as genetic markers, such as Mendelian inheritance, codominant expression, complete penetrance, and consistency of expression under a wide range of environmental conditions (Weeden and Wendel, 1989). Boyle et al. (1994) used isozymes to demonstrate that S_1 progeny had been recovered from a highly self-incompatible cultivar of *H. gaertneri*. Isozymes have also been analyzed in other Cactaceae genera, including *Lophocereus* (Parker and Hamrick, 1992), *Opuntia* (Wallace and Fairbrothers, 1986), and *Pachycereus* (García-Carreño, 1993; Murawski et al., 1994). However, there have been no formal genetic studies on isozyme inheritance for any Cactaceae taxa. The Cactaceae are not particularly amenable to genetic studies due to their long juvenility period, which can last for 5 years or longer (Cullman et al., 1987). Hence, the absence of isozyme inheritance studies on Cactaceae taxa is not suprising. By growing Easter cactus seedlings under optimal environmental

conditions, however, the juvenility period can be reduced to ≈2½ years, which makes them more suitable than most other Cactaceae taxa for genetic studies. This paper reports on the inheritance of six polymorphic isozyme loci in Easter cactus.

Materials and Methods

Plant material. Plants were propagated and grown in glasshouses at the University of Massachusetts, Amherst. Parental cultivars were obtained from the clonal germplasm collection maintained in the Department of Plant and Soil Sciences at the University of Massachusetts. F_1, F_2, BC_1, and S_1 populations were generated for inheritance studies. Cross-compatible clones were used as parents for producing F_1, F_2, and BC_1 seed, and crosses were performed in a glasshouse maintained at 18°C nights/22°C days. S_1 seed of 'Crimson Giant', a highly self-incompatible cultivar, was produced by incubating flowering plants for 12 hours in a growth chamber set at 40°C and selfing immediately after incubation (Boyle et al., 1994). Techniques used in emasculation, pollination, seed germination, and plant culture were similar to those reported previously (Boyle et al., 1994). Henceforth, all crosses will be presented as pistillate parent x pollen parent unless specified otherwise.

Protein extraction and sample preparation. Isozymes were extracted from mature phylloclades or pollen. Tissue was removed from mature 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-HCl buffer (pH 7.5), 0.014 M *B*-mercaptoethanol, 5% (w/v) polyvinylpyrrolidone (PVP-40), and 5% (w/v) sucrose (Wendel and Weeden, 1989)]. Crude homogenates were centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant (≈60 µl) was collected and mixed with 10 µl of cold sample mix [50% (v/v) glycerol and 0.1% (w/v) bromphenol blue (Shields et al., 1983)].

Flowers were obtained on the day of anthesis from glasshouse-grown plants, and pollen was removed from the anthers with a vortex and collected in scintillation vials. Soluble proteins were extracted by soaking ≈5 mg of pollen in 500 μl pollen extraction buffer (0.050 M Tris-HCl buffer (pH 7.5), 0.014 M 2-mercaptoethanol, and 1 mM EDTA) for 4 hours at 4^{o}C (Weeden and Gottlieb, 1980). Pollen extracts were then centrifuged at 10,000 rpm for 10 minutes at 4^{o}C, and the resulting supernatant was treated as described earlier for phylloclade samples.

Electrophoresis. Native proteins were separated by polyacrylamide gel electrophoresis (PAGE) using a Mini-Protean II cell (Bio-Rad Laboratories, Hercules, CA). Single percentage (5-10%) polyacrylamide gels were prepared using 0.375 M Tris-HCl (pH 8.8) as the gel buffer (Hames, 1981). Details on acrylamide concentrations and sample loading volumes are provided in Table 3.1. 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^{o}C under constant voltage (200 V). Running times varied according to the enzyme system assayed, but typically required 45 minutes for completion.

Gel staining. 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), phosphoglucomutase (PGM, E.C. 5.4.2.2), and triosephosphate isomerase (TPI, E.C. 5.3.1.1). The staining procedures of Wendel (1989) for assaying enzyme activity. Enzyme stains were prepared immediately prior to use. After the appropriate staining intensities were achieved, the gels were rinsed with water and fixed in 7% acetic acid (Hames, 1981).

Data analysis. R_f values were calculated for each band by dividing the band's migration distance by the tracking dye's migration distance (Hames, 1991). Data were collected immediately after gels were fixed, i.e., when the staining intensities were maximal. For each enzyme, loci were numbered sequentially, with *1* denoting the

most anodally migrating locus. Alleles at individual loci were designated by letters assigned sequentially from the anode.

Isozyme inheritance was determined by evaluating the segregation ratios in S_1, full-sib F_1, F_2, and BC_1 families. Data from reciprocal crosses were pooled for chi-square analysis when populations did not differ significantly from the expected ratios in homogeneity testing. The computer program LINKAGE-1 (Suiter et al., 1983) was used to test goodness-of-fit for the expected segregation ratios at single loci and possible linkage relationships among loci.

Results and Discussion

Aspartate aminotransferase. Two polymorphic zones of activity were observed for AAT (Figure 3.1). Five phenotypes were observed at *Aat-1*: four single-banded phenotypes (aa, bb, cc and dd) and one triple-banded phenotype (cd). The R_f values for cc, and dd were 0.45 and 0.38, respectively. Bands aa and bb (with R_f values 0.50 and 0.48) were not subjected to genetic analysis. Presence of cd in phylloclade samples and its absence in pollen samples suggests that AAT is a dimer composed of two subunits. Crosses between a single-banded phenotype and a triple-banded phenotype yielded, in most cases, progenies which exhibited the expected 1 : 1 segregation ratio (Table 3.3). One BC_1 family ('Crimson Giant' x R891-2), however, deviated significantly from the expected 1 : 1 ratio (Table 3.3). Progeny recovered from crosses between two triple-banded phenotypes exhibited significant departures from the expected 1 : 2 : 1 ratio in all three familes that were examined (Table 3.3). In two of these families ('Crimson Giant' x R891-6 and the S_1 progeny of 'Crimson Giant'), one single-banded phenotype was deficient whereas the other single-banded phenotype was in surplus. Interestingly, the ratio of single-banded phenotypes (cc and dd) to triple-banded phenotypes did not deviate from the expected 1 : 1 ratio in either

of these families (X^2 = 0.200, $P > 0.50$ for 'Crimson Giant' x R891-6 ; X^2 = 0.005, $P > 0.90$ for 'Crimson Giant' S_1 progeny).

The single band of *Aat-2* (= cc) had an R_f value of 0.32 (The bands aa and bb with R_f values 0.36 and 0.34 were not analyzed in this study). Since the R_f value of cc did not change when different banding patterns were observed at *Aat-1*, the two zones of activity were considered to be separate loci. Interlocus heterodimers between *Aat-1* and *Aat-2* were not observed, suggesting that these two loci code for proteins which have different subcellular locations (Weeden and Marx, 1987). The existence of two activity zones for AAT along with the dimeric structure is consistent with findings on other plant species (Nagamine et al., 1989).

Glucose-6-phosphate isomerase. GPI exhibited two polymorphic zones of activity (Figure 3.1). The bands of *Gpi-1* migrated close together and had R_f values of 0.43, 0.39, and 0.36. At *Gpi-2* only one band (ee) was subjected to genetic analysis, bands aa, bb, cc, and dd with R_f values 0.32, 0.30, 0.27, and 0.25 respectively, were not analyzed in this study. Five phenotypes were observed at *Gpi-1*: two single-banded phenotypes (aa and bb) and three triple-banded phenotypes, each of which displayed a band of intermediate mobility (ab, ac, and bc). The intermediate bands were observed in phylloclade extracts but absent in pollen extracts, indicating that *Gpi-1* is a dimer. When a triple-banded phenotype was crossed with a single-banded phenotype, progenies were obtained in the expected 1 : 1 segregation ratio, except for one F_1 family ('Crimson Giant' x 'Evita') which deviated significantly from the expected ratio (Table 3.4). Progeny recovered from crossing two identical triple-banded phenotypes did not significantly differ from the expected 1 : 2 : 1 segregation ratio (Table 3.4). When a triple-banded phenotype was crossed with a different triple-banded phenotype with one allele in common, progeny were obtained in the expected 1 : 1 : 1 : 1 ratio (Table 3.4). Presence of two zones of enzymatic activity and a dimeric quaternary

structure for GPI has been been demonstrated in several other plant species (Gottlieb, 1982; Weeden and Wendel, 1989).

Malate dehydrogenase. Electrophoretic separation of MDH isozymes from phylloclades revealed four zones of activity: *Mdh-1*, *Mdh-2*, *Mdh-3*, and *Mdh-4* (Figure 3.2). *Mdh-1* and *Mdh-4* were polymorphic whereas *Mdh-2* and *Mdh-3* were monomorphic. Bands of *Mdh-4* were too faint to interpret consistently and therefore were not subjected to genetic analysis.

Mdh-1 displayed three single-banded phenotypes (aa bb and cc) and one triple-banded phenotype with a band of intermediate mobility (ac). The bb band with an R_f value 0.58, was not subjected to genetic analysis. Bands aa, ac, and cc had R_f values of 0.63, 0.58, and 0.53, respectively. Band ac was present in phylloclade extracts but absent in pollen extracts, suggesting that *Mdh-1* has a dimeric structure. Crosses between a single-banded phenotype and a triple-banded phenotype yielded five families which segregated in the expected 1 : 1 ratio, but three other families deviated significantly from the expected 1 : 1 ratio (Table 3.5). Crosses between two triple-banded phenotypes resulted in the expected 1 : 2 : 1 segregation ratio in all three families that were examined.

Addition of 8.3% (w/v) ascorbic acid to the phylloclade extraction buffer (Goodman et al., 1980) inhibited the activity of *Mdh-1* but did not affect the activity of *Mdh-2* and *Mdh-3*, indicating that *Mdh-1* is a cytosolic form of MDH whereas *Mdh-2* and *Mdh-3* are organellar forms of this enzyme (Goodman and Stuber, 1983). The single bands of *Mdh-2* and *Mdh-3* had R_f values of 0.39 and 0.34, respectively, and were designated as aa-2 and aa-3, respectively. A band of intermediate mobility between aa-2 and aa-3 was tenatively identified as an interlocus heterodimer of *Mdh-2* and *Mdh-3*; this band was present along with aa-2 and aa-3 in MDH assays of pollen extracts, providing further evidence it is an intelocus heterodimer of *Mdh-2* and *Mdh-3*. Presence of an interlocus heterodimer between two mitochondrial forms of MDH have

been reported in *Cucumis sativus* L. (Knerr and Staub, 1992) and *Zea mays* L. (Goodman et al., 1980).

Phosphoglucose mutase. Two polymorphic zones of PGM activity were observed in PAGE gels (Figure 3.2). At *Pgm-1*, six single-banded phenotypes (aa, bb, cc, dd, ee, and ff) and one one double-banded phenotype were observed. Bands dd and ff had R_f values of 0.62 and 0.55, respectively. Bands aa, bb, cc and ee with R_f values 0.70, 0.67, 0.65, and 0.57 respectively, were not subjected to genetic analysis. Crosses between a single-banded phenotype and a double-banded phenotype resulted in the expected 1 : 1 segregation ratio in four families, but significant departures from expectations were observed in three other families (Table 3.6). Crosses between two double-banded phenotypes yielded three families that exhibited the expected 1 : 2 : 1 segregation ratio (Table 3.6). In one F_2 family (R891-2 x R891-3), however, dd phenotypes were were deficient and ff phenotypes were in excess (Table 3.6).

At *Pgm-2*, three single-banded phenotypes (aa, bb, and dd) and three double-banded phenotypes were observed (Figure 3.2). Bands aa, bb, and dd had R_f values of 0.48, 0.46, and 0.42, respectively. Band *cc-1* (R_f value 0.43) was not subjected to genetic analysis. When a double-banded phenotype was crossed with a single-banded phenotype, progenies were obtained in the expected 1 : 1 segregation ratio (Table 3.7). Crosses between clones with identical double-banded phenotypes resulted in progeny which fit the expected 1 : 2 : 1 ratio, and crosses between clones with different double-banded phenotypes yielded progeny in the expected 1 : 1 : 1 : 1 ratio (Table 3.7). Segregations at *Pgm-2* fit the expected ratios in all families except for the S_1 progeny of 'Crimson Giant' (Table 3.7). These results generally support the hypothesis that PGM is a monomeric enzyme that is specified by two loci in Easter cactus. Previous studies (Navot and Zamir, 1986; Weeden and Gottlieb, 1980) have shown that PGM is a monomer. Two PGM loci have been identified in several other plant species (Gottlieb, 1982).

Triosephosphate isomerase. TPI displayed two zones of activity: a more anodal monomorphic zone (*Tpi-1*) and a more cathodal polymorphic zone (*Tpi-2*) (Figure 3.2). The single band observed at *Tpi-1* (= aa) had an R_f value of 0.52. Three phenotypes were observed at *Tpi-2*: two single-banded phenotypes (aa and bb) and one triple-banded phenotype with a band of intermediate mobility (ab). The R_f values for bands aa, ab, and bb were 0.38, 0.35, and 0.32, respectively. Band ab was present in phylloclade extracts but was absent in pollen extracts, confirming that TPI has a dimeric structure and that ab was an intralocus heterodimer. When a triple-banded phenotype was crossed with a single-banded phenotype, most families segregated in the expected 1 : 1 ratio, but significant departures from expectations were observed in two families ('Crimson Giant' x 'Red Pride' and 'Crimson Giant' x 'Evita') (Table 3.8). Crosses between two triple-banded phenotypes yielded two families which fit the expected 1 : 2 : 1 ratio, and another family with no aa individuals but a surplus of bb individuals (Table 3.8).

Bands that formed between *Tpi-1* and *Tpi-2* were presumed to be interlocus heterodimers (Figure 3.2). Clones with single-banded phenotypes at *Tpi-2* produced only one interlocus heterodimer, whereas clones with triple-banded phenotypes at *Tpi-2* exhibited two interlocus heterodimers (Figure 3.2). Presence of these band(s) in pollen extracts supports that contention that they are interlocus heterodimers. Interlocus heterodimers between *Tpi-1* and *Tpi-2* have also been reported in *Annona cherimola* Mill. (Pascual et al., 1993; Patty et al., 1988).

Segregation distortion. Deviations from expected Mendelian ratios were observed in at least one segregating family for all six isozyme loci. *Hatiora gaertneri*, *H. rosea*, and *H.* x *graeseri* are outcrossers (Boyle et al., 1994; Ganders, 1976; Taylor, 1976), and inbreeding of a normally-outbred taxon would be expected to result in some weak or inviable progeny, thus leading to the attrition of some genotypes prior to PAGE analysis. Easter cactus exhibits self-incompatibility (Boyle et al., 1994), and

linkage between isozyme loci and the self-incompatiblity (*S*) locus may have also caused unequal segregation, as has been reported in *Malus pumila* Mill. (Manganaris and Alston, 1987), *Camellia japonica* L. (Wendel and Parks, 1984) and *Lycopersicon* (Tanksley and Figueroa, 1985). Segregation distortion may also have been caused by interspecific hybridization. All of the F_2 and BC_1 families used in this study were derived from the interspecific cross *H. gaertneri* 'Crimson Giant' x *H. rosea*. Zamir and Tadmor (1986) analyzed monogenic segregation ratios in three plant genera (*Capsicum*, *Lens*, and *Lycopersicon*) and found that the proportion of loci deviating from expected Mendelian ratios was 13% for progeny of intraspecific crosses, but was significantly higher (54%) for progeny of interspecific crosses. The above hypotheses are speculative, and further research is needed to discern the mechanism(s) underlying segregation distortion in Easter cactus.

Linkage analysis. Independent assortment of *Aat-1*, *Gpi-1*, *Mdh-1*, *Pgm-1* *Pgm-2*, and *Tpi-2* was tested for all possible pairs of loci using the LINKAGE-1 computer program (Suiter et al., 1983). All six loci segregated independently except for *Aat-1* and *Pgm-1* which displayed a mean recombination fraction of 0.26 and standard error of 0.07.

This study demonstrates that considerable isozyme polymorphism is present in Easter cactus. The high degree of isozyme polymorphism observed in Easter cactus is consistent with other plant species that are primarily outbreeding (Ellstrand and Roose, 1987; Hamrick and Godt, 1989). The six isozyme loci identified in this study will be useful for distinguishing cultivars, confirming hybridity, and may aid in selecting superior genotypes in the seedling stage, thus expediting the development of new cultivars.

Table 3.1. Polyacrylamide gel concentrations and sample loading volumes used for electrophoresis.

Enzyme	Gel concentration (%)	Loading volume (ul)
Aspartate aminotransferase (AAT)	5	10
Glucose-6-phosphate isomerase (GPI)	7.5	5
Malate dehydrogenase (MDH)	7.5	10
Phosphoglucomutase (PGM)	7.5	10
Triosephosphate isomerase (TPI)	10	5

Table 3.2. *Hatiora* clones used for generating F_1, F_2, BC_1, and S_1 progenies for isozyme analysis.

Clone	Parentage	Source [z]
Andre	unknown	RG
Crimson Giant	unknown	MD
Evita	unknown	JV
MD861	unknown	MD
Red Pride	unknown	JV
R891-2	Crimson Giant x MD681	MA
R891-3	Crimson Giant x MD681	MA
R891-6	Crimson Giant x MD681	MA
R891-8	Crimson Giant x MD681	MA
R891-9	Crimson Giant x MD681	MA
R891-1	MD681 *x* Crimson Giant	MA

[z] JV=J. de Vries Potplantencultures bv, Aalsmer, The Netherlands; MA=University of Massachusetts, Amherst; MD=University of Maryland, College Park; RG=Rainbow Gardens, Vista, CA.

Table 3.3. Segregation and X^2 analysis for *Aat-1*.

Cross or self	Parental phenotypes	Progeny phenotypes	Expected ratio	X^2	*P* value
		F_1 progeny			
Crimson Giant x MD681	cd x cc	18 cc 23 cd	1 : 1	0.39	0.60-0.50
Crimson Giant x Red Pride [a]	cd x cc	31 cc 32 cd	1 : 1	0.00	>0.99
Crimson Giant x Evita [a]	cd x cc	34 cc 36 cd	1 : 1	0 01	0.90-0.80
Evita x Red Pride [a]	cc x cc	61 cc	----	----	----
		F_2 progeny			
R891-2 x R891-3 [a]	cc x cd	13 cc 19 cd	1 : 1	0.78	0.40-0.30
R891-2 x R891-9	cc x cd	51 cc 44 cd	1 : 1	0.38	0.60-0.50
R891-8 x R891-6	cc x cd	6 cc 3 cd	1 : 1	0.44	0.60-0.50
		BC_1 progeny			
Crimson Giant x R891-2 [a]	cd x cc	18 cc 36 cd	1 : 1	5.35	< 0.05
Crimson Giant x R891-3 [a]	cd x cd	1 cc 17 cd 9 dd	1 : 2 : 1	6.55	< 0.05
Crimson Giant x R891-6 [a]	cd x cd	21 cc 21 cd 3 dd	1 : 2 : 1	14.60	< 0.001
Crimson Giant x R892-1 [a]	cd x cc	25 cc 24 cd	1 : 1	0.00	>0.99
R891-2 x MD681	cc x cc	12 cc	----	----	----
R891-8 x Crimson Giant	cc x cd	5 cc 5 cd	1 : 1	0.00	>0.99
		S_1 progeny			
Crimson Giant	cd x cd	5 cc 53 cd 49 dd	1 : 2 : 1	36.19	< 0.001

[a] Pooled data from reciprocal crosses

Table 3.4. Segregation and X^2 analysis for *Gpi-1*.

Cross or self	Parental phenotypes	Progeny phenotypes	Expected ratio	X^2	*P* value
		F_1 progeny			
Andre x Evita [a]	bc x ab	25 ab 12 ac 17 bb 14 bc	1 : 1 : 1 : 1	5.76	0.20-0.10
Andre x MD681	bc x ab	21 ab 22 ac 25 bb 27 bc	1 : 1 : 1 : 1	0.96	0.90-0.80
Crimson Giant x MD681	bb x ab	19 ab 21 bb	1 : 1	0.03	0.90-0.80
Crimson Giant x Red Pride [a]	bb x ab	25 ab 37 bb	1 : 1	1.95	0.20-0.10
Crimson Giant x Evita [a]	bb x ab	26 ab 44 bb	1 : 1	4.13	< 0.05
Evita x Red Pride [a]	ab x ab	15 aa 23 ab 23 bb	1 : 2 :1	5.79	0.10-0.05
Red Pride x MD681	ab x ab	25 aa 50 ab 25 bb	1 : 2 : 1	0.00	> 0.99
		F_2 progeny			
R891-2 x R891-3 [a]	bb x bb	32 bb	----	----	----
R891-8 x R891-6	bb x ab	4 ab 5 bb	1 : 1	0.00	>0.99
		BC_1 progeny			
Crimson Giant x R891-2 [a]	bb x bb	56 bb	----	----	----
Crimson Giant x R891-3 [a]	bb x bb	26 bb	----	----	----
Crimson Giant x R891-6 [a]	bb x ab	20 ab 25 bb	1 : 1	0.36	0.60-0.50
Crimson Giant x R892-1 [a]	bb x ab	29 ab 20 bb	1 : 1	1.31	0.30-0.20
R891-2 x MD681	bb x ab	8 ab 4 bb	1 : 1	0.75	0.40-0.30
R891-8 x Crimson Giant	bb x bb	10 bb	-----	---	---
		S_1 progeny			
Crimson Giant	bb x bb	100 bb	-----	-----	-----

[a] Pooled data from reciprocal crosses

Table 3.5. Segregation and X^2 analysis for *Mdh-1*.

Cross or self	Parental phenotypes	Progeny phenotypes	Expected ratio	X^2	*P* value
		F_1 progeny			
Crimson Giant x MD681	ac x aa	18 aa 22 ac	1 : 1	0.23	0.60-0.50
Crimson Giant x Red Pride [a]	ac x aa	30 aa 33 ac	1 : 1	0.06	0.90-0.80
Crimson Giant x Evita [a]	ac x aa	42 aa 25 ac	1 : 1	3.82	0.10- 0.05
Evita x Red Pride [a]	aa x aa	61 aa	-----	-----	-----
		F_2 progeny			
R891-2 x R891-3 [a]	aa x ac	9 aa 22 ac	1 : 1	4.65	< 0.05
R891-8 x R891-6	ac x aa	6 aa 3 ac	1 : 1	0.44	0.60-0.50
R891-8 x R891-2	ac x aa	6 aa 9 ac	1 : 1	0.27	0.50-0.40
		BC_1 progeny			
Crimson Giant x R891-2 [a]	ac x aa	15 aa 41 ac	1 : 1	11.16	< 0.001
Crimson Giant x R891-3	ac x ac	5 aa 9 ac 8 cc	1 : 2 : 1	1.54	0.20-0.10
Crimson Giant x R891-6 [a]	ac x aa	16 aa 29 ac	1 : 1	3.20	0.10-0.05
Crimson Giant x R892-1 [a]	ac x ac	10 aa 23 ac 16 cc	1 : 2 : 1	1.65	0.50-0.40
R891-2 x MD681	aa x aa	12 aa	-----	-----	-----
R891-8 x Crimson Giant	ac x ac	5 aa 3 ac 2 cc	1 : 2 : 1	3.40	0.20-0.10
		S_1 progeny			
Crimson Giant	ac x ac	30 aa 52 ac 19 cc	1 : 2 : 1	2.49	0.30-0.20

[a] Pooled data from reciprocal crosses

Table 3.6. Segregation and X^2 analysis for *Pgm-1*.

Cross or self	Parental phenotypes	Progeny phenotypes	Expected ratio	X^2	*P* value
		F_1 progeny			
Andre x MD681	dd x dd	96 dd	----	----	----
Crimson Giant x MD681	ff x dd	40 df	----	----	----
Crimson Giant x Red Pride [a]	ff x dd	62 df	----	----	----
Crimson Giant x Evita [a]	ff x dd	67 df	-----	-----	----
Evita x Red Pride [a]	dd x dd	59 dd	----	----	----
Evita x Shocking Pink	dd x df	15 dd 17 df	1 : 1	0.03	0.90-0.80
Red Pride x MD681	dd x dd	100 dd	----	----	----
		F_2 progeny			
R891-2 x R891-3 [a]	df x df	4 dd 13 df 14 ff	1 : 2 : 1	7.26	< 0.05
R891-2 x R891-9	df x df	20 dd 53 df 22 ff	1 : 2 : 1	1.36	0.60-0.50
R891-8 x R891-2	df x df	5 dd 9 df 1 ff	1 : 2 : 1	2.73	0.30-0.20
R891-8 x R891-6	df x df	2 dd 7 df 0 ff	1 : 2 : 1	3.66	0.20-0.10
		BC_1 progeny			
Crimson Giant x R891-2 [a]	ff x df	40 df 16 ff	1 : 1	9.45	< 0.01
Crimson Giant x R891-3 [a]	ff x df	9 df 18 ff	1 : 1	2.37	0.10-0.05
Crimson Giant x R891-6 [a]	ff x df	33 df 12 ff	1 : 1	8.89	< 0.01
Crimson Giant x R892-1 [a]	ff x df	35 df 14 ff	1 : 1	8.16	< 0.01
R891-2 x MD681	df x dd	5 dd 7 df	1 : 1	0.08	0.90-0.80
R891-8 x Crimson Giant	df x ff	8 df 2 ff	1 : 1	2.50	0.20-0.10
		S_1 progeny			
Crimson Giant	ff x ff	100 ff	-----	-----	-----

[a] Pooled data from reciprocal crosses

Table 3.7. Segregation and X^2 analysis for *Pgm-2*.

Cross or self	Parental phenotypes	Progeny phenotypes	Expected ratio	X^2	*P* value
		F_1 progeny			
Andre x MD681	ad x ab	29 aa 22 ab 22 ad 23 bd	1 : 1 : 1 : 1	1.42	0.70-0.60
Crimson Giant x MD681	ad x ab	9 aa 12 ab 11 ad 8 bd	1 : 1 : 1 : 1	1.00	0.90-0.80
Crimson Giant x Red Pride [a]	ad x ab	16 aa 9 ab 21 ad 16 bd	1 : 1 : 1 : 1	4.71	0.20-0.10
Crimson Giant x Evita [a]	ad x aa	31 aa 36 ad	1 : 1	0.24	0.60-0.50
Evita x Red Pride [a]	aa x ab	27 aa 34 ab	1 : 1	0.59	0.40-0.30
Evita x Shocking Pink	aa x aa	32 aa	-----	-----	-----
Red Pride x MD681	ab x ab	33 aa 39 ab 28 bb	1 : 2 : 1	5.34	0.10-0.05
		F_2 progeny			
R891-2 x R891-3 [a]	ad x aa	16 aa 15 ad	1 : 1	0.00	>0.99
R891-2 x R891-9	ad x ad	28 aa 42 ad 25 dd	1 : 2 : 1	1.46	0.50-0.40
R891-8 x R891-2	aa x ad	8 aa 7 ad	1 : 1	0.00	>0.99
R891-8 x R891-6	aa x ad	5 aa 4 ad	1 : 1	0.00	>0.99
		BC_1 progeny			
Crimson Giant x R891-2 [a]	ad x ad	17 aa 26 ad 13 dd	1 : 2 : 1	0.86	0.70-0.60
Crimson Giant x R891-3 [a]	ad x aa	9 aa 17 ad	1 : 1	1.88	0.20-0.10
Crimson Giant x R891-6 [a]	ad x ad	9 aa 25 ad 11 dd	1 : 2 : 1	0.73	0.70-0.60
Crimson Giant x R892-1 [a]	ad x ad	13 aa 25 ad 11 dd	1 : 2 : 1	0.18	0.95-0.90
R891-2 x MD681	ad x ab	4 aa 3 ab 3 ad 2 bd	1 : 1 : 1 : 1	0.66	0.90-0.80
R891-8 x Crimson Giant	aa x ad	6 aa 4 ad	1 : 1	0.10	0.70-0.60
		S_1 progeny			
Crimson Giant	ad x ad	37 aa 51 ad 12 dd	1 : 2 : 1	12.54	< 0.01

[a] Pooled data from reciprocal crosses

Table 3.8. Segregation and X^2 analysis for *Tpi-2*.

Cross or self	Parental phenotypes	Progeny phenotypes	Expected ratio	X^2	*P* value
		F_1 progeny			
Andre x MD681	ab x ab	21 aa 55 ab 20 bb	1 : 2 : 1	2.06	0.40-0.30
Crimson Giant x MD681	bb x ab	14 ab 26 bb	1 : 1	3.60	0.10-0.05
Crimson Giant x Red Pride	bb x ab	5 ab 26 bb	1 : 1	12.90	< 0.01
Red Pride x Crimson Giant	ab x bb	14 ab 17 bb	1 : 1	0.13	0.70-0.60
Crimson Giant x Evita [a]	bb x ab	11 ab 59 bb	1 : 1	31.56	< 0.001
Evita x Red Pride [a]	ab x ab	0 aa 33 ab 27 bb	1 : 2 : 1	24.9	< 0.001
Red Pride x MD681	ab x ab	22 aa 56 ab 22 bb	1 : 2 : 1	1.44	0.50-0.40
		F_2 progeny			
R891-2 x R891-3 [a]	bb x bb	31 bb	-----	-----	-----
R891-2 x R891-9	bb x ab	50 ab 45 bb	1 : 1	0.17	0.60-0.50
R891-8 x R891-2	ab x bb	7 ab 8 bb	1 : 1	0.00	>0.99
R891-8 x R891-6	ab x bb	4 ab 5 bb	1 : 1	0.00	>0.99
		BC_1 progeny			
Crimson Giant x R891-2 a	bb x bb	56 bb	-----	-----	-----
Crimson Giant x R891-3 a	bb x bb	26 bb	-----	-----	-----
Crimson Giant x R891-6 a	bb x bb	45 bb	-----	-----	-----
Crimson Giant x R892-1 a	bb x bb	49 bb	-----	-----	-----
R891-2 x MD681	bb x ab	4 ab 8 bb	1 : 1	0.75	0.30-0.20
R891-8 x Crimson Giant	ab x bb	6 ab 4 bb	1 : 1	0.10	0.70-0.60
		S_1 progeny			
Crimson Giant	bb x bb	100 bb	-----	-----	-----

[a] Pooled data from reciprocal crosses

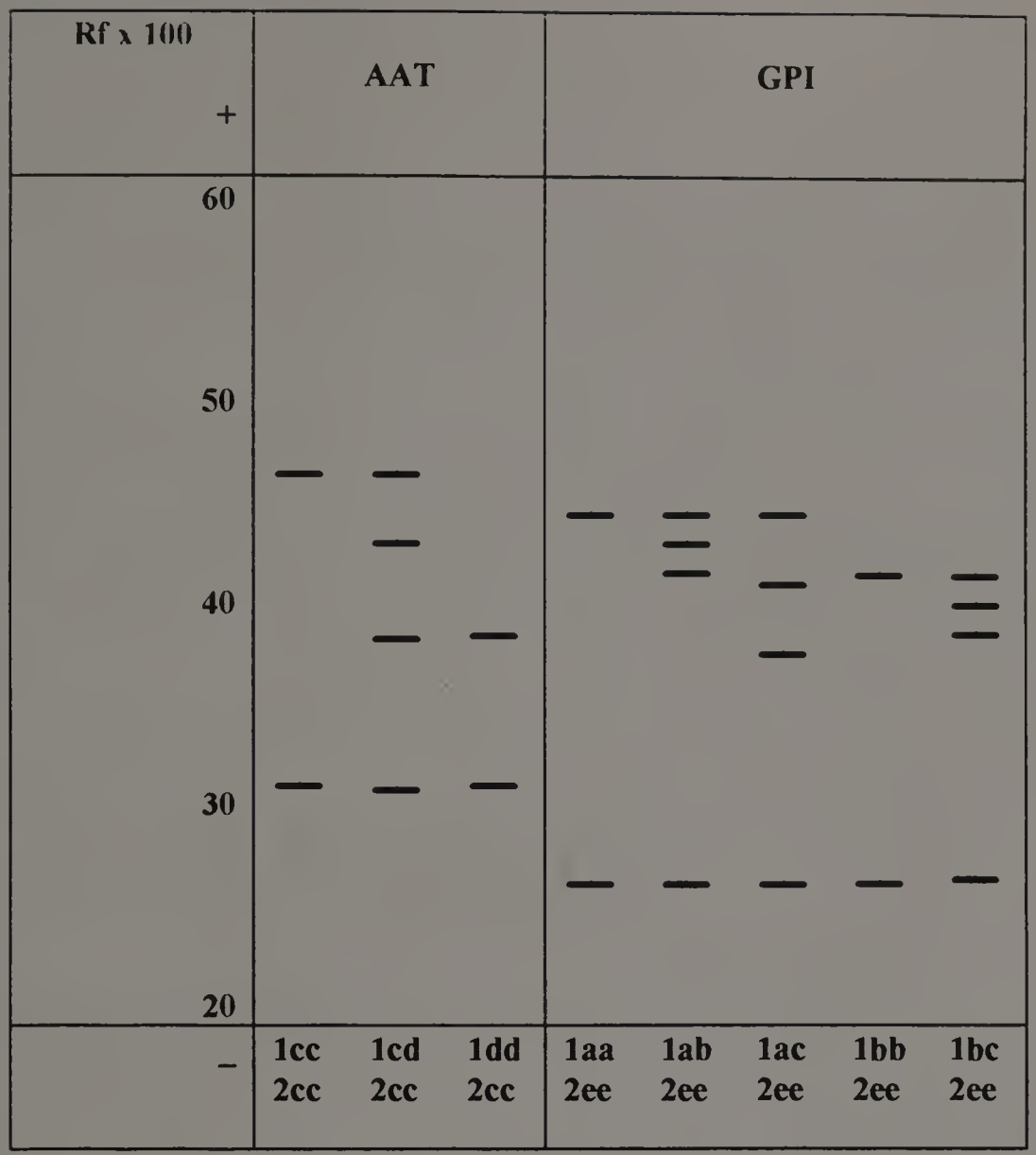

Figure 3.1. Schematic illustrations of enzyme phenotypes for AAT and GPI in *Hatiora*.

Rf x 100 +	PGM						MDH			TPI		
70												
60												
50												
40												
30												
–	1dd 2aa	1df 2ab	1ff 2ad	1dd 2bd	1df 2bb	1ff 2dd	1aa 2aa 3aa	1ac 2aa 3aa	1cc 2aa 3aa	1aa 2aa	1aa 2ab	1aa 2bb

Figure 3.2. Schematic illustrations of enzyme phenotypes for PGM, MDH, and TPI in *Hatiora*.

CHAPTER 4
INHERITANCE OF THREE POLYMORPHIC ISOZYME LOCI IN *SCHLUMBERGERA*

Abstract

Polyacrylamide gel electrophoresis (PAGE) was performed to investigate the inheritance of leucine aminopeptidase (LAP), phosphoglucomutase (PGM), and shikimate dehydrogenase (SKD) in the genus *Schlumbergera* (Cactaceae). F_1, F_2, and BC_1 populations were examined to determine the mode of inheritance. The results of inheritance studies for three polymorphic loci (*Lap-1*, *Pgm-1*, and *Skd-1*) were generally as expected for single loci with codominant alleles. However, significant segregation distortion was observed in at least one segregating family for all three isozyme loci. Segregation ratios for *Lap-1* were significantly distorted in 15 out of the 22 families that were examined. Further analysis revealed that disturbed segregation at *Lap-1* was due to tight linkage with the locus controlling gametophytic self-incompatibility (*S*). Linkage analysis indicated that all three loci assorted independently of each other. The monomeric structure of *Lap-1*, *Pgm-1*, and *Skd-1* was consistent with the quaternary structures previously reported for other plant species.

Introduction

The genus *Schlumbergera* consists of six species of epiphytic or lithophytic shrubs that are native to southeastern Brazil (Hunt, 1969; Huxley et al., 1992). *Schlumbergera truncata* (Haw.) Moran, also known as zygocactus or Thanksgiving cactus, is the most commonly cultivated species and is an economically important floricultural crop in northern Europe and North America (Cobia, 1992). The other *Schlumbergera* species are rare in cultivation, but have been used for breeding. Hybridization between *S. truncata* and *S. russelliana* (Hook.) Britton & Rose has

yielded many cultivars, including the well-known Christmas cactus (Hunt, 1981). The collective name for interspecific hybrids of *S. truncata* and *S. russelliana* is *S.* x *buckleyi* (T. Moore) Tjaden (Tjaden, 1966). *Schlumbergera truncata* has also been crossed with *S. orssichiana* Barthlott & McMillan, and the resulting interspecific hybrids have been given the collective name *S.* x *reginae* McMillan & Orssich (Horobin and McMillan, 1985). *Schlumbergera orssichiana*, *S. russelliana*, and *S. truncata* are diploid species with a chromosome number of $2n = 2x = 22$ (Barthlott, 1976; Remski, 1954; Stockwell, 1935).

Breeding of *Schlumbergera* was initiated at the University of Massachusetts in 1987, and the program's objective is to develop novel and superior clones for commercial production. To achieve this objective, commercial cultivars (*S. truncata* and *S.* x *buckleyi*) have been intercrossed with wild species clones (*S. orssichiana*, *S. russelliana*, and *S. truncata*) and F_1 interspecific hybrids to create a diverse gene pool. A set of genetic markers would be highly desirable for identifying clones, and also for selecting progeny in the seedling stage. Currently, morphological traits are used to distinguish *Schlumbergera* clones, but these traits can vary with environmental conditions. Furthermore, crossing studies have revealed that few simply-inherited morphological markers exist in *Schlumbergera* (T.H. Boyle, unpublished data).

Isozymes are widely used in agriculture for identifying cultivars and as genetic markers for breeding programs (Nielsen, 1985). In most cases, isozymes are simply inherited and exhibit codominant expression, complete penetrance, and consistency of expression under a wide range of environmental conditions (Weeden and Wendel, 1989), and thus are particularly useful as genetic markers. To date, there have been no published studies of isozyme polymorphism or inheritance in *Schlumbergera*. This paper reports on the inheritance of three polymorphic isozyme loci in *Schlumbergera*.

Materials and Methods

Plant material. Plants were propagated and grown in glasshouses at the University of Massachusetts, Amherst. Parental cultivars were obtained from the clonal germplasm collection maintained in the Department of Plant and Soil Sciences at the University of Massachusetts. Cross-compatible clones were used as parents for producing F_1, F_2, and BC_1 seed, and crosses were performed in a glasshouse maintained at 18°C nights/22°C days. Techniques used in emasculation, pollination, seed germination, and plant culture were similar to those previously reported (Boyle et al., 1994). Henceforth, all crosses will be presented as pistillate parent x pollen parent unless specified otherwise.

Protein extraction and sample preparation. Tissue was removed from mature phylloclades with a no. 1 cork borer. Soluble proteins were extracted by homogenizing ≈8 mg tissue in 100 μl cold extraction buffer [0.050 M Tris-HCl buffer (pH 7.5), 0.014 M *B*-mercaptoethanol, 5% (w/v) polyvinylpyrrolidone (PVP-40), and 5% (w/v) sucrose (Wendel and Weeden, 1989)]. Crude homogenates were centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant (≈60 μl) was collected and mixed with 10 μl of cold sample mix [50% (v/v) glycerol and 0.1% (w/v) bromphenol blue (Shields et al., 1983)].

Electrophoresis. Native proteins were separated by polyacrylamide gel electrophoresis (PAGE) using a Mini-Protean II cell (Bio-Rad Laboratories, Hercules, CA). Single percentage (7.5%) polyacrylamide 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). Running times varied according to the enzyme system assayed, but typically required 45 minutes for completion.

Gel staining. Three enzyme systems were examined: leucine aminopeptidase (LAP, E.C. 3.4.11.1), phosphoglucomutase (PGM, E.C. 5.4.2.2), and shikimate dehydrogenase (SKD, E.C. 1.1.1.25). The staining procedures of Wendel (1989) were used to assay LAP, PGM, and SKD. Enzyme stains were prepared immediately prior to use. After the appropriate staining intensities were achieved, the gels were rinsed with water and fixed in 7% acetic acid (Hames, 1981).

Data analysis. R_f values were calculated for each band by dividing the band's migration distance by the tracking dye's migration distance (Hames, 1981). Data were collected immediately after gels were fixed, i.e., when the staining intensities were maximal. For each enzyme, loci were numbered sequentially, with *1* denoting the most anodally migrating locus. Alleles at individual loci were designated by letters assigned sequentially from the anode.

Isozyme inheritance was determined by evaluating the segregation ratios in full-sib F_1, F_2, and BC_1 families. Reciprocal crosses were pooled for chi-square analysis in the following circumstances: 1) one or both families consisted of relatively few individuals; and 2) parents and progeny were homozygous for the same isozyme phenotype. The computer program LINKAGE-1 (Suiter et al., 1983) was used to test goodness-of-fit for single locus segregation ratios, and to test for linkage relationships among the enzyme loci.

Results and Discussion

Shikimate dehydrogenase. One polymorphic region of SKD activity was observed on PAGE gels, which was designated *Skd-1* (Fig. 4.1). Plants produced either a single-banded pattern (aa, bb, or cc) or a double-banded pattern (cd or ce). The a and b alleles with R_f values 0.47 and 0.44 respectively, were not subjected to genetic analysis. Crosses between two different clones with cc phenotypes yielded only cc progeny (Table 4.2). Crosses between cc and cd phenotypes yielded, in most cases,

progenies which exhibited the expected 1 : 1 segregation ratio (Table 4.2). Progeny from the cross *S.* x *buckleyi* S881-6 (cc) x *S.* x *reginae* 'Madame Butterfly' (ce) deviated significantly from the expected 1 : 1 ratio ($P < 0.001$), with only one plant exhibiting the e allele from the pollen parent. The observed segregation ratios at *Skd-1* generally conform to the expected ratios for a simply-inherited monomeric enzyme, and are consistent with previous studies on SKD (Weeden and Wendel, 1989).

Phosphoglucomutase. Two polymorphic regions of PGM activity were observed on PAGE gels (Fig. 4.1). At the slower region, designated as *Pgm-2,* only one band cc was subjected to genetic analysis, bands aa and bb (R_f values 0.47 and 0.45) were not analyzed in this study. At *Pgm-1* three single-banded patterns (aa, bb, and cc) and one double-banded pattern (bc) were observed. The a allele was not subjected to genetic analysis and had an R_f value of 0.64. Crosses between two bb phenotypes yielded exclusively bb progeny (Table 4.3). As expected, only bc progeny were obtained when a bb phenotype was crossed with a cc phenotype. Crosses between bb and bc phenotypes yielded, in all cases, progenies which exhibited the expected 1 : 1 ratio (Table 4.3). Crosses between two bc phenotypes yielded five families which segregated in the expected 1 : 2 : 1 ratio, and one F_1 family (*S. truncata* 'Linda' x *S.* x *buckleyi* S881-4) which exhibited a deficiency of bb progeny and an excess of cc progeny (Table 4.3). The observed segregation ratios at *Pgm-1* generally conform to those expected for a simply inherited gene. Navot and Zamir (1986) and Weeden and Gottlieb (1980) have shown that PGM is a monomer, and the current results are consistent with a monomeric enzyme.

Leucine aminopeptidase. One polymorphic region of LAP activity was observed on PAGE gels (Fig. 4.1). This region (designated *Lap-1*) exhibited four single-banded patterns (aa, bb, cc, and dd) and three double-banded patterns (bc, bd, and cd). Inheritance of the a allele with an R_f value 0.60 was not examined. Progenies exhibited significant departures from the expected single-locus segregation

ratios in 15 of the 22 full-sib families that were examined (Table 4.4). Analysis of reciprocal crosses revealed that segregation distortion was due to differential transmission of *Lap-1* alleles (Table 4.4). Maternally-derived alleles were present at the expected frequency (0.5) in most progenies, whereas specific paternally-derived alleles were present at a higher-than-expected frequencies. These results are consistent with linkage between *Lap-1* and the locus controlling gametophytic self-incompatibility (SI). Distorted segregation results from differential transmission of pollen tubes in semi-compatible crosses, i.e., those in which both parents share one *S* allele in common, whereas fully compatible crosses (those in which both parents share no *S* alleles in common) exhibit normal segregation ratios (Leach, 1988). This phenomenon has been reported in several species with gametophytic SI systems (Leach, 1988; Manganaris and Alston, 1987; Tanksley and Loaiza-Figueroa, 1985). SI is reported to occur in all *Schlumbergera* species except for *S. obtusangula* (Schum.) D. Hunt (McMillan, 1991) and *S. kautskyi* (Horobin & McMillan) N.P. Taylor (McMillan and Horobin, 1992). Recent studies (T.H. Boyle, unpublished data) indicate that the SI system in *Schlumbergera* is gametophytic and controlled by a single locus. The cross 'Rocket' x 'Buckleyi' is fully compatible and yields four cross-compatible but intra-incompatible progeny classes, showing that 'Rocket' and 'Buckleyi' have totally different *S* phenotypes. From these results, the following phenotypes for the *S* locus and *Lap-1* can be proposed: S_1 b/S_2 d for 'Rocket', S_3 c/S_4 c for 'Buckleyi', and S_1 b/S_3 c, S_1 b/S_4 c, S_2 d/S_3 c, and S_2 d/S_4 c for the F_1 progeny (Table 5). Simple 1 : 1 segregation was observed for 'Rocket' x 'Buckleyi' and the reciprocal cross, which is consistent with a fully-compatible cross (Table 5). All five F_2 families and most of the BC_1 families exhibited distorted segregation ratios, results which agree with those expected from semi-compatible crosses (Table 5). Three BC_1 progenies that were produced using 'Buckleyi' as the paternal parent segregated in a 1 : 1 Mendelian ratio, whereas the reciprocal crosses exhibited disturbed segregation ratios (Table 5).

Differences between reciprocal crosses occurred because 'Buckleyi' was homozygous at *Lap-1* (cc) whereas the other BC_1 parents were heterozygous at *Lap-1* (bc or cd). These data show that disturbed segregation ratios occur only when the cross is semi-compatible and the paternal parent is heterozygous at *Lap-1*. Distorted segregation ratios in two F_1 families (S881-6 x 'Madame Butterfly' and 'Linda' x S881-6) is most likely due to semi-compatible parents. It is worth noting that most of the F_2 and BC_1 families which displayed distorted segregation ratios for *Lap-1* were found to have normal segregation ratios for *Skd-1* and *Pgm-1* (Tables 1 and 2), thus supporting the hypothesis that segregation distortion is due to linkage between *Lap-1* and the *S* locus and not due to clonal variation in pollen viability.

Seven F_1 and BC_1 families exhibited segregation ratios at *Lap-1* that are consistent with a simply inherited isozyme with a monomeric structure (Tables 3 and 5). Scandalios and Espiritu (1969) reported that LAP is a monomer in *Pisum sativum* L.

Linkage analysis. Linkage relationships among *Skd-1*, *Pgm-1*, and *Lap-1* were estimated in all possible pairwise combinations using the LINKAGE-1 computer program (Suiter et al., 1983). The recombination frequencies (r) and X^2 values for the three pairs of isozyme loci suggest that the three loci segregate independently. The maximum likelihood methods described by Leach (1988) were used to estimate linkage between the *S* locus and *Lap-1*. The recombination frequency between the *S* locus and *Lap-1* varied from 0.02 to 0.13 for the 13 segregating families, and was 0.07 ± 0.01 for pooled data (Table 5).

Hatiora (Boyle et al., 1994), *Lophocereus* (Parker and Hamrick, 1992), *Opuntia* (Wallace and Fairbrothers, 1986), and *Pachycereus* (Garcia-Carreño, 1993; Murawski et al., 1994) are the only Cactaceae genera that have been subjected to isozyme analysis. The Cactaceae are not particularly amenable to genetic studies due to their long juvenility period, which can last for 5 years or longer (Cullman et al., 1987).

Hence, the paucity of isozyme inheritance studies on Cactaceae taxa is not suprising. By growing *Schlumbergera* seedlings under optimal environmental conditions, however, the juvenility period can be reduced to ≈2½ years, which makes them more suitable than most other Cactaceae taxa for genetic studies.

The three monomeric isozymes examined in *Schlumbergera* demonstrated considerable polymorphism (Fig. 1), results which are consistent with other plant species that are primarily outbreeding (Ellstrand and Roose, 1987; Hamrick and Godt, 1989). These three isozymes can aid in distinguishing cultivars and confirming hybridity in *Schlumbergera*, and may facilitate selection of superior phenotypes in the seedling stage, thus expediting the development of new cultivars.

Table 4.1. *Schlumbergera* clones used for generating F_1, F_2, and BC_1 progenies for isozyme analysis.

Clone	Parentage	Source [z]
Buckleyi	unknown	RG
Dark Marie	unknown	PM
Frida	unknown	GT
Linda	unknown	PM
Madame Butterfly	*S. orssichiana* x White Christmas	LN
Rocket	unknown	RG
S881-2	Rocket x Buckleyi	MA
S881-4	Rocket x Buckleyi	MA
S881-6	Rocket x Buckleyi	MA
S881-7	Rocket x Buckleyi	MA
S881-15	Rocket x Buckleyi	MA

[z] GT=Gartneriet Thoruplund, Odense, Denmark; LN=Leavitt's Nursery, Lehighton, Penn. MA=University of Massachusetts, Amherst; PM=Gartneriet PKM, Odense, Denmark; RG=Rainbow Gardens, Vista, CA.

Table 4.2. Segregation and X^2 analysis for *Skd-1*.

Cross	Parental phenotypes	Progeny phenotypes	Expected ratio	X^2	P value
		F_1 Progeny			
Buckleyi x Rocket [a]	cc x cd	96 cc 120 cd	1 : 1	2.45	0.20-0.10
Frida x S881-2	cd x cc	14 cc 22 cd	1 : 1	1.36	0.20-0.10
S881-6 x M. Butterfly	cc x ce	27 cc 1 ce	1 : 1	22.32	< 0.001
Linda x S881-4	cd x cc	11 cc 19 cd	1 : 1	1.63	0.20-0.10
S881-7 x Linda	cc x cd	12 cc 21 cd	1 : 1	1.94	0.20-0.10
		F_2 Progeny			
S881-4 x S881-6 [a]	cc x cc	148 cc	-----	-----	-----
S881-4 x S881-15 [a]	cc x cc	127 cc	-----	-----	-----
S881-2 x S881-7 [a]	cc x cd	38 cc 12 cd	1 : 1	12.50	< 0.001
S881-2 x S881-6	cc x cc	18 cc	-----	-----	-----
		BC_1 Progeny			
Buckleyi x S881-4 [a]	cc x cc	100 cc	-----	-----	-----
Buckleyi x S881-6 [a]	cc x cc	100 cc	-----	-----	-----
Buckleyi x S881-15 [a]	cc x cc	100 cc	-----	-----	-----
S881-4 x Rocket	cc x cd	25 cc 25 cd	1 : 1	0.00	>0.99
Rocket x S881-6 [a]	cd x cc	50 cc 50 cd	1 : 1	0.00	>0.99
Rocket x S881-15 [a]	cd x cc	49 cc 51 cd	1 : 1	0.01	0.90-0.80

[a] Pooled data from reciprocal crosses

Table 4.3. Segregation and X^2 analysis for *Pgm-1*.

Cross	Parental phenotypes	Progeny phenotypes	Expected ratio	X^2	P value
		F_1 Progeny			
Buckleyi x Rocket [a]	bb x bc	107 bb 109 bc	1 : 1	.01	0.90-0.80
Frida x S881-2	cc x bb	36 bc	-----	-----	-----
S881-6 x M. Butterfly	bc x bc	9 bb 13 bc 6 cc	1 : 2 : 1	0.79	0.70-0.60
Linda x S881-4	bc x bc	0 bb 15 bc 15 cc	1 : 2 : 1	15.0	< 0.001
S881-7 x Linda	bc x bc	8 bb 15 bc 10 cc	1 : 2 : 1	0.52	0.80-0.70
		F_2 Progeny			
S881-4 x S881-6 [a]	bc x bc	40 bb 74 bc 34 cc	1 : 2 : 1	0.49	0.80-0.70
S881-4 x S881-15 [a]	bc x bb	54 bb 73 bc	1 : 1	2.55	0.20-0.10
S881-2 x S881-7 [a]	bb x bb	50 bb	-----	-----	-----
S881-2 x S881-6	bb x bc	11 bb 7 bc	1 : 1	0.50	0.40-0.30
		BC_1 Progeny			
Buckleyi x S881-4 [a]	bb x bc	52 bb 48 bc	1 : 1	0.09	0.90-0.80
Buckleyi x S881-6 [a]	bb x bc	47 bb 53 bc	1 : 1	0.25	0.60-0.50
Buckleyi x S881-15 [a]	bb x bb	100 bb	-----	-----	-----
S881-4 x Rocket	bc x bc	15 bb 23 bc 12 cc	1 : 2 : 1	0.68	0.80-0.70
Rocket x S881-6 [a]	bc x bc	26 bb 56 bc 18 cc	1 : 2 : 1	2.72	0.30-0.20
Rocket x S881-15 [a]	bc x bb	45 bb 55 bc	1 : 1	0.81	0.40-0.30

[a] Pooled data from reciprocal crosses

Table 4.4. Segregation and X^2 analysis for *Lap-1*.

Cross (female x male)	Parental phenotypes	Progeny phenotypes	Expected ratio	X^2	P value
		F_1 Progeny			
Buckleyi x Rocket	cc x bd	59 bc 57 cd	1 : 1	0.01	0.90-0.80
Rocket x Buckleyi	bd x cc	41 bc 59 cd	1 : 1	2.89	0.20-0.10
Dark Marie x S881-2 [a]	cd x bc	14 bc 17 bd 13 cc 13 cd	1 : 1 : 1 : 1	0.75	0.90-0.80
Frida x S881-2	cd x bc	9 bc 3 bd 10 cc 11 cd	1 : 1 : 1 : 1	4.70	0.20-0.10
S881-6 x M. Butterfly	cd x cd	1 cc 18 cd 9 dd	1 : 2 : 1	6.60	< 0.05
Linda x S881-4	bd x bc	0 bb 9 bc 2 bd 19 cd	1 : 1 : 1 : 1	29.46	< 0.001
S881-7 x Linda	bc x bd	5 bb 5 bc 10 bd 11 cd	1 : 1 : 1 : 1	4.03	0.30-0.20
		F_2 Progeny			
S881-4 x S881-6	bc x cd	4 bc 28 bd 42 cd 1 cc	1 : 1 : 1 : 1	61.80	< 0.001
S881-6 x S881-4	cd x bc	34 bc 33 bd 3 cd 5 cc	1 : 1 : 1 : 1	46.55	< 0.001
S881-4 x S881-15	bc x bc	1 bb 24 bc 27 cc	1 : 2 : 1	26.31	< 0.001
S881-15 x S881-4	bc x bc	3 bb 40 bc 32 cc	1 : 2 : 1	22.76	< 0.001
S881-2 x S881-7	bc x bc	3 bb 16 bc 21 cc	1 : 2 : 1	17.80	< 0.001
		BC_1 Progeny			
Buckleyi x S881-4	cc x bc	47 bc 3 cc	1 : 1	36.98	< 0.001
S881-4 x Buckleyi	bc x cc	20 bc 30 cc	1 : 1	1.62	0.20-0.10
Buckleyi x S881-6	cc x cd	1 cc 49 cd	1 : 1	44.18	< 0.001
S881-6 x Buckleyi	cd x cc	26 cc 24 cd	1 : 1	0.01	0.90-0.80
Buckleyi x S881-15	cc x bc	48 bc 2 cc	1 : 1	40.50	< 0.001
S881-15 x Buckleyi	bc x cc	23 bc 27 cc	1 : 1	0.09	0.90-0.80
S881-4 x Rocket	bc x bd	0 bb 1 bc 20 bd 29 cd	1 : 1 : 1: 1	49.36	< 0.001
Rocket x S881-6	bd x cd	16 bc 3 bd 28 cd 3dd	1 : 1 : 1 : 1	34.64	< 0.001
S881-6 x Rocket	cd x bd	12 bc 35 bd 1 cd 2 dd	1 : 1 : 1 : 1	59.92	< 0.001
Rocket x S881-15	bd x bc	1 bb 18 bc 3 bd 28 cd	1 : 1 : 1 : 1	39.44	< 0.001
S881-15 x Rocket	bc x bd	1 bb 2 bc 26 bd 21 cd	1 : 1 : 1 : 1	39.76	< 0.001

[a] Pooled data from reciprocal crosses

Table 4.5. Influence of reciprocal crosses on phenotypic frequencies at *Lap-1* in *Schlumbergera*.

Cross (female x male)	Parental phenotypes	Progeny phenotypes	Phenotype frequency Observed	Expected
S881-4 x S881-6	bc x cd	b_	0.43	0.50
		d_	0.93	0.50
S881-6 x S881-4	cd x bc	b_	0.89	0.50
		d_	0.48	0.50
Buckleyi x S881-4	cc x bc	b_	0.94	0.50
S881-4 x Buckleyi	bc x cc	b_	0.40	0.50
Buckleyi x S881-6	cc x cd	d_	0.98	0.50
S881-6 x Buckleyi	cd x cc	d_	0.48	0.50
Buckleyi x S881-15	cc x bc	b_	0.96	0.50
S881-15 x Buckleyi	bc x cc	b_	0.46	0.50
Rocket x S881-6	bd x cd	b_	0.38	0.50
		c_	0.88	0.50
S881-6 x Rocket	cd x bd	b_	0.94	0.50
		c_	0.26	0.50
Rocket x S881-15	bd x bc	c_	0.92	0.50
		d_	0.62	0.50
S881-15 x Rocket	bc x bd	c_	0.46	0.50
		d_	0.94	0.50

Table 4.6. Parental phenotypes for the *S* locus and *Lap-1*, and estimates of the recombination frequency between *S* and *Lap-1*.

Cross (female x male)	Proposed *S* locus and *Lap-1* phenotypes	Progeny *Lap-1* phenotypes	Recombination [z] r	SE
		F_1 progeny		
Rocket x Buckleyi	S_1b/S_2d x S_3c/S_4c	41 bc 59 cd	-----	-----
Buckleyi x Rocket	S_3c/S_4c x S_1b/S_2d	59 bc 57 cd	-----	-----
		F_2 progeny		
S881-4 x S881-6	S_1b/S_3c x S_3c/S_2d	4 bc 28 bd 42 cd 1cc	0.07	0.03
S881-6 x S881-4	S_3c/S_2d x S_1b/S_3c	34 bc 33 bd 3 cd 5 cc	0.11	0.04
S881-4 x S881-15	S_1b/S_3c x S_1b/S_4c	1 bb 24 bc 27 cc	0.04	0.04
S881-15 x S881-4	S_1b/S_4c x S_1b/S_3c	3 bb 40 bc 32 cc	0.09	0.05
S881-2 x S881-7	S_1b/S_4c x S_1b/S_3c	3 bb 16 bc 21 cc	0.13	0.07
		BC_1 progeny		
Buckleyi x S881-4	S_3c/S_4c x S_1b/S_3c	47 bc 3 cc	0.06	0.03
S881-4 x Buckleyi	S_1b/S_3c x S_3c/S_4c	20 bc 30 cc	-----	-----
Buckleyi x S881-6	S_3c/S_4c x S_3c/S_2d	1 cc 49 cd	0.02	0.02
S881-6 x Buckleyi	S_3c/S_2d x S_3c/S_4c	26 cc 24 cd	-----	-----
Buckleyi x S881-15	S_3c/S_4c x S_1b/S_4c	48 bc 2 cc	0.04	0.03
S881-15 x Buckleyi	S_1b/S_4c x S_3c/S_4c	23 bc 27 cc	-----	-----
S881-4 x Rocket	S_1b/S_3c x S_1b/S_2d	0 bb 1 bc 20 bd 29 cd	0.02	0.02
S881-6 x Rocket	S_3c/S_2d x S_1b/S_2d	12 bc 35 bd 1 cd 2 dd	0.06	0.03
Rocket x S881-6	S_1b/S_2d x S_3c/S_2d	16 bc 3 bd 28 cd 3 dd	0.12	0.05
S881-15 x Rocket	S_1b/S_4c x S_1b/S_2d	1 bb 2 bc 26 bd 21 cd	0.06	0.03
Rocket x S881-15	S_1b/S_2d x S_1b/S_4c	1 bb 18 bc 3 bd 28 cd	0.08	0.04
Pooled data			0.07	0.01

[z] Maximum likelihood estimates (Leach, 1988).

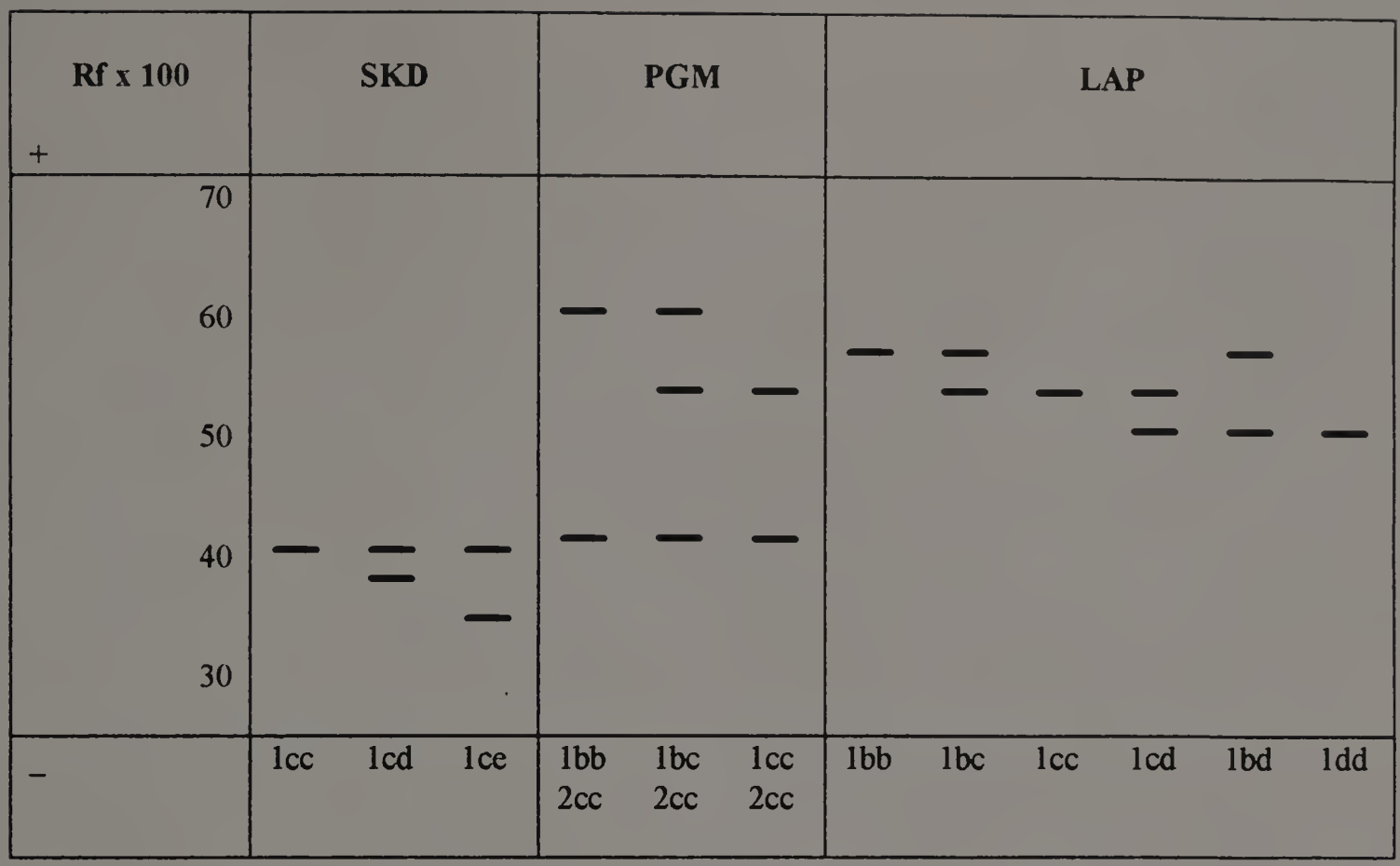

Figure 4.1. Schematic illustrations of enzyme phenotypes for SKD, PGM, and LAP in *Schlumbergera*.

CHAPTER 5
USING ISOZYME MARKERS TO IDENTIFY CULTIVARS AND ESTIMATE THE GENETIC DIVERSITY IN A *HATIORA* CLONAL GERMPLASM COLLECTION

Abstract

A clonal germplasm collection representing four *Hatiora* species and containing 49 commercial cultivars and field-collected specimens was evaluated for seven enzyme systems: aspartate aminotransferase (AAT), glucose-6-phosphate isomerase (GPI), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucomutase (PGM), shikimate dehydrogenase (SKD), and triosephosphate isomerase (TPI). Isozymes were extracted from phylloclades and were separated by polyacrylamide gel electrophoresis. Thirteen putative loci and 42 putative alleles were identified, and 9 of the 13 loci (69%) were polymorphic. Twenty-two clones (45%) could be distinguished solely on the basis of their isozyme profiles, but the other 27 clones shared isozyme profiles with one to five other clones. Among clones that shared isozyme profiles, 21 could be distinguished by flower and/or phylloclade traits. A group of 13 modern *H.* x *graeseri* cultivars exhibited less genetic diversity than a group of 40 *H. gaertneri*, *H.* x *graeseri*, and *H. rosea* clones representing older and modern cultivars plus field-collected specimens. The difference in genetic diversity was primarily attributed to a loss of alleles during breeding. The percentage of heterozygous loci ranged from 22 to 56% for eleven field-collected clones of the predominantly outcrossing species *H. salcornioides*, and was 0% for a field-collected clone of primarily selfing species *H. herminiae*, results which conform to expectations. However, field-collected clones of *H. gaertneri* and *H. rosea* contained few (0 to 23%) heterozygous loci, i.e., less than expected for these predominantly outcrossing species, suggesting that these plants originated from populations composed of relatively few individuals.

Introduction

The genus *Hatiora* consists of five species of epiphytic or epilithic shrubs that are endemic to southeastern Brazil (Barthlott, 1987; Barthlott and Taylor, 1995). Plants have a determinate growth pattern and produce a series of leafless stem segments (phylloclades) with a composite areole (Moran, 1953). As currently conceived, the genus is comprised of two subgenera - Hatiora and Rhipsalidopsis (Barthlott, 1987). Subgenus Hatiora contains two species - *H. salcornioides* (Haworth) Britton & Rose and *H. herminiae* (Campos-Porto & Castellanos) Backeberg ex Barthlott - which have terete or cylindric 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 - which have flattened or angular phylloclades and angled pericarpels. *Hatiora epiphylloides* and *H. herminiae* are difficult to cultivate and are represented in only a few living collections. *Hatiora salcornioides* is cultivated to some extent as a foliage plant. The most horticulturally important *Hatiora* species are *H. gaertneri* and *H. rosea*. *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, along with their interspecific hybrid [= *H.* x *graeseri* Barthlott ex D. Hunt (Hunt, 1992)], are commonly referred to as "Easter cactus" because they flower primarily in the spring, i.e., near Easter.

Easter cactus is a popular flowering potted plant in northern Europe, and has been increasing in popularity in North America (Boyle, 1991). Cultivars of Easter cactus, like many other ornamentals, are clonally propagated. More than 100 cultivars of Easter cactus are in existence, the majority of which are complex *H.* x *graeseri*

hybrids (Boyle, 1995; Meier, 1992). A rapid and accurate means of identifying specific cultivars is important to breeders and propagators. Presently, Easter cactus cultivars are identified by their flower and phylloclade morphology, flower color, and plant habit. Identifying cultivars becomes difficult when plants are in a vegetative state because there are fewer distinguishing features that can be used for identification. Isozymes are widely used in agricultural crops for identifying cultivars (Nielsen, 1985; Weeden, 1989). Isozymes can also be used to quantify the genetic diversity within germplasm collections (Weeden, 1989), data which may be useful in germplasm evaluation and amelioration. Several attributes of isozymes make them particularly useful as biochemical markers, including Mendelian inheritance, codominant expression, complete penetrance, lack of epistatic or pleiotropic interactions among loci, and consistency of expression under a wide range of environmental conditions (Weeden and Wendel, 1989). Isozymes can be extracted from a variety of tissues and can be separated using relatively inexpensive techniques.

The likelihood of identifying *Hatiora* cultivars by their isozyme phenotypes depends on the level of isozyme polymorphism present within the germplasm (Weeden, 1989). Previously, it was shown that several isozyme loci are polymorphic in *Hatiora* (Chapter 3). The objectives of the present study were to determine the isozyme phenotypes for a diverse collection of *Hatiora* clones, and to ascertain if isozymes could be used to identify cultivars and estimate the genetic diversity within the germplasm collection.

Materials and Methods

Plant material. A total of 49 clonal accessions were used for this study (Table 1). Accessions were obtained from commercial sources, botanical gardens, and universities, and included both commercial clones (cultivars) and field-collected clones.

Hatiora epiphylloides could not be obtained for analysis, but clones of the other four *Hatiora* species were included in this study. All species except for *H. herminiae* were represented by two or more accessions.

Protein extraction and sample preparation. Isozymes were extracted from immature or mature phylloclades. Immature phylloclades (3-5 mm in length) were removed with tweezers. Tissue samples were removed from mature phylloclades with a no. 1 cork borer. Soluble proteins were extracted by homogenizing 5-7 immature phylloclades (or ≈8 mg mature phylloclade tissue) in 100 μl cold extraction buffer [0.050 M Tris-HCl buffer (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 10,000 rpm for 10 minutes at 4^{o}C. The supernatant (≈60 μl) was collected and mixed with 10 μl of cold sample mix [50% (v/v) glycerol and 0.1% (w/v) bromphenol blue (Shields et al., 1983)].

Electrophoresis. Native proteins were separated by polyacrylamide gel electrophoresis (PAGE) using a Mini-Protean II cell (Bio-Rad Laboratories, Hercules, CA). Single percentage (5-10%) polyacrylamide gels were prepared using 0.375 M Tris-HCL (pH 8.8) as the gel buffer (Hames, 1981). Details on acrylamide concentrations and sample volumes for each enzyme system are provided in Table 2. 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^{o}C under constant voltage (200 V). Running times varied according to the enzyme system assayed, but typically required 45 minutes for completion.

Gel staining. 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), phosphoglucomutase (PGM, E.C. 5.4.2.2), shikimate dehydrgenase

(SKD, E.C. 1.1.1.25) and triosephosphate isomerase (TPI, E.C. 5.3.1.1). The staining procedures of Wendel (1989) for assaying enzyme activity. Enzyme stains were prepared immediately prior to use. After the appropriate staining intensities were achieved, the gels were rinsed with water and fixed in 7% acetic acid (Hames, 1981).

Gels were scored for the presence or absence of specific allelic bands of known migration distances. Data were collected immediately after gels were fixed, i.e., when the staining intensities were maximal. For each enzyme, loci were numbered sequentially, with *1* denoting the most anodally migrating locus. Alleles at individual loci were designated by letters assigned sequentially from the anode. Rf values were calculated by dividing the band's migration distance by the tracking dye's migration distance (Hames, 1991). The Rf values presented in this paper represent the average of three gels that were run under identical conditions.

Data analysis. Isozyme data were used to assess genetic diversity in the germplasm collection. The percentage of polymorphic loci, mean number of alleles per locus, average heterozygosity, and allelic diversity [(number of alleles observed ÷ total number of alleles at all loci) x 100] were calculated separately for the following four groups: 1) clones of *H. herminiae* and *H. salcornioides* (= subgenus Hatiora); 2) clones of *H. gaertneri*, *H.* x *graeseri*, and *H. rosea* (= subgenus Rhipsalidopsis); 3) modern cultivars of *H.* x *graeseri*; and 4) all *Hatiora* clones. Mean number of alleles per locus and average heterozygosity were calculated for all isozyme loci. The percentage of heterozygous loci was determined for several clones to determine if heterozygosity was correlated with the breeding system.

Results and Discussion

The inheritance of AAT, GPI, MDH, PGM, and TPI isozymes has been reported for *Hatiora* (Chapter 3). There have been, however, no published accounts on

the inheritance of LAP and SKD in *Hatiora*, and therefore the loci and alleles for these two enzyme systems are reported as putative. The inheritance of LAP and SKD has been reported for the closely related genus *Schlumbergera* (Chapter 4). These two studies (Chapters 3 and 4) have shown that LAP, PGM, and SKD are monomeric enzymes whereas AAT, GPI, MDH, and TPI are dimeric enzymes.

Considerable isozyme polymorphism was present in the *Hatiora* germplasm collection (Table 3 and Figure 1). A total of 13 putative loci and 42 putative alleles were identified among the 49 accessions, and 9 of the 13 loci (69%) were polymorphic (Table 4). 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 4).

Aspartate aminotransferase. Two polymorphic zones of AAT activity were observed on the gels. Three single-banded patterns (aa, cc, ee) and four triple-banded patterns (ae, be, cd, ce) were observed at *Aat-1* (Table 3 and Figure 1). *Aat-1* was dimorphic in *H. gaertneri*, *H.* x *graeseri*, and *H. rosea* but was tetramorphic in *H. herminiae* and *H. salcornioides*. *Aat-1* phenotypes were useful for identifying clones of *H. salcornioides*. Three single-banded patterns (aa, bb, cc) were observed at *Aat-2*. *Aat-2* phenotypes for *H. salcornioides* and *H. herminiae* were aa and bb, respectively. All clones of *H. gaertneri*, *H.* x *graeseri*, and *H. rosea* exhibited a cc phenotype for *Aat-2*.

Glucose-6-phosphate isomerase. GPI activity was detected in two zones on the zymograms. Clones of *H. gaertneri*, *H.* x *graeseri*, and *H. rosea* yielded well-defined *Gpi-1* bands, but clones of *H. herminiae* and *H. salcornioides* produced *Gpi-1* bands that were too faint to be scored. Two single-banded patterns (aa, bb) and three triple-banded patterns (ab, ac, bc) were observed at *Gpi-1* (Table 3 and Figure 1). The most common *Gpi-1* phenotypes for *H. gaertneri*, *H.* x *graeseri*, and *H. rosea* were ab and bb. The c allele of *Gpi-1* was restricted to three accessions ('Andre', 'Crystal

Lake No. 1', 'Crystal Lake No. 32'). *Gpi-2* displayed three single-banded patterns (bb, cc, ee) and three triple-banded patterns (ac, bc, cd). *Gpi-2* was monomorphic in *H. gaertneri*, *H.* x *graeseri*, and *H. rosea*, but was polymorphic in *H. salcornioides*. *Gpi-1* may be useful for distinguishing clones in subgenus Rhipsalidopsis whereas *Gpi-2* may have value for differentiating clones in subgenus Hatiora.

Leucine aminopeptidase. Gels contained one polymorphic zone of LAP activity, tenatively designated as *Lap-1* (Figure 3). Four single-banded patterns (aa, cc, dd, ee) and six double-banded patterns (ad, bd, cd, ce, de) were observed among the clones (Table 3). Alleles a and b were unique to *H. herminiae* and *H. salcornioides*, whereas alleles c and e were restricted to *H. gaertneri*, *H.* x *graeseri*, and *H. rosea*. The *H.* x *graeseri* clones exhibited six different *Lap-1* phenotypes (cc, cd, ce, dd, de, ee). The *H. rosea* clones, however, had the same *Lap-1* phenotype (ee). *Lap-1* is a highly polymorphic locus which is useful for distinguishing many *Hatiora* clones.

Malate dehydrogenase. Four zones of MDH activity were observed. The most anodal zone (*Mdh-1*) was polymorphic (Figure 2). Clones displayed either a single-banded pattern (aa, bb) or a triple-banded pattern (ac) at *Mdh-1* (Table 3). *Mdh-1* was monomorphic in *H. salcornioides* and *H. herminiae*, but was dimorphic in *H. gaertneri*, *H.* x *graeseri*, and *H. rosea*. Most clones of *H. gaertneri*, *H.* x *graeseri*, and *H. rosea* exhibited a single-banded (aa) pattern at *Mdh-1*; the triple-banded (ac) pattern was observed in only three clones ('Bliss', 'Crimson Giant', 'Crystal Lake No. 2'). *Mdh-2* and *Mdh-3* were monomorphic in all clones that were analyzed (Table 3). *Mdh-3* bands for *H. herminiae* and *H. salcornioides* were very faint, and therefore were not scored. Initially, it was thought that *Mdh-2* and *Mdh-3* were one zone of activity due to the band of intermediate mobility that formed between the two loci. However, the intermediate band was also observed on gels of pollen extracts, indicating

that *Mdh-2* and *Mdh-3* were discrete loci and the intermediate band was actually was an interlocus heterodimer. The most cathodal zone, *Mdh-4*, contained very faint bands and was not evaluated in this study.

Phosphoglucomutase. Two polymorphic zones of PGM activity were detected on the gels. Eight *Pgm-1* phenotypes were observed, including four single-banded patterns (aa, cc, dd, ff) and four double-banded patterns (ab, ac, df, ef) (Table 3 and Figure 2). The more anodal *Pgm-1* bands (a, b, and c) were present only in *H. herminiae* and *H. salcornioides* whereas the more cathodal *Pgm-1* bands (d, e, and f) were present only in *H. gaertneri*, *H.* x *graeseri*, and *H. rosea*. Several of the *H. salcornioides* clones could be distinguished solely on the basis of their *Pgm-1* phenotypes. Most *H.* x *graeseri* clones were either a dd or df phenotype. The ef phenotype was limited to five *H.* x *graeseri* accessions ('Rainbow', 'Sutter's Gold', 'Crystal Lakes No. 2', 'Crystal Lakes No. 3', 'Crystal Lakes No. 16') and the ff phenotype was restricted to two *H. gaertneri* accessions ('Bliss', 'Crimson Giant'). All *H. rosea* accessions were a dd phenotype. A total of ten *Pgm-2* phenotypes were observed, including four single-banded patterns (aa, bb, cc, ee) and six double-banded patterns (ab, ad, bc, bd, be, ce) (Figure 2). Alleles a and d were observed only in clones of *H. gaertneri*, *H.* x *graeseri*, and *H. rosea*; alleles c and e were limited to *H. herminiae* and *H. salcornioides*. The d allele was uncommon and was observed in only five accessions ('Andre', 'Bliss', 'Crimson Giant', 'Thor-Ina', 'Crystal Lake No. 32'). The high level of polymorphism at *Pgm-1* and *Pgm-2* makes these loci extremely useful for identifying *Hatiora* clones.

Shikimate dehydrogenase. Two zones of SKD activity were observed, but only the more anodal zone (tenatively designated *Skd-1*) was resolved clearly. In addition, the *Skd-1* bands for the *H. herminiae* and *H. salcornioides* accessions were

blurred and therefore were not scored. *Skd-1* was monomorphic in all *H.gaertneri*,*H.* x *graeseri*, and *H. rosea* accessions that were surveyed (Table 3 and Figure 3).

Triosephosophate isomerase. Two zones of TPI activity were detected on the gels (Figure 3). The more anodal zone (*Tpi-1*) was monomorphic in accessions of *H. gaertneri*, *H.* x *graeseri*, and *H. rosea*. *Tpi-1* bands were not consistently resolved for accessions of *H. herminiae* and *H. salcornioides* and therefore were not scored. The less anodal zone (*Tpi-2)*, however, was resolved clearly for all 49 accessions.
Clones exhibited either a single-banded pattern (aa, bb) or a triple-banded pattern (ab, ac) at *Tpi-2* (Table 3 and Figure 3). The most common *Tpi-2* alleles were a and b; the c allele was detected in only one clone (*H. salcornioides* LG891).

Distinguishing clones by isozyme profiles. A total of 33 isozyme profiles, each with a distinctive combination of alleles, were identified among the 49 accessions that were surveyed (Table 3). Twenty-two accessions (45%) could be distinguished solely on the basis of their isozyme profiles. The other 27 accessions shared isozyme profiles and could be classified into one of eleven groups (Table 5). Clones within eight of these groups could be distinguished using other phenotypic characters, i.e., flower and/or phylloclade morphology (profiles 2, 7, 9, 10, 11, 12, 19, and 21). Clones within three groups (profiles 5, 6, and 33) could not be distinguished either by isozyme profiles or morphological characters, and most likely represent identical clones. These results demonstrate that isozyme phenotypes can aid in identification of *Hatiora* clones, especially when used in conjunction with morphological characters.

Substantiating parentage by isozyme analysis. Isozymes have proven useful for confirming parentage in several crops (Weeden, 1989). In the current study, isozymes were used to verify the parentage of *H. salcornioides* H921-2. This clone was a seedling of the cross *H. salcornioides* LG891 (female parent) x UM875. The *Pgm-1* phenotypes for LG891 and UM875 are cc and aa, respectively, and the

phenotype for H921-2 (ac) indicates that it is indeed an F_1 hybrid. Boyle et al. (1994) used AAT and PGM phenotypes to verify that the progeny obtained when a highly self-incompatible *Hatiora* clone ('Crimson Giant') was selfed were in fact S_1 progeny. These results demonstrate that isozymes can be useful for confirming hybridity in *Hatiora*.

Genetic diversity in the germplasm collection. Among the 13 loci that were surveyed, 54% were polymorphic in *H. gaertneri*, *H.* x *graeseri*, and *H. rosea*, but only 38% were polymorphic in the modern *H.* x *graeseri* cultivars (Table 6). Among the *H. herminiae* and *H. salcornioides* accessions, 78% of the 9 loci surveyed were polymorphic. Mean number of alleles per locus was 2.67 for *H. herminiae* and *H. salcornioides*, 1.85 for *H. gaertneri*, *H.* x *graeseri*, and *H. rosea*, and 1.54 for the modern *H.* x *graeseri* cultivars. Mean heterozygosity for the *H. herminiae* and *H. salcornioides* clones was nearly twice that of the *H. gaertneri*, *H.* x *graeseri*, and *H. rosea* clones. Among the 42 putative alleles that were identified in this study, 24 (57%) were present in *H. gaertneri*, *H.* x *graeseri*, and *H. rosea* and 20 (48%) were detected in the modern *H.* x *graeseri* cultivars. The allelic diversity present in the *H. herminiae* and *H. salcornioides* clones was comparable to that observed in the *H. gaertneri*, *H.* x *graeseri*, and *H. rosea* clones.

The statistics presented in Table 6 indicate that the group of 40 *H. gaertneri*, *H.* x *graeseri*, and *H. rosea* clones exhibit greater genetic diversity than the 13 modern *H.* x *graeseri* cultivars. This to be expected, since the former group contains field-collected clones, older cultivars, and modern cultivars and thus represents a broader genetic base than the modern cultivars alone. However, an examination of the isozyme phenotypes in Table 5.3 reveals that many of the uncommon alleles found in older *H. gaertneri* and *H.* x *graeseri* cultivars were not present in the modern *H.* x *graeseri* cultivars. Thus, the decline in genetic diversity results from the loss of alleles during

breeding. The primary objective of commercial breeding programs is towards the production of superior clones, and, as a result, the parental material for crossing programs is nearly always modern cultivars rather than wild species or older cultivars. Utilization of field-collected clones and older cultivars in breeding programs will broaden the genetic base and may yield clones exhibiting novel physiological and morphological traits.

Relationship between genetic diversity and a species' breeding system. Several *Hatiora* species exhibit self-incompatibility (SI), including *H. gaertneri* (Boyle et al., 1994; Ganders, 1976), *H. rosea* (Taylor, 1976), and *H. salcornioides* (Ross, 1981). As a consequence, these three species would be expected to be predominantly outcrossed. However, *H. herminiae* is self-compatible and undisturbed plants set seed freely, suggesting that this species is predominantly selfed (T.H. Boyle, unpublished data). Generally, species that are predominantly outcrossed exhibit greater genetic diversity than species that are primarily selfed (Hamrick and Godt, 1990). One measure of intrapopulational genetic diversity is the mean proportion of loci that are heterozygous per individual (Hamrick, 1989). Table 7 shows the percentage of heterozygous isozyme loci for ten *Hatiora* clones that presumably represent unimproved (field-collected) material. The percentage of heterozygous loci ranged from 22 to 56% for the four *H. salcornioides* clones and was 0% for the single *H. herminiae*, results that generally conform to expectations. Contrary to expectations, the percentage of heterozygous loci was low for all *H. gaertneri* and *H. rosea* clones except for MD861. Morphologically, MD861 appears to be pure *H. rosea* but its *Gpi-1*, *Pgm-2*, and *Tpi-2* phenotypes differ from the other *H. rosea* clones, suggesting it is actually a man-made or natural interspecific hybrid. What then may account for the low levels of heterozygosity in the *H. gaertneri* and *H. rosea* clones? The most likely answer is that these clones originated from populations composed of relatively few

individuals. Genetic diversity is rapidly lost from small populations due to inbreeding and random changes in allelic frequencies (genetic drift) (Wilcox, 1984). *Hatiora gaertneri* and *H. rosea* are native to montane forests in the southeastern Barzilian states of Paraná, Santa Catarina, and Rio Grande do Sul (Barthlott and Taylor, 1995). The low levels of heterozygosity in the *H. gaertneri* and *H. rosea* clones may indicate habitat disturbance or other factors which can threaten the long-term survival of the species. Quantitative estimates of biological diversity such as those provided here may prove useful for in situ conservation of *Hatiora* species.

Summary. This study demonstrates that substantial isozyme polymorphism is present in *Hatiora*. The high level of polymorphism permitted the unambiguous identification of many clones and quantification of genetic diversity present in individual accessions and the collection as a whole. The information presented in this study may have practical applications for plant breeders, ecologists, and taxonomists. The data may also be useful for resolving long-standing questions regarding these taxa.

Table 5.1. The *Hatiora* accessions used for study and their sources, botanical names, and status.

Clone	Binomial	Status[y]	Source[z]
	Named accessions		
Andre	*H. x graeseri*	CV	RG
Andromeda	*H. x graeseri*	CV	PM
Annika	*H. x graeseri*	CV	BF
Auriga	*H. x graeseri*	CV	PM
Bliss	*H. gaertneri*	CV	BF
Caprice	*H. x graeseri*	CV	RG
Cassiopeia	*H. x graeseri*	CV	PM
Cetus	*H. x graeseri*	CV	PM
China Rose	*H. x graeseri*	CV	RG
Christine	*H. x graeseri*	CV	RG
Crimson Giant	*H. gaertneri*	CV	MD
Evita	*H. x graeseri*	CV	JV
Flash	*H. x graeseri*	CV	RG
France	*H. x graeseri*	CV	RG
Gaertneri Giant	*H. x graeseri*	CV	RG
Hatherton Star	*H. x graeseri*	CV	RG
Laura Ann	*H. x graeseri*	CV	RG
Leo's Pink	*H. x graeseri*	CV	RG
Mira	*H. x graeseri*	CV	PM
Monarch	*H. x graeseri*	CV	RG
Orion	*H. x graeseri*	CV	PM
Pink Perfection	*H. x graeseri*	CV	RG
Purple Pride	*H. x graeseri*	CV	JV
Rainbow	*H. x graeseri*	CV	RG
Red Pride	*H. x graeseri*	CV	JV
Rood	*H. x graeseri*	CV	JV
Shocking Pink	*H. x graeseri*	CV	RG
Sutter's Gold	*H. x graeseri*	CV	RG
Thor-Anne	*H. x graeseri*	CV	GT
Thor-Ina	*H. x graeseri*	CV	GT
Vista Delight	*H. x graeseri*	CV	RG
Yvonne Pelham	*H. x graeseri*	CV	RG
	Numbered accessions		
06524	*H. gaertneri*	FC	BG
No. 1	*H. x graeseri*	CV	CL
No. 2	*H. x graeseri*	CV	CL
No. 3	*H. x graeseri*	CV	CL
No. 16	*H. x graeseri*	CV	CL
No. 32	*H. x graeseri*	CV	CL

Continued next page

Table 5.1 continued

36170	*H. herminiae*	FC	BH
AG941	*H. rosea*	CV; FC	AG
AG942	*H. rosea*	CV; FC	AG
EC500	*H. rosea*	CV; FC?	RG
MD861	*H. rosea*	CV; FC?	MD
FN931	*H. salcorniodes*	CV; FC?	FN
H921-2	*H. salcorniodes*	CV	MA
LG891	*H. salcorniodes*	CV; FC?	LG
UM875	*H. salcorniodes*	CV; FC?	MA
01625	*H. salcorniodes*	FC	BG
52257	*H. salcorniodes*	FC	HG

[z]AG= Abbey Gardens, Carpinteria, CA; BF= Bliss Farms Inc., Woodcliff Lake, NJ; BG= Botanischer Garten Bonn, Bonn, Germany; BH= Botanischer Garten Heidelberg, Heidelberg, Germany; CL= Crystal Lake Greenhouses, Carver, Mass; FN= Franks Nursery and Crafts, Hadley, Mass; GT= Gartneriet Thoruplund, Odense, Denmark; HG= Huntington Botanical Gardens, San Marino, CA; JV= J. de Vries Potplantencultures bv, Aalsmeer, The Netherlands; LG= Logees Greenhouses, Danielson, CT; MA= Univ. of Massachusetts, Amherst, Mass; MD= Univ. of Maryland, College Park; PM= Gartneriet PKM, Odense, Denmark; RG= Rainbow Gardens, Vista, CA.

[y] CV= commercial cultivar; FC= field-collected clone or grown from field-collected seed.

Table 5.2. Polyacrylamide gel concentrations and sample loading volumes used for electrophoresis.

Enzyme	Gel concentration (%)	Loading volume (ul)
Aspartate aminotransferase (AAT)	5	10
Glucose-6-phosphate isomerase (GPI)	7.5	5
Leucine aminopeptidase (LAP)	7.5	5
Malate dehydrogenase (MDH)	7.5	10
Phosphoglucomutase (PGM)	7.5	10
Shikimate dehydrogenase (SKD)	7.5	10
Triosephosphate isomerase (TPI)	10	5

Table 5.3. Phenotypes for thirteen isozyme loci in 49 *Hatiora* clones.

	Isozyme profile (no.)	Isozyme locus												
		Aat-1	*Aat2*	*Gpi-1*	*Gpi-2*	*Lap-1*	*Mdh-1*	*Mdh-2*	*Mdh-3*	*Pgm-1*	*Pgm-2*	*Skd-1*	*Tpi-1*	*Tpi-2*
Andre	1	cc	cc	bc	ee	ee	aa	aa	aa	dd	ad	aa	aa	ab
Andromeda	2	cc	cc	ab	ee	de	aa	aa	aa	dd	ab	aa	aa	ab
Annika	3	cc	cc	bb	ee	cd	aa	aa	aa	dd	aa	aa	aa	bb
Auriga	4	cc	cc	bb	ee	ee	aa	aa	aa	dd	aa	aa	aa	ab
Bliss	5	cd	cc	bb	ee	ee	ac	aa	aa	ff	ad	aa	aa	bb
Caprice	6	cc	cc	ab	ee	dd	aa	aa	aa	dd	aa	aa	aa	ab
Cassiopeia	7	cc	cc	bb	ee	cd	aa	aa	aa	df	ab	aa	aa	bb
Cetus	8	cc	cc	bb	ee	ee	aa	aa	aa	df	bb	aa	aa	ab
China Rose	9	cc	cc	ab	ee	ee	aa	aa	aa	dd	ab	aa	aa	ab
Christine	10	cc	cc	ab	ee	ee	aa	aa	aa	df	ab	aa	aa	aa
Crimson Giant	5	cd	cc	bb	ee	ee	ac	aa	aa	ff	ad	aa	aa	bb
Evita	6	cc	cc	ab	ee	dd	aa	aa	aa	dd	aa	aa	aa	ab
Flash	11	cc	cc	ab	ee	de	aa	aa	aa	df	ab	aa	aa	aa
France	12	cc	cc	ab	ee	ee	aa	aa	aa	df	ab	aa	aa	bb
Gaertneri Giant	2	cc	cc	ab	ee	de	aa	aa	aa	dd	ab	aa	aa	ab
Hatherton Star	12	cc	cc	ab	ee	ee	aa	aa	aa	df	ab	aa	aa	bb
Laura Ann	9	cc	cc	ab	ee	ee	aa	aa	aa	dd	ab	aa	aa	ab
Leo's Pink	13	cc	cc	ab	ee	ee	aa	aa	aa	dd	ab	aa	aa	ab
Mira	14	cc	cc	ab	ee	ee	aa	aa	aa	df	ab	aa	aa	ab
Monarch	11	cc	cc	ab	ee	de	aa	aa	aa	df	ab	aa	aa	aa
Orion	15	cc	cc	bb	ee	ee	aa	aa	aa	dd	ab	aa	aa	ab
Pink Perfection	16	cc	cc	bb	ee	de	aa	aa	aa	df	ab	aa	aa	aa
Purple Pride	17	cc	cc	bb	ee	de	aa	aa	aa	dd	ab	aa	aa	bb
Rainbow	18	cc	cc	ab	ee	de	aa	aa	aa	cf	aa	aa	aa	aa
Red Pride	2	cc	cc	ab	ee	de	aa	aa	aa	dd	ab	aa	aa	ab
Rood	19	cc	cc	bb	ee	cc	aa	aa	aa	df	bb	aa	aa	bb

Continued next page

Table 5.3 continued

Shocking Pink	20	cc	cc	ab	ee	ce	aa	aa	aa	df	aa	aa	aa	ab
Sutter's Gold	21	cc	cc	bb	ee	ce	aa	aa	aa	ef	aa	aa	aa	aa
Thor-Anne	7	cc	cc	bb	ee	cd	aa	aa	aa	df	ab	aa	aa	bb
Thor-Ina	22	cc	cc	bb	ee	cc	aa	aa	aa	dd	bd	aa	aa	ab
Vista Delight	9	cc	cc	ab	ee	ee	aa	aa	aa	dd	ab	aa	aa	ab
Yvonne Pelham	10	cc	cc	ab	ee	ce	aa	aa	aa	df	ab	aa	aa	aa
H. gaertneri 06524	19	cc	cc	bb	ee	cc	aa	aa	aa	df	bb	aa	aa	bb
Crystal Lake No. 1	23	cc	cc	bc	ee	ce	aa	aa	aa	dd	aa	aa	aa	ab
Crystal Lake Scarlet No. 2	24	cd	cc	bb	ee	ce	ac	aa	aa	ef	aa	aa	aa	ab
Crystal Lake No. 3	25	cc	cc	bb	ee	ce	aa	aa	aa	cf	aa	aa	aa	ab
Crystal Lake No. 16	21	cc	cc	bb	ee	ce	aa	aa	aa	ef	aa	aa	aa	aa
Crystal Lake No. 32	26	cc	cc	ac	ee	ce	aa	aa	aa	df	ad	aa	aa	ab
H. herminiae 36170	27	ee	bb	----	bb	aa	bb	aa	----	aa	cc	----	----	bb
H. rosea AG941	9	cc	cc	aa	ee	ee	aa	aa	aa	dd	bb	aa	aa	aa
H. rosea AG942	9	cc	cc	aa	ee	ee	aa	aa	aa	dd	bb	aa	aa	aa
H. rosea EC500	9	cc	cc	aa	ee	ee	aa	aa	aa	dd	bb	aa	aa	aa
H. rosea MD861	28	cc	cc	ab	ee	ee	aa	aa	aa	dd	ab	aa	aa	ab
H. salcorniodes FN931	29	aa	aa	----	bc	aa	bb	aa	----	ab	cc	----	----	bb
H. salcorniodes H921-2	30	ae	aa	----	cc	bd	bb	aa	----	ac	ce	----	----	aa
H. salcorniodes LG891	31	ce	aa	----	cc	dd	bb	aa	----	cc	ee	----	----	ac
H. salcorniodes UM875	32	ae	aa	----	cd	bd	bb	aa	----	aa	bc	----	----	ab
H. salcorniodes 01625	33	be	aa	----	ac	ad	bb	aa	----	aa	be	----	----	aa
H. salcorniodes 52257	33	be	aa	----	ac	ad	bb	aa	----	aa	be	----	----	aa

Table 5.4. Number of alleles present at 13 isozyme loci among the 49 *Hatiora* accessions.

Locus	Alleles	Locus	Alleles
Aat-1	a,b,c,d,e	*Mdh-3*	a
Aat-2	a,b,c	*Pgm-1*	a,b,c,d,e,f
Gpi-1	a,b,c	*Pgm-2*	a,b,c,d,e
Gpi-2	a,b,c,d,e	*Skd-1*	a
Lap-1	a,b,c,d,e	*Tpi-1*	a
Mdh-1	a,b,c	*Tpi-2*	a,b,c
Mdh-2	a		

Table 5.5. *Hatiora* clones with identical isozyme patterns.

Isozyme profile (no.)	Clones	Clonal differentiation by 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, Vista Delight, AG941, AG942, EC500	yes
10	Christine, Yvonne Pelham	yes
11	Flash, Monarch	yes
12	France, Hatherton Star	yes
19	Rood, 06524	yes
21	Sutter's Gold, No. 16	yes
33	01625, 52257	no

Table 5.6. Diversity statistics for the *Hatiora* clonal germplasm collection.

Group	No. clones analyzed	% polymorphic loci[Z]	Mean # alleles/ locus	Mean heterozygosity	Allelic diversity [y]
H. gaertneri *H. rosea* *H. x graseri* [x]	40	54	1.85	0.21	57
H. salcornioides *H. herminae* [x]	6	78	2.67	0.42	57
Modern cultivars *H. x graseri* [xw]	13	38	1.54	0.17	48
All clones[x]	46	69	3.23	0.30	-----

[Z] Calculations based on thirteen loci for all groups except *H. salcornioides/H. herminiae*, which were based on 9 loci.

[y] (No. alleles observed in group ÷ total no. alleles at all loci for all accessions) x 100.

[x] Excluding duplicate accessions.

[w] Andromeda, Annika, Auriga, Cassiopiea, Cetus, Evita, Mira, Orion, Purple Pride, Red Pride, Rood, Thor-Anne, Thor-Ina.

Table 5.7. A comparison of percent heterozygous loci and breeding systems among four *Hatiora* species.

Species	Clone	Heterozygous loci (%)	Breeding system[z]
H. gaertneri	06524	8	outbreeding (SI)
H. herminiae	36170	0	unknown
H. rosea	AG941	0	outbreeding (SI)
	AG942	0	outbreeding (SI)
	EC500	0	outbreeding (SI)
	MD861	23	outbreeding (SI)
H. salcorniodes	FN931	22	outbreeding (SI)
	LG891	22	outbreeding (SI)
	UM875	56	outbreeding (SI)
	01625	44	outbreeding (SI)

[z] SI= self-incompatible. Presence of SI confirmed by pollination tests.

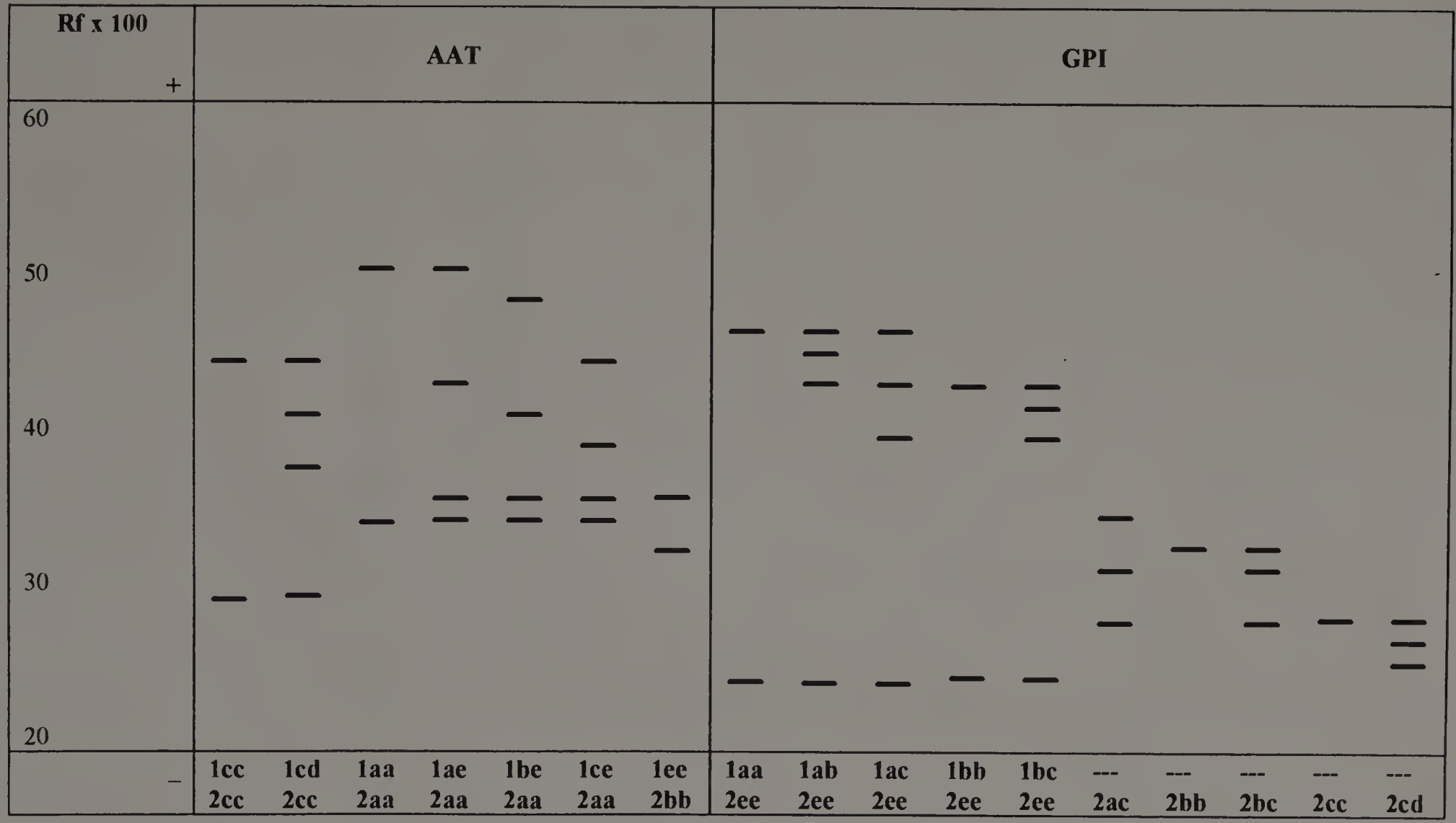

Figure 5.1. Schematic illustrations of enzyme phenotypes for AAT and GPI in *Hatiora*.

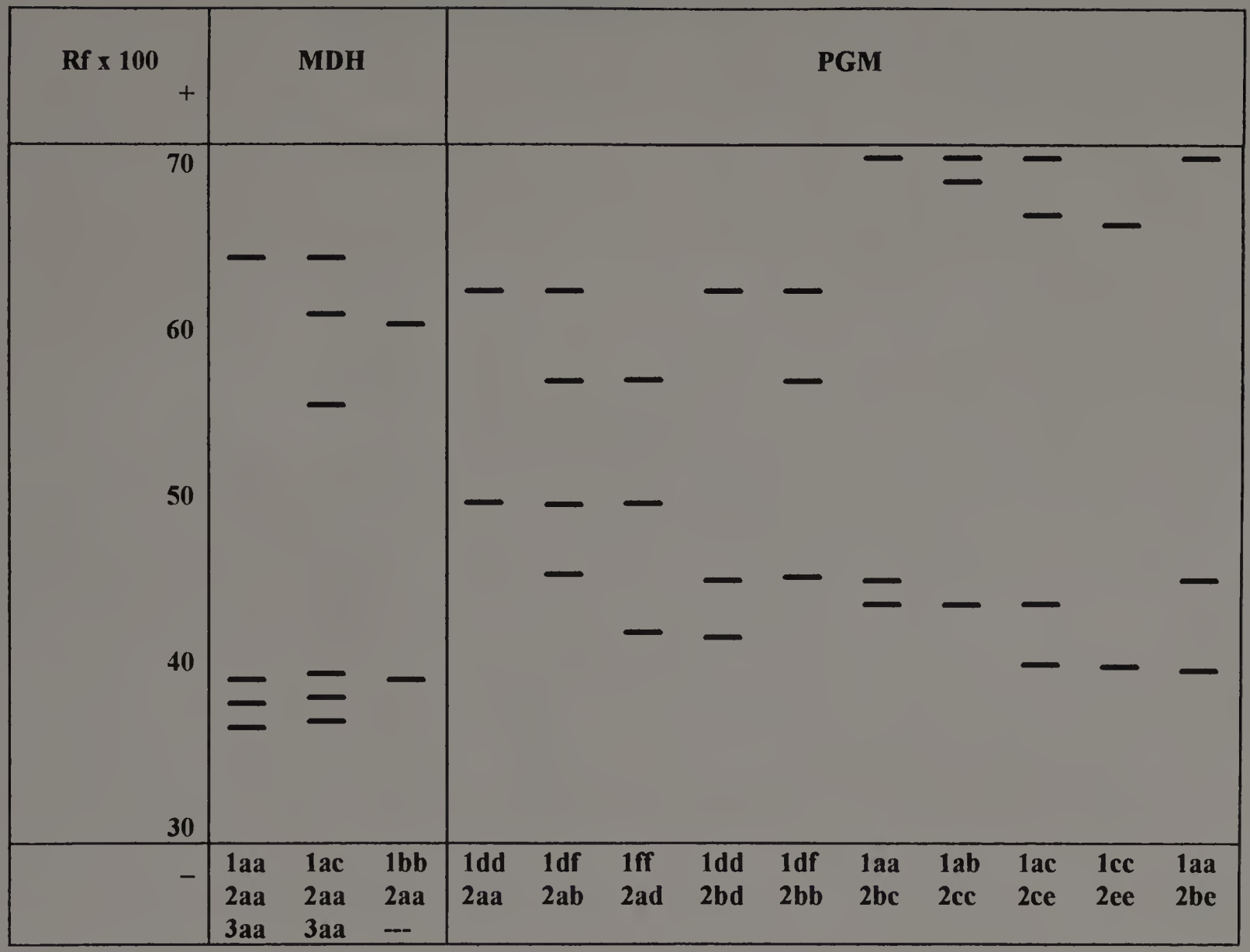

Figure 5.2. Schematic illustrations of enzyme phenotypes for MDH and PGM in *Hatiora*.

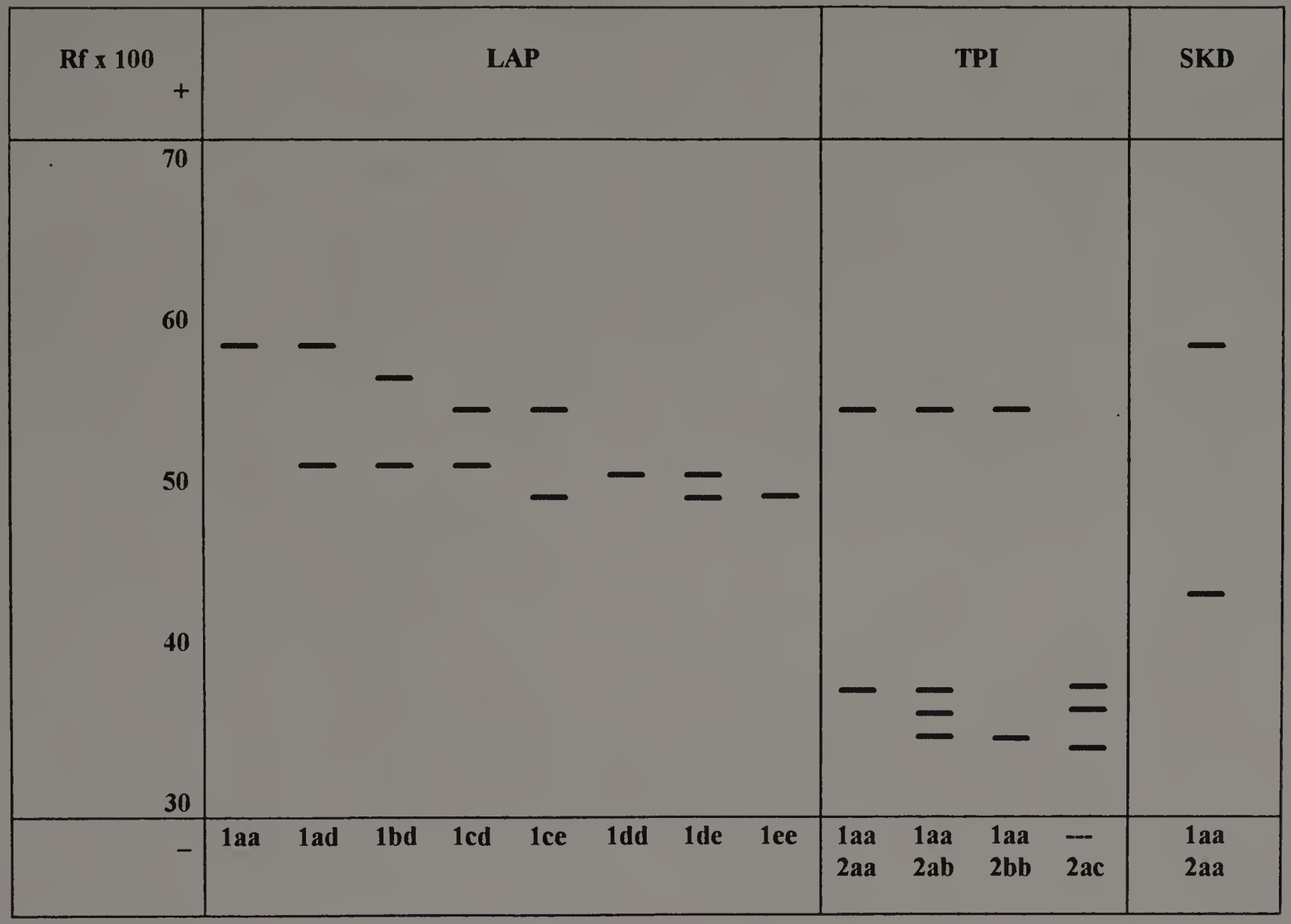

Figure 5.3. Schematic illustrations of enzyme phenotypes for LAP, TPI and SKD in *Hatiora*.

CHAPTER 6
USING ISOZYME MARKERS TO IDENTIFY CLONES AND ESTIMATE GENETIC DIVERSITY IN A *SCHLUMBERGERA* CLONAL GERMPLASM COLLECTION

Abstract

A clonal germplasm collection representing five *Schlumbergera* species and containing 59 commercial cultivars and field-collected specimens was evaluated for seven enzyme systems - aspartate aminotransferase (AAT), glucose-6-phosphate isomerase (GPI), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucomutase (PGM), shikimate dehydrogenase (SKD), and triosephosphate isomerase (TPI). Isozymes were extracted from phylloclades and were separated by polyacrylamide gel electrophoresis. Twelve putative loci and 36 putative alleles were identified, and 10 of the 12 loci (83%) were polymorphic. Forty-one clones (69%) could be distinguished solely on the basis of their isozyme profiles, but the other 18 clones shared isozyme profiles with one or two other clones. Among clones that shared isozyme profiles, 13 could be distinguished by flower and/or phylloclade traits. A group of 42 commercial clones of *S. truncata*, *S.* x *buckleyi*, and *S.* x *exotica* exhibited less genetic diversity than a group of 14 field-collected clones of *S. kautskyi*, *S. opuntioides*, *S. orssichiana*, *S. russelliana*, and *S. truncata*. The difference in genetic diversity was attributed to limited sampling of germplasm from wild populations and during breeding a loss of alleles. The percentage of heterozygous loci ranged from 17 to 42% for field-collected clones of the predominantly outcrossing species *S. opuntioides*, *S. orssichiana*, and *S. truncata*, and was 0% for a field-collected clone of primarily selfing species *S. kautskyi*, results which conform to expectations. However, field-collected *S. russelliana* clones contained few (0 to 17%) heterozygous loci, i.e., less than

expected for a predominantly outcrossing species, suggesting that these plants were obtained from populations composed of relatively few individuals.

Introduction

The genus *Schlumbergera* consists of six species epiphytic or lithophytic cacti that are endemic to the states of São Paulo, Rio de Janeiro, Espírito Santo, and Minas Gerias in southeastern Brazil (Barthlott and Taylor, 1995; Hunt, 1969, 1992). *Schlumbergera truncata* (Haworth) Moran, also known as zygocactus or crab cactus, is the most commonly cultivated species and is an economically important floricultural crop in northern Europe, Japan, and North America (Cobia, 1992). *Schlumbergera* species other than *S. truncata* are rare in cultivation but have been used for breeding. Hybridization between *S. truncata* and *S. russelliana* (Hooker) Britton & Rose has yielded the well-known Christmas cactus (Hunt, 1981), along with many other cultivars. The collective name for *S. truncata* x *S. russelliana* hybrids is *S.* x *buckleyi* (T. Moore) Tjaden (Tjaden, 1966). *Schlumbergera truncata* has also been crossed with *S. opuntioides* (Löfgren & Dusén) Hunt (Barthlott and Rauh, 1977) and *S. orssichiana* Barthlott & McMillan (Horobin and McMillan, 1985). Cultivars of *S. truncata* x *S. opuntioides* parentage have been given the collective name *S.* x *exotica* Barthlott & Rauh (Barthlott and Rauh, 1977). Most of the *Schlumbergera* cultivars that are grown currently are either *S. truncata* or *S.* x *buckleyi* (Cobia, 1992; McMillan, 1985).

Schlumbergera is a clonally propagated crop, and a rapid and accurate means of identifying clones is important to breeders and propagators. Currently, clones are identified by their flower and phylloclade morphology, flower color, and time of flowering under natural photoperiods. Clones that are in a vegetative state are difficult to identify due to a paucity of morphological traits. Isozymes have proven useful for identifying cultivars in many horticultural crops (Nielsen, 1985; Weeden, 1989). Isozymes can also be used to estimate the genetic diversity in germplasm collections

(Weeden, 1989). Estimates of genetic diversity can aid breeding programs in the evaluation of samples for potential sources of new traits, in the conservation of genetic resources, and in elucidating taxonomic relationships among species (Mowrey and Werner, 1990; Tanksley, 1983). Several attributes of isozymes make them particularly useful as biochemical markers, including Mendelian inheritance, codominant expression, complete penetrance, lack of epistatic or pleiotropic interactions among loci, and consistency of expression under a wide range of environmental conditions (Weeden and Wendel, 1989). Isozymes can be extracted from a variety of tissues and separated using relatively inexpensive procedures.

A high level of isozyme polymorphism must be present within a crop in order to identify cultivars by their isozyme phenotypes (Weeden, 1989). Previous studies (Chapter four) indicated that *Schlumbergera* clones were polymorphic for leucine aminopeptidase, phosphoglucomutase, and shikimate dehydrogenase. The objectives of the present study were to determine the isozyme phenotypes for a diverse collection of *Schlumbergera* clones, and to ascertain if isozymes could be used to identify clones and estimate genetic diversity within the germplasm collection.

Materials and Methods

Plant material. A total of 59 accessions were used for this study (Table 1). Accessions were obtained from commercial sources, botanical gardens, and private collections, and included both commercial clones (cultivars) and field-collected clones. *Schlumbergera microsphaerica* (Schumann) Hövel could not be obtained for analysis, but clones of the other five *Schlumbergera* species were included in this study. All species except for *S. kautskyi* (Horobin & McMillan) Taylor were represented by two or more accessions.

Protein extraction and sample preparation. Tissue samples were removed from mature phylloclades using a no. 1 cork borer. Soluble proteins were extracted by

homogenizing ≈8 mg tissue in 100 μl cold extraction buffer [0.050 M Tris-HCl buffer (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 microcentrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant (≈60 μl) was collected and mixed with 10 μl of cold sample mix [50% (v/v) glycerol and 0.1% (w/v) bromphenol blue (Shields et al., 1983)].

Electrophoresis. Native proteins were separated by polyacrylamide gel electrophoresis (PAGE) using a Mini-Protean II cell (Bio-Rad Laboratories, Hercules, CA). Polyacrylamide gels were prepared using 0.375 M Tris-HCL (pH 8.8) as the gel buffer (Hames, 1981). Table 2 lists the gel concentrations and sample loading volumes for each enzyme system. 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). Running times varied according to the enzyme system assayed, but typically required 45 minutes for completion.

Gel staining. 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), phosphoglucomutase (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). The staining procedures of Wendel (1989) were used for all seven enzyme systems. Enzyme stains were prepared immediately prior to use. After the appropriate staining intensities were achieved, the gels were rinsed with water and fixed in 7% acetic acid (Hames, 1981).

Gels were scored for the presence or absence of specific allelic bands of known migration distances. Data were collected immediately after gels were fixed, i.e., when the staining intensities were maximal. For each enzyme, loci were numbered sequentially, with *1* denoting the most anodally migrating locus. Alleles at individual

loci were designated by letters assigned sequentially from the anode. Rf values were calculated for each band by taking the average of three measurements and dividing the band's migration distance by the tracking dye's migration distance (Hames, 1991).

Data analysis. Isozyme data were used to assess genetic diversity in the germplasm collection. The percentage of polymorphic loci, mean number of alleles per locus, average heterozygosity, and allelic diversity [(number of alleles observed ÷ total number of alleles at all loci) x 100] were calculated separately for the commercial clones (cultivars) and the field-collected clones. Mean number of alleles per locus and average heterozygosity were calculated for all isozyme loci (monomorphic as well as polymorphic). The percentage of heterozygous loci was determined for several field-collected clones to determine if heterozygosity was correlated with a species' breeding system.

Results and Discussion

The inheritance of LAP, PGM, and SKD in *Schlumbergera* was reported previously (Chapter 4). AAT, GPI, MDH, and TPI were not subjected to genetic analysis, and therefore the loci and alleles designated for these four enzyme systems are reported as putative. However, the inheritance of AAT, GPI, MDH, and TPI has been reported for the closely related genus *Hatiora* (Chapter 3). These two studies (Chapter 3 and 4) have shown that LAP, PGM, and SKD are monomeric enzymes whereas AAT, GPI, MDH, and TPI are dimeric enzymes.

Considerable isozyme polymorphism was present among the accessions (Table 3 and Figure 1). A total of 12 putative loci and 36 putative alleles were identified among the 59 accessions, and 10 of the 12 loci (83%) were polymorphic (Table 4). 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*) for the polymorphic loci (Table 4).

Aspartate aminotransferase. Gels exhibited two zones of AAT activity, designated *Aat-1* and *Aat-2* (Figure 1). *Aat-1* homodimers exhibited greater mobilities than the *Aat-2* homodimers on 5% polyacrylamide gels, thus permitting the two loci to be differentiated readily. Two homodimers (aa or bb) and one heterodimer (ab) were observed at *Aat-1*. The aa homodimer was the most common *Aat-1* phenotype. The b allele of *Aat-1* was present in only five accessions: *S. opuntioides* clones 01537 and CC615 (= bb), *S. russelliana* 33160 (= bb), and the *S.* x *exotica* cultivars 'Exotica' and 'Minibelle' (= ab) (Table 3). 'Exotica' and 'Minibelle' are F_1 interspecific hybrids of *S. truncata* and *S. opuntioides* (Barthlott and Rauh, 1977), and their AAT phenotypes confirm their hybridity. Three homodimers (aa, bb, cc) and four heterodimers (ab, ac, bc, bd) were observed at *Aat-2*. The *Aat-2* alleles a, b, and c were relatively common, whereas the d allele of *Aat-2* was present only in the *S. orssichiana* clones. The high level of polymorphism at *Aat-2* makes this locus exceptionally useful in distinguishing *Schlumbergera* clones, and may have value in distinguishing *Schlumbergera* species.

Glucose-6-phosphate isomerase. Two areas of GPI activity (designated *Gpi-1* and *Gpi-2*) were identified on the gels (Figure 1). *Gpi-1* was monomorphic whereas *Gpi-2* was polymorphic. The *Gpi-1* homodimer (aa) displayed a greater mobility than *Gpi-2* homodimers on 7.5% polyacrylamide gels, so that *Gpi-1* was clearly distinguishable from *Gpi-2* (Figure 1). Six phenotypes were observed at *Gpi-2*, including three homodimers (aa, bb, cc) and three heterodimers (ab, ac, bc). The most common *Gpi-2* alleles were a and b. The c allele of *Gpi-2* was restricted to nine clones of *S. truncata* and *S.* x *buckleyi*. Clones of *S. kautskyi*, *S. orssichiana*, and *S. russelliana* possessed either an aa or bb phenotype for *Gpi-2*. The ab phenotype was observed in both *S. opuntioides* clones and several clones of *S. truncata* and *S.* x *buckleyi*. Many *Schlumbergera* accessions could be distinguished by their *Gpi-2* phenotypes, which makes this locus quite useful for identifying clones.

Leucine aminopeptidase. Gels stained for LAP activity contained one polymorphic zone of activity, designated *Lap-1* (Figure 2). Three single-banded phenotypes (bb, cc, dd) and five double-banded phenotypes (ac, bc, bd, cd, ce) were observed at *Lap-1* (Table 3). The most common *Lap-1* alleles were b, c, and d. The a allele was detected only in *S. opuntioides* CC615 and *S.* x *exotica* 'Exotica', whereas the e allele was present only in *S. russelliana* JFH clone 1. The high level of polymorphism at *Lap-1* permitted many *Schlumbergera* clones to be distinguished from clones with similar phenotypes. For example, *S. opuntioides* clones 01537 and CC615 were distinguishable based on their *Lap-1* genotypes. Also, two of the five *S. russelliana* clones could be separated based on their *Lap-1* genotypes (Table 3).

Malate dehydrogenase. *Mdh-1* and *Mdh-2* were dimorphic whereas *Mdh-3* was monomorphic (Tables 3 and 4). The clones produced either a single-banded pattern (bb) or a triple-banded pattern (ab) at *Mdh-1* (Figure 2). The a allele was present in many *S. truncata* and *S.* x *buckleyi* clones, but was not observed in any of the field-collected clones except for *S. orssichiana* 05584 (Table 3). Two single-banded patterns (aa and bb) were observed at *Mdh-2* (Figure 2). The *Mdh-2* bb phenotype was restricted to *S. truncata* Abendroth No. 2 (Table 3). *Mdh-1* and *Mdh-2* could be distinguished readily on 7.5% polyacrylamide gels due to the distinct mobility differences between the slow migrating (b) band of *Mdh-1* and the fast migrating (a) band of *Mdh-2* (Figure 2). Initially it was thought that *Mdh-2* and *Mdh-3* were one zone of activity due to the band of intermediate mobility that formed between the two loci. However, the intermediate band was also observed on gels of pollen extracts, indicating that *Mdh-2* and *Mdh-3* were distinct loci and the intermediate band was actually was an interlocus heterodimer.

Phosphoglucomutase. Two zones of PGM activity were observed on PAGE gels (Figure 3). Both zones (designated *Pgm-1* and *Pgm-2*) were polymorphic. Three single-banded phenotypes (aa, bb, cc, dd) and two double-banded phenotypes (ad, bc)

were observed at *Pgm-1* (Table 3). The *S. truncata* clones were either bb, bc, or cc phenotypes. All of the *S. kautskyi*, *S. orssichiana*, and *S. russelliana* clones were bb phenotypes. The most common *Pgm-1* alleles were b and c. The a allele was limited to *S. opuntioides* and its interspecific progeny ('Exotica' and 'Minibelle'), whereas the d allele was found only in a few *S.* x *buckleyi* clones ('Kris Kringle II', 'Lavender Doll II', 'Sonja', 'Thor-Louise') (Table 3). *Pgm-1* and *Pgm-2* could be differentiated easily on 7.5% polyacrylamide gels due to differences in band mobility (Figure 3). One single-banded pattern (cc) and four double-banded patterns (ac, bc, cd, ce) were observed at *Pgm-2* (Table 3). Most clones exhibited a single-banded cc phenotype for *Pgm-2*. The a, d, and e alleles of *Pgm-2* were limited to a few clones. Alleles a and d were observed only in *S. truncata* Abendroth No. 2 and *S.* x *buckleyi* 'Species B', respectively. Only the two *S. opuntioides* clones and *S.* x *exotica* 'Exotica' and 'Minibelle' contained the e allele.

Shikimate dehydrogenase. Gels contained one polymorphic zone of SKD activity, designated *Skd-1* (Figure 3). Two single-banded patterns (cc, ee) and five double-banded patterns (ab, bc, cd, ce, de) were observed at *Skd-1* (Tables 3 and 4). Most of the named accessions were either cc or cd phenotypes. *Skd-1* alleles a, b, and e were restricted in distribution. Only the *S. russelliana* clone 34533 and the two *S. opuntioides* clones contained the a allele. The b allele was detected in *S. opuntioides* and *S.* x *exotica* ('Exotica' and 'Minibelle'). Only *S. kautskyi* and *S. orssichiana* contained the e allele. *Skd-1* is a highly polymorphic locus in *Schlumbergera* may be useful for studying taxonomic relationships within the genus.

Triosephosphate isomerase. Two zones of TPI activity were detected on the gels. The more anodal zone, designated *Tpi-1*, was not consistently resolved and therefore was not scored. The less anodal zone (*Tpi-2*) was resolved clearly and was scored (Figure 2). Three *Tpi-2* phenotypes were observed, including two homodimers (aa, bb) and one heterodimer (ab). Among the species clones, *S. kautskyi* and *S.*

truncata Abendroth No. 2 and 6 were a bb phenotype, *S. opuntioides* and *S. orssichiana* were an ab phenotype, and the *S. russelliana* clones were either aa, ab, or bb phenotypes. The *S.* x *buckleyi* clones were either aa or ab phenotypes whereas the all of the *S. truncata* clones surveyed exhibited a bb phenotype (Tables 1 and 3). Hence, the a allele of *Tpi-2* was common in *S.* x *buckleyi* and one of its parents, i.e., *S. russelliana*, but was notably absent in the other parent, i.e., *S. truncata*. These data suggest that *Tpi-2* may be a useful locus for systematic studies of *Schlumbergera*.

Distinguishing clones by isozyme profiles. Sufficient isozyme polymorphism was present at ten loci to distinguish 41 of the 59 clones (69%) solely on the basis of their isozyme profiles. Clones that shared isozyme profiles could be classified into one of eight groups (Table 5). Clones within six of these groups could be distinguished using other characters such as flower morphology/color (profiles 4, 12, 15, 27, and 49) or phylloclade morphology (profiles 7, 15, 27, and 49). 'Dark Marie' is a flower color sport of 'Marie', and these two cultivars could not be distinguished based on their isozyme profiles (Tables 3 and 5). Bud sports result from minor genetic changes that do not produce discernible differences in isozyme patterms (Weeden and Lamb, 1985; Mendenez, 1986). Clones within two groups (profiles 20 and 42) could not be distinguished by their isozyme profiles or morphological characters, and most likely represent duplicate accessions. These results demonstrate that isozyme markers can aid in identifying *Schlumbergera* clones. Isozyme data are best used in conjunction with morphological descriptors to resolve questions of identity.

Substantiating parentage by isozyme analysis. The isozyme profiles presented in Table 3 can be used to ascertain the parentage of some *Schlumbergera* clones. The commonly-grown Christmas cactus 'Buckleyi' has been in cultivation for over a century, and its parentage is not known. It has been suggested 'Buckley' is a F_1 hybrid of *S. russelliana* and *S. truncata*, but McMillan (1990) proposed that it may be a hybrid between *S. russelliana* and a *S.* x *buckleyi* clone. As noted previously, the a

allele of *Tpi-2* is common in clones of *S.* x *buckleyi* and *S. russelliana*, but was not detected in any of the *S. truncata* clones (Table 3). The *Tpi-2* phenotype for 'Buckleyi' (aa) is inconsistent with that expected for an F_1 interspecific hybrid of *S. russelliana* and *S. truncata*, which lends support McMillan's (1990) theory. Clone 50447 was a field-collected specimen tenatively identified as *S. truncata*, but its flower and phylloclade morphology suggested that it was a hybrid of *S. russelliana* and *S. truncata*. The *Tpi-2* phenotype for clone 50447 (aa) indicates that its parentage includes *S.russelliana*, thus corroborating the morphological data. Thus, the correct binomial for clone 50447 is *S.* x *buckleyi*.

Isozyme expression and ploidy level. Previous studies (Barthlott, 1976; Remski, 1954; Stockwell, 1935) have shown that the genus *Schlumbergera* consists of diploid species with a chromosome number of $2n = 2x = 22$. However, several commercial cultivars included in this study are reported to be polyploid, i.e., 'Bridgeport', 'Cambridge', 'Gold Charm', and 'Santa Cruz' (Cobia, 1992; Wade et al., 1985). Isozyme profiles of individual plants may provide evidence of polyploidy. 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. As a consequence, polyploids that have three or more alleles per locus usually exhibit greater numbers of isozyme bands than would be expected for diploids. The ploidy level of 'Bridgeport', 'Cambridge', 'Gold Charm', 'Santa Cruz', however, could not be deduced from their isozyme phenotypes (Table 3). These four cultivars were heterozygous at several isozyme loci, but at most two bands were detected for the monomeric enzymes LAP, PGM, and SKD and three bands for the dimeric enzymes (AAT, GPI, MDH, and TPI), as would be expected for diploid taxa (Figures 1, 2, and 3). Chyi and Weeden (1984), Murawski et al. (1994), and Nielsen (1980) reported that relative intensity of isozyme bands can be used to distinguish specific genotypes in some polyploid taxa. 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 specific *Schlumbergera* clones from the relative intensities of isozyme bands.

Genetic diversity in the germplasm collection. The percentage of polymorphic loci was 75% for the 42 commercial clones, but was slightly higher (83%) for the 14 field-collected clones (Table 6). On average, the commercial clones contained fewer alleles per locus and displayed less allelic diversity than the field-collected clones. However, the mean heterozygosity values for the commercial clones and the field-collected clones were similar (Table 6).

The isozyme data presented here indicate that the field-collected clones are a more genetically diverse group than the commercial clones. It should be noted that the level of genetic diversity in the 14 field-collected clones nearly matched that of the entire germplasm collection (Table 6). Crop species typically exhibit less genetic diversity than their wild relaives (see review by Doebley, 1989). The decline in genetic diversity results from limited sampling of germplasm from wild populations and loss of alleles during breeding. The commercial clones undoubtedly represent only a small fraction of the genetic variation in *Schlumbergera*, whereas the field-collected clones represent a more diverse gene pool which may be useful in future breeding efforts.

Relationship between genetic diversity and a species' breeding system. *Schlumbergera truncata*, *S. russelliana*, *S. opuntioides*, and *S. orssichiana* are self-incompatible, predominantly outcrossed species whereas *S. kautskyi* and *S. microsphaerica* are self fertile, primarily selfing species (Boyle, 1995; Ganders, 1976; Horobin and McMillan, 1990; McMillan, 1991). Species that are predominantly outcrossed often exhibit higher levels of genetic diversity than species that are primarily selfed (Hamrick and Godt, 1990). Hence, one would expect *Schlumbergera* species

that are predominantly outcrossed to exhibit greater genetic diversity than those species that are primarily selfed. One measure of intrapopulational genetic diversity is the mean proportion of loci that are heterozygous per individual (Hamrick, 1989). Table 7 shows the percentage of heterozygous isozyme loci for 12 field-collected *Schlumbergera* clones. The *S. kautskyi* clone was homozygous at all 12 loci, as would be expected for a selfing species. The percentage of heterozygous loci ranged from 17 to 42% for the *S. opuntioides*, *S. orssichiana*, and *S. truncata* clones, and ranged from 0 to 17% for the *S. russelliana* clones. Most *S. russelliana* clones contained fewer heterozygous loci than expected for an outcrossing species (Hamrick, 1989). *Schlumbergera russelliana* is endemic to the Serra dos Orgãos in the Brazilian state of Rio de Janeiro, and is found only in cloud forests at 1350 to 2200 m altitude (Barthlott and Taylor, 1995). Low levels of heterozygosity may indicate that the *S. russelliana* clones were sampled from relatively small populations. Genetic diversity is rapidly lost from small populations due to inbreeding and random changes in allelic frequencies (genetic drift) (Wilcox, 1984). Low levels of heterozygosity in the *S. russelliana* clones may indicate habitat disturbance or other factors which can threaten the long-term survival of the species. Quantitative estimates of biological diversity such as those provided in Table 7 could prove useful in conservation efforts for *Schlumbergera* species.

Summary. This study demonstrates the utility of isozyme markers for distinguising clones, identifying duplicate accessions, substantiating parentage, and quantifying genetic diversity for the entire germplasm collection and for specific clones. The information obtained from this study may aid in systematic studies of *Schlumbergera* species and in plant conservation efforts.

Table 6.1. The *Schlumbergera* accessions used for study and their sources botanical names, and status.

Clone	Binomial	Status[y]	Source[z]
	Named accessions		
Alexis	*S.* x *buckleyi*	CV	PM
Apricot	*S.* x *buckleyi*	CV	DK
Barbara	*S.* x *buckleyi*	CV	PM
Bridgeport	*S. truncata*	CV	CO
Buckleyi	*S.* x *buckleyi*	CV	MA
Cambridge	*S. truncata*	CV	CO
Christmas Charm	*S.* x *buckleyi*	CV	CO
Christmas Cheer	*S. truncata*	CV	JV
Christmas Fantasy	*S. truncata*	CV	CO
Claudia	*S.* x *buckleyi*	CV	PM
Dark Marie	*S. truncata*	CV	PM
Eva	*S. truncata*	CV	JV
Exotica	*S. x exotica*	CV	DK
Gina	*S.* x *buckleyi*	CV	PM
Gold Charm	*S. truncata*	CV	CO
Kris Kringle	*S.* x *buckleyi*	CV	CO
Kris Kringle II	*S.* x *buckleyi*	CV	CO
Lavender Doll	*S.* x *buckleyi*	CV	CO
Lavender Doll II	*S.* x *buckleyi*	CV	CO
Lilofee	*S.* x *buckleyi*	CV	DK
Linda	*S. truncata*	CV	PM
Madisto	*S.* x *buckleyi*	CV	PM
Madonga	*S.* x *buckleyi*	CV	PM
Majestic	*S.* x *buckleyi*	CV	DK
Marie	*S. truncata*	CV	PM
Minibelle	*S. x exotica*	CV	RG
Naomi	*S.* x *buckleyi*	CV	JV
Noris	*S.* x *buckleyi*	CV	DK
Peach Parfait	*S. truncata*	CV	CO
Pinkie	*S.* x *buckleyi*	CV	RG
Purple Devil	*S.* x *buckleyi*	CV	DK
Red Radiance	*S.* x *buckleyi*	CV	CO
Rocket	*S. truncata*	CV	RG
Salmoneum Rubrum	*S.* x *buckleyi*	CV	DK
Santa Cruz	*S. truncata*	CV	CO
Sonja	*S.* x *buckleyi*	CV	JV
Starbright	*S.* x *buckleyi*	CV	LN
Thor-Britta	*S. truncata*	CV	GT
Thor-Louise	*S.* x *buckleyi*	CV	GT

Continued next page

Table 6.1 continued

Twilight Tangerine	*S.* x *buckleyi*	CV	CO
Voll B	*S.* x *buckleyi*	FC	DK
White Christmas	*S. truncata*	CV	CO
Yantra	*S.* x *buckleyi*	CV	JV
Zaraika	*S.* x *buckleyi*	CV	JV

Numbered accessions

50447	*S.* x *buckleyi*	FC	HU
Clone 1	*S. kautskyi*	FC	DK
01537	*S. opuntioides*	FC	BG
CC615	*S. opuntioides*	FC	RG
O5584	*S. orssichiana*	FC	BG
CC617	*S. orssichiana*	FC	RG
UM921	*S. orssichiana*	FC	DK
32959	*S. orssichiana*	FC	BH
34533	*S. russelliana*	FC	HU
33160	*S. russelliana*	FC	BH
Hunt 6484	*S. russelliana*	FC	DK
JFH Clone 1	*S. russelliana*	FC	DK
02636	*S. russelliana*	FC	BG
Abendroth No. 2	*S. truncata*	FC	HG
Abendroth No. 6	*S. truncata*	FC	HG

[z] BG= Botanischer Garten Bonn, Bonn, Germany; BH= Botanischer Garten Heidelberg, Heidelberg, Germany; CO= B.L. Cobia Inc., Winter Garden, FL; DK= Dolly Kolli, Mashpee, MA; GT= Gartneriet Thoruplund, Odense, Denmark; HG= Holly Gate Cactus Nursery, West Sussex, England; HU= Huntington Botanical Gardens, San Marino, CA; JV= J. de Vries Potplantencultures bv, Aalsmeer, The Netherlands; LN= Leavitt's Nursery, Lehighton, PA; MA= University of Massachusetts, Amherst, MA; PM= Gartneriet PKM, Odense, Denmark;RG= Rainbow Gardens, Vista, CA.
[y] CV= commercial cultivar; FC= field-collected clone.

Table 6.2. Polyacrylamide gel concentrations and sample loading volumes used for electrophoresis of seven enzyme systems.

Enzyme	Gel concentration (%)	Loading volume (ul)
Aspartate aminotransferase (AAT)	5	10
Glucose-6-phosphate isomerase (GPI)	7.5	10
Leucine aminopeptidase (LAP)	7.5	10
Malate dehydrogenase (MDH)	7.5	10
Phosphoglucomutase (PGM)	7.5	10
Shikimic dehydrogenase (SKD)	7.5	10
Triosephosphate isomerase (TPI)	10	5

Table 6.3. Phenotypes for twelve isozyme loci in 59 *Schlumbergera* clones.

		Isozyme locus											
	Isozyme profile (no.)	*Aat-1*	*Aat-2*	*Gpi-1*	*Gpi-2*	*Lap-1*	*Mdh-1*	*Mdh-2*	*Mdh-3*	*Pgm-1*	*Pgm-2*	*Skd-1*	*Tpi-2*
Alexis	1	aa	bc	aa	ab	cc	bb	aa	aa	bb	cc	cd	ab
Apricot	2	aa	ab	aa	bc	cc	bb	aa	aa	bb	cc	cc	aa
Barbara	3	aa	cc	aa	ab	cc	bb	aa	aa	bb	cc	cd	ab
Bridgeport	4	aa	bc	aa	bb	bb	ab	aa	aa	cc	cc	cc	bb
Buckleyi	2	aa	ab	aa	bc	cc	bb	aa	aa	bb	cc	cc	aa
Cambridge	5	aa	cc	aa	bc	bb	ab	aa	aa	bc	cc	cc	bb
Christmas Charm	6	aa	bc	aa	ab	bb	ab	aa	aa	bb	cc	cd	bb
Christmas Cheer	7	aa	bc	aa	bb	bb	ab	aa	aa	bc	cc	cd	bb
Christmas Fantasy	8	aa	bc	aa	ab	bb	ab	aa	aa	cc	cc	cc	bb
Claudia	9	aa	ab	aa	ab	cc	bb	aa	aa	bb	cc	cc	ab
Dark Marie	10	aa	bc	aa	bb	cd	bb	aa	aa	bb	cc	cc	bb
Eva	11	aa	bb	aa	bb	dd	ab	aa	aa	bc	cc	cc	bb
Exotica	12	ab	bb	aa	bc	ac	bb	aa	aa	aa?	ce	bc	ab
Gina	13	aa	bc	aa	bb	bb	bb	aa	aa	bb	cc	cd	bb
Gold Charm	5	aa	cc	aa	bc	bb	ab	aa	aa	bc	cc	cc	bb
Kris Kringle	14	aa	ab	aa	ab	cc	ab	aa	aa	bb	cc	cd	ab
Kris Kringle II	15	aa	ab	aa	bb	cc	ab	aa	aa	dd	cc	cd	bb
Lavender Doll	16	aa	ac	aa	ab	bc	bb	aa	aa	bb	cc	cc	bb
Lavender Doll II	17	aa	bc	aa	bb	bb	ab	aa	aa	bd	cc	cd	bb
Lilofee	18	aa	bc	aa	ab	bd	bb	aa	aa	bc	cc	cd	bb
Linda	19	aa	ab	aa	bb	bd	ab	aa	aa	bc	cc	cd	bb
Madisto	20	aa	ac	aa	ab	cd	bb	aa	aa	bb	cc	cc	ab
Madonga	21	aa	bc	aa	ab	cc	bb	aa	aa	bc	cc	cc	bb
Majestic	22	aa	bc	aa	ab	cd	bb	aa	aa	bc	cc	cd	ab
Marie	10	aa	bc	aa	bb	cd	bb	aa	aa	bb	cc	cc	bb
Minibelle	23	ab	bb	aa	ab	cc	bb	aa	aa	ad	ce	bc	ab
Naomi	24	aa	cc	aa	bb	dd	bb	aa	aa	bc	cc	cd	bb

Continued next page

Table 6.3 continued

Peach Parfait	26	aa	cc	aa	ab	cc	ab	aa	aa	bc	cc	cc	bb
Pinkie	20	aa	ac	aa	ab	cd	bb	aa	aa	bb	cc	cc	ab
Purple Devil	25	aa	bc	aa	ab	cc	bb	aa	aa	bb	cc	cc	aa
Red Radiance	27	aa	ac	aa	aa	cc	bb	aa	aa	bb	cc	cd	bb
Rocket	28	aa	bb	aa	bb	bd	bb	aa	aa	bc	cc	cd	bb
Salmoneum Rubrum	29	aa	ac	aa	bb	cc	bb	aa	aa	bb	cc	cc	aa
Santa Cruz	30	aa	ab	aa	ab	bb	ab	aa	aa	cc	cc	cd	bb
Sonja	31	aa	ac	aa	ab	cd	bb	aa	aa	bd	cc	cd	ab
Starbright	32	aa	ab	aa	ab	bc	ab	aa	aa	bc	cc	cc	ab
Species B	33	aa	bb	aa	ac	cc	bb	aa	aa	bb	cd	cd	ab
Thor-Britta	34	aa	bb	aa	bb	dd	bb	aa	aa	bb	cc	cc	bb
Thor-Louise	35	aa	bc	aa	ab	dd	bb	aa	aa	dd	cc	cc	ab
Twilight Tangerine	13	aa	bc	aa	bb	bb	bb	aa	aa	bb	cc	cd	bb
White Christmas	36	aa	bb	aa	bb	bb	ab	aa	aa	cc	cc	cc	bb
Yantra	37	aa	bb	aa	bb	bb	bb	aa	aa	bb	cc	cc	bb
Zaraika	37	aa	bb	aa	bb	bb	bb	aa	aa	bb	cc	cc	bb
S. x buckleyi 50447	2	aa	ab	aa	bc	cc	bb	aa	aa	bb	cc	cc	aa
S. kautskyi Clone1	38	aa	bb	aa	bb	dd	bb	aa	aa	bb	cc	ce	bb
S. opuntioides 01537	39	bb	bb	aa	ab	cc	bb	aa	aa	aa	ce	ab	ab
S. opuntioides CC615	40	bb	bb	aa	ab	ac	bb	aa	aa	aa	ce	ab	ab
S. orssichiana 05584	41	aa	bd	aa	bb	bc	ab	aa	aa	bb	cc	ce	ab
S. orssichiana CC617	42	aa	bd	aa	bb	cd	bb	aa	aa	bb	bc	de	ab
S. orssichiana UM921	42	aa	bd	aa	bb	cd	bb	aa	aa	bb	bc	de	ab
S. orssichiana 32958	42	aa	bd	aa	bb	cd	bb	aa	aa	bb	bc	de	ab
S. russelliana 34533	43	aa	bb	aa	bb	dd	bb	aa	aa	bb	cc	ab	ab
S. russelliana 33160	44	ab	bb	aa	aa	cc	bb	aa	aa	bb	cc	cc	aa
S. russelliana Hunt 6484	45	aa	bb	aa	bb	cc	bb	aa	aa	bb	cc	cc	aa
S. russelliana JFH Clone1	46	aa	bb	aa	bb	ce	bb	aa	aa	bb	cc	cc	aa
S. russelliana 02636	47	aa	bb	aa	bb	cc	bb	aa	aa	bb	cc	cc	bb
S. truncata Aben. No. 2	48	aa	bb	aa	cc	cd	bb	aa	aa	bc	ac	cc	bb
S. truncata Aben. No. 6	49	aa	bc	aa	bc	bb	bb	bb	aa	bb	cc	cc	bb

?= doubtful determination.

Table 6.4. Number of alleles present at 12 isozyme loci among the 59 *Schlumbergera* accessions.

Locus	Alleles	Locus	Alleles
Aat-1	a,b	*Mdh-2*	a,b
Aat-2	a,b,c,d	*Mdh-3*	a
Gpi-1	a	*Pgm-1*	a,b,c,d
Gpi-2	a,b,c	*Pgm-2*	a,b,c,d,e
Lap-1	a,b,c,d,e	*Skd-1*	a,b,c,d,e
Mdh-1	a,b	*Tpi-2*	a,b

Table 6.5. *Schlumbergera* clones with identical isozyme patterns.

Isozyme profile (no.)	Clones	Clonal differentiation by phenotypic characters
2	Apricot, Buckleyi, 50447	yes
5	Cambridge, Gold Charm	yes
10	Dark Marie, Marie	yes
13	Gina, Twilight Tangerine	yes
20	Madisto, Pinkie	no
25	Noris, Purple Devil	yes
37	Yantra, Zaraika	yes
42	CC617, UM921, 32959	no

Table 6.6. Diversity statistics for the *Schlumbergera* clonal germplasm collection.

Group	No. clones analyzed	% Polymorphic loci	Mean # alleles/ locus	Mean heterozygosity	Allelic diversity[z]
Commercial clones[y]	42	75	2.33	0.28	78
Field-collected clones[y]	14	83	2.92	0.30	97
All clones[y]	56	83	3.00	0.30	100

[z] (No. alleles observed ÷ total no. alleles at all loci) x 100.
[y] Excluding duplicate accessions.

Table 6.7. A comparison of percent heterozygous loci and breeding systems among five *Schlumbergera* species.

Species	Clone	Heterozygous loci (%)	Breeding system[z]
S. kautskyi	1	0	inbreeding (SC)
S. opuntioides	10537	33	outbreeding (SI)
	CC615	42	outbreeding (SI)
S. orssichiana	05584	42	outbreeding (SI)
	32959	42	outbreeding (SI)
S. russelliana	34533	17	outbreeding (SI)
	33160	8	outbreeding (SI)
	Hunt 6484	0	outbreeding (SI)
	JFH clone 1	8	outbreeding (SI)
	02636	0	outbreeding (SI)
S. truncata	Abendroth No. 2	25	outbreeding (SI)
	Abendroth No. 6	17	outbreeding (SI)

[z] SC= self-compatible; SI= self-incompatible. Breeding system confirmed by pollination tests.

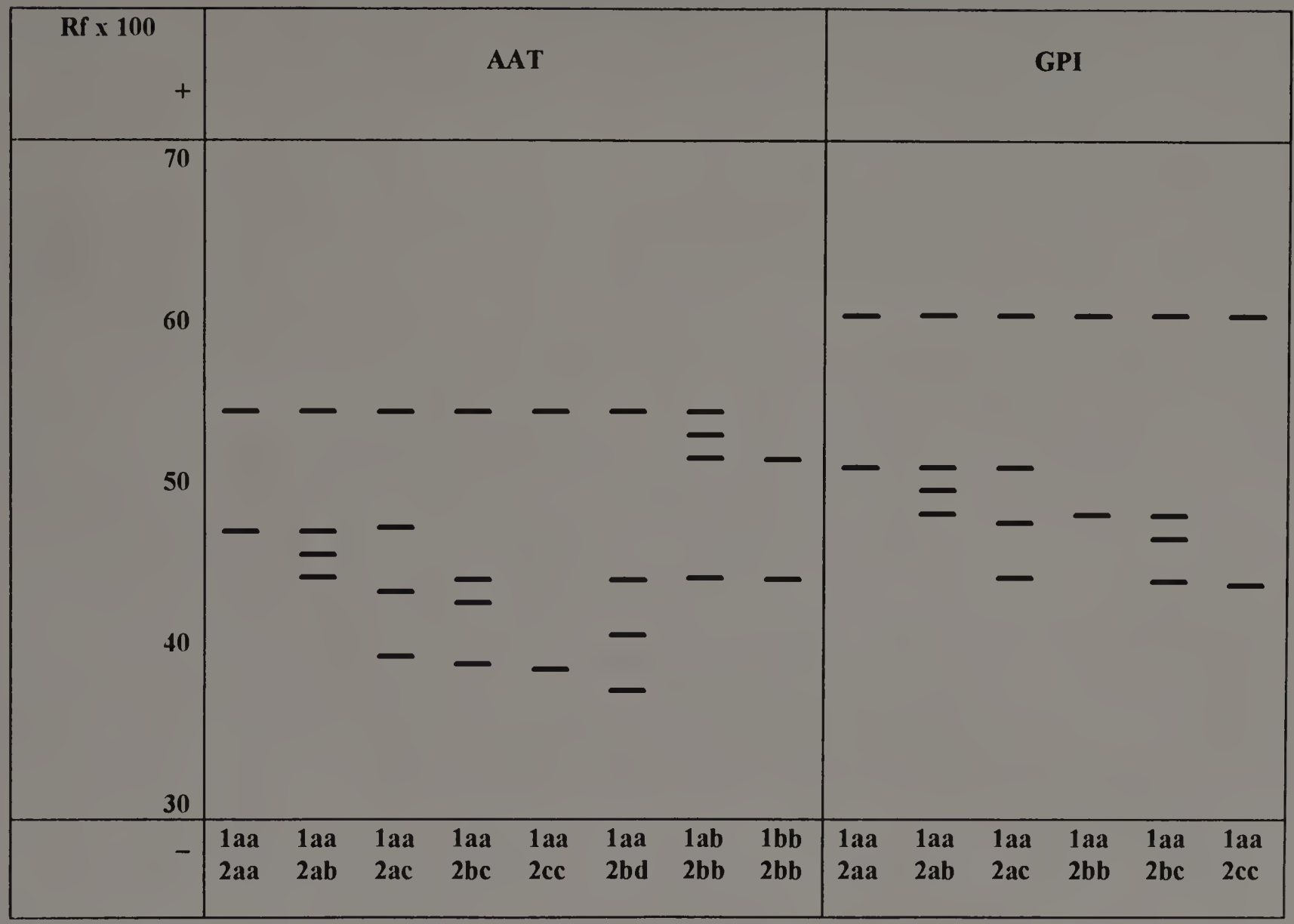

Figure 6.1. Schematic illustrations of enzyme phenotypes for AAT and GPI in *Schlumbergera*.

Rf x 100 +	LAP	MDH	TPI
70			
60			
50			
40			
30			
–	1ac 1bb 1bc 1bd 1cc 1cd 1ce 1dd	1ab 2aa 3aa; 1bb 2aa 3aa; 1bb 2bb 3aa	2aa; 2ab; 2bb

Figure 6.2. Schematic illustrations of enzyme phenotypes for LAP, MDH and TPI in *Schlumbergera*.

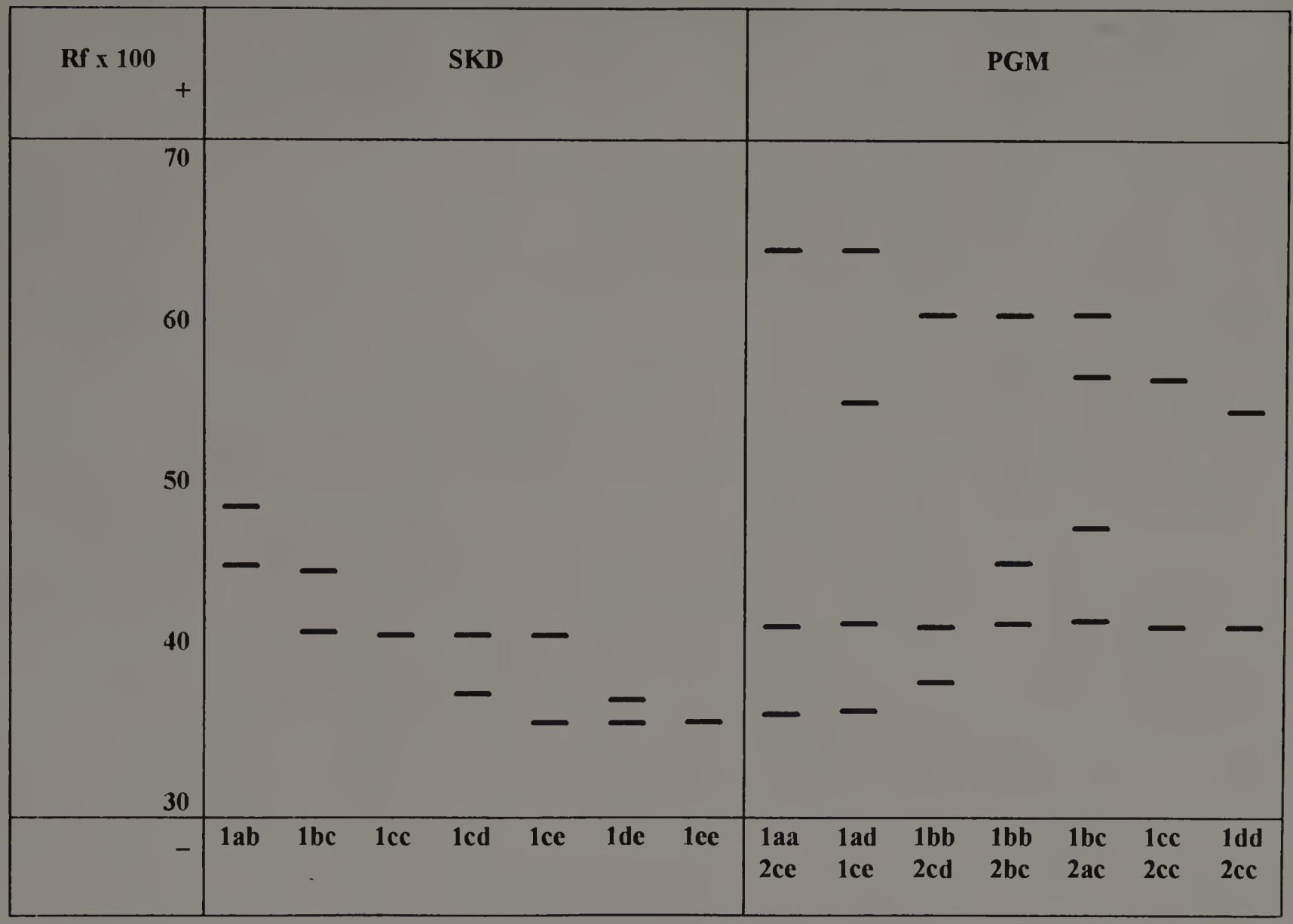

Figure 6.3. Schematic illustrations of enzyme phenotypes for SKD and PGM in *Schlumbergera*.

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