Pergamon

0305-1978(95)00065-8

Biochemical Systematics and Ecology, Vol. 23, No. 7/8, pp. 793–797, 1995

0305-1978/95 $9.50+0.00

Genetic Variation in *Haworthia pumila* and *H. herbacea*

GAEL CAMPBELL,* HERMAN VAN DER BANK† and BEN-ERIK VAN WYK*

*Department of Botany, Rand Afrikaans University, P.O. Box 524, Auckland Park 2006, Republic of South Africa;

†Research Unit for Aquatic and Terrestrial Ecosystems, Rand Afrikaans University, P.O. Box 524, Auckland Park 2006, Republic of South Africa

Key Word Index—Aloaceae; *Haworthia*; allozymes; electrophoresis; genetic variation.

Abstract—Horizontal starch gel electrophoresis was used to examine genetic diversity within and differences between one population each of two morphologically different species of *Haworthia*, namely *H. pumila* and *H. herbacea*. Twenty-five leaf samples of each species were surveyed for 24 proteins of which 13 were useful for routine analysis, and gene products of 16 protein coding loci revealed genetic variation at 7 (43.8%) thereof in both species. Values of 1.63 (±0.20) and 1.56 (±0.18) were obtained for the mean number of alleles per locus and the average heterozygosity per locus was calculated at 0.168 (±0.058) and 0.144 (±0.048) for *H. herbacea* and *H. pumila* respectively. The malate dehydrogenase-2 locus is a potential genetic marker to identify the species studied electrophoretically. The mean genotypic distance index between the populations studied was 0.184, an indication of a large degree of differentiation between the species. These values are much higher than values obtained for other members of the Aloaceae, showing that normal levels of genetic variation can be expected in at least some succulent monocotyledons.

Introduction

Haworthia is a genus of some 68 species of succulent plants endemic to southern Africa (Bayer, 1976, 1982; Scott, 1985). Bayer (1976) highlighted the problems associated with the taxonomy of the group. Many species are poorly defined and it is often difficult to associate different populations when they are strung out in isolated localities. This is why the taxonomic accounts of Bayer (1976, 1982) and Scott (1985) differ in both the application of rank and in the circumscription of taxa. Ecological requirements are proposed as a likely selection mechanism in speciation (Bayer 1976) but the biological relationships between various populations, varieties and species have not yet been studied. Species of *Haworthia* and other genera of the Aloaceae are much sought after by local and overseas plant collectors. According to Hilton-Taylor and Smith (1994), at least 17 species of *Haworthia* have become endangered through agricultural development, overgrazing, collection by succulent enthusiasts and overexploitation for traditional medicine. Some species are on the brink of extinction and sound decisions concerning the conservation of specific populations have been difficult to make. When a rare species or infraspecific entity occurs in a few isolated populations, it is important to know if each population is genetically unique, or whether a particular population can be sacrificed to development without eroding the genetic integrity of the species as a whole.

This study was undertaken to evaluate the feasibility of using horizontal starch gel electrophoresis to determine the amount of genetic variation within and between different *Haworthia* species. For our study, we chose one population each of *H. herbacea* (Mill.) Stearn and *H. pumila* (L.) Duval, from different subgenera and representing the extremes of the range of morphological variation found in the genus. Previous results for *Aloe ferox* Miller and *A. marlothii* Berger (Van der Bank *et al.*

(*Received* 30 *January* 1995; *accepted* 29 *June* 1995)

1995a) suggested that succulent plants may have low levels of genetic variation but a wider survey of other taxa from the Aloaceae seemed desirable.

Materials and Methods

Plant material. Twenty-five leaf samples each of *H. herbacea* and *H. pumila* were collected from two natural populations (one of each species) at Worcester (33°38′ S, 19°27′ E; altitude *ca* 300 m) in the south-western Cape Province of South Africa. *Haworthia pumila*, from the subgenus *Robustipedunculares* Uitewaal ex Bayer, was chosen because it is the largest of all *Haworthia* species (up to 300 mm high), with large, firm-textured leaves. It represents one extreme of the range of variation found in the genus. *Haworthia herbacea*, on the other hand, is one of 45 species from the subgenus *Haworthia* (Bayer, 1982) and the small habit (up to 80 mm in diameter) and soft, fleshy leaves are quite unlike those of *H. pumila*. *Haworthia herbacea* was also chosen because it is part of a complicated species complex where genetic information may eventually contribute to a better understanding of species circumscriptions.

Procedure. Mature leaves were collected in cryotubes and immediately submerged in liquid nitrogen (−196°C). Approximately 2 g of leaf tissue were manually ground with a glass mortar and pestle in 2 ml of Tris–HCl extraction buffer (pH = 7.5), as described by Soltis *et al.* (1983). The supernatant was then transferred to eppendorf test tubes and centrifuged at 4000 *g* for 5 mins, after which different methods of applying samples to gels were used in order to obtain optimal enzyme concentrations from the succulent leaves with their high water content. We modified the method described by Swart *et al.* (1994), by adding 200 µl of the supernatant and by mixing it with another 200 µl of a water-based gel consisting of 20% sucrose and 4.5% Sephadex G-200. The samples were applied to preformed slots in the starch gel, about 2.5 cm from the cathodal end. Alternatively, the supernatant was absorbed directly onto paper wicks and recommendations by Kephart (1990) were taken into consideration to optimise electrophoretic conditions.

Twelve per cent starch (Sigma: S-4501) gels were used and 13 (Table 1) of the 24 protein systems stained for produced interpretable banding patterns, resulting in a total of 16 loci that could be scored and analyzed. Genetic interpretation of enzyme banding patterns was based on the subunit structure of the enzymes (Gottlieb, 1981, 1982; Kephart, 1990). Locus nomenclature followed Harris and Hopkinson (1976), Soltis and Soltis (1989), Hillis and Moritz (1990) and Shaklee *et al.* (1990). Locus abbreviations, monomorphic loci, buffers used and enzyme commission numbers are given in Table 1. Buffer systems are described in Van der Bank *et al.* (1995a,b) and statistical analysis of allozyme data was executed using *BIOSYS-1* (Swofford and Selander, 1981).

Results

Sixteen protein-coding loci provided interpretable results in *H. pumila* and *H. herbacea*, of which 43.75% displayed polymorphism (Table 1). Nine of the 16 loci displayed monomorphic gel banding patterns and products of the following loci migrated cathodally: *GDA, GPD, ME* and *PGD-2*. In addition to these loci, the following proteins were stained for: acid phosphatase (E.C. 3.1.3.2); adenylate kinase

TABLE 1: LOCUS ABBREVIATIONS, BUFFER SYSTEMS AND ENZYME COMMISSION NUMBERS (E.C. NO.) ARE LISTED AFTER EACH ENZYME

Enzyme	Locus	Buffer (pH)	E.C. No.
Aspartate aminotransferase	**AAT*	P (8.2)	2.6.1.1
Esterase	**EST*	MF (8.6)	3.1.1.
Glyceraldehyde-3-phosphate dehydrogenase	**GAPDH*	HC (5.7)	1.2.1.12
Guanine deaminase	*GDA*	HC (5.7)	3.5.4.3
Glucose-3-phosphate dehydrogenase	*GPD*	MF (8.6)	1.1.1.8
Glucose-6-phosphate isomerase	*GPI-1*	RW (8.0)	3.5.1.9
	GPI-2	RW (8.0)	
Isocitrate dehydrogenase	*IDH*	HC (6.5)	1.1.1.42
Malate dehydrogenase	*MDH-1*	HC (6.5)	1.1.1.37
	**MDH-2*	HC (6.5)	
Malic enzyme	*ME*	HC (6.5)	1.1.1.3
Menadione reductase	**MNR*	HC (6.5)	1.6.99.-
Mannose-6-phosphate isomerase	**MPI*	MCT (6.1)	5.3.1.8
6-Phosphogluconate dehydrogenase	*PGD-1*	RW (8.0)	1.1.1.44
	PGD-2	RW (8.0)	
Phosphoglucomutase	*PGM*	MF (8.6)	5.4.2.2

* = Polymorphic loci; gel and electrode buffers and abbreviations used according to Van der Bank *et al.* (1995a,b)

(E.C. 2.7.4.3); dihydrolipoamide dehydrogenase (E.C. 1.8.1.4); peptidase (E.C. 3.4.-.-) using glycyl-L-leucine, leucine–alanyl, leucylglycylglycine, leucyl–tyrosine and L-phenylalanyl-L-proline as substrates; leucine aminopeptidase (E.C. 3.4.11.1); general (unidentified) protein and shikimate dehydrogenase (E.C. 1.1.1.25). These proteins did not show sufficient activity or resolution to score them satisfactorily in *Haworthia* samples.

A comparison of the two methods used to apply the samples to gels showed that the greater amount of extract loaded using preformed slots resulted in higher enzyme activity, which was beneficial for all the enzymes except *GPI-2*, for which the method using paper wicks produced better results.

Table 2 presents allele frequencies and Chi-square (χ^2) values for polymorphic loci in the two species studied. Loci where significant ($P<0.05$) deviations of alleles from expected Hardy–Weinberg proportions occurred and individual heterozygosities are also listed in Table 2. Allozyme phenotypes of putative heterozygotes at loci were congruent with those expected on the basis of the quaternary structure of the enzyme (Ward, 1977). Thus heterozygotes at *GPI-2* and *MDH-2* were triple banded, as expected for dimeric enzymes and double banded at the monomeric enzymes: *AAT, EST, MNR* and *MPI*. The heterozygote found at the *GAPDH* locus was four-banded.

Genotypic frequencies at four loci (*GPI-2, MDH-2, MNR* and *MPI*) in *H. pumila* and at all seven polymorphic loci in *H. herbacea* (Table 2) closely approximated Hardy–Weinberg expectations. Heterozygote deficiencies were encountered at the *AAT, EST, GAPDH* and *MPI* enzyme-coding loci in *H. herbacea*, and additionally at the *MNR* locus in *H. pumila* and deviations of allele frequencies from expected Hardy–Weinberg proportions were encountered at the former three loci in *H. pumila* (Table 2). The relative mobility of alleles at the *MDH-2* locus differed between the species studied (Table 2). The percentage of polymorphic loci (*P*) was 43.75% for both *Haworthia* species. The mean number of alleles per locus (*A*) was 1.63 (±0.20) in *H. pumila* and 1.56 (±0.18) in *H. herbacea*; average heterozygosity values (*H*) were 0.168 (±0.058) and 0.144 (±0.048) respectively and individual heterozygosity values (*h*) ranged from 0.095 to 0.611 in *H. pumila* and from 0.117 to 0.469 in *H. herbacea*. The

TABLE 2: SAMPLE SIZE, ALLELE FREQUENCIES, OBSERVED NUMBER OF HETEROZYGOTES (OBS), COEFFICIENTS FOR HETEROZYGOSITY DEFICIENCY OR EXCESS (D), χ^2 VALUES, DEGREES OF FREEDOM (DF) AND INDIVIDUAL HETEROZYGOSITIES (*h*) FOR POLYMORPHIC LOCI

Locus		*n*	*fA*	*fB*	*fC*	*fD*	OBS	*D*	χ^2	DF	*h*
AAT	(1)	21	0.143	0.452	0.405	—	5	−0.61	25.116*	3	0.611
	(2)	15	0.267	0.7	0.033	—	3	−0.543	6.601	3	0.438
EST	(1)	20	0.625	0.375	—	—	5	−0.467	4.356*	1	0.469
	(2)	4	0.625	0.375	—	—	1	−0.467	0.871	1	0.469
GAPDH	(1)	9	0.111	0.833	0.056	—	1	−0.617	9.04*	3	0.29
	(2)	7	0.071	0.786	0.143	—	1	−0.6	7.058	3	0.357
GPI-2	(1)	13	0.346	0.539	0.115	—	8	0.067	2.686	3	0.577
	(2)	3	0.167	0.833	—	—	1	0.2	0.12	1	0.278
MDH-2	(1)	10	0.95	0.05	—	—	1	0.053	0.028	1	0.095
	(2)	10	—	—	0.9	0.1	2	0.111	0.123	1	0.18
MNR	(1)	8	0.375	0.625	—	—	2	−0.467	1.742	1	0.469
	(2)	8	0.063	0.937	—	—	1	0.067	0.036	1	0.117
MPI	(1)	5	0.1	0.9	—	—	1	0.111	0.062	1	0.18
	(2)	8	0.625	0.375	—	—	2	−0.467	1.742	1	0.469

(1) = H. pumila; (2) = H. herbacea.

* = Loci where significant ($P<0.05$) deviations of alleles from expected Hardy–Weinberg proportions occurred.

mean genotypic distance (*D*) value between the species studied was 0.184 (Nei, 1978).

Discussion

Deviations of allele frequencies from expected Hardy–Weinberg proportions occurred at the *AAT, EST* and *GAPDH* loci in *H. pumila* (Table 2) due to deficiencies of heterozygotes at these loci. Heterozygote deficiencies may be due to non-random mating, selection, gene flow, mutations and genetic drift (Soltis and Soltis, 1988). However, since heterozygote deficiencies (Table 2) were observed at three of the 14 loci (less than 22%), non-random mating, gene flow and drift were probably not factors, since these processes should affect all loci equally. Thus the heterozygote deficiencies encountered may be the result of selection, mutations, sample error or a combination thereof.

Sufficient genetic variation and differentiation ($H = 14.4–16.8\%$; $D = 0.18$) were obtained to justify further studies in *Haworthia,* which may provide valuable new insight into the genetic basis of the rather intricate patterns of morphological and geographical variation present in the species (mainly habit and leaf morphology). Allele mobilities at the *MDH-2* locus were nearly twice as fast in *H. pumila* compared to *H. herbacea* and it is a potential biochemical marker for the species studied. Furthermore, the genetic variation was relatively high compared to that obtained in *A. ferox* and *A. marlothii,* as reported by Van der Bank *et al.* (1995a). In the family Aloaceae, it appears that only the data on *Aloe* are available for comparison. Here, there was genetic variation at only one locus (4.55%) in the *A. marlothii* compared to seven loci (43.8%) in the two *Haworthia* species studied (Table 2). The value of *P* for *A. marlothii* was 4.7%, and 0% for *A. ferox*, compared to 43.75% obtained for the *Haworthia* species analysed in the present study. The value of *A* was 1.05 (± 0.05) and an *H* value was calculated at 0.022 (± 0.022) for *A. marlothii* compared to $A = 1.0$ ($H = 0$) for *A. ferox.* These values are higher in the two *Haworthia* species. The *h* value for *A. marlothii* was 0.461, which compares favourably with the values obtained for the *Haworthia* species (0.61 and 0.47 in *H. pumila* and *H. herbacea* respectively). The *D* value was 0.056 between the *Aloe* species, whereas it was 0.184 between the *Haworthia* species. This suggests a high degree of differentiation between the two *Haworthia* species studied. This is not unexpected, since the two *Haworthia* species are from two different subgenera and are more distantly related than the two *Aloe* species, which are morphologically and even chemically closely similar.

Van der Bank *et al.* (1995a) suggested that the lack of allozyme variation in the two *Aloe* species may be related to the xerophytic habit of the plants, perhaps making them less sensitive to drought stress, one of the most important selection pressures in the arid parts of southern Africa. Since there were high levels of genetic variation in the two *Haworthia* species studied, the previous results seem to apply only to some members of the Aloaceae and generalizations are not valid. The ecological theory, which was proposed by Van der Bank *et al.* (1995a) to explain the low levels of variation in two *Aloe* species clearly does not apply to all xerophytic succulents. The *H* value (0.062) calculated for other monocotyledonous flowering plants (Nevo *et al.*, 1984) was also considerably lower than that found in the *Haworthia* species (0.168 in *H. pumila* and 0.144 in *H. herbacea*), suggesting a relatively high degree of outbreeding in the *Haworthia* species studied. The frequency of polymorphic loci was also found to be lower in other monocotyledons: 30.3% compared to a value of 43.75% in *Haworthia.*

According to Hamrick (1989) and Ayala (1976), the average *P* value for plant species is 36.8–46.4%, which is comparable to that found for the *Haworthia* species (43.75%). Similarly, the *A* value is 1.69, which in this case corresponds rather closely in both species, 1.63 (± 0.20) and 1.56 (± 0.18), and *H* values of 0.141–0.17%, which

also corresponds closely to the values obtained for *Haworthia* (0.168 and 0.144%, respectively). From these values, there appears to be a great deal of genetic variation within and between the two species of *Haworthia*. The variation is comparable to that found in other plant groups and is not exceptionally low as was found in the two *Aloe* species studied by Van der Bank *et al.* (1995a).

In conclusion, this is the first account of electrophoretic variants in the genus *Haworthia* and the success of the technique may have rather far-reaching applications in that it may be of value in taxonomic studies of *Haworthia*, both at the species and infra-specific levels. Such data would be of use to study microevolution and speciation, to determine relationships and to gain a better understanding of genetic and evolutionary characters of members of the genus. It may also be of practical and theoretical value in conservation management.

Acknowledgements—SASOL, the Rand Afrikaans University and the Foundation for Research Development provided financial support for this project. We thank the curator and staff of the Karoo Botanic Garden at Worcester, for help with the sampling.

References

Ayala, F.J. (1976) *Molecular Evolution*. Sinauer Association Inc., Massachusetts.

Bayer, M.B. (1976) *Haworthia Handbook*. National Botanic Gardens of South Africa, Cape Town.

Bayer, M.B. (1982) *The New Haworthia Handbook*. National Botanic Gardens of South Africa, Cape Town.

Gottlieb, L.D. (1981) Electrophoretic evidence and plant populations. *Prog. Phytochem.* **7**, 1–46.

Gottlieb, L.D. (1982) Conservation and duplication of isozymes in plants. *Science* **216**, 373–380.

Hamrick, J.L. (1989) Isozymes and the analysis of genetic structure in plant populations. In: *Isozymes in Plant Biology* (Soltis, D.E. and Soltis, P.S., eds), Chapter 4, pp. 87–105. Chapman and Hall, London.

Harris, H. and Hopkinson, D.A. (1976) *Handbook of Enzyme Electrophoresis in Human Genetics*. Elsevier, New York.

Hillis, D.M. and Moritz, G. (1990) *Molecular Systematics*. Sinauer Associates Inc., Massachusetts.

Hilton-Taylor, C. and Smith, G.F. (1994) The conservation status of Aloaceae in southern Africa. *Strelitzia* **1**, 287–303.

Kephart, S.R. (1990) Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. *Am. J. Bot.* **77**, 693–712.

Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* 89, 583–590.

Nevo, E., Beiles, H. and Ben-Shlomo, R. (1984) The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. In: *Lecture Notes in Biomathematics: Evolutionary Dynamics of Genetic Diversity* (Mani, G.S., ed.), pp. 13–213. Springer, New York.

Scott, C.L. (1985) *The Genus Haworthia: A Taxonomic Revision*. Aloe Books, Johannesburg. Shaklee, J.B., Allendorf, F.W., Moritz, D.C. and Whitt, G.S. (1990) Gene nomenclature for protein-coding loci in fish. *Trans. Am. Fish. Soc.* **119**, 2–15.

Soltis, D.E. and Soltis, P.S. (1989) *Isozymes in Plant Biology*. Chapman and Hall, London.

Soltis, D.E., Haufler, C.H., Darrow, D.C. and Gastony, G.J. (1983) Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. *Am. Fern J.* **73**, 9–27.

Soltis, P.S. and Soltis, D.E. (1988) Estimated rates of intragametophytic selfing in lycopods. *Am. J. Bot.* **75**, 248–256.

Swart, M.K.J., Bissbort, S., Ferguson, J.W.H. and Ungerer, J.P.J. (1994) Polymorphism of glucose-6-phosphate dehyrogenase in black rhinoceroses: a possible link with haemolytic anaemia. *S. Afr. J. Sci.* **90**, 4–16.

Swofford, D.L. and Selander, R.B. (1981) *BIOSYS-1:* A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *J. Hered.* **72**, 281–283.

Van der Bank, H., Van Wyk, B.-E. and Van der Bank, M. (1995a) Genetic variation in two economically important *Aloe* species (Aloaceae). *Biochem. Syst. Ecol.* **23**, 251–256.

Van der Bank, H., Van der Bank, M. and Van Wyk, B.-E. (1995b) Allozyme variation in *Virgilia oroboides* (Tribe Podalyrieae, Fabaceae). *Biochem. Syst. Ecol.* **23**, 47–52.

Ward, R.D. (1977) Relationships between enzyme heterozygosity and quaternary structure. *Biochem. Genetics* **15**, 123–135.