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Genetic Polymorphism in Wild and Cultivated *Siphonochilus aethiopicus* (Zingiberaceae)

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Key Word Index—Zingiberaceae; *Siphonochilus aethiopicus*; African wild ginger; genetic diversity; allozyme fingerprinting; electrophoresis.

Abstract—Horizontal starch gel-electrophoresis was utilized to estimate genetic diversity within a natural population of wild ginger (*Siphonochilus aethiopicus*) and individuals of the species cloned for commercial purposes. The aim of this study was to determine if electrophoresis is useful in studying genetic variation in *S. aethiopicus*. In the wild population, 50 plants revealed genetic variation at 11 (50%) of the 22 enzyme coding loci studied. The percentage of polymorphic loci (*P*) was 50, a value of 1.55 (±0.13) was obtained for the mean number of alleles per locus (*A*) and the average heterozygosity per locus (*H*) was calculated at 0.177 (±0.044). These values were 4.55, 1.05 (±0.05), and 0.023 (±0.023), respectively, for the cultivated clones. Allozyme data for the wild population is compared with that of two sources of cultivated specimens, showing that genetic fingerprinting of *S. aethiopicus* clones can be achieved by using only 11 polymorphic loci and that the two cultivated clones probably originated from the same source. The exceptionally high allelic heterogeneity obtained for individuals (clonal polymorphism) may be due to the synergistic effects of vegetative and sexual reproduction. © 1997 Elsevier Science Ltd

Introduction

The family Zingiberaceae is well known for its spice plants which include ginger (*Zingiber officinale* Roscoe), tumeric (*Curcuma longa* L.), and others (Nayar and Ravindran, 1995). *Siphonochilus aethiopicus* (Schweinf.) B. L. Burtt, commonly known as "wild ginger", is a rhizomatous herb that occurs in Africa, southwards from Senegal and Ethiopia to the northern and eastern parts of South Africa (Lock, 1985; Smith, 1997). It is the only member of the family indigenous to South Africa, although some species of *Hedychium* have become naturalized (Smith, 1997). The rhizomes are used extensively in traditional African medicine and in witchcraft: they have a strong ginger smell and are chewed to clear nasal passages and their infusion is used in the treatment of malaria and horse-sickness (Watt and Breyer-Brandwijk, 1962). No published information is available on the chemistry and pharmacology of wild ginger.

Although *S. aethiopicus* is a rare plant in South Africa that has become extinct in some provinces (Hilton-Taylor, 1996), no information is available regarding its genetic variation. If we are to save the South African populations of this species, such information will be of practical and theoretical value. From a conservation point of view it is important to guard against the erosion of genetic diversity in wild populations. Since the plant is used medicinally, studies of this nature may be useful in the selection and breeding of superior biotypes for commercial cultivation.

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This paper is the first attempt at documenting genetic diversity in *S. aethiopicus* and is aimed at the assessment of genetic diversity by the separation of isoenzymes using starch gel-electrophoresis. Electrophoresis is often used to study genetic variation of populations for distinct allelic forms of isozymes. This is of great advantage because isozymes are unlinked characters which are often hardly or not environmentally influenced. Environmental variation is quantitative only—it may influence the expression of the genotype, i.e. the concentration of the band on the gel, but not the genotype itself. Thus once fertilization is complete, the genotype is fixed (e.g. in a leaf sample, an A-allele cannot change to a B-allele). Since many isozymes show codominance, identification of heterozygotes is possible (Hillis and Moritz, 1990). Allozyme electrophoresis remains the method of choice for characterizing populations because it is informative and technically simple (Mitton, 1994).

Materials and Methods

Leaf samples of six plants propagated by tissue culture at Kirstenbosch National Botanic Garden (samples A_1–A_6) were collected from a farm at Pietersburg. Samples B_1–B_6, of unknown origin, are material similar to A, with larger leaves, growing in containers at the above locality. Leaf samples from a wild population (50 individuals) were collected near Tzaneen, on the farm Duplex (23 30 CC). We collected young leaves from actively growing shoots, which were placed in cryotubes, submerged in liquid nitrogen and transported to the laboratory.

Sample preparation, electrophoretic procedures, buffers (except buffer A), staining of gels, interpretation of results and locus nomenclature are as referred to in Van der Bank *et al.* (1995). Statistical analysis of allozyme data was done using BIOSYS-1 (Swofford and Selander, 1981). Five buffer systems were used (see Table 1).

Results

Eleven of the 22 enzyme coding loci studied (50%), displayed monomorphic gel banding patterns (Table 1). Table 2 presents allele frequencies, observed and expected

TABLE 1. A LIST OF ALL ENZYMES USED IN THIS STUDY, TOGETHER WITH LOCUS ABBREVIATIONS, ENZYME COMMISSION NUMBERS (E.C. No.) AND BUFFER SYSTEMS WHICH GAVE THE BEST RESULTS (* = MONOMORPHIC LOCI)

Enzyme	Locus	E.C. No.	Buffer
Acid phosphate	ACP*	3.1.3.2	TC
Aspartate aminotransferase	AAT-1,-2*	2.6.1.1	PO
Creatine kinase	CK	2.7.3.2	TC
Esterase	EST-1,-2*	3.1.1.-	PO,MF
Glucose-6-phosphate isomerase	GPI-1*,-2	3.5.1.9	A
Isocitrate dehydrogenase	IDH*	1.1.1.42	TC,HC
Leucine aminopeptidase	LAP	3.4.11.1	A
Malate dehydrogenase	MDH-1,-2*,-3	1.1.1.37	HC
Menadione reductase	MNR-1,-2	1.6.99.-	HC
Peroxidase	PER-1,-2	1.11.1.7	PO
Peptidase:		3.4.-.-	
substrate: Leucylglycylglycine	PEP-B*		MF
Leucyl-tyrosine	PEP-S*		MF
6-Phosphogluconate dehydrogenase	PGD*	1.1.1.44	MF
Phosphoglucomutase	PGM*	5.4.2.2	PO
Superoxide dismutase	SOD*	1.15.1.1	MF

A = tris-EDTA-borate (pH 8.6) (Goncharenko *et al.*, 1992).
MF = a continuous tris, boric acid, EDTA buffer (pH 8.6).
TC = a continuous tris, citric acid (pH 6.9) buffer system.
PO = Poulik, a discontinuous buffer (electrode pH = 8.0; gel pH = 8.7).
HC = histidine-citrate (pH 6.5).

TABLE 2. SAMPLE SIZE (*N*), ALLELE FREQUENCIES (*f*), OBSERVED NUMBER OF HETEROZYGOTES (OBS), EXPECTED NUMBER OF HETEROZYGOTES (EXP), COEFFICIENTS OF HETEROZYGOSITY DEFICIENCY OR EXCESS (*D*), CHI-SQUARE VALUES (χ^2), DEGREES OF FREEDOM (d.f), AND INDIVIDUAL HETEROZYGOSITY FOR POLYMORPHIC LOCI (*h*)

Locus	*N*	*f*	OBS	EXP	*D*	χ^2	d.f	*h*
AAT-1	50	A=0.080 B=0.920	2	7.360	−0.728	26.518*	1	0.147
CK	25	A=0.560 B=0.440	14	12.320	0.136	0.465	1	0.493
EST-1	44	A=0.773 B=0.227	4	15.455	−0.741	24.171*	1	0.351
GPI-2	50	A=0.490 B=0.510	1	24.990	−0.960	46.078*	1	0.500
LAP	50	A=0.880 B=0.120	12	10.560	0.136	0.930	1	0.211
MDH-1	50	A=0.940 B=0.060	6	5.640	0.064	0.204	1	0.110
MDH-3	50	A=0.520 B=0.480	20	24.960	−0.199	1.974	1	0.499
MNR-1	42	A=0.357 B=0.643	20	19.286	0.037	0.058	1	0.450
MNR-2	49	A=0.245 B=0.724 C=0.031	1	20.296	−0.951	69.867*	3	0.414
PER-1	43	A=0.628 B=0.372	18	20.093	−0.104	0.467	1	0.467
PER-2	50	A=0.860 B=0.140	6	12.040	−0.502	12.583*	1	0.241

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

numbers of heterozygotes, Chi-square values for polymorphic loci, loci where significant ($P<0.05$) deviations of alleles from expected Hardy–Weinberg proportions occurred and individual heterozygosity values (*h*). Genotypic frequencies deviated from Hardy–Weinberg proportions at the following loci (Table 2); AAT-1, EST-1, GPI-2, MNR-2 and PER-2. The *h* values ranged from 0.110 to 0.500. Examples of polymorphism are shown in Fig. 1, for the MDH-3 (Fig. 1a) and PER-2 (Fig. 1b) loci. The cultivated clones from both sources (A and B) displayed the dominant alleles as reported for the wild population except at ACP where the alternate allele was found, and PER-2 (Fig. 1b) where only the B-alleles were obtained. The remarkably high allelic heterogeneity within the 50 individuals from the wild population is shown in Table 3. Missing data is shown for individuals where enzyme activity was insufficient to score phenotypes with confidence. Similar genotypes were found for individual numbers 1, 13 and 29, individuals 27 and 34, individuals 5 and 48, and individuals 37 and 46, respectively (marked with superscripts in Table 3).

Discussion

Genotypic frequencies deviated from Hardy–Weinberg proportions at five loci, AAT-1, EST-1, GPI-2, MNR-2, and PER-2, due to deficiencies of heterozygotes (Table 2). There was a slight excess of heterozygotes at the other polymorphic loci (Table 2). Van der Bank (1995) discussed various factors that can shift the equilibrium to disrupt the stability of a population giving rise to change in the genetic structure. The effects such

factors could have had on *S. aethiopicus* is unknown since this is the first study of its genetic diversity and very little is known of its biology (Gordon-Gray *et al.*, 1989).

In the wild population of *S. aethiopicus* a low genetic diversity was expected because it appeared to propagate vegetatively, as we had observed the presence of asexual propagules on its rhizomes. Populations of clonal plants are expected to contain only a few clones and therefore have a low genetic diversity (Ellstrand and Roose, 1987; Carter and Robinson, 1993). We obtained 1.55 for the A value, $P = 50$ and $H = 0.177$ for the natural wild ginger population, which corresponds with values obtained by several other authors. Van der Bank *et al.* (1995) for example, obtained a P value of 46.51%, a value of 1.49 ($\pm$ 0.08) for A and H was calculated at 0.207 ($\pm$ 0.034) in *Virgilia oroboides*. The P value was 4.55, $A = 1.05$ ($\pm$ 0.05) and the H value was calculated at 0.023 ($\pm$ 0.023) for the cultivated wild ginger population. This shows that there was a wide genetic variation in the wild population whilst there was virtually no variation at all in the cultivated plants that were sampled. The individual genetic diversity expressed at 12 polymorphic (11 polymorphic loci for the wild population and an additional locus, ACP, for the cultivated clones) enzymes is summarized in Table 3 indicating that allelic heterogeneity is remarkably high and that 45 of the 50 wild individuals had unique genotypes. The data in Table 2 show that all loci except the MNR-2 have two alleles (MNR-2 has three), which means that the total number of possible allele combinations for the 11 polymorphic loci in the wild population are $3^{10} \times 6^1 = 354\,294$. Missing data for six individuals prevent further resolution. Because standard statistics used by various authors to quantify population variation gives a poor measure of heterogeneity within populations, we propose: Allelic Heterogeneity (AH) = number of different genotypic individuals at all polymorphic loci over total number of individuals. This gives a value of 0.9, which is an underestimate since missing data was responsible for the lumping of individuals 1, 13 and 29, individuals 27 and 34, individuals 5 and 48, and individuals 37 and 46. Similar high levels of genetic diversity were observed by Carter and Robinson (1993), within a population of *Setaria incrassata*. The high levels of heterozygosity and polymorphism within this clonal grass were attributed to extensive recombination through outbreeding, and a possible synergistic effect between vegetative and sexual reproduction. Ellstrand and Roose (1987), in their special review of this subject have observed that species with clonal reproduction often possess considerable variation within and between populations. They attributed this genetic variation to several factors, including mutations, multiple origins of the clones and outcrossing.

The high allelic heterogeneity (Table 3) may be ascribed to the following factors (see reviews by Hamrick *et al.* (1979) and Loveless and Hamrick (1984)):

(1) Selfing promotes divergence. Although no new genes are incorporated into the gene pool, those that are there are effectively fixed within the clones. Evidence for selfing in *Siphonochilus* comes from the interesting review of the reproductive biology of *S. aethiopicus* by Gordon-Gray *et al.* (1989).

(2) Limited dispersal promotes divergence. Gordon-Gray *et al.* (1989) found that germination occurred *in situ*, with the seedling emerging through the fruit wall. Our own observations also suggest that fruit are borne below ground level, so that dispersal seems unlikely in *S. aethiopicus*. It is interesting to consider why local domination by one or a few clones failed to occur in the *S. aethiopicus* population. Burdon (1980) ascribed this phenomenon (in *Trifolium repens* L.) to microevolution and competitive interactions in a temporally and spatially variable environment.

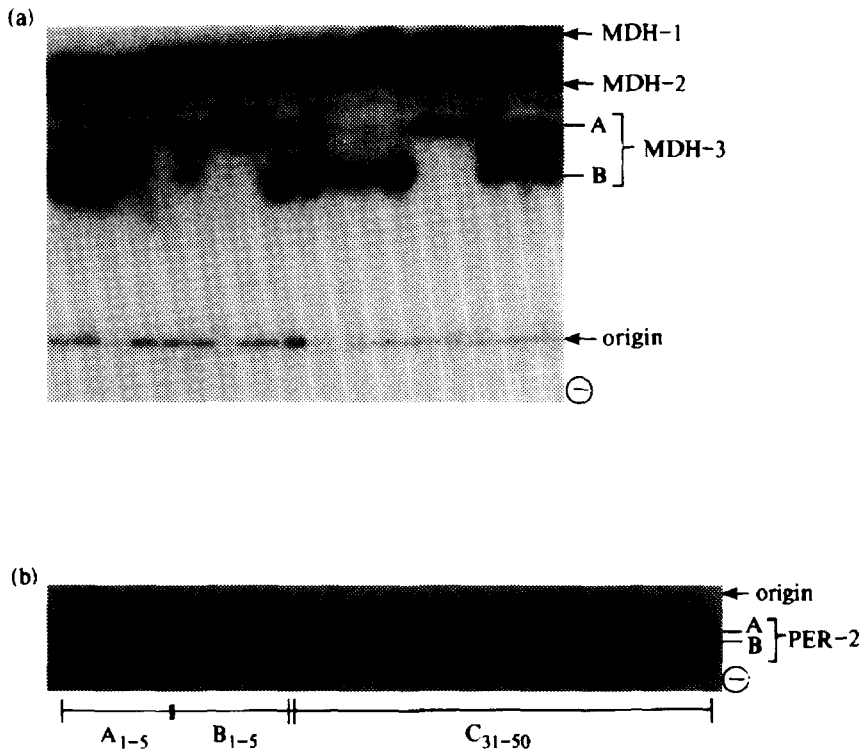

FIG. 1. ZYMOGRAMS OF (a) THE MALATE DEHYDROGENASE ENZYME CODING LOCUS SHOWING POLYMORPHISM AT THE THIRD LOCUS FOR WILD GINGER (INDIVIDUALS NOS 2–19) AND (b) THE PEROXIDASE-2 ENZYME CODING LOCUS SHOWING THE SCARCE (B) ALLELE IN THE WILD GINGER POPULATION (C). A and B are clonal individuals from Kirstenbosch and Pietersburg, respectively.

TABLE 3. CLONAL POLYMORPHISM IN *SIPHONOCHILUS AETHIOPICUS*: ALLOZYME COMBINATIONS (GENOTYPES) OF 12 CULTIVATED (A_1–A_6, B_1–B_6) AND 50 WILD PLANTS (C_1–C_{50}) AT 12 POLYMORPHIC ENZYME CODING LOCI

	Locus											
	ACP	AAT-1	CK	EST	GPI-2	LAP	MDH-1	MDH-3	MNR-1	MNR-2	PER-1	PER-2
A_1–A_6	AA	BB	AA	AA	BB	AA	AA	AB	BB	BB	AA	BB
B_1–B_6	AA	BB	AA	AA	BB	AA	AA	AB	BB	BB	AA	BB
C1[1]	BB	BB	??	AA	BB	AA	AA	BB	AB	BB	AB	AA
C2	BB	BB	BB	AA	BB	AA	AA	AB	AB	BB	AA	AB
C3	BB	BB	BB	AA	BB	AA	AA	BB	BB	BB	AB	AA
C4	BB	BB	AB	AA	BB	AA	AA	BB	AA	BB	AB	AB
C5[3]	BB	BB	AB	AA	AA	AA	AA	AB	BB	BB	AB	AA
C6	BB	BB	AB	AA	BB	AA	AA	AA	AB	BB	AA	AA
C7	BB	BB	AA	AA	BB	AA	AA	AB	AB	BB	AB	AA
C8	BB	BB	AB	AA	AA	AA	AA	AA	BB	AA	??	BB
C9	BB	BB	AB	BB	BB	AB	AA	AA	AB	BB	AB	AA
C10	BB	BB	AB	AA	BB	AA	AA	BB	AB	BB	BB	AA
C11	BB	BB	BB	AA	BB	AA	AA	BB	AA	AA	AA	AA
C12	BB	BB	AA	AA	BB	AA	AA	BB	BB	BB	BB	AA
C13[1]	BB	BB	??	AA	BB	AA	AA	BB	AB	??	??	AA
C14	BB	BB	??	AA	BB	AA	AA	BB	BB	BB	AB	BB
C15	BB	BB	??	AA	AA	AA	AA	AA	BB	AA	BB	AA
C16	BB	BB	??	AA	BB	AB	AA	AA	AB	BC	AB	AA
C17	BB	BB	AB	AA	BB	AA	AA	AB	AB	AA	AA	AA
C18	BB	BB	AA	AA	AA	AB	AA	AB	BB	AA	AB	AB
C19	BB	BB	AB	AA	BB	AA	AA	AB	BB	AA	AB	AA
C20	BB	BB	AA	AA	AA	AB	AA	AB	BB	BB	BB	AB
C21	BB	BB	AB	AA	BB	AA	AA	BB	AB	AA	AA	AA
C22	BB	BB	AA	AA	AA	AA	AA	AA	AA	AA	AB	AA
C23	BB	BB	AB	AA	BB	AA	AA	AA	BB	AA	AA	AA
C24	BB	BB	BB	AA	BB	AA	AA	AA	AB	AA	AA	AA
C25	BB	BB	AB	AA	AB	AA	AA	AB	AB	BB	AA	AA
C26	BB	BB	AB	AA	BB	AA	AA	AB	AB	AA	AA	AA
C27[2]	BB	BB	AA	AA	AA	AA	AA	AB	AB	BB	AA	AA
C28	BB	BB	AB	AA	BB	AA	AA	BB	BB	CC	AA	AA
C29[1]	BB	BB	AB	AA	BB	AA	AA	BB	AB	BB	AB	AA
C30	BB	BB	AA	AA	BB	AA	AA	BB	AB	AA	AB	BB
C31	BB	BB	??	??	AA	AA	AB	AB	??	BB	AB	AB
C32	BB	AA	??	??	AA	AA	AB	AB	??	BB	AB	AA
C33	BB	AA	??	??	AA	AA	AB	AA	??	BB	AA	AB
C34[2]	BB	BB	??	AA	AA	AA	AA	AB	??	BB	??	AA
C35	BB	BB	??	BB	AA	AB	AB	AA	??	BB	??	AA
C36	BB	BB	??	AA	AA	AA	AB	AB	??	BB	AA	AA
C37[4]	BB	BB	??	BB	AA	AB	AA	AA	??	BB	AB	AA
C38	BB	BB	??	AB	AA	AA	AA	AA	AB	BB	AA	AA
C39	BB	BB	??	AA	AA	AB	AA	BB	AA	BB	??	AA
C40	BB	AA	??	AB	AA	AA	AA	AB	AB	BB	AA	BB
C41	BB	BB	??	AB	AA	AA	AA	AB	BB	BB	BB	AA
C42	BB	BB	??	AB	AA	AB	AB	BB	BB	BB	??	AA
C43	BB	BB	??	BB	AA	AB	AA	AB	BB	BB	BB	AA
C44	BB	AB	??	BB	AA	AA	AA	AA	??	BB	AA	AA
C45	BB	BB	??	??	BB	AB	AA	AB	AB	BB	AB	AA
C46[4]	BB	BB	??	BB	AA	AB	AA	AA	BB	BB	AB	AA
C47	BB	BB	??	??	AA	AA	AA	AA	BB	BB	AA	AA
C48[3]	BB	BB	??	??	AA	AA	AA	AB	BB	BB	AB	AA
C49	BB	AB	??	BB	BB	AA	AA	BB	AB	BB	AA	AA
C50	BB	BB	??	BB	BB	AA	AA	AA	AA	BB	??	AA

?? = missing data, superscript numbers = similar genotypes.

(3) Facultative apomixis leads to moderate divergence. The breeding system of *Siphonochilus* was studied by Gordon-Gray *et al.* (1989). They found no evidence of apomixis but confirmed that *S. aethiopicus* produces two types of flowers (hermaphrodite and female); the latter do not form viable seeds, hence their contribution to gene transfer is nil. The possibility of a temporal change from a female to a dioecious reproductive mode should also be considered, as it would result in higher diversity.

The degree of genetic diversity found within this wild population may rule out the possibility of vegetative propagation alone and shows that sexual reproduction must have contributed to the genetic structure of the population. This study provides a good basis for future genetic studies of *Siphonochilus*, especially for estimating the amount and pattern of genetic variation within the species. Our data shows that genetic fingerprinting of clones is possible. It may be useful, for example, to determine the exact origins of the material used by Gordon-Gray *et al.* (1989). The fact that samples A and B (the 12 cultivated specimens, see Table 3) had identical alleles has led us to believe that sample B most likely originated from sample A (the Kirstenbosch tissue culture material) or vice versa. These cultivated clones possessed the unique and rare alleles at two protein coding loci (ACP and PER-2, respectively) indicating that they originated from a different source than the wild population. However from a conservation point of view it would be advisable to preserve individuals with dominant alleles, as individuals with rare alleles may not have the capacity to adapt to changing environmental conditions.

Since this is the first account of electrophoretic variants in the family Zingiberaceae, no information was available to draw comparisons or to evaluate the taxonomic value of the data. Nevertheless, our study provides a good basis for future work in this economically important family. Genetic data, combined with a better understanding of microspatial dynamics and pollination (degree of outcrossing) may help explain the remarkably high allelic heterogeneity in *S. aethiopicus*.

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