Genet Resour Crop Evol (2007) 54:947–958
DOI 10.1007/s10722-006-9155-8

RESEARCH ARTICLE

Analysis of genetic diversity in the endangered tropical tree species *Hagenia abyssinica* using ISSR markers

Tileye Feyissa · Hilde Nybom · Igor V. Bartish · Margareta Welander

Received: 29 November 2005 / Accepted: 3 May 2006 / Published online: 9 February 2007

Abstract Genetic diversity within and among 12 populations of the dioecious tropical tree species *Hagenia abyssinica* (Bruce) J.F. Gmel. in Ethiopia was examined with eight inter simple sequence repeat (ISSR) primers. A total of 104 clearly scorable bands were generated, among which 84 (81%) were polymorphic. Jaccard similarity coefficient was calculated for pairwise comparisons among all 120 individuals and ranged from 0.30 to 0.88 while average within-population similarity ranged from 0.53 to 0.66. Within-population variability was estimated as percentage polymorphic loci (ranging from 52% to 87%), Shannon's information index (0.30–0.50) and Nei's genetic diversity (0.21–0.35). The highest variability values were obtained for one recently planted population and for one wild population growing in an undisturbed primary forest area. Significant overall differentiation among populations was detected by both Shannon's information index (0.26) and G_{ST} (0.25). Relatedness among samples was estimated with a principal coordinate analysis, and relatedness among populations was estimated with a cluster analysis (UPGMA). A Mantel test indicated a significant association between genetic and geographic distances, and an autocorrelation analysis showed significant evidence of gene flow over distances up to 30 km. This study is the first of its kind for *H. abyssinica*, which has decreased recently in Ethiopia and now must be regarded as an endangered species. Both within-population and between-population diversity estimates are typical of outcrossing, longlived and late successional species, suggesting that recent anthropogenic disturbances have not yet had much impact on population genetic parameters. DNA marker data can, however, be used to identify the most suitable sites for *in situ* conservation and for collection of material for establishment of genebanks and plant improvement programs.

Keywords DNA marker · Genetic diversity · *Hagenia abyssinica* · ISSR · Kosso

T. Feyissa
Department of Biology, Faculty of Science, Addis Ababa University, 1176, Addis Ababa, Ethiopia

H. Nybom
Balsgård – Department of Crop Science, Swedish University of Agricultural Sciences, Fjälkestadsvägen 459, SE-291 94 Kristianstad, Sweden

I. V. Bartish
Department of Phanerogamic Botany, Swedish Museum of Natural History, 50007, SE-104 05 Stockholm, Sweden

M. Welander · T. Feyissa (✉)
Department of Crop Science, Swedish University of Agricultural Sciences, 44, SE-230 53 Alnarp, Sweden
e-mail: Tileye.Feyissa@vv.slu.se

Introduction

Hagenia abyssinica belongs to a monotypic genus in the family Rosaceae. This dioecious tree species is known by its common name 'kosso' in Ethiopia. It was once abundant in the semi-humid mountain woodlands of Ethiopia from 2450 to 3250 m a.s.l. (Hedberg 1989). Currently, it is however sparsely distributed in mountainous areas in Central, South-western and South-eastern parts of Ethiopia (Negash 1995). The species is also found in Kenya, Tanzania, Uganda, Sudan, Democratic Republic of Congo, Malawi, Burundi and Rwanda. Being restricted to the mountains of tropical Africa, it is a typical example of Afromontane endemism (Friis 1992). It is reported to be wind-pollinated (Negash 1995) and the seeds are dispersed by wind.

Historically, *H. abyssinica* has been one of the most famous plants of Africa since it was used by Merck of Germany already in 1870 for the production of kosins (phloroglucinols), a crystalline substance derived from the female flowers. The species thus became incorporated in the European Pharmacopoeia (Lounasmaa et al. 1973) and several papers have since reported on the medical aspects of kosins which have e.g. antitumour activity (Abegaz et al. 1999; Lounasmaa et al. 1974; Woldemariam et al. 1992). Although no longer marketed in Europe, *H. abyssinica* is still widely used in Ethiopia for the treatment of tapeworm. This species is also extremely valuable for formation and preservation of fertile soil, and for rainwater conservation. Recently, it has become one of the most endangered tree species of East Africa due mainly to the high demand for its timber. Micropropagation (Feyissa et al. 2005a) and in vitro regeneration (Feyissa et al. 2005b) protocols of *H. abyssinica* have been developed but no reports have as yet been published on genetic diversity of the species.

Several DNA marker systems have been developed to measure genetic diversity in plant species. Widely used PCR-based marker systems are random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSRs) or microsatellites (Gupta and Varshney 2000; Staub et al. 1996). However, the sometimes low reproducibility of RAPD and high cost of AFLP, and the need to develop species-specific primers for SSR analysis are major limitations, all of which are overcome by the inter simple sequence repeats (ISSR) technique (Gupta et al. 1994; Reddy et al. 2002; Zietkiewicz et al. 1994). ISSR is a PCR-based technique that involves amplification of a DNA segment between two inversely oriented identical microsatellite repeat regions (Reddy et al. 2002). In plants, dinucleotide repeats are most prevalent (Wang et al. 1994) and often used in the primers (Moreno et al. 1998) which are usually also anchored at the 3′ or 5′ end with 1–4 degenerate bases (Zietkiewicz et al. 1994). Longer primers (16–25 mer) permit the use of high annealing temperatures (45–60°C), which is probably the reason for the improved reproducibility of ISSR compared to RAPD where 10-mer primers are used (Reddy et al. 2002).

Conservation and improvement of the ecologically and economically valuable *H. abyssinica* would have significant effects for sustainable agriculture in the highlands of East Africa. The objective of this study was to analyse genetic diversity for conservation and genetic improvement of this species.

Materials and methods

Plant material

A total of 120 leaf samples from 12 populations were collected from southwestern, central and southeastern parts of Ethiopia (Fig. 1). Each population was represented by 10 individual trees (Table 1). Two populations, Sigmo and Gedo, consist of trees planted about 15 years ago within a reforestation programme. The other populations grow in natural montane rain forests and therefore mainly contain older trees.

DNA isolation

About 1.0 g of young leaves were harvested from the tip of new shoots on the stem or branches of the trees and dried with silica gel (Chase and Hills 1991). Total genomic DNA was isolated from

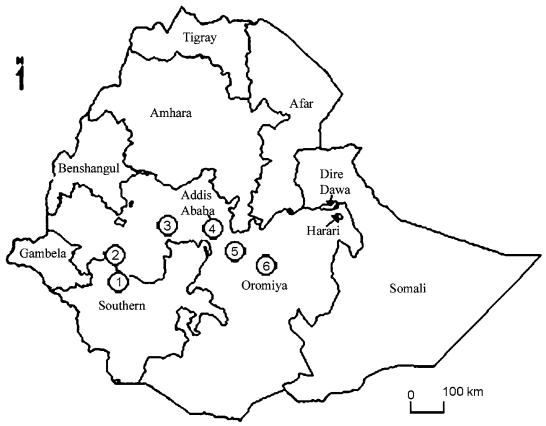

Fig. 1 Map of Ethiopia showing the sampled populations of *Hagenia abyssinica*. 1 = Bonga, 2 = Sigmo, 3 = Gedo and Jibat, 4 = Menagesha and Intoto, 5 = Chilalo and Kersa, 6 = Adaba, Dinsho-1, Dinsho-2 and Goba

silica gel-dried leaves using the CTAB method (Wang et al. 1996) with minor modifications. Quality of the DNA was checked by electrophoresis of the samples on 1% agarose gel and staining with ethidium bromide. DNA concentration was determined by spectrophotometry.

PCR amplification and electrophoresis

Fifteen primers (Invitrogen) were screened on individuals of different populations and regions, and eight primers that produced clear and polymorphic band pattern were selected for further study (Table 2).

DNA amplification was carried out in a total volume of 25 μl containing 10 ng sample DNA, 1× reaction buffer (75 mM Tris–HCl pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20), 2 mM $MgCl_2$, 0.2 mM of each dNTP (Amersham Biosciences), 2% formamide, 0.198 μM primer, 0.04 U/μl Taq polymerase (Sigma). The water used in the reaction contained 0.1% (v/v) diethyl pyrocarbonate (DEPC) before autoclaving. Amplification was performed using a Thermal Cycler GeneAmp® PCR System 9700, version 3.01 (Applied Biosystems) under the following temperature profiles: an initial denaturation step of 1 min at 94°C, followed by 40 cycles of 1 min at 94°C (denaturation) followed by 2 min at 55°C (annealing) at a ramp rate of 0.5°C s^{-1} and then 30 s at 72°C (extension) at a ramp rate of 1.3°C, this ramp rate was also used to rise to 94°C. The last cycle was followed by a final extension step of 5 min at 72°C.

The PCR products were electrophoresed on polyacrylamide gel (CleanGel 48S, ETC Electrophorese-Technik). The gel was rehydrated for 1 h face down on rehydration buffer (ETC Electrophorese-Technik). Loading buffer (5 μl) was added to each PCR product and 6 μl of the mix was loaded in each slot of the gel. The loading buffer consisted of 20% (w/v) sucrose, 10% (w/v) Ficoll, 0.05% (w/v) bromophenol blue, 5 M urea

Table 1 Description of *H. abyssinica* samples collected from different sites in Ethiopia

No.	Population	Zone/ Province	Altitude (m)	Position	Description
1	Bonga	Kaffa	2438	N7°18′, E36°22′	About 24 km south east of Bonga town near Baqa village
2	Sigmo	Jimma	2494	N7°49′, E36°07′	About 15 km south east of Sigmo town
3	Gedo	West Shewa	2376	N9°02′, E37°26′	4 km north west of Gedo town
4	Jibat	West Shewa	2653	N8°55′, E37°31′	About 3 km south east of the road from Gedo town to Ambo town
5	Menagesha	West Shewa	2460	N9°00′, E38°35′	About 35 km south west of Addis Ababa
6	Intoto	Addis Ababa	2900	N9°06′, E38°47′	About 12 km north of Addis Ababa on Intoto Mountain
7	Chilalo	Arsi	3067	N7°56′, E39°12′	7 km east of Assela town on Chilalo Mountain
8	Kersa	Arsi	2850	N7°30′, E38°59′	About 3 km south west of Kersa town
9	Adaba	Bale	3062	N7°02′, E39°34′	19 km south east of Adaba town
10	Dinsho-1	Bale	3047	N7°06′, E39°49′	On the south side of Dinsho town
11	Dinsho-2	Bale	3066	N7°08′, E39°45′	About 2 km north of Dinsho town in Dinsho Park
12	Goba	Bale	3298	N6°56′, E39°57′	2 km south of Goba town

Table 2 Primers used in ISSR analysis, number of loci scored, number of polymorphic loci and size range of the bands

Primer		Total no. of loci	No. of polymorphic loci	Size range
Code	Sequence 5′–3′			
811	GAG AGA GAG AGA GAG AC	11	8	300–1350
825	ACA CAC ACA CAC ACA CT	15	15	325–2200
827	ACA CAC ACA CAC ACA CG	15	14	450–2200
834[a]	AGA GAG AGA GAG AGA GYT	15	11	225–3000
868	GAA GAA GAA GAA GAA GAA	17	10	250–>3000
878	GGA TGG ATG GAT GGA T	10	9	350–>3000
880	GGA GAG GAG AGG AGA	10	7	225–>3000
890[b]	VHV GTG TGT GTG TGT GT	11	10	325–1700
Total		104	84	–
Mean		13	10.5	–

[a] Y = Pyrimidine (C or T)

[b] V = Non-T (i.e. A, C or G), H = Non-G (i.e. A, C or T)

and 1 mM EDTA. A 100 bp ladder was loaded in left and right border slots of the gel. Electrode strips (ETC Electrophorese-Technik) were soaked in 25 ml of electrode buffer (ETC Electrophorese-Technik) and placed on the edges of the gel at anodal and cathodal ends. The electrophoresis was performed on a horizontal Multiphor II Electrophoresis unit (LKB Bromma) at 450 V and 25 mA current for 25 min and then at 50 mA current of same voltage. When the electrophoresis dye reached the strip at the anodal end, the gel was taken off and the PCR products were visualized by silver staining using a Hoefer Automated Gel Stainer (Pharmacia Biotech).

Data scoring and analysis

Each ISSR band was considered as an independent locus and polymorphic bands were scored as absent (0) or present (1) for all the 120 individual samples. Only clearly reproducible bands were scored and differences in band intensity were not considered. Data analyses were conducted using only the polymorphic bands. A pair-wise genetic similarity matrix was generated using Jaccard similarity coefficient. A principal coordinate analysis was performed based on similarity for qualitative data (SIMQUAL) for all individuals and a plot was generated using NTSYS-PC version 2.1 (Rohlf 2000).

Estimations of within- and between-population variation were obtained with POPGENE software version 1.31 (Yeh et al. 1999) assuming Hardy–Weinberg equilibrium since *H. abyssinica* is an obligately outbreeding species. For estimation of within-population variation, we calculated percentage polymorphic loci, Nei's (1973) gene diversity (H_S), average Jaccard similarity coefficient and Shannon's information index (H_{pop}). These parameters were then compared using Pearson correlation analysis.

For estimation of between-population genetic variation, total genetic diversity (H_T), mean within-population genetic diversity (H_S), among populations genetic diversity (D_{ST}), and the coefficient of genetic differentiation (G_{ST}) were calculated using the expressions $H_T = H_S + D_{ST}$, and $G_{ST} = D_{ST}/H_T$ (Nei 1977). We also calculated Shannon-index based genetic diversity for the species (H_{sp}), mean within- population genetic diversity (H_{pop}), proportion of genetic variation within populations (H_{pop}/H_{sp}), and proportion of genetic variation between populations (H_{sp}–H_{pop})/H_{sp} (King and Schaal 1989).

Similarities among the 12 populations were quantified with the Jaccard similarity coefficient and visualized using a cluster analysis (unweighted pair-group method with arithmetic averages, UPGMA) and illustrated in a phenogram using NTSYS-PC version 2.1.

A matrix of geographical distances among populations was obtained by the use of http://www.jan.ucc.nau.edu/~cvm/latlongdist.html and compared with the corresponding matrix of Jaccard similarity coefficients to investigate the possible association between genetic and geographic distances (Mantel test, MXCOMP in NTSYS, 1000 permutations). To further examine the associations between genetic and geographic distances at different distance intervals, a modified version of spatial autocorrelation analysis (Oden and Sokal 1986) was conducted following

Rohlf (2000), as implemented in Bartish et al. (2000). To carry out the analysis, we first produced a series of binary matrices corresponding to different geographic distance classes. In each of these matrices, we coded comparisons between pairs of populations within a certain distance class as 0, and all other pairwise comparisons as 1. We set distance classes to span about 43 km so that 10 distance classes of approximately equal length could be created (the largest distance between populations is 434 km). We then computed an autocorrelation coefficient (R_a) for each of the distance classes as a Pearson correlation coefficient, by comparing a binary matrix from each distance class to the matrix of Jaccard coefficients between populations. A random set of binary matrices (1000 permutations) was created for each distance class to obtain a random distribution of autocorrelation coefficients, which could allow us to test for significance of our values of association between genetic and geographic distances. All computations in the autocorrelation analyses were performed by ACORMAN program (created by Mark Bartish in collaboration with Igor V. Bartish). The program is available from the third author upon request.

Results

The eight ISSR primers produced a total of 104 bands that could be unambiguously scored. Of these bands, 84 (81%) were polymorphic in the 120 *H. abyssinica* samples although some bands were polymorphic only in certain populations. The total number of scored bands varied from 10 for primers 878 and 880 to 17 for primer 868 with a mean of 13 bands per primer (Table 2). The number of polymorphic bands ranged from 7 for primer 880 to 15 for primer 825 with a mean of 10.5 bands per primer, and the size of the bands ranged from 225 to more than 3000 (Fig. 2).

Within-population variation

Number of polymorphic bands within populations varied from 52% for Adaba to 87% for Gedo, with a mean of 73% (Table 3). Adaba also had the lowest values for Shannon index-based genetic diversity, 0.30, and Nei's genetic diversity, 0.21. Bonga and Sigmo had the highest Shannon index value, 0.50, and the highest Nei's genetic diversity, 0.35. Pearson correlation analyses showed that all three parameters yielded closely correlated estimates of within-population variation, $r = 0.918$–0.990, $P < 0.001$. Jaccard similarity index values were, as expected, negatively correlated with the other three parameters, $r = -0.732$ to -0.882, $P < 0.01$.

Variation among populations

Calculations of total genetic diversity (H_T), average within-population diversity (H_S), among-population diversity (D_{ST}), and coefficient of

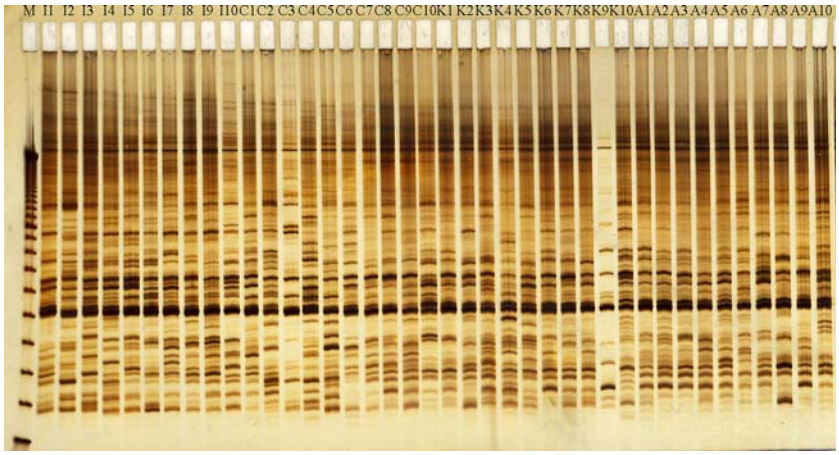

Fig. 2 ISSR band patterns of *Hagenia abyssinica* from Intoto (I1–I10), Chilalo (C1–C10), Kersa (K1–K10) and Adaba (A1–A10) using primer 834. M is a molecular size marker

Table 3 Measures of genetic diversity in the 12 populations of *Hagenia abyssinica* assuming Hardy–Weinberg equilibrium

Population	Polymorphic loci		Jaccard's coefficient	Shannon's index	Gene diversity
	Number	Percent		*I**	*H**
Bonga	68	81	0.59	0.50	0.35
Sigmo	72	86	0.56	0.50	0.35
Gedo	73	87	0.53	0.48	0.32
Jibat	60	71	0.61	0.40	0.28
Menagesha	64	76	0.60	0.44	0.30
Intoto	64	76	0.61	0.45	0.31
Chilalo	67	80	0.59	0.46	0.31
Kersa	62	74	0.64	0.43	0.30
Adaba	44	52	0.66	0.30	0.21
Dinsho-1	52	62	0.65	0.38	0.26
Dinsho-2	54	64	0.65	0.41	0.29
Goba	55	65	0.61	0.41	0.28
Mean	61	73	0.61	0.43	0.30

among-population differentiation (G_{ST}) yielded 0.40, 0.30, 0.10 and 0.25, respectively. Corresponding analyses of Shannon's information index for mean genetic variation for all the 120 individual samples (H_{sp}), average within-population diversity (H_{pop}), proportion of genetic variation within populations (H_{pop}/H_{sp}), and proportion of genetic variation between populations (H_{sp}–H_{pop})/H_{sp} yielded 0.58, 0.43, 0.74 and 0.26, respectively. Interestingly, the two parameters for estimation of population differentiation, i.e. G_{ST} and (H_{sp}–H_{pop})/H_{sp}, yielded highly similar values; 0.25 and 0.26, respectively.

Jaccard similarity coefficient-based pairwise comparisons of the 12 populations showed that Dinsho-1 and Adaba are the closest with 0.72, whereas Chilalo and Sigmo as well as Menagesha and Gedo are the most distant populations with a similarity coefficient of 0.50 (Table 4).

Principal coordinate analysis

Jaccard similarity coefficients for pairwise comparisons among all the 120 individuals ranged from 0.30 between the most distant genotypes C-7 from Chilalo and M-4 from Menagesha to 0.88 between the most similar genotypes A-3 and A-5, both from Adaba. A principal coordinate analysis based on the Jaccard values was undertaken; the first principal coordinate accounted for 6.6% of the variation, the second for 5.1% and the third for 4.1% (Fig. 3).

Although there was considerable overlapping between samples from different populations and regions, some patterns could still be discerned. Samples from the southwestern and central-western populations were mostly found in the upper and right side of the diagram, samples from the southeastern populations in the upper and left

Table 4 Pairwise Jaccard similarity coefficient-based comparisons among 12 populations of *Hagenia abyssinica*

	1	2	3	4	5	6	7	8	9	10	11	12
Bonga	–											
Sigmo	0.580	–										
Gedo	0.521	0.532	–									
Jibat	0.607	0.580	0.529	–								
Menagesha	0.580	0.559	0.502	0.609	–							
Intoto	0.597	0.540	0.512	0.606	0.588	–						
Chilalo	0.528	0.502	0.510	0.562	0.544	0.560	–					
Kersa	0.569	0.566	0.519	0.623	0.597	0.616	0.618	–				
Adaba	0.595	0.563	0.527	0.620	0.590	0.622	0.629	0.700	–			
Dinsho-1	0.594	0.573	0.544	0.628	0.607	0.625	0.601	0.666	0.715	–		
Dinsho-2	0.637	0.589	0.546	0.632	0.620	0.651	0.604	0.641	0.668	0.682	–	
Goba	0.598	0.590	0.550	0.623	0.635	0.638	0.586	0.644	0.667	0.665	0.684	–

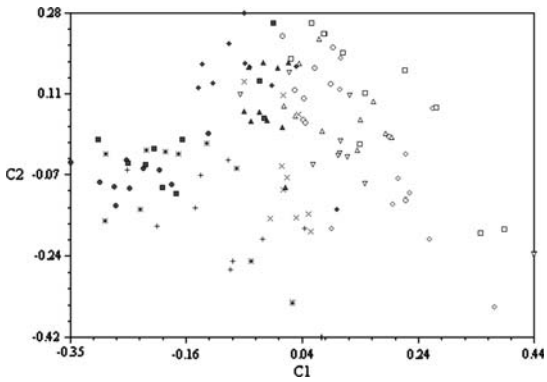

Fig. 3 Principal coordinate analysis of 120 individual samples of *Hagenia abyssinica* investigated with ISSR: open circle = Bonga, solid circle = Adaba, open square = Sigmo, solid square = Dinsho-1, open diamond = Gedo, solid diamond = Dinsho-2, open triangle pointing upwards = Jibat, solid triangle pointing upwards = Goba, open triangle pointing downwards = Menagesha, cross = Intoto, plus = Chilalo, star = Kersa

side of the plot, and samples from the central populations in the center of the plot. Differentiation was found also within these groups. Looking at the southwestern and central-western populations, we find Bonga and Jibat samples mainly in the upper part while samples from Sigmo and Gedo occurred along the whole right side of the plot. Looking at the southeastern populations, we find Dinsho-2 in the upper left part of the plot, Goba in the upper and central parts, Dinsho-2 stretching from the top downwards along the left side, and Adaba and Kersa in the left-most part. Finally, among the central populations, we find Intoto in the center of the plot, Menagesha in the central-right part and Chilalo in the central-left part. This plot clearly suggests that there is a relationship between geographical locations and the ISSR data.

Cluster analysis

A UPGMA-based cluster analysis of the 12 sampled populations showed that Kersa, Adaba, Dinsho-1, Dinsho-2 and Goba formed a tight cluster, thus grouping all four populations from the Bale province together with one from neighbouring Arsi (Fig. 4). Although much more loosely affiliated, another four populations also clustered with this inner group, namely Intoto (in Addis Ababa), Jibat and Menagesha (both in West Shewa) and Bonga (in Kaffa). The Gedo population (West Shewa) is genetically the most distant from all others, followed by Sigmo (Jimma) and Chilalo (Arsi). Interestingly, Gedo and Sigmo are much younger than the remainder and were planted about 15 years ago using unknown source material.

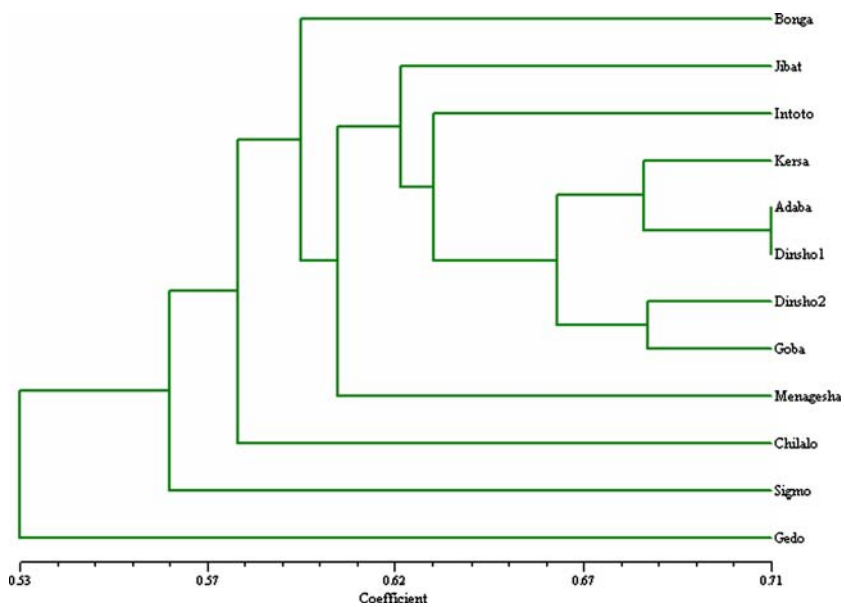

Fig. 4 ISSR-based UPGMA dendrogram for 12 populations of *Hagenia abyssinica*

Mantel test and autocorrelation analysis

A significant overall association was found between geographic and genetic distances among populations, $r = -0.375$, $P < 0.01$ for all the 12 populaitons and $r = -0.547$, $P < 0.01$ when the populations of uncertain origin (Sigmo and Gedo) were excluded, indicating that genetic similarity generally decreases over increasing geographic distances between the sampled populations. In the spatial autocorrelation analysis, the geographic distances were divided into 10 different distance classes for the 12 populations (Table 5). In the first of these, with pairwise geographic distances of 8–31 km, we found a significant negative deviation and in the second, 44–83 km, R_a was marginally significant, suggesting that over these distances, populations have above-average similarity. A marginally significant positive deviation of the R_a value from a random distribution was detected also in one of the large-distance classes, 308–347 km, indicating that populations have below-average similarity and that their differentiation may have been non-random also at a larger geographic scale. R_a was significant only in the first class (up to 44 km) among the nine distance classes of the 10 natural populations (Table 6). We decreased the number of the distance classes because of a low number of values to compare after the two populations were excluded.

Discussion

Has *Hagenia abyssinica* suffered from anthropogenic disturbance?

Molecular marker-based studies of genetic diversity in plant species have become important tools in plant breeding, genecology and conservation (Nybom and Bartish 2000; Weising et al. 2005). Generally, genetic diversity of a species is strongly associated with phyletic group, life form, geographic range, regional distribution, breeding system, seed dispersal mechanism, mode of reproduction and successional status (Bhat et al. 1999; Hamrick and Godt 1989). Of these parameters, breeding system appears to be the most important factor, followed (in no particular order) by life form, seed dispersal and successional status (Nybom 2004).

The present study reports genetic diversity parameters for the tropical tree species *H. abyssinica* for the first time. ISSR-based analysis revealed values for within-population diversity (mean $H_S = 0.30$) that are somewhat higher than the overall value of 0.22 for a set of four ISSR-based studies compiled in Nybom (2004). In this and other compilations (Weising et al. 2005), ISSR-based estimates have been shown to be very similar to estimates based on AFLP (average for 13 studies = 0.23) or RAPD (average for 60 studies = 0.22). For 60 RAPD-based studies

Table 5 Autocorrelation analysis of genetic versus geographic distances between *Hagenia abyssinica* populations

Dist. Class (km)	0–31	44–83	94–126	137–173	179–215	220–251	273–302	308–347	355–382	391–434
R_a	–0.357	–0.233	–0.123	0.178	0.117	0.045	–0.024	0.236	0.008	0.075
0.05*	0.24	0.246	0.25	0.238	0.24	0.235	0.237	0.235	0.235	0.229
–0.05	–0.24	–0.246	–0.25	–0.238	–0.24	–0.235	–0.237	–0.235	–0.235	–0.229

* Lines denoted 0.05 and –0.05 report the critical values for a positive (0.05) or a negative correlation (–0.05) at the $P < 0.05$ level

Table 6 Autocorrelation analysis of genetic versus geographic distances of 10 natural populations of *Hagenia abyssinica*

Dist. Class in km	8–44	54–94	102–141	173–179	197–226	243–275	302–327	340–347	391–434
R_a	–0.519	–0.238	0.110	0.037	0.156	–0.101	0.207	0.253	0.251
P	0.297	0.264	0.303	0.278	0.276	0.298	0.323	0.271	0.314

Critical values of probability (P) of random autocorrelation coefficients (both positive and negative) are presented at 0.05 level

compiled in Nybom (2004), values similar to the ISSR-based values of 0.30 for *H. abyssinica* were encountered for long-lived perennials (0.25) that are outcrossed (0.27), dispersed by wind or water (0.27) and belong to late successional species (0.30)—all of which is typical for *H. abyssinica*.

For DNA marker-based between-population differentiation, the most commonly reported parameters in the literature are F_{ST} (usually calculated using AMOVA) and G_{ST} which generally produce highly similar values (Nybom 2004), and Shannon's index which produces values that vary more (in either direction) from the other two (Nybom et al. 2001). In our study, *H. abyssinica* populations had almost the same value for G_{ST} (0.25) and Shannon's index (0.26). In a compilation of ISSR-based studies, 9 studies had an average F_{ST} value of 0.35 and 6 studies had an average G_{ST} value of 0.34 (Nybom 2004). These values are quite similar to overall values obtained in studies where RAPD was used instead (0.34 and 0.27, respectively), therefore suggesting that ISSR and RAPD produce comparable results (Nybom 2004; Weising et al. 2005). When compared to RAPD-based G_{ST} and F_{ST} values, the presently obtained G_{ST} (0.25) is most similar to values obtained for longlived perennials (0.19 and 0.25) that are outcrossing (0.22 and 0.27), wind or water-dispersed (0.17 and 0.25) and late successional (0.22 and 0.23).

To summarize the population genetic analysis of *H. abyssinica*, both within- and between-population diversity appears to be quite typical of a perennial, outcrossing, wind-dispersed and late successional species. Since *H. abyssinica* grows only in mountain areas of tropical Africa, at a narrow altitudinal range of 2450–3250 m a.s.l (Hedberg 1989) in areas suffering from anthropogenic disturbance, a reduction in genetic diversity could have been expected. According to Semagn et al. (2001), among three altitudinal groups of *Phytolacca dodecandra*, the highland group (above 2500 m) showed significantly less diversity than the lowland (1600–2100 m) and the central-highland (2101–2500 m) populations.

Obviously, however, neither the restricted habitat nor the recent decrease in tree numbers due to logging activities appear to have caused any major reduction in within-population genetic diversity of *H. abyssinica* or an increase in population differentiation. In keeping with our results, a review on neotropical tree species report of no significant consequences from habitat loss and degradation in most of the cited studies (Lowe et al. 2005) and remarkable resilience has been shown also for tropical tree species in Africa (Allaye Kelly et al. 2004). Studies that instead assess progeny inbreeding, reproductive output and fitness usually, however, show adverse effects of anthropogenic disturbance (Lowe et al. 2005). The reason for this discrepancy is that although inbreeding may have been initiated, genetic diversity is lost slowly over the generations. Therefore, the pre-existing genetic structure is still visible when the time lapse between disturbance and genetic analysis is short compared to the generation span of the species.

Is within-population diversity associated with anthropogenic activities?

The various parameters used in our study for estimating within-population diversity in *H. abyssinica* yielded closely correlated results. Three populations stand out as being overall more variable than the others. Two of these variable populations, Sigmo and Gedo, were planted only 15 years ago using unknown source material although it would probably have been obtained from nearby populations. Presumably the material used for planting was more variable than the trees growing in a typical wild population. The third variable population, Bonga, is geographically rather close to Sigmo but it grows in what appears to be the best preserved forest habitat when compared to all other sampled populations in our study.

The lowest level of genetic within-population diversity was exhibited by Adaba followed by Dinsho-1, Dinsho-2 and Goba, all of which are geographically very close in the province of Bale. Of these, Dinsho-1, Dinsho-2 and Goba populations appeared to be less disturbed as compared to the other populations (save Bonga). Lower within-population diversity values were reported also for forest *Coffea arabica* populations when the Bale region was compared to populations collected from other parts of Ethiopia (Aga et al. 2003). The forest coffee from this region was

considered to be part of a primary forest ecosystem and the authors suggested that the low genetic diversity could be due to a very narrow genetic base, aggravated by the fact that *C. arabica* reproduces mostly by selfing.

By contrast, Semagn et al. (2001) reported that populations of the outcrossing *Phytolacca dodecandra* collected from well-preserved forest areas in Ethiopia showed more variation than those collected from degraded areas. Studies on wild populations of *Ensete ventricosum* from southwestern Ethiopia showed that samples from dense and natural forest areas revealed higher genetic diversity than those from more disturbed areas (Birmeta et al. 2004). Effects of agroforestry procedures of course depend on e.g. the origination of plant material when new populations are planted. Thus five planted stands of the Amazonian tree species *Inga edulis* showed less genetic diversity than five natural stands (Hollingsworth et al. 2005).

The high rate of deforestation in Ethiopia during the last century has caused an alarmingly rapid decline in forest coverage (Eshetu and Högberg 2000; Taddese 2001). This is true particularly for *H. abyssinica*, which is under severe pressure of destruction in Ethiopia mainly due to the high demand for timber. In some of the collection sites, many trees appear to have been cut down recently without replacement, resulting in populations that consist of a few scattered trees only. In our study of *H. abyssinica*, we could however not find a clear relationship between level of within-population diversity and present-day degree of human-induced habitat disturbance. Possibly other environmental factors like growth habitat and microclimate have been more important for shaping the differences in variability in these longlived forest trees. It was also interesting to notice that contrary to expectations, the newly planted populations actually harbored more variability than almost any of the natural populations. In keeping with our results, Allaye Kelly et al. (2004) report that extensive agroforestry activities in Mali has not yet affected population structure of the tree species *Vitellaria paradoxa*, probably due to the buffering effect of extensive gene flow between unmanaged and managed populations.

In order to preserve this endangered, yet economically and ecologically valuable tree species, both *in situ* and *ex situ* conservation should be undertaken. For *ex situ* conservation, live plants need to be preserved since the small, light and fleshy seeds of *H. abyssinica* appear to loose their viability relatively quickly. For collection of plant material, particular attention should be given to the Bonga population, as it grows in undisturbed primary forest and, at the same time, showed the highest within-population variation.

Is between-population variability associated with gene flow and isolation-by-distance?

Gene flow caused by the movement of pollen and seeds is highly associated with the development of genetic structure within and between populations. DNA-marker loci appear to be particularly well suited for investigation of isolation-by-distance, a concent which was developed on the assumption of neutrality of markers (Slatkin 1993). Application of spatial autocorrelation analysis allows us to define over what distances similarities and dissimilarities are most prominent. For *H. abyssinica*, a strong and highly-significant negative relationship was indicated at distances up to around 30 km. This result suggests that gene flow among populations of this species played an important role at this relatively short-range scale, but differentiation among more distant populations was to a large extent spatially independent.

Populations collected in the southeastern highlands of the country (Kersa, Adaba, Dinsho-1, Dinsho-2, Goba) grouped closely together both in the dendrogram and in the PCO. When compared pairwise using the Jaccard similarity index, this group of southeastern populations exhibited values that were higher than for any other comparison, except for one between Dinsho-2 and Intoto. Interestingly, these populations were also characterized by the lowest within-population diversity. A negative relationship between within-population diversity and between-population variability has been noted both with allozymes (Hamrick and Godt 1989) and RAPD (Nybom and Bartish 2000). Here, however, variability is low both within and between the southeastern populations.

Populations collected in the southwestern and central-western parts of the country (Bonga, Sigmo, Gedo, Jibat) grouped loosely together in the PCO but showed no similarities in the dendrogram. Probably the high within-population variation in these populations made them less amenable to cluster analysis and therefore they occurred as outliers.

Finally, three populations collected in the central part of the country (Menagesha, Intoto, Chilalo) took intermediate positions in the PCO and clustered loosely with the group from the southeastern area. They also showed intermediate levels of within-population diversity.

The obviously spatially-independent differentiation of populations at distances above 30 km, revealed by our study, suggests that gene flow is sparse between most of the *H. abyssinica* populations in Ethiopia. Gene flow is, however, likely to decrease even further because of genetic isolation of the extant populations due to the increasing range fragmentation brought about by current logging practices. Due to small sample sizes, the presently available data do not allow us to draw any strong conclusions regarding the observed genetic structure and our results must be regarded as preliminary. Still, there is no reason to expect our estimates to be biased in one or another direction since previous DNA marker data compilations have shown that sample size is not correlated with within-population genetic diversity (Nybom and Bartish 2000; Nybom 2004). These findings should be taken into account when conservation management policies for the species is developed.

Acknowledgements This work was supported by BIOEARN (East African Regional Programme and Research Network for Biotechnology, Biosafety and Biotechnology Policy Development) funded by SIDA/SAREC, Sweden. The authors acknowledge Department of Biology, Addis Ababa University for the help during sample collections from the field.

References

Abegaz BM, Ngadjui BT, Bezabih M, Mdee LK (1999) Novel natural products from marketed plants of eastern and southern Africa. Pure Appl Chem 71:919–926

Aga E, Bryngelsson T, Bekele E, Salomon B (2003) Genetic diversity of forest arabica coffee trees (*Coffea arabica* L.) in Ethiopia as revealed by random amplified polymorphic DNA (RAPD) analysis. Hereditas 138:36–46

Allaye Kelly B, Hardy OJ, Bouvet J-M (2004) Temporal and spatial genetic structure in *Vitellaria paradoxa* (shea tree) in an agroforestry system in southern Mali. Mol Ecol 13:1231–1240

Bartish IV, Jeppsson N, Bartish GI, Lu R, Nybom H (2000) Inter- and intraspecific genetic variation in *Hippophae* (Elaeagnaceae) investigated by RAPD markers. Plant Syst Evol 225:85–101

Bhat KV, Babrekar PP, Lakhanpaul S (1999) Study of genetic diversity in Indian and exotic sesame (*Sesamum indicum* L.) germplasm using random amplified polymorphic DNA (RAPD) markers. Eupytica 110:21–33

Birmeta G, Nybom H, Bekele E (2004) Distinction between wild and cultivated enset (*Ensete ventricosum*) gene pools in Ethiopia using RAPD markers. Hereditas 140:139–148

Chase MW, Hills HH (1991) Silica gel: an ideal material for field preservation of leaf samples for DNA studies. Taxon 40:215–220

Eshetu Z, Högberg P (2000) Reconstruction of forest site history in Ethiopian highlands based on ^{13}C natural abundance of soils. Ambio 29:83–89

Feyissa T, Welander M, Negash L (2005a) Micropropagation of *Hagenia abyssinica*: a multipurpose tree. Plant Cell Tissue Organ Cult 80:119–127

Feyissa T, Welander M, Negash L (2005b) In vitro regeneration of *Hagenia abyssinica* (Bruce) JF Gmel. (Rosaceae) from leaf explants. Plant Cell Rep 24:392–400

Friis I (1992) Forest and forest trees of northeast tropical Africa. Their natural habitats and distribution patterns in Ethiopia, Djibouti and Somalia. Her Majesty's Stationary Office, London

Gupta M, Chyi Y-S, Romero-Severson J, Owen JL (1994) Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theor Appl Genet 89:998–1006

Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113:163–185

Hamrick JL, Godt MJW (1989) Allozyme diversity in plant species. In: Brown HD, Clegg MT, Kahler AL, Weir BS (eds) Plant population genetics, breeding, and genetic resources. Sinauer Associates Inc., Sunderland, pp 43–63

Hedberg O (1989) Flora of Ethiopia, Pittosporaceae to Araliaceae. In: Hedberg I, Edwards S (eds) Rosaceae, vol. 3. Addis Ababa University, Addis Ababa, pp 1–659

Hollingsworth PM, Dawson IK, Goodall-Copestake WP, Richardson JE, Weber JC, Sotelo Montes C, Pennington T (2005) Do farmers reduce genetic diversity when they domesticate tropical trees? A case study from Amazonia. Mol Ecol 14:497–501

King LM, Schaal BA (1989) Ribosomal-DNA variation and distribution in *Rudbeckia missouriensis*. Evolution 43:1117–1119

Lounasmaa M, Widen C-J, Huhtikangas A (1973) Phloroglucinol derivatives of *Hagenia abyssinica*. Phytochemistry 12:2017–2025

Lounasmaa M, Widen C-J, Huhtikangas A (1974) Phloroglucinol derivatives of *Hagenia abyssinica* II. The structure determination of kosotoxin and protokosin. Acta Chem Scand B 28:1200–1208

Lowe AJ, Boshier D, Ward M, Bacles CFE, Navarro C (2005) Genetic resource impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. Heredity 95:255–273

Moreno S, Martin JP, Ortiz JM (1998) Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm. Euphytica 101:117–125

Negash L (1995) Indigenous trees of Ethiopia: Biology, uses and propagation techniques. SLU Reprocentralen, Umeå

Nei M (1973) Analysis of genetic diversity in subdivided populations (population structure/genetic variability/heterozygosity/gene differentiation). Proc Natl Acad Sci USA 70:3321–3323

Nei M (1977) F-statistics and analysis of gene diversity in subdivided populations. Ann Hum Genet 41:225–233

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13:1143–1155

Nybom H, Bartish IV (2000) Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Persp Plant Ecol Evol Syst 3:93–114

Nybom H, Werlemark G, Olsson ÅME (2001) Between- and within population diversity in dogrose species. Acta Hort 546:139–144

Oden NL, Sokal RR (1986) Directional autocorrelation: an extension of spatial correlograms to two dimensions. Syst Zool 35: 608–617

Reddy MP, Sarla N, Siddiq EA (2002) Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128:9–17

Rohlf FJ (2000) NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System, Version 2.1. Exeter Software, New York

Semagn K, Stedje B, Björnstad A (2001) Analysis of genetic diversity and structure in Ethiopean populations of *Phytolacca dodecandra* using RAPD. Hereditas 135:51–60

Slatkin M (1993) Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264–279

Staub JE, Serquen FC, Gupta M (1996) Genetic markers, map construction, and their application in plant breeding. HortScience 31:729–739

Taddese G (2001) Land degradation: a challenge to Ethiopia. Env Manag 27:815–824

Wang M, Oppedijk BJ, Lu X, Duijn BV, Schilperoort RA (1996) Apoptosis in barley aleurone during germination and its inhibition by abscisic acid. Plant Mol Biol 32: 1125–1134

Wang Z, Weber JL, Zhong G, Tanksley SD (1994) Survey of plant short tandem DNA repeats. Theor Appl Genet 88:1–6

Weising K, Nybom H, Wolf K, Kahl G (2005) DNA fingerprinting in plants: principles, methods and applications. CRC Press, Boca Raton USA

Woldemariam TZ, Fell AF, Linley PA, Bibby MC, Phillips RM (1992) Evaluation of the anti-tumour action and acute toxicity of kosins from *Hagenia abyssinica*. J Pharm Biomed Anal 10:555–560

Yeh FC, Yang RC, Boyle TJ, Ye ZH, Mao JX (1999) POPGENE 1.31, The user friendly Shareware for population genetic analysis. Molecular Biology and Biotechnology Centre University of Alberta, Edmonton

Zietkiewicz E, Rafalski A, Labuda D (1994) Genomic fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176–183