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RESEARCH ARTICLE

Genetic diversity and structure of Ethiopian, Yemen and Brazilian *Coffea arabica* L. accessions using microsatellites markers

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Abstract Genetic diversity among 115 coffee accessions from the *Coffea* Germplasm Collection of IAC was assessed using SSR markers. The germplasm represents 73 accessions of *Coffea arabica* derived from spontaneous and subspontaneous plants in Ethiopia and Eritrea, species center of origin and diversity, 13 commercial cultivars of *C. arabica* developed by the Breeding Program of IAC, 1 accession of *C. arabica* cv. 'Geisha', 13 accessions of *C. arabica* from Yemen, 5 accessions of *C. eugenioides*, 4 accessions of *C. racemosa* and 6 accessions of *C. canephora*. Genetic analysis was performed using average number of alleles per locus (A), proportion of polymorphic loci (P), Shannon's genetic index (H'; and G'_{ST}) and clustering analysis. All evaluated species were distinguished by a cluster analysis based on Jaccard's coefficient. Differentiation between the cultivated plants of *C. arabica* and accessions derived from spontaneous and subspontaneous plants was observed. Spontaneous and subspontaneous accessions from Ethiopia were separated according to the geographical origin: east and west of the Great Rift Valley. Cultivated plants showed a low genetic diversity with a division in two groups: accessions from Yemen ($\underline{H'}$=0,028) and Brazilian commercial cultivars ($\underline{H'}$=0,030). The results agreed with previously reported narrow genetic basis of cultivated plants of *C. arabica* and supported the hypotheses about domestication of the species. This study also showed a significant genetic diversity among accessions from Ethiopia and Eritrea present in the Germplasm Collection of IAC. This diversity is specially observed in accessions from Sidamo ($\underline{H'}$=0,143), Kaffa ($\underline{H'}$=0,142) and Illubabor ($\underline{H'}$=0,147) indicating their importance as source of genetic variability for coffee breeding programs.

Keywords *Coffea arabica* · Domestication · Germplasm · Molecular markers

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Introduction

Although the genus *Coffea* contains approximately 80 taxa (Bridson and Verdcourt, 1988), only two species are commercially exploited,

Coffea arabica L. and *Coffea canephora* Pierre ex Froehner. *C. arabica* is an autogamous species and the only allotetraploid ($2n = 4x = 44$) of the genus while the other species are diploids ($2n = 2x = 22$) and generally self-incompatible (Krug and Carvalho, 1951). *C. arabica* is native of the highlands of Ethiopia (Sylvain, 1955), but there are also records of wild arabica coffee in the Boma Plateau of Sudan (Thomas, 1942) and Mount Marsabit of Kenya (Berthaud and Charrier, 1988).

The arabica coffee cultivation started in Arabia, specifically in Yemen, five centuries ago. In the early 18th century, progenies from a single Indonesia plant cultivated in Europe were spread out to South America and turned out to be the genetic basis of main cultivars of Brazil and other countries (Chevalier and Dagron, 1928; Carvalho, 1945).

In view of this narrow genetic basis and the necessity of genetic resources conservation, FAO organized in 1964–65 a mission to collect spontaneous and subspontaneous coffee germplasm in probable native regions of the species (FAO, 1968). This initiative resulted in 621 samples of seeds from various collecting sites in Ethiopia and the Republic of Eritrea which were carefully documented and sent to six institutions in India, Tanzania, Ethiopia, Costa Rica, Peru and Portugal for further research (FAO, 1968).

The accessions represented in the Coffee Germplasm Collection of Instituto Agronômico de Campinas (IAC) include plant material originated from 308 of those seed sample, obtained from plants of the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) at Turrialba, Costa Rica, collected in the years 1973 and 1987. There is also a substantial number of cultivated coffee germplasm in the IAC's collection. Besides this, several samples of plants from Yemen were introduced by A.B. Eskes, a researcher from IRCC, Montpellier, after a detailed morphologic and agronomic characterization (Eskes and Mukred, 1990).

Since their introduction at IAC, all those materials are under continuous evaluation regarding botanic, agronomic, and technologic aspects (Carvalho et al., 1983; Mazzafera et al., 1990; Silvarolla et al., 2000). However, despite all these studies, the degree of genetic diversity and structure of the IAC collection is poorly understood. In order to fulfill this analysis, molecular markers could help reveal the structure of genetic diversity, which is an important information for setting up a core collection and to improve the conservation, accessibility and use of genetic resources of this collection (Hamon et al., 1999).

Previous studies have already evaluated the genetic diversity of some Ethiopian and FAO accessions through morphologic and agronomic characteristics (Montagnon and Bouharmont, 1996), RAPD markers (Lashermes et al., 1996; Anthony et al., 2001; Chaparro et al., 2004; Aga et al., 2003) and ISSR markers (Aga et al., 2005). However, in these studies only few accessions from Yemen were analyzed (Montagnon and Bouharmont, 1996; Lashermes et al., 1996; Anthony et al., 2002) and were reported different results regarding grouping and genetic structure of those accessions collected in different regions from Ethiopia and Yemen. Chaparro et al. (2004) did not found an association between grouping and the site of origin in Ethiopia. Montagnon and Bouharmont (1996) showed that there is a separation between coffee trees growing east and west of the Great Rift Valley, with accessions from south–eastern and southern of Ethiopia being grouped together with cultivated plants from Yemen. Anthony et al. (2001) and Aga et al. (2003, 2005) also distinguished eastern and western groups in accessions from Ethiopia, but with a low genetic differentiation between them and cultivated plants arising as a group apart (Anthony et al., 2001).

Microsatellites markers (SSR) have shown good efficiency to assess genetic diversity and relationships among coffee trees (Moncada and McCouch 2004; Maluf et al., 2005). Thus, we thought SSR could be an alternative tool to elucidate the questions about genetic structure of *C. arabica* as well to characterize diversity levels of genetic resources included in the *Coffea* Germplasm Collection of IAC. Furthermore, this is the first study analyzing a representative sample of both materials collected from the center of the origin and diversity of the *C. arabica* species and cultivated plants from Yemen and Brazil through microsatellites markers.

Material and methods

Plant material

The evaluated plant material consisted of 115 coffee accessions from the *Coffea* Germplasm Collection of IAC. These included 73 accessions of *C. arabica* L. derived from spontaneous and subspontaneous trees from various regions of Ethiopia and Eritrea (Fig. 1), 13 accessions of *C. arabica* from Yemen, 1 accession of *C. arabica* cv. 'Geisha' (Table 1), and 13 commercial cultivars of *C. arabica* developed by the IAC Breeding Program (Table 2). Also, 5 accessions of *C. eugenioides* S. Moore, 4 accessions of *C. racemosa* Lour. and 6 accessions of *Coffea canephora* Pierre ex Froehner were included as outgroup species due to their importance in the evolutionary history of coffee and as source of target genes for breeding programs. Sampling of spontaneous and subspontaneous accessions from Ethiopia and Eritrea was representative of total accession number at the Germplasm Collection of IAC.

Genomic DNA extraction

Total genomic DNA was extracted from frozen young leaves according to Stewart Jr. and Via (1993), using CTAB as detergent. All DNA samples were diluted to a final concentration of 20 ng/μl.

SSR amplification

Primer sequences to amplify SSR locus were obtained from Combes et al. (2000) and Rovelli et al. (2000) (Table 3). Final reaction conditions were 40 ng of genomic DNA, 1.5× reaction buffer, 0.1 mmol l^{-1} of dNTP, 2 mmol l^{-1} of $MgCl_2$, 5 ρmol of each primer and 1.5 U of *Taq* DNA polymerase. The complete thermal cycle program was 5 min at 95°C, followed by 30 cycles of 1 min at 95°C, 1 min at 60°C and 1 min at 72°C, and a final 5 min of elongation time at 72°C.

Primers were fluorescently labelled and amplified products were separated on a 5% acrylamide gel using an ABI 377 automated sequencer

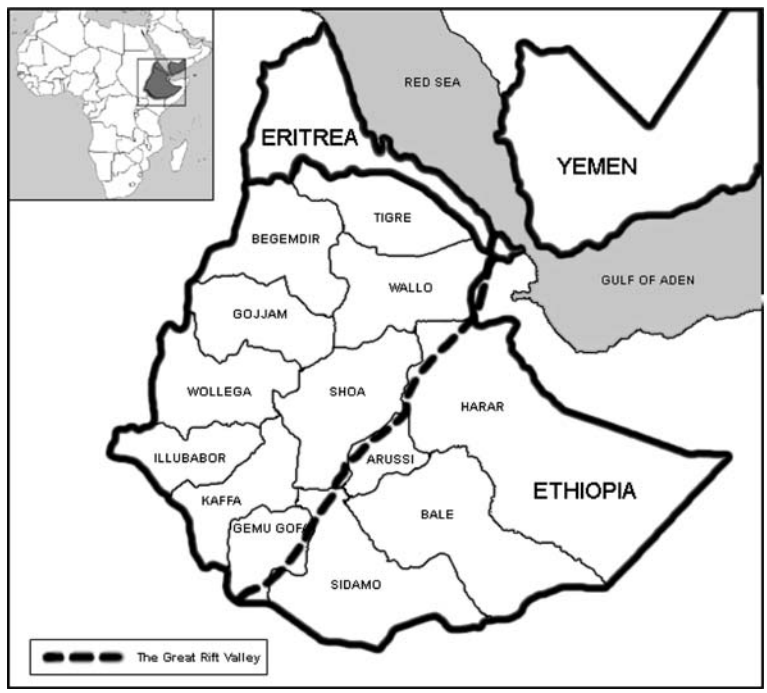


Fig. 1 Collecting sites of *Coffea arabica* accessions evaluated. Ethiopia political organization is according to FAO (1968), except by separation of Eritrea

Table 1 List of *Coffea arabica* accessions from the *Coffea* Germplasm Collection of IAC analyzed through SSR markers. ''E'' identifies material collected in Ethiopia and Eritrea by FAO and ''T'' identifies material from the CATIE's collection. Spontaneous (spont) and subspontaneous-derived (sub) material originated from single plant, random or representative sample according to FAO (1968)

Origin	Sample Code	Accession	Collection number	Type	Origin	Sample Code	Accession	Collection number	Type
Harar	HA1	IAC 2026	E12/T4950	Random sample sub	Kaffa	KA23	IAC 3929	E336/T4594	Single sub
	HA2	IAC 2025	E7/T4472	Random sample sub		KA24	IAC 3930	E345/T4603	Single sub
Sidamo	SI1	IAC 2029	E18/T4474	Random sample sub		KA25	IAC 3948	E142/T4663	Single spont
	SI2	IAC 2102	E237/T4758	Representative sample sub		KA26	IAC 3949	E143/t4664	Single spont
	SI3	IAC 2032	E22/T4476	Random sample sub		KA27	IAC 3952	E468/T4691	Single sub
	SI4	IAC 2030	E19/T4475	Random sample sub		KA28	IAC 3950	E144/T4665	Single spont
	SI5	IAC 2031	E21/T4953	Random sample sub		KA29	IAC 3954	E188/T4693	Two plants sub
	SI6	IAC 2028	E17/T4473	Random sample sub		KA30	IAC 3955	E311/T4699	Single sub
Shoa	SH1	IAC 2027	E16/T4951	Random sample sub		KA31	IAC 3959	E385/T4784	Single sub
	SH2	IAC 2035	E36/T4500	Random sample sub		KA32	IAC 3960	E388/T4787	Single sub
	SH3	IAC 2036	E37/T4501	Random sample sub		KA33	IAC 3965	E396/T4803	Single sub
Eritrea	ER	IAC 2207	E579/T4945	Random sample sub		KA34	IAC 3968	E411/T4811	Single sub
Gojjam	GO1	IAC 2205	E571/T4939	Single sub		KA35	IAC 3972	E418/T4818	Single sub
	GO2	IAC 2202	E558/T4926	Single sub	Illubabor	IL1	IAC 3986	E453/T4853	Single spont
	GO3	IAC 2206	E572/T4940	Single sub		IL2	IAC 3985	E448/T4848	Single spont
	GO4	IAC 2204	E565/T4933	Single sub		IL3	IAC 3984	E446/T4846	Single spont
	GO5	IAC 2203	E563/T4931	Single sub		IL4	IAC 3983	E445/T4845	Single spont
	GO6	IAC 4008	E559/T4927	Single sub		IL5	IAC 3981	E438/T4838	Single spont
	GO7	IAC 4009	E560/T4928	Single sub		IL7	IAC 3979	E436/T4836	Single spont
	GO8	IAC 4010	E561/T4929	Single sub		IL8	IAC 3976	E425/T4825	Single spont
	GO9	IAC 4011	E562/T4930	Single sub		IL9	IAC 3975	E424/T4824	Single spont
	GO11	IAC 4013	E578/T4944	Single sub		IL10	IAC 3843	E431/T4831	Single spont
	GO12	IAC 4015	E577/T4959	Single sub		IL11	IAC 3849	E465/T4865	Single spont
Kaffa	KA1	IAC 3814	E134/T4568	Random sample sub		IL12	IAC 3934	E358/T4631	Single sub
	KA2	IAC 3818	E328/T4586	Single sub		IL13	IAC 3935	E361/T4634	Single sub
	KA3	IAC 3832	E226/T4749	Single sub		IL14	IAC 3936	E366/T4639	Single sub
	KA4	IAC 3856	E531/T4900	Single sub		IL15	IAC 3937	E367/T4640	Single sub
	KA5	IAC 3838	E380/T4795	Single sub		IL16	IAC 3938	E369/T4642	Single sub
	KA6	IAC 3995	E522/T4889	Single sub		IL17	IAC 3940	E373/T4646	Single sub
	KA7	IAC 3998	E536/T4905	Single sub					
	KA8	IAC 3999	E539/T4908	Single spont	Geisha (cv)	GE	IAC 2210	T 4305	Cultivated from Malawi
	KA9	IAC 4000	E542/T4911	Single sub	Yemen	YE1	IAC 4113		Cultivated - Local type
	KA10	IAC 4001	E543/T4912	Single sub		YE2	IAC 4114		Cultivated - Local type
	KA11	IAC 4002	E547/T4916	Single sub		YE4	IAC 4116		Cultivated - Tessawi
	KA12	IAC 3804	E307/T4485	Single sub		YE5	IAC 4117		Cultivated - Tessawi
	KA13	IAC 3839	E412/T4812	Single sub		YE6	IAC 4118		Cultivated - Essaii
	KA14	IAC 3913	E495/T4533	Single sub		YE8	IAC 4120		Cultivated - Essaii
	KA15	IAC 3915	E300/T4546	Single sub		YE10	IAC 4122		Cultivated - Tessawi
	KA16	IAC 3916	E474/T4547	Single sub		YE11	IAC 4123		Cultivated - Odaynii
	KA17	IAC 3917	E475/T4548	Single sub		YE12	IAC 4124		Cultivated - Local variant
	KA18	IAC 3919	E480/T4553	Single sub		YE13	IAC 4125		Cultivated - Local variant
	KA19	IAC 3920	E488/T4560	Single sub		YE14	IAC 4126		Cultivated - Tessawi
	KA20	IAC 3921	E491/T4564	Single sub		YE15	IAC 4127		Cultivated - Katii
	KA22	IAC 3926	E150/T4573	Single sub		YE16	IAC 4128		Cultivated - Katii

(Applied Biosystems). Products were detected by the applicative Gene Scan (Applied Biosystems) using internal molecular size markers (GENESCAN-500 ROX), and analyzed by the applicative Genotyper v 2.0 (Applied Biosystems).

Table 2 List of commercial *Coffea arabica* cultivars developed by IAC evaluated through SSR markers

Cultivar	Sample code	Origin
Acaiá IAC 474-4	AC474-4	Bourbon Vermelho × Sumatra (Typica)
Mundo Novo IAC 388-17	MN388-17	Bourbon Vermelho × Sumatra (Typica)
Bourbon Amarelo IAC J19	BA	Bourbon Vermelho (mutation)
Catuaí Vermelho IAC 81	CV81	Caturra Amarelo 476 × Mundo Novo 374-19
Catuaí Vermelho IAC 144	CV144	Caturra Amarelo 476 × Mundo Novo 374-19
Catuaí Amarelo IAC 100	CA100	Caturra Amarelo 476 × Mundo Novo 374-19
Icatu Vermelho IAC 4042	IV4042	(*C. canephora* cv. Robusta × Bourbon Vermelho) × Mundo Novo
Icatu Vermelho IAC 4045	IV4045	(*C. canephora* cv. Robusta × Bourbon Vermelho) × Mundo Novo
Icatu Vermelho IAC 4046	IV4046	(*C. canephora* cv. Robusta × Bourbon Vermelho) × Mundo Novo
Icatu Amarelo IAC 2944	IA	Icatu Vermelho × Bourbon Amarelo
Ouro Verde IAC H5010-5	OV	Catuaí Amarelo × Mundo Novo 515
Obatã IAC 1669-20	OB	(Villa Sarchi × Hybrid of Timor) × Catuaí Vermelho
Tupi IAC 1669-33	TP	(Villa Sarchi × Hybrid of Timor) × Catuaí Vermelho

Table 3 Access number of microsatellite loci in the ''Genbank'', locus code and respective forward (F) and reverse (R) primer sequences

Acession number	Locus code	Sequence of primers (5 '–3')	Repeated sequence
AJ308738	4-1CTG	F= AAAAAGCTGGTCCATGTCAA	$(TG)_8$
	(SSR5)	R= GGGGCGTTCAGTTATAAACA	
AJ308746	17-2CTG	F= AGGCCTTCATCTCAAAAACC	$(TC)_{14}/(CA)_{11}/(CA)_{16}$
	(SSR6)	R= AGCGTTACTTGAGGCAAAGA	
AJ308762	E6-3CTG	F= CTGGGTTGGTTCTGATTTTG	$(TG)_{16}$
	(SSR7)	R= GGTTCCCAGAGATTCTCTCC	
AJ308767	E12-3CTG	F= TGCTTAGGCACTTGATATAGGA	$(CA + TA)_{38}$
	(SSR9)	R= CACGTGCAAGTCACATACTTTA	
AJ308785	I9-3CTG	F= TGGCCGTGATAATAAACAGC	$(TG)_{21}$
	(SSR11)	R= ATGTGGCAATCTAAAGCCAA	
AJ250258	M32	F= AACTCTCCATTCCCGCATTC	$(CA)_3/(CA)_3/(CA)_{18}$
	(SSR14)	R= CTGGGTTTTCTGTGTTCTCG	
–	C2-2CATC	F= CTCTCCCTCAGTCAATTCCA	$(ATC)_{14}$
	(SSR15)	R= CTTGGTCTCCCTCCTTTTTC	
AJ308754	32-2CTG	F= AAGGGGAGTGGATAAGAAGG	$(CA)_{12}$
	(SSR17)	R= GGCTGGATTTGTGCTTTAAG	
AJ308764	E8-3CTG	F= CACTGGCATTAGAAAGCACC	$(CA)_{14}$
	(SSR18)	R= GGCAAAGTCAATGATGACTC	
AJ250251	M3	F= ATTCTCTCCCCCTCTCTGC	$(CA)_6/(CA)_3/(CA)_3/(CA)_3/$
	(SSR20)	R= TGTGTGCGCGTTTTCTTG	$(CA)_4/(CA)_3/(CA)_3/(CA)_3$
AJ250252	M11	F= ACCCGAAAGAAAGAACCAAG	$(GT)_4/(GA)_4/(GT)_4/(GT)_6$
	(SSR21)	R= CCACACAACTCTCCTCATTC	
AJ250253	M20	F= CTTGTTTGAGTCTGTCGCTG	$(GA)_5/(GT)_8/TT(GT)_4/TT$
	(SSR22)	R= TTTCCCTCCCAATGTCTGTA	$(GT)_7/(GA)_{11}/(TC)_2/(CT)_3GT$
AJ250255	M25	F= CCCTCCCTGCCAGAAGAAGC	$(GT)_5/CT(GT)_2/(GT)_{12}$
	(SSR24)	R= AACCACCGTCCTTTTCCTCG	
AJ250256	M27	F=AGGAGGGAGGTGTGGGTGAAG	$(GT)_{11}$
	(SSR25)	R= AGGGGAGTGGATAAGAAGG	
AJ250257	M29	F= GACCATTACATTTCACACAC	$(CTCACA)_4/(CA)_9$
	(SSR26)	R= GCATTTTGTTGCACACTGTA	
AJ250260	M47	F= TGATGGACAGGAGTTGATGG	$(CT)_9/(CA)_8/(CT)_4/(CA)_5$
	(SSR27)	R= TGCCAATCTACCTACCCCTT	

Data analysis

Gels were scored by presence or absence of bands or alleles. Due to the tetraploid condition of *C. arabica*, it is impossible to distinguish between diallelic duplex and simplex and among different types of triallelic combinations of SSR loci. Therefore, for each individual plant, fragment frequencies were analyzed as multilocus fingerprints, in which each allele was either scored present or absent.

Genetic diversity within groups of *C. arabica* and within diploids species was evaluated by average number of alleles per locus (A), proportion of polymorphic loci (P) and the Shannon's genetic index (H') (Bussel, 1999). Groups of *C. arabica* consisted of accessions of the same collecting region (Table 1) and commercial cultivars developed by IAC. Cultivar 'Geisha' was excluded of the genetic diversity analysis because it was represented by only one accession/group. Estimation of A was an exception to the multilocus fingerprints approach once amplified products of each pair of primers were considered as alleles of the same locus. P was calculated dividing the number of polymorphic bands by total number of amplified bands in each group. Shannon's genetic index for each marker was calculated for each group as:

$$H' = - \sum p_i * \log_2 p_i$$

where p_i is the frequency of the presence or absence of a band in that group.

Following the method of Bussel (1999), the partitioning of genetic variation within and between groups of *C. arabica* was estimated. The average diversity over all populations for each locus (H'_{pop}) and the total diversity in the 99 accessions of *C. arabica* for each locus (H'_{sp}) were calculated (for more details see Bussel, 1999). Then the component of diversity within populations (H'_{pop}/H'_{sp}) and the component between populations ($G'_{ST} = (H'_{sp}–H'_{pop})/H'_{sp}$) were estimated.

$\underline{H'}$, $\underline{H'}_{pop}$, $\underline{H'}_{sp}$ and $\underline{G'}_{ST}$ were the average values per locus calculated over all loci, including monomorphic ones, according to Bussel (1999). We categorized the partitioning of genetic diversity analysis according to the groups of accessions analyzed. These groups included all accessions (Analysis 1), all accessions without Eritrea group (Analysis 2), spontaneous and subspontaneous accessions (Analysis 3) and spontaneous and subspontaneous accessions without Eritrea group (Analysis 4). Eritrea group was excluded from Analysis 2 and 4 to improve understanding of species genetic structure. This was carried out because Eritrea group contains just one individual and this could lead to bias $\underline{G'}_{ST}$ values by decreasing $\underline{H'}_{pop}$.

Genetic distance among all accessions (including *C. arabica* cv. 'Geisha') was estimated as the complement of Jaccard's (1908) coefficient (Link et al., 1995). Genetic distances were also estimated using Dice coefficient (Dice, 1945), which is equivalent to Nei and Li (1979), in order to compare values with other studies. Cluster analysis was performed using the matrix distance based on the complement of Jaccard's coefficient employing the UPGMA method. Bootstrap analysis (Felsenstein, 1985) was performed to evaluate the tree topology reliability for 1,000 simulations using the software Treecon (Van de Peer and Watcher, 1994).

Results

The multilocus fingerprints approach was used to analyze the genetic diversity and structure of coffee accessions. In spite of this, specific results for each primers pairs were pointed out for characterization of individual SSR locus.

SSR locus characterization

The E12-3CTG (SSR9) locus showed a profile with 3 and 4 peaks even in diploid species such as *C. canephora* and *C. eugenioides*. The 4-1CTG (SSR5) locus was monomorphic (band of 97 bp) in *C. arabica* and *C. eugenioides* and did not amplify any fragment in *C. racemosa* and *C. canephora* species. Primers E6-3CTG, E8-3CTG and M3 also amplified monomorphic bands within *C. arabica* accessions (a total of 12 bands), and those were polymorphic among evaluated species.

Genetic diversity

Sixteen SSR primers pairs produced a total of 121 bands or alleles. All tested markers detected polymorphisms among the accessions evaluated, being 54 bands among *C. arabica* accessions and 15 bands among cultivated plants (Yemen, 'Geisha' and Brazilian cultivars). Also, 7 out of 54 polymorphic bands of *C. arabica*, were present at high frequencies (0.8 or more), 31 as rare alleles in low frequency (0.2 or less) and 16 with frequencies between 0.2 and 0.8.

Genetic diversity analysis showed the highest values of $\underline{H}'$ in diploid species (Table 4). Values for *A* in diploid species were similar or even lower than those observed in *C. arabica* groups, probably due to the allotetraploid nature of *C. arabica* which results in duplicate *A* values per plant. $\underline{H}'$ values were relatively high in accessions from Kaffa, Illubabor and Sidamo provinces, moderate in Gojjam and Harar, and very low in cultivated plants. The *P* index was higher in diploid species and within Kaffa and Illubabor groups. The discrepancy of $\underline{H}'$ relative to *A* and *P* values in Kaffa and Illubabor groups occurred due to the presence of rare alleles (Table 4).

Genetic distances ranged from 0 to 0.88 between all possible pairs of genotypes, from 0 to 0.37 among *C. arabica* accessions, from 0 to 0.30 among spontaneous and subspontaneous accessions from Ethiopia and Eritrea, and from 0 to 0.19 among cultivated accessions (Fig. 1). Genetic distances among *C. arabica* accessions were also calculated using only the polymorphic bands exclusive to *C. arabica* (44.6% of total fragments). Results showed that this distance ranged from 0 to 0.65 using Jaccard's coefficient or its complement and from 0 to 0.49 using Dice's coefficient.

Genetic structure

$\underline{G}'_{ST}$ values showed a strong genetic structure in all accessions of *C. arabica* (Table 5). A strong genetic structure was also observed in spontaneous and subspontaneous accessions from Ethiopian. Exclusion of group Eritrea, which contains just one individual, increased $\underline{H}'_{pop}$ values and decreased $\underline{G}'_{ST}$ values but yet results showed the strong genetic structure in the evaluated accessions (Table 5).

The hierarchical clustering analysis presented in Fig. 2 showed four major clusters comprising grouped accessions of each species. The analysis of species relationships showed *C. canephora* closer to *C. arabica*, followed by *C. eugenioides*. Also, *C. racemosa* was distantly related to *C. arabica*.

Grouping of *C. arabica* accessions (Fig. 2) revealed two main clusters. The first contains only cultivated plants, including the accessions of Yemen, cultivar 'Geisha' and commercial cultivars from Brazil. An exception was the accession of Sidamo (SI4) also included in this group with materials from Yemen. Yemen accessions of type Tessawi (Table 1) were distinguished from the others in a separated cluster. The second group encompassed basically all spontaneous and subspontaneous accessions from Ethiopia and Eritrea. Despite the low boostrap values (Fig. 2), there was a clear separation of these accessions in two large subgroups: the first included mainly

Table 4 Genetic diversity within species and groups of coffee species assessed by average number of alleles per locus (*A*), proportion of polymorphic loci (*P*) and Shannon's genetic index averaged over all loci ($\underline{H}'$)

Species	Group	*A*	*P* (%)	$\underline{H}'$
Coffea arabica	Harar (HA)	2.3	5.0	0.050
	Sidamo (SI)	2.7	16.5	0.143
	Shoa (SH)	2.5	12.4	0.115
	Eritrea (ER)	2.0	–	–
	Gojjam (GO)	2.5	13.2	0.087
	Kaffa (KA)	3.2	24.8	0.142
	Illubabor (IL)	3.1	24.0	0.147
	Yemen (YE)	2.2	5.0	0.028
	Commercial cultivars	2.0	4.1	0.030
Coffea eugenioides		2.5	29.8	0.242
Coffea canephora		2.8	24.6	0.197
Coffea racemosa		2.1	20.7	0.200

Table 5 Partitioning of genetic diversity generated by 121 SSR bands within and between groups of *Coffea arabica* accessions. $\underline{H'}_{pop}$, $\underline{H'}_{sp}$ and $\underline{G'}_{ST}$ are the average per locus values calculated over all loci

Groups analyzed	$\underline{H'}_{pop}$	$\underline{H'}_{sp}$	$\underline{G'}_{ST}$
(1) All accessions	0.082	0.210	0.577
(2) All accessions without Eritrea	0.093	0.210	0.526
(3) Spontaneous and subspontaneous accessions	0.098	0.179	0.464
(4) Spontaneous and subspontaneous accessions without Eritrea	0.114	0.175	0.349

accessions from Sidamo and the second subgroup included all other accessions from the west side of the Great Rift Valley.

Discussion

SSR locus characterization

In general, the amplification patterns for each SSR locus evaluated corresponded to those previously reported. The profile of E12-3CTG (SSR9) locus with 3 and 4 peaks in the diploid species *C. canephora* and *C. eugenioides* confirmed that this pair of primers amplified a number of independent loci (Rovelli et al., 2000). Considering this, E12-3CTG locus has not been used to estimate number of alleles per locus (*A*).

The locus 4-1CTG (SSR5) was monomorphic in *C. arabica* and *C. eugenioides* and did not amplify any fragment in *C. racemosa* and *C. canephora* species. According Rovelli et al. (2000) this locus showed diploid type segregation in *C. arabica*. Thus, it is probable that the amplification has just occurred in putative genome provided by *C. eugenioides* (Lashermes et al., 1999). However, in other analysis Poncet et al. (2004) using different primer sequences to amplify this same SSR locus identified a monomorphic band of 239 bp also in *C. canephora* and other diploid species, including *C. eugenioides.* This new primer pair amplified the total SSR sequence present at GENBANK, while Rovelli et al. (2000)'s primers amplified only part of the SSR sequence.

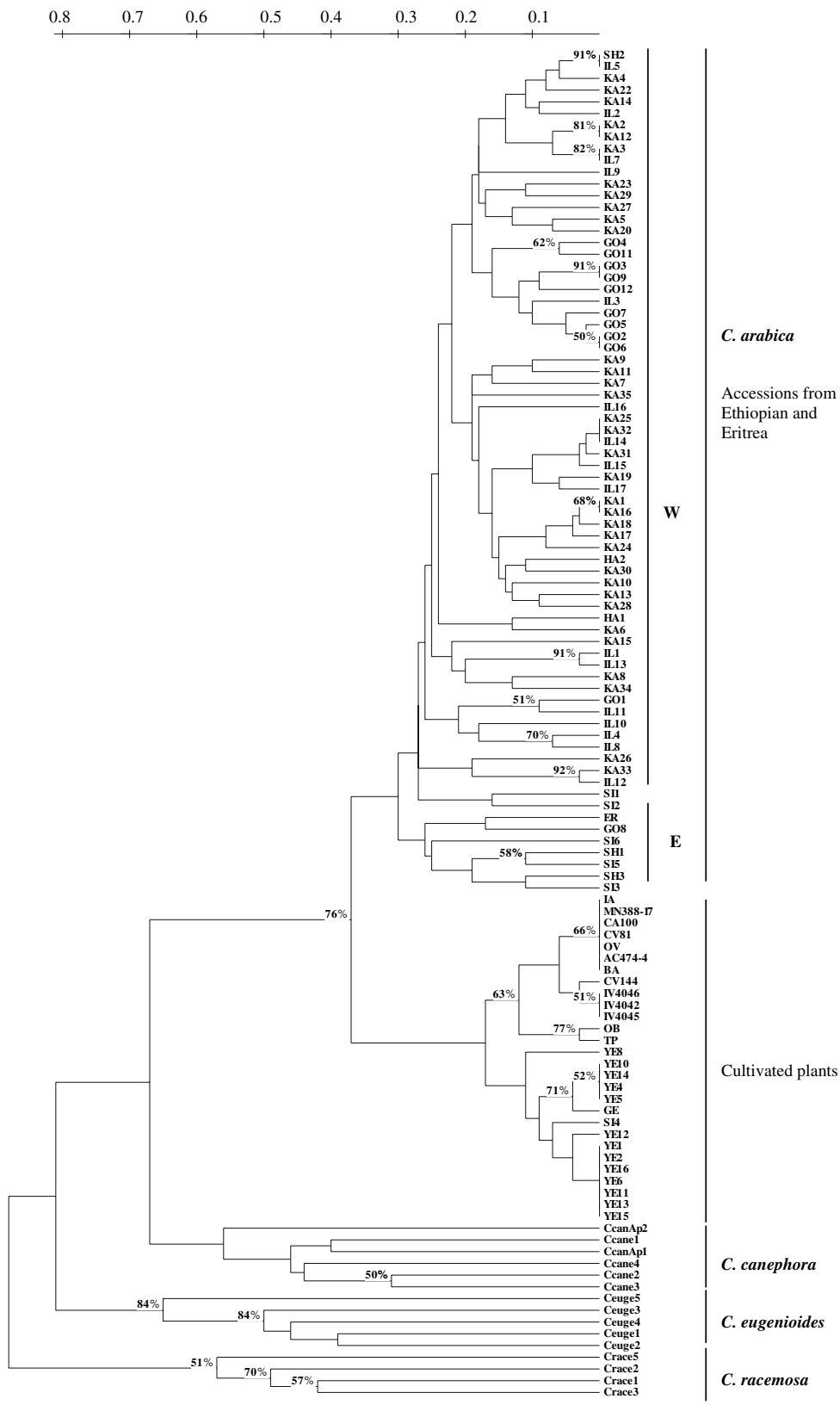
These results suggested that the 4-1CTG locus is present in *C. arabica*, *C. eugenioides* and *C. canephora* and other *Coffea* species (see Poncet et al., 2004). However, there is an interspecific polymorphism in the flanking regions of SSR that prevent amplification in *C. canephora*, *C. racemosa* and in the putative *C. canephora* genome of *C. arabica* (Lashermes et al., 1999). Interestingly, Rovelli et al. (2000) identified heterozygotes in the accessions of *C. arabica* var. Caturra showing a polymorphism in repeat motifs within cultivated plants of *C. arabica* that was not observed in our study.

Genetic diversity

The high variability detected in spontaneous and subspontaneous accessions of *C. arabica* was observed mainly in coffee trees from Sidamo, Kaffa and Illubabor provinces although it also must be noted that 50% of accessions were sampled from Kaffa and Illubabor regions. Significant levels of genetic diversity in coffee plants from Kaffa and Illubabor were also reported by Anthony et al. (2001) and Chaparro et al. (2004). Indeed, the high variability among accessions from these regions in the collections is a consequence of the great effort in collecting samples with as much visual, botanical and agronomical diversity as possible (FAO, 1968). The high genetic diversity detected in Sidamo accessions could be visualized by the proportion of shared alleles among genotypes within both divergent groups of *C. arabica*, cultivated plants and spontaneous and subspontaneous accessions from Ethiopia.

Comparing the genetic distance values among *C. arabica* accessions, we found values similar to those reported by Orozco-Castillo et al. (1994), Anthony et al. (2001, 2002), but lower values than Moncada and McCouch (2004). Genetic diversity evaluated by allele distribution (29.6% of alleles

Fig. 2 Dendrogram of the 115 *Coffea* accessions listed in Tables 1 and 2 based on Jaccard genetic distance obtained from SSR markers using the UPGMA method. Numbers (%) on the branches correspond to bootstrap values above 50% (1,000 replications). Letters indicate the geographical origin of accessions: W (West of the Great Rift Valley) and E (East of the Great Rift Valley)


0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
91%
SH2
IL5
KA4
KA22
KA14
IL2
81%
KA2
KA12
82%
KA3
IL7
IL9
KA23
KA29
KA27
KA5
KA20
62%
GO4
GO11
91%
GO3
GO9
GO12
IL3
GO7
GO5
50%
GO2
GO6
KA9
KA11
KA7
KA35
IL16
KA25
KA32
IL14
KA31
IL15
KA19
IL17
68%
KA1
KA16
KA18
KA17
KA24
HA2
KA30
KA10
KA13
KA28
HA1
KA6
KA15
91%
IL1
IL13
KA8
KA34
51%
GO1
IL11
IL10
70%
IL4
IL8
KA26
92%
KA33
IL12
SI1
SI2
ER
GO8
SI6
58%
SH1
SI5
SH3
SI3
76%
IA
MN388-I7
CA100
66%
CV81
OV
AC474-4
BA
CV144
63%
51%
IV4046
IV4042
IV4045
77%
OB
TP
YE8
YE10
52%
YE14
YE4
71%
YE5
GE
SI4
YE12
YE1
YE2
YE16
YE6
YE11
YE13
YE15
CcanAp2
Ccane1
CcanAp1
Ccane4
50%
Ccane2
Ccane3
84%
Ceuge5
84%
Ceuge3
Ceuge4
Ceuge1
Ceuge2
51%
Crace5
70%
Crace2
57%
Crace1
Crace3
W
E
C. arabica
Accessions from Ethiopian and Eritrea
Cultivated plants
C. canephora
C. eugenioides
C. racemosa

with frequencies between 0.2 and 0.8) revealed a diversity degree intermediate between that identified by Anthony et al. (2001) and that found by Chaparro et al. (2004), with 17 and 39.6% of alleles with frequencies between 0.2 and 0.8, respectively.

Proportion of polymorphic loci and Shannon's index values estimated in this study can be considered in the same range of a similar analysis of arabica coffee in natural populations from Ethiopia (Aga et al., 2003). These authors determined P and H' values ranging from 37% to 73% and 0.2 to 0.4, respectively, in 9 plants per population. However, P and H' were calculated based on *C. arabica* amplified bands while in the present study all amplified bands, including those specific of diploid species and monomorphic to *C. arabica* were included in the index calculation. On the other hand, if only *C. arabica* amplified bands were considered for calculating P and H' values, these would be for Kaffa group, for example, P= 55.6% and $\underline{H'}$= 0.328, identical to that observed by Aga et al. (2003). Besides this, observed values of genetic distances among accessions were similar in both studies.

Therefore, these results suggest that there is a significant genetic diversity in the coffee collection of IAC. Furthermore, the genetic diversity estimated for Ethiopian accessions was higher than that of cultivated plants. Thus there is a large variation that can be source for introgression of desirable characteristics in commercial cultivars that has already been used by the coffee breeding program of IAC (Bettencourt and Carvalho, 1968; Moraes et al., 1974; Fazuoli, 1981; Silvarolla et al., 2004).

Genetic structure

Results of amplification of 4-1CTG locus in *C. arabica*, *C. eugenioides* and *C. canephora* and analysis of species relationship observed in this work are in agreement with the hypothesis about the botanic origin of *C. arabica* species as a natural hybrid between *C. canephora* and *C. eugenioides* (Lashermes et al., 1999). Despite contrasting results concerning which of both species is closer to *C. arabica*, several molecular markers as well as phylogenetics studies have recognized *C. canephora* and *C. eugenioides* closer related to *C. arabica* than other *Coffea* species (Lashermes et al., 1993, 1997; Cros et al., 1998; Ruas et al., 2000, 2003). These diversity analyses also identified *C. racemosa* as the most genetically distant species of *C. arabica.*

According to Bussel (1999) calculation of G'_{ST} using the Shannon's index are in accordance with G_{ST} estimated by other methods, such as AMOVA and modified F-statistics. Also, G'_{ST} is a good estimate to evaluate genetic structure of tetraploid, autogamous collections such as this of *C. arabica* plants, where heterozygosity cannot be assessed and Hardy–Weinberg equilibrium assumptions cannot be considered. G'_{ST} values of *C. arabica* observed in this work fitted into expected values based on the breeding system. In that case, according to Bussel (1999), G'_{ST} values for autogamous species from natural populations is around 60% and around 15% for allogamous species.

Results of $\underline{G'}_{ST}$ for *C. arabica* accessions associated with cluster analysis indicated a strong genetic structure in the species. Therefore, the genetic diversity was observed among groups rather than within groups. Cluster analysis clearly showed the separation of cultivated plants from Ethiopian and Eritrean spontaneous and subspontaneous accessions. Besides, the analysis allowed distinguishing a morphologic type of Yemen (Tessawi) and Yemen accessions from Brazilian cultivars, although both groups exhibited a very low genetic diversity. These results are in agreement with the well-described narrow genetic basis of cultivated plants of *C. arabica* (Lashermes et al., 1996; Anthony et al., 2001; Moncada and McCouch 2004; Maluf et al., 2005) and historical data. Brazilian coffee originated from a few plants introduced in the early 18th century and these plants were originated from the first cultivated plants in Yemen (Chevalier and Dagron, 1928; Carvalho, 1945).

Partitioning of genetic diversity of the spontaneous and subspontaneous accessions showed a lower value of $\underline{G'}_{ST}$ (0.464) than that observed when all accessions were analyzed. However, the $\underline{G'}_{ST}$ value still indicated a strong genetic structure in these accessions. Hence, accessions of

Gojjam were grouped altogether and there was a separation between Sidamo accessions and the others from the opposite side of Great Rift Valley: Kaffa, Gojjam, and Illubabor. However, these grouping associations were not supported by high bootstrap values indicating that the cluster was established based on a few distinct markers.

A genetic structure and low differentiation between southern/south–eastern and south–western coffee trees of Ethiopia were also verified by Anthony et al. (2001) and Aga et al. (2003, 2005). All together, these analyses may support the hypothesis that southern and south–eastern coffee trees of Ethiopia were introduced from the South West (Montagnon and Bouharmont, 1996; Anthony et al., 2001).

Based on morphological and agronomical traits, Montagnon and Bouharmont (1996) were the first to point out a genetic structure in *C. arabica* accessions, with a division between accessions from west and east side of the Great Rift Valley. However, accessions from east side of Ethiopia were grouped with cultivated plants of Yemen. According to Montagnon and Bouharmont (1996), most of the evaluated characteristics were affected by domestication and the similarity found could be the result from the fact that plants collected from Sidamo, Harar and other provinces of the East are cultivated rather than wild-type once primary forests were eradicated in this region. The authors suggested then two hypotheses to explain the origin of the cultivated plants of *C. arabica*: eastern plants could have been introduced from the West or could have been selected from wild-type plants situated to the East of the Rift. Nevertheless, the authors claimed unlikely the existence of a single center of wild coffee trees in Ethiopia located west of the Great Rift Valley. They also suggested that arabica plants transferred to Yemen could be originated from south–eastern of Ethiopia.

In the present study, cluster analysis showed accessions from Sidamo closer related to the cultivated plants, with one accession from Sidamo (SI4) being grouped within Yemen group. Similar results were achieved by Moncada and McCouch (2004). It is interesting that the accession from Eritrea and two accessions from Shoa were clustered with Sidamo's accessions (Fig. 2). According to FAO (1968), E579 (ER) was introduced in Eritrea probably from Yemen, E37 (SH3) was a cultivated plant and E16 (SH1) was also a cultivated plant that is originated from Harar seeds. This observed similarity between accessions from Yemen and from eastern Ethiopia agrees with results from Montagnon and Bouharmont (1996). In the same way, these relationships corroborate the putative separation between Ethiopian accessions from east and west of the Great Rift Valley, but with a low differentiation between both groups (Anthony et al., 2001).

The cultivar 'Geisha', derived of plants from Kaffa province (Jones, 1956; FAO, 1968), was grouped with accessions from Yemen and was very distant from Kaffa accessions. This suggests that plants of the same origin were genetically separated by the domestication process and that the West is the primary origin of plants from the East.

Hence, our results suggest that there was a straight evolutionary pathway in domestication of *C. arabica* trees. The species origin is the Southwest highlands of Ethiopia, and thereafter it was introduced into the South and South East (natural colonization or/and by humans). Later on, some plants were transferred by Arabs to Yemen. After a long period in Yemen, these cultivated plants were spread out around the world. Also, possible occurrences of wild plants in south and south–east of Ethiopia cannot be ruled out.

SSR markers confirmed previously reported phylogenetic relationships among *Coffea* species as well as they demonstrated to be an efficient method to analyze genetic diversity and structure within *C. arabica* species. Our results agreed with the well-described narrow genetic basis of cultivated plants of *C. arabica* and showed a significant genetic diversity of accessions from Ethiopia and Eritrea included in the *Coffea* Germplasm Collection of IAC. These results indicate the importance of non-cultivated accessions as source of genetic variability for coffee improvement.

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