EJAST 1(1): 63-79 (2010)

ORIGINAL ARTICLE

Genetic diversity of Arabica coffee (*Coffea arabica* L.) collections

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(Received in revised form: September 26, 2010)

ABSTRACT

Arabica coffee (*Coffea arabica* L.) has its centre of origin and diversity in south-western Ethiopian highlands. Its populations exist as wild and under production systems. There is limited use of molecular genetic diversity information of Ethiopian Arabica coffee in the improvement programs. Thus, generating genetic diversity information is an important parameter in the future efforts of Arabica coffee genetic resources conservation and sustainable utilization. Hence, in this study the genetic diversity of Arabica coffee collections were studied using 32 microsatellite (SSRs) markers. The result indicated high genetic variability reserve with a lot of specificity in Ethiopian Arabica coffees. More than 90% of the total alleles were detected in Ethiopian Arabica coffee. Of the total alleles detected in Ethiopian Arabica coffee, about 83.7 % and 46.4 % were polymorphic and specific, respectively. The cultivated cultivars contained only 53.6 % of the total alleles detected in indicating the genetic diversity bottleneck due to early human impacts. In the cluster analysis, Ethiopian Arabica coffees with larger within population genetic distances were clearly separated from the cultivated cultivars. The result suggests the potential application of SSRs in genetic diversity study of Arabica coffee according to its origin and the possibility of high potential to use Ethiopian Arabica coffee gene pool in the improvement programs.

Keywords: *Coffea arabica*, Ethiopian Arabica coffee, Genetic diversity, SSR markers

INTRODUCTION

Coffee belongs to the genus *Coffea* in the Rubiaceae family, and is mostly grown in the tropical and subtropical regions (Berthaud and Charrier, 1988). Of the 100 species in the genus *Coffea*, *Coffea arabica* L. (Arabica coffee) and *Coffea canephora* P. (Robusta coffee) are the two most important commercial species with *C. arabica* considered as a high quality coffee and contributes more than 70 percent of the world coffee production (Lashermes *et al.,* 1997; Carneiro, 1999; Anthony *et al.,* 2001a; Anthony *et al.,* 2002; Stieger *et al.,* 2002). Economically, coffee is the most important agricultural commodity which stands second only to oil in terms of international trading on the world market. In many producing countries, besides contributing a tremendous amount to the foreign exchange currency as a main cash crop, it serves as a means of livelihood for millions of people and plays a vital role in their socio-economic values (Orozco-

Castillo *et al.,* 1994; Carneiro, 1999; Anthony *et al.,* 2001a; Stieger *et al.,* 2002). Geographically, most of the coffee species are originated from tropical African countries: Ethiopia for the tetraploid *Coffea arabica*, and Central and West African countries for other coffee species (Berthaud and Charrier, 1988). During the early centuries, these coffee species were disseminated to other parts of the world where they are produced in mass nowadays. However, the Arabica coffee plants in major producing areas such as Latin and Central America, and Asian countries are believed to have a narrow genetic bases attributed to the few seeds/plants used for dissemination, the successive genetic reduction due to human impacts and reproduction nature, especially for Arabica coffee which is autogamous (Orozco-Castillo *et al.,* 1994; Lashermes *et al.*, 1996; Carneiro, 1999; Anthony *et al.,* 2002; Stieger *et al*., 2002; Raus *et al*., 2003).

Even though, the overall genetic diversity of *Coffea arabica* is believed to be less polymorphic as compared to its diploid relative species, the populations in its place of origin and diversity, particularly south-western Ethiopia, have a lot of genetic variability for many agronomic characters. This fact has been supported by many studies based on different techniques such as morphological (Ameha and Belachew, 1987; Carvalho, 1988), biochemical (Silvarolla *et al* ., 2000; Silvarolla *et al*., 2004) and DNA-based molecular markers techniques (Lashermes *et al*., 1995; Lashermes *et al*., 1996; Lashermes *et al.,* 1997; Anthony *et al.,* 2001a; Anthony *et al.,* 2001b; Moncada, 2004). According to Bellachew (1997), indigenous cultivars of Arabica coffee in Ethiopia are location specific for adaptability demonstrating the existence of wide genetic variability in natural Arabica coffee populations for the development of location and agro-climate specific improved varieties. Denich and Gatzweiler (2006) had reported site-specificity of wild-coffee for drought tolerance. These populations exist in different forms: as wild coffee that are inaccessible and non-used, forest and/or semi-forest coffee and garden (landraces) coffees. The within population genetic diversity decreases as we go from wild population to landraces (Senbeta and Denich, 2006).

Genetic diversity of coffee can be assessed using different techniques that range from the traditional morphological techniques to the modern DNA-based molecular markers. The use of morphological techniques in diversity study of plants is limited by the influence of environmental factors and growth stage of the plant (Weising *et al.,* 2005). In addition, they are also few in number and require lengthy follow-up during the whole growth stage especially in perennial plants like coffee. In response to the limitation of morphological techniques, the more effective technique based on protein, isozymes, was developed. However, its application was limited due to inefficiency to detect within species differences in Arabica coffee (Berthou and Trouslot, 1979; Orozco-Castillo *et al.,* 1994; Berthaud and Charrier, 1998). Today, a number of DNA-based techniques are in use in different coffee genetic studies. These include the conventional RFLP method (Herrera *et al.,* 2001; Crouzillat *et al*., 2004) and the different PCR-based methods such as RAPD (Orozco-Castillo *et al.,* 1994; Lashermes *et al.,* 1996; Anthony *et al.,* 2001a; Anthony *et al.,* 2001b; Aga *et al.,* 2003; Cristancho *et al.,* 2004a; Cristancho *et al.,* 2004b), AFLP (Anthony *et al.,* 2001a; Anthony *et al.,* 2001b; Coulibaly *et al.*, 2001; Prakash *et al* ., 2001; Coulibaly *et al*., 2002; Steiger *et al*., 2002) and microsatellite (SSRs) markers (Lashermes *et al.,* 1995; Lashermes *et al.,*

1997; Dufour *et al.*, 2001; Prakash *et al .,* 2001 ; Herrera *et al.,* 2001; Anthony *et al.,* 2002; Crouzillat *et al.*, 2004 ; Moncada, 2004; Moncada and McCouch. 2004; Lin *et al.,* 2005 ; Geletu *et al.*,2006). These molecular marker techniques have many advantages such as: not subjected to environmental factors and growth stage of the plant, and the potential of existing in unlimited numbers, covering the entire genomes (Weising *et al.*, 2005). Of the different DNA based techniques, microsatellite (SSRs) markers are the recently used techniques in the genetic study of plants. They are short tandem repeats of DNA sequence of one to six base pairs. Their use as a molecular marker has advantages over other techniques as it fulfills most of the good characteristics of genetic markers such as highly polymorphic and reproducible, locus-specific and "co-dominant". Because of this, today SSRs are the markers of choice for many genetic studies.

However, the coffee genetic study program, particularly the wild Arabica coffee populations in Ethiopia, has not benefited a lot from the development of the recent molecular markers such as SSRs as compared to other cash crops (Orozco-Castillo *et al.,* 1994). Most of the studies, so far using these technologies, were done on commercial cultivars of Arabica coffee or the out-crossing diploid species, *C. canephora*. Because of this, little is known about the genetic structure and pattern of wild Arabica coffee (Aga *et al.,* 2003), which in turn has limited the use of its diverse genepool in the improvement program (Cristancho *et al.,* 2004a). In addition to this, the wild coffee populations are under threat due to its natural habitat disturbance mainly by deforestation and land use change (Bellachew, 1997; Gole *et al.*, 2002; Gole *et al.,* 2003; Schmitt et al., 2005; Senbeta and Denich, 2006). This has been aggravated by the high population pressure in need of more arable land to produce more food crops and low coffee price on the world market forcing farmers to replace their coffee plants with other high cash value crops. On the other hand, the current conservation efforts both at ex-*situ* field gene banks, and *in-situ* on farm (landraces) or in its natural forest ecosystem (wild forest coffee population) is very low as compared to the economic importance of coffee, the great threat to its genetic diversity and the ample genetic diversity in its populations in Ethiopia which one cannot find anywhere else on the world. According to Bellachew (1997), the accessions available in the gene bank are too few to represent the high genetic variability available within the natural coffee populations in Ethiopia. Unless immediate protective measures are taken at large scale for long-term benefits, the pressure could lead to the total irreversible loss of a significant part of the available genetic resources in less than a couple of decades. This could have a high consequential cost both at national and international level to the coffee production and marketing chain.

For the future benefit of coffee economy, it is important to plan and decide a strategy to conserve these populations at its very beginning place. However, conserving the whole populations is practically impossible due to resources limitation. Thus, there is a need to identify and conserve potential populations with the maximum possible genetic diversity, which depends on the availability of genetic diversity information**.** Hence, any effort towards generating information on the genetic pattern of Ethiopian Arabica coffee populations, especially using DNA molecular techniques, is very important. Microsatellite (SSRs) markers were used to study the genetic diversity of Arabica coffee collections with different geographical origin and historical

backgrounds. Genetic relationships among Arabica coffee collections and commercial Arabica cultivars were also estimated. The potential application of SSRs in Arabica coffee genetic study according to its tetraploid origin was also evaluated considering the previously suggested putative parental diploid species: *C. canephora* and *C. eugenioides*.

MATERIALS AND METHODS

Plant material

A total of 133 genotypes of *C. arabica* were used in this study (Table 1). It includes 78 Ethiopian Arabica accessions and 55 cultivated genotypes. Of the total 78 Ethiopian Arabica accessions, 54 accessions were obtained from Jimma Agricultural Research Centre, Ethiopian Institute of Agricultural Research. The rest of 24 Ethiopian accessions and the cultivated genotypes were obtained from Nestlé R&D Tours, France. They are representative of Nestlé R&D Arabica coffee core collection. DNA samples from six *Coffea eugenioides* and 21 *Coffea canephora* genotypes were also included to help in determining the species origin of amplified alleles in the tetraploid Arabica coffee.

DNA extraction and SSRs marker selection

Total DNA was isolated from frozen leaves of each genotype following DNeasy plant Maxi Kit procedure (QIAGEN, 2006). Thirty two SSRs markers were used to assess the overall genetic diversity of Arabica coffee collections. Most of these markers were already used in coffee genetic mapping by Nestlé R&D Tours and their genetic map locations is known (Table 2).

PCR Amplification

PCR was performed in 22µl reaction volume containing 9µl of AmpliTaq Gold® PCR Master Mix (Applied Biosystems product), 9µl of deionised water, 1µL of forward primers (10nM) and 0.5µL of reverse primer (20nM) and 2.5 µl of extracted DNA. Amplification was carried out in a GeneAmp® PCR System 9700 using the following program: 10 min initial denaturation step at 94°C, followed by 35 cycles of denaturing at 94°C for ½ min, annealing at 50°C for ½ min and extension at 72°C for 1 min with a final extension step at 72°C for 7 min.

Table 1. Lists of 133 Arabica coffee genotypes with their collection area

No	Code	Collection area	Sample type	Source[a]	No	Code	Collection area	Sample type	Source
1	ETH-83	Wollega	Ethiopian accessions	JARC/EIAR[b]	42	ETH-9	Jimma	Ethiopian accessions	JARC/EIAR
2	ETH-111	Wollega	Ethiopian accessions	JARC/EIAR	43	ETH-76	Jimma	Ethiopian accessions	JARC/EIAR
3	ETH-112	Wollega	Ethiopian accessions	JARC/EIAR	44	ETH-66	Jimma	Ethiopian accessions	JARC/EIAR
4	ETH-113	Wollega	Ethiopian accessions	JARC/EIAR	45	ETH-62	Jimma	Ethiopian accessions	JARC/EIAR
5	ETH-73	Illuababor	Ethiopian accessions	JARC/EIAR	46	ETH-106	Jimma	Ethiopian accessions	JARC/EIAR
6	ETH-79	Illuababor	Ethiopian accessions	JARC/EIAR	47	ETH-107	Jimma	Ethiopian accessions	JARC/EIAR
7	ETH-110	Illuababor	Ethiopian accessions	JARC/EIAR	48	ETH-108	Jimma	Ethiopian accessions	JARC/EIAR
8	ETH-11	Illuababor	Ethiopian accessions	JARC/EIAR	49	ETH-109	Jimma	Ethiopian accessions	JARC/EIAR
9	ETH-14	Illuababor	Ethiopian accessions	JARC/EIAR	50	ETH-65	Sidamo	Ethiopian accessions	JARC/EIAR
10	ETH-21	Illuababor	Ethiopian accessions	JARC/EIAR	51	ETH-74	Sidamo	Ethiopian accessions	JARC/EIAR
11	ETH-4	Kaffa	Ethiopian accessions	JARC/EIAR	52	ETH-64	--[c]	Ethiopian accessions	JARC/EIAR
12	ETH-37	Kaffa	Ethiopian accessions	JARC/EIAR	53	ETH-50	--	Ethiopian accessions	JARC/EIAR
13	ETH-40	Kaffa	Ethiopian accessions	JARC/EIAR	54	ETH-51	--	Ethiopian accessions	JARC/EIAR
14	ETH-45	Kaffa	Ethiopian accessions	JARC/EIAR	55	ArCl06-04	--	Wild-Ethiopian (FAO)	Nestlé R&D
15	ETH-48	Kaffa	Ethiopian accessions	JARC/EIAR	56	ArCl19-2	--	Wild - Ethiopian(FAO)	Nestlé R&D
16	ETH-53	Kaffa	Ethiopian accessions	JARC/EIAR	57	ArCl23-4	--	Wild - Ethiopian (FAO)	Nestlé R&D
17	ETH-55	Kaffa	Ethiopian accessions	JARC/EIAR	58	ArCl27-3	--	Wild – Ethiopian (FAO)	Nestlé R&D
18	ETH-58	Kaffa	Ethiopian accessions	JARC/EIAR	59	ArCl33-2	--	Wild - Ethiopian (FAO)	Nestlé R&D
19	ETH-31	Kaffa	Ethiopian accessions	JARC/EIAR	60	ArCl53-1	--	Wild – Ethiopian (FAO)	Nestlé R&D
20	ETH-33	Kaffa	Ethiopian accessions	JARC/EIAR	61	ArCl61-4	--	Wild - Ethiopian (FAO)	Nestlé R&D
21	ETH- 67	Kaffa	Ethiopian accessions	JARC/EIAR	62	Et39-1	--	Diploid Wild - Ethiopian	Nestlé R&D
22	ETH-1	Kaffa	Ethiopian accessions	JARC/EIAR	63	CCCA31	--	Wild - Ethiopian (FAO)	Nestlé R&D
23	ETH-2	Kaffa	Ethiopian accessions	JARC/EIAR	64	CCCA32	--	Wild - Ethiopian (FAO)	Nestlé R&D
24	ETH-7	Kaffa	Ethiopian accessions	JARC/EIAR	65	C CCA33	--	Wild - Ethiopian (FAO)	Nestlé R&D
25	ETH-68	Kaffa	Ethiopian accessions	JARC/EIAR	66	CCCA34	--	Wild - Ethiopian (FAO)	Nestlé R&D
26	ETH-28	Kaffa	Ethiopian accessions	JARC/EIAR	67	CCCA35	--	Wild - Ethiopian (FAO)	Nestlé R&D
28	ETH-114	Kaffa	Ethiopian accessions	JARC/EIAR	69	CCCA37	--	Wild - Ethiopian (FAO)	Nestlé R&D
29	ETH-16	Kaffa	Ethiopian accessions	JARC/EIAR	70	CCCA38	--	Wild - Ethiopian (FAO)	Nestlé R&D
30	ETH-18	Kaffa	Ethiopian accessions	JARC/EIAR	71	CCCA39	--	Wild - Ethiopian (FAO)	Nestlé R&D
31	ETH-23	Kaffa	Ethiopian accessions	JARC/EIAR	72	GPFA25	--	Wild - Ethiopian (FAO)	Nestlé R&D
32	ETH-24	Kaffa	Ethiopian accessions	JARC/EIAR	73	GPFA27	--	Wild - Ethiopian (FAO)	Nestlé R&D
33	ETH-77	Kaffa	Ethiopian accessions	JARC/EIAR	74	GPFA30	--	Wild - Ethiopian (FAO)	Nestlé R&D
34	ETH-78	Kaffa	Ethiopian accessions	JARC/EIAR	75	GPFA47	--	Wild - Ethiopian (FAO)	Nestlé R&D
35	ETH-80	Kaffa	Ethiopian accessions	JARC/EIAR	76	GPFA96	--	Wild - Ethiopian (FAO)	Nestlé R&D
40	ETH-60	Jimma	Ethiopian accessions	JARC/EIAR					
36	ETH-81	Kaffa	Ethiopian accessions	JARC/EIAR	77	GPFA97	--	Wild - Ethiopian (FAO)	Nestlé R&D
37	ETH-29	Kaffa	Ethiopian accessions	JARC/EIAR	78	GPFA103	--	Wild - Ethiopian (FAO)	Nestlé R&D
38	ETH-71	Kaffa	Ethiopian accessions	JARC/EIAR	79	CCCA1*	--	Bourbon-Cultivated	Nestlé R&D
39	ETH-72	Kaffa	Ethiopian accessions	JARC/EIAR	80	CCCA2	--	Bourbon-Cultivated	Nestlé R&D

Continued on next page

No	Code	Collection area	Sample type	Source[a]	No	Code	Collection area	Sample type	Source
81	CCCA3	--	Bourbon-Cultivated	Nestlé R&D	108	GPFA5	--	Bourbon-Cultivated	Nestlé R&D
82	CCCA4	--	Bourbon-Cultivated	Nestlé R&D	109	GPFA14	--	Bourbon-Cultivated	Nestlé R&D
83	CCCA5	--	Typica-Cultivated	Nestlé R&D	110	GPFA15	--	Yellow Bourbon-Cultivated	Nestlé R&D
84	CCCA6	--	Bourbon-Cultivated	Nestlé R&D	111	GPFA21***	--		Nestlé R&D
85	CCCA7	--	Typica-Cultivated	Nestlé R&D	112	GPFA23***	--		Nestlé R&D
86	CCCA8	--	Typica-Cultivated	Nestlé R&D	113	GPFA46	--	Typica or Bourbon cultivated	Nestlé R&D
87	CCCA9	--	Typica-Cultivated	Nestlé R&D	114	GPFA50	--	Bourbon X Typica-Cultivated	Nestlé R&D
88	CCCA10	--	Typica-Cultivated	Nestlé R&D	115	GPFA54	--	Catimor-Cultivated	Nestlé R&D
89	CCCA11	--	Typica-Cultivated	Nestlé R&D	116	GPFA55	--	Catimor X Catuai-Cultivated	Nestlé R&D
90	CCCA12	--	Bourbon-Cultivated	Nestlé R&D	117	GPFA61	--	Hybrido de Timor-Cultivated	Nestlé R&D
91	CCCA13	--	Bourbon-Cultivated	Nestlé R&D	118	GPFA67	--	Catimor-Cultivated	Nestlé R&D
92	CCCA14	--	Typica-Cultivated	Nestlé R&D	119	GPFA71	--	Catimor-Cultivated	Nestlé R&D
93	CCCA15	--	Typica or Bourbon –Cult.	Nestlé R&D	120	GPFA73	--	Catimor-Cultivated	Nestlé R&D
94	CCCA16	--	Typica or Bourbon –Cult.	Nestlé R&D	121	GPFA74	--	Catimor-Cultivated	Nestlé R&D
95	CCCA17	--	Bourbon-Cultivated	Nestlé R&D	122	GPFA76	--	Catimor-Cultivated	Nestlé R&D
96	CCCA18	--	Bourbon-Cultivated	Nestlé R&D	123	GPFA77	--	Catimor-Cultivated	Nestlé R&D
97	CCCA19	--	Sarchimor-Cultivated	Nestlé R&D	124	GPFA78	--	Yellow Catimor-Cultivated	Nestlé R&D
98	CCCA20	--	Cavimor-Cultivated**	Nestlé R&D	125	GPFA79	--	Catimor-Cultivated	Nestlé R&D
99	CCCA21	--	Bourbon-Cultivated	Nestlé R&D	126	GPFA80	--	Catimor-Cultivated	Nestlé R&D
100	CCCA22	--	Catimor-Cultivated	Nestlé R&D	127	GPFA81	--	Catimor-Cultivated	Nestlé R&D
101	CCCA23	--	Sarchimor-Cultivated	Nestlé R&D	128	GPFA84	--	Catuai-cultivated	Nestlé R&D
102	CCCA24	--	Catimor-Cultivated	Nestlé R&D	129	GPFA98	--	Catimor-Cultivated	Nestlé R&D
103	CCCA25	--	Catimor-Cultivated	Nestlé R&D	130	GPFA99	--	Catimor-Cultivated	Nestlé R&D
104	CCCA26	--	Catimor-Cultivated	Nestlé R&D	131	GPFA100	--	Catimor-Cultivated	Nestlé R&D
105	CCCA28	--	Catuai-Cultivated	Nestlé R&D	132	GPFA101	--	Catimor-Cultivated	Nestlé R&D
106	CCCA29	--	Yellow Catuai-Cultivated	Nestlé R&D	133	GPFA102	--	Catimor-Cultivated	Nestlé R&D
107	CCCA30	--	Typica-Cultivated	Nestlé R&D					

a Source = Institution(s) from where the sample materials were obtained
bJARC/EIAR = Jimma Agricultural Research Centre, Ethiopian Institute of Agricultural Research
c unknown source ; *CCCA= Core Collection of Coffea Arabica
**Hybrid between Catimor and Hybrido de Timor; ** *Probably introgression with C. canephora

Table 2. List of SSR markers used: locus code, forward/reverse primer sequence and repeat types

No	Gene Bank accession	SSR Locus code	5'.3' Forward primer/5'.3' Reverse primer	Repeat type	Linkage group	Origin	Source Organism	References	Sequence source
1		ssrR105	CACCAATTCCACTGACAATG/ TCCCTGCCAACACACTTC	GA(18)	E	Genomic	*C. canephora*	R&D Nestlé Tours	Nestlé
2		ssrR126	GCACAATCACTCCCAAAG / TGACGGCCTACTACTTACAG	GA(23)	C	Genomic	*C. canephora*	R&D Nestlé Tours	Nestlé
3		ssrR175	GCAGTGACGCAGCAATG/ AAAAGGAGAGCCAAAGCAGT	GA(20)	F	Genomic	*C. canephora*	R&D Nestlé Tours	Nestlé
4		ssrR209	CGGGGGTAAAAAGATTGTAA/ TTGGTGGGAGGGGAGTA	GA(16)	D	Genomic	*C. canephora*	R&D Nestlé Tours	Nestlé
5		ssrR268	GTATCCCACAATGAAATCAC/ AGTAGAATTTTCAACATATAAG	GA(19)	G	Genomic	*C. canephora*	R&D Nestlé Tours	Nestlé
6		ssrR278	TGTAGATTTGAAACCCAATC/ AAGTCTCGACAAGTTTTGAC	GA(16)	E	Genomic	*C. canephora*	R&D Nestlé Tours	Nestlé
7		ssrR325	CCTTGTTGTTGGGGAATGTC/ GGCTGTTCTGGGCTTTGTG	GA(23)	F	Genomic	*C. canephora*	R&D Nestlé Tours	Nestlé
8		ssrR338	CGAAGGCTGTCAACAACTGG/ GGGATAAACAAGTTAAAGGA	GA(17)	E	Genomic	*C. canephora*	R&D Nestlé Tours	Nestlé
9		ssrR339	ATTATGCTCGCTGGGCTGTT/ TGGGATCACTCCTGTGTCGC	CT(12)	G	Genomic	*C. canephora*	R&D Nestlé Tours	Nestlé
10	AJ308783	ssrA8783	CTTCGTATGGTTGTCTGTGT/ AATGATAGGAGGCACTTGAC	GT(16)	B	Genomic	*C. arabica*	Rovelli et al. 2000	Genbank
11	AJ308837	ssrA8837	AAAAGTGAGCACGTCATGTG/ GCGTGAGAGGGACCAT	GT(16) & GA(11)	D	Genomic	*C. arabica*	Rovelli et al. 2000	Genbank
12	AJ308847	ssrA8847	GCACACATGAAAAAGATGCT/ GATGGACAGGAGTTGATGG	GT(18) GA(18)	K	Genomic	*C. arabica*	Rovelli et al. 2000	Genbank
13	AY102434	ssrAY2434	CGCAAATGTTTATGTCAATC/ GCAACTTATGAGCCTAATCC	GA(20) & CA(11)	C	Genomic	*C. arabica*	Cristancho et al. 2002	Genbank
14	AY102449	ssrAY2449	CGAAAATATGCTGCCCATTG/ CCGAACCCATAAGGTGTGAC	CT(20)	?	Genomic	*C. arabica*	Cristancho et al. 2003	Genbank
15	AJ250255	ssrZAP25	GCGAAATCTTTCTCCCTCCC/ CCGTCCTTTTCCTCGAACTC	GT(12)	K	Genomic	*C. arabica*	Combes et al. 2000	Genbank
16		ssrCMA008	CATTCTGGTCCTGATGCTCT/ TCATTCACTTATTAACGTCCATC	(CT)14..(TG)10	C	Genomic	*C.arabica* -Caturra	Université Trieste	Genbank

Continue on next page

No	Gene Bank accession	SSR Locus code	5'.3' Forward primer/5'.3' Reverse primer	Repeat type	Linkage group	Origin	Source Organism	References	Sequence source
17		ssrCMA055	TTGAGCAAAAACCCTATTCC/ TAAACCCAAAAAGACCACAA	(TG)18	F	Genomic	*C.arabica* -Caturra	Université Trieste	Genbank
18		ssrCMA059	GATGGACAGGAGTTGATGGT/ TTTTAACACTCATTTTGCCAAT	(CT9)(CA)8	K	Genomic	*C.arabica* -Caturra	Université Trieste	Genbank
19		ssrCMA151	GCCAGAAGAAGCTGGATGAC/ ACCGTCCTTTTCCTCGAACT	(GT)8	K	Genomic	*C.arabica* -Caturra	Université Trieste	Genbank
21		ssrCMA198	AGCAACTCCAGTCCTCAGGT/ TGGAAGCCCGCATATAGTTT	(TG)9(AG)18	I	Genomic	*C.arabica* -Caturra	Université Trieste	Genbank
22		ssrCMA199	CATGCCATCATCAATTCCAT/ CTAGCTAGCTGGATCAGTACCC	(CT)11	K	Genomic	*C.arabica* -Caturra	Université Trieste	Genbank
23		ssrCMA233	CAACGAGATAACTGGCAGGTC/ CAAACCAATATTAGGAATAAAGAACG	(CA)13(TA)5	B	Genomic	*C.arabica* - Caturra	Université Trieste	Genbank
24		ssrCMA263	TGCTTGGTATCCTCACATTCA/ ATCCAATGGAGTGTGTTGCT	(CT)18	K	Genomic	*C.arabica*- Bourbon Tekisic	Université Trieste	Genbank
25		SSR124577	GATGGCTTTTCTCCGTTATCC/ GGATTCGACTGCTGGATGAT	AAG(6)	?	EST	*C. canephora*	CGN	CGN
26		SSR122850	TCCAGTTTGATCAGCAACCA/ CCATCTTGGGGATAGAGCAA	(AGAG)3	?	EST	*C. canephora*	CGN	CGN
27		SSR124195	ATCCCCATCAGAAGACCTCA/ CCTCCACCGCCTGTTTATTA	(AGC)6	?	EST	*C. canephora*	CGN	CGN
28		SSR119699	GCCGTGGTGGAAGATGTACT/ CGAGTTCACCAAGAACGTCA	AT(5)	A	EST	*C. canephora*	CGN	CGN
29		SSR129793	CTTGTAGCGGGGAAAATTGA/ GCGATGGAAAAACCGATTAC	CACA(5)	E	EST	*C. canephora*	CGN	CGN
30		SSR123909	AGGCTTGCTGGAACTCTTGA/ GAAAGACTTGTCCTTTGCCG	CTCT(7)	B	EST	*C. canephora*	CGN	CGN
31		SSR124161	TGCGAAACCATTGAGAACAG/ CCGGAGGATGAGATTGAAAA	CT(5)	A	EST	*C. canephora*	CGN	CGN
32		SSR123557	ATCTCCTCGTTCTTCCCCAT/ GCTTGTAGCAGGCAGGAAAC	CTCT(4)	B	EST	*C. canephora*	CGN	CGN

Data Scoring and Analysis

Data scoring

The amplified PCR products were separated and detected by capillary electrophoresis using an ABI Prism 310 Genetic Analyzer in built with analysis software (www.appliedbiosystem.com). Sample DNA amplified with four microsatellite markers of specific fluorescent labels (FAM: VIC: PET: NED in a ratio of 1:1:2:1) were combined at each run and data were collected using data collection software (version 3.0). The size of amplified PCR products was estimated using internal size standard by Genscan analysis software (version 3.7). Then, the individual fragments were assigned as 'alleles' of the appropriate microsatellite loci with Genotyper software (version 3.7) according to their size (bp) and area of the peaks based on the tetraploid origin of *C. arabica*. In polyploidy plants like Arabica coffee, SSR markers can potentially amplify more than two alleles where it is able to detect alleles from the two genomic origins: *C. canephora* and *C. eugenioides* genome in this case. Hence, amplified alleles in tetraploid arabica were assigned to their species origin (where possible) depends on the size of alleles in the two diploid species.

Data analysis

All the detected alleles were used to calculate the following genetic parameters: allelic richness (A), percent of polymorphic alleles (rP), average number of alleles per SSRs locus, and Polymorphism information contents (PIC). PIC, also known as heterozygosity index, was calculated for each microsatellite marker based on the allele frequencies in all analysed accessions taking into account the species origin of alleles in the tetraploid Arabica coffee. Many authors used the formula: PIC = 1- $\sum P_i^2$, where P*i* is the frequency of each allele at a given SSR locus (Moncada, 2004; Moncada and McCouch, 2004; Manifesto *et al*, 2001; Selvi *et al*, 2003). When calculated in this way, PIC is similar to the term 'gene diversity' described by Nei (1973). PIC was calculated using the same formula for each genome part based on the allele frequencies in the two genomes of the tetraploid Arabica coffee (i.e., two PIC values were calculated for each SSRs where it was possible to assign alleles to their putative parental species). Ward's minimum variance grouping method using euclidian distance was used to generate dendrogram utilizing all detected "polymorphic alleles" to estimate the genetic relationship and similarity among collections using ncss v.2007 software (Hintze, 2006). The diversity distribution was also represented on a principal component analysis (pca).

RESULTS AND DISCUSSION

SSR diversity

A total of 209 alleles were detected for 32 SSR markers across 133 Arabica accessions (Table 3). Out of 209 alleles, 200 alleles were polymorphic for all samples. The number of observed alleles per SSRs varied from two to fourteen with an average of 6.5 alleles for all Arabica collection (Table 3). The number of alleles in Ethiopian Arabica coffee ranged from two to 12 with an average of 5.9 alleles per SSRs locus while it ranged from one to eight with an average of 3.5 in cultivated group. Larger values were obtained for the different genetic parameters analysed than previous studies. Anthony *et al.* (2002) reported an average number of 4.7 alleles per SSRs using only six SSRs in Arabica coffee collections containing four Typica, five Bourbon and 10 sub-spontaneous derived accessions. Using 34 SSRs, Moncada and McCouch (2004) reported an average of 2.5 and 1.9

amplified alleles per SSRs in 11 wild Arabica coffee genotypes and 12 cultivated Arabica coffee, respectively, with the number of alleles ranging from one to eight. Maluf *et al. (*2005) also reported an average number of 2.87 alleles in 28 cultivated Arabica lines using 23 SSRs. One reason for such difference could be due to the small sample size and the type of coffee genotypes (Ethiopian vs Cultivated) used in the previous studies as compared to the present study. The other reason could be the number of SSRs used and their genome coverage *i.e.,* a total 32 SSRs covering nine of the 11 linkage groups of coffee genetic map were used. Larger coverage of the total genome was/is one of the limiting factors in diversity study of coffee due to lack of enough number of SSR markers.

Table 3. Number of total and polymorphoric alleles and rate of polymorphism (*rP,* %) for 32 SSR markers in Arabica coffee collections

No	SSR Code	PCR size range(bp)	Ethiopian accessions		Cultivated CCCAs group		Overall	
			Number of alleles		Number of alleles		Number of alleles	
			Total	Polymorphic	Total	Polymorphic	Total	Polymorphic
1	SSR124577	138-157	3	2	3	2	3	3
2	SSR122850	132-141	3	2	2	0	3	2
3	SSR124195	83-101	3	2	2	0	3	2
4	SSR119699	98-110	3	2	4	4	4	4
5	SSR129793	200-236	11	10	5	5	12	12
6	SSR124161	161-177	4	4	4	4	5	5
7	SSR123909	252-263	2	2	1	0	2	2
8	SSR123557	206-270	4	4	2	2	4	4
9	ssrCMA008	106-128	4	4	5	4	6	6
10	ssrCMA055	82-97	6	6	4	4	7	7
11	ssrCMA059	129-165	10	10	3	2	10	10
12	ssrCMA151	168-177	4	4	3	2	4	4
13	ssrCMA159	147-174	12	12	3	3	13	13
14	ssrCMA198	195-236	7	6	5	4	9	8
15	ssrCMA199	122-153	11	11	8	8	14	14
16	ssrCMA233	255-270	5	4	4	3	6	5
17	ssrCMA263	178-200	7	7	2	2	8	8
18	ssrAY2434	178-199	4	3	4	3	4	3
19	ssrAY2449	273-294	6	6	4	3	8	8
20	ssrA8783	106-126	8	8	5	5	8	8
21	ssrA8837	148-165	5	5	3	3	5	5
22	ssrA8847	159-192	10	10	3	2	10	10
23	ssrR105	187-222	9	9	5	5	10	10
24	ssrR126	206-142	11	10	4	3	11	10
25	ssrR175	214-217	3	3	2	2	4	4
26	ssrR209	161-173	3	2	2	0	3	2
27	ssrR268	131-147	3	2	4	4	5	5
28	ssrR278	123-141	7	7	2	0	7	7
29	ssrR325	224-262	10	10	6	6	10	10
30	ssrR338	221-233	3	2	2	0	3	2
31	ssrR339	215-226	5	4	4	4	5	5
32	ssrZAP25	185-193	3	2	2	0	3	2
Total			189	175	112	89	209	200
Rate of Polymorphism (rP, %)			92.6		79.5		95.7	
No SSRs			32	32	32	32	32	32
Average			5.9	5.5	3.5	2.8	6.5	6.2

SSR Allelic assignment to genomic origin of Arabica coffee

From the total of 32 SSRs, the detected alleles were assigned according to their putative species origin (C. canephora or C. eugenioides) for 13 SSRs. Two SSRs (R175 and R263) showed no amplification for the Eu*genioides* genome. For 17 SSRs, it was difficult to assign alleles to their putative species origin due to size overlapping for the alleles of the two genomes. The different genetic parameters for 13 SSRs were calculated taking into account the species origin of the amplified alleles in the Arabica coffee (Table 4). The result indicated higher values in the Robusta genome part. The total numbers of detected alleles in tetraploid Arabica coffee were 36 and 42 for *Eugenioides* and Robusta genomes, respectively. The number of alleles per marker ranged from one to eight with an average of 2.77 for *Eugenioides* genome while it ranged from one to nine for Robusta genome with an average of 3.23 alleles per marker. Thirty alleles out of 36 alleles and 41 alleles out of 42 alleles were polymorphic in the *Eugenioides* and Robusta genome part, respectively. The average polymorphism information contents were 0.22 and 0.25 for *Eugenioides* and Robusta genomes, respectively. In addition the different genetic parameters for each SSRs also showed differences between Ethiopian accessions and cultivated commercial cultivars with the larger values in the former group. These larger values for Robusta genome in the Arabica coffee could be either due to the high polymorphoric nature of the *C. canephora* species and/or due to the involvement of a limited number of *C. eugenioides* species in the natural crossing during early speciation time of *C. arabica*.

Genetic diversity of Arabica coffee

Allelic richness and uniqueness

Genetic richness of Arabica coffee in Ethiopian accessions was observed that can be used as potential source in improvement programs, while the commercial cultivars showed less SSRs polymorphism (Table 3). Of the total 209 alleles, 189 alleles were detected in Ethiopian accessions, while only 112 alleles were detected in cultivated group. Of the total alleles detected in Ethiopian accessions, 175 alleles were polymorphic alleles, while only 90 alleles were polymorphic in cultivated group. Ninety seven alleles of the total alleles were specific to Ethiopian accessions while only 20 alleles were specific to cultivated group. Eleven of the 20 specific alleles in the cultivated group were detected in Catimor, Sarchimor, Hybrido de Timor and other introgressed genotypes. They are robusta gene introgressed Arabica genotypes (Eskes and Leroy, 2004). When the introgressed alleles were not taken into consideration, only 101 alleles were observed in cultivated varieties. These specific introgressed alleles also contribute about 5.3 % of the total polymorphic alleles in the commercial cultivars. The size of these introgressed specific alleles is similar to the size of alleles in *C. canephora* species outside the genome region of those alleles detected in Ethiopian accessions and other non-introgressed cultivated genotypes. Probably these genotypes have obtained these specific alleles from Robusta species during the introgression process to improve one of their agronomic characters of interests.

This result is in agreement with the early history of Arabica coffee distribution when the commercial cultivars have undergone successive genetic reductions (Anthony *et al.*, 2002). Historical data indicated that the Arabica coffee populations in major

producing countries were derived from few plants and/or seeds taken from Ethiopia during the early centuries. This could be the main factor for the low allelic richness and less polymorphism of the commercial cultivars. According to Moncada and McCouch (2004), the cultivated tetraploids were embodied approximately three fourth the amount of SSR diversity as the wild tetraploids based on the number of alleles, PIC values and similarity coefficients. Relatively high level of SSR polymorphism and genetic richness were also reported in Arabica coffee from Ethiopia (Anthony *et al.,* 2002; Moncada and McCouch, 2004). Similar results were also reported using other marker techniques such as RAPD (Lashermes *et al.,* 1996), AFLP (Lashermes *et al.,* 1996) and ISSR (Lashermes *et al.,* 1996).

Cluster analysis

Diversity representation of the overall studied coffee collections was also generated using the first and second components of principal component analysis (Figure 1). The first and the second principal components covered 28.7 and 8.2 % of the total variation, respectively, with a total of 36.9 %. The genetic relationship analysis revealed two clearly separated main clusters: one cluster for Ethiopian accessions (Cluster I) and the other for cultivated group (Cluster II). The Arabica coffee from Ethiopia were distributed in all scatter plots (with most the accessions in the third and fourth components) by making wider distribution as compared to the cultivated group. The cultivated grouped closely indicating their close similarity genetic similarity. The observed diversity representation supported the hierarchical clustering (Dendrogram*)* generated using the Ward's minimum variance by grouping the individual coffee collections into two main clusters: one cluster for Ethiopian accessions and the other cluster for cultivated group (Figure 2). According to Moncada and McCouch (2004), the wild Ethiopian tetraploids were scattered in three of the four quadrants of the PCA and most were genetically differentiated from the cluster of cultivated cultivars. Lashermes *et al.* (1996) also indicated the narrow genetic variations of commercial varieties using RAPD. The result from this study also confirms the presence of wide genetic variation in Ethiopian accessions: genetically distant from each other and from their cultivated relatives, and the very closure similarity of the commercial cultivars.

Table 4. Number of total alleles and polymorphism information contents (PIC) in the two genome of Arabica coffee

No	SSR Code	PCR size range (bp)	Sample types													
			Ethiopian accessions					Cultivated CCCAs group					Overall				
			Number of alleles			PIC		Number of alleles			PIC		Number of alleles			PIC	
			EG[a]	RG[b]	Total	EG	RG	EG	RG	Total	EG	RG	EG	RG	Total	EG	RG
1	SSR124577	138-157	2	1	3	0.44	0	2	2	3	0.45	0.02	2	2	3	0.49	0.01
2	SSR122850	132-141	1	2	3	0	0.37	1	1	2	0	0	1	2	3	0	0.5
3	SSR124195	83-101	2	1	3	0.25	0	1	1	2	0	0	2	1	3	0.16	0
4	SSR123557	206-270	3	2	4	0.49	0.17	2	1	2	0.03	0	4	2	4	0.37	0.12
5	ssrCMA008	106-128	1	4	4	0	0.43	1	4	5	0	0.3	1	6	7	0	0.41
6	ssrCMA059	129-165	8	2	10	0.58	0.02	2	1	3	0.02	0	8	2	10	0.6	0.01
7	ssrCMA198	195-236	1	6	7	0	0.6	1	4	5	0	0.15	1	8	9	0	0.49
8	ssrAY2434	178-199	1	3	4	0	0.52	1	3	4	0	0.47	1	3	4	0	0.54
9	ssrA8847	159-192	8	2	10	0.58	0.02	2	1	3	0.04	0	8	2	10	0.6	0.01
10	ssrR105	187-222	2	7	9	0.5	0.54	2	4	6	0.02	0.02	2	9	11	0.45	0.59
11	ssrR209	161-173	1	2	3	0	0.35	1	1	2	0	0	1	2	3	0	0.25
12	ssrR268	131-147	2	1	3	0.3	0	1	2	3	0.02	0.07	4	2	5	0.23	0.02
13	ssrR338	221-233	1	2	3	0	0.33	1	1	2	0	0	1	2	3	0	0.24
Total alleles			33	35	66	3.14	3.35	19	27	42	0.58	1.03	36	42	75	2.9	3.19
Number of non-polymorphic alleles			6	3	9			7	7	14			6	1	7		
Number of polymorphic alleles			27	32	56			12	20	28			30	41	68		
Rate of Polymorphism (%)			81.8	91.4	86.4			63.2	74.1	66.7			83.3	97.7	90.4		
No. SSRs			13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
Average			2.5	2.7	5.1	0.24	0.26	1.5	2.1	3.2	0.04	0.08	2.8	3.2	5.6	0.22	0.25

[a] EG=*C. eugenioides* genome in Arabica coffee, [b] RG=*C. robusta* Genome in Arabica coffee

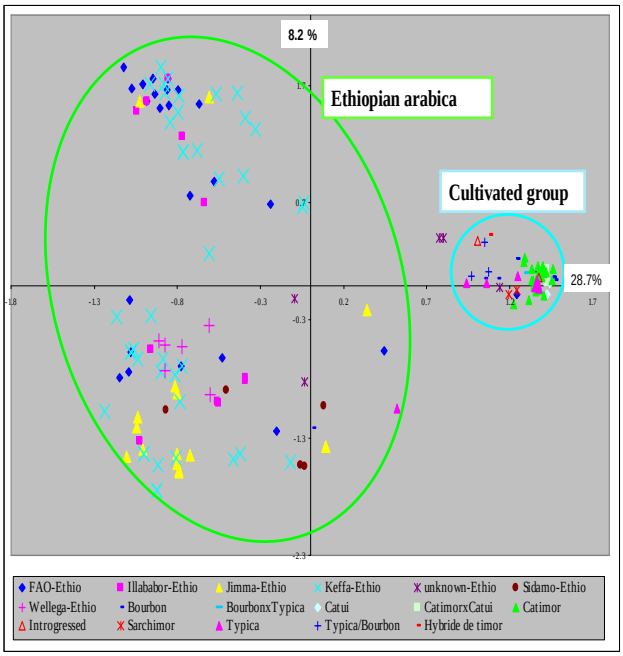

Figure 1. Diversity of Arabica coffee individuals based on the first and second components of PCA.

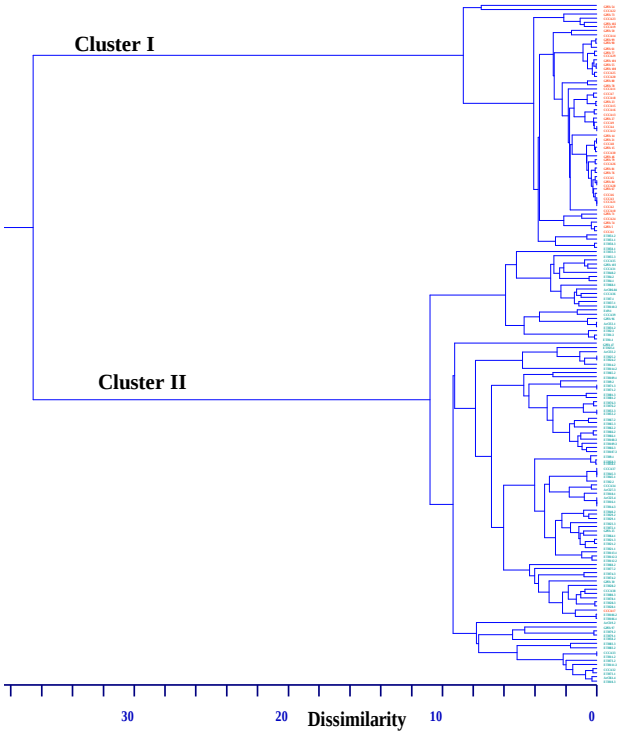

Figure 2. Dendrogram obtained by Ward's minimum variance among Arabica coffee collections based on 32 SSR markers (green colour = Ethiopian Arabica coffee, and red colour = cultivated varieties).

In conclusion, the genetic diversity of Arabica coffee collections (Ethiopian accessions and commercial cultivars) were studied using SSR markers. The result indicated that Arabica coffee accessions collected from its centre of origin, Ethiopia, are genetically more diverse and rich with a lot of specific alleles than commercial cultivars. The potential of SSRs markers to clearly differentiate coffee genotypes from different geographical origin suggests the possibility to use in quality control (DNA-based traceability) of Ethiopian premium specialty coffees known by their areas of production in Ethiopia. The information from this study can be used to utilise coffee genetic resources in sustainable ways, which can be conservation in its wild natural habitat or field gene banks, development of core collection and improved varieties, and other applications at molecular level such as Arabica coffee genetic map development and QTL detections to be used in marker assisted selection. In addition to SSR marker, other molecular marker techniques such as RAPD, AFLP and SNPs should also be used to get the maximum benefits from the application of molecular techniques.

ACKNOWLEDGMENTS

The authors duly acknowledge Nestle R&D Tours, France for the financial support, laboratory facilities and provision of plant materials. We also greatly thank JARC to provide us the Ethiopian Arabica coffee accessions used in this study.

REFERENCES

Aga, E, Bryngelsson, T, Bekele E. and Solomon B. 2003. Genetic diversity of forest Arabica coffee (*Coffea arabica* L.) in Ethiopia as revealed by random amplified polymorphic DNA (RAPD) analysis. *Hereditas* **138(1)**:36-46.

Amaha, M and Bellachew, B.1987. Genotype-Environmental interactions in coffee (*Coffea arabica* L.). *ASIC 12th*. pp. 476-481. Montreux.

Anthony, F, Bertrand, B, Quiros, O, Wilches, A, Lashermes, P, Berthaud. J and Charrier A. 2001a. Genetic diversity of wild coffee (*Coffea arabica* L.) using molecular markers. *Euphytica* **118**:53–65.

Anthony, F, Combes, MC, Astorga. C, Bertrand, B, Graziosi, G and Lashermes, P. 2002. The origin of cultivated *Coffea arabica* L. varieties revealed by AFLP and SSR markers. *Theory of Applied Genetics* **104**:894– 900.

Anthony, F, Combes, MC, Herrera, JC, Prakash, NS, Berthand, B and Lashermes, P. 2001b. Genetic Diversity and introgression analysis in coffee (*Coffea arbica* L.) using molecular markers. *ASIC 19th*. Trieste, Italy.

Bellachew, B. 1997. Arabica coffee breeding in Ethiopia: A Review. *ASIC 17th*. Nairobi, Kenya.

Berthaud, J and Charrier A. 1998. Genetic Resources of Coffea. pp. 1-42. *In* Clarke RJ and Marcrae R (eds). Coffee Vol.4 Agronomy, Elsevier Applied Science, London and New York.

Berthou, F and Trouslot P. 1979. Analysis of Enzymatic polymorphism in the genus Coffea: Adaptation of an electrophoresis method in serious: first results. *ASIC 18th*. Abidjan. pp. 373 – 383.

Carneiro, M.F. 1999. Advances in coffee biotechnology. *AgBiotechNet* **1**:1-8.

Carvalho, V.P. (1988). Principles and practices of coffee plant breeding for productivity and quality factors: *Coffea arabica*. pp.1-42. *In* Clarke RJ and Marcrae R (eds). Coffee Vol.4 Agronomy. Elsevier Applied Science, London and New York.

Charrier, A. 1980. Conservation of the genetic resources of the Genus Coffea. *ASIC 9th*. Londres. pp. 507-518.

Charrier, A and Eskes, AB. 2004. Botany and Genetics of coffee. pp. 25-55. *In* Wintgens JN (ed). Coffee: growing, processing, sustainable production. Wiley.VCH, Weiheim.

Combes, MC, Andrzejewski, S, Anthony, F, Bertrand, B, Rovelli, P, Graziosi, G and

Lashermers, P. 2000. Characterization of microsatellite loci in *Coffea arabica* and related coffee species. *Molecular Ecology* **9**:1171-1193.

Coulibaly, I, Louarn, J, Lorieux, M, Hamon, S and Noirot, M. 2001. Genetic map of a backcross between *C. canephora* P. and *C. heterocalyx* and autogamy gene location. *ASIC 19th*. Trieste, Italy.

Coulibaly, I, Noirot, M, Lorieux, M and Charrier, A. 2002. Introgression of self-compatibility from *Coffea heterocalyx* to cultivated species *Coffea canephora*. *Theory of Applied Genetics* **105**:994-999.

Cristancho, MA, Chaparro, AP, Cortina, HA and Gaitàn, AL. 2004a. Genetic variability of *Coffea arabica* L. accessions from Ethiopia evaluated with RAPDs. *ASIC 20th*. Bangalore, India.

Cristancho, MA, Gaitàn, AL, and McCouch, SR. 2004b. Analysis of expressed sequence tags (ESTs) and Microsatellite sequences in coffee (Coffea arabica). *ASIC 20th*. Bangalore, India.

Crouzillat, D, Rigoreau, M, Bellanger, L, Priyono, P, Mawardi, S, Ahrudi, SY, McCarthy, J, Tanksley, S, Zaenudin, I and Petiard, V. 2004. A Robusta consensus genetic map using RFLP and microsatellite markers for the detection of QTL. *ASIC 20th*. Bangalore, India.

Denich, M and Gatzweiler, F. 2006. Wild Arabica coffee in the montane rainforests of Ethiopia: An invaluable resource for coffee breeding. *ASIC 21st*. Montpellier, France.

Dufour, M, Hamon, P, Noirot, M, Risterucci, AM, Brottier, P, Vico, V and Leroy,T. 2001. Potential use of SSR markers for *Coffea Spp*. Genetic mapping. *ASIC 19th*. Trieste, Italy.

Eskes, AB and Leroy, Th. 2004. Coffee Selection and Breeding. Pp. 57-86. *In* Wintgens J N (ed). Coffee: Growing, Processing, Sustainable Production: A Guidebook for Growers, Processors, Traders, and Researchers. Welley-VCH Verlag GmbH & KGaA.

Geletu, KT, Govers, K, Bekele, E and Borsch, T. 2006. Genetic Diversity of Wild *Coffea arabica* in Ethiopia: Analyses based on plastid, ISSR and Microsatellite markers. *ASIC 21st*. Montpellier, France.

Gole, TW. 2003. Vegitation of the Yayu Forest in SW Ethiopia: Impacts of Human Use and Implication for *In Situ* Conservation of Wild *Coffea arabica L.* Populations. Ecology and Development Series No.10, Cuvillier Verlag, Gottingen.

Gole, T.W, Denich, M, Teketay, D and Vlek, P.L.G. 2002. Human impacts on *Coffea arabica* genetic pool in Ethiopia and the need for its *in-situ* conservation. pp. 237-247. *In* Engels, T, Ramanatha Rao,V, Brown, AHD and Jackson M (eds). Managing plant genetic diversity. CAB International/ IPGRI.

Herrera, JC, Combes, MC, Anthony, F and Lashermes, P. 2001. Efficient use of coffee genetic resources: Molecular analyses of genome interactions in the arbusta hybrid (*Coffea arabica* X C. *canephora*). *ASIC 19th*. Trieste, Italy.

Hintze, J. (2006). NCSS, PASS and GESS. NCSS. Kaysville, Utah.

Lashermes, L, Trouslot, P, Anthony, F, Combes, MC and Charrier, A. 1996. Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica. Euphytica* **87**:59-64.

Lashermes, P, Combes, MC, Cros, J, Trouslot, P, Anthony, F and Charrier, A. 1995. Origin and genetic diversity of *Coffea Arabica* L. based on DNA molecular markers. *ASIC, 16th*. Kyoto.

Lashermes, P, Comes, MC, Trouslot, P, Anthnony, F, Hamon, S and Charrier, A. 1999. Molecular characterization and origin of the Coffea Arabica L. genome. *Molecular Genomics and Genetics* **261**:259-266.

Lashermes, P, Agwanda, CO, Anthony, F, Combes, MC, Trouslot, P and Charrier, A.1997. Molecular marker-assisted selection: A powerful approach for coffee improvement. *ASIC 17th*. Nairobi, Kenya.

Lin, C, Mueller, LA, McCarthy, J, Crouzillat, D, Petiard, V and Tanksley, SD. 2005. Coffee and tomato share common gene repertoires as revealed by deep sequencing of seed and cherry transcripts. *Theory of Applied Genetics* **112**:114-130.

Maluf, MP, Silvestrini, M, Ruggiero, Ruggiero, LM. de C, Filho, OG and Colombo, CA. 2005. Genetic diversity

of cultivated *Coffea Arabica* inbred lines assessed by RAPD, AFLP and SSR marker systems. *Science Agriculture (Piracicaa, Braz.)* **62(4)**:373 -373.

Manifesto, MM, Schlatter, AR, Hopp, HE. Suarez, EY and Dubcovsky, J. 2001. Quantitative Evaluation of genetic diversity in Wheat germplasm using molecular markers. *Crop Science* **4**:682-690.

Moncada, P. 2004. Characterization of simple sequence length polymorphisms (SSLP) in a sample of *Coffea spp*. Germplasm. *ASIC 20th*. Bangalore, India.

Moncada, P and McCouch S. 2004. Simple sequence repeats diversity in diploid and tetraploid *Coffea* species. *Genome* **47**:501-509.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci.* USA. Vol. 70, No. 12, pp. 3312-3323.

Orozco-Castillo, C, Chalmers, K.J, Waugh, R and Powell, W. 1994. Detection of genetic diversity and selective gene introgression in coffee using RAPD markers. *Theory of Applied Genetics* **87**:934-940.

Prakash, SN, Combes, MC, Naveen, SK, Graziosi, G and Lashermes, P. 2001. Application of DNA marker technologies in characterizing genome diversity of selected coffee varieties and accessions from India. *ASIC 19th*. Trieste, Italy.

QIAGEN. 2006. DNeasy® Plant Handbook **(http://www.qiagen.com).**

Raus, PM, Raus, CF, Rampim, L, Carvalho, VP, Raus, EA and Sera, T. 2003. Genetic relationship in *Coffea* species and parentage determination of interspecific hybrids using ISSR (Inter-Simple Sequence Repeat) markers. *Genetics and Molecular Biology* **26(3)**:319-327.

Rovelli, P, Mettulio, R, Anthony, F, Anzueto, F, Lashermes, P, and Graziosi, G. 2000. Microsatellite in *Coffea arabica* L. pp.123–133. *In* Sera T, Soccol CR, Pandey A and Roussos S (eds) Coffee biotechnology and quality. Kluwer Academic Publishers, Dordrecht, Netherlands.

Schmitt, C, Senbeta, F, Denich, MN, Preisinger, H, Woldemariam, T and Demissew, S. 2005. Sustainable management of the montane rainforests with wild coffee *(Coffea arabica* L.) in the Bonga region of southwest Ethiopia. Conference on International Agricultural Research for development. October 11-13, 2005. Tropentag, Stuttgart-Hohenheim, Germany.

Selvi, A, Nair, NV, Balasundaram, N and Mohapatra, T. 2003. Evaluation of maize microsatellite markers for genetic diversity analysis and fingerprinting in sugarcane. *Genome* **46**:393-403.

Senbeta, F and Denich, M. 2006. Effects of wild coffee management on species diversity in the Afromontane rainforests of Ethiopia. *Forest Ecology and Management* **23(2)**:68-74.

Silvarolla, MB, Mazzafera, P and Alves de Lima, MM. 2000. Caffeine content of Ethiopian *Coffea arabica* beans. *Genetics and Molecular Biology*. **23(1)**:213-215.

Silvarolla, MB, Mazzafera, P and Fazvoli, LC. 2004. A naturally decaffeinated Arabica coffee. *Nature.* 429. Pp 826.(www.nature.com/naturre).

Steiger, DL, Nagai, C, Moore, PH, Morden, CW, Osgoog, RV and Ming, R. 2002. AFLP analysis of genetic diversity within and among *Coffea arabica* cultivars. *Theory of Applied Genetics* **185**:189-215.

Weising, K, Nybom, H, Wolff, K and Kahl, G. 2005. DNA Fingerprinting in Plants: Principles, Methods, and Applications. 2nd ed. CRC Press, Taylor and Francis Group.