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AFLP analysis of genetic diversity within and among *Coffea arabica* cultivars

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Abstract Genetic diversity of *Coffea arabica* cultivars was estimated using amplified fragment length polymorphism (AFLP) markers. Sixty one *Coffea* accessions composed of six arabica cultivars, including Typica, Bourbon, Catimor, Catuai, Caturra and Mokka Hybrid, plus two diploid *Coffea* species, were analyzed with six *Eco*RI–*Mse*I primer combinations. A total of 274 informative AFLP markers were generated and scored as binary data. These data were analyzed using cluster methods in the software package NTSYSpc. The differences among cultivars at the DNA level were small, with an average genetic similarity of 0.933. Most accessions within a cultivar formed a cluster, although deviant samples occurred in five of the six cultivars examined due to residual heterozygosity from ancestral materials. Among the six cultivars fingerprinted, the highest level of genetic diversity was found within the cultivar Catimor, with an average genetic similarity of 0.880. The lowest level was found within Caturra accessions, with an average genetic similarity of 0.993. Diversity between *C. arabica* and two other *Coffea* species, *Coffea canephora* and *Coffea liberica*, was also estimated with average genetic similarities of 0.540 and 0.413, respectively, suggesting that *C. canephora* is more closely related to *C. arabica* than is *C. liberica*. The genetic variation among arabica cultivars was similar to the variation within cultivars, and no cultivar-specific DNA marker was detected. Although arabica cultivars appear to have a narrow genetic base, our results show that sufficient polymorphism can be found among some arabica cultivars with a genetic similarity as low as 0.767 for genetic/QTL mapping and breeding. The assessment of genetic diversity among arabica cultivars provided the necessary information to estimate the potential for using marker-assisted breeding for coffee improvement.

Keywords *Coffea arabica* · Genetic diversity · AFLP · Molecular phylogeny

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Introduction

Coffee belongs to the genus *Coffea* in the Rubiaceae family, and is mostly grown in tropical and subtropical regions (Berthaud and Charrier 1988). Commercially important coffee species are *Coffea arabica* L. (arabica coffee) and *Coffea canephora* P. (robusta coffee). Of the approximately 100 species within the genus, only *C. arabica* is tetraploid (2n = 44) and self-fertile, while all other *Coffea* species, including *C. canephora*, are diploids (2n = 22) and self-sterile. Arabica coffee is known to produce a high quality beverage and originated in southwestern Ethiopia with the center of genetic diversity remaining in that region. Robusta coffee is used to make instant coffee and originated in central and western equatorial Africa (Ferwerda 1976).

The first migration of *C. arabica* was from Ethiopia to Yemen as part of the prehistoric trade. The introduction of *C. arabica* to the other continents first occurred from Yemen to the Malabar coast of India, and from there to Ceylon and Java in the last decade of the 17th century. A single *C. arabica* plant from Java was taken to and grown at the botanical garden of Amsterdam in 1706. Seedlings from this plant, subsequently named "Typica", were brought to Martinique and from there to South America. Other *C. arabica* materials collected by the French from Yemen were brought to Reunion (previously Bourbon Island) and from there also on to South America as the cultivar "Bourbon". These introductions involved small numbers of plants that resulted in a nar-

row genetic base for arabica coffee cultivars cultivated worldwide (Ferwerda 1976).

Molecular markers have been used to study the phylogenetic relationship of *Coffea* germplasm. Isozymes were the first molecular markers applied to *coffea* with moderate success, which could not distinguish between types within the species *C. arabica* (Berthou and Trouslot 1979; Berthaud and Charrier 1988). Taxonomic relationships within the genus *Coffea* have since been studied using chloroplast and mitochondria DNA variation (Berthou et al. 1983), random amplified polymorphic DNA (RAPD) and organelle-specific PCR markers (Orozco-Castillo et al. 1996). Limited studies have been published about the genetic relationships in *C. arabica* genotypes that are of the most commercial interest. Orozco-Castillo et al. (1994) distinguished the arabica cultivar groups Typica and Bourbon using RAPD markers. RAPD markers have also been used for detecting genetic diversity among wild arabica coffee accessions, six Ethiopian cultivars, and two Typica- and Bourbon-derived accessions (Lashermes et al. 1996; Anthony et al. 2001).

Amplified fragment length polymorphism (AFLP) markers are extensively used for studying genetic diversity in different plant species (Vos et al. 1995; Maughan et al. 1996; Ellis et al. 1997; Breyne et al. 1999; Erschadi et al. 2000). Comparative studies using restriction fragment length polymorphism (RFLP), RAPD, AFLP and microsatellites have shown that AFLP is the most-efficient method to estimate genetic diversity because of their high reproducibility and multiplex ratio (Powell et al. 1996; Russell et al. 1997; Pejic et al. 1998). These authors also reported that the estimates based on RFLP, AFLP and microsatellites are highly correlated, whereas the correlations of RAPD marker data with the other three types of markers were significantly lower.

Seventy percent of the world coffee production is from *C. arabica*. A thorough understanding of the genetic diversity of arabica cultivars is critical to future coffee improvement. This project was designed to explore the genetic variation within and between arabica cultivars using AFLP markers because of their increased yield of genetically variable loci and high level of reliability. The objectives of this coffee DNA fingerprinting project were: (1) to evaluate genetic diversity among arabica cultivars using AFLP markers; (2) to explore the possibility of using AFLP markers for cultivar identification; (3) to estimate the genetic distances among parental coffee cultivars for a coffee breeding program in order to evaluate the potentials for cultivar improvement through breeding, and (4) assess relative levels of genetic similary among *C. arabica*, *C. canephora*, and *Coffea liberica*.

Materials and methods

Plant material

A total of 58 accessions of arabica coffee and three accessions of diploid *Coffea* species were collected in Hawaii for DNA fingerprinting. Most samples studied are cultivated on different islands, and detailed collection sites of these samples are listed in Table 1. More than two accessions of each arabica cultivar were used in the analysis to allow for the estimation of standard deviation within a cultivar, except for Caturra (only two accessions) because it is one of the parental cultivars of Catuai. The coffee germplasm maintained at the Hawaii Agriculture Research Center's Kunia and Maunawili Stations on the Island of Oahu was collected worldwide by scientists from the University of Hawaii in the early 1960s or imported by various growers in 1980s (Nagai et al. 2001).

DNA Isolation

Young coffee leaves were collected and lyophilized over a period of 2–3 days for DNA extraction. A modified version of the extraction protocol described by Chitenden et al. (1994) was followed. Lyophilized tissue was ground to a fine powder with a Udy sample mill (Udy Corp, Ft. Collins, Colo. USA). Ground leaf tissue was added to a 50-ml centrifuge tube to the 7-ml mark, and 30-ml of 65 °C extraction buffer (100 mM Tris, pH 8.0, 50 mM EDTA, pH 8.0, 500 mM NaCl, 1.25% SDS, 2% PVP-40, and 27.5 mM $NaHSO_3$) was added. Samples were mixed thoroughly by vortexing and placed in a 65 °C water-bath for 1 h with periodic mixing. Nine microliters of 5 M KoAc was added to each tube. The tube was inverted several times and placed on ice for 20 min. Samples were then centrifuged at 2,800 g for 20 min at 4 °C. After centrifugation, the supernatant was removed from the cellular debris by filtering through Miracloth (Calbiochem) to a new tube containing 20 ml of ice-cold isopropanol (–20 °C). Samples were stored at –20 °C for 2 h. The DNA was then spooled out and placed in 1 ml of purifying buffer (70% ethanol, 0.3 M NaOAc) and stored at –20 °C overnight. After removing the purifying buffer, the pellets were rinsed in 70% ethanol, air dried, and resuspended in 300–500 µl of TE. RNA was removed with 50 µg of RNAse A and incubated at 37 °C for 30 min. Further purification was achieved by a phenol:chloroform:isoamyl alcohol (25:24:1) extraction followed by a chloroform:isoamyl alcohol (24:1) extraction. Two vol of 95% ethanol and 1/25 vol of 5 M NaCl were added to the extracted DNA then incubated at –20 °C for 30 min and centrifuged at 15,300 g for 15 min. The pellets were rinsed in 70% ethanol, air-dried, and resuspended in TE. DNA concentration was estimated by comparison to serial dilutions of a lambda DNA standard in a 1.0% agarose gel.

AFLP analysis

Genomic DNA digestion

AFLP reactions were performed according to the protocol of Vos et al. (1995) with the modification that 250 ng of DNA were digested at 37 °C for 3 h with 5 U each of *Eco*RI and *Mse*I.

Table 1 List of 61 *Coffea* accessions analyzed by AFLP markers

Accession	Identity	Collection site	Cultivar/species
Bourbon1	Bourbon Vermelho	Maunawili, Oahu	Bourbon Vermelho
Bourbon2	Bourbon selection	Maunawili, Oahu	Bourbon
Bourbon3	Pink Bourbon	Maunawili, Oahu	Bourbon
Bourbon4	Yellow Bourbon	Kunia, Oahu	Bourbon
Catimor1	5175-6-8	Kunia, Oahu	Catimor
Catimor2	5175-7	Kunia, Oahu	Catimor
Catimor3	5175-7-1	Kunia, Oahu	Catimor
Catimor4	5175-7-4	Kunia, Oahu	Catimor
Catimor5	5175-7-5	Kunia, Oahu	Catimor
Catimor6	8667-5	Kunia, Oahu	Catimor
Catimor7	8667-5-3	Kunia, Oahu	Catimor
Catimor8	8667-6-4	Kunia, Oahu	Catimor
Catuai1	Catuai-92	Maunawili, Oahu	Red Catuai
Catuai2	KA19-3	Kunia, Oahu	Red Catuai
Catuai3	KA19-C4	Kunia, Oahu	Red Catuai
Catuai4	MA7-1	Kunia, Oahu	Red Catuai
Catuai5	MO21-C4	Kunia, Oahu	Red Catuai
Catuai6	Catuai	Kunia, Oahu	Red Catuai
Catuai7	KA16-C4	Kunia, Oahu	Yellow Catuai
Catuai8	KA17-C3	Kunia, Oahu	Yellow Catuai
Catuai9	KA17-5	Kunia, Oahu	Yellow Catuai
Catuai10	Yellow Catuai	Kunia, Oahu	Catuai
Caturra1	MO29-7	Kunia, Oahu	Caturra
Caturra2	Red Caturra	Kunia, Oahu	Caturra
MH1	OA12-15	Kunia, Oahu	Mokka hybrid
MH2	OA12-C6	Kunia, Oahu	Mokka hybrid
MH3	OA13-13	Kunia, Oahu	Mokka hybrid
MH4	MA1-10	Kunia, Oahu	Mokka hybrid
MH5	MA1-12	Kunia, Oahu	Mokka hybrid
MH6	MA1-9	Kunia, Oahu	Mokka hybrid
MH7	OA11-C4	Kunia, Oahu	Mokka hybrid
MH8	OA13-C3	Kunia, Oahu	Mokka hybrid
Typica-G1	Guatemalan-1	Kunia, Oahu	Typica
Typica-G2	Guatemalan-2	Kunia, Oahu	Typica
Typica-H1	Farm1-1	Hilo, Hawaii	Typica
Typica-H2	Farm1-2	Hilo, Hawaii	Typica
Typica-H3	Farm1-3	Hilo, Hawaii	Typica
Typica-H4	Farm1-4	Hilo, Hawaii	Typica
Typica-H5	Farm1-5	Hilo, Hawaii	Typica
Typica-H6	Farm2-1	Hilo, Hawaii	Typica
Typica-H7	Farm2-2	Hilo, Hawaii	Typica mutant
Typica-H8	Farm2-3	Hilo, Hawaii	Typica
Typica-K1	Farm3-1	Kona, Hawaii	Typica
Typica-K2	Farm4-1	Kona, Hawaii	Typica
Typica-K3	Farm5-1	Kona, Hawaii	Typica
Typica-K4	Farm5-2	Kona, Hawaii	Typica
Typica-K5	Farm6-1	Kona, Hawaii	Typica
Typica-K6	Farm6-2	Kona, Hawaii	Typica
Typica-K7	Farm7-1	Kona, Hawaii	Typica
Typica-K8	Farm7-2	Kona, Hawaii	Typica
Typica-K9	Farm8-1	Kona, Hawaii	Typica
Typica-K10	Farm8-2	Kona, Hawaii	Typica
Typica-K11	Farm8-3	Kona, Hawaii	Typica mutant
Typica-K12	Farm9-1	Kona, Hawaii	Typica
Typica-K13	Farm9-2	Kona, Hawaii	Typica
Typica-K32	KO32-C2	Kunia, Oahu	Typica
Typica-K33	KO33	Kunia, Oahu	Typica
Typica-K34	KO34-9	Kunia, Oahu	Typica
C. canephora	*C. canephora*	Kunia, Oahu	Diploid species
C. liberica	*C. liberica*	Maunawili, Oahu	Diploid species
Deweveri	*C. liberica*	Kona, Hawaii	Diploid species

Table 2 List of AFLP primers used in DNA fingerprinting of coffee cultivars

Code	Primer combination	Polymorphic bands
E01M01	E-AAC, M-CAA	57
E01M07	E-AAC, M-CTG	60
E02M02	E-AAG, M-CAC	42
E03M01	E-ACA, M-CAA	67
E04M03	E-ACC, M-CAG	27
E08M07	E-AGG, M-CTG	21
Total polymorphic bands		274

Adapter ligation and pre-amplification

Adapters and primers (listed in Table 2) were synthesized by Operon Technologies. Preamplification was performed as described by Vos et al. (1995) except that 1 unit of *Taq* polymerase (Promega) was used.

Primer labeling and selective amplification

*Eco*RI and *Mse*I primers containing three selective nucleotides were obtained from Life Technologies. Reactions were performed according to the manual in the AFLP Analysis System I (Life Technologies) except that gamma ^{32}P-ATP (6,000 Ci/mmol) was used. An initial screen of 64 primer combinations was performed and those yielding banding patterns with good resolution and a high rate of polymorphism were selected for analysis (Table 2).

Gel analysis

To each PCR product was added 20 µl of formamide dye (98% formamide, 10 mM EDTA, 0.005% xylene cyanol FF, and 0.005% bromophenol blue), and the samples were denatured at 95 °C for 3 min. Three microliters of sample were loaded onto a pre-warmed 5% polyacrylamide gel and run for 2.5 h at 105 W. Gels were transferred to 3 MM Whatman paper and vacuum dried for 1–1.5 h at 80 °C, then exposed to X-ray film at –80 °C for 1–2 days with one intensifying screen.

Data analysis

Polymorphic AFLP markers were manually scored as binary data with presence as "1" and absence as "0". Monomorphic markers were not scored. The data for the 61 accessions were used to compute pair-wise simple matching coefficients among cultivars and species (Sokal and Michener 1958). Cluster analysis was performed on the similarity matrix employing the "unweighted pair group method using arithmetic means" (UPGMA) algorithm (Sneath and Sokal 1973) provided in the computer program NTSYSpc, version 2.1 (Exeter Software Co., New York). The cophenetic correlation coefficient was calculated to test the goodness of fit between the similarity and the cophenetic matrices.

Results

The polymorphism rates of AFLP primers were evaluated using five arabica accessions: Typica, Yellow Bourbon, Mokka Hybrid, Yellow Catuai and Catimor. Among the 24 sets of *Eco*RI/*Mse*I primers with the three nucleotides extension surveyed, the six most polymorphic sets were selected for genotyping 61 coffee accessions. Each primer set generated 21 to 67 markers (Table 2), for a total of 274.

Pair-wise comparison of genetic similarity (percentage of matched markers) among coffee cultivars and species revealed narrow genetic diversity within arabica cultivars (Fig. 1). About 86% of the pair-wise comparisons among 58 arabica accessions exhibited a genetic similarity greater than 0.9, and less than 1% showed a genetic similarity lower than 0.8 (Fig. 2). The mean genetic similarities within each cultivar varied from 0.880 to 0.969 with the similarity among specific pairs of individuals ranging from 0.802 to 1.0 (Table 3). The least-variable cultivar appears to be Typica, with nearly 97% of the genetic content shared among 26 accessions, and complete genetic identity for the 274 markers was found among Typica samples K2 and K3, as well as samples

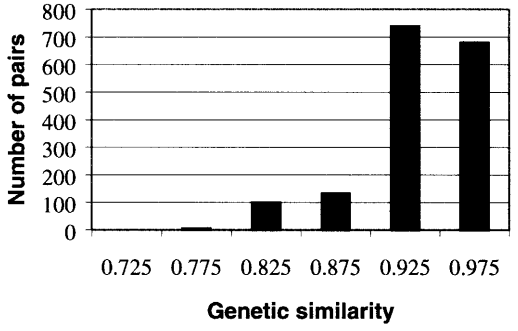

Fig. 1 Pair-wise comparison of genetic similarity among arabica cultivars

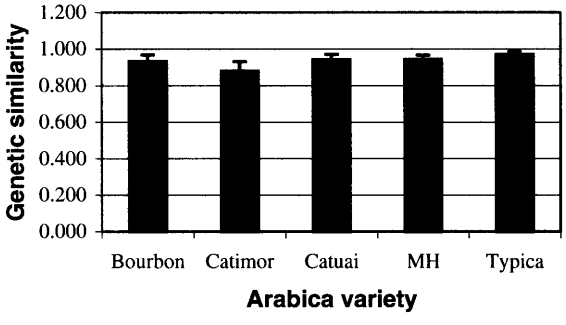

Fig. 2 Average genetic similarity within each cultivar

Table 3 Average genetic similarity within each arabica coffee cultivar

Item	Bourbon	Catimor	Catuai	MH	Typica	Overall
Average	0.933	0.880	0.942	0.943	0.969	0.935
Standard deviation	0.036	0.051	0.030	0.023	0.017	0.040
Min.	0.886	0.802	0.855	0.884	0.891	0.767
Max.	0.996	0.977	0.993	0.985	1.000	1.000

Table 4 Average genetic similarity among coffee cultivars

Item	Bourbon	Catimor	Catuai	Caturra	MH
Catimor	0.890				
Catuai	0.935	0.887			
Caturra	0.949	0.907	0.958		
MH	0.925	0.878	0.928	0.937	
Typica	0.945	0.902	0.944	0.954	0.944

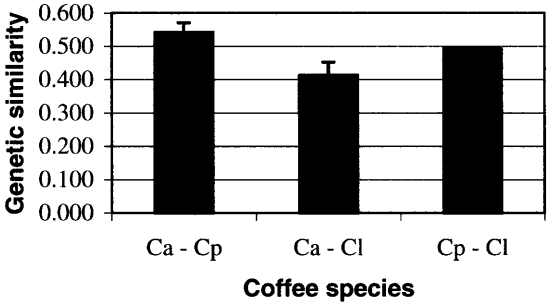

Fig. 3 Average genetic similarity between *Coffea* species

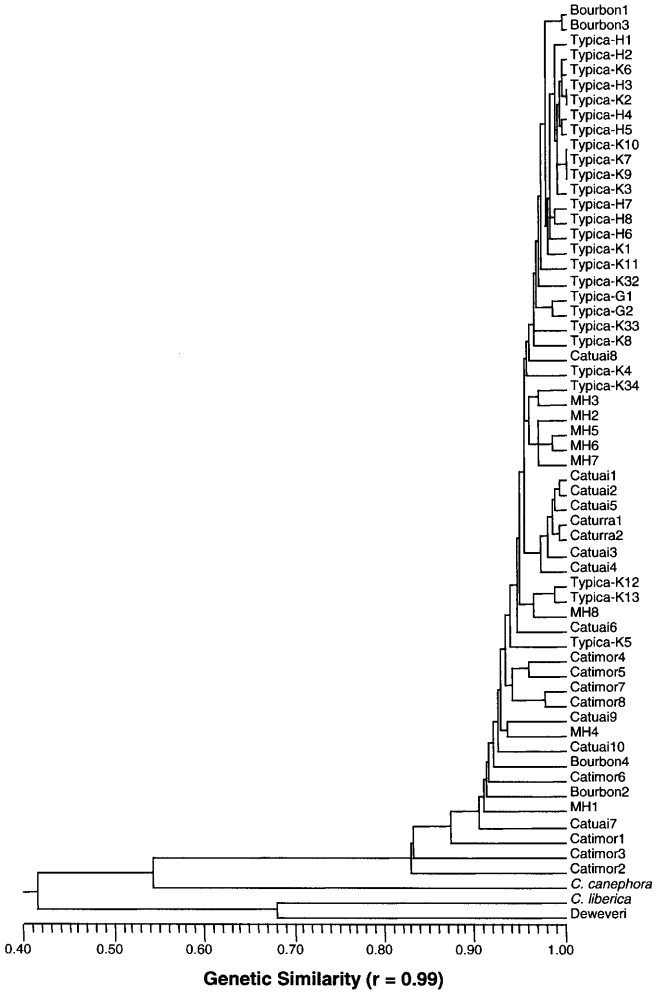

Fig. 4 Phenogram based on a simple matching coefficient of similarity among 61 *Coffea* accessions. Cophenetic correlation coefficient = 0.99

K7, K9 and K10. The most-variable cultivar was Catimor, with a mean genetic similarity of 0.880 among eight samples. Mean genetic similarities within Bourbon, Catuai and Mokka Hybrid were 0.933, 0.942 and 0.943, respectively.

The differences among cultivars at the DNA level were determined by comparing the genetic similarity. The mean genetic similarity among all 58 arabica accessions was 0.935 ranging from 0.767 to 1.0 (Table 4). Among the six cultivars fingerprinted, Caturra and Catuai are most similar with an average genetic similarity of 0.958. The genetic similarities between Caturra and Typica, Bourbon and Caturra, Bourbon and Typica, and Typica and Mokka Hybrid were at similar levels with averages of 0.954, 0.949, 0.945 and 0.944, respectively. The least similar cultivars were Catimor and Mokka Hybrid with an average genetic similarity of 0.878, but the genetic similarities between Catimor and both Catuai and Bourbon were also relatively low (0.887 and 0.890, respectively).

The differences between species were substantial (Fig. 3). *C. arabica* and *C. canephora* share the highest genetic similarity among the three species, with an average of 0.541. *C. arabica* and *C. liberica* share the lowest genetic similarity, with an average of 0.413. The genetic similarity between diploid species *C. canephora* and *C. liberica* fell in between at 0.492.

Cluster analysis of 61 coffee samples showed a clear separation of the coffee species. However, the differences among arabica cultivars were evident, but subtle. Six clusters that consist of two or more accessions can be distinguished (Fig. 4). The first cluster consists of 22 Typica accessions, two Bourbon accessions and one Catuai accession. The second cluster consists of five Mokka Hybrid accessions and one Typica accession. The third cluster consists of five Catuai and two Caturra accessions. The fourth cluster consists of two Typica accessions and one Mokka Hybrid accession. The fifth cluster includes four Catimor accessions. The sixth consists of two *C. liberica* accessions. A total of 12 accessions did not fall within any cluster, including two Bourbon, three Catuai, four Catimor, one Mokka Hybrid, one Typica and one *C. canephora*.

Discussion

Among the established arabica cultivars, distinctive and uniform morphological characters were observed, such as plant height, leaf shape and size, leaf tip color, branch angle and tree stature. However, the differences between cultivars at the DNA level were as limited as the differences within each cultivar. This is because many of the established arabica cultivars originated from single gene mutations (Krug and Carvalho 1951) or hybrids of established arabica cultivars. Typica is believed to be the primitive type of the species *C. arabica*, and Bourbon is very closely related to Typica. The cultivar Caturra is named after the single dwarf mutant derived from the Bourbon stock in Brazil (Krug et al. 1949). Mokka is derived from a complete recessive mutant *laurina* (*lr*) and an incomplete recessive mutant *mokka* (*mo*) with the double-mutant genotype *lrlrmomo* having small leaves, short internodes, a conical tree shape and the smallest seeds of any cultivar of *C. arabica* (Krug 1949; Carvalho et al. 1965). Maragogipe is derived from a dominant mutant found on a plantation in Maragogipe county, Brazil, in 1870. Yellow Catuai is a hybrid between Mundo Novo and Yellow Caturra that maintained the vigor of Mundo Novo and the dwarf gene *Caturra* (Bisco and Logan 1987). Catimor is derived from a cross between Caturra and Hybido de Timor, while the latter is a hybrid between *C. arabica* and *C. canephora* (Bisco and Logan 1987). The close relationship of these cultivars resulted in the high degree of genetic similarity detected by the DNA markers.

The genetic diversity among examined arabica cultivars was small. Nevertheless, six clusters were formed representing Typica, Mokka Hybrid, Catuai/Caturra and Catimor, and a diploid species *C. liberica*. Deviant samples were observed in five of the six cultivars most likely due to outcrossing and/or residual heterozygosity in ancestral materials. The high degree of genetic similarity between the two Caturra accessions (0.993) is probably not typical for this cultivar as a whole because of the limited number of samples included in this experiment. Typica samples collected from Kona and Hilo, on the Island of Hawaii, are believed to be primarily the progeny of Typica from Guatemala, with possibly some infusion of the old Typica cultivar that was imported earlier from Brazil. This cultivar showed the highest genetic similarity among the six arabica cultivars studied, indicating the narrow genetic base of the progenitor materials. Mokka Hybrid appears to be close to Typica, most likely because Typica is one of the parents from which Mokka Hybrid is derived (H.P. Medina-Filho, personal communication). The two Caturra accessions were clustered with five Catuai accessions; consistent with Caturra being one of the parents of Catuai. Catimor was derived from interspecific crosses between *C. canephora* (doubled chromosomes) and *C. arabica*, and is the most-variable group even though the samples were progeny of only two trees. The four Bourbon samples included in this project exhibited a significant amount of divergence among them. This divergence could have originated from the different sources from where these Bourbon plants were imported. Orozco-Castillo et al. (1994) reported clear separation between the Bourbon group (including Catimor, Catuai, Caturra, Mundo Novo) and the Typica group (including Typica, Timor, Blue Mountain, Pache) with the number of shared bands at about 50% using RAPD markers. Our results with AFLP markers suggested over 93% genetic similarity between the Typica and Bourbon groups.

The genetic variation between arabica cultivars was similar to the variation within cultivars. The genetic similarities between some varieties (most notably between Typica and Bourbon and between Typica and Catuai) were higher than the similarity values within those varieties. Two Bourbon accessions (Bourbon 1 and Bourbon 2) and one Catuai accession (Catuai 8) were similar to the Typica group as demonstrated by cluster analysis. The average genetic similarities between Bourbon 1, Bourbon 2 and Catuai 8, and the 26 Typica accessions were 0.972, 0.967 and 0.956, respectively, much higher than the average genetic similarities within Bourbon and Catuai. The similar level of genetic variation within and among arabica cultivars posed a challenge for cultivar identification based on DNA markers. Under this circumstance, multiple samples of each cultivar are certainly necessary to assess the genetic diversity and establish genetic relationship among arabica cultivars.

Among the three coffee species evaluated, the genomic relationship between *C. arabica* and *C. canephora* is closer than either of them is to *C. liberica*. These data support the conclusion that *C. canephora* is one of the ancestral progenitors of *C. arabica* (Lashermes et al. 1999). Lashermes et al. (1997a, b) also detected a similar genomic relationship among these three species based on the sequences of the internal transcribed spacer region of nuclear ribosomal DNA.

Cultivar-specific DNA markers were not detected. Identification of such markers might be a difficult task even with an increased number of DNA markers since the pedigree of these cultivars is intermingled. One alternative to using DNA markers to identify cultivars would be to tag the major mutant genes that are characteristic of one or more cultivars, such as *Ct*, *mo* and *Mg* (Carvalho 1939). DNA markers linked to these genes would separate Catuai and Caturra, Mokka and Maragogipe from the rest of the coffee cultivars, although Catuai and Caturra would not be separated.

Although genetic variation within and among arabica cultivars was limited; sufficient DNA polymorphism was found among some arabica accessions to allow differentiation. Among the parental varieties of 18 crosses made in 1999, the highest polymorphic rate was between Catimor and Mokka Hybrid at 23.3% (data not shown). Of the 58 arabica accessions examined, 103 pair-wise combinations exhibited a polymorphic rate higher than 15%. A mapping population derived from the cross between Catimor and Mokka Hybrid was planted and maintained at Kunia Stations, Oahu, Hawaii. Work is currently underway to construct a linkage map of the arabica genome using AFLP markers. A complete genetic map will facilitate QTL mapping for agronomic traits related to coffee quality and productivity, which is the foundation of marker-assisted breeding in coffee (Lashermes et al. 1997).

The AFLP technique used in this study is widely recognized as the most-efficient marker system when compared with RFLP, SSR and RAPD markers. It is as reliable as RFLP and SSR at a lower cost, and it is more reliable than RAPD markers (Powell et al. 1996; Pejic et al. 1998). Five coffee trees were selected for testing the repeatability of AFLP markers and ranged from 97% to 99%. Hansen et al. (1999) reported an overall reproducibility of 97.7% for AFLP markers, and traced the sources of errors as 0.3% due to the human factor, 1.5% due to gel resolution, and 0.5% from the AFLP protocol.

One error of AFLP marker data could arise from partial digestion of the genomic DNA. This type of error can be suspected when the banding pattern of a sample is distinctively different from its close relatives, often with fewer bands overall but more bands at the high-molecular-weight region. Thirteen coffee accessions with a higher frequency of high-molecular-weight bands were selected to test whether the restriction digestion was complete. Leaf samples from the same tree were collected and DNA isolation was carried out to avoid repeating the errors attributed to DNA quality. Three of the 13 samples were found to be only partially digested. Although it is impractical to run every sample twice, it would reduce errors if samples from different species or genera and the samples with suspicious banding patterns were reanalyzed using the same AFLP protocol.

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