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Full Length Research Paper

AFLP analysis among Ethiopian arabica coffee genotypes

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Knowledge of genetic diversity is vital for genetic improvement and germplasm conservation. The genetic relationship of 28 *Coffea arabica* genotypes from Ethiopia was assessed using 10 Amplified Fragment Length Polymorphism (AFLP) primer combinations. The level of polymorphism was 30.9% and 18 markers were unique to 10 genotypes. Pair-wise genetic similarity coefficients ranged from 0.851 to 0.982, AD1491 being the most divergent and AD5091 the most closely related to all genotypes. All genotypes were independently distinguished and did not cluster according to collection region, demonstrating the presence of coffee genetic resource diversity within each region. Results of this study will be utilised for germplasm collection, conservation as well as in variety development efforts.

Key words: *Coffea arabica* L., DNA-based markers, fingerprinting, genetic diversity.

INTRODUCTION

Coffee, one of the most important beverage crops in the world and a valuable agricultural export commodity, is cultivated in over 80 countries (Pearl et al., 2004). On average its trade generates about US $9.7 billion annually (ITC, 2002) and provides a livelihood for 25 million coffee farming families around the world (ICO, 2004). About 7.2 billion ton coffee was harvested from 10.4 million ha land in 2003 (FAO, 2004). Arabica coffee accounts for about 70% of the world coffee production and known for the preparation of high quality beverage (Anthony et al., 2002). It is the only tetraploid (2n =4x= 44) and self-fertile (over 95%) species in the genus *Coffea* (Silvarolla et al., 2004) as well as the most widely cultivated and the longest known coffee species (Coste, 1992; Moncada and McCouch, 2004).

Arabica coffee is native to the highlands of Southwestern Ethiopia (Sylvain, 1955). It is cultivated almost throughout the country (Yemane-Berhan, 1998). Several researchers reported the presence of phenotypic variation among *Coffea arabica* genotypes grown in Ethiopia (Sylvain, 1955; FAO, 1968; Ameha, 1986; Selvakumar and Sreenivasan, 1989; Montagnon and Bouharmont, 1996; Bellachew et al., 2000; Anzueto et al., 2001). Since 1928, several teams as well as individual researchers from international and national organizations have collected a large number of accessions from different parts of the country (Anthony et al., 2001). Consequently, over 4500 accessions are conserved in a field genebank by the Ethiopian Biodiversity Conservation and Research Institute (ITC, 2002). However, the genetic relationship of these accessions, especially recent ones, was not investigated using DNA-based markers.

A variety of DNA marker techniques are available for measuring genetic diversity. The AFLP technique has several advantages over other DNA marker systems. The most important of these are that it does not require sequence information, produces a large number of informative polymorphic markers per primer, requires a small amount of DNA, inspects an entire genome and is highly reproducible (Portis et al., 2004; Shan et al., 2004). In arabica coffee, the AFLP technique was employed to identify the origin of cultivated cultivars (Anthony et al., 2002), analyse genetic diversity within and among cultivars (Steiger et al., 2002), detect genetic introgression (Prakash et al., 2004) and construct a genetic linkage map (Pearl et al., 2004). In this study AFLP was employed to assess the level of genetic relationships among arabica coffee genotypes currently grown in Northwestern and Southwestern parts of Ethiopia and explore

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Table 1. List of 28 *C. arabica* genotypes used for AFLP characterisation.

Accession number	Collection region	Altitude (masl)	Characters
AD0391	Southwestern Ethiopia	1900	CBD resistant, average in cup quality
AD0491	Southwestern Ethiopia	1920	CBD resistant, weak apical dominance
AD0591	Southwestern Ethiopia	1700	Vigorous, average in cup quality
AD0791	Southwestern Ethiopia	1700	Vigorous, good in cup quality
AD0991	Southwestern Ethiopia	1710	Dwarf, poor in cup quality
AD1191	Southwestern Ethiopia	1710	Dwarf , poor in cup quality
AD1491	Southwestern Ethiopia	1575	Branchy, long bean
AD1291	Northwestern Ethiopia	1820	Bronze young leaf, bold bean
AD2191	Northwestern Ethiopia	1940	Poor in cup quality
AD4391	Northwestern Ethiopia	1960	Green young leaf
AD4491	Northwestern Ethiopia	2290	Bronze young leaf
AD2291	Northwestern Ethiopia	2210	Green young leaf
AD2691	Northwestern Ethiopia	ND	Green young leaf
AD4591	Northwestern Ethiopia	ND	Bronze young leaf
AD4691	Northwestern Ethiopia	ND	Bronze young leaf
AD4791	Northwestern Ethiopia	1850	Green young leaf
AD3891	Northwestern Ethiopia	2060	Bold, heavy and uniform bean
AD4091	Northwestern Ethiopia	ND	Green young leaf
AD4891	Northwestern Ethiopia	ND	Green young leaf
AD2991	Northwestern Ethiopia	2040	Rust susceptible, bear on main stem
AD3991	Northwestern Ethiopia	1900	Green young leaf
AD3591	Northwestern Ethiopia	2000	CBD susceptible
AD3791	Northwestern Ethiopia	1880	Bold, heavy and uniform bean
AD4191	Northwestern Ethiopia	2000	Green young leaf, round and bold bean
AD4991	Northwestern Ethiopia	2020	Green young leaf
AD5091	Northwestern Ethiopia	1940	Green young leaf
AD1891	Northwestern Ethiopia	1930	Green young leaf, long bean
AD3491	Northwestern Ethiopia	1500	Bronze young leaf, round bean

CBD = coffee berry disease; ND = no data; masl = metre above sea level.

the possibility of utilising AFLP markers for cultivar identification.

MATERIALS AND METHODS

Genotypes

A total of 28 arabica coffee genotypes collected from the Northwestern and Southwestern parts of Ethiopia were used for this study (Table 1). These genotypes were obtained from Adet Agricultural Research Centre, Ethiopia and differ in disease resistance, growth habits, cup quality, green bean biochemical composition and ecological adaptation.

DNA isolation

Total genomic DNA was isolated using the CTAB (hexadecyltrimethylammonium bromide) method (Saghai-Maroof et al., 1984). A representative sample of each genotype was obtained by isolating DNA from lyophilized leaves of five plants (Herselman, 2003). The concentration and purity of isolated DNA was determined using spectrophotometer by measuring absorbance at 260 and 280 nm. The integrity of isolated DNA was also assessed through electrophoresis in 0.8% (w/v) agarose gels and 1x UNTAN (40 mM Tris-Cl, 2 mM EDTA, pH adjusted to 7.4 with acetic acid) buffer at 60 volts for 45 min.

AFLP analysis

AFLP analysis was performed following the protocol of Vos et al. (1995) with minor modifications. Genomic DNA (500 ng) was digested with 5 U of *Mse*I at 37°C for 5 h followed by overnight digestion with *Eco*RI. The restricted DNA fragments were ligated to double-stranded *Eco*RI- and *Mse*I- adapters using T4 DNA Ligase by overnight incubation at 16°C. Adaptor-ligated DNA fragments were pre-amplified using primers with one added selective nucleotide (*Eco*RI+A and *Mse*I+C) for 30 cycles of: 30 s at 94°C, 1 min at 56°C and 1 min at 72°C.

Selective amplifications were performed using 10 primer combinations with three selective nucleotides (Table 2). Primer combinations were selected based on results of Anthony et al. (2002) and Steiger et al. (2002). The reaction was performed in a total volume of 20 µl containing 5 µl of 1:10 diluted preamplified product, 1x Promega polymerase buffer, 2 mM $MgCl_2$, 200 µM of each dNTP, 100 µg/ml bovine serum albumin (BSA), 30 ng *Mse*I primer, 30 ng *Eco*RI primer and 0.75 U Promega *Taq* DNA polymerase. The PCR programme for selective amplification consisted of an initial 10 cycles of denaturing at 94°C for 30 s, annealing at 65°C

Table 2. Level of genetic information generated by each primer combination.

Primer combination	TNF	NPF	P (%)	H`	PIC	MI	NGI	NUF	Pair-wise Genetic similarity coefficient		
									Minimum	Maximum	Mean
*Eco*RI-ACA/*Mse*I-CAA	90	25	27.8	0.620	0.52	3.61	26	1	0.839	1.000	0.915
*Eco*RI-AAC/*Mse*I-CAA	108	39	36.1	0.473	0.35	4.93	26	2	0.788	1.000	0.921
*Eco*RI-ACT/*Mse*I-CAA	71	21	29.6	0.530	0.39	2.42	20	0	0.843	1.000	0.929
*Eco*RI-ACC/*Mse*I-CAA	67	19	28.4	0.610	0.42	2.27	24	0	0.821	1.000	0.909
*Eco*RI-ACT/*Mse*I-CAG	70	31	44.3	0.653	0.63	8.65	28	1	0.714	0.981	0.841
*Eco*RI-ACC/*Mse*I-CAG	69	24	34.8	0.517	0.73	6.10	25	6	0.754	1.000	0.906
*Eco*RI-ACA/*Mse*I-CTG	50	16	32.0	0.376	0.42	2.15	8	2	0.809	1.000	0.948
*Eco*RI-AAC/*Mse*I-CTG	69	17	24.6	0.529	0.45	1.88	17	3	0.864	1.000	0.939
*Eco*RI-ACT/*Mse*I-CTG	72	18	25.0	0.716	0.65	2.93	22	2	0.794	1.000	0.905
*Eco*RI-ACC/*Mse*I-CTG	46	10	21.7	0.494	0.37	0.80	9	1	0.844	1.000	0.951
Total	712	220						18			
Mean	71	22	30.9	0.552	0.49	3.57	20.5	1.8	0.851	0.982	0.915

TNF = total number of fragments; NPF = number of polymorphic fragments; P (%) = percentage of polymorphic fragments; H` = Shannon-Weaver diversity index; PIC = polymorphic information content; MI = marker index; NGI = number of genotypes identified; NUF = number of unique fragments.

for 30 s and elongation at 72°C for 1 min by lowering the annealing temperature by 1°C at each cycle. This was followed by 25 cycles of 30 s at 94°C, 30 s at 56°C and 1 min at 72°C followed by a final elongation cycle for 5 min at 72°C. All polymerase chain reactions (PCR) were performed in a Hybaid Thermal Cycler. Finally, 5 µl of each selective amplification reaction product was added to 24 µl formamide and 0.75 µl Rox size standard marker, denatured at 94°C for 10 min, cooled in ice slurry for 5 min and resolved on a Perkin Elmer ABI Prism 310 automated capillary sequencer (PE Bio-systems).

Data analysis

DNA fragments amplified by 10 primer combinations were scored for each genotype as present (1) or absent (0). Pair-wise genetic similarity coefficients were calculated according to Jaccard (1908). The similarity coefficient was used to construct a dendrogram by the unweighted pair group method with arithmetic averages (UPGMA) with NTSYS-pc software version 2.02i (Rohlf, 1998).

Shannon diversity index (H`), marker index (MI) and polymorphic information content (PIC) were calculated to compare the efficiency of each primer combination in generating genetic information (Lanteri et al., 2004). Shannon diversity index was calculated over all loci of each primer combination as follows: $H` = -\Sigma pilogpi$, where pi is the frequency of the i^{th} fragment in the sample. MI was calculated according to Lanteri et al. (2004) as the product of expected heterozygosity (H_n) and effective multiplex ratio (EMR). H_n of a locus is defined as: $1-\Sigma pi^2$, where pi is the frequency of the i^{th} allele (DNA fragment). EMR of a primer is defined as: ßn, where ß is the percentage of polymorphic loci (DNA fragments) and n is the number of polymorphic loci detected per primer combination (Lanteri et al., 2004). In addition, PIC was calculated according to Smith et al. (2000) as follows: $PIC = 1-\Sigma fi^2$, where fi is the percentage of genotypes in which the fragment is present. Spearman's rank correlation coefficients were calculated among genetic similarity coefficient matrices resulted from 10 primer combinations.

RESULTS

Efficiency of primer combinations

A total of 712 fragments were amplified using 10 primer combinations, of which 220 (30.9%) were polymorphic among evaluated arabica coffee genotypes (Table 2). The number of polymorphic fragments per primer combination ranged from 10 to 39 with an average of 22. Among the 10 primer combinations tested, *Eco*RI-ACC/*Mse*I-CTG and *Eco*RI-ACC/*Mse*I-CAA amplified the lowest and highest number of total as well as polymorphic fragments, respectively.

Shannon diversity indexes, PIC and MI values per primer combinations ranged from 0.376-0.716, 0.35-0.73 and 0.80-8.65, respectively (Table 2). Primer combination *Eco*RI-ACT/*Mse*I-CTG showed the highest value for H`, while *Eco*RI-ACT/*Mse*I-CAG showed the highest value for MI and independently distinguished all genotypes. The highest PIC value was recorded for primer combination *Eco*RI-ACC/*Mse*I-CAG. On the other hand, primer combinations *Eco*RI-ACA/*Mse*I-CTG, *Eco*RI-AAC/*Mse*I-CAA and *Eco*RI-ACC/*Mse*I-CTG showed the lowest values for H`,

Table 3. List of primer combinations which amplified unique fragment(s), size and name of the genotype identified with unique fragment(s).

Primer combination	Unique fragments (bp) and identified genotypes
*Eco*RI-ACA/*Mse*I-CAA	168bp (AD1291)
*Eco*RI-AAC/*Mse*I-CAA	156bp (AD4791) and 325bp (AD1491)
*Eco*RI-ACT/*Mse*I-CAG	105bp (AD4591)
*Eco*RI-ACC/*Mse*I-CAG	122bp, 211bp and 241bp (AD1491), 59bp (AD2691), 279bp (AD3991) and 272bp (AD4991)
*Eco*RI-ACA/*Mse*I-CTG	198bp (AD1891) and 226bp (AD3491)
*Eco*RI-AAC/*Mse*I-CTG	137bp (AD1491), 332bp (AD2291) and 77bp (AD3491)
*Eco*RI-ACT/*Mse*I-CTG	103bp (AD1291) and 115bp (AD3491)
*Eco*RI-ACC/*Mse*I-CTG	137bp (AD1491)

Table 4. Correlation coefficients among 10 primer combinations in estimating genetic similarity among genotypes.

No.	Primer combination	2	3	4	5	6	7	8	9	10	Combined data
1	*Eco*RI-ACA/*Mse*I-CAA	0.447**	0.378**	0.376**	0.269**	0.279**	0.096	0.330**	0.401**	0.236**	0.725**
2	*Eco*RI-AAC/*Mse*I-CAA		0.348**	0.186**	0.153**	0.372**	0.299**	0.321**	-0.001	0.193**	0.608**
3	*Eco*RI-ACT/*Mse*I-CAA			0.293**	0.315**	0.262**	0.129*	0.222**	0.353**	0.350**	0.623**
4	*Eco*RI-ACC/*Mse*I-CAA				0.129*	0.248**	0.206**	0.207**	0.314**	0.202**	0.525**
5	*Eco*RI-ACT/*Mse*I-CAG					0.387**	0.171**	0.109*	0.230**	0.207**	0.558**
6	*Eco*RI-ACC/*Mse*I-CAG						0.333**	0.162**	0.125*	0.425**	0.622**
7	*Eco*RI-ACA/*Mse*I-CTG							0.073	-0.095	0.225**	0.348**
8	*Eco*RI-AAC/*Mse*I-CTG								0.118*	0.110*	0.456**
9	*Eco*RI-ACT/*Mse*I-CTG									0.224**	0.501**
10	*Eco*RI-ACC/*Mse*I-CTG										0.449**

* and ** indicates significant correlations at p≤0.05 and 0.01, respectively.

PIC and MI, respectively. A total of 18 unique fragments were recorded from eight primer combinations. As a result, 10 arabica coffee genotypes were uniquely characterised using these distinctive fragments (Table 3). *Eco*RI-ACC/*Mse*I-CAG amplified the highest number (six) of unique fragments on a large number (four) of genotypes. Of the 10 genotypes with distinctive fragments, genotype AD1491 had the maximum number (six) of unique fragments.

Correlation coefficients among genetic similarity matrices estimated by different primer combinations ranged from -0.001 to 0.447 (Table 4). The lowest correlation coefficient was recorded between genetic similarities estimated by primer combinations *Eco*RI-AAC/*Mse*I-CAA and *Eco*RI-ACT/*Mse*I-CTG, while the highest value was recorded between genetic similarities estimated by primer combinations *Eco*RI-ACA/*Mse*I-CAA and *Eco*RI-AAC/*Mse*I-CAA. Except for *Eco*RI-AAC/*Mse*I-CAA and *Eco*RI-ACT/*Mse*I-CTG as well as *Eco*RI-ACA/*Mse*I-CTG and *Eco*RI-ACT/*Mse*I-CTG, all other primer combinations showed positive correlations in estimating genetic similarity among tested genotypes. This illustrated the similarity of most primer combinations in estimating pair-wise genetic similarity coefficients among genotypes. Of all tested primer combinations, *Eco*RI-ACA/*Mse*I-CTG had the lowest (0.348) and *Eco*RI-ACA/*Mse*I-CAA the highest (0.725) correlation coefficients with the genetic similarity estimated from a combined data of all primer combinations. No primer combination had genetic similarity identical to that estimated from combined data of all primer combinations. Hence, each primer combination probably scanned different parts of a genome and provided complementary information.

Genetic relationships among genotypes

Pair-wise genetic similarity coefficients varied from 0.851 to 0.982 with an average of 0.915. AD1291 and AD1491 were the most dissimilar, whereas AD3591 and AD3991 were the most similar genotypes. Of the 378 pairs of genotypes, 82 had genetic similarity coefficients less than 0.900 and AD1491 was one of the genotypes for most of these pairs of genotypes. Pair-wise genetic similarity coefficients ranged from 0.877 to 0.982 and 0.861 to 0.956 among coffee genotypes collected from the Northwestern and Southwestern parts of Ethiopia, respectively. Of the 220 polymorphic AFLP markers detected among 28 coffee genotypes, 24 were absent and 53 were monomorphic across all coffee genotypes collected from

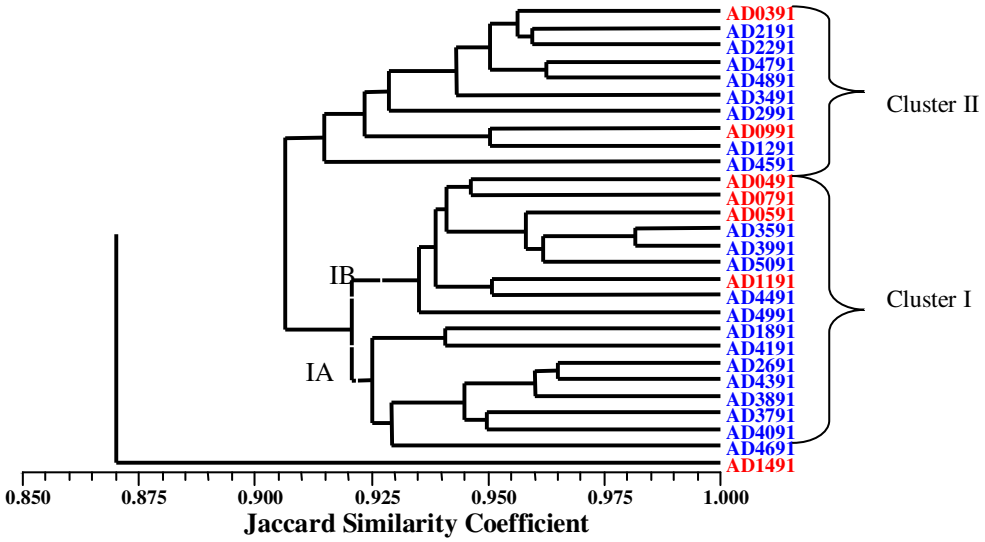

Figure 1. Dendrogram of 28 *C. arabica* genotypes constructed by UPGMA method of cluster analysis using AFLP data.

Southwestern Ethiopia. On the other hand, six were absent and 28 were monomorphic among all coffee genotypes collected from Northwestern Ethiopia. Genotypes from Northwestern Ethiopia contained 97.3% of the AFLP markers.

A dendrogram constructed using UPGMA method of cluster analysis from a combined data of all primer combinations classified genotypes into two main clusters and a singleton (Figure 1). Genotype AD1491 clustered separately from all other coffee genotypes tested in this study. Cluster I contained 17 coffee genotypes, of which four were collections from Southwestern Ethiopia. Cluster II comprised 10 coffee genotypes, of which two were collections from Southwestern Ethiopia. Genotypes did not cluster according to collection regions. The cophenetic correlation coefficient was high (0.87), suggesting a very good fit of the cluster analysis performed.

DISCUSSION

Screening and selection of primer combinations which detect maximum genetic variation is vital to establish dependable genetic relationships among genotypes. The efficiency of 10 AFLP primer combinations were compared based on number of polymorphic fragments, Shannon diversity index, polymorphic information content, marker index, number of genotypes identified, number of distinctive fragments and correlation of pair-wise genetic similarity coefficients calculated from data of each primer combination and combined data of all primer combinations. Although a primer combination superior for all parameters was not identified, primer combinations *Eco*RI-ACA/*Mse*I-CAA, *Eco*RI-ACC/*Mse*I-CAG, *Eco*RI-ACT/*Mse*I-CAG and *Eco*RI-AAC/*Mse*I-CAA were superior for most of the parameters considered. Anthony et al. (2002) reported the superiority of the last three primer combinations in evaluating genetic diversity among wild and cultivated *C. arabica* genotypes. Furthermore, *Eco*RI-AAC/*Mse*I-CAA and *Eco*RI-ACC/*Mse*I-CAG were recommended as more informative primer combinations by Steiger et al. (2002). Therefore, the above mentioned four primer combinations are identified as efficient primer combinations for genetic diversity analysis in arabica coffee.

Using 10 primer combinations, 220 polymorphic fragments were amplified and all genotypes were distinctly distinguished. Moreover, all genotypes were differentiated using 31 polymorphic fragments amplified by a primer combination, *Eco*RI-ACT/*Mse*I-CAG. In addition, 18 markers that can be exploited for cultivar fingerprinting of 10 genotypes were identified during the present study. Anthony et al. (2002) differentiated 26 arabica coffee accessions using 107 AFLP markers while Steiger et al. (2002) identified 58 arabica coffee accessions using 274 informative AFLP markers. The AFLP technique was successfully employed by Noir et al. (2003) and Prakash et al. (2004) to identify introgressed root-knot nematode and leaf rust resistance genes, respectively, from *Coffea canephora* to the *C. arabica* genome. Pearl et al. (2004) constructed a genetic map of arabica coffee with a total length of 1802.8 cM and an average distance of 10.2 cM between adjacent markers using AFLP analysis. All these studies demonstrated the efficiency of the AFLP technique for different genetic studies in arabica coffee.

Pair-wise, genetic similarity coefficients computed for

28 coffee genotypes using 712 AFLP markers ranged from 0.851 to 0.982 with an average of 0.915. Steiger et al. (2002) reported an average genetic similarity coefficient of 0.933 among 58 arabica coffee accessions. Therefore, results of the present study were in agreement with previous research results and confirmed the low genetic diversity within *C. arabica,* which is attributable to its reproductive biology and evolution (Lashermes et al., 2000).

The two most similar genotypes, AD3591 and AD3991, were collected from the Northwestern parts of Ethiopia whereas the two most dissimilar genotypes, AD1291 and AD1491, were collected from different regions. Despite this observation, AD0591 and AD5091 are collections from different regions but had high genetic similarity coefficient (0.960). This could be attributed to the unrestricted movement of planting materials from region to region, high self-pollination habit (Silvarolla et al., 2004) and perennial nature of the crop. Genotypes AD1191 and AD1491 were collected from the Southwestern part of Ethiopia, but were genetically dissimilar. The low genetic similarity between genotypes collected from the same region could be attributed to the vast agro-ecological diversity present within each region that can contribute to the development of genetically diverse genotypes through natural selection. Therefore, similarity in collection locality may not necessarily imply genetic similarity in coffee, in Ethiopia.

The average genetic similarity among coffee genotypes from Southwestern Ethiopia (0.912) was relatively lower than from Northwestern Ethiopia (0.918). This illustrated the presence of higher genetic diversity among coffee genotypes in the Southwestern than in the Northwestern Ethiopia. This was expected, since arabica coffee originated in the Southwestern part of Ethiopia (Sylvain, 1955). In agreement to this result, Mengesha (1975) reported the presence of low levels of arabica coffee genetic resource diversity in northwestern Ethiopia. Similarly, Anthony et al. (2001) observed narrow genetic diversity among coffee accessions collected from Northwestern Ethiopia. However, the present study detected unique AFLP markers for Northwestern Ethiopia genotypes. Therefore, coffee genotypes in Northwestern Ethiopia should be collected and conserved for unforeseen future uses.

Cluster analysis classified the 28 genotypes into two clusters and a singleton. AD1491 was identified as the most genetically divergent genotype and can be exploited for hybrid variety development by crossing with other genotypes. Genotypes in the same cluster may represent members of one heterotic group. Therefore, crossing between genotypes from different clusters must be considered for hybrid variety development.

It was not possible to consistently correlate genetic clustering with morphological characters or growth habits usually adopted for cultivar identification. For example, AD0991 and AD1191 are morphologically similar and characterised by dwarf and compact growth habits but were assigned in different clusters. Similarly, AD4591 and AD4691 have bronze young leaves but were assigned in different clusters. Therefore, morphological markers are not sufficient to characterise the genetic relationship of coffee genotypes. Similar results were reported by Portis et al. (2004) and Guzman et al. (2005) on oleander and capsicum, respectively. Ruiz et al. (2000) attributed such observations to randomness of the identified AFLP markers on a genome compared to the possibility that these morphological characteristics are controlled by a small portion of the genome. The chance that any of the identified markers target a genomic region involved in the control of the differentiating characteristics will be low. Charcosset and Moreau (2004) attributed the above observation to the neutrality of molecular markers which implies that detected polymorphisms did not contribute directly to the variation in traits of interest.

Results of the present study as well as studies by Anthony et al. (2002) and Moncada and McCouch (2004) indicated the presence of genetic variation among Ethiopian arabica coffee genotypes. Several coffee breeders also identified some highly desirable characteristics including CBD resistance (Bellachew et al., 2000), coffee leaf rust resistance (Wondimu, 1998), nematode resistance (Anzueto et al., 2001), naturally decaffeinated coffee (Silvarolla et al., 2004) and excellent cup quality coffee (Selvakumar and Sreenivasan, 1989) among arabica coffee genotypes collected from Ethiopia. This confirms the importance of conservation of the Ethiopian arabica coffee gene pool for the world coffee industry.

The longevity of coffee seed is insufficient to use in the preservation of genetic resources of coffee (Van der Vossen, 1985). Consequently, coffee germplasms are conserved in field genebanks. Since coffee is a tree and a perennial crop, it demands large area and year round management which is expensive. Moreover, morphological markers require evaluation over long periods of time and the present study indicated the inadequacy of morphological characters for characterisation of closely related genotypes. Hence, genetic diversity analysis using DNA-based marker techniques is recommended for cost effective and efficient conservation of coffee germplasm.

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