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GENETIC CHARACTERIZATION OF ARABUSTA COFFEE HYBRIDS AND THEIR PARENTAL GENOTYPES USING MOLECULAR MARKERS

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ABSTRACT

Twenty coffee genotypes were characterized comprising of eleven F_1 interspecific Arabusta hybrids, three induced tetraploid Robusta parental genotypes, four Arabica parental genotypes, one diploid Robusta accession and one Hybrido de Timor (HDT), a natural interspecific hybrid between Arabica and Robusta. Characterization was conducted using thirteen Simple Sequence Repeats (SSR) and ten Random Amplified Polymorphic DNA (RAPD) molecular markers. The molecular data were analyzed for polymorphism and also subjected to cluster analysis using Unweighted Pair Group Method with Arithmetic Average (UPGMA) to estimate the diversity among the genotypes. SSR markers revealed a genetic diversity of 51.5%, 50% and 6.9% while RAPD markers revealed genetic diversity of 51.6%, 47.4% and 3.5% within induced tetraploid Robusta, F_1 interspecific Arabusta hybrids and Arabica genotypes respectively. The SSR primers separated the genotypes into 3 distinct clusters unlike RAPDs which separated them into 7 distinct clusters. The study therefore confirmed the narrow genetic base within Arabica coffee and successfully portrayed the possibility of broadening it through interspecific hybridization. These results can be used to select parents with high combining ability in a hybridization program between Arabica and tetraploid Robusta.

Keywords: *Coffea arabica*, *Coffea canephora*, Arabusta, SSR, RAPD, genetic diversity.

INTRODUCTION

Coffee is one of the world's most important agricultural commodities with more than 125 million people worldwide deriving their income directly or indirectly from its products (Lashermes et al., 2011). It is grown in about 80 countries spanning over 10.2 million hectares of land in the tropical and sub-tropical regions of the world especially in Africa, Asia and Latin America whereby the economies of these coffee growing countries depends heavily on the earnings from this crop (Mishra and Slater, 2012). Coffee belongs to the genus *Coffea* in the Rubiaceae family (Berthaud and Charrier, 1988) with some 640 genera and about 10000 species (Lashermes et al., 1997). The genus Coffea has been reorganized into two subgenera, *Coffea* and *Paracoffea* with more attention having been paid to the Coffea subgenus that has two cultivated species of economic importance, namely Arabica (*Coffea arabica* L.) and Robusta (*Coffea canephora* Pierre) (Lashermes et al., 1997). Coffee trees differ greatly in morphology, size and ecological adaptation, leading to the description of a large number of species (Lashermes et al., 1997). The basic chromosome number for the genus *Coffea* is n = 11. Arabica coffee is the only polyploid and self-fertile (over 95%) species of the genus *Coffea,* with chromosome number 2n = 4x = 44, while others are diploid (2n = 2x = 22) and self-infertile (Silvarolla et al., 2004). Based on molecular and cytological investigation, Arabica was shown to be an allotetraploid formed by hybridization between two diploids, *C. canephora* and *C. eugenoides* (Lashermes et al., 2011).

Arabica coffee is known for the production of very high quality beverage but is more susceptible to major diseases of coffee (Gichuru et al., 2008; Kathurima et al., 2009). *Coffea canephora* is indigeneous to tropical African forests and covers a large area stretching from West Africa through Cameron, central Africa Republic, Congo, Democratic Republic of Congo, Uganda, northern Tanzania and Nothern Angola. *C. canephora* populations are generally small, disconnected population with small number of mother trees and a few offspring's that are scattered. (Musoli et al., 2009). *C. canephora's* area of distribution is variable and corresponds to hot and humid tropical climatic regions with its range of production found in low and middle altitudes areas in Africa (Tshilenge et al., 2009). Robusta is more tolerant to major coffee diseases and insect pests but with inferior cup quality (Bertrand et al., 2003). Transfer of disease resistance genes from the diploid species like *C. canephora* and *C. liberica* into tetraploid Arabica cultivars without adversely affecting quality traits has been the main objective of Arabica breeding. However, ploidy difference between *C. arabica* and diploid species is one of the major bottlenecks for interspecific gene transfer (Lashermes et al., 1997; Ky et al., 2001).

Despite these challenges, interspecific hybrids between *C. arabica* and various diploid species including *C. canephora* have been successfully produced (Lashermes et al., 2011). Viable and reasonably fertile interspecific hybrids between the allotetraploid *C. arabica* L. (2n = 44) and *C. canephora* Pierre (2n = 22) can easily be obtained through induced autotetraploid forms of *C. canephora* (Owour and Van Der Vossen, 1981). Doubling of the chromosome number in coffee can be achieved through colchicine treatment and the first successful interspecific hybrids between induced tetraploid *C. canephora* and *C. arabica* were made in Brazil in 1950 (Owour and Van Der Vossen, 1981). These hybrids have been applied in coffee breeding programs to introgress resistance to coffee leaf rust (*Hemileia vastatrix* Berk. & Br.) and coffee berry disease (*Colletotrichum kahawae* Waller and Bridge) from *C. canephora* into *C. arabica* or to improve the quality of Robusta coffee by direct use of the F_1 hybrids (Owour and Van Der Vossen, 1981). Occurrence of spontaneous hybrids between tetraploid Arabica and other diploid species is also common. A good example of such natural tetraploid interspecific hybrids is Hibrido De Timor (HDT) from Timor Island which gained priority in coffee breeding and still assumes greater significance (Prakash et al., 2002).

Genetic diversity of coffee can be assessed using different techniques that range from traditional morphological techniques to the modern DNA based molecular markers (Teressa et al., 2010). Several studies on genetic diversity of coffee using Simple Sequence Repeats (SSR) and Random Amplified Polymorphic DNA (RAPD) markers have been carried out. SSR markers were applied in coffee to identify *C. arabica, C. canephora* and related coffee species (Combes et al., 2000); isolation and characterization of microsatellite markers from *C. arabica* (Baruah et al., 2003) and to establish the frequency of several microsatellite motifs in the coffee genome (Critancho and Gaitan, 2008). SSR markers were also used to analyze the introgression of DNA fragments from *C. canephora* and *C. liberica* into *C. arabica* (Gichuru et al., 2008; Lashermes et al., 2011); to study genetic diversity among commercial coffee varieties and selected museum collection in Kenya (Kathurima et al., 2012) and to study the genetic diversity of Arabica coffee collections with different geographical origin and historical backgrounds (Teressa et al., 2010).

RAPD makers were used to construct a linkage map in coffee (Lashermes et al., 1996a), to detect markers for resistance to coffee berry disease (Agwanda et al., 1997); to study genetic diversity amongst coffee accessions (Lashermes et al., 1996b; Sera et al., 2003; Dinesh et al., 2011; Kathurima et al., 2012) and to examine intraspecific variation in *C. arabica, C. canephora* and genetic relationships among a set of coffee accessions representing major *Coffea* species (Lashermes et al., 1993). RAPD markers were also used for the identification of the genetic variability within and among coffee populations (Silveira et al., 2003) and for detection of genetic diversity and selective gene intogression in coffee (Castillo et al., 1994). This study was carried out to determine the genetic diversity of interspecific F_1 Arabusta hybrids and their parental genotypes.

MATERIALS AND METHODS

Description of the Study Site

Coffee Research Station (CRS), in Kenya lies within the upper midland 2 (UM2) at latitude 1^0 06'S and longitude 36^0 45'E and is approximately 1620m above sea level. The area receives a bimodal rainfall of 1063mm annually with mean temperature of 19^0 C (minimum 12.8^0C, maximum 25.2^0C). The soils are classified as complex humic nitisols and plinthic ferrasols. They are well drained, deep, reddish brown, slightly friable clays with murram sections occasionally interrupting. The soil pH ranges from 5 to 6 (Kathurima et al., 2009; Gichimu and Omondi, 2010).

Test Genotypes

The study genotypes comprised of 11 interspecific Arabusta F_1 hybrids, four Arabica cultivars: three induced tetraploid Robusta genotypes, one natural interspecific hybrid (HDT) and one diploid Robusta accession (Table 1). The F_1 interspecific hybrids were obtained by crossing four induced tetraploid *C. canephora* clones, UT3, UT6, UT8 and UT10 (ex France, introduced from Uganda) and four *C. arabica* cvs. SL 28, SL 34, N 39 and Caturra (Owour and Van Der Vossen, 1981).

Table 1. Description of genotypes used in the study

Genotypes	Description	Grouping
SL28 x UT3	Interspecific Arabusta F_1 Hybrid	Group 3
Caturra x UT3	Interspecific Arabusta F_1 Hybrid	Group 3
SL28 x UT6	Interspecific Arabusta F_1 Hybrid	Group 3
SL34 x UT6	Interspecific Arabusta F_1 Hybrid	Group 3
Caturra x UT6	Interspecific Arabusta F_1 Hybrid	Group 3
SL28 x UT8	Interspecific Arabusta F_1 Hybrid	Group 3
SL34 x UT8	Interspecific Arabusta F_1 Hybrid	Group 3
N39 x UT8	Interspecific Arabusta F_1 Hybrid	Group 3
Caturra x UT8	Interspecific Arabusta F_1 Hybrid	Group 3
SL28 x UT10	Interspecific Arabusta F_1 Hybrid	Group 3
N39 x UT10	Interspecific Arabusta F_1 Hybrid	Group 3
SL34	Arabica cultivar	Group 2
SL28	Arabica cultivar	Group 2
N39	Arabica cultivar	Group 2
Caturra	Arabica cultivar	Group 2
UT3	Induced tetraploid Robusta	Group 1
UT8	Induced tetraploid Robusta	Group 1
UT10	Induced tetraploid Robusta	Group 1
HDT	Spontaneous Arabusta Hybrid	-
Robusta	Diploid Robusta	-

DNA Extraction

Young coffee leaves were picked from the growing tips of the test genotypes and genomic DNA was extracted following the method of Diniz et al. (2005) with modification of the extraction buffer by adding MATAB (Mixed Alkyltrimethylammonium Bromide) instead of CTAB. About five hundred (500) miligram of fresh leaves were ground in a 2 mL Eppendorf tube. After grinding, 1 mL extraction solution was added and the tubes shaken vigorously for 5 min and immediately put in a 65 ^{0}C water bath for 40 min. The samples were centrifuged for 5 min at 1300 rpm and the supernatant transferred to a new tube, to which 1 mL CIA (chloroform: isoamyl 24:1) was added. The tubes were softly shaken for 10 min and centrifuged for 5 min at 12000 rpm. The supernatant were transferred to another tube and the same volume of frozen Isopropanol added to it and maintained at -20 ^{0}C for 1 hour and after which the resultant material was centrifuged at maximum speed for 5 min, the supernatant was discarded and the pellet washed with 70% ethanol. This step was repeated twice and after drying, the pellets were treated with 190 µL TE (Tris-EDTA buffer plus RNAse 10 mg µL-1) for 30 min at 37 ^{0}C and 65 ^{0}C for 5 min. The DNA was purified with the addition of 100 µL TE, 100 µL water, 100 µL NaCl 5 M and 100 µL EDTA 0.5 M. The

samples were homogenized and incubated on ice for 30 min and centrifuged for 5 min at maximum speed. The supernatant was transferred to another tube and the same volume of frozen isopropanol added. After drying, the pellet was diluted in 60 µL TE buffer.

PCR Amplification of SSR Primers

PCR reactions were performed in a final volume of 25 µL, containing 100 ng (10ng/µL) template of genomic DNA, 0.4 µM of primer, 75 µM dNTPs (each), 2.5 µM $MgCl_2$, PCR buffer 1 x TBE and 1 unit Taq DNA polymerase (Gene-on, Germany). Amplification was carried out in a Eurogene thermocycler (TECHNE, UK). The SSR amplification program started with one cycle of initial denaturation at 94 ^{0}C for 5 minutes followed by 35 cycles of 30 seconds at 94^{0}C (denaturation), 30 seconds at 55 ^{0}C for primer annealing, and 90 seconds at 72 ^{0}C for elongation. The final extension was done at 72 ^{0}C for 10 minutes to ensure that the primer extension reaction was completed. Thirteen SSR primers were amplified (Table 2). Selection of these primers was guided by previous work by Combes et al. (2000) and Lashermes et al. (2011)

Table 2. SSR primers information (Forward and Reverse)

Primer	Forward primer (5'>3')	Reverse primer (5'>3')
Sat 11	ACCCGAAAGAAAGAACCAA	CCACACAACTCTCCTCATTC
Sat 32	AACTCTCCATTCCCGCATTC	CTGGGTTTTCTGTGTTCTCG
Sat 172	ACGCAGGTGGTAGAAGAATG	TCAAAGCAGTAGTAGCGGATG
Sat 207	GAAGCCGTTTCAAGCC	CAATCTCTTTCCGATGCTCT
Sat 227	TGCTTGGTATCCTCACATTCA	ATCCAATGGAGTGTGTTGCT
Sat 229	TTCTAAGTTGTTAAACGAGACGCTTA	TTCCTCCATGCCCATATTG
Sat 235	TCGTTCTGTCATTAAATCGTCAA	GCAAATCATGAAAATAGTTGGTG
Sat 240	TGCACCCTTCAAGATACATTCA	GGTAAATCACCGAGCATCCA
Sat 254	ATGTTCTTCGCTTCGCTAAC	AAGTGTGGGAGTGTCTGCAT
Sat 255	AAAACCACACAACTCTCCTCA	GGGAAAGGGAGAAAAGCTC
Sat 262	CTGCGAGGAGGAGTTAAAGATACCAC	GCCGGGAGTCTAGGGTTCTGTG
Sat 283	AGCACACACCCATACTCTCTT	GTGTGTGATTGTGTGTGAGAG
M24	GGCTCGAGATATCTGTTTAG	TTAATGGGCATAGGGTCC

Origin of primer -Invitrogen Corporation, Grand Island, NY, USA

PCR Amplification of RAPD Primers

PCR reactions for RAPD primers were performed in a final volume of 25 µL, containing 2 ng template of genomic DNA, 0.4 µM of primer, 75 µM dNTPs, 1.0 mM $MgCl_2$ (thermo), PCR reaction buffer 1 x TBE and 1 unit Taq DNA polymerase (Biolabs, England). The RAPD amplification was carried out in a Eurogene thermocycler (TECHNE, UK). The amplification program started with one cycle of initial denaturation at 94°C for 5 minutes followed by 43 cycles of 1 minute at 94°C (denaturation), 1 minute at 32°C (annealing), and 2 minutes at 72°C (elongation). The final extension was at 72°C for 7 minutes to ensure that the primer extension reaction was completed. Ten RAPD primers were amplified (Table 3). The primers were not screened for polymorphism as they had been used for diversity studies in coffee by Lashermes et al. (1996b)

Table 3. RAPD primers amplified and their nucleotide sequence

Primer	Nucleotide sequence
OP I-7	5'- CAGCGACAAG
OP I-20	5' - AAAGTGCGGG
OP J-19	5' - GGACACC ACT
OP L-18	5' - ACCACCCACC
OP M-4	5' - GGCGGTTGTC
OP N-18	5' - GGTG AGGTCA
OP X-16	5' - CTCTGITCGG
OP X-20	5' - CCCAGCTAGA
OP Y-10	5' - CAAACGTGGG
OP Y-15	5' – AGTCGCCCTT

NB: Origin of primer sequence: OP = Operon, from Operon Technologies Inc. (Ca, USA)

SSR and RAPD products were electrophoresed in 2.3% (w/v) agarose gel with 1 x TBE buffer system and then visualized in a UV trans-illuminator after staining with ethidium bromide.

Band Scoring and Data Analysis

Both SSR and RAPD data were scored for presence (1) or absence (0) of bands in the various genotypes. The 20 genotypes were first categorized into three groups namely induced tetraploid Robusta, Arabica and interspecific F_1 Arabusta hybrids and each group analyzed for total number of alleles and polymorphic alleles (Prakash et al., 2002) for both SSR and RAPD primers. The data was further organized into a matrix and subjected into cluster analysis using XLSTAT software, 2012 version. Cluster dendrograms were constructed using Unweighted Pair-Group method with Arithmetic Averages (UPGMA) (Anthony et al., 2002; Silveira et al., 2003; Kathurima et al., 2012) and used to estimate the genetic diversity among the genotypes.

RESULTS

Diversity Generated by SSR Primers

The co-dominance feature of SSR markers was not taken into account in this study due to difficulties in using the SSR markers as co-dominant in polyploid genome species such as *C. arabica* (Missio et al., 2009a). The SSR data was formatted and scored as dominant data for presence (1) or absence (0) of DNA bands. All the thirteen primers amplified the DNA with bands that could be clearly scored. The total number of alleles observed among the coffee genotypes based on the thirteen SSR primers was 46. Ten out of the thirteen primers were polymorphic among the genotypes studied while three primers (Sat 11, Sat 229 and Sat 262) were monomorphic. The number of alleles produced per primer ranged from 1 to 6 and the average number of alleles per primer was 3.6.

The genotypes were further classified into three groups' namely induced tetraploid Robusta, Arabica and F_1 interspecific Arabusta hybrids. Induced tetraploid Robusta accessions amplified a total of 33 alleles out of which 17 alleles were polymorphic. This group had 51.5% diversity among its genotypes (Table 4). Arabica genotypes amplified a total of 29 alleles out of which only 2 alleles were polymorphic resulting in 6.9% within group diversity (Table 4). Interspecific F_1 Arabusta hybrids had a total of 36 alleles out of which 18 alleles were polymorphic resulting in 50% within group diversity (Table 4). This implied that genetic diversity was low for Arabica genotypes and higher within induced tetraploid Robusta and interspecific F1 Arabusta hybrids.

Table 4. Results of SSR Primers Amplification

Primer	Tetraploid Robusta (3)		Arabica varieties (4)		F1 Arabusta Hybrids (11)	
	Number of alleles		Number of alleles		Number of alleles	
	Total	Polymorphic	Total	Polymorphic	Total	Polymorphic
Sat 11	1	0	1	0	1	0
Sat 32	3	2	3	1	3	2
Sat 172	2	1	1	0	2	1
Sat 207	3	2	4	0	6	4
Sat 227	3	1	2	0	3	2
Sat 229	1	0	1	0	1	0
Sat 235	1	0	1	0	2	0
Sat 240	3	2	2	0	3	1
Sat 254	2	1	1	0	2	2
Sat 255	4	1	3	0	4	2
Sat 262	2	0	2	0	2	0
Sat 283	4	3	4	0	2	0
M24	4	4	4	1	5	4
Total	33	17	29	2	36	18
Average	2.5	1.7	2.2	0.2	2.8	1.8
Polymorphic rate	**51.5%**		**6.9%**		**50%**	

Key: The number in brackets represents individuals per group

A cluster dendrogram constructed using the thirteen SSR primers were used to estimate the genetic diversity among the twenty coffee accessions (Fig. 1). The genotypes separated into three supported clusters. All the Arabica varieties (SL28, N39, SL34 and Caturra) which are also the female parents of the F_1 interspecific hybrids clustered together in the first cluster, an indication of close relatedness among them. HDT, Diploid Robusta and Caturra x UT8 hybrid clustered together in the second cluster. The remaining thirteen genotypes most of which were Arabusta hybrids clustered together in the third cluster. This cluster further separated into two unsupported sub clusters, one with seven genotypes and the other with six genotypes. This was an indication of more diversity among the genotypes but the primers used were not enough to significantly separate them in more supported clusters. The dendrogram clustered the Arabica accessions together and at a distance from all other genotypes. The maximum dissimilarity index observed was 4.9 (49%) which was also the diversity between Arabica and the other genotypes (Robusta and Arabustas). The dendrogram was truncated at 4.4 (44%) to define the three distinct clusters.

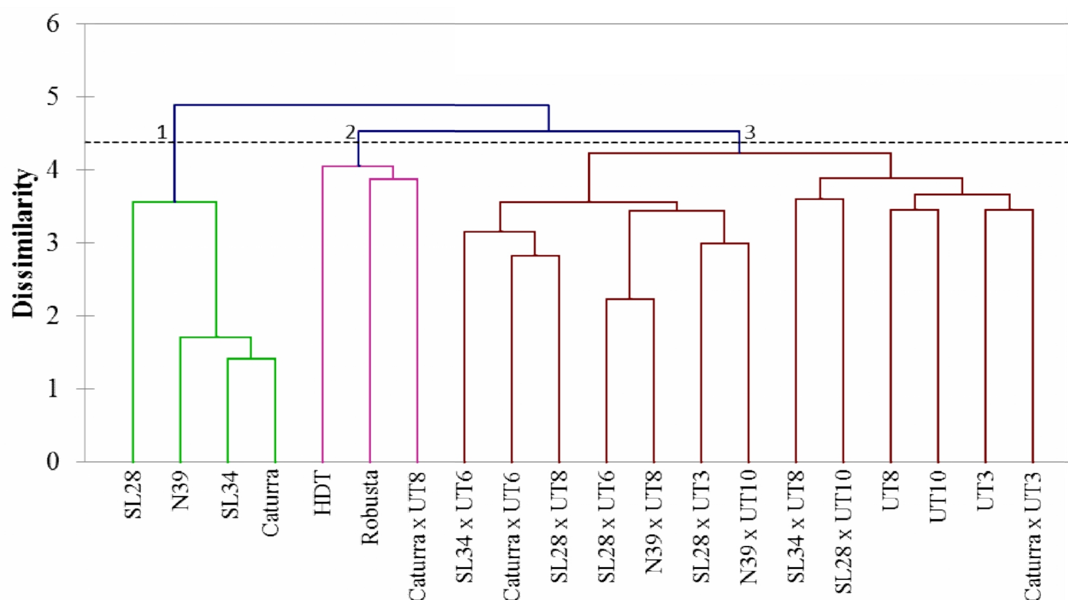

Fig. 1. Cluster dendrogram illustrating genetic diversity among the twenty coffee genotypes characterized using thirteen SSR primers. The broken line shows the point at which the dendrogram was truncated to define distinct clusters

Diversity Generated by RAPD Primers

Among the ten RAPD primers analyzed, seven showed amplification with clear bands. Three primers, I-7, X-16 and L-18 did not amplify the DNA. The total number of fragments observed among the twenty coffee genotypes based on the seven RAPD primers was 41 with an average of 5.9. The number of fragments produced per primer ranged from 3 to 8. Six of the seven primers generated polymorphic fragments. Primer N-18 did not exhibit polymorphism and generated the lowest number (3) of bands while Y-15 amplified the highest number (8) of bands (Table 5). The genotypes were further grouped into three classes namely induced tetraploid Robusta, Arabica and interspecific F_1 Arabusta hybrids. Induced tetraploid Robusta genotypes amplified a total of 31 fragments out of which 16 were polymorphic. The average number of amplified fragments per primer was 4.4 while the average number of polymorphic fragments per primer was 2.3.

Genetic diversity observed within induced tetraploid Robusta was 51.6%. A total of 29 fragments were amplified in Arabica genotypes with an average of 4.1 fragments per primer. Only one primer (X-20) was polymorphic among the Arabica genotypes resulting in a 3.5% polymorphism. The highest number of fragments was amplified among interspecific F_1 Arabusta hybrids recording a total of 38 fragments with an average of 5.4 fragments per primer, out of which 15 fragments were polymorphic. The rate of polymorphism for this group was 47.4%. Therefore, Arabica had the lowest genetic diversity of 3.5% while the induced tetraploid robusta and interspecific F1 Arabusta hybrids had a high genetic diversity of 51.6% and 47.4% respectively.

Table 5. The results of RAPD Primers Amplification

Primer	Tetraploid Robusta (3)		Arabica varieties (4)		F1 Arabusta Hybrids (11)	
	Number of alleles		Number of alleles		Number of alleles	
	Total	Polymorphic	Total	Polymorphic	Total	Polymorphic
OP I-20	4	1	5	0	6	3
OP J-19	6	3	5	0	6	1
OP M-4	5	3	2	0	4	2
OP N-18	2	0	3	0	3	0
OP X-20	7	5	3	1	6	5
OP Y-10	4	2	4	0	5	2
OP Y-15	3	2	5	0	8	2
Total	31	16	29	1	38	15
Average	4.4	2.3	4.1		5.4	2.1
Polymorphic rate	51.6%		3.5%		47.4%	

Key: The number in brackets represents individuals per group

A cluster dendrogram constructed using the RAPD primers was used to estimate the genetic diversity of the twenty (20) coffee accessions (Fig. 2). The genotypes separated into seven clusters. Induced tetraploid Robusta accessions: UT3, UT8, UT10; diploid Robusta and interspecific F_1 hybrid N39 x UT10 separated into cluster 1, 2, 3, 4, and 5 respectively. All the other nine (9) interspecific F_1 Arabusta hybrids clustered together. This cluster separated further into three unsupported sub clusters. This was an indication of more diversity among the genotypes but the primers used were not enough to significantly separate them in more supported clusters. HDT, interspecific F_1 hybrid SL28 x UT3 and all the Arabica varieties (SL28, SL34, N39 and Caturra) clustered together, an indication that the two Arabustas are more genetically related to Arabica than Robusta. The maximum dissimilarity index observed was 4.4 (44%) which was also the diversity between induced tetraploid Robustas and the other genotypes (Arabica and Arabustas). The dendrogram was truncated at 2.8 (28%) to define the seven distinct clusters.

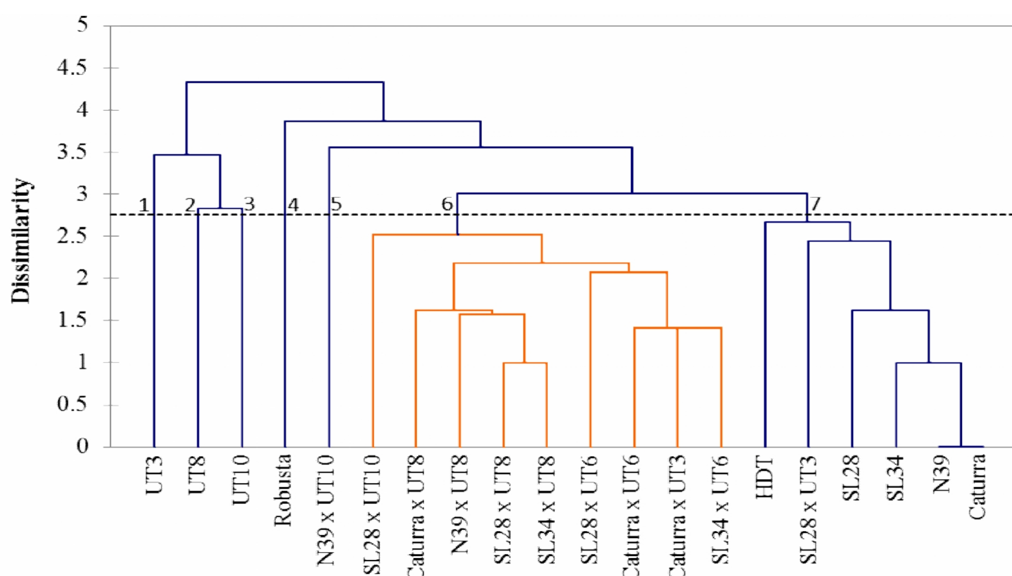

Fig. 2. Cluster dendrogram illustrating genetic diversity among the twenty coffee genotypes characterized using seven RAPD primers. The broken line shows the point at which the dendrogram was truncated to define distinct clusters

DISCUSSION

Microsatellite markers have been developed for a large number of plant species and are increasingly being used for germplasm diversity, linkage analysis and molecular breeding (Baruah et al., 2003). Although several studies have been carried out on interspecific F_1 Arabusta hybrids in Kenya (Owour and Van Der Vossen, 1981), there is little information about their genetic diversity. In this study, out of thirteen SSR primers used to characterize the 20 coffee accessions, ten (77%) amplified polymorphic alleles. This result was similar to Cristancho and Gaitan (2008) who reported amplification profile of 12 coffee SSR markers in diploid and tetraploid genotypes whereby 9 out of 12 (75%) markers were polymorphic in diploid genotypes while 5 were polymorphic in tetraploid genotypes. Combes et al. (2000) reported 5 (45%) polymorphic microsatellites primers out of 11 that were analyzed. Missio et al. (2009a) studied 33 primer pairs and reported that 22 of them (67%) exhibited polymorphism among 24 accessions.

The number of alleles per primer varied from 1 to 6 with an average of 3.6 alleles; 2.5 for induced tetraploid Robusta, 2.5 for diploid Robusta, 2.2 for Arabica and 2.8 for interspecific F_1 Arabusta hybrids. Similar result was reported by Moncada and McCouch (2004) using 34 SSR primers where an average of 2.5 and 1.9 amplified alleles per primer in 11 wild Arabica coffee genotypes and 12 cultivated Arabica coffee respectively, with the number of alleles ranging from 1 to 8. Maluf et al. (2005) reported 2.9 alleles per locus on 23 SSR primers for 28 accessions of *C. arabica* and *C. canephora*. Anthony et al. (2002) reported an average number of 4.7 alleles per primer using only six primers in Arabica coffee collections containing four Typicas, five Bourbons and 10 sub spontaneous derived accessions. Missio et al. (2010) reported an average of 5.1 alleles per primer for 17 SSR primers. Teressa et al. (2010) reported an average of 6.5 alleles, 5.9 alleles and 3.5 alleles per primer using 32 SSR primers for Arabica collections, Ethiopian collections and cultivated varieties respectively. The reason for such a difference may be due to differences in sample sizes and the type of coffee genotypes used in the previous studies as compared to the present study or number of SSRs primers used and their genome coverage (Teressa et al., 2010). These findings demonstrated that the total number of alleles in a population depends highly on the genetic constitution assessed (Missio et al.,

2009b). Induced tetraploid Robusta and interspecific F_1 Arabusta hybrids recorded higher polymorphism than Arabica genotypes. These results was similar to Missio et al. (2009a) who reported polymorphism of 46% for *C. canephora*, 22% for Arabica and 22% for triploid accessions using 33 SSR primers. Missio et al. (2011) reported polymorphism of 89.2% for Robusta and 39.5% in Arabica. Missio et al. (2010) reported 88.2% polymorphism for *C. canephora* genotypes and 35.3% within six rust resistant commercial varieties of *C. arabica* and the lowest level of 11.8% within *Coffea arabica* genotypes using ESTR- SSR primers. The higher molecular polymorphism for Robusta genome could be either due to the high polymorphic nature of the *Coffea canephora* species (Teressa et al., 2010) while for the interspecific F_1 hybrids may be due to Robusta genome introgression. The low molecular polymorphism in Arabica cultivars is attributed to the allotetraploid origin and mode of speciation of *C. arabica* and the restricted genetic base of the original population from which the varieties evolved (Agwanda et al., 1997)

In the cluster dendrogram generated from SSR data, all Arabica genotypes clustered together. One interspecific F_1 hybrid (Caturra x UT8) clustered with HDT and Robusta, implying that the SSR markers used were able isolate the gene of introgression from its *C. canephora* parents. All the other 9 interspecific F_1 hybrids clustered together with all the 3 induced tetraploid Robusta genotypes. This implied that from the general analysis, the coffee genotypes clustered according to the two different species of origin namely Robusta and Arabica. Similar finding was reported by Kathurima et al. (2012) where Robusta introgressed genotypes (HDT, Ruiru 11 and Catimor clustered together with Robusta. Anthony et al. (2002) also reported similar findings on Typica and Bourbon accessions where the two were classified according to their genetic origin. In the same study, Yemen cultivars were grouped together with Typica and di-haploid with Bourbon accessions. Missio et al. (2011) reported similar results on traditional Arabicas, resistant Arabicas, triploids, HDT and Robusta. Broadening the narrow genetic base of Arabica coffee is therefore achievable through interspecific hybridization.

RAPD markers also successfully revealed variability among the coffee accessions studied. This was in agreement with other researchers (Lashermes et al., 1993; Lashermes et al., 1996b; Agwanda et al., 1997; Anthony et al., 2002; Sera et al., 2003; Masumbuko et al., 2003; Dinesh et al., 2011 and Kathurima et al., 2012) who reported the successful use of RAPDs in genetic characterization of *Coffea* species. The seven polymorphic RAPD primers generated 41 bands with an average of 5.9 bands and number of bands ranging from 3 to 8 per primer. These results was similar to Lashermes et al (1993); Lashermes et al (1996b) and Agwanda et al. (1997). Lashermes et al. (1993) analyzed 23 primers that amplified 112 fragments with the number of amplified fragments varying from 1 to 8 and an average of 4.3 fragments per primer. Agwanda et al. (1997) reported an average of 8 fragments per primer in Arabica coffee with number of fragments ranging from 1 to 15. Similarly, Lashermes et al. (1996b) reported polymorphism in wild and cultivated *Coffea arabica* accessions with RAPD primers that generated between 1 and 15 bands.

The rate of polymorphism for induced tetraploid Robusta, interspecific F_1 hybrids and Arabica was 51.6%, 47.4% and 3.5% respectively. This study further observed close proximity between four non introgressed Arabica cultivars, namely SL28, SL34, N39, and Caturra. In a similar study, comprising of Robusta, Eugenioides and Arabica genotypes, Kathurima et al. (2012) reported a polymorphism of 42% among 24 coffee interspecific accessions with minimal diversity among non introgressed Arabica genotypes. Agwanda et al. (1997) also reported a polymorphismof 5.2% in Arabica coffee and similarly very close proximity among non introgressed Arabica cultivars, SL28, K7, and Caturra. Unlike these authors, Sera et al. (2003) reported higher polymorphism of 68% in 14 elite *Coffea arabica* varieties. Silveira et al. (2003) also reported a higher genetic diversity of 38.5% and 61.5% among and within Arabica coffee populations, respectively. In this study, it was also observed that the genotypes clustered according to the different species of origin i.e. Robusta and Arabica. This corroborated the earlier finding by Kathurima et al. (2012) where coffee accessions

clustered according to the three different species namely Eugenoides, Robusta and Arabica.

CONCLUSION

The study demonstrated the existence of varying genetic diversity within species and among the genotypes evaluated. It also confirmed the narrow genetic base within Arabica coffee and successfully portrayed the possibility of broadening it through interspecific hybridization. Although the genetic diversity was high for both induced tetraploid Robusta and interspecific F_1 hybrids genotypes, both SSR and RAPD markers revealed a relatively higher diversity for the former as compared to the later. Both SSR and RAPD markers efficiently detected the genetic diversity among the genotypes characterized with minimum variations indicating that both markers can be reliably employed in genetic diversity studies. However, use of more primers of both markers may enable more accurate estimation of genetic diversity and possibly reveal some markers linked to some desirable traits. These results can be used to select parents with high combining ability in a hybridization program between Arabica and tetraploid Robusta thus ensuring hybrid vigour.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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