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Genetic diversity of bitter and sweet African bush mango trees (*Irvingia* spp., Irvingiaceae) in West and Central Africa

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Economically important food tree species in sub-Saharan Africa should be domesticated to enhance their production within agro forestry systems. The African bush mango trees (*Irvingia* species) are highly preserved and integrated in agro forestry systems in tropical Africa. However, the taxonomic debate related to the species or varietal status of the bitter and sweet fruited African bush mango trees hinders their domestication process and rational use. Amplified fragment length polymorphisms (AFLPs) and chloroplast simple sequence repeats (cpSSRs) were used in this study to assess the genetic diversity of African bush mango trees and to test the distinction between bitter and sweet fruited trees, sampled across Togo, Benin, Nigeria and Cameroon. Both the AFLPs and cpSSRs showed low genetic diversity for the Dahomey Gap bitter trees population. This is due to the higher fragmentation and the continuous reduction of this small sized population occurring in a limited forest ecosystem. The higher polymorphism and genetic diversity of the sweet mango tree populations in Benin and Togo showed the effects of domestication of materials of different geographical origin coupled with the frequent long distance transfer of genetic materials. When used separately, the AFLPs and cpSSRs failed to consistently discriminate the populations and type of trees. From the combined dataset, both markers differentiated geographically recognizable groups; bitter from sweet mango trees. However, Nigerian sweet mango trees clustered with the bitter ones. The suitability of AFLPs and cpSSRs to test our hypotheses within *Irvingia* needs to be thoroughly reassessed.

Key words: AFLP, Benin, cpSSR, Togo, Dahomey Gap, *Irvingia*, taxonomy, domestication.

INTRODUCTION

The food tree species in African tropical forests are important sources of food in sub Saharan Africa (Hladik et al., 1996; Malaisse, 1997; FAO, 2008; Augustino et al., 2011). Despite the increased food deficits, ecosystems face very high destruction (Laurance, 1999; Archard et al., 2002). Thus, domestication of the most important food tree species used by local communities in their daily diets remains a logical policy. This will strengthen

traditional and regional strategies for agrobiodiversity maintenance while enhancing the global production of agro forestry systems.

The International Centre for Research in Agroforestry (ICRAF, now called the World Agroforestry Centre) has become a leading institution in traditional food tree species domestication in West and Central Africa. Since decades, the African bush mango trees (ABMTs) are systematically preserved and integrated in various traditional agroforestry systems in humid sub-Saharan Africa (Okafor and Fernandes, 1987; Franzel et al., 1996; Tabuna, 2001; Okunomo and Egho, 2010). ABMTs are widely distributed and taxonomically ambiguous taxa within the family of Irvingiaceae thus the bitter fruited and sweet fruited forms are difficult to be distinguished. The only way to easily differentiate them is by assessing the bitterness versus sweetness of the mesocarp because there are limited morphological differences between the ABMTs (Harris, 1996).

However, these mangoes exhibit a high morphological and phenological diversity, which is vital for domestication and selection programs (Harris, 1996; Atangana et al., 2002). There is also no clear relation between biochemical properties and the type of ABMT (Tchoundjeu and Atangana, 2007). Due to this overlap of morphological, phenological and biochemical properties, the correct taxonomy of ABMTs needs to be re-visited. Okafor (1975) proposed the variety level for sweet and bitter ABMTs, respectively, *Irvingia gabonensis* (Aubry-LeComte ex O'Rorke) Baill. var. *gabonensis* and *Irvingia gabonensis* var. *excelsa* (Mildbr.) Okafor. Although, based on a thorough taxonomic revision (Harris 1996) and a random amplified polymorphic DNA (RAPD) analysis (Lowe et al., 2000), a distinction at species level was suggested: *I. gabonensis* and *I. wombolu* Vermoesen, for sweet and bitter trees, respectively. The latter taxonomic grouping was not supported due to the low reproducibility of the RAPD analysis.

Moreover, a sound quantitative morphological comparison between bitter and sweet ABMTs is still lacking. Still, in order to ensure proper *in-* and *ex situ* conservation of the genetically diverse material and to support its development and genetic improvement (Grace et al., 2008), a clear taxonomic identity of the material is essential. Furthermore, the geographic origin of ABMTs that occur in the Dahomey gap (which is the wide savannah area separating the West African forest into the Upper and Lower Guinean forest blocks in Benin and Togo) remains an important topic. Like RAPDs, the amplified fragment length polymorphisms (AFLPs) are dominant markers. However, the power of AFLPs to reveal genetic diversity and difference even for closely related species are demonstrated better than that of the RAPDs. Thus, AFLPs were successfully used by Ude et al. (2006) to separate population of the Lower Guinean and Congolian forest block provenances of the sweet ABMTs. Simple sequence repeat (SSR) are PCR based-co-dominant markers, consist of repeats of short nucleotide sequences and are proven to have great importance in the studies of genome (Stafne and Clark, 2005). The chloroplast simple sequence repeats (cpSSRs) are genetic markers with uniparental inheritance used particularly to investigate phenomena of evolution of species and to assess the phylogenetic relations between populations of species (Wills et al., 2005).

This study intends to revisit the pattern of genetic diversity of ABMTs in answering two main questions: 1) What is the genetic diversity and differentiation within and between bitter and sweet ABMTs, and what do the patterns tell us about the geographical origin of the Dahomey Gap material? 2) Are our markers (AFLPs and cpSSRs) suitable to consistently discriminate populations and types of ABMTs?

MATERIALS AND METHODS

Sampling

Sweet and bitter ABMTs were sampled throughout the Dahomey Gap and in Cameroon. Materials were also sampled in four gene banks: IITA-Ibadan (International Institute for Tropical Agriculture) and NAGRAB (National Centre for Genetic Resources and Biotechnology) both in Nigeria and Kolbison and Mbalmayo both established by the World Agroforestry Centre in Cameroon (Table 1). For each sampled tree, young leaves were collected and stored in silica gel. DNA was extracted from each sample following the protocol described in Fulton et al. (1995). An AFLP analysis was first carried out on 33 samples (10 bitter and 23 sweet trees). This was followed by an independent cpSSR analysis carried out with 47 samples (14 bitter and 33 sweet trees), including more individuals from the Dahomey Gap and the regions postulated as ABMTs genetic diversity centres by Lowe et al. (2000) and Ude et al. (2006) in the Lower Guinean forest and the Congolian forest blocks. Thus, in total, 59 accessions (39 in the Dahomey Gap and 20 from Nigeria and Cameroon) were used in this study with 21 samples common to both analyses (Table 1).

Genetic diversity

The AFLPs are more reliable genetic markers that generate large number of bands and have high reproducibility. Therefore, they are

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Table 1. ABMT accessions and types of analysis applied.

Accession	Type of ABMT	Provenance: Site of collection and country	Population	Types of analysis applied
P2	Sweet	Pobè, South Benin	SDG	AFLP
POB21	Sweet	Pobè, South Benin	SDG	AFLP
IP2	Sweet	Pobè, South Benin	SDG	AFLP
Ip4	Sweet	Pobè, South Benin	SDG	AFLP
Coco6	Sweet	Calavi, South Benin	SDG	AFLP
NPA4	Sweet	Parakou, North Benin	SDG	AFLP
NPA6	Sweet	Parakou, North Benin	SDG	AFLP
MBM1	Sweet	Sangmelima, South Cameroon	STA	AFLP
IW3BAD5	Bitter	Badou, Southwest Togo	BDG	AFLP
FNGB	Bitter	NAGRAB Gene bank, Nigeria	BTA	AFLP
DNGB	Bitter	NAGRAB Gene bank, Nigeria	BTA	AFLP
IWSAK1	Bitter	Centre Cameroon, ICRAF Kolbison Gene Bank	BTA	AFLP
POB20	Sweet	Pobè, South Benin	SDG	AFLP + SSR
Coco1	Sweet	Calavi, South Benin	SDG	AFLP + SSR
Coco5	Sweet	Calavi, South Benin	SDG	AFLP + SSR
NPA7	Sweet	Parakou, North Benin	SDG	AFLP + SSR
NPA9	Sweet	Parakou, North Benin	SDG	AFLP + SSR
TG1	Sweet	Atakpamè, Centre Togo	SDG	AFLP + SSR
TG18	Sweet	Lomé, South Togo	SDG	AFLP + SSR
BAD1	Sweet	Badou, Southwest Togo	SDG	AFLP + SSR
WAMP2	Sweet	Badou, Southwest Togo	SDG	AFLP + SSR
IGIBDGB2	Sweet	IITA Gene bank, Nigeria	STA	AFLP + SSR
IGIBDGB1	Sweet	IITA Gene bank, Nigeria	STA	AFLP + SSR
Limb	Sweet	Limbé, Southwest Cameroon	STA	AFLP + SSR
Limbe6	Sweet	Limbé, Southwest Cameroon	STA	AFLP + SSR
IGGBWAC	Sweet	ICRAF Kolbison Gene Bank, Yaoundé	STA	AFLP + SSR
NGMK1	Sweet	Sangmelima, South Cameroon	STA	AFLP + SSR
NMKIW02	Bitter	Mamfé, South Cameroon, Mbalmayo Gene bank	BTA	AFLP + SSR
BSIW07	Bitter	Mamfé, South Cameroon, Mbalmayo Gene bank	BTA	AFLP + SSR
IWSAK2	Bitter	Centre Cameroon, ICRAF Kolbison Gene Bank	BTA	AFLP + SSR
CENRAD	Bitter	CENRAD Gene bank	BTA	AFLP + SSR
BAD4kiw	Bitter	Badou, Southwest Togo	BDG	AFLP + SSR
TGIW2	Bitter	Badou, Southwest Togo	BDG	AFLP + SSR
P2	Sweet	Pobè, South Benin	SDG	SSR
POB21	Sweet	Pobè, South Benin	SDG	SSR
CALI	Sweet	Calavi, South Benin	SDG	SSR
TORI13J	Sweet	Calavi, South Benin	SDG	SSR
TORI25	Sweet	Calavi, South Benin	SDG	SSR
Djot6	Sweet	Couffo, South Benin	SDG	SSR
LALO1G	Sweet	Couffo, South Benin	SDG	SSR
VODassa	Sweet	Dassa Centre Benin	SDG	SSR
Djoug	Sweet	Djougou, North Benin	SDG	SSR
Peninsou	Sweet	Peninsoulou, North Benin	SDG	SSR
Lom1	Sweet	Lomé, South Togo	SDG	SSR
L2	Sweet	Lomé, South Togo	SDG	SSR
TG4	Sweet	Lomé, South Togo	SDG	SSR
TG12	Sweet	Lomé, South Togo	SDG	SSR
Atak	Sweet	Atakpamè, Centre Togo	SDG	SSR

Table 1. Contd.

BAD5	Sweet	Badou, Southwest Togo	SDG	SSR
IGGBWACII	Sweet	Centre Cameroon, ICRAF Kolbison Gene Bank	STA	SSR
MBUM	Sweet	Sangmelima, South Cameroon	STA	SSR
KGH1	Bitter	Kougnonhou, Southwest Togo	BDG	SSR
KGH2	Bitter	Kougnonhou, Southwest Togo	BDG	SSR
KGH3	Bitter	Kougnonhou, Southwest Togo	BDG	SSR
KGH4	Bitter	Kougnonhou, Southwest Togo	BDG	SSR
NKIW19	Bitter	Mamfé, South Cameroon, Mbalmayo Gene bank	BTA	SSR
BSIW324	Bitter	Mamfé, South Cameroon, Mbalmayo Gene bank	BTA	SSR
T2BSIW16	Bitter	Mamfé, South Cameroon, Mbalmayo Gene bank	BTA	SSR
IWSAK3	Bitter	Centre Cameroon, ICRAF Kolbison Gene Bank	BTA	SSR

AFLP, Amplified fragment length polymorphisms; cpSSRs, chloroplast simple sequence repeats (or microsatellites), SDG, sweet ABMTs from the Dahomey Gap, BDG, bitter ABMTs from the Dahomey Gap, STA, sweet ABMTs from Tropical Africa, BTA, bitter ABMTs from Tropical Africa.

widely used in genetic analysis (Berchowitz et al., 2001; Assogbadjo et al., 2010) as opposed to the RAPDs with low reproducibility (Powell et al., 1996). Microsatellites (or simple sequence repeats, SSRs) and especially chloroplast microsatellites (cpSSRs), have the power to reveal genetic diversity as well as phylogenetic relationships and hybridization between plant species (Wills et al., 2005; Panwar et al., 2010). Thus AFLPs and cpSSRs were used for these analyses.

AFLP data

The AFLP analysis was carried out at the Biosystematics Group, University of Wageningen, The Netherlands, following the procedure of Vos et al. (1995) with minor modifications. Three primer combinations previously identified to amplify sweet mango tree material (Ude et al., 2006) were used to analyze all the 33 samples. These included: E38M59 (Eco ACT/ se CTA), E40M62 (Eco AGC/Mse CTT) and E33M48 (Eco AAG/Mse CAC). PCR reactions were performed using a MJ PTC200 thermocycler. Prior to the selective amplification, the EcoR1 primer was fluorescently labelled with IRD700. AFLP fragments were separated on a LYCOR 4300 (Westburg, The Netherlands), and the resulting profiles were scored using the Quantar software (Key Gene Products, Wageningen, The Netherlands 2000) to produce the presence/absence data matrix.

cpSSR data

The cpSSR analysis was carried out in the Laboratory of Genetics and Biotechnology of the University of Abomey-Calavi, Benin. Eighteen (18) chloroplast SSR primers were tested on independent samples (bitter and sweet mango tree accessions). They included: CCMP 2, NTCP 8, NTCP 9, NTCP 30, NTCP 37, NTCP 39, NTCP 40, NTCP 5, NTCP 16, NTCP 19, NTCP 25, NTCP 26, NTCP 27, NTCP 29, NTCP 32, NTCP 33, NTCP 34 and NTCP 38. Among the 18 primers, seven of them (CCMP 2, NTCP 8, NTCP 9, NTCP 30, NTCP 37, NTCP 39 and NTCP 40) that amplified the chloroplast DNA of ABMT material were retained and used to test the amplification and polymorphism of complete set of 47 samples. PCR reaction was performed using a Peltier-Effect Cycling PTC 100 thermocycler programmed for an initial denaturation at 94°C for 4 min, followed by 35 cycles at 94°C for 30 s per cycle, annealing temperature (55 - 60°C) for 1 min, a step at 72°C for 1 min, and a final extension step at 72°C for 5 min. Migration of the PCR products was visualized with denaturing polyacrylamide gel (5%) electrophoresis and then revealed with silver nitrate in accordance with Chair et al. (2005). The electrophoresis bands were scored to generate a presence/absence data matrix.

Data analysis

Genetic diversity and structure

Three datasets were considered in this study: the AFLP set, the cpSSRs set and the one containing the accessions that showed a result for both AFLP and cpSSR. An analysis of the genetic diversity and population structure based on allele frequency using AFLP-SURV version 1.0 (Vekemans, 2002) was performed on each dataset. The type of ABMT (sweet versus bitter) was considered as well as the geographical origin of the sample. Four geographic 'populations' were considered: (i) bitter mango trees from the Dahomey Gap (Benin and Togo = population BDG), (ii) bitter mango trees from Tropical Africa (population BTA), (iii) sweet mango trees from the Dahomey Gap (Benin and Togo = population SDG) and (iv) sweet mango trees from Tropical Africa (population STA; Table 1). For each dataset, we computed the mean Nei genetic diversity (Nei, 1973) for each population, the global genetic differentiation (Fst statistics) and the pair wise genetic distance among populations and between sweet and bitter mango trees. Assuming there was no genetic structure among populations under Hardy-Weinberg equilibrium (Vekemans, 2002), the significance of the genetic differentiation was assessed by comparing the observed F_{st} with the distribution of obtained F_{st} using 100 random individual permutations.

Identification of populations and distinction between sweet and bitter ABMTs

To assess the effectiveness of the genetic markers used in the discrimination of the four geographically recognized populations as well as the two types of ABMT, a cluster analysis was carried out on each dataset and a dendrogram was produced using the well as

Table 2a. Results of the genetic diversity analysis with AFLP-SURV.

Genetic diversity	Population level				Type level	
	BTA	BDG	SDG	STA	Bitter	Sweet
AFLPs						
Segregating fragments (%)			96.5			97.2
Polymorphism (%)	22	66.7	**85.1**	60.3	66.7	**76.6**
Nei's genetic diversity	0.091	0.263	0.304	0.234	0.221	**0.264**
cpSSRs						
Segregating fragments (%)			100			100
Polymorphism (%)	55	70	65	**90**	60	**80**
Nei's genetic diversity	0.202	0.273	**0.289**	0.235	0.240	**0.278**
AFLPs + cpSSRs						
Segregating fragments (%)			83.9			85.1
Polymorphism (%)	24.8	50.9	**70.8**	63.4	50.9	**67.1**
Nei's genetic diversity	0.105	0.232	**0.251**	0.211	0.210	**0.245**

AFLP, Amplified fragment length polymorphisms; cpSSRs, chloroplast simple sequence repeats (or microsatellites), SDG, sweet ABMTs from the Dahomey Gap, BDG, bitter ABMTs from the Dahomey Gap, STA, sweet ABMTs from Tropical Africa, BTA, bitter ABMTs from Tropical Africa. *Highest figures in bold.

the two types of ABMT, a cluster analysis was carried out on each dataset and a dendrogram was produced using the unweighted pair group method with arithmetic mean (UPGMA) method based on Jaccard similarity index (Jaccard, 1908) in Past software (Hammer et al., 2001):

$$I_j = {}^{a}/_{(a+b+c)} \quad (1)$$

Where, for a random pair of individuals, a = number of totally loci scored present for the two individuals, b = number of loci scored present exclusively present for only one individual and c = number of loci exclusively present for only the second individual.

When classifying individuals using the principal component analysis (PCA), there is unclear grouping pattern due to the abundance of factors of low contribution to an existing pattern in the dataset. Therefore, the PCA axes that explained a high percentage of the total variance within the dataset or the factors correlated with those PCA often used in a subsequent multivariate analysis to get a better signal from the dataset (Mohammadi and Prasanna, 2003; Bidogeza et al., 2009). Because the separate use of the AFLPs and cpSSRs data generated more confusing patterns, only the combined AFLPs + cpSSRs dataset was used in the rest of the analysis. First, all the alleles with no variability (totally shared presence and absence) in the AFLPs + cpSSRs dataset were excluded. A principal coordinate analysis (PCoA) was performed in order to highlight the main groups yielded in the cluster analysis. The AFLPs + cpSSRs datasets without the totally shared presence and absence alleles were used to detect relationship among our sample. First, a PCA was performed and the axes that accumulated at least 70% of the total variation within the dataset were retained. The loci that were highly correlated (at least 70%) with those axis were used to produce a Neighbor Joining (NJ) tree using Kulczynski similarity index. Like Jaccard index, the Kulczynski similarity index (Kulczynski, 1927) does not integrate totally shared absent alleles and is one of the most consistent similarity index used in systematic and taxonomy (Boyce and Ellison, 2001). Especially, this index is influenced by the total number of loci that make the different individuals between two randomly chosen individuals (Kronberg, 1987).

$$I_K = 0.5 * \left(\frac{a}{a+b} + \frac{a}{a+c}\right) \quad (2)$$

RESULTS

For the AFLPs analysis, a total of 141 polymorphic alleles were scored for all the 33 individuals. The cpSSR analysis yielded 20 polymorphic alleles (one to six per locus). Thus, a total of 161 polymorphic alleles were available for the set of 21 samples with both AFLP and cpSSR results.

Genetic diversity and differentiation of ABMTs

The AFLP analysis indicated that the mean number of fragments scored as present for an individual tree was 47. The number of segregating fragments in the dataset was high (96.5%). The polymorphism was higher within the Dahomey Gap sweet mango tree population and lowest in the Dahomey Gap bitter mango trees population. The same tendency was observed regarding the within population Nei genetic diversity. The sweet mango tree population in the Dahomey Gap showed significantly highest genetic diversity, while the bitter ones in this eco-region displayed the lowest diversity (Table 2a). The test for genetic differentiation among

Table 2b. Pairwise F_{st} statistics among populations.

Parameter	BTA	BDG	SDG	STA
AFLP				
BTA	0	-	-	-
BDG	0.149	0	-	-
SDG	0.0731	0.2407	0	-
STA	0	0.1587	0.0789	0
cpSSRs				
BTA	0	-	-	-
BDG	0.0772	0	-	-
SDG	0	0.1244	0	-
STA	0.0437	0.3268	0.0411	0
AFLPs + cpSSRs				
BTA	0	-	-	-
BDG	0.2511	0	-	-
SDG	0.132	0.1618	0	-
STA	0.1646	0.2916	0.096	0

AFLP, Amplified fragment length polymorphisms; cpSSRs, chloroplast simple sequence repeats (or microsatellites), SDG, sweet ABMTs from the Dahomey Gap; BDG, bitter ABMTs from the Dahomey Gap; STA, sweet ABMTs from Tropical Africa; BTA, bitter ABMTs from Tropical Africa. *Highest figures in bold.

populations indicated a global F_{st} value of 0.108 (P = 0.024). The pairwise F_{st} values among populations was higher between bitter and sweet mango tree populations within the Dahomey Gap and no genetic difference was found between bitter and sweet tree populations outside of the Dahomey Gap (Table 2b). Sweet mango trees showed a higher polymorphism and Nei genetic diversity than bitter mango trees (Table 2a). However, the difference based on this AFLP data was not significantly different (global F_{st} = 0.034; P = 0.0639). The F_{st} between bitter and sweet mango tree populations were very low (0.011).

The cpSSR results showed that the mean number of fragments scored as present at individual tree level was 8 and all the 20 scored loci have segregation power. The polymorphism was highest in the sweet trees population outside the Dahomey Gap and lowest for the Dahomey Gap bitter trees. The sweet tree populations in the Dahomey Gap and the bitter one in tropical Africa presented the highest genetic diversity, while the bitter tree population in the Dahomey Gap presented the lowest diversity again (Table 2a). No clearly significant genetic differentiation was found among populations (global F_{st} = 0.105; P = 0.048 ≅ 0.05) even though the highest pairwise F_{st} was found between bitter tree population of the Dahomey Gap and the sweet trees of Tropical Africa (Table 2b).

Considering bitter versus sweet trees, the genetic diversity was highest in the sweet mango trees (Table 2a). However, based on the cpSSRs there was no significant genetic differentiation amongst the two types (F_{st} = 0.0537; P = 0.077), and the F_{st} between bitter and sweet tree populations was low (0.0540).

The combined AFLPs + cpSSRs data also indicated a high number of alleles with segregating power (83.9%), with 24 alleles presenting no variability. The within population proportion of polymorphism and the Nei genetic diversity showed the same tendency as the separate AFLPs and cpSSRs results: the highest value was calculated for the sweet trees population from the Dahomey Gap and the lowest in the bitter trees population from this eco-region (Table 2a). A significant genetic differentiation was detected among populations (global F_{st} = 0.0176; P = 0.016). The bitter tree population in the Dahomey Gap and the sweet one in Tropical Africa had the highest pairwise F_{st} while the lowest distance was shown between the sweet tree population in the Dahomey Gap and that in Tropical Africa (Table 2b). Considering sweet and bitter mango trees, 85.1% of the combined AFLP and SSR alleles had segregation power. The proportion of polymorphic loci was higher within sweet trees than within bitter trees (Table 2b).

The Nei genetic diversity was 0.2453 and 0.21 for sweet and bitter trees, respectively. The genetic differentiation between sweet and bitter trees was low (F_{st} = 0.0335) and not significantly different (global F_{st} =

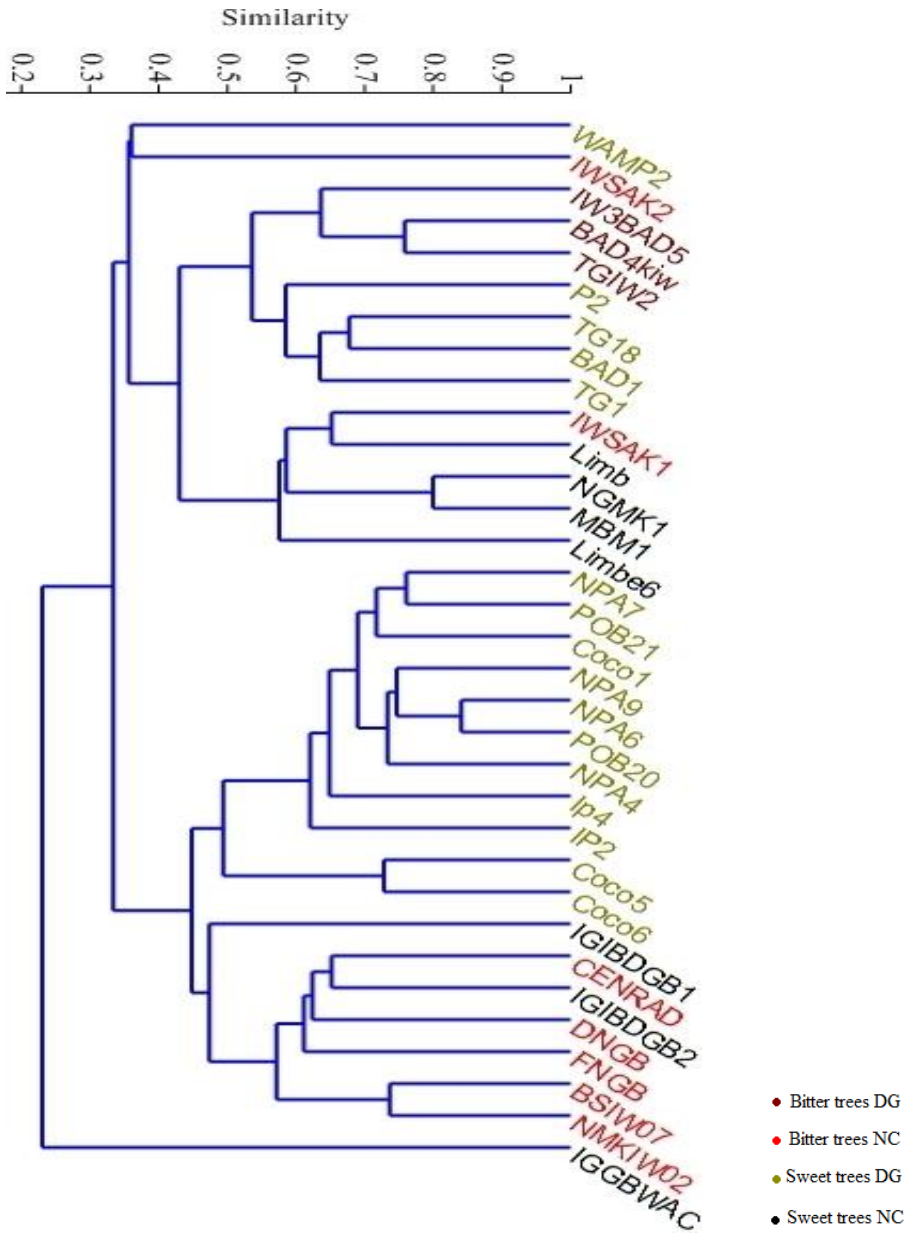

Figure 1a. UPGMA dendrogram for AFLP data of the 33 accessions based on Jaccard's similarity index.

0.0333; P = 0.064).

Cluster analysis

The dendrogram based on the AFLP results (Figure 1a) showed no clear pattern among populations and no clear distinction between bitter and sweet mango trees. The majority of individuals from each considered population were spread across many clusters. However, apart from few accessions, there is a tendency for the Togo (bitter and sweet) and Benin (sweet) materials to cluster together (Figure 1a and Table 1).

The dendrogram based on the cpSSR results (Figure 1b and Table 1) showed an even less clear pattern with higher similarity among individuals and completely failed to discriminate between the two types of ABMTs or geographically defined populations.

The dendrogram resulting from the cluster analysis of the combined APLP + cpSSR data (Figure 1c and Table

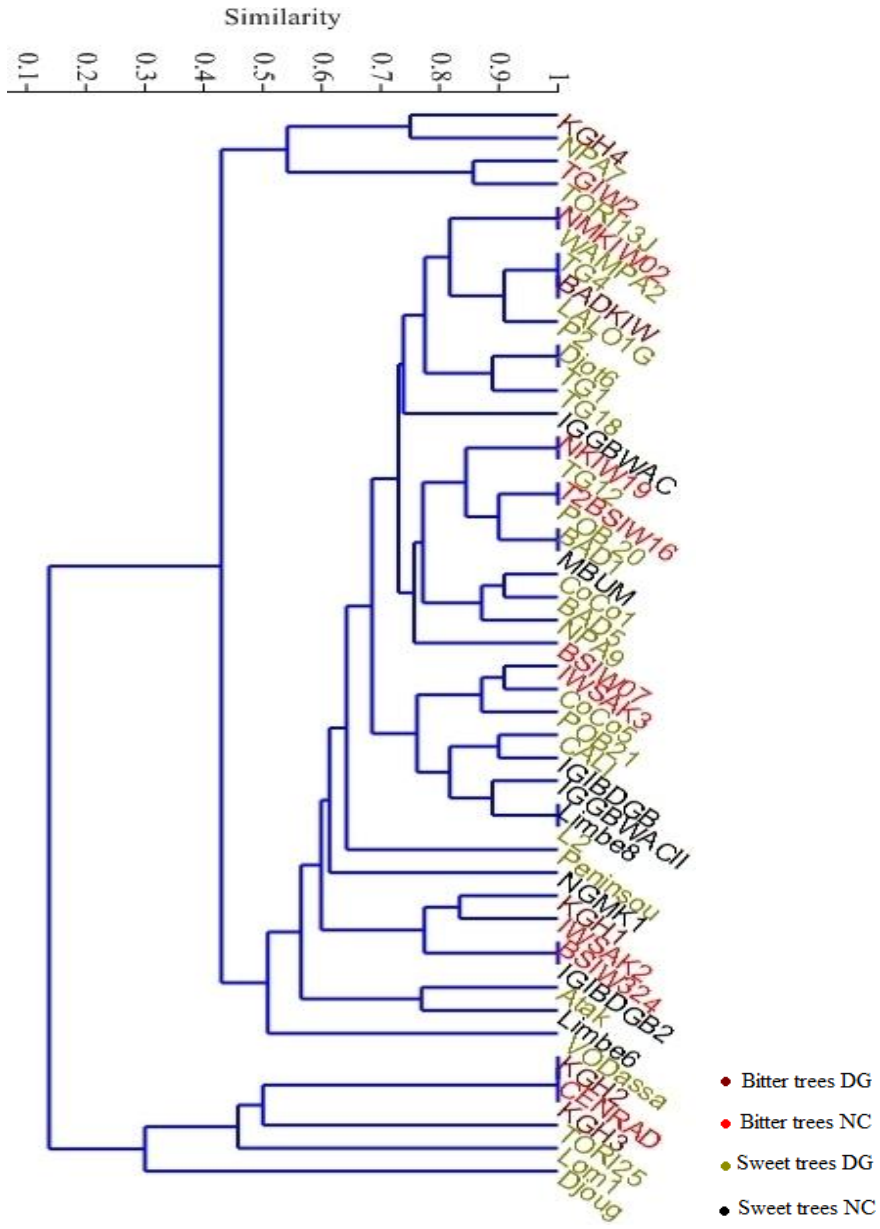

Figure 1b. UPGMA dendrogram for cpSSR data of the 47 accessions based on Jaccard's similarity index.

1) was more discriminative than those obtained from the separate AFLP and cpSSR datasets. Apart from one accession from Togo (WAMP2), four geographically distinct groups could be distinguished from the lower to upper position: (i) all sweet trees from Benin, (ii) sweet and bitter trees from the Lower Guinean forest bloc (southern Nigeria and Mamfé region in South-west Cameroon), (iii) sweet and bitter trees from Central and South Cameroon, and (iv) bitter and sweet trees from Togo.

The first two axes of the PCoA on the combined dataset with 137 alleles (Figure 2 and Table 1) accounted for 62.5% of the variance (46.13 and 16.38% for coordinate 1 and 2, respectively). The PCoA tended to separate the bitter from sweet ABMTs, with the two sweet trees from Nigeria (IGIBGB1 and IGIBGB2) falling within the bitter tree group. Within each of the two groups, the populations were not clearly distinguishable apart from the sweet trees from Benin as shown in Figures 1a and c.

Forty-eight (48) alleles were highly correlated (at least

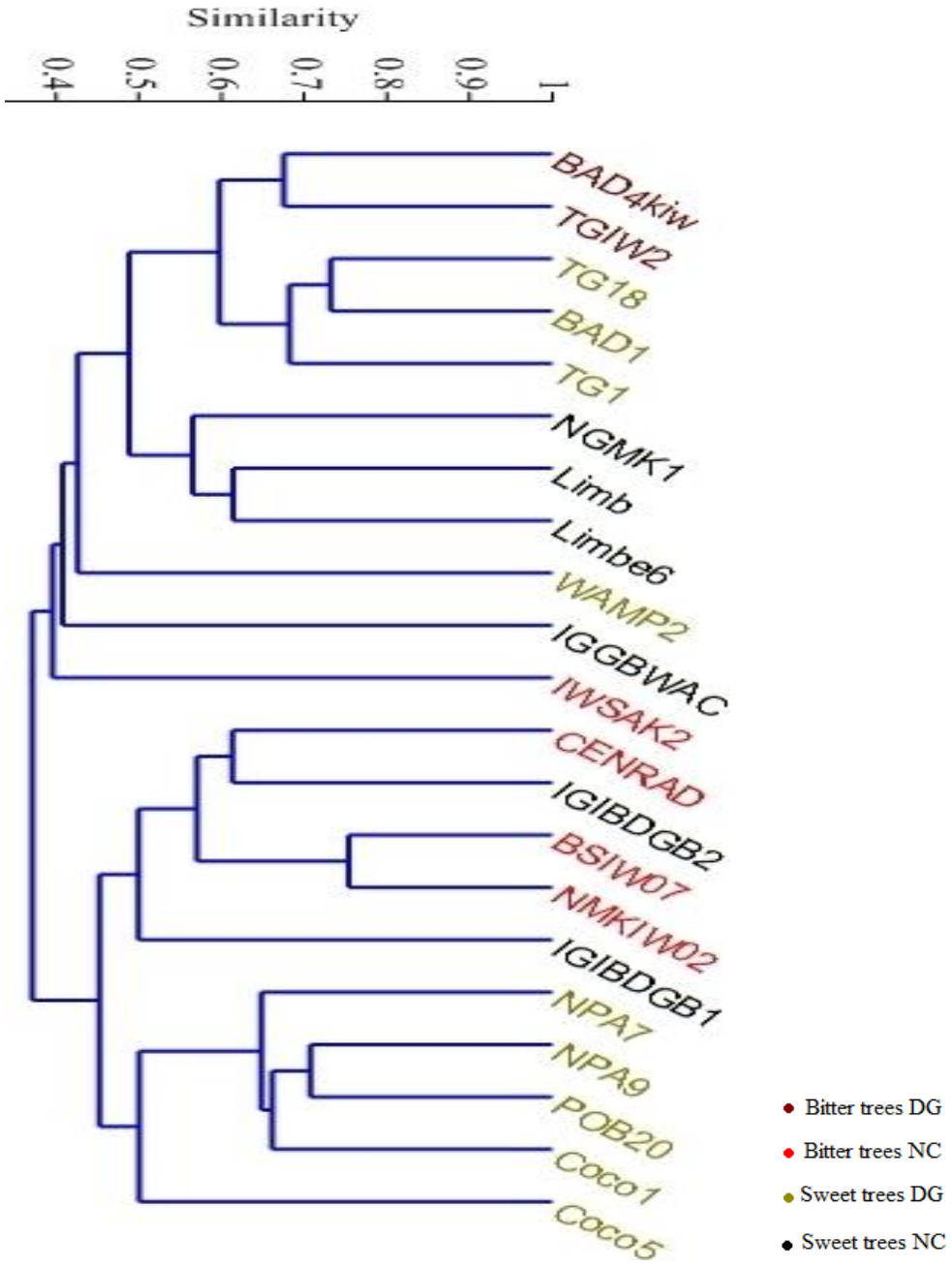

Figure 1c. UPGMA dendrogram for AFLP + cpSSR data of the 21 accessions based on Jaccard's similarity index.

70%) with the first 8 PCA axes accounting for 72.8% of the total variation. The clustering obtained with NJ based on the Kulczynski similarity index of these alleles (Figure 3) confirmed the pattern in the PCA. The inability of the combined AFLP + cpSSR data to accurately separate populations and the clustering of the sweet trees from Nigeria within the bitter tree cluster was also confirmed.

DISCUSSION

ABMTs genetic diversity: Failure of AFLPs and cpSSRs or influence of domestication

For all the three datasets considered, the lowest polymorphism and genetic diversity was observed in the

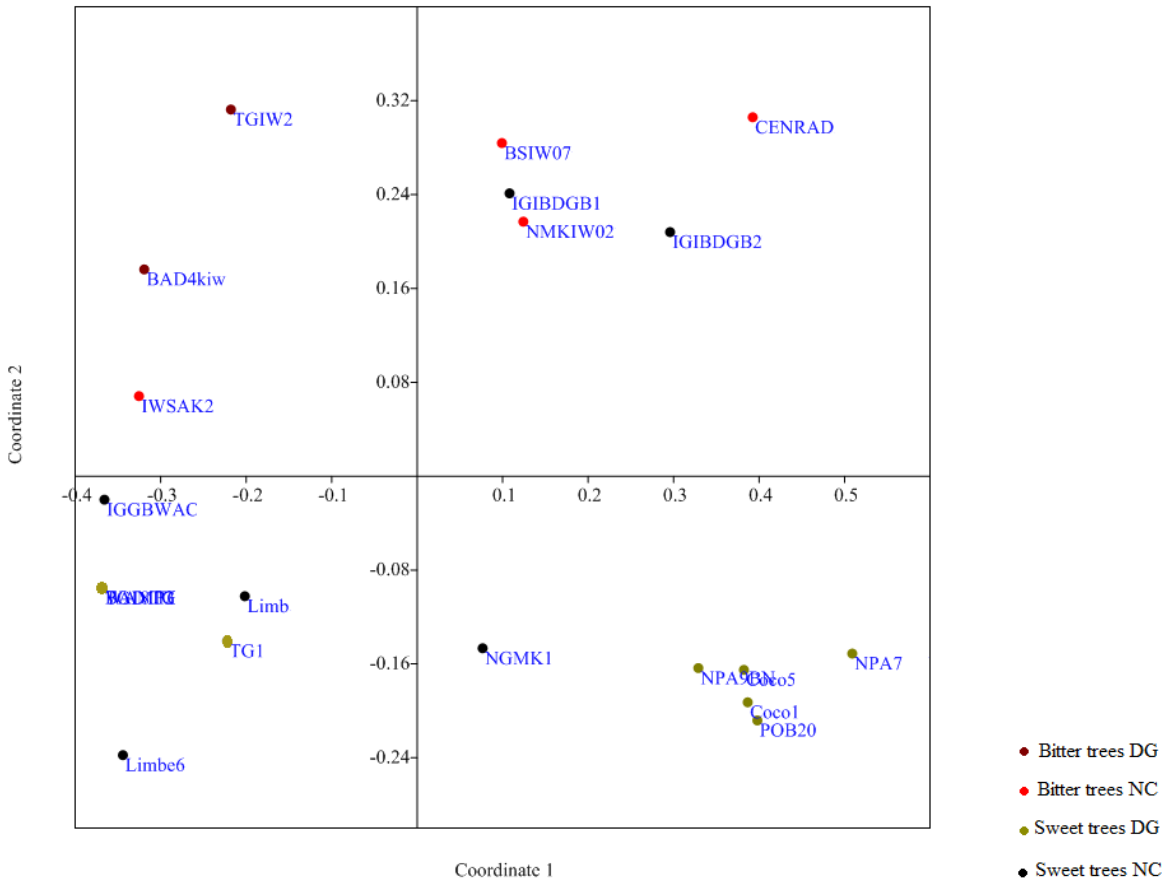

Figure 2. Plot of first two principal coordinates based on Jaccard's similarity index with the 48 AFLPs + cpSSRs for the 21 common accessions. DG, Dahomey Gap; NC, Nigeria and Cameroon.

bitter mango tree population in the Dahomey Gap, while the highest values for these parameters was noted among the cultivated sweet tree population from the same eco-region. These results are not consistent with those of Lowe et al. (2000) and Ude et al. (2006) who indicated a higher genetic diversity for ABMTs in Cameroon and Nigeria. Environmental transformation through logging, extension of agricultural productive space through yearly bush fires, and urbanisation are the main causes of biodiversity loss in Tropical Africa (FRIG, 2003; Sodhi, 2007; Jose, 2012). Fragmentation and decreased population size have changed the climatic characteristics of the Volta forest region; a unique ecosystem in which bitter trees are found in the wild in the Dahomey Gap (Vihotogbé et al., 2014a). Additionally, the economic potential of bush mangoes' seed highly threatens the ABMTs, since the market of this non timber forest product relies mostly on natural populations (Agbor, 1994; Lowe et al., 2000). Thus, the population size of wild bitter ABMTs has decreased in the Volta forest region in their entire distribution range due to a lack of sufficient natural regeneration (Agbor, 1994; Zapfack and Ngobo-Nkongo, 2002; Vihotogbé et al., 2014a). Consequently, the reduction of their ecological variability has narrowed their morphological and genetic diversity. Nevertheless, the domestication and cultivation of sweet trees in various climatic zones in the Dahomey Gap, has helped to preserve or increase the existing diversity (Casas et al., 2005; Jose, 2012). Although, the provenance of sweet ABMTs in the Dahomey Gap is unknown (Harris, 1996; Asaah et al., 2003; Lesley and Brown, 2004; Ude et al., 2006, Vihotogbe et al., 2014a), their higher diversity in this eco-region may well be due to the fact that the ongoing traditional domestication process in this region includes material from geographically different origins: the Upper and Lower Guinean forest blocks as well as the Congolian forest region. We conclude that this is a consequence of the random genetic material transfer between and within local communities, not only for ABMTs but related to any economically important food tree species in agroforestry systems (Jose, 2012).

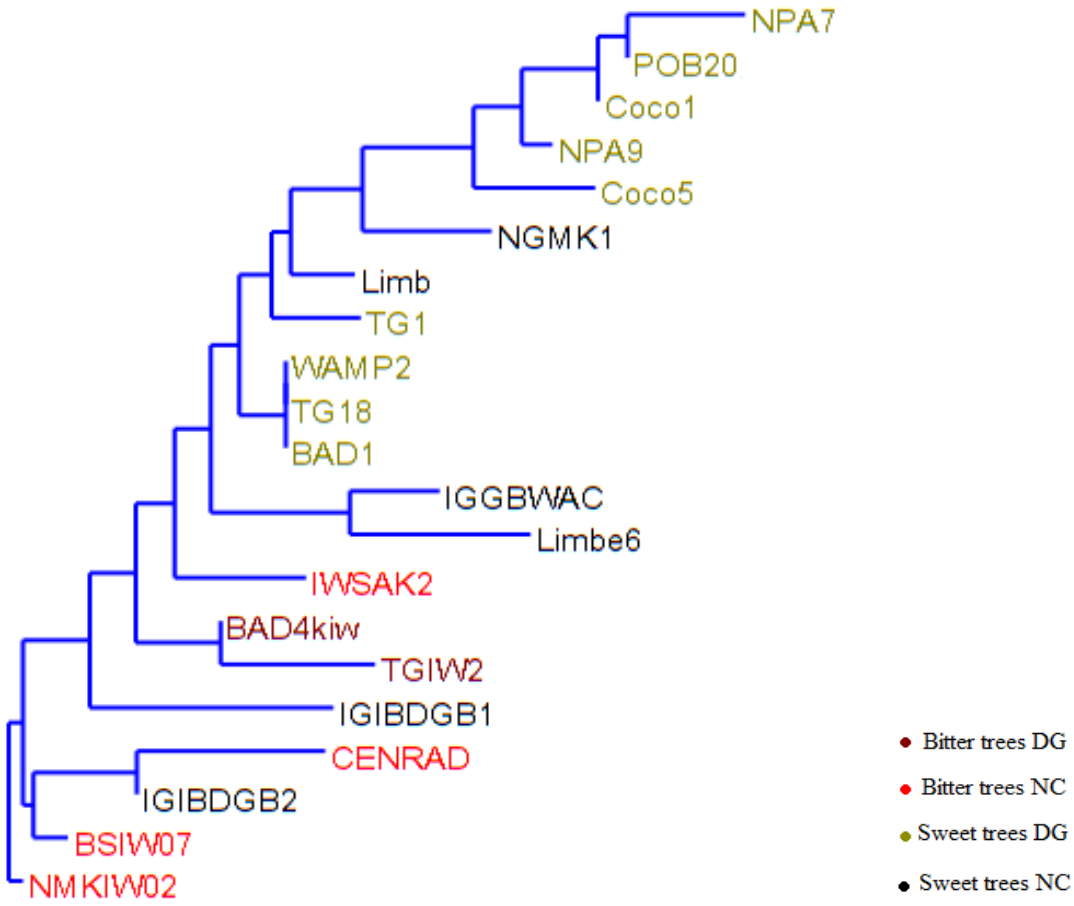

Figure 3. Kulczynski similarity-based Neighbor Joining tree of the 48 AFLPs + cpSSRs loci. DG, Dahomey Gap; NC, Nigeria and Cameroon.

In general, for the three datasets, no significant genetic differentiation was found between bitter and sweet ABMTs. Similarly, no genetic differentiation among populations was observed with the AFLP dataset. Genetic differentiation was detected within the cpSSR and AFLP + cpSSR datasets but the pattern was not the same for the two datasets. The dominance of cultivated material in our samples (collected in the field as well as in gene banks) resulted in the expression of artificially generated variation.

Comparatively few sharp bands could be scored with the AFLP and the cpSSR products. This weakness was noted during an AFLP study by Ude et al. (2006) who used 12 pairs of primers. The genetic diversity among populations and most importantly between bitter and sweet mango trees posed important questions concerning the suitability of the genetic markers used in our study. Thus, including wild materials from every eco-region in the entire distribution range of ABMTs and using sound genetic analyses' methods would be of great importance in the evaluation of the genetic diversity, the influence of domestication and the genetic adaptability of ABMTs.

Suitability of the markers

Apart from the sweet mango tree population from Benin, which formed the most consistent and distinct clusters throughout our analyses, none of the methods used in this study clearly separated the ABMTs into geographically distinct populations (Figures 1 to 3). The PCA and NJ (Figures 2 and 3) showed a distinction between the two types, with the exception of the sweet tree samples from Nigeria which clustered with the bitter trees (Figures 2 and 3). This was also observed in the study of Lowe et al. (2000), but was explained in terms of inaccuracy of sampling. In our study, the fact that neither the PCA plots nor the NJ dendrogram were able to discriminate either geographical populations or fruit types

implied that the markers used to achieve this goal were probably unsuitable. This idea corroborated with the fact that we know that in the area where bitter and sweet ABMTs co-occur (in the Volta forest region) successful gene flow between bitter and sweet trees is hardly expected for phenological reasons: (i) very short co-flowering time, (ii) flowers abortion on all sweet trees after this co-flowering time and (iii) consistent overall difference in phenology between both types (Vihotogbé et al., 2014b). Further support comes from the presence of ecological differences between both types (Ainge and Brown, 2004; Vihotogbé et al., 2014a). Therefore, the unclear species distinction from the AFLP and cpSSR data might be attributed to the high level of sweet ABMTs diversity (Kelleher et al., 2005). So, in conclusion, we attributed the failure of AFLPs, cpSSRs and AFLPs + cpSSRs to distinguish populations to the effect of domestication and large scale transfer of genetic material via seeds of the economically and nutritionally appreciated ABMT morphotypes.

Conflict of interests

The authors have not declared any conflict of interest.

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Abbreviations: AFLP, Amplified fragment length polymorphisms; **cpSSRs,** chloroplast simple sequence repeats (or microsatellites); **SDG,** sweet ABMTs from the dahomey gap; **BDG,** bitter ABMTs from the dahomey gap; **STA,** sweet ABMTs from Tropical Africa; **BTA,** bitter ABMTs from Tropical Africa; **UPGMA,** unweighted pair group method with arithmetic mean.

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