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Genetic relationships among accessions of African indigenous melons (*Cucumis melo* L. ssp. *agrestis*) using AFLP markers

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A R T I C L E   I N F O

A B S T R A C T

Cucumis melo ssp. *agrestis* is among the most important cucurbit consumed in Côte d'Ivoire. Two varieties were identified and used in different areas. One is eaten as soup thickener and the second is used as vegetable. In spite of the socio cultural importance of this species, knowledge of its genetic diversity and differentiation is very poor. Little information is available on the variety itself or relationships between the both varieties. Amplified fragment length polymorphism (AFLP) markers were employed to determine the genetic relationships between these varieties in Côte d'Ivoire. Five AFLP primer combinations were used for this study. A total of 75 loci were observed in the 24 accessions with 82.16% of polymorphic loci ranging between 13.3% and 100%. The averages of total gene diversity (H_T) and within accessions genetic diversity (Hs) were 0.313 and 0.273 respectively. The inter accessions genetic diversity (D_{ST}) and the coefficient of gene differentiation among cultivars (F_{ST}) were 0.040 and 0.129 respectively. Cluster analysis using Unweighted Pair-Group Method using Néi's genetic distance highlighted two main clusters indicating clearly separation between vegetable and thickener cultivars of *Cucumis melo* L. ssp. *agrestis* consumed in Côte d'Ivoire. These data are useful for best management of genetics resources of *Cucumis melo* L. ssp. *agrestis*.

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1. Introduction

Melon family is an economically important crop that includes wild types and numerous varieties, consumed worldwide either as desert fruits, vegetable or sauce ingredients, depending on the type of fruit. Varieties vary widely in fruit size, morphology and taste, as well as vegetative traits and climatic adaptation (Pitrat et al., 1999). *Cucumis melo* L. is reported to be the most variable species in the genus *Cucumis* (Akashi et al., 2002). The diversity in cultivation areas, uses, and morphology certainly reflect the growing number of research programs conduced namely on evolution and genetics (Silberstein et al., 1999) of *C. melo*. These studies are mainly aimed at assessing the intra-genus diversity and the identification of the closest

relatives of *C. melo*. Such investigations are of practical interest for germplasm management and for plant breeders. Ongoing investigations on evolution, systematics and genetics of melon are based either on morphological traits, isozymes- or PCR-based techniques (Djè et al., 2006; Staub et al., 1997, 2000). Relevant results have been obtained from these studies, allowing authors to refine melon species classification. Thus, it has been established that the genus *Cucumis* is subdivided into two subgenera designated *Cucumis* ($n = 7$) and *Melo* ($n = 12$), with 4 and 5 species, respectively. Africa, where wild and free-living species occur has been identified as the most likely centre of origin for the genus *Cucumis*. It has been suggested that the probable sites of domestication of melon and cucumber is Middle East and Southern Asia (Pitrat et al., 1999). To date, melon intra-species classification is still unclear. Further investigations are needed to elucidate their evolutionary genetics and phylogeny. Varieties of melon cultivated in temperate zones have been relatively well studied using morphological and molecular analysis. So far, the classification within the African indigenous melon has not been investigated using similar approaches. To our knowledge only a few studies have been conducted on intraspecific genetic variability and phylogeny of the African indigenous melon using morphological traits (Djè et al., 2006), isozymes (Staub et al., 1997) and RAPD (Staub et al., 2000).

The African *Cucumis* species (wild or domesticated) represent reservoirs of potential useful genes for the improvement of current cultivars of cucumber and melon. The edible types, widely adopted in many tropical regions of the world are important for food security (Badifu and Ogunsua, 1991; de Mello et al., 2001; Loukou et al., 2007). In Côte d'Ivoire, a 6-years (2000–2006) country level prospecting resulted in the collection of 101 accessions of *C. melo*. (taxon *agrestis*) composed of two andromonoecious types classified by peasants as varieties. Preliminary studies have established morphological differentiation between both varieties. This work is carried out to investigate the genetic diversity and relationship within and among the two presumed varieties of *C. melo*. (taxon *agrestis*) using DNA variations.

2. Material and methods

2.1. Plant material

Twenty four accessions of *C. melo* ssp. *agrestis* were selected from indigenous cucurbits germplasm collection maintained at Nangui Abrogoua University (Abidjan, Côte d'Ivoire). The selected accessions identified by alpha-numeric codes (Table 1), were representative of the main agro-ecological zones of Côte d'Ivoire in which this species is regularly produced (Zoro Bi et al., 2006). Accessions were selected from the collection of 101 introductions to represent the two cultivars of *C. melo* var. *agrestis* produced in Côte d'Ivoire and to maximize the range of morphological diversity and geographical origin.

Two andromonoecious variety of *C. melo* ssp. *agrestis*, with dark green leaves, yellow flowers, and small oval fruits (3–10 cm length) occur in Côte d'Ivoire. The most common type is cultivated. The seeds of this variety are toasted, ground, and eaten as soup thickener. The flesh of fruits is light green, lacks aroma, and has bitter taste. The second variety, less widely cultivated, is often found along the roads, on rubbish sites or on unbuilt plots in villages and cities. Its mature fruits are orange, possess aroma and are exclusively used as vegetable. The fruits are cut into slices and added to soup.

Table 1
Accession codes, cultivar, collection site, and sample sizes for *Cucumis melo* ssp *agrestis* used for AFLP analysis.

Codes	Cultivars	Collection site	Geographic coordinate
NI057	Thickener	Assiè-Assasso	6°39'N–4°11'W
NI064	Thickener	Assiè-Koumassi	6°43'N–4°21'W
NI104	Thickener	Assiè-Assasso	6°39'N–4°11'W
NI136	Thickener	Assiè-Assasso	6°39'N–4°11'W
NI139	Thickener	Assiè-Assasso	6°39'N–4°11'W
NI142	Thickener	Assiè-Koumassi	6°43'N–4°21'W
NI150	Thickener	Assiè-Koumassi	6°43'N–4°21'W
NI158	Thickener	Assiè-Assasso	6°39'N–4°11'W
NI159	Thickener	Assiè-Assasso	6°39'N–4°11'W
NI168	Thickener	Assiè-Assasso	6°39'N–4°11'W
NI169	Thickener	Assiè-Assasso	6°39'N–4°11'W
NI186	Vegetable	Riviera Lauriers 9	5°21'N–3°56'W
NI197	Thickener	Korhogo	9°23'N–5°33'W
NI253	Thickener	Lobouakouzin	5°45'N–3°46'W
NI378	Thickener	Korhogo	9°23'N–5°33'W
NI380	Thickener	Korhogo	9°23'N–5°33'W
NI400	Thickener	Boflèkro	6°38'N–4°42'W
NI405	Thickener	Assié Assaso	6°39'N–4°11'W
NI413	Vegetable	Yakasse Me	5°49'N–3°56'W
NI415	Vegetable	Abié	5°49'N–3°56'W
NI416	Vegetable	Akin	5°42'N–3°32'W
NI417	Vegetable	Awakoua	5°42'N–3°52'W
NI418	Vegetable	Awakoua	5°42'N–3°52'W
NI424	Vegetable	Awakoua	5°42'N–3°52'W

Table 2

Mean (±SE) of intra- and inter-accession gene diversity indices for 24 indigenous oilseed melon (*Cucumis melo* L. ssp. *agrestis*) accessions from Côte d'Ivoire.

Genetic diversity indices	Collection ($n = 24$)	Thickener ($n = 14$)	Vegetable ($n = 7$)
Total number of loci	75	75	75
Number of polymorphic loci	70.42 ± 2.04	60.29 ± 27.35	68.71 ± 2.29
Percent of polymorphic loci (5%)	93.90 ± 2.72	80.39 ± 36.47	91.63 ± 3.05
Total gene diversity (H_T)	0.313	0.279	0.310
Within accessions gene diversity (Hs)	0.273 ± 0.010	0.252 ± 0.017	0.293 ± 0.023
Among accessions gene diversity (D_{ST})	0.040 ± 0.007	0.027 ± 0.017	0.016 ± 0.004
Among accessions gene differentiation (F_{ST})	0.129 ± 0.167	0.097 ± 0.160	0.052 ± 0.280

2.2. DNA extraction and AFLP analysis

Ten to thirteen seeds of each accession were sown in a greenhouse (IITA, Ibadan, Nigeria) and after 4–6 days, total genomic DNA was extracted from 0.15 to 0.2 g of young leaves collected from 4 seedling of each accession following the CTAB extraction procedure according to Doyle & Doyle method (Doyle and Doyle, 1988) with minor modification. The DNA final concentration was determined by 2% agarose-gel electrophoresis. AFLP was performed with partial modifications of Keygene method (Vos et al., 1995). In brief, 0.5 μg of genomic DNA was restricted with 5 U *Eco*RI and 1 U *Pst*I (New England Biolabs) for 2 h at 37 °C and then ligated to *Eco*RI and *Pst*I adapters using 1 U T4 DNA ligase (New England Biolabs). Restriction and ligation were carried out in a single reaction containing $1 \times$ T4 ligase buffer with ATP; 0.05 M NaCl, 0.5 μg bovine serum albumin (BSA), and 1 μL each of *Eco*RI and *Pst*I adapter pairs. The diluted restricted-ligated DNA was amplified employing *Eco*RI and *Pst*I preselective primers using AFLP core mix supplied with the kit; $0.05 \times$ diluted amplified product was used for selective amplification. Five AFLP primer combinations were employed for the generation of markers: *Eco*RI-AAC + *Pst*I-CAC; *Eco*RI-ACC + *Pst*I-CAA; *Eco*RI-ACT + *Pst*I-CTA; *Eco*RI-AGG + *Pst*I-CAG and *Eco*RI-AGC + *Pst*I-CAC. Amplified fragments were detected by silver staining. The resulting gels were scored manually.

2.3. Data collection and analysis

The number of monomorphic and polymorphic AFLP fragments was determined for each primer pair. Presence or absence of each fragment was scored as 1 = present, 0 = absent. Bands of similar size and intensity were assumed to be homologous, since paternity and mating system studies have highlighted the Mendelian inheritance of AFLP markers. Genetic variation within each accession was estimated, using AFLPSURV (Vekemans et al., 2002), by the percentage of polymorphic loci (*PPL*) at the 5% level based on the approach of Lynch and Milligan (1994) and the expected heterozygosity (*H*j), using the Bayesian method with nonuniform prior distribution of allele frequencies. Estimates of the partitioning of total genetic variation were calculated by F_{ST} using aflpsurv and by analysis of molecular variance (AMOVA) following the methods of Excoffier et al. (1992) and using GenAlex version 5 (Peakall and Smouse, 2001). A phylogenetic tree was constructed by the neighbour-joining method (Saitou and Nei, 1987) using the neighbour program in Phylip version 3.63 (Felsenstein, 1993). One thousand bootstraps, performed over loci, of Nei's unbiased genetic distance (*D*) from AFLP polymorphism were constructed using the Bayesian method with nonuniform prior distribution of allele frequencies, and an assumption of Hardy–Weinberg equilibrium, in aflpsurv (Vekemans et al., 2002), to infer confidence levels for tree branches.

3. Results

Primer pairs used in this study generated an average 69 bands per individual, ranging from 12 to 96, and 83.65% polymorphic bands were observed in the total of 253 bands. A total of 75 loci were observed in the 24 accessions. The proportion of polymorphic loci (*PPL*) evaluated at 95% criterion varied from 13.3% to 100% with a mean of 82.16%. At the cultivar level, the proportion of polymorphic loci varied from 13.3% to 100% for thickener cultivars and 14.7%–100% for vegetable cultivars with respective mean of 80.39% and 86.49%. The averages of total gene diversity (H_T) and within accessions genetic diversity (Hs) were 0.313 and 0.273, respectively. These values were 0.279 and 0.252 in thickener cultivars and 0.310 and 0.293 for vegetable cultivars. The inter accessions genetic diversity (D_{ST}) and the coefficient of gene differentiation among cultivars (F_{ST}) were 0.040 and 0.129, respectively. At the cultivar level, among accessions gene diversity (D_{ST}) and among accessions gene differentiation (F_{ST}) were 0.027 and 0.097 for thickener cultivar (Table 2). These indices were 0.016 and 0.052 for vegetable cultivar.

The UPGMA phylogenetic tree based on the Nei's genetic distances matrix from AFLP data is shown in Fig. 1. A clear accessions grouping supported by relatively high number of bootstrap was observed. The phenogram subdivided the accessions analysed into two major groups: group I composed of vegetable (V) cultivars and group II composed of thickener (T) cultivars.

4. Discussion

Cucumis melo, is one of the members of cucurbit family for which classification is unclear because of the high number of varieties or cultivars described (Pitrat, 2013; Stepansky et al., 1999), stirring controversy between scientist (Achigan and
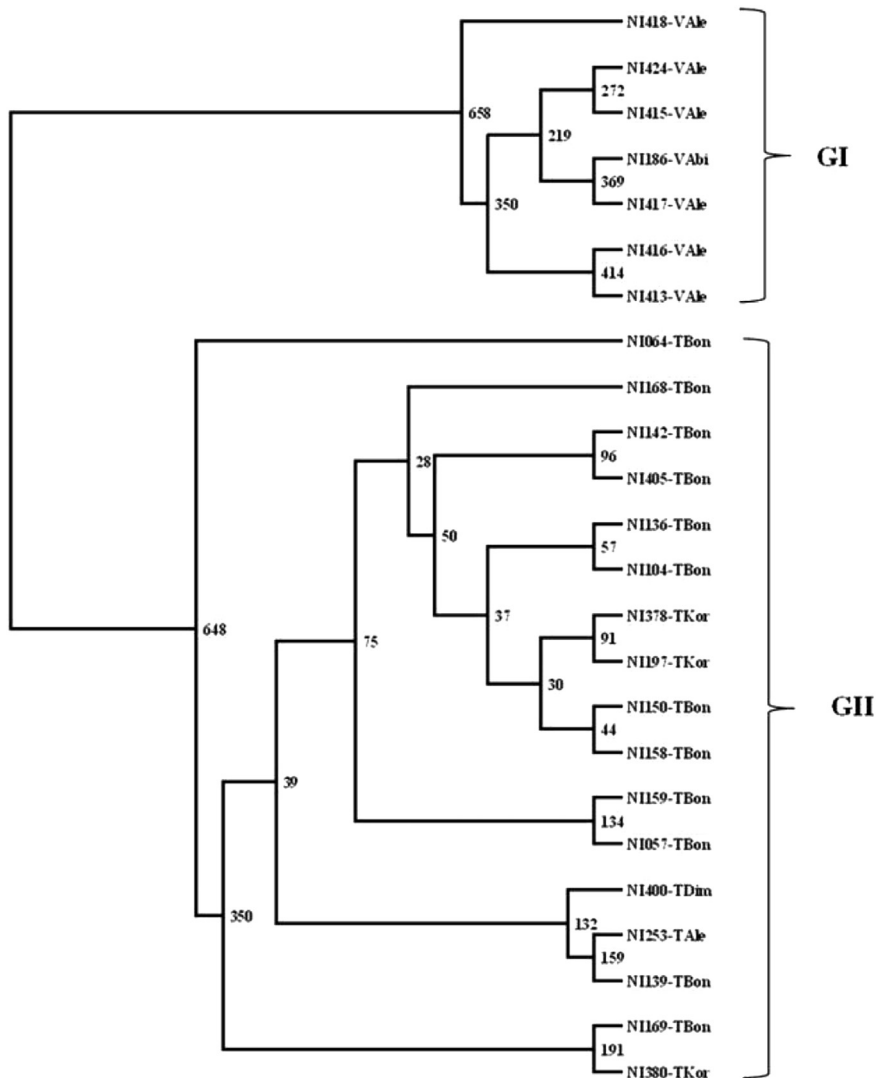
Fig. 1. UPGMA dendrogram for 24 accessions of the indigenous melon, using Nei's genetic distance and based on 1000 bootstraps. "T" and "V" in the accessions' ID indicate thickener and vegetable, respectively. Numbers on each branch are bootstrap values.

Baudoin, 2007). In Côte d'Ivoire two varieties have been described as *Cucumis melo var agrestis* based of morphological traits (Djè et al., 2006; Zoro Bi et al., 2006). The most common variety is cultivated for its seeds eaten as sauce thickener. This variety is similar to those described by Pitrat (2013) as typical wild melons. The second variety less widely cultivated in the sampled zones, is often found along the roads. Except for the yellow skin colour, they look like the "wild" melons described above. In this study we compared at the molecular level in order to evaluate intraspecific variability and to describe the relationships amongst them. The assessment of genetic diversity is important not only for crop improvement but also for efficient management and conservation of germplasm resources. Ability of AFLP analysis to display genetic relationships among accessions has been widely demonstrated (Stepansky et al., 1999; Talebi et al., 2008; Zhang et al., 2012). Morphological characterization is important to genetic diversity evaluation but it is not enough to appreciate the variability within a species. Elucidating the genetic relationships within the genus *Cucumis* is of great importance because the closest relatives and natural composition can provide valuable information to genetics resources management.

Genetics analysis carried out revealed a high proportion of polymorphic loci (82.16%). The high level of polymorphism detected indicates that these AFLP markers provide a useful tool for estimating genetic variation in melon. This result indicated also that Ivoirian accessions of *Cucumis melo* exhibited great genetic diversity. Our result was similar to those reported by McCreight and Staub (2004) for certain group of melon in India and China *Cucumis melo* germplasm collections using isozyme variation and agreed, broadly, with intraspecific variability of this species reported by Stepansky et al. (1999). On the other hand, estimates of the genetic structure indicated that *C. melo* maintained a high level of its variability within accessions. The same trend is observed in each variety. This result suggests that to capture variability effort must be

concentrated on the number of individual per accession. The relatively high level of the within accessions and cultivars genetic diversity component compared to the genetic variation among accessions and cultivars was also in accordance with the mating system coupled with farmers' seed management approaches. The low genetic differentiation between accessions ($F_{ST} = 0.129$) was observed. It indicated the important rate of gene exchange between accessions.

A study based on life cycle duration, seed size and seed weight analysis exhibited the morphological distinction established between the two types *C. m.* subsp. *agrestis* by peasants (Zoro Bi et al., 2006). The UPGMA dendrogram generated from AFLP data was supported by high bootstrap values, thereby implying the high reliability of the pattern found. The dendrogram divided accessions into two highly supported clusters, both of which consisting of the two varieties. This result confirmed morphological distinction and rural communities separation of Ivoirian *Cucumis melo* (Zoro Bi et al., 2006). The neighbour-joining tree constructed based on AFLP fragments supports the view that cultivars are distinct. It could be indicated that Ivoirian cultivars of *Cucumis melo* might belong to different botanical groups.

Acknowledgement

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