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# Genetic diversity and structure of kola tree (*Cola nitida*) clones germplasm in Côte d'Ivoire using Single Nucleotide Polymorphism markers

**YAYA OUATTARA[1,♥], KOUAMÉ GUILLAUME KOFFI[2], DROLET JEAN-MARC SÉRY[1], DIDIER MARTIAL SARAKA[3], N'DA DÉSIRÉ POKOU[4], KARINE KOSSIA MANZAN GBA[1], BOUADOU BONSSON[1], DOFFOU SÉLASTIQUE AKAFFOU[5], RAOUL SYLVÈRE SIÉ[2]**

[1]Centre National de Recherche Agronomique (CNRA), Station de Recherche de Man. Abidjan, Côte d'Ivoire. Tel./fax. +225-07-59-32-98-83, [♥]email: yaya.ouattara@cnra.ci / ouatt_yaya@yahoo.fr
[2]Université Nangui Abrogoua. Abidjan, Côte d'Ivoire
[3]Université Péléforo Gbon Coulibaly. Korhogo, Côte d'Ivoire
[4]Centre National de Recherche Agronomique (CNRA), Laboratoire Central de Biotechnologies. Abidjan, Côte d'Ivoire
[5]Université Jean Lorougnon Guédé. Daloa, Côte d'Ivoire

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**Abstract.** *Ouattara Y, Koffi KG, Séry DJ-M, Saraka DM, Pokou ND, Gba KKM, Bonsson B, Akaffou DS, Sié RS. 2022. Genetic diversity and structure of kola tree (*Cola nitida*) clones germplasm in Côte d'Ivoire using Single Nucleotide Polymorphism markers. Biodiversitas 23: 4677-4685.* The kola nut comes from the cola tree (*Cola nitida* [Vent.] Schott & Endl.), a product of economic interest to Côte d'Ivoire. The country is the leading producer and exporter. However, much of its collection has been destroyed due to urbanization and deforestation. This has resulted in the loss of much of its genetic diversity. This study aimed to determine genetic diversity and structure of *C. nitida* in Côte d'Ivoire using Single Nucleotide Polymorphism (SNPs) markers. Thirty clones from Côte d'Ivoire (14 clones) and Nigeria (16 clones) cultivated in CNRA (Centre National de Recherche Agronomique) were analyzed using 145 SNPs. GBS (Genotyping by Sequencing) technique was used for the genotyping. The results confirmed the existence of both origins (Côte d'Ivoire and Nigeria) by analyzing clones' genetic structure and the principal coordinate analysis. The genetic diversity obtained was higher within origins than among origins. The two origins showed moderate differentiation ($F_{ST}$ = 0.11) and the genetic distance was 0.46. The 30 clones were subdivided into six subgroups partially overlapping the geographical origins. Genetic differentiation among subgroups was moderate to very strong ($F_{ST}$ ranging from 0.10 to 0.26). Four clones from Nigeria were close to those from Côte d'Ivoire. This genetic structuring highlights the possibilities for genetic improvement of kola trees in Côte d'Ivoire.

**Keywords:** *Cola nitida*, genetic improvement, genetic structure, germplasm, SNPs markers

## INTRODUCTION

The economy of Côte d'Ivoire has been sustained by agricultural sector. Indeed, it accounts for 22% of GDP and 60% of the country's overall export earnings by 2021 (Fratmat.info 2022). The main export crops are coffee, cocoa, oil palm, rubber, cashew nuts, and kola nut (Koffi and Oura 2019). Kola nut comes from kola tree, a typically African tree (Savi et al. 2019; Adesida et al. 2021) belonging to the Malvaceae family (Whitlock et al. 2001). The genus *Cola* consists of more than 140 species, two species of which are cultivated, namely *C. nitida* (Vent.) Schott & Endl. and *C. acuminata* (P. Beauv.) Schott & Endl. (Adenuga et al. 2012). The *C. nitida* is commonly cultivated in Côte d'Ivoire with the average annual production is 260,000 tons since 2016 (FAO 2019). This production has enabled the country to become the world's leading producer and exporter of kola nuts. Therefore, kola nuts are the main agricultural product traded among ECOWAS countries (Gestrich et al. 2021).

Kola nut (*C. nitida*) has an important role in several areas (Amon-Armah et al. 2021). It is a valued chewable for its many virtues, such as calming hunger, thirst, and fighting against sleep. Kola nut is used in several domains like traditional medicine (treatment of indigestion, diarrhea, vomiting) (Ekalu and Habila 2020), pharmaceutical industry (manufacture of medicines), food industry (carbonated and energy drinks, wines etc.) (Asogwa et al. 2012; Nyadanu et al. 2020), and chemical industry (manufacture of dyes). Some African societies recognize it as having strong symbolic values, as it appears to be a tool for integration, hospitality, solidarity, and social cohesion (Ouattara 2013; Dah-Nouvlessounon et al. 2015).

The Centre National de Recherche Agronomique (CNRA) built up collections of kola trees with genotypes from Côte d'Ivoire and Nigeria. However, politico-military crises from 2002 to 2011, urbanization and land pressures have led to huge losses of kola trees' genetic resources (Ouattara 2021). In spite of the performances achieved both at the production and research levels, the kola nut cultivation in Côte d'Ivoire is confronted with major difficulties, such as the lack of performance plant material, the problems of conservation, and post-harvest treatments (Ouattara 2021). To overcome some of these difficulties, the kola research program in Côte d'Ivoire has focused on plant material by exploiting the intra-specific diversity of

*C. nitida*. The deforestation due to intensive agriculture (1.44 to 3.44% per year), logging and urbanization (Traoré 2018; Kouassi et al. 2021) makes it imperative to modernize and stabilize kola cultivation through the creation and selection of high-performance plant material. This activity requires a diversified collection for genetic recombination.

The analysis of the diversity and structure of a collection involves using molecular markers, such as SNPs (Single Nucleotide Polymorphism) which are co-dominant and bi-allelic markers. Their polymorphism is generated by point mutations that create different alleles at the same locus (Robert-Granié et al. 2011). They are the most frequent form of variation in the genome. Thus, SNPs have been developed in many species, such as cocoa (Ji et al. 2012), soybean (Chander et al. 2021), and Bambara groundnut (Uba et al. 2021).

The genetic diversity and structure of remaining germplasm remains unknown in Côte d'Ivoire to this day. However, germplasm diversity knowledge to assess the levels of variability among clones is a prerequisite for the implementation of a breeding program on one hand, and the management and safeguarding of the genetic heritage of kola trees in Côte d'Ivoire on the other. This study aimed to determine genetic diversity and structure of *C. nitida* in Côte d'Ivoire using SNPs markers.

## MATERIALS AND METHODS

### Plant materials

Kola trees (*C. nitida*) were collected from CNRA (Centre National de Recherche Agronomique) station (5°50'27.8''N; 5°21'30.1''W) in Lôh Djiboua region, 17 km from Divo, about 200 km northwest of Abidjan in Côte d'Ivoire. It covers 1.36 ha with tropical climate, the average annual rainfall is estimated at 1200 to 1600 mm/year, the average temperature is 27°C, and the humidity level is 85% (Ehounou et al. 2019a,b).

CNRA cultivates kola tree germplasm from two origins, namely Côte d'Ivoire and Nigeria. Plant materials studied are composed of 30 clones of *C. nitida* of which 14 clones from Côte d'Ivoire and 16 clones from Nigeria (Table 1). As the plants were propagated by cuttings, only one individual per clone was sampled.

### Procedures

*Genomic DNA isolation*

Fresh young leaves were collected from each clone for DNA extraction. These leaves were wrapped with aluminium foil and stored in a cooler with ice. This step is important to avoid oxidation and to preserve the leaves samples and the quality of DNA to be extracted (Adesoye et al. 2014). Genomic DNA was extracted from leaves material using MATAB (Mixed Alkyl Trimethyl Ammonium Bromide) protocol described by Adje et al. (2016). The 0.15 g of leaf fragment was ground in liquid nitrogen and transferred into 800 µL of MATAB buffer. The reaction mixture was incubated at 65°C for 15 min, vortexed, and centrifuged at 13,000 rpm for 15 min. The supernatant was collected in a tube to which 500 µL of chloroform was added. The tubes were shaken by inverting and then centrifuged again at 12,000 rpm for 10 min. The aqueous upper phase was recovered in a 1.5 mL Eppendorf tube to which cold isopropanol was added. Then, the tubes were shaken by inverting them to homogenize the mixture. The extracts were stored in a refrigerator at 4°C for 24 hours, after which the supernatant was drained. Ethanol at 70% was added and the mixture was centrifuged one last time at 12,000 rpm for 10 min to purify the pellet. Finally, the last supernatant was drained and the pellet was air-dried to evaporate ethanol and then recovered in pure sterile water.

The DNA quality and concentrations were detected by a Nanodrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Each DNA sample was dissolved in a final volume of 50 µL water with a concentration of 300 ng/µL and transferred to 96-well plates and shipped to SEQART laboratory (Nairobi, Kenya) for genotyping.

**Table 1.** List of the 30 clones of *C. nitida* used in the study

| No. | Clones | Country of origin |
|---|---|---|
| 1 | NIG-329 | Nigéria |
| 2 | NIG-330 | Nigéria |
| 3 | NIG-331 | Nigéria |
| 4 | NIG-332 | Nigéria |
| 5 | NIG-341 | Nigéria |
| 6 | NIG-342 | Nigéria |
| 7 | NIG-345 | Nigéria |
| 8 | NIG-346 | Nigéria |
| 9 | NIG-350 | Nigéria |
| 10 | NIG-351 | Nigéria |
| 11 | NIG-352 | Nigéria |
| 12 | NIG-356 | Nigéria |
| 13 | NIG-359 | Nigéria |
| 14 | NIG-364 | Nigéria |
| 15 | NIG-366 | Nigéria |
| 16 | NIG-379 | Nigéria |
| 17 | CIV-305 | Côte d'Ivoire |
| 18 | CIV-306 | Côte d'Ivoire |
| 19 | CIV-308 | Côte d'Ivoire |
| 20 | CIV-311 | Côte d'Ivoire |
| 21 | CIV-313 | Côte d'Ivoire |
| 22 | CIV-314 | Côte d'Ivoire |
| 23 | CIV-315 | Côte d'Ivoire |
| 24 | CIV-316 | Côte d'Ivoire |
| 25 | CIV-318 | Côte d'Ivoire |
| 26 | CIV-321 | Côte d'Ivoire |
| 27 | CIV-323 | Côte d'Ivoire |
| 28 | CIV-A3 | Côte d'Ivoire |
| 29 | CIV-CR | Côte d'Ivoire |
| 30 | CIV-CV | Côte d'Ivoire |

*SNP genotyping*

Genomic DNA from each tree was subjected to GBS (Genotyping by Sequencing) (De Donato et al. 2013; He et al. 2014; Chung et al. 2017). GBS libraries were prepared according to Elshire et al. (2011) at the Genomic Analysis Platform of SEQART laboratory (Nairobi, Kenya). Each DNA sample of 36 samples was digested with the enzyme ApeKI in the wells of a 96-well PCR plate. The digestion products were ligated to adapters with cohesive ends, one with a unique barcode consisting of 4-8 bases and used in a single well, while the second was identical in all wells. Subsequently, samples were subjected to PCR amplification. The PCR products obtained were purified and sequenced by the Illumina method. The sequences obtained were aligned with the cocoa genome (*Theobroma cacao* L.), as the kola tree does not have a reference genome. Indeed, cocoa and kola trees belong to the same family and have the closest genomes (Lanaud et al. 1999; Whitlock et al. 2001). Subsequently, SNPs were determined with Freebayes software. Sequencing generated 151 SNPs that were filtered to keep only the most informative (PIC (Polymorphism Information Content) ≥ 0.3), and to exclude genotypes that presented more than 30% missing data (Nazareno et al. 2017). After this selection filter, 145 SNPs were retained.

**Data analysis**

Data analysis consisted of assessing the genetic diversity within and among origins and determining the genetic structure of each clone. Thus, genetic diversity within each origin was estimated through the average alleles number per locus (A), the average effective alleles number per locus (Ae), and the inbreeding coefficient (FIS) mean gene diversity within origin (Hs) using GenAlEx 6.5 software (Peakall and Smouse 2012). The percentage of polymorphic loci at the 95% threshold was also calculated from each origin. The percentage of polymorphic loci represents the probability of observing at least two alleles at the same locus, and the PIC is used as an indicator to evaluate the discriminatory power of a marker. It varies from 0 to 1. Finally, the search for possible duplicate genotypes was carried out using the multiple pair matching method in GenAlEx 6.5 software.

Differentiation among the two origins was determined through diversity among origin ($D_{ST}$), the total diversity ($H_T = H_S + D_{ST}$), the pairwise Nei's genetic distances (Nei 1972), and genetic differentiation index ($F_{ST}$). These parameters were calculated using DnaSP v6.12.03 software (Rozas et al. 2017). Molecular analysis of variance (AMOVA) was performed to estimate diversity distribution within and among origins, and to quantify genetic variation among origins and clones with the Genalex 6.5 Software (Peakall and Smouse 2012). The statistical significance of the differences observed was assessed using a resampling of 1000 permutations.

A principal coordinate analysis (PCoA) was performed to graphically represent individuals of each clone in a two-dimensional plane and to determine the contributions of each component to the variation that existed in the germplasm. This analysis was performed using a similarity matrix obtained after calculating genetic distances using Distance procedure implemented in GenAlEx 6.5 software. $F_{ST}$ and genetic distances were also calculated between the subgroups derived from the PCoA. The purpose of this analysis was to determine the structure and genetic distance of the subgroups from each other. The relationship among clones was analyzed by imputing the full set of data into Blast-Explorer (ngphylogeny.fr) (Lemoine et al. 2019). This analysis consisted of a dendrogram drawing using a Bayesian algorithm based on sequential clustering of neighboring genotypes. The robustness of tree nodes was tested by applying 1000 resamplings based on the repeatability of individuals' groupings.

The SNPs markers data was subjected to the evaluation of clone's genetic structure using STRUCTURE software 2.3.4 (Porras-Hurtado et al. 2013). The optimal group number K was determined using Evanno et al. (2005) method. It was assessed in Structure Harvester platform (http://taylor0.biology.ucla.edu/structureHarvester/) (Earl and von Holdt 2012) applying the admixture model (Lawson et al. 2018). In this analysis, we varied K from 1 to 5, with five iterations in the analysis program execution. Then, an analysis probabilistically assigned each individual to a group using a Bayesian algorithm.

## RESULTS AND DISCUSSION

**Percentage of polymorphic loci and PIC values**

The percentage of polymorphic loci at 95% threshold was 100% for Côte d'Ivoire and 99% for Nigeria. Its global value was 99% for the two origins. According to these results, all markers were polymorphic on Côte d'Ivoire's origin, whereas only one marker was monomorphic on Nigerian's origin. The polymorphic information content (PIC) was 0.38 (Table 2). In addition, there were no duplicated genotypes across 30 clones analyzed. It means that each clone showed a unique profile.

**Genetic diversity within origin**

The average 2 alleles per locus were found in each origin. The average number of effective alleles (Ae) was almost similar in both origins, i.e., 1.74 ± 0.02 for Côte d'Ivoire and 1.73 ± 0.02 for Nigeria (Table 3). Similarly, mean gene diversity ($H_S$) values were very close in each origin, i.e., 0.42 ± 0.01 for Côte d'Ivoire and 0.41 ± 0.01 for Nigeria. This result indicates the existence of a significant allelic diversity in each origin. As for fixation index ($F_{IS}$), the value obtained within Côte d'Ivoire origin ($F_{IS}$ = 0.12 ± 0.02) was higher than Nigeria origin ($F_{IS}$ = 0.07 ± 0.02). These values indicate there was inbreeding within original trees.

**Table 2.** Percentage of polymorphic loci and PIC values within Côte d'Ivoire and Nigeria origins

| Clones origins | $P_{(0,95)}$ | PIC |
|---|---|---|
| Côte d'Ivoire (CIV) | 1 | 0.38 |
| Nigeria (NIG) | 0.99 | |
| Mean | 0.99 | 0.38 |

Note: $P_{(0.95)}$: Percentage of polymorphic loci at 95% threshold; PIC: Polymorphic Loci Information Content

**Genetic diversity among origin**

The total diversity value was 0.46, and the diversity among origin ($D_{ST}$ = 0.05) was lower than the diversity within origin as presented in Table 3, suggesting a high diversity among the 30 clones. $F_{ST}$ value among the two origins was 0.11 (Table 4), indicating that there was moderate genetic differentiation among the origins in Côte d'Ivoire and Nigeria. Furthermore, the average Nei's genetic distance among both origins was 0.46, suggesting that clones from Côte d'Ivoire and Nigeria are genetically distant.

**Genetic differentiation revealed by the molecular analysis of variance (AMOVA)**

The AMOVA result showed significant genetic differences among origin and clones. This result showed that 18% of total genetic variation originated from the origin, whereas 82% came from clones (Table 5). Both origins showed a highly significant level (p = 0.001). The results were consistent with the previous analysis results, suggesting that the genetic diversity revealed was mainly caused by genetic differences among clones.

**Principal coordinate analysis (PCoA) and dendrogram**

The results of principal coordinate analysis (PCoA) based on genetic similarity values from the proportion of shared alleles showed that axes 1 and 2 explained 27.76% of the total variability, i.e., 18.57% for axis 1 and 9.19% for axis 2. These axes allowed to separation of the clones from Nigeria into four homogeneous subgroups (SGN1, SGN2, SGN3, and SGN4), and clones from Côte d'Ivoire into two subgroups (SGC1 and SGC2). The subgroup SGC2 was heterogeneous and englobe the clone NIG-366 from Nigeria (Figure 1). The six subgroups from the 30 *C. nitida* clones are superimposed on their geographical origins. The groupings are as follows: (i) subgroup SGN1 consists of 4 clones from Nigeria, namely NIG-329, NIG-346, NIG-351, and NIG-356; (ii) subgroup SGN2 consists of 4 clones from Nigeria, namely NIG-332, NIG-342, NIG-359, and NIG-364; (iii) subgroup SGN3 consists of 4 clones from Nigeria, namely NIG-352, NIG-341, NIG-330, and NIG-331; (iv) subgroup SGN4 consists of 3 clones from Nigeria, namely NIG-350, NIG-379, and NIG-345; (v) subgroup SGC1 consists of 7 clones from Côte d'Ivoire, namely CIV-314, CIV-A3, CIV-305, CIV-313, CIV-323, CIV-318, and CIV-CR; (vi) subgroup SGC2 consists of 7 clones from Côte d'Ivoire, namely CIV-316, CIV-311, CIV-308, CIV-321 CIV-CV, CIV-306, and CIV-315 and one clone from Nigeria (NIG-366).

The results from dendrogram (Figure 2) were consistent with the PCoA results. Thus, the six subgroups overlap in the geographical origins of the clones. In addition, the branch of the dendrogram was supported by high bootstrap values (83 to 100%). This indicates reliable grouping of clones into the groups revealed by the principal coordinate analysis and the phylogenetic relationship between them.

**Genetic structure**

The genetic structure analysis showed a delta K peak at K = 2 (Figure 3). This confirms existence of two genetic groups, i.e. Côte d'Ivoire and Nigeria. The genetic structure designed by the 30 clones indicates that clones from Côte d'Ivoire and those from Nigeria are quite distinct. The barplot showed the genotype with mixed and pure alleles (inferred by the color in every line) (Figure 4). Thus, the 14 clones from Côte d'Ivoire are grouped with affiliation rates of more than 80%, and the proportion of genotypes with pure alleles was higher than the mixed one. This is not the case with the clones from Nigeria, where clones NIG-379, NIG-366, NIG-350, and NIG-345 were closer to those from Côte d'Ivoire, and had low affiliation rates with their original group (5 to 20%). In addition, mixed genotypes were more frequent than homogeneous one.

**Table 3.** Values of genetic diversity parameters assessed within each *C. nitida* clones from Côte d'Ivoire and Nigeria

| Genetic diversity parameters within origin | Côte d'Ivoire (N=14) | Nigeria (N=16) | Means |
|---|---|---|---|
| Alleles number per locus (A) | 2 ± 0 | 2 ± 0 | 2 ± 0 |
| Effective alleles number (Ae) | 1.74 ± 0.02 | 1.73 ± 0.02 | 1.74 ± 0.01 |
| Mean gene diversity ($H_S$) | 0.42 ± 0.01 | 0.41 ± 0.01 | 0.41 ± 0.01 |
| Fixation index ($F_{IS}$) | 0.12 ± 0.02 | 0.07 ± 0.02 | 0.09 ± 0.02 |

Note: N: number of clones

**Table 4.** Values of genetic diversity parameters assessed among *C. nitida* origin (Côte d'Ivoire and Nigeria)

| Genetic diversity parameters among origin | Mean values |
|---|---|
| $D_{ST}$ | 0.05 |
| $H_T$ | 0.46 |
| $F_{ST}$ | 0.11 |
| D | 0.46 |

**Table 5.** Analysis of molecular variance (AMOVA) in the 30 *C. nitida* clones from Côte d'Ivoire and Nigeria using 145 SNP markers

| Sources of variation | DF | Sum of squares of deviations | Mean square | Variance components | Percentage of variation (%) | *p value* |
|---|---|---|---|---|---|---|
| Among origin | 1 | 300.07 | 300.07 | 15.47 | 18 | 0.001 |
| Among clones | 28 | 1934.43 | 69.087 | 69.09 | 82 | |
| Total | 29 | 2234.5 | 369.87 | 84.56 | 100 | |

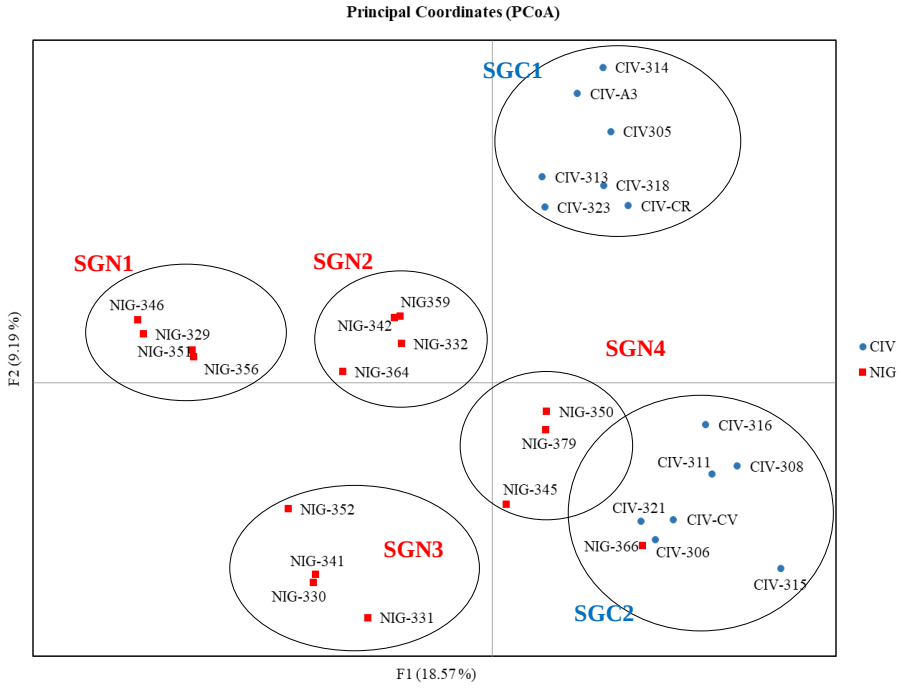


**Figure 1.** Scatter plot showing the relationships among the 30 clones according to the principal coordinate analysis (PCoA) based on the 145 SNPs

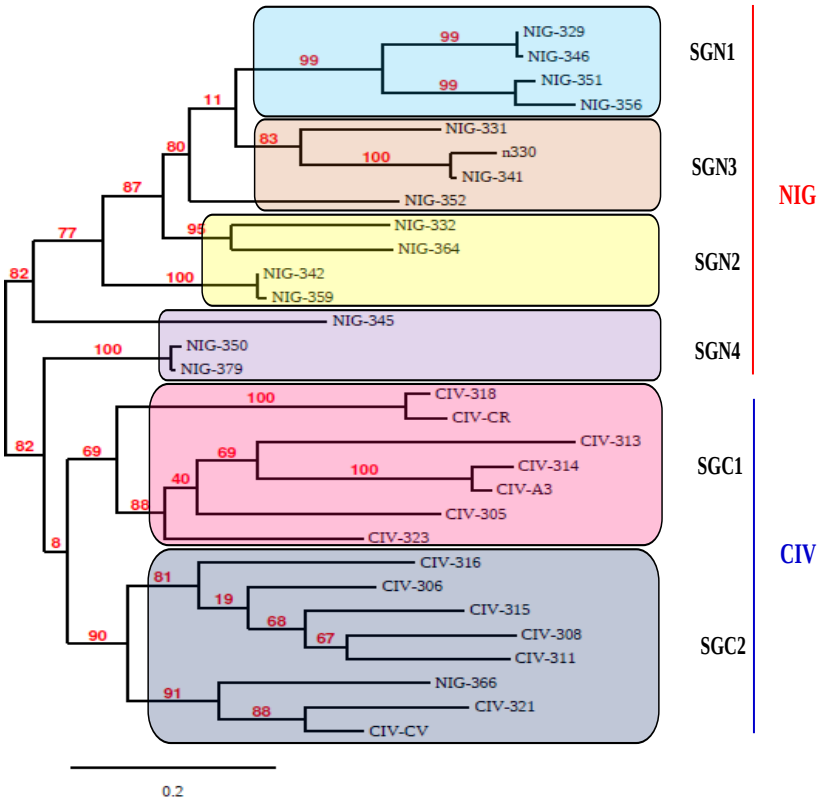


**Figure 2.** Dendrogram generated from 30 kola trees clones using a Bayesian algorithm based on sequential clustering of neighboring genotypes analyzed using 145 SNPs

**Genetic differentiation and genetic distance between subgroups revealed by the PCoA**

Genetic differentiation coefficients $F_{ST}$ ranged from 0.10 to 0.26, and genetic distance from 0.17 to 0.37 (Table 6). Subgroups from Côte d'Ivoire (SGC1 and SGC2) presented a very high differentiation among them ($F_{ST}$ = 0.25) and a high genetic distance (D = 0.28). In subgroups from Nigeria, couples SGN2-SGN3 and SGN3-SGN4 presented a moderate differentiation with $F_{ST}$ = 0.12. The genetic distance calculated between these two couples was almost similar, there are 0.18 and 0.17, respectively. In the same origin, other subgroups presented a high differentiation, namely SGN1-SGN2 ($F_{ST}$ = 0.18); SGN1-SGN3 ($F_{ST}$ = 0.23); SGN1-SGN4 ($F_{ST}$ = 0.23); SGN2-SGN4 ($F_{ST}$ = 0.15). The genetic distance calculated between these couples ranged from 0.19 (SGN2-SGN4) to 0.29 (SGN1-SGN3).

The SGC2 subgroup showed very strong differentiation and the greatest genetic distance from SGN1 ($F_{ST}$ = 0.37; D = 0.37). The SGC1 subgroup showed strong differentiation with SGN1 ($F_{ST}$ = 0.19), SGN3 ($F_{ST}$ = 0.18) and SGN4 ($F_{ST}$ = 0.17), and moderate differentiation with SGN2 ($F_{ST}$ = 0.14). The calculated genetic distances ranged from 0.17 to 0.26. Similarly, SGC2 and SGN2 were very strongly differentiated ($F_{ST}$ = 0.16) with a genetic distance estimated to 0.23. Data's analysis further revealed that SGC2 showed moderate differentiation with SGN3 ($F_{ST}$ = 0.10) and SGN4 ($F_{ST}$ = 0.12), with a genetic distance estimated to 0.15.

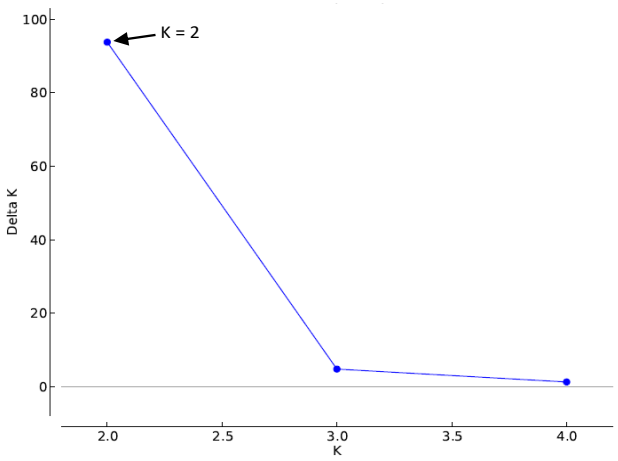


**Figure 3.** Relations between the number of K and ΔK, based on the method developed by Evanno et al. (2005) show a prominent peak at K = 2

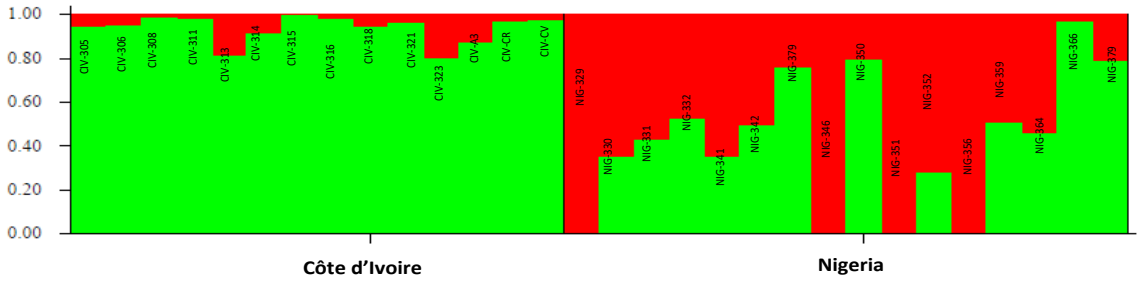


**Figure 4.** Genetic structure of 30 kola tree clones from Côte d'Ivoire and Nigeria of defined by the Bayesian method using 145 SNP loci. Each color represents a genetic group (two groups) and each barplot is a clone with a probability of belonging to a genetic group ranging from 0 to 1

**Table 6.** Matrix of genetic differentiation coefficients $F_{ST}$ (below diagonal) and genetic distances (above diagonal) calculated among subgroups

| | | Genetics distances | | | | | |
|---|---|---|---|---|---|---|---|
| | **Subgroups** | **SGN1** | **SGN2** | **SGN3** | **SGN4** | **SGC1** | **SGC2** |
| $F_{ST}$ | SGN1 | 0 | 0.20 | 0.29 | 0.28 | 0.19 | 0.37 |
| | SGN2 | 0.18 | 0 | 0.18 | 0.19 | 0.17 | 0.23 |
| | SGN3 | 0.22 | 0.12 | 0 | 0.17 | 0.26 | 0.15 |
| | SGN4 | 0.23 | 0.15 | 0.12 | 0 | 0.22 | 0.15 |
| | SGC1 | 0.19 | 0.14 | 0.18 | 0.17 | 0 | 0.28 |
| | SGC2 | 0.26 | 0.16 | 0.10 | 0.12 | 0.25 | 0 |

**Discussion**

Genetic diversity and structure analysis of germplasm is a crucial step in plant breeding and selection of new varieties. The results showed that the average percentage of polymorphic loci (PIC) obtained was 0.38. This is similar to the average obtained in plantain *Musa* sp. (Koffi et al. 2022) and *Sesamum indicum* L. (Wei et al. 2014) with the same kind of markers. These 145 markers were reasonably informative according to the classification scale of Hayden et al. (2010), with a PIC value range of 0.25 to 0.5.

Furthermore, percentage of polymorphic loci estimated at 99% confirms a high level of polymorphism of SNPs as stated by Ji et al. (2012) and Forrest et al. (2014). This percentage of polymorphic loci is higher than that obtained with RAPD markers in *C. nitida* (71.47%), and on five other *Cola* species (28.5%) in Nigeria (Akinro et al. 2019; Wood and Ohimain 2020). These results illustrate the effectiveness of SNPs for genetic diversity analysis in kola tree compared to other molecular markers including RAPDs. In addition, no duplicated genotypes across the 30 clones analyzed have been found. It means that 145 SNPs would be adequate to uniquely identify a kola clone in a population, as demonstrated by Ji et al. (2012) at the cocoa tree. Therefore, a high percentage of polymorphic loci found means that markers used to provide a reliable estimate of genetic diversity parameters.

The number of effective alleles (Ae), reflecting gene diversity, was 1.74 on both origins, showing a high variation of genes on overall loci (Nazareno et al. 2017). The inbreeding coefficient ($F_{IS}$) was low through the 30 clones (0.09). This result reflects a low rate of homozygous individuals at 145 loci analyzed. In other words, almost all clones involved in this study were heterozygous at different loci. This heterozygosity is thought to be favored by the breeding system of kola tree, involving different alleles between pollen and ovary tissue during fertilization. Cross-fertilization would have favored seed formation from which mother plants of genotypes studied originate. Indeed, the kola tree is a self-incompatible plant (Nyadanu et al. 2021). This self-incompatibility is sporophytic and presumes the existence of incompatibility alleles (Claessen et al. 2019). In this self-incompatibility system, the pollen tube does not develop correctly on stigmas producing the same allele contained in pollen. Furthermore, the presence of a large number of heterozygous individuals contributes to increased diversity (Porth and El-Kassaby 2014).

The genetic diversity within the origin value ($H_S$ = 0.41) obtained was relatively high. This relatively high genetic diversity within the origin result could be related to the equally high migration due to the geographical distance. In addition, reproductive system and self-incompatibility of kola tree could promote diversity within and between origins. Indeed, kola tree is allogamous due to self-incompatibility (Nyadanu et al. 2020) and reproduces naturally by seed. Plants that reproduce sexually are generally more diverse than those that preferentially multiply vegetatively (Ribeiro and Lovato 2014). Given this high diversity, clones studied could constitute an important gene pool for many selection criteria for improving kola tree.

According to the results of AMOVA, a strong contribution of intra-origin diversity to total diversity ($H_T$) has been observed. This diversity within origins ($H_S$) was much higher than diversity among origins ($D_{ST}$ = 0.05). According to Ouattara (2021), survey method adopted when collections were created contributed to their diversification. Indeed, plant material used for collection establishment was collected on several different and isolated sites, namely Divo, Issia, Oumé (Centre-West), Bingerville (South), and Toumodi (Centre) in Côte d'Ivoire. Nigerians' clones were introduced through the Cocoa Research Institute of Nigeria (CRIN). This provides a large and effective diversity for implementation of a long-term improvement strategy.

Genetic structure analysis allowed is to discriminate between two main genetic groups, namely clones originating from Côte d'Ivoire and those originating from Nigeria. These results are consistent with those of Montagnon et al. (2021) on *Coffea arabica* L. showing differentiation between different accessions from Ethiopia and Yemen. This differentiation was further confirmed by differentiation index ($F_{ST}$) value and genetic distance obtained between the two origins. Genetic differentiation can be reflected by the group member's degree of similarity or dissimilarity (Rahayu et al. 2022). It could also be attributed to geographical distance (Côte d'Ivoire and Nigeria) that separates the two countries (Islam et al. 2014), limiting gene flow. Indeed, gene flow between populations can be reduced by geographical distance (isolation-by-distance or IBD) or by divergent selection resulting from local adaptation (isolation-by-ecology or IBE) (Mallet et al. 2014). IBD expresses genetic differentiation by distance. Thus, pairs of populations that are geographically close to each other may be genetically closer than those that are distant by distance because of the limited dispersal capacity of seeds and pollen. Similar results were reported by Xiong et al. (2016) and Seo et al. (2020) on populations of cowpea (*Vigna unguiculata* (L.) Walp.) from different countries, and by Liu et al. (2017) on soybean (*Glycine max* (L.) Merr.) accessions from China and United States. In addition, divergence between both origins could result in the fragmentation of the West African rainforests into two batches by the Dahomey gap. Divergence among plant populations, probably due to forest fragmentation has been demonstrated in forests in Australia (Bradshaw 2012). Furthermore, the grouping of clones according to geographic origins suggests that a probable pattern of isolation by distance may exist. In addition, analysis grouped clones with similar geographical origins together except 4 clones (NIG-379, NIG-366, NIG-350, and NIG-345) that were grouped into Côte d'Ivoire. Indeed, these clones had low affiliation rates with their original group (Nigeria). It could be due to previous exchanges of plant material between the IFCC (Institut Français du Café et de Cacao) and the CRIN within framework of a partnership, which could lead to the reintroduction of genotypes originating from Côte d'Ivoire.

The PCoA subdivided the 30 clones into six subgroups (SGN1, SGN2, SGN3, SGN4, SGC1, and SGC2) partially overlapped with the geographical origins of the clones. This result corroborates those of Uba et al. (2021) on 270 Bambara groundnut (*Vigna subterranea* (L.) Verdc.) landraces collected in Nigeria, Cameroon and the United Kingdom, and Garot et al. (2019) at *Coffea mauritiana* Lam. The genetic differentiations among these subgroups were moderates ($0.05 < F_{ST} < 0.1$), high ($0.15 < F_{ST} < 0.25$) or very high ($F_{ST} > 0.25$) according to the classification of Wright (1978). The genetic differentiation coefficient distance calculated between subgroups from Côte d'Ivoire (SGC1 and SGC2) was higher than the values obtained

between subgroups from Nigeria. This result could be explained by the fact that Côte d'Ivoire is the center of origin of *C. nitida* species, where the level of variability is high (Bodard 1960). Given its economic importance, it has been introduced into other countries in Africa, such as Nigeria and Cameroon from the Ivory Coast (Babatunde 1977). This would explain the similarity of the clones NIG-379, NIG-366, NIG-350, and NIG-345 with those of Côte d'Ivoire.

In conclusion, the 30 cola clones from Côte d'Ivoire and Nigeria cultivated in CNRA showed high genetic diversity. The levels of diversity in both origins were almost similar, with moderate genetic differentiation. The genetic variation was mainly from clones of different origins. Geographical isolation would be the main reason for the differences between the origins. The 30 clones were subdivided into six subgroups, partially overlapping the geographical origins. The genetic differentiation between these subgroups was moderate, strong, or very strong. Based on the results obtained, possibilities for the development of a breeding program to improve the productivity of the cola tree in Côte d'Ivoire can be envisaged. It would also be interesting to determine the agronomic parameters of the 30 clones involved in the study for the introgression of traits by hybridization.

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## REFERENCES

Adenuga OO, Mapayi EF, Olasupo FO, Olaniyi OO, Oyedokun AV. 2012. Nigeria's *Cola* genetic ressources: The need for renewed exploration. Asian J Agric Sci 4: 177-182.

Adesida FA, Oluyole KA, Agulanna FT, Oladokun YO, Adelusi AA, Agboola LO, Ogunwolu QA, Ujunwa EI, Mustopha FB. 2021. Kola as an indispensable article of trade in West Africa. World J Adv Res Rev 12 (02): 324-331. DOI: DOI: 10.30574/wjarr.2021.12.2.0533.

Adesoye AI, Fasola TR, Akinro LA. 2014. Extraction of high-quality DNA from *Cola nitida* and *Cola acuminata*. Res Plant Biol 4 (4): 21-26.

Adje OAC, Achigan DEG, Agbangla C. 2016. Optimizing genomic DNA isolation in pineapple (*Ananas comosus* L.). J Plant Breed Genet 04 (01): 11-18.

Akinro LA, Adesoye AI, Fasola TR. 2019. Genetic diversity in *Cola acuminata* and *Cola nitida* using RAPD primers. Genetika 51 (1): 227-236. DOI: 10.2298/GENSR1901227A.

Amon-Armah F, Oduro SS, Doe EK, Asani M, Nyadanu D, Konlan S. 2021. Supply-side practices and constraints of the kola nut (*Cola nitida* (Vent) Schott. and Endl.) value chain in Ghana: Descriptive evidence. Int J Agron 1: 1-16. DOI: 10.1155/2021/9942699.

Asogwa EU, Adedeji AR, Oyedokun AV, Otuonye AH, Mokwunye FC, Agbongiarhuoyi AE. 2012. Strategies for improving production and storage of kolanuts in Nigeria. Am-Eur J Agric Environ Sci 12 (1): 37-43. DOI: 10.5402/2012/492394.

Babatunde A. 1977. The introduction of *Cola nitida* into Nigerian agriculture, 1880-1920. Afr Econ His 3: 1-14. DOI: 10.2307/3601136.

Bodard M. 1960. Contribution à l'étude systématique du genre Cola en Afrique occidentale. Thèse de la Faculté des Sciences de l'Université de Dakar, Dakar.

Bradshaw CJA. 2012. Little left to lose: Deforestation and forest degradation in Australia since European colonization. J Plant Ecol 5 (1): 109-120. DOI: 10.1093/jpe/rtr038.

Chander S, Garcia-Oliveira AL, Gedil M, Shah T, Otusanya GO, Asiedu R, Chigeza G. 2021. Genetic diversity and population structure of soybean lines adapted to Sub-Saharan Africa using Single Nucleotide Polymorphism (SNP) markers. Agronomy 11 (604) : 1-11. DOI: 10.3390/agronomy11030604.

Chung YS, Choi SC, Jun T-H, Kim C. 2017. Genotyping-by-sequencing: A promising tool for plant genetics research and breeding. Hortic Environ Biotechnol 58 (5): 425-431. DOI 10.1007/s13580-017-0297-8.

Claessen H, Keulemans W, Van de Poel B, De Storme N. 2019. Finding a compatible partner: Self-incompatibility in European pear (*Prunus communis*): Molecular control, genetic determination, and impact on fertilization and fruit set. Front Plant Sci 10 (401): 1-17. DOI: 10.3389/fpls.2019.00407.

Dah-Nouvlessounon D, Adoukonou-Sagbadja H, Diarrasouba N, Adjanohoun A, Baba-Moussa F, Sina H, Sezan A, Baba-Moussa L. 2015. Indigenous knowledge and socioeconomic values of three kola species (*Cola nitida*, *Cola acuminata* and *Garcinia kola*) used in southern Benin. Eur Sci J 11 (36): 206-227. DOI: 10.1155/2015/493879.

De Donato M, Peters SO, Mitchell SE, Hussain T, Imumorin IG. 2013. Genotyping-by-Sequencing (GBS): A novel, efficient and cost-effective genotyping method for cattle using next-generation sequencing. PLoS One 8 (5): 1-9. DOI: 10.1371/journal.pone.0062137.

Earl DA, von Holdt BM. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4: 359-361. DOI 10.1007/s12686-011-9548-7.

Ehounou JN, Kouamé B, Tahi GM, Kassin KE, Dékoula SC, Guy YF, Kouadio KH, N'guessan EBA, Soro N. 2019a. Impact of local global warming on rainfall and annual cocoa water requirements in the regions of Lôh-Djiboua and Gôh in West-central Côte d'Ivoire. Intl J Environ Clim Change 9 (9): 503-517. DOI: 10.9734/IJECC/2019/v9i930135.

Ehounou JN, Kouamé B, Tahi GM, Kassin KE, Kotaix JA, Dékoula SC, Guy YF, Kouadio KH, N'guessan EBA, Soro N. 2019b. Global warming influence on major seasonal and intra-seasonal rainfall indicators for sustainable cocoa production in West-central Côte d'Ivoire. J Exp Agric Intl 40 (5): 1-10. DOI: 10.9734/JEAI/2019/v40i530378.

Ekalu A, Habila JD. 2020. Phytochemistry, pharmacology and medicinal uses of *Cola* (Malvaceae) family: A review. Med Chem Res 29: 2089-2105. DOI: 10.1007/s00044-020-02637-x.

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE. 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6 (5): 1-10. DOI: 10.1371/journal.pone.0019379.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Mol Ecol 14: 2611-2620. DOI: 10.1111/j.1365-294X.2005.02553.x.

FAO. 2019. Recensement des exploitants et exploitations agricoles 2015-2016. Résultats de l'analyse des données du module communautaire du recensement des exploitants et exploitations agricoles (REEA). Rapport FAO/Ministère de l'agriculture et du développement rural, Vol. 2, Abidjan 128 p.

Forrest K, Pujol V, Bulli P, Pumphrey M, Wellings C, Herrera-Foessel S, Huerta-Espino J, Singh R, Lagudah E, Hayden M, Spielmeyer W. 2014. Development of a SNP marker assay for the Lr67 gene of wheat using a genotyping by sequencing approach. Mol Breed 34: 2109-2118. DOI 10.1007/s11032-014-0166-4.

Fratmat.info. 2022. Agriculture: 22% du PIB et 60% des recettes d'exportation de la Côte d'Ivoire. FratMat. Accessed on April 26th 2022 at 09 h 21 mn.

Garot E, Joët T, Combes MC, Lashermes P. 2019. Genetic diversity and population divergences of an indigenous tree (*Coffea mauritiana*) in Reunion Island: Role of climatic and geographical factors. Heredity 122 (6): 833-847. DOI: 10.1038/s41437-018-0168-9

Gestrich N, Champion L, Keïta D, Coulibaly N, Fuller DQ. 2021. Evidence of an eleventh-century AD *Cola nitida* trade into the Middle

Niger Region. Afr Archaeol Rev 38: 403-418. DOI: 10.1007/s10437-021-09445-7.

Hayden MK, Tabone TL, Nguyen TM, Coventry S, Keiper F, Fox R, Chalmers K, Mather D, Eglinton J. 2010. An informative set of SNP markers for molecular characterisation of Australian barley germplasm. Crop Pasture Sci 61: 70-83. DOI: 10.1071/CP09140.

He J, Zhao X, Laroche A, Lu Z, Liu H, Li Z. 2014. Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Front Genet 5 (484): 1-9. DOI: 10.3389/fpls.2014.00484.

Islam M, Lian C, Kameyama N, Hogetsu T. 2014. Low genetic diversity and limited gene flow in a dominant mangrove tree species (*Rhizophora stylosa*) at its northern biogeographical limit across the chain of three Sakishima islands of the Japanese archipelago as revealed by chloroplast and nuclear SSR analysis. Plant Syst Evol 300 (5): 1123-1136. DOI: 10.1007/s00606-013-0950-3.

Ji K, Zhang D, Motilal LA, Boccara M, Lachenaud P, Meinhardt LW. 2012. Genetic diversity and parentage in farmer varieties of cacao (*Theobroma cacao* L.) from Honduras and Nicaragua as revealed by Single Nucleotide Polymorphism (SNP) markers. Genet Resour Crop Evol 60: 441-453. DOI: 10.1007/s10722-012-9847-1.

Koffi KGC, Pokou ND, Traoré S, Kouadio DL, Atsin GJO, Aby N, N'guetta A, Kobenan K, Sié RS, Thiémélé DF, Dapeng Z. 2022. Genetic diversity of plantain (*Musa* sp.) collection in Côte d'Ivoire revealed by Single Nucleotide Polymorphism (SNP) markers. Adv Crop Sci Technol 10 (2): 1-8.

Koffi SY, Oura KR. 2019. Les facteurs de l'adoption de l'anacarde dans le bassin cotonnier de Côte d'Ivoire. Cah Agric 28 (24): 6-8. DOI: 10.1051/cagri/2019025.

Kouassi JL, Gyau A, Diby L, Bene Y, Kouamé C. 2021. Assessing land use and land cover change and farmers' perceptions of deforestation and land degradation in South-West Côte d'Ivoire, West Africa. Land 10 (429): 1-25. DOI: 10.3390/land10040429.

Lanaud C, Risterucci AM, Pieretti I, Falque M, Bouet A, Lagoda PJL. 1999. Isolation and characterization of microsatellites in *Theobroma cacao* L. Mol Ecol 8: 2141-2152. DOI: 10.1046/j.1365-294x.1999.00802.x.

Lawson JD, Van Dorp L, Falush D. 2018. A tutorial on how not to over-interpret STRUCTURE and admixture bar plots. Nat Commun 9 (3258): 1-11. DOI: 10.1038/s41467-018-05257-7.

Lemoine F, Correia D, Lefort V, Doppelt-Azeroual O, Mareuil F, Cohen-Boulakia S, Gascuel O. 2019. NGPhylogeny.fr: new generation phylogenetic services for non-specialists. Nucleic Acids Res 47: 1-6. DOI: 10.1093/nar/gkz303.

Liu Z, Li H, Wen Z, Fan X, Li Y, Guan R, Guo Y, Wang S, Wang D, Qiu L. 2017. Comparison of genetic diversity between Chinese and American soybean (*Glycine max* (L.)) accessions revealed by High-Density SNPs. Front Plant Sci 8: 1-13. DOI: 10.3389/fpls.2017.02014.

Mallet B, Martos F, Blambert L, Pailler T, Humeau L. 2014. Evidence for isolation by-habitat among populations of an epiphytic orchid species on a small oceanic island. PLoS One 9 (2): 1-15. DOI: 10.1371/journal.pone.0087469.

Montagnon C, Mahyoub A, Solano W, Sheibani F. 2021. Unveiling a unique genetic diversity of cultivated *Coffea arabica* L. in its main domestication center: Yemen. Genet Resour Crop Evol 68 (6): 2411-2422. DOI: 10.1007/s10722-021-01139-y.

Nazareno AG, Bemmels JB, Dick CW, Lohmann LG. 2017. Minimum sample sizes for population genomics: An empirical study from an Amazonian plant species. Mol Ecol Resour 17: 1136-1147. DOI: 10.1111/1755-0998.12654.

Nei M. 1972. Genetic distance between populations. Am Nat 106: 283-292. DOI: 10.1086/282771.

Nyadanu D, Lowor ST, Akpertey A, Tchokponhoue DA, Pobee P, Dogbatse JA, Okyere D, Amon-Armah F, Brako-Marfo M. 2020. Genetic variability of bioactive compounds and selection for nutraceutical quality in kola [*Cola nitida* (Vent) Schott. and Endl.]. PLoS One 15 (12): 1-19. DOI: 10.1371/journal.pone.0242972.

Nyadanu D, Lowor ST, Tchokponhoue DA, Pobee P, Nunekpeku W, Brako-Marfo M, Okyere D, Owusu-Ansah F, Ofori A. 2021. Combining ability and gene action for sexual compatibility and pattern of nut colour segregation among ten elite clones of kola (*Cola nitida* (Vent) Schott and Endl.). Euphytica 217 (62): 1-19. DOI: 10.1007/s10681-021-02801-3.

Ouattara B. 2013. Vertus et usages de la noix de cola dans les sociétés Ouest Africaines. Rev Afr Anth, Nyansa-Pô 14: 5-21.

Ouattara Y. 2021. Caractérisation agro-morphologique et moléculaire de colatiers (*Cola nitida* Vent.) Schott & Endl.) en collection et de plants en pépinière. Thèse Unique, Université Nangui Abrogoua, Abidjan (Côte d'Ivoire).

Peakall R, Smouse P. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28: 2537-2539. DOI: 10.1093/bioinformatics/bts460.

Porras-Hurtado L, Ruiz Y, Santos C, Phillips C, Carracedo Á, Lareu MV. 2013. An overview of STRUCTURE: applications, parameter settings, and supporting software. Front Genet 4 (98): 1-13. DOI: 10.3389/fgene.2013.00098.

Porth I, El-Kassaby YA. 2014. Assessment of the genetic diversity in forest tree populations using molecular markers. Diversity 6: 283-295. DOI: 10.3390/d6020283.

Rahayu MS, Setiawan A, Maskromo I, Purwito A, Sudarsono A. 2022. Genetic diversity analysis of Puan Kalianda kopyor coconuts (*Cocos nucifera*) from South Lampung, Indonesia based on SSR markers, Biodiversitas 23 (1): 205-211. DOI: 10.13057/biodiv/d230126.

Ribeiro RA, Lovato MB. 2014. Mating system in a neotropical tree species, *Senna multijuga* (Fabaceae). Genet Mol Biol 27 (3): 418-424. DOI: 10.1590/S1415-47572004000300018.

Robert-Granié C, Legarra A, Ducrocq V. 2011. Principes de base de la sélection génomique. INRA Prod Anim 24 (4): 331-340. DOI: 10.20870/productions-animales.2011.24.4.3265.

Rozas J, Ferrer-Mata A, Sànchez-Del Barrio JC, Librado P, Ramos-Onsins E, Sànchez-Gracia A. 2017. DNA sequence polymorphism analysis of large datasets. Mol Biol Evol 34: 3299-3302. DOI: 10.1093/molbev/msx248.

Savi MK, Noumonvi R, Chadaré FJ, Daïnou K, Salako VK, Idohou R, Assogbadjo AE, Kakaï RG. 2019. Synergy between traditional knowledge of use and tree population structure for sustainability of *Cola nitida* (Vent.) Schott. & Endl in Benin (West Africa). Environ Dev Sustain 21: 1357-1368. DOI: 10.1007/s10668-018-0091-5.

Seo E, Kim K, Jun TH, Choi J, Kim SH, Muñoz-Amatriaín M, Sun H, Ha BK. 2020. Population structure and genetic diversity in Korean cowpea germplasm based on SNP markers. Plants 9 (9): 1-14. DOI: 10.3390/plants9091190.

Traoré K. 2018. Le couvert forestier en Côte d'Ivoire : une analyse critique de la situation de gestion des forêts (classées, parcs et réserves). Intl J Soc Sci Humanit Invent 5 (02): 4387-4397. DOI: 10.18535/ijsshi/v5i2.02.

Uba CU, Oselebe HO, Tesfaye AA, Abtew WG. 2021. Genetic diversity and population structure analysis of bambara groundnut (*Vigna subterrenea* L) landraces using DArT SNP markers. PLoS One 16 (7): 1-18. DOI: 10.1371/journal.pone.0253600.

Wei L, Miao H, Li C, Duan Y, Niu J, Zhang T, Zhao Q, Zhang H. 2014. Development of SNP and InDel markers via de novo transcriptome assembly in *Sesamum indicum* L. Mol Breed 34: 2205-2217. DOI: 10.1007/s11032-014-0174-4.

Whitlock BA, Bayer C, Baum DA. 2001. Phylogenetic relationships and floral evolution of the Byttnerioideae (Sterculiaceae or Malvaceae s.l.) based on sequences of the chloroplast gene, ndhF. Syst Bot 26 (2): 420-437.

Wood TT, Ohimain EI. 2020. Molecular and morphological diversity studies of five *Cola* (Schott & Endl.) species in Ibadan, Nigeria. Ann Res Rev Biol 35 (9): 1-13. DOI: 10.9734/ARRB/2020/v35i930266.

Wright S. 1978. Variability Within and among Natural Populations. University of Chicago Press, Chicago.

Xiong H, Shi A, Mou B, Qin J, Motes D, Lu W, Ma J, Weng Y, Yang W, Wu D. 2016. Genetic diversity and population structure of cowpea (*Vigna unguiculata* L. Walp). PLoS One 11 (82): 1-15. DOI: 10.1371/journal.pone.0160941.