Assessing genetic diversity in a germplasm collection of kola trees (*Cola nitida* (Vent.) Schott and Endl.) using enzymatic markers

R.S. Sié,[1] J.A.K. N'Goran,[2] C. Montagnon,[3] D.S. Akaffou,[1] C. Cilas[3] and F. Mondeil[4]

[1] UFR-SN, Université d'Abobo-Adjamé, 02 BP 801 Abidjan 02, Côte d'Ivoire. Email: sieraoul@yahoo.fr
[2] CNRA, 01 BP 1827 Abidjan 01, Côte d'Ivoire
[3] CIRAD-Montpellier, BP 5035-34032, Montpellier cedex 1, France
[4] UFR Biosciences, Université de Cocody, 22 BP 582 Abidjan 22, Côte d'Ivoire

Summary

Assessing genetic diversity in a germplasm collection of kola trees (*Cola nitida* (Vent.) Schott and Endl.) using enzymatic markers

The genetic diversity of a germplasm collection of kola trees (*Cola nitida* (Vent.) Schott and Endl.) maintained in Côte d'Ivoire and consisting of accessions originating from Côte d'Ivoire, Guinea and Nigeria, was assessed using 6 enzyme systems and 11 loci. These enzyme systems were GPI with 3 loci and 17 alleles, PGM (2 loci, 4 alleles), IDH (2 loci, 3 alleles), EST (1 locus, 3 alleles), ACP (1 locus, 2 alleles) and MDH (2 loci, 5 alleles). Nine of the 11 loci were polymorphic. A hierarchical ascendant classification (cluster analysis) revealed that the total population was structured into three genetically distinct and diversified groups: A, B and C. The fixation index (F_{st}) and the inbreeding coefficient within subpopulation (F_{is}) were found to be +0.20 and −0.15, respectively. The three groups (A, B and C) differ at allele *f* of the locus *Gpi-2* and the alleles *e*, *f* and *g* of the locus *Gpi-3*. Each group is heterogeneous and contained both Ivorian, Guinean and Nigerian genotypes. The originality of these groups was confirmed by a factorial discriminant analysis. The results led to the conclusion that the three populations studied would have had a common centre of dispersion.

Key words: *Cola nitida*, electrophoresis, enzymes, genetic diversity

Résumé

Évaluation de la diversité génétique dans une collection de matériel génétique de kolatiers (*Cola nitida* (Vent.) Schott et Endl.) en utilisant des marqueurs enzymatiques

La diversité génétique dans une collection de matériel génétique de kolatiers (*Cola nitida* (Vent.) Schott et Endl.) conservée en Côte d'Ivoire et constituée d'accessions originaires de Côte d'Ivoire, Guinée et Nigeria, a été évaluée en utilisant 6 systèmes enzymatiques et 11 loci, dans lesquels GPI représente 3 loci et 17 allèles, PGM (2 loci, 4 allèles), IDH (2 loci, 3 allèles), EST (1 locus, 3 allèles), ACP (1 locus, 2 allèles) et MDH (2 loci, 5 allèles). Neuf des 11 loci sont polymorphes. Une classification hiérarchique ascendante (analyse de clusters) révèle que la population totale est structurée en trois groupes génétiquement distincts et diversifiés : A, B et C. L'indice de fixation (F_{st}) et le coefficient d'endogamie dans les sous-populations (F_{is}) sont de +0,20 et −0,15, respectivement. Les trois groupes (A, B et C) diffèrent au niveau de l'allèle *f* du locus *Gpi-2* et des allèles *e*, *f* et *g* du locus *Gpi-3*. Chaque groupe est hétérogène et contient à la fois des génotypes ivoiriens, guinéens et nigérians. L'originalité de ces groupes est confirmée par une analyse factorielle discriminante. Les résultats permettent de conclure que les trois populations étudiées ont un centre de dispersion commun.

Resumen

Evaluación de la diversidad genética en una colección de germoplasma de árboles de cola (*Cola nitida* (Vent.) Schott y Endl.) empleando marcadores enzimáticos

Se evaluó la diversidad genética de una colección de germoplasma de árboles de cola (*Cola nitida* (Vent.) Schott y Endl.) conservada en Costa de Marfil y que consiste de accesiones provenientes de Costa de Marfil, Guinea y Nigeria, empleando 6 sistemas de enzimas y 11 loci. Estos sistemas de enzimas eran GPI con 3 loci y 17 alelos, PGM (2 loci, 4 alelos), IDH (2 loci, 3 alelos), EST (1 locus, 3 alelos), ACP (1 locus, 2 alelos) y MDH (2 loci, 5 alelos). Nueve de los 11 loci eran polimórficos. Una clasificación jerárquica ascendente (análisis de conglomerados) reveló que toda la población se estructuraba en tres grupos genéticamente distintos y diversificados: A, B y C. Se determinó que el índice de fijación (F_{st}) y el coeficiente de intracruzamiento en la subpoblación (F_{is}) eran de +0,20 y −0,15 respectivamente. Los tres grupos (A, B y C) diferían en el alelo *f* del locus *Gpi-2* y en los alelos *e*, *f* y *g* del locus *Gpi-3*. Cada grupo es heterogéneo y contiene genotipos guineanos, nigerianos y marfilenses. El carácter original de estos grupos quedó confirmado por un análisis discriminante factorial. Los resultados llevan a la conclusión de que las tres poblaciones estudiadas habrían tenido un centro común de dispersión.

Introduction

The kola tree, *Cola nitida* (Vent.) Schott and Endl. is indigenous to West Africa (Bodard 1960). Its fruits contain seeds known as kola nuts. The nuts are consumed for their stimulant properties and their taste and are traditionally used in western and central Africa during weddings, funerals and ritual sacrifices. They are also used in the pharmaceutical and food industries to produce cardiac stimulants, laxatives, sedatives and sodas (Egbe and Oladokun 1987).

Kola cultivation is relatively recent in Côte d'Ivoire and most of the kola nuts sold in the markets come from old and low-yielding wild or semi-wild trees. Because the use of high-yielding genotypes is essential for the development of kola production in Côte d'Ivoire, a kola improvement programme was designed by the national agricultural research institute, Centre National de Recherche Agronomique de Côte d'Ivoire (CNRA), and a germplasm collection of about hundred genotypes of diverse

origins (Côte d'Ivoire, Guinea and Nigeria) was set up. The first step consisted in simple phenotypic assessments of the genotypes and their progenies (Bonsson 1983) and this led to the selection of a few parents whose progenies gave relatively good yields. No prior genetic diversity study had been conducted before the different crosses were carried out, although it was known that such study would have enabled a better choice of the crosses. To assess the genetic diversity of the germplasm established, enzymatic markers that are more stable in relation to environmental effects were used.

Enzymatic markers have been already used to study genetic diversity in many tropical plants including cocoa (Lanaud 1987), coffee (Leroy et al. 1993) and sorghum (Djè et al. 1998). For the species examined, the genetic variability and the structure obtained were useful in establishing appropriate breeding strategies.

This paper describes the organization and the genetic variability of kola germplasm maintained in Côte d'Ivoire and its consequences for kola-tree breeding.

Materials and methods

Plant material

The plant material used consisted of 79 genotypes (41 from Côte d'Ivoire, 9 from Guinea and 29 from Nigeria) collected from primary forests (material from Côte d'Ivoire and Guinea) and from smallholdings set up with local planting material (Nigerian genotypes). The germplasm was maintained at the CNRA station at Bingerville in Côte d'Ivoire. In both Côte d'Ivoire and Guinea, the survey strategy consisted in taking genotypes from several sites spread over the natural range of habitats of the plant. The trees were planted in a design consisting of totally randomized elementary plots of 1–5 replicates per genotype, with a planting density of 312 trees per hectare.

Electrophoresis method

Six enzyme systems with 11 loci were used. These were malate dehydrogenase (EC 1.1.1.37, MDH) with two loci, isocitrate dehydrogenase (EC 1.1.1.42, IDH) with two loci, acid phosphatase (EC 3.1.3.2, ACP) with one locus, glucose phosphate isomerase (EC 5.3.1.9, GPI) with three loci, phosphoglucomutase (EC 5.4.2.2, PGM) with two loci, and esterase (EC 3.1.1.1, EST) with one locus (Sié 1999).

Electrophoresis was carried out on 12.5% starch gel. Two migration systems were used: histidine–citrate pH6 and histidine–citrate pH8. For both systems we used a gel buffer (0.05 M histidine and 0.1 M Tris) and a electrode buffer (0.15 M Tris, 0.04 M citric acid). We adjusted pH with Tris. Enzymes were extracted by crushing pieces of fresh leaves in a small amount of extraction buffer consisting of 0.5 M Tris–HCl, pH7, 0.3 M ascorbic acid, 0.01 M EDTA, 2% mercaptoethanol and 1.5 10^{-5}M of potassium cyanide (KCN) at pH7. Filter paper wicks (Whatman No. 3) were dipped into the leaf extracts. The wicks were then removed, lightly blotted and loaded into a transverse cut in the gels. Information on the genetic determinism (number of loci and number of alleles) of these enzyme systems can be found in Sié (1999).

The first three enzymes (MDH, IDH and ACP) were migrated on a histidine Tris–citrate migration system with a constant current (40 mA for 6–7 h) at pH8. GPI and PGM were migrated on the histidine Tris–citrate migration system with a discontinuous current (20 mA for 1 h 30 min, 30 mA for 1 h 40 min and 40 mA for 4 h) at pH6. For EST, the histidine Tris–citrate migration system was used with a constant current (40 mA for 6 h) at pH6.

Data analysis

Wright's fixation indexes (Wright 1951), F_{st} (fixation index) and F_{is} (inbreeding coefficient within subpopulations), were used to analyse the diversity existing in the overall population of kola trees studied. Thus, F_{is} was used to analyse within-subpopulation diversity and F_{st} between-subpopulation diversity. Each geographical origin was considered as a population.

The statistical method described by Montagnon and Bouharmont (1996) was applied. A factorial correspondence analysis (FCA) was first carried out and each genotype was encoded to each locus as follows: 0 if the allele was absent, 1 if the allele was present in its heterozygous state, and 2 if the allele was present in the homozygous state. Then a hierarchical ascendant classification (HAC) was carried out from the factorial coordinates of the first six FCA axes. The aggregation criterion used was variance. Lastly, a factorial discriminant analysis (FDA) was carried out to confirm the reality of the clusters found with the HAC. The GENEPOP2 statistics program (Raymond and Rousset 1995) was used for the analysis.

Results and discussion

Structure and characterization of kola trees in the collection

The HAC indicated genetic proximity between the genotypes studied (Figure 1). Three genetic groups, A, B and C, were detected, containing 49, 20 and 10 genotypes respectively. The minority group C was the most genetically distant from the others.

The FDA carried out on groups A, B and C confirmed the reality of those groups (100% of correctly classified individuals). Differentiation between these groups occurred primarily for enzyme GPI and allele *f* of locus *Gpi*-2 and alleles *e*, *f* and *g* of *Gpi*-3. In fact, these groups differed from each other at the following alleles (Table 1): group A with alleles *Gpi*-3*g* and *Gpi*-2*f* at respective frequencies of 0.82 and 0.34, differed from group B which was characterized by allele *Gpi*-3*f* (0.92), while group C was characterized by allele *Gpi*-3*e*, which was exclusively present at a frequency of 1.

Glucose phosphate isomerase (GPI) was the most polymorphic enzyme, which revealed all the diversity of the kola trees in the collection. For any future study of the genetic diversity of the kola tree based on enzymes, GPI should be used.

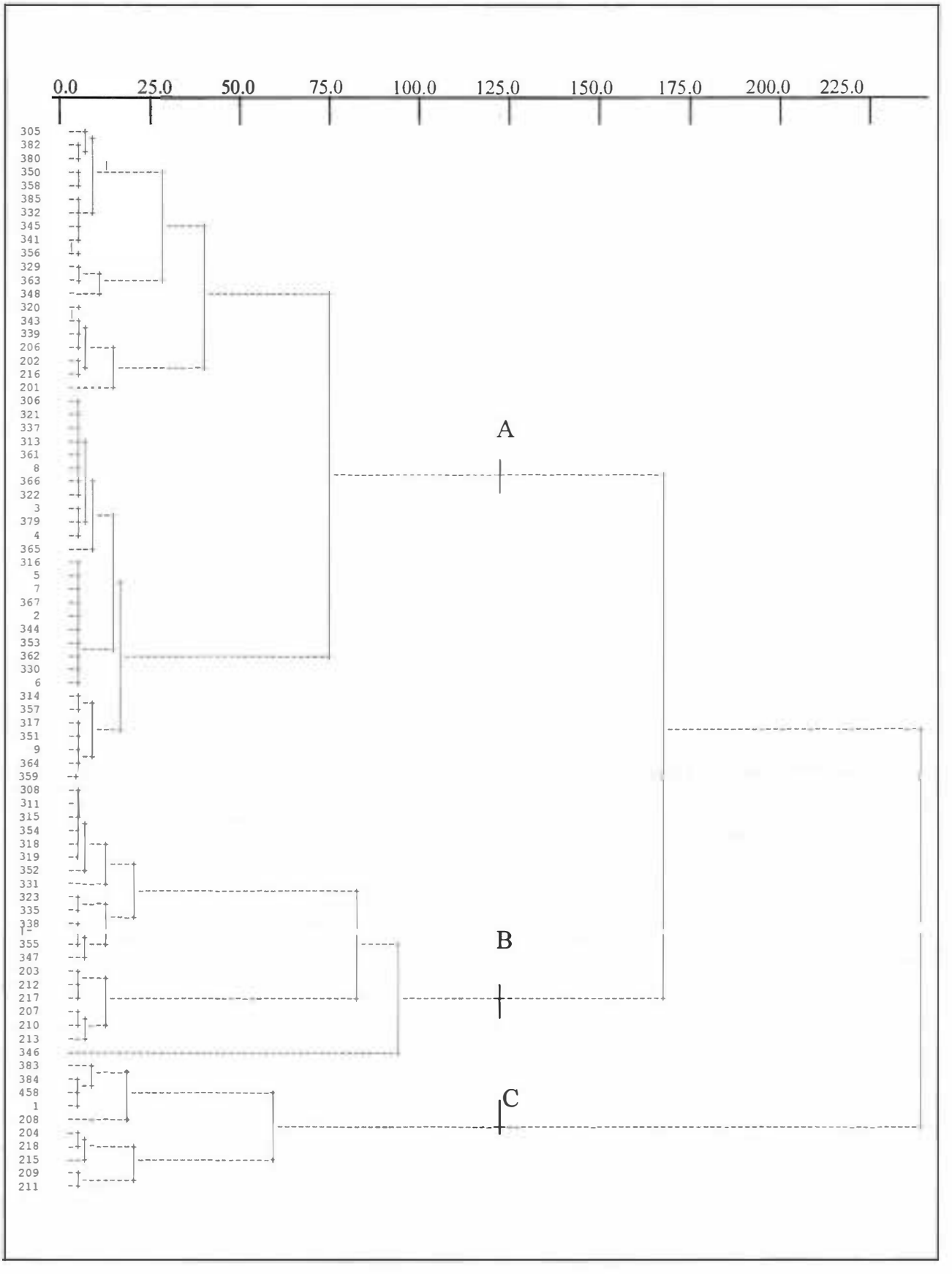

Figure 1. Dendrogram showing kola tree (*Cola nitida*) germplasm diversity.

Table 1. Allelic frequencies per polymorphic locus within the genetic groups of kola trees obtained by HAC

Loci	Alleles	Group A	Group B	Group C
Gpi-2	*a*	0	0.05	0
	b	0.01	0.26	0.28
	c	0.01	0.21	0
	d	0.63	0.45	0.22
	e	0.01	0	0.50
	f	0.34	0.03	0
Gpi-3	*a*	0.01	0	0
	b	0.01	0	0
	c	0	0.03	0
	d	0.07	0	0
	e	0	0	1
	f	0.02	0.92	0
	g	0.82	0.03	0
	h	0.07	0.03	0
Pgm-1	*a*	0.46	0.35	0.50
	b	0.54	0.65	0.50
Pgm-2	*a*	0.46	0.74	0.30
	b	0.54	0.26	0.70
Idh-2	*a*	0.09	0.11	0.06
	b	0.91	0.89	0.94
Mdh-1	*a*	0.32	0.39	0.38
	b	0.68	0.61	0.62
Mdh-2	*a*	0.56	0.58	0.50
	b	0.38	0.35	0.38
	c	0.05	0.07	0.12
Est	*a*	0.60	0.65	0.44
	b	0.18	0.15	0.28
	c	0.21	0.20	0.28
Acp	*a*	0.54	0.63	0.65
	b	0.46	0.37	0.35

Table 2. Distribution of kola tree (*Cola nitida*) geographical origins in the three genetic groups obtained by HAC

Groups	Geographical origin			Total
	Côte d'Ivoire	Guinea	Nigeria	
A	18 (37%)	8 (16%)	23 (47%)	49
B	14 (70%)	0 (0)	6 (30%)	20
C	9 (90%)	1 (10%)	0 (0%)	10
Total	41	9	29	79

The distribution of the genotypes in the three groups was not dependent on their geographical origin (Table 2). In fact, group A contained genotypes from Ivorian, Guinean and Nigerian origins, group B contained Ivorian and Nigerian genotypes, and group C contained Ivorian and Guinean genotypes. This independent distribution of the Ivorian, Guinean and Nigerian genotypes in these three genetic groups indicated that the survey sites were not independent centres of dispersion for this plant. Unlike the kola tree, other perennial plants with the same reproduction system, such as cocoa (Lanaud 1987), and the same natural range, such as coffee *Coffea canephora* (Montagnon et al. 1991), have centres of dispersion that merge with their centres of origin. The lack of allelic differentiation between these different geographical origins reveals the recent human dispersal of kola, from its centre of origin between Liberia and Côte d'Ivoire (Bodard 1960) to the rest of western Africa. According to Dublin (1965) this migration would have taken place in the 19th century.

Axis 1 of the FCA (Figure 2) accounted for 39% of total variability; it was determined by alleles *b*, *e* and *f* of locus *Gpi-2* and alleles *e* and *g* of locus *Gpi-3*. Axis 2 (23.45% of variability) was primarily characterized by *Gpi-3f*. This FCA revealed a distinction between the three groups. Group C was separated from groups A and B on axis 1. Axis 2 separated group B from groups A and C.

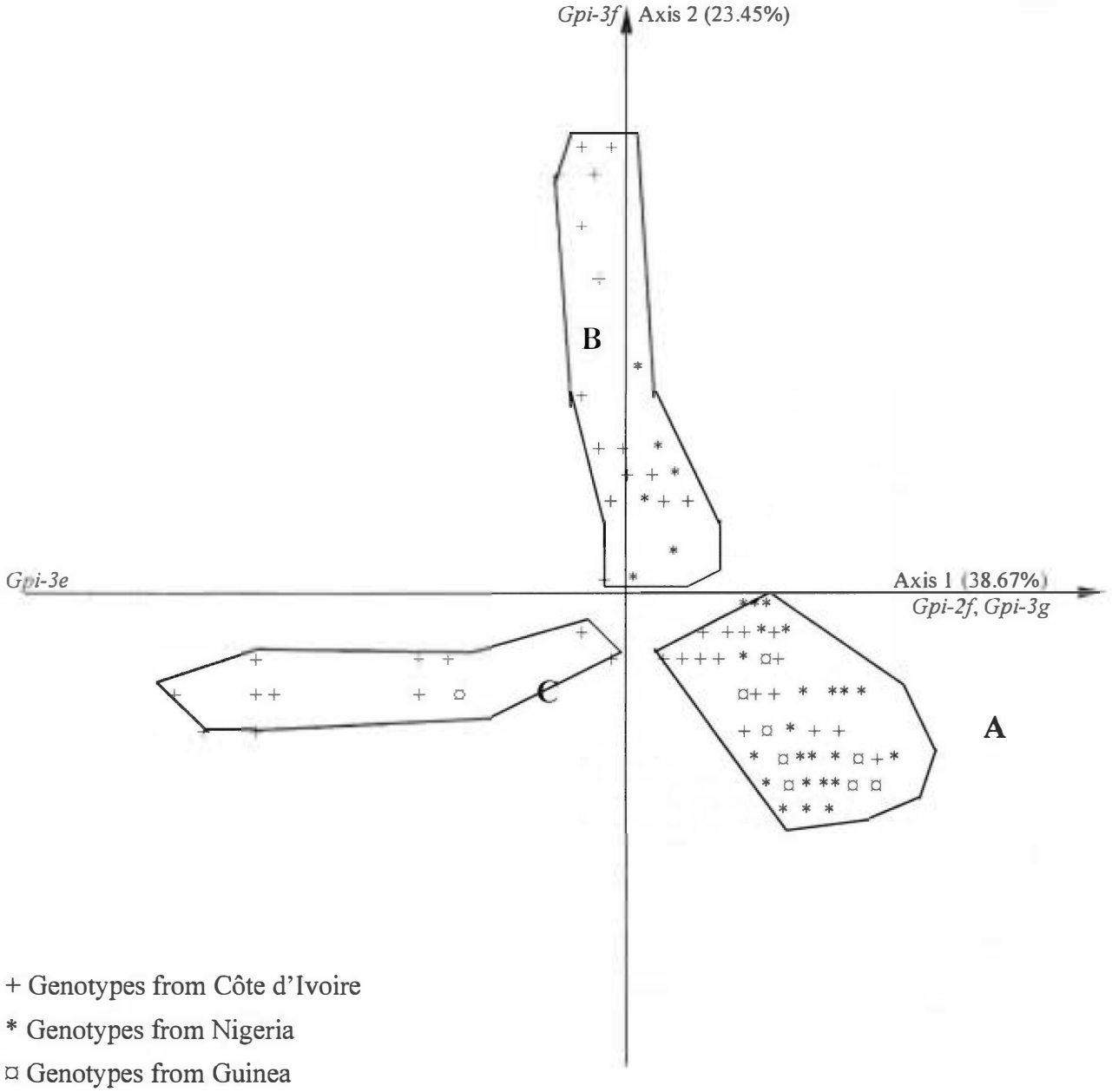

Figure 2. Distribution and clustering of kola genotypes (*Cola nitida*) of different geographical origins by factorial correspondence analysis (FCA).

The F_{st} value (+0.20) calculated from these three groups was quite high and revealed substantial genetic differentiation between the three groups. This considerable genetic diversification between genetic groups A, B and C could be used in a breeding strategy such as recurrent selection (Baudouin et al. 1997).

The relatively high value of F_{is} (–0.15) calculated within the groups revealed the existence of major diversification in each of these groups. In the case of recurrent selection, within-group genetic recombination phases could be introduced at the beginning of each selection cycle. The planting material used as a basis for launching this selection strategy would thus consist of the 79 genotypes used in this research. In comparison, the planting material used as the basis for oil palm selection is composed of 82 genotypes (Gascon and de Berchoux 1964), and of 22 trees for *Hevea* (Besse 1993).

Conclusion

This study revealed a common origin for kola trees from Côte d'Ivoire, Guinea and Nigeria. The diversity existing in these three populations of kola trees is organized in three genetically distant groups. These groups could be used as the basis for setting up a breeding scheme such as recurrent selection. However, it would be interesting to search for morphological and/or production traits specific to each of those groups.

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