Coffee (*Coffea canephora*)

Stéphane Dussert, Philippe Lashermes, François Anthony, Christophe Montagnon, Pierre Trouslot, Marie-Christine Combes, Julien Berthaud, Michel Noirot and Serge Hamon

Coffee is the primary agricultural export product (Charrier and Eskes, 1997). It is produced from two species: *Coffea arabica* L. and *C. canephora* Pierre. *Coffea arabica* is known for its gustatory qualities. It is cultivated on the high humid tropical plateaux, essentially in Latin America and East Africa. *Coffea canephora* is renowned for its agronomic hardiness, whence its common name of Robusta. It is cultivated mainly in humid tropical zones of low altitude and represents 30% of the world production of coffee. It comes mostly from Brazil, Indonesia, and Côte d'Ivoire. It is now widely produced in Southeast Asia—the Philippines and Vietnam—and in India.

During the 18th and 19th centuries, only Arabica was produced and that mainly in tropical America, the Caribbean, and Asia (Charrier and Eskes, 1997). However, this species appeared to be highly sensitive to parasitic threats, especially orange rust. That is why, in Africa, during the 19th century, the spontaneous forms of other species of coffee, especially *C. canephora*, were cultivated locally. For *C. canephora*, it was mostly in the Belgian Congo (now the Democratic Republic of Congo) and Uganda that coffee plants from local forest populations, of the Robusta type, were cultivated. They were transferred to Java, a major breeding centre of *C. canephora* from 1900 to 1930 (Montagnon et al., 1998). At the same time, in Africa, the diversity of material cultivated was extended with the use of local spontaneous forms: Kouilou in Côte d'Ivoire, Niaouli in Togo and Benin, and Nana in the Central African Republic. The material selected in Java was reintroduced in the Belgian Congo around 1916 at INEAC (Institut National pour l'Étude Agronomique du Congo Belge), which has become the major breeding centre of *C. canephora* from 1930 to 1960 (Montagnon et al., 1998). However, although the overall performance of cultivated trees has increased noticeably after a few breeding cycles at

Java and the Belgian Congo, the cultivars nonetheless have remained genetically very close to individuals of the original natural populations. Moreover, in the African countries where the species originated and where *C. canephora* is cultivated, local spontaneous forms could be crossed with the introductions and the cultivated plants could revert to the wild forms.

BOTANY AND GENETIC RESOURCES

Botany and Mode of Reproduction

Coffea canephora belongs to the family Rubiaceae, genus *Coffea* L., subgenus *Coffea* Bridson. The genus *Coffea* has an area of distribution limited to the African continent, Madagascar, and the Mascarene Islands. It contains close to 80 species, of which 25 are endemic to Africa (Bridson and Verdcourt, 1988). The species of the genus *Coffea* that are closest genetically to *C. canephora* are *C. congensis* and *C. brevipes* (Lashermes et al., 1997). Moreover, *C. canephora*, or an ancestral form of it, is one of the two parental diploid species of *C. arabica* (Lashermes et al., 1997). All the species of the genus are diploid, with the exception of *C. arabica*, which is allotetraploid. Similarly, they all have a mode of reproduction that is strictly allogamous, with the exception of *C. arabica*, which is autogamous. Studies on *C. canephora* have indicated a system of gametophytic self-incompatibility (Berthaud, 1980).

For the diploid species of the genus, the quantity of DNA per genome, measured by flow cytometry, varies from 0.95 to 1.78 pg (Cros et al., 1995). It is 1.54 pg for *C. canephora* and 2.61 pg for *C. arabica*.

Coffea canephora has one of the widest areas of distribution of the subgenus *Coffea*: it extends west to east from Guinea to Sudan, and north to south from Cameroon to Angola (Berthaud, 1986).

The growth of coffee plants of this species is dimorphic. The main stems (orthotropic axes) grow vertically and the branches (plagiotropic axes) grow horizontally. Horticultural propagation is relatively easy. The plant may flower once or twice a year, after a rainfall of at least 10 mm, which follows a period of water stress. Berries mature at 8 to 12 months depending on the variety and environment.

The seeds of *C. canephora* do not behave in an orthodox manner (Roberts, 1973) when dehydrated or stored at low temperature (Couturon, 1980). Their longevity is only one to two years in the hydrated state at ambient temperatures.

Genetic Resources

Given the behaviour of *C. canephora* seeds, the long-term conservation of genetic resources of this species is done in the field.

Collections that are more or less representative of the most widespread introductions, of material taken from plantations and of local forms, are conserved in Côte d'Ivoire, Cameroon, Uganda, India, Indonesia, and Brazil. But the only reference collection for wild forms of *C. canephora* is the Divo collection, in Côte d'Ivoire. It contains more than 700 wild genotypes collected by ORSTOM (now the IRD, Institut de Recherche pour le Développement, France) in collaboration with CIRAD, the FAO (Food and Agriculture Organization, Italy), the IPGRI (International Plant Genetic Resources Institute, Italy), and the MNHN (Muséum national d'histoire naturelle, France) between 1975 and 1987, in five African countries: Côte d'Ivoire and Guinea, in West Africa; and Cameroon, Congo, and Central African Republic, in Central Africa. Management of this collection relies on clonal duplication of each genotype in the field. Dead trees are replaced by horticultural propagation from the other representative of the same genotype. In parallel, CIRAD constituted a significant collection of cultivated material, also conserved in the Divo experimental station. This collection contains more than 600 accessions of diverse origin: local varieties and populations, forms taken from village plantations, and selected material.

STRUCTURE OF GENETIC DIVERSITY

Isozymic Variability

Primary analysis of the genetic diversity of *C. canephora* from enzymatic polymorphism was done by Berthaud (1986). Fifteen samples were classified using genetic distances calculated from allelic frequencies of each sample. Twelve out of 15 samples corresponded to the forest populations: nine populations studied in Côte d'Ivoire and three in the Central African Republic. For the three other samples, it was necessary to group individuals of different populations or origins. One sample was made up of material from Cameroon; the second combined all the genotypes cultivated in the working collection of CIRAD involved in the agronomic trials; and the third combined cultivated coffee plants of the Ebobo type, which originated in Côte d'Ivoire (today there are no more representatives of the Ebobo type in collection). This study indicated, for the first time, a genetic structure in the species *C. canephora*. Two groups were identified: the 'Guinean' group, composed of wild populations of Côte d'Ivoire, and the 'Congolese' group, which comprises the wild material of the Central African Republic and of Cameroon and the cultivated material. Subsequently, by increasing the number of genotypes analysed and classifying the collection of cultivated material into 11 samples, Montagnon et al. (1992) identified two subgroups within the Congolese group: SG1 and SG2.

In our study, we took into account individuals—60 wild and 50 cultivated—and not 'populations' (Tables 1 and 2). In total, 29 alleles were

Table 1. Origin of wild material studied: country, year of collection, number of forest populations sampled, and number of genotypes analysed

Country	Year of collection	No. of populations	No. of genotypes	Reference
Cameroon	1983	10	15	Anthony et al., 1985
Congo	1985	7	13	De Namur et al., 1988
Côte d'Ivoire	1975-1986	21	36	Berthaud 1983 Le Pierres et al., 1989
Guinea	1987	1	2	Le Pierres et al., 1989
Central African Republic	1975	6	11	Berthaud and Guillamet, 1978
Total		45	77	

Table 2. Origin of cultivated material studied: type of introduction, denomination in collection, donor institute or reference of the collection, country (of origin for donations, of cultivation for plantation samples) and number of genotypes analysed

Type of introduction	Name	Donor or collector	Country	No.
Donation	Aboisso	Aboisso,[1] Côte d'Ivoire	Gabon	6
	Niaouli	Bingerville,[2] Côte d'Ivoire	Togo	3
	Kouilou of Madagascar	Bingerville,[3] Côte d'Ivoire	Gabon	4
	C10 Man	—	Rep. of Congo	2
	INEAC	INEAC,[4] Rep. of Congo	Rep. of Congo	12
Plantation sample	Côte d'Ivoire	Berthaud, 1983, Le Pierres et al., 1989	Côte d'Ivoire	7
	Guinea	Le Pierres et al., 1989	Guinea	9
	Togo		Togo	2
	Hybrids		Côte d'Ivoire	6
Unknown	Robusta A1	Unknown	Unknown	4
Total				55

[1] Introduction at Aboisso (Côte d'Ivoire) by Beynis in 1910, of material cultivated in Gabon (Cordier, 1961).

[2] Introduction at the trial garden at Bingerville (Côte d'Ivoire), in 1914, of material cultivated in Togo (Cordier, 1961).

[3] Introduction at Bingerville (Côte d'Ivoire), in 1951, of material selected at Madagascar and originating in Gabon (Cordier, 1961).

[4] Introduction in Côte d'Ivoire, in 1935, of material selected at INEAC in the Belgian Congo (Cordier, 1961).

detected for the 8 polymorphic loci, with 2 to 6 alleles per locus and an average of 3.6 alleles per locus. There is no significant difference between the wild and cultivated individuals for mean number of alleles per locus.

The classification of the 60 wild genotypes indicates a structure in two groups (Fig. 1a). Group 1 contains only individuals collected in West Africa (Côte d'Ivoire and Guinea). Group 2 combines all the individuals originating from Central Africa (Cameroon, Congo, and Central African Republic) and

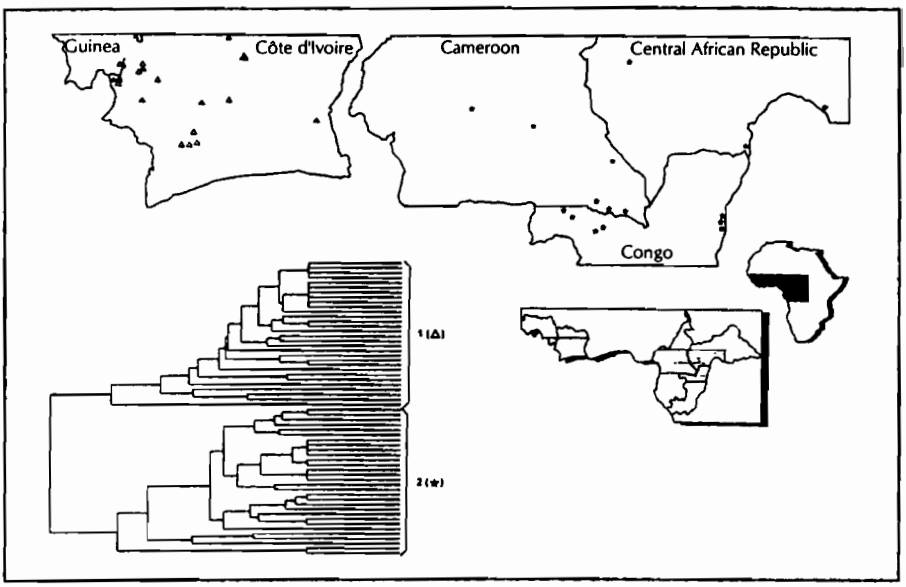

Fig. 1a. Classification of 60 wild genotypes according to the Dice similarity index and the UPGMA method of aggregation from data observed for 29 isozyme markers.

two genotypes from Côte d'Ivoire. These two groups correspond, in their composition, to the Guinean and Congolese groups of Berthaud (1986).

The dendrogram obtained with all the genotypes studied, wild and cultivated, is structured in three groups (Fig. 1b). No separation appears between wild and cultivated forms. The group most distant from the other two (group 1) contains all the individuals of the wild group 1 (West Africa) and genotypes taken from plantations in Côte d'Ivoire. The second group (group 2) comprises the genotypes of the wild group 2 (with the exception of three genotypes), all the cultivated material originating in the Republic of Congo, the individuals of the 'hybrid' groups, and those that were taken from the plantations in Guinea. The cultivated material originating in Gabon (except three genotypes), the individuals of the Robusta A1 group, two genotypes taken from the plantations of Côte d'Ivoire, a genotype taken from Togo, and three individuals of the wild group 2 form the third group (group 3). With regard to the origin of the material they contain, our group 2 corresponds to subgroup SG2 of Montagnon et al. (1992) and our group 3 corresponds to their subgroup SG1.

Molecular Variability

The use of molecular markers for the study of genetic diversity of the genus *Coffea* is recent. The first studies covered the analysis of the diversity of

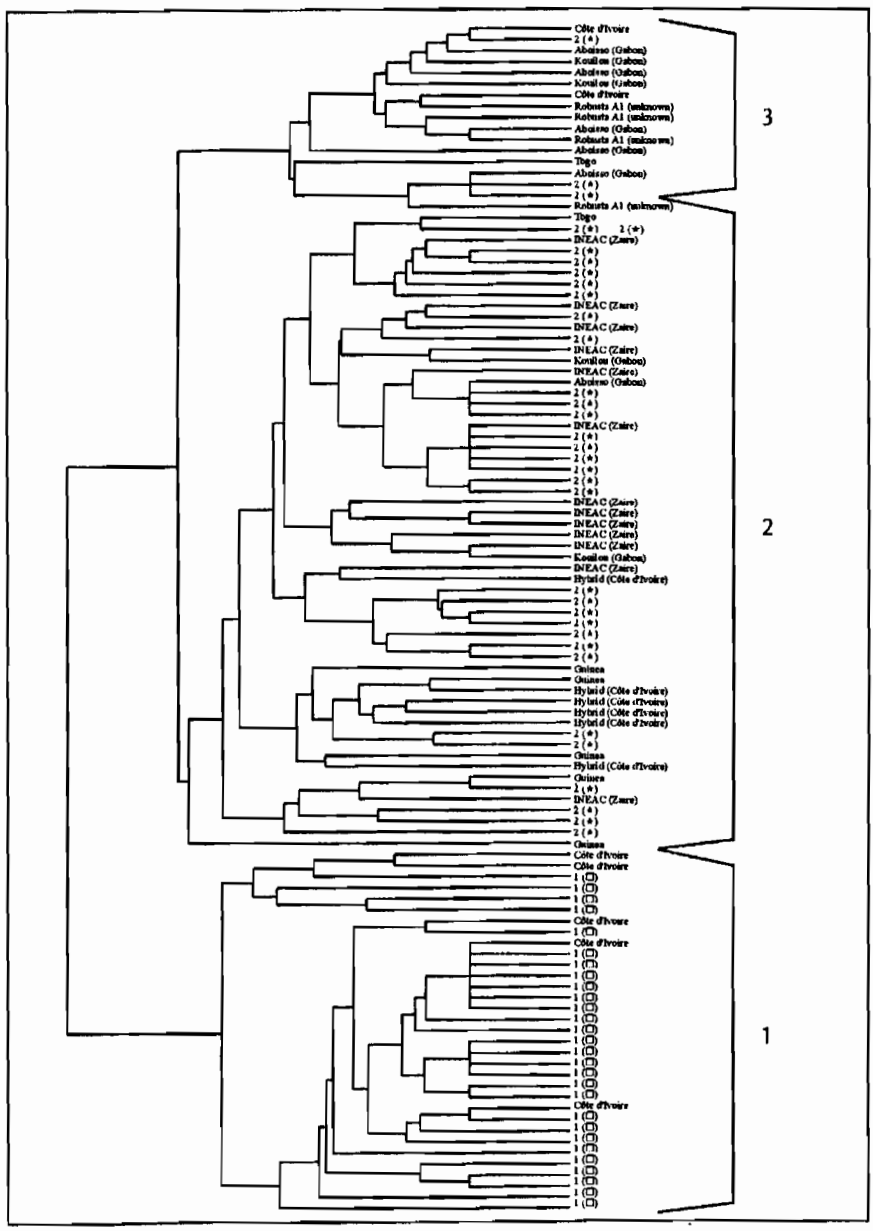

Fig. 1b. Classification of 110 wild and cultivated genotypes according to the Dice similarity index and the UPGMA method of aggregation from data observed for 29 isozyme markers.

C. arabica using RAPD markers (Lashermes et al., 1996). The results that we present are the first data on the molecular variability of *C. canephora*.

Out of the 26 homologous probes tested, 10 were found to be mono-locus and polymorphic. They allowed the detection of 2 to 14 alleles per locus or 66 alleles in total and an average of 6.6 alleles per polymorphic locus. The total number of alleles observed for the wild genotypes (62) is significantly higher ($\chi^2 = 4.55$; $P = 0.0329$) than that of cultivated genotypes (54).

The dendrogram obtained from molecular data indicates a structure of wild material into five groups (Fig. 2a). The genotypes of a population of northwest Congo and a population of southwest Cameroon make up group A. Group B comprises all the genotypes collected along the southern frontier of the Central African Republic. The individuals of group C are distributed in the three countries of Central Africa: northwest Congo, southwest Cameroon, and southwest Central African Republic. Group D is made up of all the genotypes collected in Guinea and Côte d'Ivoire, with the exception of four individuals of western Côte d'Ivoire. Group E contains the genotypes collected in northeast Congo, those that belong to populations of northwest

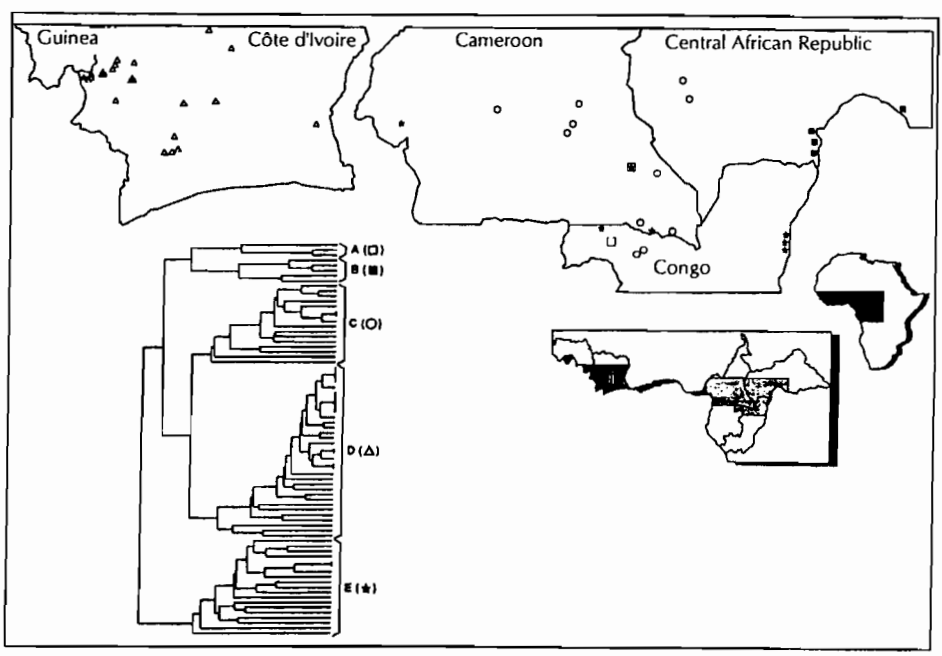

Fig. 2a. Classification of 77 wild genotypes according to the Dice similarity index and the UPGMA method of aggregation from data observed for 66 RFLP markers.

Congo and southern Cameroon, and individuals from three populations in western Côte d'Ivoire.

A global analysis of the dendrogram indicates that the wild material originating from West Africa is classified in a single group, while the material collected in Central Africa is structured into four groups. Group E is the most distant from the other four groups. The group of Central Africa (group C) closest to that of West Africa (group D) has the widest geographic distribution.

When the cultivated material is taken into account in the analysis, the structure of the species in five groups is conserved (Fig. 2b). Similarly, the position of groups with respect to each other remains unchanged. For each group, the composition of wild material remains identical to that defined previously. However, no cultivated material is present in the wild groups B and C. The individuals from the Republic of Congo, with the exception of one individual, are included in group E, as are individuals collected in the plantations of Guinea and a hybrid taken from the plantations of Côte d'Ivoire. In addition to the wild individuals of group A, most of the cultivated genotypes originating from Gabon, those of Togo (sampled from plantations and the Niaouli group), and individuals of the Robusta A1 group are classified in group A. Finally, almost all of the hybrid individuals, the genotypes taken from the plantations of Côte d'Ivoire, and most of the genotypes from the Guinea plantations are included in group D.

Agromorphological Variability

To our knowledge, only two studies have been done on the analysis of agromorphological diversity of *C. canephora* (Montagnon et al., 1992; Leroy et al., 1993). In the two cases, the principal components analysis did not reveal a high level of organization of the species. On the other hand, subsequent comparisons between the groups established on the basis of isozymes were done in several studies and for various combinations of agromorphological variables (Berthaud, 1986; Montagnon et al., 1992, 1993; Leroy et al., 1993; Montagnon and Leroy, 1993; Moschetto et al., 1996). Significant differences between the means of isozyme groups have been shown with some traits: leaf morphology, length of internodes, ramification, drought-sensitivity, phenology of fructification, and sensitivity to orange rust due to *Hemileia vastatrix*. On the other hand, in a plantation it is not possible to determine what genetic group a coffee plant belongs to on the basis of its morphology.

In our study, analysis of agromorphological data leads to a classification of wild genotypes into two major groups and a third group comprising two individuals relatively close to each other but very distant from other wild genotypes (Fig. 3a). This classification is not geographical: all the countries studied have representatives of group I and group II. Moreover, for around one third of the populations, the individuals from a single population are

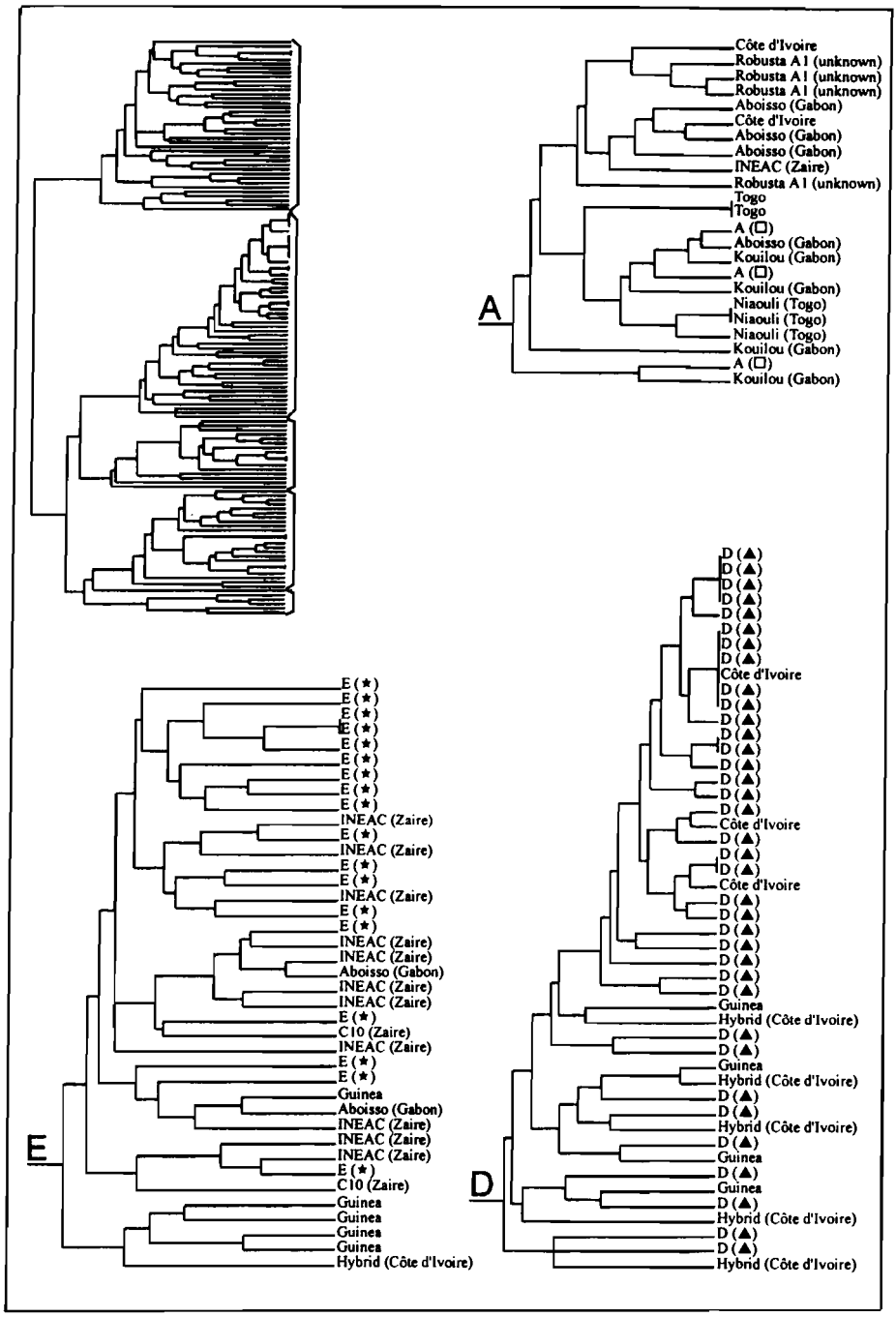

Fig. 2b. Classification of 132 wild genotypes according to the Dice similarity index and the UPGMA method of aggregation from data observed for 66 RFLP markers.

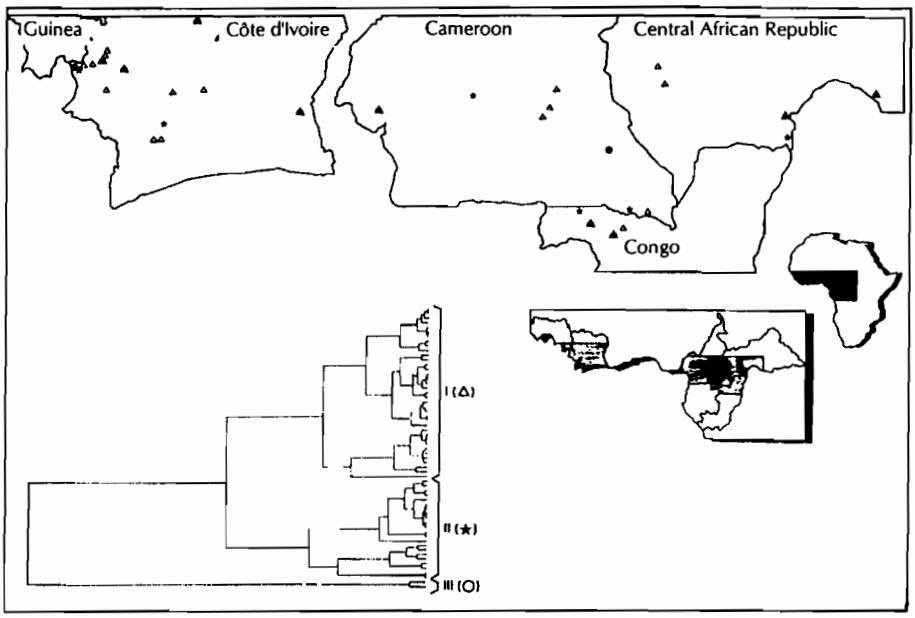

Fig. 3a. Classification of 61 wild genotypes according to the Euclidean distance and the UPGMA method of aggregation from data observed for 11 agromorphological markers.

distributed between the two major groups I and II. Analyses of variance for each of the agromorphological markers studied indicate a significant difference between the means of groups I and II for the berry development duration, bean weight, and leaf morphology.

When the cultivated forms are taken into account, the structure of the diversity is overall similar to that obtained when only the wild genotypes are analysed (Fig. 3b). The distribution of wild individuals in the groups remains unchanged with the exception of two genotypes of the wild group II, which are included within group III. No cultivated form is found in group III. All the genotypes originating in Gabon, with the exception of a genotype of the Aboisso group, as well as the individuals of groups Robusta A1 and hybrids are included in group I. The individuals of the Republic of Congo, except one genotype, and the genotypes taken from plantations in Côte d'Ivoire are found in group II.

Relationships between Various Levels of Diversity

The distribution of individuals of the biochemical groups within molecular and agromorphological groups (Table 3) allows us to compare the structures

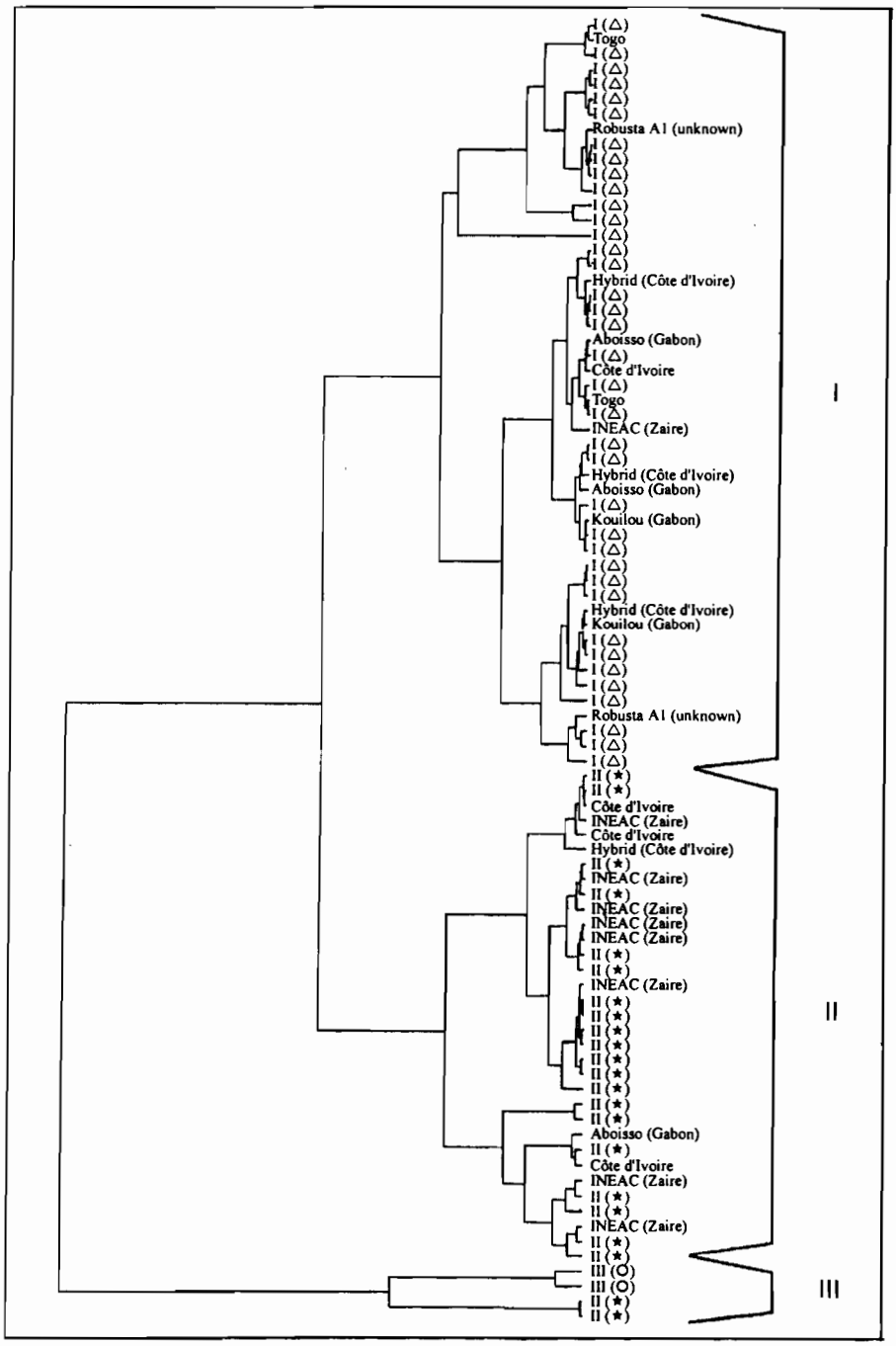

Fig. 3b. Classification of 87 wild and cultivated genotypes according to the Euclidean distance and the UPGMA method of aggregation from data observed for 11 agromorphological markers.

Table 3. Distributions of wild genotypes, on the one hand, from wild and cultivated genotypes, on the other, classified according to their relationship to biochemical groups within molecular groups and agromorphological groups. For each comparison, only the genotypes common to analyses corresponding to two compared markers have been taken into account

	Biochemical groups	Molecular groups					Agromorphological groups		
		A	B	C	D	E	I	II	III
Wild genotypes	1	0	0	0	30	0	14	7	0
	2	2	4	12	1	12	7	7	1
Wild and cultivated genotypes	1	0	0	0	32	1	17	9	0
	2	6	4	12	8	29	16	20	2
	3	13	0	0	0	1	8	1	0

observed. The biochemical groups present different levels of diversity. Biochemical group I, relatively homogeneous in molecular terms, corresponds only to molecular group D. Biochemical group 2 has wide diversity and comprises representatives of the five molecular groups. When only wild genotypes are taken into account, a nearly perfect agreement (excepting one individual) is observed between biochemical group 1 and molecular group D. The individuals of biochemical group 2 are distributed among molecular groups A, B, C, and E. When the wild and cultivated genotypes are considered simultaneously, the agreements indicated with the wild genotypes alone are not modified in their essentials. The biochemical variability of cultivated genotypes is greater than that of wild genotypes. Biochemical group 3, containing mainly cultivated genotypes, corresponds essentially to molecular group A.

The agromorphological markers indicate a relatively strong organization of the diversity of *C. canephora* in two major groups. However, no agreement could be established between the two biochemical groups and the three agromorphological groups. Similarly, the agromorphological structure does not coincide with that drawn from RFLP markers. The fact that each morphological group includes representatives of different biochemical and molecular groups makes it impossible to distinguish the Guinean and Congolese groups according to their morphological characteristics. Similar selection pressures are probably exerted in West Africa and Central Africa, which has led to morphologically undifferentiated forms.

The high agreement of structures observed with the two types of neutral marker used, isozymes and RFLP, conforms to the classification within *C. canephora* (Table 4). Moreover, the results of our study prove the utility of molecular markers in the analysis of the genetic structure of *C. canephora*. On the one hand, for an equivalent number of loci, RFLP markers allowed detection of a higher number of alleles per polymorphic locus than did biochemical markers (6.6 against 3.6). On the other hand, RFLP markers

Table 4. Agreement of structures of the wild and cultivated diversity observed using isozyme markers and RFLP markers during three successive studies and composition of wild material, taken from plantations and selected, from observed groups

Berthaud, 1986	Montagnon et al., 1992	Our study				
	Markers			Material		
Isozymes	Isozymes	Isozymes	RFLP	Wild	Plantation	Selected
Guinea	Guinea	1	D	Côte d'Ivoire Guinea	Côte d'Ivoire Guinea	Hybrid (Côte d'Ivoire)
			B	Central African E		
	SG2	2	C	Central African O Cameroon Congo NO		
Congo			E	Congo Cameroon S	Guinea	INEAC (Rep. of Congo) C10 (Rep. of Congo)
	SG1	3	A	Congo NO Cameroon SE	Togo Côte d'Ivoire	Kouilou (Gabon) Aboisso (Gabon) Niaouli (Togo) Robusta A1 (unknown)

allowed us to refine the analysis by indicating an intragroup structure: the Congolese group defined by Berthaud (1986) corresponds to four molecular groups.

DEVELOPMENT AND MANAGEMENT OF GENETIC RESOURCES

Structure of Diversity and Use of Genetic Resources

Using isozymic loci that discriminate between the Guinean and Congolese groups, Berthaud (1986) identified intergroup hybrids in the cultivated clones. Among the 12 intergroup clones identified, 6 are the highest-yielding cultivars. From this observation, Berthaud proposed a reciprocal recurrent selection procedure for *C. canephora* based on the use of the Guinean and Congolese groups. The efficiency of this procedure was demonstrated subsequently (Leroy et al., 1993) and confirmed that it is important to know the structure of the diversity to best exploit the genetic resources.

The results of our study with molecular markers could be used to improve this selection procedure. Indeed, it should first be verified that there are no combinations within the Congolese group presenting a heterosis higher than the mean heterosis between the Guinean and Congolese groups. Second, if the hybrids between Guinean and Congolese groups remain the most promising, the preliminary results seem to indicate that the value of the heterosis between the two groups depends on the molecular group to which the individual of the Congolese group belongs. Thus, based only on the genetic distances established using RFLP markers, the heterosis between individuals in groups D and C could be lower than that between the individuals in groups E and C.

Structure of the Diversity and Management of Genetic Resources

From the results of this study we can propose a certain number of recommendations to ensure better *ex situ* management of genetic resources of *C. canephora*.

Most collections of *C. canephora* contain cultivated material and are highly redundant. Our study shows that a large part of the diversity existing in the wild material is not represented in the cultivated material. Moreover, the cultivated material does not contain an original diversity in relation to the wild material. Consequently, the wild material collected in many expeditions and conserved in Côte d'Ivoire presently constitutes the largest source of variability available for this species. Efforts must thus be made to preserve this collection, either by duplicating plant material or by other means of conservation.

In Côte d'Ivoire, the two collections of cultivated and wild coffee trees have so far been managed independently, which increases the task of management. Our results indicate that it is possible to consolidate the collection and hierarchize it on the basis of molecular groups. Moreover, the use of algorithms of sampling that maximize the intragroup diversity, such as that proposed by Noirot et al. (1996), would, by defining a core collection, enable optimal management of the global collection and help establish priorities for conservation as well as for evaluation, use, and diffusion of the genetic resources.

At present, the plant material is maintained only in the form of field genotypes. The creation of stratified, small core collections could allow conservation of genes rather than genotypes by rationally constituting bulks of seeds within each of the genetic groups indicated in our study. *In vitro* conservation of microcuttings established from such seed bulks has shown its limits: some genetic groups are quickly lost, especially within *C. canephora* (Dussert et al., 1997). On the other hand, the cryopreservation of seeds, already attempted with *C. arabica* (Dussert et al., 1998), is a promising alternative to field conservation.

CONCLUSION

Our study shows that molecular markers of the RFLP type can be used to increase our knowledge of the organization of the diversity of coffee *C. canephora*. This organization agrees to a great extent with that obtained with biochemical markers. However, the molecular markers show a higher differentiation than other markers.

Even though our analysis was done on a reduced sample of the genotypes conserved in Côte d'Ivoire, it is interesting to observe that the cultivated material is not generally differentiated from the wild material and that only part of the diversity of this wild material has so far been exploited in *C. canephora* cultivation. Moreover, there is a differentiation within the Congolese group, the material originating from Central Africa being structured in several molecular groups. On the other hand, the material originating in West Africa, of the Guinean group and classified in a single molecular group, is not more distant from the Central African groups than the latter are among themselves.

Thus, the present results enable us to consider new strategies for varietal selection as well as for rational management of genetic resources of *C. canephora*.

APPENDIX

Plant Material

A sampling of 132 genotypes was done within collections of wild and cultivated material conserved in Côte d'Ivoire (Tables 1 and 2). The 77 wild genotypes were sampled in order to have a representation of each of 45 forest populations studied (Table 1). For the cultivated material, a random proportional sampling was done for each of the 10 principal origins identified in collection (Table 2). The grouping is highly heterogeneous. For the material that was donated, the denomination of the groups corresponds to the name, the location, and the donor experimental station or to the varietal type of the material (Robusta or Kouilou). For these groups, the country of origin could correspond to the cultivation zone or the breeding centre. For the material collected in the plantations, the country mentioned is that in which the material was collected. The group called 'hybrids' includes genotypes for which it was later shown that they are hybrids between the forms originating in West Africa and those originating in Central Africa (Berthaud, 1983). Finally, the history of the introduction of the Robusta A1 group could not be traced. The origin of this group thus remains unknown.

RFLP Analysis

The total genomic DNA was extracted according to the method described by Agwanda et al. (1997). The technique of molecular marker analysis used is that described by Lashermes et al. (1995). Two restriction enzymes were used: *Eco*RI and *Hind*III. The 26 probes tested come from a genome bank of *C. arabica*. Among these, 10 were retained for their polymorphic and mono-locus characteristics. Each probe was used after restriction by one or the other restriction enzyme. The presence and absence of 66 bands corresponding to 66 alleles were coded 1 and 0, respectively.

Enzymatic Analysis

Within the total sampling of 132 individuals (Tables 1 and 2), the analysis of isozymic polymorphism was done on 60 wild individuals and 50 cultivated individuals. Among the 60 wild individuals, 48 were common to analysis done with agromorphological markers. For the cultivated material, the number of individuals common to isozyme and agromorphological analyses was 26. The techniques of extraction, electrophoresis, and detection of isozymes are those of Berthaud (1986). The analyses were done on 5 enzymatic systems revealing 8 loci: esterases a and b (3 loci), 6-phosphogluconate dehydrogenase (2 loci), isocitrate dehydrogenase (1 locus), phospho-glucomutase (1 locus), and phosphoglucoisomerase (1 locus). The 29 alleles identified were coded as present or absent (1 or 0).

Agromorphological Study

Within the132 genotypes studied (Tables 1 and 2), 61 wild genotypes and 26 cultivated genotypes were evaluated for 11 agromorphological markers. Four classes of markers could be distinguished: morphological (length, width, area and shape of leaves, length of acumen, length of petiole); technological (100 bean weight, percentage of peaberries, outturn or bean weight to berry weight ratio, percentage of empty loges, for the wild material only); phenological (for the wild genotypes only, berry development duration and extension of maturation of berries); and agronomic (yield). For a detailed description of markers, see Anthony (1992).

Statistical Analyses of Classification

For the RFLP and isozyme markers the distance matrixes between individuals were calculated using the Dice similarity index (1945). The Euclidean distance was used for agromorphological markers. For the three types of markers, the method of aggregation used to construct the dendrograms was the UPGMA method.

REFERENCES

Agwanda, O.A., Lashermes, P., Trouslot, P., Combes, M.C., and Charrier, A. 1997. Identification of RAPD markers for resistance to coffee berry disease, *Colletotrichum kahawae*, in arabica coffee. *Euphytica*, 97: 241-248.

Anthony, A. 1992. Les ressources génétiques des caféiers: collecte, gestion d'un conservatoire et évaluation de la diversité génétique. Montpellier, France, Orstom, collection Travaux et documents, 320 p.

Anthony, F., Couturon, E., and de Namur, C. 1985. Les caféiers sauvages du Cameroun: résultats d'une mission de prospection effectuée par l'Orstom en 1983. In: *XI^e Colloque Scientifique International sur le Café*. Paris, ASIC, pp. 495-501 .

Berthaud, J. 1980. L'incompatibilité chez *Coffea canephora*: méthode de test et déterminisme génétique. *Café, Cacao, Thé*, 24: 267-274.

Berthaud, J. 1983. Liste du matériel provenant des prospections de Côte d'Ivoire. Paris, Orstom (document interne).

Berthaud, J. 1986. Les ressources génétiques pour l'amélioration des caféiers africains diploïdes. Montpellier, France, Orstom, collection Travaux et documents, 379 p.

Berthaud, J. and Guillaumet, J.L. 1978. Les caféiers sauvages en Centrafrique: résultats d'une mission de prospection (janvier-février 1975). *Café, Cacao, Thé*, 3: 171-186.

Bridson, D.M. and Verdcourt, B. 1988. Rubiaceae (Part 2). In: *Flora of Tropical East Africa*. R.M. Polhill, eds., Rotterdam, Balkema, 727 p.

Charrier, A. and Eskes B. 1997. Les caféiers. In: *L'Amélioration des Plantes Tropicales*. A. Charrier et al., eds., Montpellier, France, CIRAD-Orstom, collection Repères, pp. 171-196.

Cordier, L. 1961 . Les objectifs de la sélection caféière en Côte d'Ivoire. *Café, Cacao, Thé*, 5: 147-159.

Couturon, E. 1980. Le maintien de la viabilité des graines de caféiers par le contrôle de leur teneur en eau et de la température de stockage. *Café, cacao, thé*, 24: 27-32.

Cros, J., Combes, M.C., Chabrillange, N., Duperray, C., Monnot des Angles, A., and Hamon, S. 1995. Nuclear DNA content in the subgenus *Coffea*: inter- and intra-specific variation in African species. *Canadian Journal of Botany*, 73: 14-20.

de Namur, C., Couturon, E., Sita, P., and Anthony, F. 1988. Résultats d'une mission de prospection des caféiers sauvages du Congo. In: XII^e Colloque Scientifique International sur le Café. Paris, ASIC, pp. 397-404.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. *Ecology*, 26: 297-302.

Dussert, S., Chabrillange, N., Anthony, F., Engelmann, F., Recalt C., and Hamon, S. 1997. Variability in storage response within a coffee (*Coffea* spp.) core collection under slow growth conditions. *Plant Cell Report*, 16: 344-348.

Dussert, S., Chabrillange, N., Engelmann, F,, Anthony, F., Louarn, J. and Hamon, S. 1998. Cryopreservation of seeds of four coffee species (*Coffea arabica, C. costatifructa, C. racemosa* and *C. sessiliflora*): importance of water content and cooling rate. *Seed Science Research*, 8: 9-15.

Lashermes, P., Combes, M.C., and Cros, J. 1995. Use of non-radioactive digixigenin-labelled DNA probes for RFLP analysis in coffee. In: *Techniques et Utilisations des Marqueurs Moléculaires*. A. Berville and M. Tressac, eds., Paris, INRA, pp. 21-25.

Lashermes, P., Combes, M.C., Trouslot, P., and Charrier, A. 1997. Phylogenetic relationships of coffee-tree species (*Coffea* L.) as inferred from ITS sequences of nuclear ribosomal DNA. *Theoretical and Applied Genetics*, 94: 947-955.

Lashermes, P., Trouslot, P., Anthony, F., Combes, M.C., and Charrier, A. 1996. Genetic diversity for RAPD markers between cultivated and wild accessions of *C. arabica. Euphytica*, 87: 59-64.

Le Pierres, D., Charmetant, P., Yapo, A., Leroy, T., Couturon, E., Bontems, S., and Tehe, H. 1989. Les caféiers sauvages de Côte d'Ivoire et de Guinée: bilan des missions de prospection effectuées de 1984 à 1987. In: XIII[e] Colloque Scientifique International sur le Café. Paris, ASIC, pp. 420-428.

Leroy, T., Montagnon, C., Charrier, A., and Eskes, A. 1993. Reciprocal recurrent Selection applied to *Coffea canephora* Pierre. 1. Characterization and evaluation of breeding populations and value of intergroup hybrids. *Euphytica*, 67: 113-125.

Montagnon, C. and Leroy, T. 1993. Réaction à la secheresse de jeunes caféiers *Coffea canephora* de Côte d'Ivoire appartenant à différents groupes génétiques. *Café, cacao, thé*, 37: 1 79-190.

Montagnon, C., Leroy, T., Cilas, C., and Eskes, A.B. 1993. Differences among clones of *Coffea canephora* in resistance to the scolytid coffee twig-borer. *International Journal of Pest Management*, 39: 204-209.

Montagnon, C., Leroy, T., and Eskes, A.B. 1998. Amélioration variétale de *Coffea canephora*. 2. Les programmes de sélection et leurs résultats. *Plantations, recherche, développement*, 5: 89-98.

Montagnon, C., Leroy, T., and Yapo, A. 1992. Etude complémentaire de la diversité génotypique et phénotypique des caféiers de l'espèce *C. canephora* en collection en Côte d'Ivoire. In: XIV[e] Colloque Scientifique International sur le Café. Paris, ASIC, pp. 444-450.

Moschetto, D., Montagnon, C., Guyot, B., Perriot J.J., Leroy T., and Eskes, A.B. 1996. Studies on the effect of genotype on cup quality of *Coffea canephora*. *Tropical Science*, 36: 18-31.

Noirot, M., Anthony, F., and Hamon, S. 1996. The principal component scoring: a new method of constituting a core collection using quantitative data. *Genetic Resources and Crop Evolution*, 43: 1-6.

Roberts, E.H. 1973. Predicting the storage life of seeds. *Seed Science and Technology*, 1: 499-514.

Dussert Stéphane, Lashermes Philippe, Anthony François, Montagnon C., Trouslot Pierre, Combes Marie-Christine, Berthaud Julien, Noirot Michel, Hamon Serge.

Coffee (Coffea canephora).

In : Hamon P. (ed.), Seguin M. (ed.), Perrier X. (ed.), Glaszmann J.C. (ed.). Genetic diversity of cultivated tropical plants. Montpellier (FRA), Enfield : CIRAD, Science Publ., 2003, p. 239-258.

(Repères). ISSN 1251-7224