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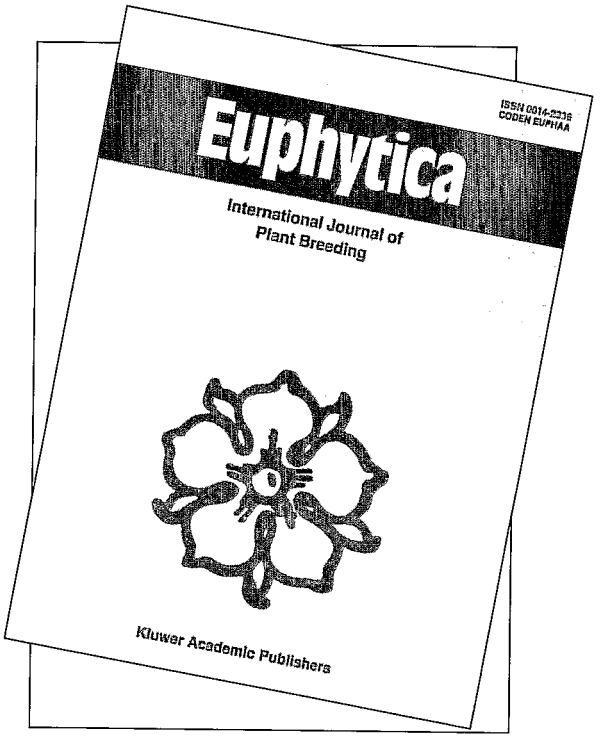

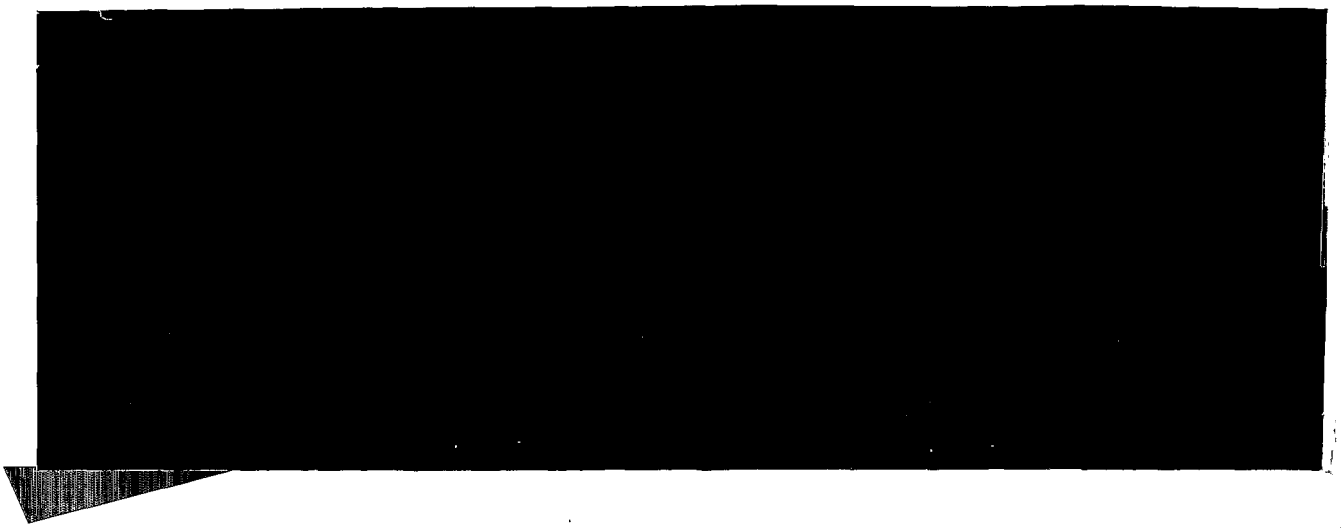


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# Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*

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**Summary**

Random amplified polymorphic DNA (RAPD) markers have been successfully employed to analyse the genetic diversity among cultivated and subspontaneous accessions of *Coffea arabica*. The narrow genetic base of commercial cultivars was confirmed. On the other hand, a relatively large genetic diversity was observed within the germplasm collection demonstrating the importance of collecting missions. Results suggested an East-West differentiation in Ethiopia, the primary centre of diversification of *C. arabica*. The large heterosis effect reported in intergroup hybrids could be related to such genetic differentiation. RAPD method appeared to be effective in resolving genetic variations and in grouping germplasm in *C. arabica*.

## Introduction

*Coffea arabica* L., apart from being the only tetraploid species in the genus, is the most widely cultivated *Coffea* species. Arabica coffee has its primary centre of genetic diversity in the highlands of South West Ethiopia, and the Boma Plateau of Sudan (Sylvain, 1958; Meyer, 1969). Populations of *C. arabica* have been also reported (Berthaud & Charrier, 1988) in Mount Imatong (Sudan) and Mount Marsabit (Kenya). The large number of named varieties and selections of arabica coffee belies the actually very narrow genetic diversity of the base populations from which they were selected (van der Vossen, 1985). Historical evidence indicates that these base populations all descended from the few trees that survived various efforts to spread arabica coffee from Southern Arabia, now Yemen, into the main coffee producing areas in Latin America, East Africa and Asia. Arabica coffee was introduced for cultivation in Yemen from Ethiopia in earlier time by the Arabs (Smith, 1985). The coffee trees from Yemen gave rise to two distinct botanical types (Krug et al., 1939): 1) *C. arabica* var. *typica* Cramer, which was the earliest grown coffee in Asia and Latin America, and 2) *C. arabica* var. *bourbon* (B. Rodr.) Choussy, which came to South America through the island of La Réunion, formerly called Bourbon. The genetic uniformity within these populations is further enhanced by the predominantly self-pollinating nature of *C. arabica*. The subsequently encountered variation, which gave rise to so many cultivars, is generally believed to be more the result of spontaneous mutations of major genes conditioning plant, fruit and seed characters than of residual heterozygosity (Carvalho, 1988).

Enlarging the genetic base has become a priority for further crop improvement and has prompted several collecting missions. In particular, two expeditions to explore and collect wild arabica material were undertaken in 1964–65 to South West Ethiopia under the auspices of the FAO (FAO, 1968), and in 1966 by ORSTOM in the Illubabor and Kaffa provinces of Ethiopia (Guillaumet & Hallé, 1978). A number of collections have been established and provide a new source of germplasm which is expected to be of great value for coffee breeding. This material may have been subjected to human interference and the truly wild or spontaneous origin of accessions is questionable as mentioned by the collectors; the term subspontaneous seems therefore more appropriate to describe this material. Observations of agromorphological characters indicated considerable phenotypic

*Table 1.* Type, country of origin and plant material source of *C. arabica* genotypes studied

| Accession | Type | Origin (collecting site) | Source[a] | Code |
|---|---|---|---|---|
| 1-Caturra | Variety (bourbon type) | Brazil | ORSTOM-F | Caturra |
| 2-Blue Mountain | Variety (typica type) | Jamaica | CATIE | T977 |
| 3-Bourbon | Variety (introduction) | Ethiopia/Yemen | CATIE | T995 |
| 4-Typica | Variety (introduction) | Ethiopia/Yemen | CATIE | T996 |
| 5-Mbirizi | Variety (bourbon type) | Congo | CATIE | T2702 |
| 6-I-60 | Variety (bourbon type) | Tanzania | CATIE | T2723 |
| 7-K-7 | Variety (selection) | Kenya | CATIE | T2737 |
| 8-Et 2 | Subspontaneous | Ethiopia (Gojeb valley) | ORSTOM-F | Et 2-5 |
| 9-Et 12 | Subspontaneous | Ethiopia (Limu) | ORSTOM-F | Et 12-5 |
| 10-Et 5 | Subspontaneous | Ethiopia (Wush-Wush1) | CATIE | T16693 |
| 11-Et 16 | Subspontaneous | Ethiopia (Gimma-Goré) | ORSTOM-F | Et 16-5 |
| 12-Et 24 | Subspontaneous | Ethiopia (Tippi) | ORSTOM-F | Et 24-5 |
| 13-Et 30 | Subspontaneous | Ethiopia (Tippi) | ORSTOM-CI | Ar 30 |
| 14-Et 36 | Subspontaneous | Ethiopia (Kollo) | ORSTOM-F | Et 36b |
| 15-Et 40 | Subspontaneous | Ethiopia (Wush-Wush2) | ORSTOM-F | Et 40-6 |
| 16-Et 41 | Subspontaneous | Ethiopia (Decchia) | ORSTOM-F | Et 41-5 |
| 17-Et 46 | Subspontaneous | Ethiopia (Decchia-Bonga) | ORSTOM-F | Et 46-5 |
| 18-Et 59 | Subspontaneous | Ethiopia (Bonga) | CATIE | T16739 |
| 19-3058 | Subspontaneous | Kenya (Marsabit) | ORSTOM-CI | Ar 3058 |
| 20-3099 | Subspontaneous | Kenya (Marsabit) | ORSTOM-CI | Ar 3099 |

[a] CATIE: Centro Agronomico Tropical de Investigacion y Ensenanza; ORSTOM: Institut français de recherche scientifique pour le développement en coopération.

diversity among the collected material (Charrier, 1978; Bouharmont & Montagnon, 1995). Random amplified polymorphic DNA markers (RAPD; Williams et al., 1990; Welsh & McClelland, 1990) have been reported to provide an effective method to identify coffee accessions (Lashermes et al., 1993; Orozco-Castillo et al., 1994).

In this study, RAPD analysis was used to estimate the level of genetic diversity among the collected germplasm, and the relatedness of cultivated and wild accessions of *Coffea arabica*.

## Material and methods

### *Plant material*

The plant material (Table 1) involved varieties and wild (or subspontaneous) accessions of *C. arabica*. The two cultivated coffee types (*typica* and *bourbon*) were represented by six varieties. The cultivar K-7 resulting from a selection work in Kenya within the Kent variety (Walyaro, 1983) was also included. The subspontaneous material consisted of 11 samples representing the different collecting sites of the ORSTOM mission in Ethiopia (Guillaumet & Hallé, 1978), and of two accessions collected in Kenya (Berthaud et al., 1980).

### *DNA amplification*

Genomic DNA was isolated from lyophilised leaf as previously reported (Lashermes et al., 1993) except that CTAB was replaced by MATAB (Mixed Alkyltrimethylammonium Bromide, Sigma) in the extraction buffer. A series of 140 arbitrary decamer oligonucleotides purchased from Operon Technologies (CA, USA) were used for the amplification of random DNA sequences. The reactions were performed in a volume of 25 $\mu$l containing 10 mM Tris Hcl, pH 9.0, 0.1% triton X-100, 1.5 mM $MgCl_2$, 0.2 mg/ml BSA, 150 $\mu$M each dATP, dCTP, dGTP, dTTP, 0.4 $\mu$M primer, 5–10 ng of genomic DNA, and 0.5 U of Taq polymerase (Appligene, Illkirch, France). Amplifications were performed in a PTC-100 thermal cycler (MJ Research). After 4 min heating at 94° C, 43 cycles were run. Each cycle consisted of 1 min at 94° C, 1 min at 37° C and 2 min at 72° C. This was followed by 6 min

*Table 2.* Nucleotide sequence of 12 primers generating polymorphic patterns among the 20 genotypes of *C. arabica* studied

| Primer code | Nucleotide sequence |
|---|---|
| I-7 | 5'-CAGCGACAAG |
| I-20 | 5'-AAAGTGCGGG |
| J-19 | 5'-GGACACCACT |
| L-18 | 5'-ACCACCCACC |
| M-4 | 5'-GGCGGTTGTC |
| N-18 | 5'-GGTGAGGTCA |
| N-20 | 5'-GGTGCTCCGT |
| X-9 | 5'-GGTCTGGTTG |
| X-16 | 5'-CTCTGTTCGG |
| X-20 | 5'-CCCAGCTAGA |
| Y-10 | 5'-CAAACGTGGG |
| Y-15 | 5'-AGTCGCCCTT |

at 72° C. Amplified DNA fragments were separated by electrophoresis in a 1.8% agarose gel with a TBE buffer system. Gels were stained with ethidium bromide and fragment patterns were photographed for further analyses.

*Data analysis*

Data were scored on the basis of the presence (1) or absence (0) of the amplified products. Pairwise comparisons were calculated from the data matrix table using the Manhattan (City-block) distance (Darlu & Tassy, 1993): distance $(x, y) = \Sigma_i |x_i - y_i|$. A distance matrix between the 20 accessions was constructed. A multidimensional scaling (MDS) analysis (Kruskal, 1964a, b) was performed using the STATISTICA computer program. MDS permits the use of metric as well as non-metric similarity measures. A co-ordinate representation is sought using only the ordinal properties of the distance measures as to arrive at a configuration that best approximates the observed distance matrix (Shepard, 1973). The similarity measures were also used to construct a dendrogram by the group average strategy (UPGMA).

## Results

Initially the level of polymorphism detected with RAPD markers was assayed in four accessions: Caturra, Typica, Et 5 and Et 39. The number of fragments generated by one primer varied from as few as one to as many as 15. Of the 140 primers of arbitrary nucleotide sequence used, only 12 detected polymorphism (Table 2). The primers which exhibited polymorphisms among these four genotypes were tested against the remaining accessions identified in Table 1. Examples of polymorphism are shown in Fig. 1.

The RAPDs generated (15 polymorphic bands) were used to determine the relationships between the 20 accessions (Table 1). Non-metric multidimensional scaling was applied, resulting in the three-dimensional representation shown in Fig. 2. The value of the stress for this configuration was 0.0025 indicating a fairly reasonable fit. The wild accessions gave rise to most of the diversity observed, although both *bourbon* and *typica* cultivated types showed significant differences. In addition, we were not able to distinguish the cultivars belonging to the same type either *bourbon* or *typica*. Similarly, the accessions Et-24 and Et-30 collected in the same site appeared identical. The cultivar K7 appeared related to one accession from Kenya. Distribution of the accessions from Ethiopia did not seem related to their geographical origin (collecting site).

A dendrogram constructed by cluster analysis is presented in Fig. 3. The grouping-association identified by classification reflected the relationships among the accessions identified by the ordination. There is a clear separation of the accessions collected in Ethiopia from the accessions collected in Kenya and the varieties.

## Discussion

The low molecular polymorphism detected in *C. arabica* is most probably related to the genesis of this allotetraploid species (Lashermes et al., 1995). However, the relatively large genetic diversity within the arabica germplasm collection observed in this study demonstrates the importance of collecting missions.

The cultivars belonging to the same type, either *bourbon* or *typica* appeared identical. Such result could have been expected since the different varieties derived from the few tree introduced from Yemen and are believed to result from gene mutations affecting characters of economic importance (Carvalho et al., 1991). The cultivar K7, which has been selected in Kenya and grew in the sixties on a large scale (Walyaro, 1983), appeared closely related to one of the accessions collected in the north of Kenya (Marsabit Mountain). The

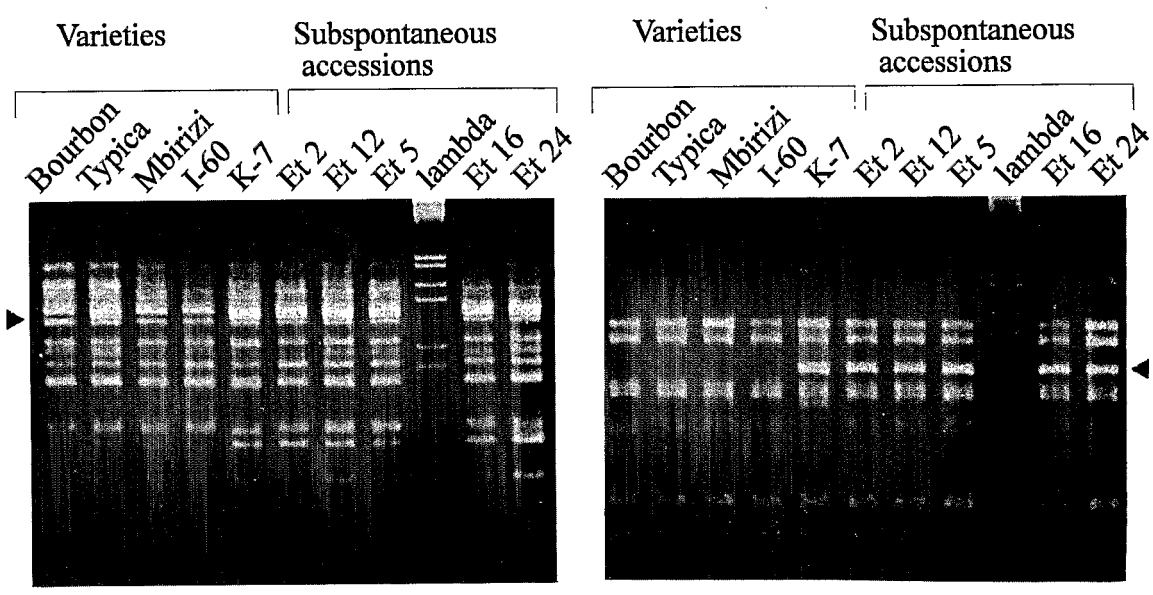


*Fig. 1.* Examples of DNA polymorphisms detected between accessions of *C. arabica*. Ethidium bromide stained agarose gel of amplification fragments produced with primers M4 (left) and N20 (right).

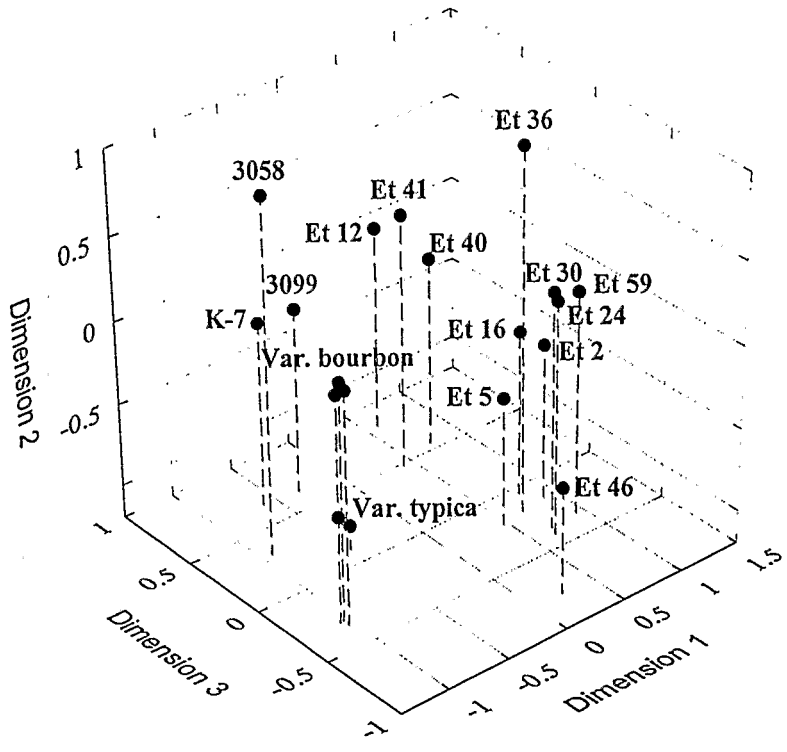


*Fig. 2.* Three dimensional solution given by non-metric multidimensional scaling applied to the matrix of RAPD-based genetic distances between 20 accessions of *C. arabica*. (stress value = 0.0025; Var. *bourbon* represents the accessions Caturra, Bourbon, Mbirizi and I-60; Var. *typica* represents the accessions Typica and Blue mountain)

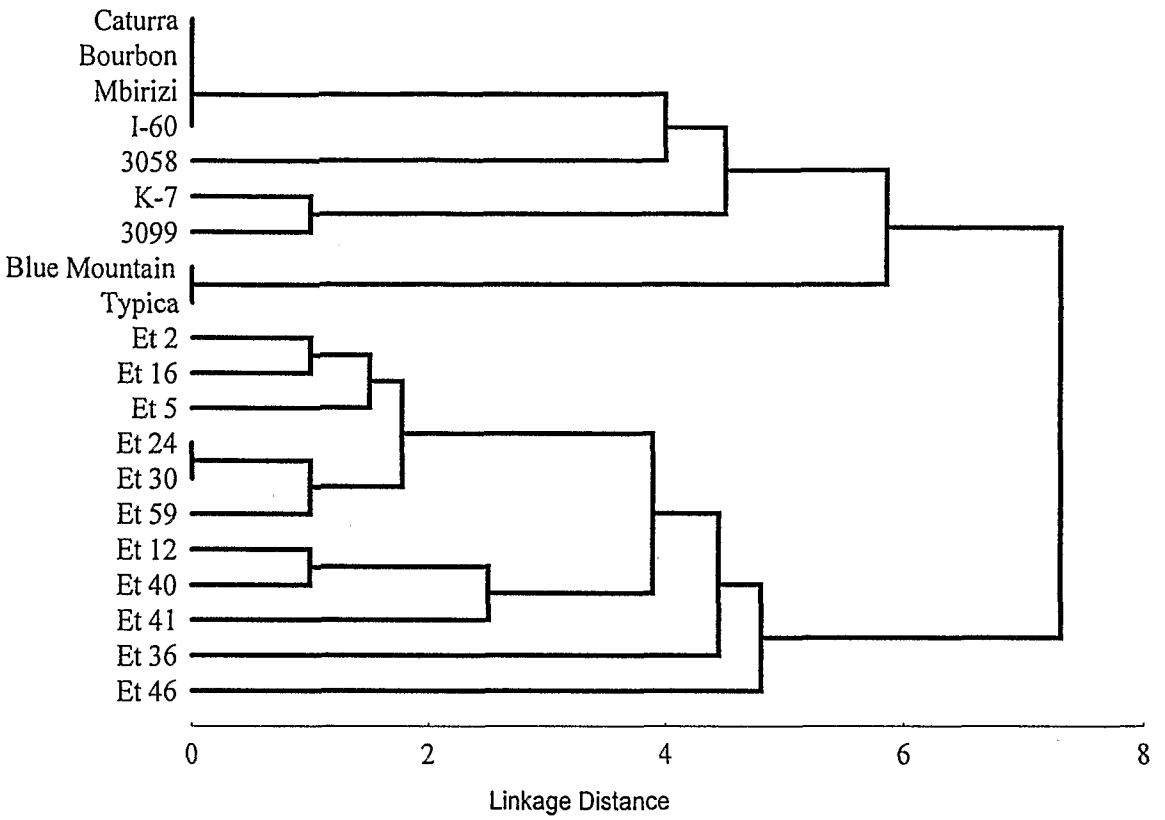


*Fig. 3.* Dendrogram of the *Coffea arabica* accessions listed in Table 1 based on single linkage cluster analysis.

truly wild origin of this accession is therefore questionable.

A relatively large difference was observed in this study between the germplasm collected in the south west highlands of Ethiopia (Illubabor and Kaffa provinces), and the cultivated material spread worldwide from Yemen and the accessions collected in North Kenya. Similar observations have been reported from agromorphological data (Bouharmont & Montagnon, 1995). Those results support the hypothesis that the arabica plants transferred to Yemen for cultivation by the Arabs originated from the south eastern part of the evergreen mountainous region of Ethiopia (Sidamo and Harar provinces). An East-West differentiation would exist in the primary centre of diversification of *C. arabica*. A large heterosis effect has been reported in F1 hybrid resulting from crosses between indigenous cultivars from the south western and south eastern parts of Ethiopia (Bayetta-Bellachew et al., 1993), and between subspontaneous Ethiopian accessions and *bourbon* type cultivars (Charrier, 1978). Such effect might be the consequence of this genetic differentiation. Therefore, the possibility of employing RAPD-based genetic distance measures for predicting hybrid performance should be considered.

The genetic variation in arabica coffee has previously been characterised using morphological and yield-related traits. However, plants cannot be fully analysed until harvest and morphological evaluation is subject to genotype × environment interaction. Alternative evaluation methods involving molecular genetic markers are especially advantageous for long-lived perennial crop such as coffee tree. Since isozymes have been shown to be useless genetic markers for estimating genetic diversity between *C. arabica* accessions (Berthou & Trouslot, 1977), DNA-based procedures appears particularly relevant. Although only a moderate degree of genetic diversity among the accessions was revealed in this study, the RAPD method seems to be effective in resolving genetic variations in arabica coffee and in grouping germplasm.

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