IV. Characterization and assessment of *Coffea arabica* L. genetic resources conserved in the CATIE field genebank

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Introduction

Awareness of the importance of plant genetic resources increased greatly in the 20th century as it became increasingly evident that this heritage was under threat. The international community responded by initiating a large-scale collection campaign with the aim of preserving as much of the available biodiversity as possible. This led to the construction of many genebanks in the 1960s. The size of these genebanks became so large that they are now very expensive to maintain and new accessions can no longer be systematically characterized. These resources will simply remain 'trite curios' if they are not characterized and assessed to enhance their use in targeted plant improvement programmes. This characterization and evaluation process is also crucial to enable efficient utilization of the genebanks' accessions and to develop core collections, thus making available the most interesting genetic resources.

The history of the CATIE coffee (*Coffea* spp.) genebank resembles that of most genebanks worldwide. Coffee genetic resources began being introduced in 1949, and this initiative continues (see Chapter 3). From 1951 to 1970, this genebank added 50 new accessions annually on average, generating several hundreds of coffee trees to plant every year for 20 years. The collection now includes 9760 coffee trees, representing 1850 accessions, more than 90% of which belong to the cultivated species *C. arabica*. It is one of the largest and most diversified genebanks for this species worldwide.

Coffee germplasm was characterized and assessed to an increasing extent as new constraints to *C. arabica* coffee tree agronomy were identified. The first introduced accessions were assessed chiefly on the basis of the size of the coffee berries and beans produced. These varieties were derived from Typica and Bourbon populations, which were disseminated throughout the world during the 18th century, as well as local varieties cropped in Ethiopia. A new selection criterion was taken into account in the 1970s, i.e. susceptibility to Coffee leaf rust, whose pathogen (*Hemileia vastatrix*) had just been identified in the Americas (Bertrand et al. 1999). Genetic factors that determine coffee resistance to this fungal disease were studied at the Portuguese *Centro de Investigação das Ferrugens do Cafeeiro* (CIFC) (see review by Avelino et al. 1999). Coffee improvement programmes then focused on the selection of the progeny of a natural interspecific hybrid (*C. arabica* × *C. canephora*), called the Timor Hybrid (Bettencourt 1973), which inherited rust resistance from its *C. canephora* parent.

This chapter presents the main characterization and assessment results concerning coffee genetic resources conserved in the CATIE field genebank. The experiments and analyses were undertaken within the framework of a coffee improvement programme that was conducted from 1993 to 2002 in Central America and the Caribbean by the *Programa Cooperativo Regional para el Desarrollo Tecnológico y Modernización de la Caficultura en Centroamérica, República Dominicana y Jamaica* (PROMECAFE) network of the *Instituto Interamericano de Cooperación para la Agricultura* (IICA), with the participation of CATIE, the *Centre de coopération internationale en recherche agronomique pour le développement* (CIRAD, France) and the *Institut de recherche pour le développement* (IRD, France). The programme was designed to broaden the genetic base of cultivated coffee varieties by tapping the diversity of wild coffee accessions collected in the centre of origin of *C. arabica*. Neutral markers (i.e. environment independent) were used for the first time to analyse genetic diversity and polymorphism in *C. arabica*. The results are presented and discussed with respect to the genetic origins of the accessions: (i) coffee accessions from the centre of origin (Ethiopia); (ii) Typica- and Bourbon-derived coffee accessions; and (iii) introgressed lines selected within interspecific hybrid progenies.

Accessions from the diversity centre of *C. arabica*

Two major coffee survey missions were conducted in Ethiopia: by FAO in 1964–65 (Fernie et al. 1968), and by ORSTOM (now IRD) in 1966 (Guillaumet and Hallé 1978). Very few evaluation data concerning these coffee trees are available in the large genebanks hosted in Brazil, Côte d'Ivoire, Ethiopia, Kenya and Tanzania. Since enzymatic markers were found to be relatively inefficient for detecting polymorphism in *C. arabica* (Berthou and Trouslot 1977), the genetic diversity structure in this species was determined after the development of polymerase chain reaction (PCR)-based markers (Lashermes et al. 1996).

Neutral diversity

A hundred and eleven coffee trees, representing 88 accessions collected in Ethiopia, were selected on the basis of their geographical origin and analysed using random amplified polymorphic DNA (RAPD) markers (Anthony et al. 2001). All accessions collected elsewhere than in Kefa and Ilubabor provinces (i.e. Gojjam, Shoa, Sidamo and Harerge provinces) were included in the sampling in order to compensate for their limited populations in the genebank. For Kefa and Ilubabor provinces, at least one accession per collection site was selected. Six accessions of varieties cropped locally in Ethiopia and two accessions of Typica- and Bourbon-derived varieties were also included in the study for comparative purposes.

Wild coffee varieties were classified into four genetic groups that clearly differ from Typica- and Bourbon-derived varieties (Figure 4.1). The Ethiopian 1 group consisted of 78 wild coffee accessions and two Ethiopian varieties. This group included all accessions collected in Gojjam, Ilubabor and Shoa provinces, virtually all of the Kefa accessions, three accessions from Sidamo and one from Harerge (Figure 4.2). This group therefore pooled almost all (apart from a few exceptions) of the accessions from south-western Ethiopia. The other groups were smaller. All accessions classified in the Ethiopian 2, 3 and 4 groups—apart from one accession from Kefa province—were collected in Harerge and Sidamo provinces, which are located east and south of the Great Rift Valley, respectively. The genetic diversity thus seems to be structured into two large complexes separated by the tectonic rift that cuts through Ethiopia from the northeast to the southwest. The Ethiopian varieties were classified in the Ethiopian 1 and 2 groups according to their geographical origins (i.e. south-west vs. south-east).

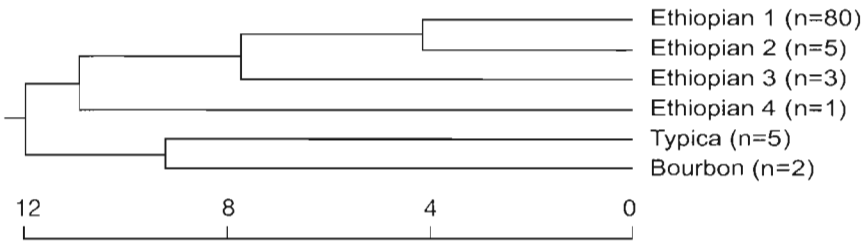

Figure 4.1. Genetic diversity structure in *C. arabica* revealed by RAPD markers (Anthony et al. 2001). The number of accessions per group is indicated in brackets.

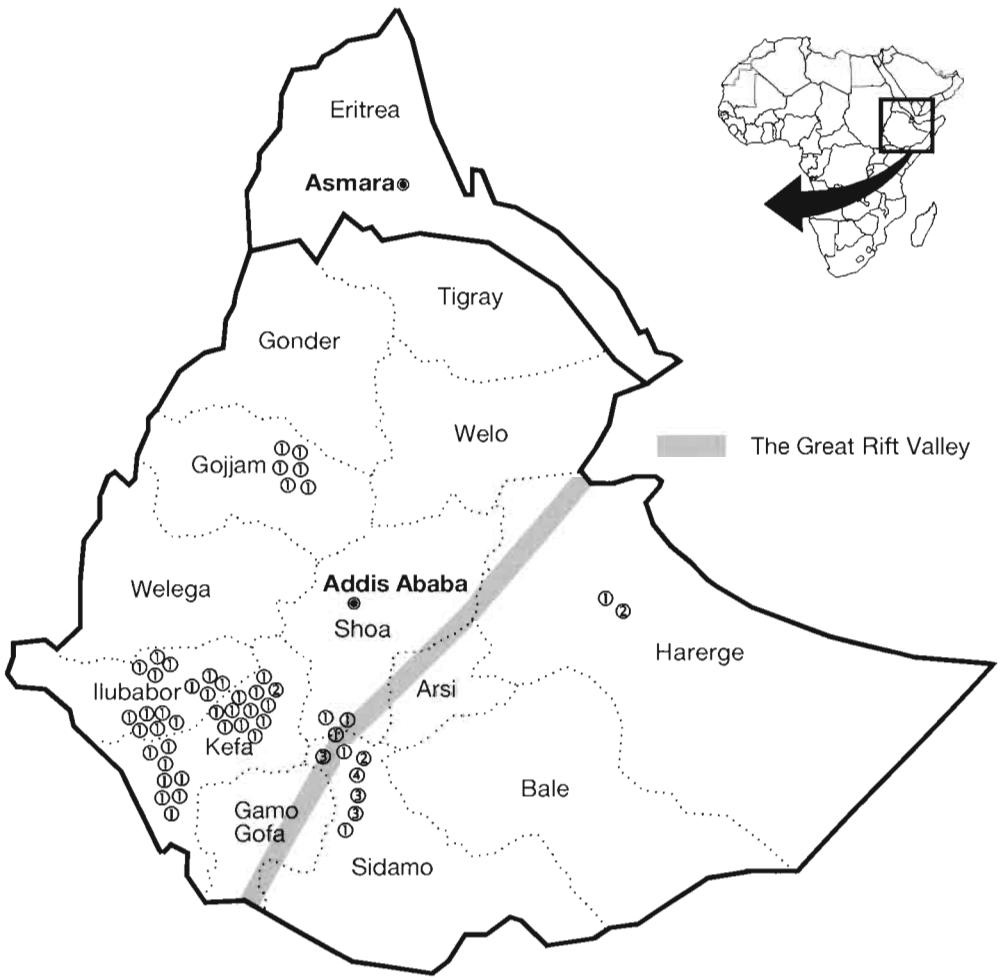

Figure 4.2. Distribution of four Ethiopian genetic groups formed on the basis of RAPD polymorphism (adapted from Anthony et al. 2001). The number of the group in which each accession was classified is circled.

Most of the diversity detected in this study was in the Ethiopian 1 group, i.e. 97% of the markers found in this group were polymorphic, whereas only 45%, 42% and 28% were detected in accessions from the Ethiopian 2, 3 and 4 groups, respectively. Only 59% of the markers produced by all accessions belonging to the three groups from the area south-east of the Great Rift Valley were polymorphic, which is much lower than the percentage noted in the Ethiopian 1 group from the south-western area. Hence, wild coffee accessions collected in Kefa and Ilubabor provinces accounted for most of the observed diversity. It is quite likely that coffee was first cultivated in this region, and this occurred around the 5th century (Lejeune 1958). Moreover, molecular marker analyses highlighted many redundancies within accessions originating from the south-western area.

Phenotypic variability

Phenotypic analyses were carried out to pinpoint wild genotypes with features that could be of interest for the regional coffee genetic improvement programme.

Morphology and fertility

Many agromorphological traits were monitored for several years in the CATIE coffee genebank (Bertrand et al. 1993; Anthony et al. 1999), including internode length, leaf size, fertility, production, and berry and bean defects. The percentage of floating berries (i.e. empty) and berries containing one bean (peaberries), rather than the usual two, were the traits that varied most (Table 4.1). Fertility was almost perfect (with two beans per berry) in some wild coffee accessions, while others produced beans about the same size as those generated by var. Maragogipe, which is famous for its large beans (Krug et al. 1939).

Table 4.1. Fertility and bean weight variability observed in 164 wild coffee trees from Ethiopia, in the CATIE field genebank in 1995 (Anthony et al. 1999).

Character	Min.	Max.	Mean	Variation
Empty fruits (%)	0.0	37.6	5.6	113%
Peaberries (%)	0.3	52.6	10.4	72%
Number of beans per fruit	1.18	2.04	1.75	8%
100-bean weight at 11% moisture (g)	11.8	23.7	17.2	13%

Pest resistance

Wild coffees in the CATIE genebank were evaluated for their resistance to root-knot nematodes (*Meloidogyne* spp.) and to two fungal diseases, Coffee leaf rust and Coffee berry disease (CBD). Resistance to three different nematode species was assessed, including two species from Costa Rica (*M. exigua* and *M. arabicida*) and one from Guatemala (*M. paranaensis*, ex *Meloidogyne* sp. or *M. incognita*). No accessions were found to be resistant to *M. exigua*, but some wild coffee trees from Ethiopia were resistant to *M. arabicida* and *M. paranaensis* (Bertrand et al. 2002; Anthony et al. 2003).

Results of tests in which coffee leaf discs were inoculated with *Hemileia vastatrix* demonstrated that 41% of the wild coffees tested were resistant to strain II, which occurs in Costa Rica (Bertrand et al. 1993). All wild coffees conserved in the genebank (1842 trees) were then screened for disease symptoms (i.e. leaf discoloration and pathogen sporulation). The trees were subsequently assessed several times a year between 1995 and 1998. More than a third of the trees showed no disease symptoms by the end of the assessment period (F. Anthony, unpublished data).

Eighty-two wild coffee accessions were screened by CIRAD for resistance to *Colletotrichum kahawae*, the causal agent of Coffee berry disease (CBD) (Berry and Bieysse, unpub. data). This

led to the identification of genotypes that presented much milder CBD symptoms in comparison with susceptible commercial coffee varieties.

Male sterility

Male sterility is also a very interesting trait for breeding programmes geared towards the selection of heterozygous hybrids (e.g. F_1 hybrids). The transfer of male sterility into a variety will allow dissemination of the heterozygous hybrids as seeds produced by crossing the male-sterile variety and a wild progenitor. Pollen production was checked in more than 7000 coffee accessions hosted in the CATIE genebank (Dufour et al. 1997). Non-pollen-producing genotypes were detected in the wild coffee population, but not in accessions that had been obtained through selection. Studies conducted in Brazil revealed that male sterility is under recessive genetic control in coffee (Mazzafera et al. 1989).

Accessions from selection

The three coffee varieties most cropped worldwide (i.e. vars. Caturra, Catuai and Mundo Novo) are derived from Typica and Bourbon populations that were first disseminated from Yemen in the 18th century. Narrative histories indicate that the Typica population was formed by progeny of a single plant that was cultivated in Amsterdam, and that the Bourbon population was formed by several coffee trees that were introduced to La Réunion (previously called Bourbon Island) (see review by Anthony et al. 1999). Coffee varieties or mutants derived from these populations thus have a narrow genetic base, which limits their breeding potential. This constraint did not, however, prevent growers and breeders from isolating an impressive number of varieties and mutants (Krug et al. 1939; Chevalier 1947). As coffee cropping intensified during the 20th century, their susceptibility to most diseases and pests that occur in coffee plantations (Coffee leaf rust, CBD, nematodes, coffee berry borers, etc.) was revealed (see reviews by Bertrand et al. 1999; Flood et al. 2001).

Neutral diversity

The genetic diversity of coffee trees selected in Typica and Bourbon populations was investigated using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) molecular markers (Anthony et al. 2002). The material assessed consisted of coffee varieties, mutants and hybrids (Typica × Bourbon), in addition to coffee trees cropped in Yemen (Eskes 1989) and a few wild Ethiopian coffee trees. Two distinct groups were revealed, matching their Typica or Bourbon genetic origins, by determining genetic distances in pairs of accessions (Figure 4.3). Catuai coffee trees were classified intermediate between Typica- and Bourbon-derived accessions, in agreement with their hybrid origin. As also noted in a previous study on the genetic diversity of wild coffee (Anthony et al. 2001), the Ethiopian coffee trees differed from Typica- and Bourbon-derived accessions, without forming well-structured groups.

Genetic diversity was found to be low in selected coffee trees. Only 51% and 55% of the markers identified in this study were detected in Typica- and Bourbon-derived accessions, respectively, while the wild accessions had 90%. This diversity was also low in coffee trees from Yemen, which contained 50% of the markers identified. Typica- and Bourbon-derived coffee accessions were distinguished by seven markers specific to each population (i.e. present in all accessions of one population but absent in all accessions of the other population). Polymorphism was substantially reduced in Typica- and Bourbon-derived accessions, with 13% and 24% polymorphic markers, respectively. By comparison, 98% of the markers identified in wild coffee accessions were polymorphic.

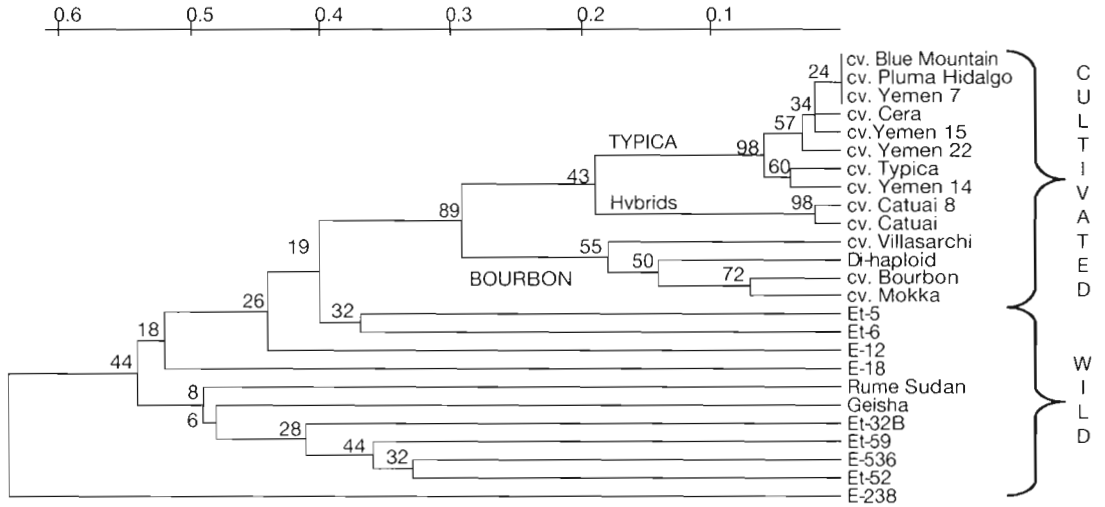

Figure 4.3. Dendrogram of 25 coffee accessions determined according to AFLP-based genetic distance (Anthony et al. 2002). Numbers on the branches are bootstrap values (%) obtained after 200 replicate analyses.

Phenotypic variability

Scientists visiting CATIE's coffee genebank are often surprised by the high variability in phenotypic traits. This feature does not seem to be related to the narrow genetic base of Typica and Bourbon populations that have been used in selection programmes for more than 150 years now. Polymorphism is especially evident in the architecture of coffee trees and in the morphology of their leaves and berries. This apparent diversity is partially due to the presence of many mutants that were isolated in different research centres worldwide and subsequently included in CATIE's coffee genebank. Mutations have had an impact on a wide range of different characters, resulting in stunted growth (e.g. vars. Caturra, San Bernardo and San Ramon), large-sized leaves and beans (e.g. var. Maragogipe), purple leaves (e.g. var. Purpurascens), erect branches (e.g. var. Erecta) and yellow endosperm (e.g. var. Cera). Most of these mutations involved only one gene, sometimes having pleiotropic effects throughout the plant (Carvalho et al. 1991), as noted with the recessive *lr* mutation in var. Laurina, which produces cone-shaped coffee trees with small narrow leaves, narrow beans that are pointed at one end, and whose caffeine content is half that of commercial varieties (Lopes 1971).

Variations observed within Typica- and Bourbon-derived accessions also account for the high apparent variability in CATIE's genebank. Significant differences in agro-morphological traits monitored in leaves, berries and beans were noted between trees of the same accession (Astorga 1999). This polymorphism likely occurred in response to suboptimal conditions when *C. arabica* trees were grown at low elevation (602 m). It was still possible to separate Typica- and Bourbon-derived accessions ($p<0.0001$) on the basis of the colour of young leaves (bronze in Typica *vs.* light green in Bourbon), as delineated by Krug et al. (1939).

Accessions from interspecific hybrid progenies

Rust-resistant introgressed lines currently disseminated worldwide are derived from three interspecific tetraploid hybrids, i.e. Timor Hybrid and Icatu obtained via a *C. arabica* × *C. canephora* cross in Latin America, and S.26 (*C. arabica* × *C. liberica*) in India. At CATIE, Timor Hybrid-derived lines (F_5–F_7) are being assessed, mainly for vigour, production and rust resistance, through

the PROMECAFE network. One advantage of interspecific tetraploid hybrids is that they can undergo recombination, which does not seem to be limited by genetic differentiation of chromosomes from different genomes (Herrera et al. 2002). Genes of these hybrids thus seem to be especially suitable for introgression into the genome of *C. arabica* varieties.

Identification of introgressed DNA

Twenty-one Timor Hybrid-derived accessions were analysed for the introgression of *C. canephora* genetic material using AFLP markers (Lashermes et al. 2000). They were compared with 23 *C. arabica* accessions and 8 *C. canephora* accessions. The Timor Hybrid-derived accessions were distinguished from the *C. arabica* accessions by 178 markers, consisting of 109 additional bands (i.e. present in *C. canephora* and absent in *C. arabica*) and 69 missing bands (i.e. present in *C. arabica* and absent in *C. canephora*). The number of additional and missing bands ranged, respectively, from 18 to 59 and from 0 to 32 among the Timor Hybrid-derived accessions (Figure 4.4). The introgressed fragments were estimated to represent 8% to 27% of the *C. canephora* genome. Assuming that a unique genotype of *C. canephora* was involved in the formation of the Timor Hybrid, the overall 109 introgressed fragments identified in the Timor Hybrid-derived accessions were estimated to represent 51% of the *C. canephora* genome. Most of the introgressed chromosome segments were not eliminated or counter-selected during the selfing and selection process. The introgression was not restricted to chromosome substitution but also involved chromosome recombination, as shown by Herrera et al. (2002).

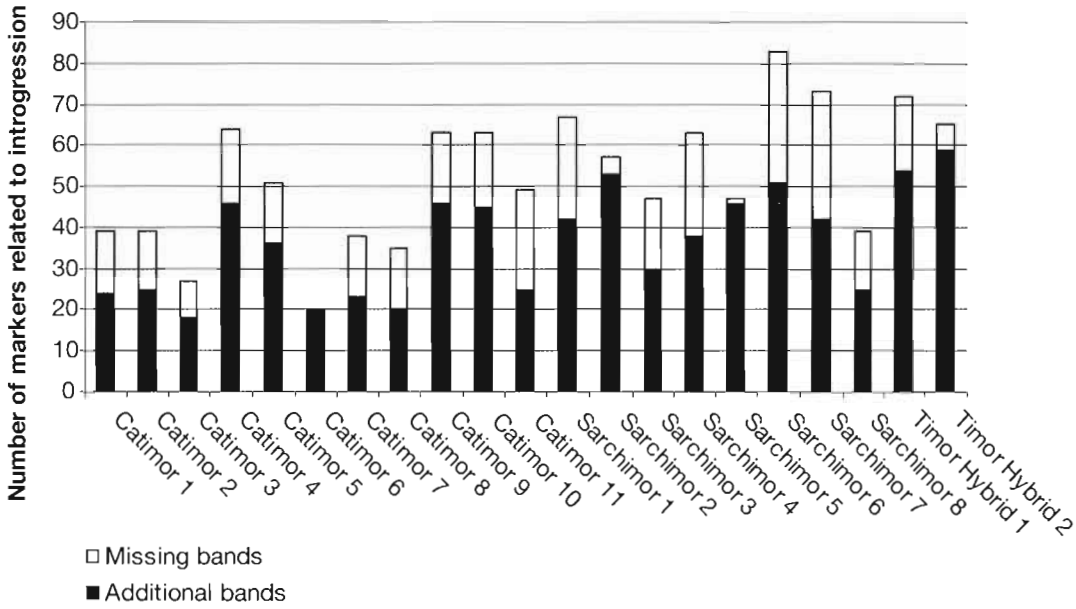

Figure 4.4. Number of AFLP markers attributable to introgression detected in Timor Hybrid-derived genotypes (Lashermes et al. 2000).

Phenotypic variability

The agronomic performances of 27 Timor Hybrid-derived lines were compared with those of two commercial coffee varieties, Caturra and Catuai (Bertrand et al. 1997a), focusing specifically on growth, production and fertility traits, as well as resistance to rust, nematodes and CBD. The results highlighted significant differences between lines for all monitored traits. Lines resistant to

rust or *M. exigua*, or both, were identified. The level of resistance to *M. exigua* was found to be as high in these lines as in *C. canephora* coffee trees (Bertrand et al. 2001). Lines resistant to corky-root, caused by *M. arabicida* and *Fusarium oxysporum* f.sp., were also detected (Bertrand et al. 2002).

Cup quality and chemical composition were studied among 22 introgressed Timor Hybrid-derived lines and compared with data from three non-introgressed varieties (Bertrand et al. 2003). Variability in the analysed characters was found to be rather high in the introgressed lines. There were significant differences between lines for all biochemical compounds analysed, and for acidity and overall standard. Two lines were significantly poorer than the controls with respect to sucrose and beverage acidity. One of them also had a higher chlorogenic acid content and had a poorer overall standard. However, two highly introgressed lines did not differ from the non-introgressed varieties.

Conclusion

C. arabica accessions conserved in the CATIE genebank were classified in three groups on the basis of their genetic origins, as determined by a review of narrative histories on the dissemination of coffee trees worldwide, along with the history of coffee improvement initiatives. The classified groups are as follows: coffee trees from the centre of origin of the species; Typica- and Bourbon-derived varieties and mutants; and introgressed lines selected within interspecific hybrid progeny (*C. arabica* × *Coffea* spp.). The results of the assessment and characterization of these resources highlighted features specific to each of these groups with respect to their innate genetic diversity and extent of polymorphism.

Neutral markers revealed high genetic diversity in coffee trees that were collected in Ethiopia, in contrast with the low diversity detected in Typica- and Bourbon-derived varieties. Polymorphism also seemed to be relatively high in wild Ethiopian coffee trees, but very low in cultivated coffee trees. Phenotypic traits of interest for improvement programmes were found in Ethiopian coffee trees, including male sterility, resistance to Coffee leaf rust and root-knot nematodes. Some of these coffee accessions were found to have almost perfect fertility or an exceptional bean size, or both. Wild coffee trees from the centre of origin thus represent a diversity reservoir that could be tapped to broaden the genetic base of cultivated coffees. Moreover, crosses between wild coffee trees and cultivated varieties generated very productive and vigorous F_1 hybrids in Costa Rica (Bertrand et al. 1997b; Bertrand 2002).

Introgressed lines differ markedly from wild and cultivated coffees. Chromosomes from different genomes can be recombined in first-generation interspecific hybrids, often giving rise to novel polymorphic traits. After five to seven selfed generations, introgressed fragments were found to vary markedly from one line to another, and high variability in the chemical contents of beans and in cup quality were observed. This organoleptic variability could be utilized by selecting lines that produce good quality coffee while also being resistant to coffee diseases and pests.

The genetic resource assessment data discussed in this chapter could now be used to build core collections for long-term germplasm conservation, evaluation and exchange purposes.

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