III. Conservation of coffee genetic resources in the CATIE field genebank

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Introduction

The importance of genebanks in crop breeding has been widely recognized since Vavilov's publications (Vavilov 1935). Genebanks are essential for preserving the genetic diversity of commercial crops and their relatives, and for characterizing the conserved accessions prior to their utilization. For many species that cannot be conserved by seeds, field genebanks became the method of choice for conservation of genetic diversity rather than botanical gardens and introduction centres, which were set up mainly to cultivate wild species, as early as the 16th century. As a part of its institutional mandate, CATIE preserves, multiplies, classifies and promotes the use of its valuable germplasm collections, which include more than 300 plant species with more than 35 000 accessions. The genetic material is available for institutions and organizations involved in plant improvement and production (for details, connect to the CATIE Web site at http://www.catie.ac.cr/research/research.htm).

As for most cultivated plants, conservation of coffee (*Coffea* spp.) genetic resources started with field collections. The sensitivity of coffee seeds to desiccation and cold (Ellis et al. 1990) has long limited the development of other conservation methods. The CATIE genebank is one of the largest and richest worldwide for *C. arabica* coffee, containing 9760 trees of 1852 accessions at the time of writing. It includes wild coffee trees collected in the centre of origin, varieties and mutants selected in various research centres, as well as intra- and interspecific hybrids. The collection is the only genebank available for Latin American and Caribbean countries. An extensive genetic evaluation was carried out in the 1990s with the aim of structuring genetic diversity and of identifying accessions that present interesting characters for the regional improvement programme (see Chapter 4). As a prerequisite to evaluation, an analysis of existing accessions in the genebank was performed in order to classify the accessions according to their genetic origin and to define possible parental linkages.

C. arabica coffee cultivation might have started in the centre of origin of the species, in the south-west of Ethiopia, around the 5th to 8th centuries. It is at that time that coffee trees were introduced to Yemen, possibly by Arabian merchants (see review by Anthony et al. 1999). Two populations, known as Typica and Bourbon, were later disseminated from Yemen to the world during the 18th century. They gave rise to a large number of mutants in Latin America, Africa and Asia (Krug et al. 1939; Chevalier 1947). During the 20th century, the extension of coffee cultivation and the intensification of production revealed that the varieties derived from Typica and Bourbon were sensitive to many pests (e.g. nematodes, Coffee Berry Borer) and diseases (e.g. Coffee leaf rust, Coffee berry disease) (see reviews by Bertrand et al. 1999; Flood et al. 2001). Natural interspecific hybrids between *C. arabica* and *C. canephora* or *C. liberica* constituted the first sources of resistance to Coffee leaf rust (aka orange rust) caused by *Hemileia vastatrix*. Other interspecific hybrids were later created. The genealogical selection of these descents has led to the diffusion of introgressed lines, resistant to rust and known under the names of Catimor, Sarchimor, Icatu, S.795, etc. Coffee genetic resources have thus varied origins.

This chapter is divided into five sections: (i) a presentation of the accessions conserved in the CATIE genebank; (ii) a description of the conditions of their conservation in the field; (iii) the data management system; (iv) an analysis of the genetic erosion; and (v) the principles of a new conservation strategy for coffee field genebanks.

CATIE field genebank constitution

The introduction of coffee genetic resources started in 1949 at IICA (*Instituto Interamericano de Ciencias Agrícolas*, now *Instituto Interamericano de Cooperación para la Agricultura*) which had available land (1000 ha) close to Turrialba, given by the government of Costa Rica (for details, consult the CATIE Web site at http://www.catie.ac.cr). The field genebank is located in the Cabiria III campus botanical garden, and covers approximately 8.5 ha. The site is situated at 9°38′ N latitude and 83°38′ W longitude, at 602 m above sea level. The average day temperature is 22.5°C and annual rainfall 2600 mm, without any marked dry season. It represents a sub-optimal zone for the culture of *C. arabica* and of the other coffee species (e.g. *C. eugenioides*) usually found at higher altitudes. Coffee produced in the Turrialba region presents normal acidity and good aroma, but small body (for details, see the ICAFE Web site at http://www.icafe.go.cr). Flowerings are multiple and of low intensity; harvest is precocious and is spread over at least four months.

The genebank became CATIE's responsibility after its creation in 1973 by IICA and the government of Costa Rica. Introduction records were maintained by world-renowned coffee researchers, such as J.B.H. Lejeune, P.G. Sylvain and F.L. Wellman. These records were then updated with the support of the German Agency for Technical Cooperation (GTZ) at the beginning of the 1980s. It is on the basis of this information that the analysis of accessions in the genebank was then carried out. Observations were performed in the genebank in order to confirm the taxonomic identification of some introductions.

History of introductions

The accessions introduced in 1949 now represent 1.5% of the total number of conserved accessions. The most massive introductions took place over 20 years, between 1951 and 1970, with an average of 52 accessions introduced annually (Figure 3.1). These introductions constitute 55.6% of the living accessions in the collection. The introduction rate decreased during the following decade (1971–80), with around 20 accessions introduced annually, and then increased between 1981 and 1990, with 43 accessions introduced annually. The 1980s introductions represent almost a quarter of accessions conserved. Since then, additions to the genebank have averaged 17.5 accessions per year.

The chronology of introductions reflects the advances of the breeding programmes which have been developed worldwide. The majority of accessions introduced in the 1950s were Typica- and Bourbon-derived varieties or varieties locally cultivated in the centre of origin (i.e. Ethiopia). These coffee trees were selected at research centres and farms. The following decade (1961–70) was marked by the first large collecting mission in Ethiopia (Fernie et al. 1968). The collected material was distributed to five field genebanks, and CATIE received the most accessions (485). The accessions introduced between 1971 and 1990 were principally introgressed lines, derived from a natural interspecific hybrid *C. arabica* × *C. canephora*, the 'Timor' hybrid (Bettencourt 1973). Finally, new coffee species (*C. brevipes* Hiern, *C. pseudozanguebariae*, *C. sessiliflora* Brid.), as well as wild *C. arabica*, *C. canephora*, *C. eugenioides* and *C. racemosa*, were introduced in the 1990s. Twenty-one accessions from the IBPGR-funded collecting mission in Yemen were also introduced (Eskes 1989).

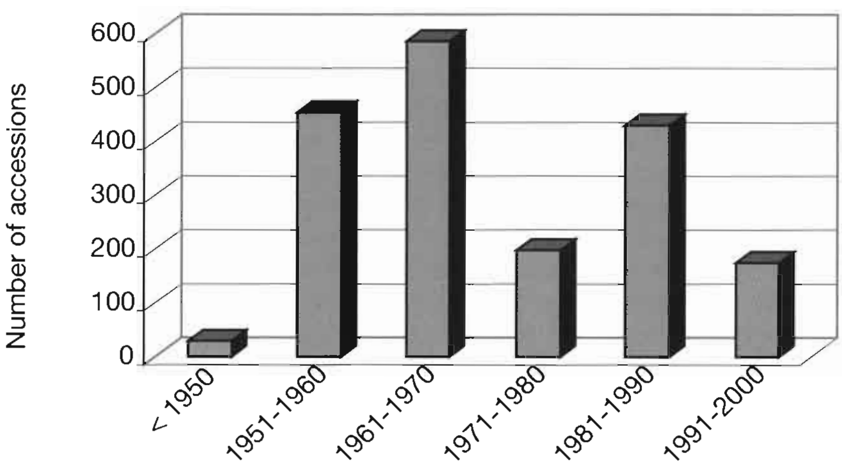

Figure 3.1. Number of coffee accessions introduced to the IICA and subsequently CATIE field genebank per decade, since 1949.

Source of introduced accessions

The coffee accessions introduced to CATIE were received from research centres and plantations located in 31 countries. Latin American (e.g. Brazil, Costa Rica) and Caribbean (e.g. Puerto Rico) countries provided 42% of the accessions (Figure 3.2). Africa provided close to 500 introduced accessions (26%). Two European countries, France and Portugal, made significant contributions, with around 200 accessions each.

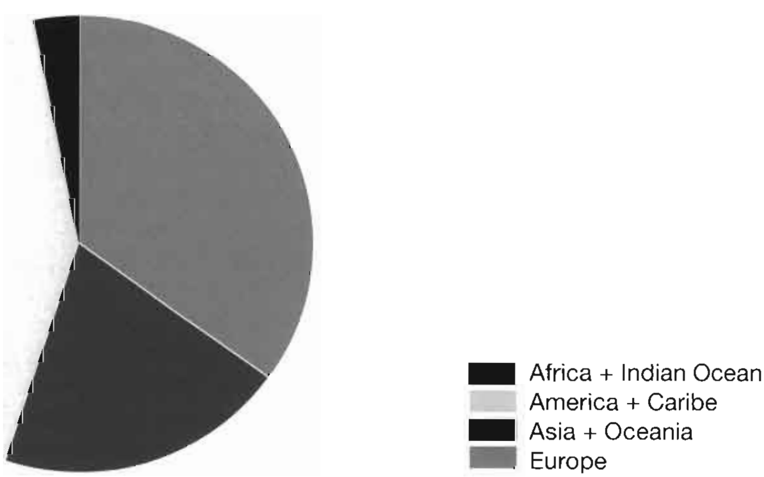

Figure 3.2. Provenance of the coffee accessions introduced to the CATIE field genebank.

Field genebank management

Classification of genetic origins

The large number of accessions in the genebank meant that, before they could be evaluated effectively, it was necessary to classify the accessions according to their genetic origin. The objective was to construct core collections for genotypic and phenotypic analyses of diversity (see Chapter 5). A hierarchical ranking was adopted by choosing the species as first criterion of classification (i.e. *C. arabica* vs. other species and hybrids). The *C. arabica* accessions were then separated into three groups as a function of the selection that had taken place: (i) 'no selection' for wild coffee trees collected in the centre of origin; (ii) 'low' for coffee trees cultivated in the centre of origin and the centre of dispersal (Yemen); and (iii) 'high' for Typica- and Bourbon-derived varieties, mutants and introgressed lines. The accessions classified in this last group often possess parental links due to the selection process.

Various observations can be made from the inventory of the genetic resources of CATIE (Table 3.1). Ninety-one percent of the conserved accessions belong to the species *C. arabica* or to interspecific hybrids involving this species. The other coffee species are under-represented in terms of number and inherent diversity (see Chapter 2). The wild coffee trees collected in Ethiopia by FAO (Fernie et al. 1968) and ORSTOM (now IRD) (Guillaumet and Hallé 1978) constitute 31.5% of the conserved accessions, but only 22.8% of the living trees. The material from the IPGRI collecting expedition in Yemen (Eskes 1989) is only represented by a few (17) trees. In contrast, the accessions originating from selection (group 3) are numerous, representing 45.8% of the total and 58.6% of all trees in the collection. Finally, many intraspecific hybrids are also conserved (15% of all trees).

Table 3.1. Number of coffee (*Coffea* spp.) accessions and corresponding trees conserved in the CATIE field genebank. Within the *C. arabica* cultivated species, three groups were defined on the basis of selection intensity: nil (0), low (+) and high (++).

Identification	Selection	Description	Accessions	Trees
C. arabica	0	Wild plants from the centre of diversity (Ethiopia)	583	2222
	+	Varieties from the centre of diversity (Ethiopia)	191	950
		Varieties from the primary dispersion centre (Yemen)	10	17
	++	Varieties derived from Typica and Bourbon	292	1818
		Introgressed lines from interspecific hybrids	303	1786
		Intraspecific hybrids	169	1467
		Mutants and other selected coffee	84	650
Diploid species		*C. canephora*	83	296
		C. liberica	15	76
		Other species	60	138
Interspecific hybrids		(*C. arabica* × *Coffea* spp.)	19	90
Not classified			43	250
Total			1852	9760

Agronomic practices

Planting

The field genebank is divided into eight sections (A to H), sub-divided into plots. The collection was maintained in a manner similar to that of commercial plantations up to 1998, when the Technical Unit for Support to Research (UTAI) took over its maintenance. Since then, a new strategy of conservation has progressively been implemented (see later in this chapter).

Most of the accessions (91%) have been received in seed form, each seed constituting a genotype. The number of genotypes planted in the genebank varies from four to eight for the majority of accessions. However, 14% of accessions are represented by larger numbers, reaching up to 46 coffee trees.

About 9% of accessions correspond to clones that were introduced as stem cuttings. These accessions are represented by one to ten trees in the genebank, produced by vegetative multiplication (i.e. cutting or grafting).

Coffee trees introduced in seed form are cultivated on their own root system. During the 1990s, grafting on vigorous rootstocks of *C. canephora* var. Nemaya, which is resistant to most root-knot nematodes of Central America (Bertrand et al. 2002), has been used in order to facilitate the adaptation of wild coffee trees (*Coffea* spp.), whose agronomic performances are rather weak.

Spacing between rows of trees and between trees within a row varies according to the section of the genebank. The most classical spacing is 2.5 × 2 m. Extreme spacing distances are 4 × 4 m for the first introductions of *C. canephora* and *C. liberica*, and 2 × 1.5 m for dwarf introgressed lines.

Coffee trees are normally grown under canopy, but shading practice in plantations varies considerably according to ecological conditions, local tradition and the level of management (Mitchell 1988). To provide shade, *Erythrina poeppigiana* trees are planted between the coffee rows, at approximately a 6 × 6 m spacing. Their relatively fast growth requires two pruning treatments annually in order to allow suitable penetration of light at the level of the coffee tree foliage.

Coffee tree maintenance

Coffee trees are maintained with at least three trunks. Pruning is performed once a year, during the dry season, in order to eliminate the oldest stems.

The most common weeds in the coffee collection are grasses, such as *Paspalum paniculatum* and *P. conjugatum*, and forbs, commonly *Bidens pilosa*, *Impatiens walleriana*, *Borreira* spp., *Mitracarpus* spp. and *Richardia scabra*. In the absence of cover plants, weeds are eliminated by applying a herbicide approximately every two months. Rotation of products is respected in order to minimize the development of resistance in the weed flora.

The soil of the coffee genebank is homogeneous for physical structure and chemical composition. However, the presence of a cemented layer makes drainage difficult. The fertility of the soil is medium, and not optimum for coffee, and so requirements are supplied by supplemental fertilizer application. Fertilizers are applied in a uniform manner to all coffee trees, wild or cultivated. Applications consist of 100 g per tree of 20-7-12-3-1.2 (N-P-K-Ca-Mg) in May, 18-5-15-6-2 in September and ammonium nitrate in December. However, variations in the budget allocated to genebank maintenance can affect the fertilization programme, as observed in most coffee field genebanks in the world (Dulloo et al. 2001).

Coffee trees conserved in the genebank are usually comparatively free from the pests and diseases encountered in commercial plantations. The most serious attacks are those provoked by *Hemileia vastatrix*, the pathogenic agent of Coffee leaf rust (orange rust).

Productive trees are more severely affected by defoliation at the time of harvest and immediately afterwards. Treatment against Coffee leaf rust is by application of classical fungicides such as triadimefon, copper hydroxide and ciproconazol. The recent arrival of Coffee Berry Borer (*Hypothenemus hampei* Ferr.) has made necessary the definition of an integrated pest management (IPM) strategy, which reduces insecticide applications. Traps containing a mixture of alcohols, as recommended by the Costa Rica Coffee Institute (ICAFE), have been set up in the genebank.

Harvesting

Harvesting is performed manually, in several passes. The spread flowering pattern implies at least four passes between July and November on each tree. The incidence of the coffee borer has been estimated to be around 5% of harvested fruits. At the last pass, all remaining berries (green, ripe and dry) are picked in order to limit possible refuges for berry borers.

Information system

Accession number

At the time of their introduction, accessions are assigned a unique number (i.e. neither repeated nor re-attributed) in CATIE's introduction records. This number is preceded by the letter 'T', which stands for Turrialba. An accession number corresponds either to several genotypes if the introduction is in the form of seeds, or to a single genotype in the case of a clone. Mixing different genotypes under one unique number is problematic because of preferential autogamy of *C. arabica*, which allows around 10% of allo-pollination at each generation (Carvalho et al. 1991). Presence of illegitimate plants (i.e. not conforming to their genetic origin) constitutes a constant risk with seed samples.

Passport data

Data on the accessions of the field genebank are maintained in a database called 'CaféBase'. This database contains two types of passport information: (i) information on the genetic origin of the accessions; and (ii) information about the provenance (i.e. source) of introduced plant material (Figure 3.3). Information on the origin corresponds to the collecting data (i.e. localization of the forest population, nature of the collected samples) for the wild coffee trees or to the genetic basis for the selection process that has taken place (i.e. Typica, Bourbon, hybrids) for cultivated coffee trees. These data have been extracted from publications and available reports. Information on the provenance of accessions has been found in the introduction records of CATIE. The source of introductions has been international organizations, national coffee research centres or private farms.

Database structure

The passport data of the accessions are stored in two tables (Figure 3.4). One has for its access key the name of the genetic resource and contains information on the genetic origin (wild or cultivated). The other table has for its access key the accession number and contains information on the accession's provenance. These tables can be linked thanks to the presence of a common field: a shortened identifier for each genetic resource (e.g. 'Caturra' instead of the complete identifier '*C. arabica* var. Caturra'). The presence of this link allows collation of data distributed between the two tables and to edit the accession passport using the format presented in Figure 3.4.

T16692

C. arabica origin ET-4 (ORSTOM collection, 1966)

Origin of the genetic resource

Collecting country:	Ethiopia
Collecting site:	Father J. Araya's farm (1720m), 10km W Bonga, Kaffa province
Collecting date:	20/11/1966
Collected material:	seeds of a spontaneous coffee
Collector(s):	J.L. Guillaumet & F. Hallé
Synonym(s):	Ar 4

Source of the accession

Donor name:	IRCC, Paris
Source country:	France
Introduction date:	1985/08
USDA number:	
Other identification:	IRCC 201
Observations:	

T3432

C. arabica var. Maragogipe

Origin of the genetic resource

Selection country:	Brazil
Selection site:	Maragogipe, Bahia
Selection date:	1870
Genealogy:	Mutation of a dominant gen (*MgMg*) in var. Typica
Breeder(s):	C.J. Fernandes
Synonym(s):	Maragogype, Pretoria

Source of the accession

Donor name:	Instituto Agronômico de Campinas
Source country:	Brazil
Introduction date:	1956/02
USDA number:	227711
Other identification:	Brasil X 47127
Observations:	red fruits

Figure 3.3. Examples of passport data of wild (T16692) and cultivated (T3432) coffee accessions.

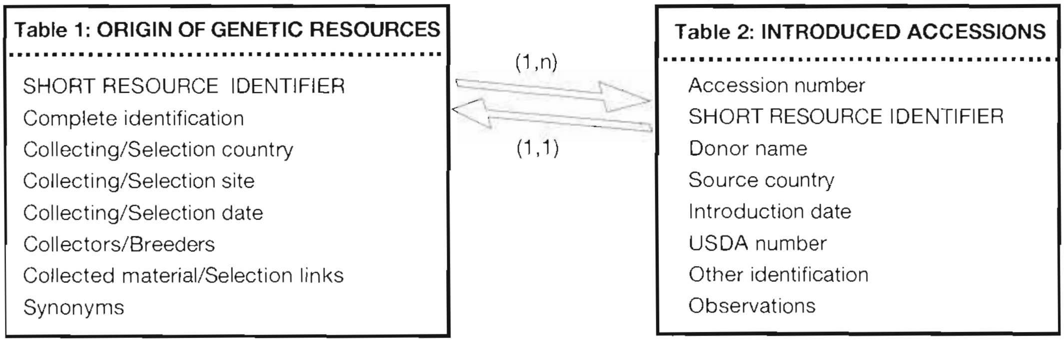

Figure 3.4. Tables of passport data with their links in the database 'CaféBase'. The access keys are indicated using bold letters. The field common to both tables is written in capital letters.

Towards computerized management

Establishment of computerized management of the CATIE genebank came up against the problem of absence of coding of genotypes and of planting sites. The problem of a single number identifying several genotypes has been already noted in this chapter. The absence of a coding system of locations for coffee trees in the field constitutes another obstacle to management computerization. It is fundamental to be able to identify the rows of coffee trees and their position within the row.

Analysis of genetic erosion

Genetic erosion between 1993 and 2002 has been estimated in three areas of the coffee genebank of CATIE:

- Section A, which contains predominantly accessions introduced in the period 1950–60, mainly coffee trees originating from selection.
- Wild coffee trees from the FAO collecting mission in Ethiopia (Fernie et al. 1968), which were planted in section C in 1965.
- Wild coffee accessions from the ORSTOM collecting mission in Ethiopia (Guillaumet and Hallé 1978), which were planted in section F in 1985–86.

Tree mortality, estimated by the number of dead trees, was slightly higher in sections A (14.9%) and F (15.7%) than in section C (11.6%) (Table 3.2). However, in terms of lost accessions, genetic erosion reached an average of 3.6%. This figure concealed significant differences between the three areas of the genebank, as erosion reached 8.2% in section F, but only 2% and 3.6% in sections A and C, respectively. Although coffee trees in section F were planted 20 years after those of section C, the higher mortality in section F cannot be explained by difference in age. The explanation lies more likely in the genetic nature of accessions. As in other large coffee genebanks (e.g. Ethiopia, Kenya, Côte d'Ivoire), cultural practices are close to those employed in commercial plantations and may not be appropriate for the conservation of wild plants collected in the forest (Dulloo et al. 2001). In section F, 45% of introduced trees were lost after eight years in the genebank (Bertrand et al. 1993). This indicates that survival of wild coffee trees was affected soon after their introduction into the field genebank. The greater survival recorded in the oldest plot (section A) can be explained by the higher initial number of trees per accession kept in the collection in this part of the genebank.

Within a given plot, genetic erosion tends to accelerate with the duration of conservation, and thus the age of plants. An analysis, using number of trees per accession as indicators, was performed for accessions represented by one or two genotypes in the CATIE genebank, i.e. those most threatened in the short term. The erosion rate of these accessions increased regularly during the period 1993–2002 in the three areas of the genebank considered, going from 23% to 29% in section A, from 28% to 33% in section C and from 30% to 51% in section F (Figure 3.5). This analysis shows the seriousness of the situation in section F, where more than one accession out of two is threatened.

Table 3.2. Genetic erosion estimated by the percentage of dead trees and lost accessions in three areas of the CATIE genebank between 1993 and 2002.

Section	Age	Genetic origin	Dead trees	Lost accessions
A	> 45 years	Cultivated	14.9%	2.0%
C	40 years	Wild	11.6%	3.6%
F	20 years	Wild	15.7%	8.2%

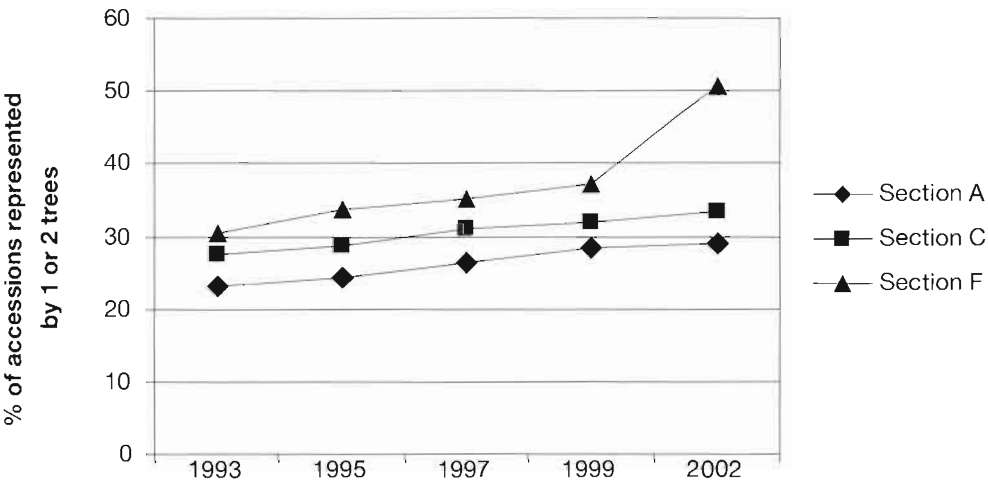

Figure 3.5. Evolution between 1993 and 2002 of the percentage of coffee accessions represented by one or two trees in sections A, C and F of the CATIE field genebank.

New strategy for sustainable field conservation

Given that the rate of genetic erosion is increasing, it has become necessary to develop a new strategy to ensure that the coffee genetic resources are safely conserved in the CATIE field genebank. A renovation project was developed, with two main objectives:

∞ to increase conservation security in the field collection, and
∞ to reduce maintenance costs for the field collection.

These objectives apply to the multiplication of living resources and preparation of fields in the new genebank. The new strategy also identifies priorities for conservation among the accessions currently conserved in the field genebank.

Defining priorities in conservation

Priorities in conservation have been defined based on the conservation cost relative to the 'genetic value' of accessions. This genetic value has been estimated using data from genetic evaluation, or from information on genetic origin for non-evaluated accessions. Three groups of accessions have been identified, corresponding to three levels of decreasing diversity (Table 3.3). The first priority is that group with the highest genetic diversity (i.e. wild coffee and interspecific hybrids). In this group, all the genotypes have to be multiplied and planted in the new genebank. Of second priority are the heterozygous varieties and introgressed lines, where it seems necessary to conserve all accessions, but the number of genotypes can be reduced to four per accession. In the last group, a total of only eight genotypes is considered to be enough to represent accessions of low diversity (i.e. homozygous varieties, mutants and intraspecific hybrids). Applying such a strategy will allow a 30% reduction in the number of coffee trees conserved in the new genebank, without loss of genetic diversity.

Table 3.3. Priority for conservation and renovation, according to the diversity estimated in the accessions.

Priority	Diversity	Genetic origin	Conservation strategy
1	+++	Wild plants (*Coffea* spp.) Interspecific hybrids	All genotypes
2	++	Heterozygous varieties Introgressed lines	4 genotypes of each accession
3	+	Homozygous varieties Mutants Intraspecific hybrids	8 genotypes maximum, sampled in all accessions

Shading to recreate forest conditions

The use of shade in coffee plantations increases tree longevity and reduces pest and disease effects (Somarriba et al. 2004). Flowerings are less intense in plantations under permanent shade than under semi-permanent shade or open sun, which reduces tree production (Mitchell 1988). Moreover, shade trees allow the recreation of a forest niche resembling the natural habitat of wild coffee, which helps the introduced germplasm to adapt to the field genebank conditions. Plantation with native forest species can also contribute to biodiversity preservation, especially for birds, which can be as populous as in forest habitats (Perfecto et al. 1996; Rappole et al. 2003).

Grafting to improve plant growth and development

Root systems poorly adapted to the conservation environment can be compensated for by grafting on vigorous rootstocks. This can also improve resistance to pests and diseases present in the soil. At the low altitude of CATIE, no incompatibility in grafting has been found between *C. arabica* and other coffee species (Couturon 1993). Grafting *C. arabica* varieties on *C. canephora* has affected neither the female fertility (i.e. occurrence of empty fruit or with a single seed) nor the biochemical content of beans (Bertrand and Etienne 2001). Since 1995, all introduced genotypes have been grafted on a *C. canephora* rootstock variety, named 'Nemaya', because of its vigour and resistance to the main root-knot nematodes in Central America (Bertrand et al. 2002). The use of var. Nemaya has proved to be also successful in conserving in the field some *C. eugenioides* and *C. stenophylla* genotypes that had not survived several attempts at growing them on their own roots (J. León, pers. comm.).

Spacing to optimize ground occupation

Coffee species exhibit large diversity in plant habit and morphology: from small shrubs (e.g. *C. brevipes*) to trees exceeding 10 m in height (e.g. *C. liberica*). Within *C. arabica* species, plants with dwarf habit due to gene mutation have to be separated from tall plants, such as wild coffee. Several dwarfism genes have been identified in *C. arabica* (Carvalho et al. 1991). The most famous gene is the *Ct* dominant gene from the variety Caturra, which has been transferred into numerous introgressed lines. Adopting a plantation scheme that allows optimal ground occupation helps to reduce the maintenance cost of the genebank. In practice, the density can vary from 1000 to 3000 trees per hectare, but this number can be greater in the case of dwarf coffee forms.

Conclusions

The management method applied to the coffee genetic resources in CATIE could be used to rationalize other large genebanks of perennial plants. Groups of accessions were defined using available data on the origin of introduced material, and then the genetic groups were submitted to genotypic and phenotypic evaluation (see Chapter 4). Information on the structure of genetic diversity was finally used to define priorities for conservation, giving more weight to genetic groups containing high diversity. Application of such an integrated strategy allows resources (financial, human, technical, spatial) allocated to conservation to be optimized, thus increasing the efficiency of conservation.

Conserving genetic resources in the field is indispensable for evaluating them. However, field genebanks appear to be very vulnerable to local hazards and consequences of global climatic change, as well as from financial resource constraints. As genotypic selection and genetic drift occur in coffee genetic resources maintained *in vitro* (Dussert et al. 1997), research efforts have been focused on the development of a cryopreservation method as a complementary conservation measure, in order to overcome the limitations of field conservation (see Chapter 6). This has been done using a core collection strategy for sampling the accessions (see Chapter 5).

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