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USE AND VALUE OF GENETIC RESOURCES OF *COFFEA* FOR BREEDING AND THEIR LONG-TERM CONSERVATION

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SUMMARY: For *Coffea*, ORSTOM has led several survey missions in tropical Africa. Plants collected during these missions have been established in a genetic resource center for *Coffea* in the Ivory Coast (20 species, 8,000 genotypes).

Many evaluation studies have been carried out on this material using different methods (isozymes, interspecific hybridization, agronomic characteristics etc.). The characterization and evaluation of coffee trees in collections demonstrate the potential diversity and its differentiation at the level of species and populations. The consequences of this genetic structure for breeding purposes will be analyzed. The genetic diversity distribution in Africa for the genus *Coffea* highlights the need for forest preservation. A patchy forest reserve distribution limits gene flow between populations. It creates a new environment for *Coffea* evolution. Conservation policies should take these traits into account.

The real cost of the establishment and maintenance of a living coffee collection was evaluated on the basis of our own research experience in Ivory Coast. The value of this plant material in a breeding programme was calculated to measure the impact of the genetic resources on coffee production in comparison with the investment.

RESUME: L'ORSTOM a conduit plusieurs missions exploratoires du genre *Coffea* en Afrique tropicale. Les caféiers collectés durant ces missions ont été introduits dans un centre de ressources génétiques de Côte d'Ivoire (20 espèces, 8.000 génotypes).

Plusieurs évaluations ont été réalisées avec ce matériel, en utilisant différentes méthodes (isozymes, hybridation interspécifique, valeur agronomique etc.). L'étude de la variabilité des caféiers sauvages en collection donne une image de leur diversité et de leur différenciation au niveau des espèces et des populations. Les conséquences de leur structure génétique sont analysées dans le cadre des programmes de sélection.

La distribution de la diversité génétique du genre *Coffea* en Afrique souligne le besoin de la conservation des forêts. La distribution des réserves forestières limite les flux de gènes entre les populations; elle crée un environnement nouveau pour l'évolution des *Coffea*. Les politiques de conservation devraient prendre en considération ces points. Le coût de création et d'entretien d'une collection de caféiers a été évalué sur la base de notre expérience ivoirienne. L'intérêt de ce matériel végétal sauvage pour la création variétale et la production caféière mondiale a été calculé comparativement aux investissements.

KEY WORDS: *Coffea*, genetic resources, breeding, *in situ* conservation, value of germplasm.

A. INTRODUCTION

The conservation and characterization of the genetic resources of coffee are based on the following major observations (BERTHAUD & CHARRIER, 1988):

1 - Coffee plantations started away from the natural distribution of the species, at the beginning of the 18^{th} century for *C. arabica* L. and of the 20^{th} century for *C. canephora* PIERRE ex FROEHNER; cultivated varieties today are not much different from the wild forms from which they originated.

2 - The limited amount of germplasm transferred between continents by man has hampered the use in plantations today of the range of variability once seen in the natural distribution of the species; the genetic variability in the autogamous species *C. arabica* is lower than in the allogamous species *C. canephora*.

3 - The natural diversity within wild coffee is still available in the distribution of the genus *Coffea*, i.e. the tropical forests of Africa and the Malagasy region, but has been undergoing drastic erosion linked to deforestation for several decades.

4 - Taxonomists have described numerous taxa within the genus *Coffea*; all of them are diploids (2n = 22 chromosomes), except for the tetraploid species *C. arabica* (2n = 44), and are crossed rather easily.

The urgency for conservation of the wild coffee diversity and the search for new agronomic characteristics motivated several collecting missions in Madagascar and Africa over the last 25 years. We shall go more deeply into the study of the conservation of the genetic resources of coffee after outlining the objectives and the strategies of variety improvement in the two cultivated species. We shall also give an evaluation of the costs of the conservation and characterization of these resources in order to compare their potential impact.

B. COFFEE PLANT BREEDING

1. BREEDING OBJECTIVES

The coffee producers, manufacturers and consumers wish to increase coffee production. However, the breeding criteria change with time, the countries concerned and the species cultivated. For example, the creation of varieties of *C. arabica* resistant to *Hemileia vastatrix* BERK. & BR. became a priority on the American continent after the outbreak of the leaf rust disease in Brazil in 1970, whereas it has been a priority in India and East Africa since the 1930s. In the latter region, the coffee berry disease (CBD) is now the main concern.

The principal objectives for selection are (Van der VOSSEN, 1985):

- a good and stable level of yield;
- resistance and tolerance to pests (nematodes, borers) and diseases (leaf rust and CBD);
- improved quality, mostly for Robusta (*C. canephora*) (reduction in caffeine content, organoleptic value);
- industrial processing criteria (e.g. extraction rate for instant coffee);
- adaptation to environment and tolerance to different stress conditions (low temperature, water stress, toxicities);
- use of reduced size coffee trees allowing high density plantations.

2. AGRONOMIC VALUE

The characterization and evaluation of coffee trees in collections demonstrate the potential diversity and its differentiation at the level of species and populations. We shall only give examples of agronomic characteristics of interest to the breeder (BERTHAUD, 1986).

* Resistance to pests and diseases
  Among the *C. arabica* strains collected in Ethiopia, some are resistant to *Hemileia vastatrix* and to *Colletotrichum coffeanum* NOACK (sensu HINDORF). In *C. canephora* some trees have been found partially resistant to *H. vastatrix* and with a fair tolerance to certain species of nematodes. Also found are species immune to leaf rust and leaf miner, such as *C. congensis* FROEHNER, *C. stenophylla* G. DON, and species from Madagascar and the Mascarenes, '*Mascarocoffea*' CHEV.

* Ecological adaptations
  Trees of *C. congensis* tolerate temporary submersion of the roots. The species originating from dry tropical regions, such as *C. racemosa* LOUR. from Mozambique area, can withstand long dry seasons by losing their leaves, by reducing the flower-fruit cycle to a maximum of 2 months.

* Caffeine content
  Caffeine content varies considerably within and between species. The highest contents are found in *C. canephora* (1.5 to 3.5%) whereas *C. arabica* has lower levels (0.7 to 1.9%). Malagasy and East African species which contain little or no caffeine have been put into our collection: *C. eugenioides* S. MOORE (0.2 to 0.7%), *C. pseudozanguebariae* BRIDSON and species of the '*Mascarocoffea*' group (caffeine free).

* Tree architecture
  Coffee plants are bushes or trees, with one or several trunks. Within *C. canephora* 3 wild sorts are found: one, monocaulous, well branched and with small fruits (originating from West Africa); a second, a vigorous bush type with several trunks and few branches bearing large fruits (originating from Central Africa); and finally, a rather small tree type, early and fertile (originating from the Central African Republic) called 'Nana coffee'.

3. GENETIC ORGANIZATION OF THE *COFFEA* GENUS

Studies at the genetic level can provide a better understanding of the relationship between populations of the same species, and group structures can be found.
Most of the information comes from isozyme analyses. For example, *C. canephora* was thoroughly studied using native material collected and clones formerly established in living collections. Two groups stand out as described previously (Fig. 1): the Guinean group includes West African forms and the Congolese group includes Central African forms (BERTHAUD, 1986).

As a result of the study of our collections, we can report the existence of multispecies populations and point out the following:

1) Interspecific hybridization in wild populations is rare.
2) Several factors limit gene flow within multispecies populations (e.g. distance of pollination, shift in the period and the timing of flowering).

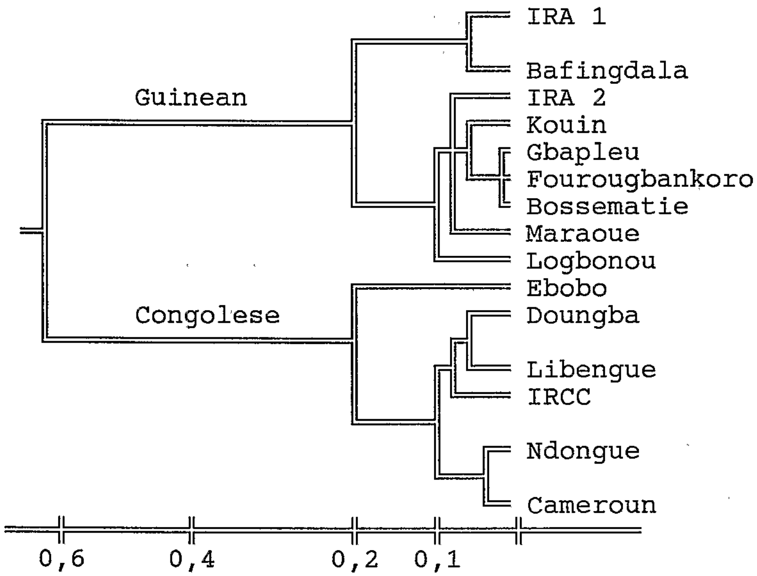

Fig. 1. Genetic organization of *C. canephora* provenances based on NEI's genetic distance. (Adapted from BERTHAUD, 1986).

Experimental interspecific hybridization helps us to characterize cytogenetic relationships between species and their reproductive barriers. Reports of extensive work done in Brazil (CARVALHO & MONACO, 1968), in India (VISHVESHWARA, 1975), in Madagascar (CHARRIER, 1978a) and in Ivory Coast (LOUARN, 1982) have been published.

Hybrids between most of the diploid species have been produced. LOUARN (1982) defined crossing ability in three categories according to their success (Fig. 2). Interspecific breeding barriers do not fit well with species groups defined by some taxonomists (e.g. CHEVALIER, 1947; LEROY, 1967). It is observed that the best results are found when *C. eugenioides* or *C. congensis* are involved in crossing; with *C. canephora*, results are clearly lower. Fertility of allodiploid hybrids varies widely and relies on several mechanisms (gene or chromosomal sterility). Nevertheless, cytological studies show only one basic genome for all diploid species.

Many interspecific hybridizations between diploid species and *C. arabica* have been tested: triploid hybrids were obtained with good cross-success, but they are all sterile. Their meiotic behaviour is similar, with 11 potential bivalents corresponding to the pairing with the common haploid genome of the diploid species (CHARRIER, 1978a).

4. BREEDING SCHEMES

The consequences of this genetic structure within species and the relationships among species for breeding purposes have to be analyzed.

C. arabica

Intraspecific variation in the self-compatible species *C. arabica* has been somewhat under-estimated and under-utilized. New information on studies conducted with wild arabica coffee gives us useful information (CHARRIER, 1978b). The most important polymorphism is observed according to geographic origins.

Nonetheless, noticeable intra-population variation due to residual allogamy exists in the centre of origin of the species. Consequently, descendants of Ethiopian coffee must be 'fixed' before using them either directly or in crosses.

Gene exchange between various diploid species and *C. arabica* has been the focus of great interest in the last twenty years. In Indonesia, natural introgression of *C. arabica* by Robusta (which gave birth to the 'Hybride de Timor') serves as a model. This hybrid being resistant to rust and to CBD has transferred these characteristics to compact shape varieties of *C. arabica* (BETTENCOURT, 1984). This method was explored in Brazil (CARVALHO, 1982), Kenya (OWUOR & Van der VOSSEN, 1981) and Ivory Coast (CHARRIER, 1982): few tetraploid plants were produced through colchicine treatment from a large gene pool of diploid species, and then crossed with *C. arabica*; the hybrids were backcrossed with *C. arabica* to obtain plants with most of the *C. canephora* characteristics but retaining some of the diploid coffee characteristics (Fig. 3).

C. canephora

C. canephora is an allogamous diploid species consisting of polymorphic populations and of strongly heterozygous individuals. Using wild coffees, BERTHAUD (1986) has carried out an analysis of enzymatic polymorphism. A synthetic representation of the interpopulation genetic distances shows a division into two geographic groups: Guinean and Congolese. This author proposed a strategy of reciprocal recurrent breeding because the best hybrid and clonal varieties are derived from intergroup hybrids. This new scheme is presently under way in Ivory Coast and will exploit the variability of wild collections.

Coffee researchers have created a large number of diploid interspecific hybrids, the usefulness in breeding of which depends on their fertility and characteristics (LOUARN, 1987). For example, the fertile 'Congusta hybrids' resulting from crossing of *C. canephora* and *C. congensis* possess some interesting agronomic characteristics, e.g. adaptability to flooded and sandy soils and green coffee similar to Arabica. Vegetative propagation of 'Congusta hybrids' in Madagascar has produced clonal varieties as productive as those of *C. canephora*. Useful characters from other diploid species can be incorporated into recurrent programmes of *C. canephora* via allodiploid hybrids after fertility restoration in the F2 generation.

C. CONSERVATION OF GENETIC RESOURCES OF *COFFEA*

1. WILD COFFEE COLLECTIONS

Wild coffee species are found in Africa, Madagascar and the Mascarenes. *C. arabica* is wild in Ethiopia. Collecting missions were organized in this country by FAO in 1964 and by ORSTOM, in 1966. After these missions, an Ethiopian national programme was set up to organize exploration and conservation of *C. arabica*. Wild coffee collecting in Madagascar began during the same period. After 1975, several surveys and collections in Africa were set up. A summary of these explorations led by ORSTOM in collaboration with other organizations is given in Table 1 (BERTHAUD & CHARRIER, 1988). It can be seen that most of the wild coffee areas have been explored.

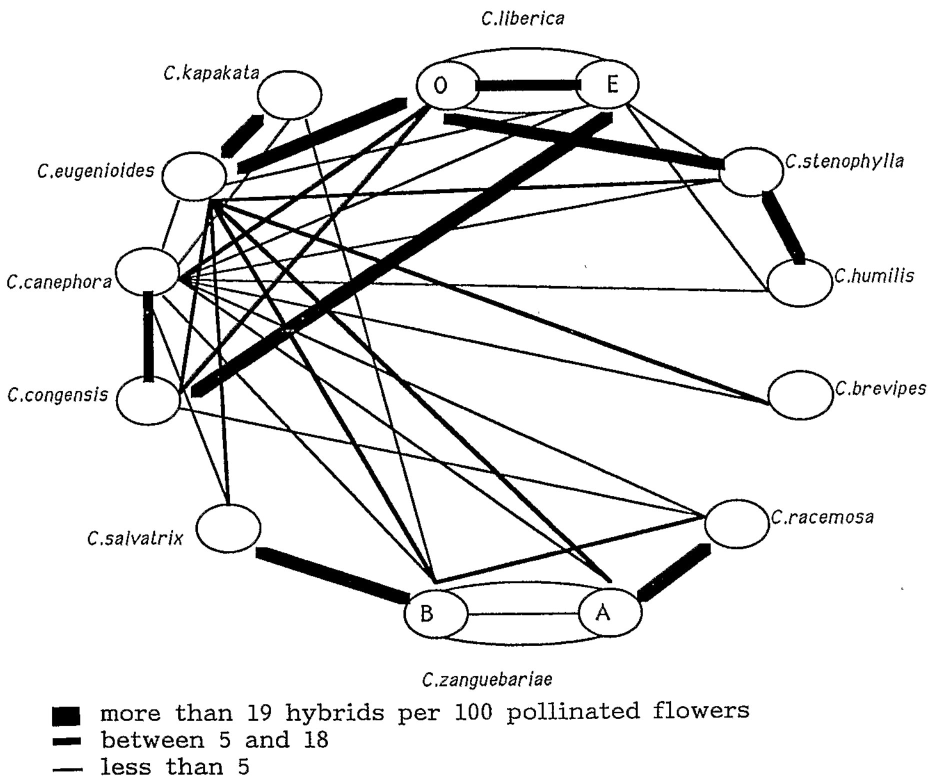

Fig. 2. Cross-success rate in diploid *Coffea* interspecific hybridization (adapted from LOUARN, 1982).

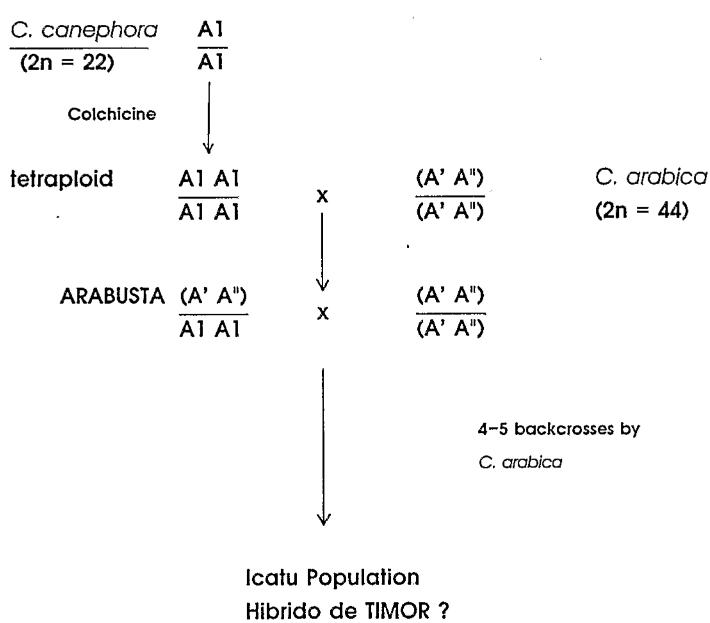

Fig. 3. Arabusta hybrids and introgressive strains with *C. arabica*.

These surveys were aimed at collecting living material. After each survey a representative sample was left in the country surveyed, for use by national research programmes. The remaining material was transferred to the Coffee Genetic Resource Unit in the Ivory Coast, through intermediate quarantine, for conservation and other studies.

In this centre, a living collection has been established and has required adaptation of many horticultural techniques (especially grafting techniques) in order to rescue interesting genotypes and to grow in one place various species with very different ecological requirements. Currently, living collections in the field have samples from more than 20 species, and representing more than 8,000 genotypes.

2. *IN SITU* CONSERVATION

We would like to think that all the genetic resources could be preserved in collections, but this is not possible since the number of samples is severely limited due to the cost of maintenance. Moreover, there is always a risk of accidental loss of samples.

It is therefore more effective to propose conservation *in situ* which allows evolution of coffee to continue in these reserves.

Conservation of natural coffee populations must be organized keeping in mind the following (BERTHAUD, 1986):

1 - Coffee has been found mostly in forest reserves (e.g. in Ivory Coast) and seldom in disturbed forests. Besides, ancient sites of wild coffee have been destroyed and replaced by plantations and crops (for example on Mount Kenya, tea plantations have replaced *C. eugenioides* S. MOORE).

2 - Distribution areas of wild coffee vary in size. Some species are widely distributed (e.g. *C. canephora, C. liberica* HIERN) whereas others are found only in very restricted zones (*C. humilis* CHEV., *C. congensis*).
The number of species is unevenly distributed throughout regions: 4 in West Africa; 10 in Central Africa; 22 in East Africa and 50 in the Malagasy region.

3 - From the phytogeographical view point, there is a clear distinction between forests of Central and West Africa on one hand, and East Africa on the other. These differences concern more specifically the species of coffee characterizing these forests and their spatial distribution within the forests. The forest zone of Central and West Africa shows a continuous distribution, whereas in East Africa we observe a patchwork distribution.

However, some problems linked with studies of wild coffee populations must be stressed (BERTHAUD, 1986):

1 - Due to forest exploitation, the existing and potential reserves have become isolated from one another and therefore, are evolving independently. The coffee species in West and Central Africa are now being exposed to conditions very different from those which have prevailed up to the present.

2 - Gene flow between and within wild populations has been traced, using genetic markers. In the Ira forest Reserve (Ivory Coast) a *C. canephora* population was observed. An analysis showed that up to 50% of the descendants of some coffee trees were produced from foreign pollen from coffee plantations surrounding the reserve: there is pollution of the wild population by cultivated forms and so a very different evolution from that prevailing when gene exchange existed only between wild populations.

Table 1. Coffee surveys led by ORSTOM since 1960. After BERTHAUD & CHARRIER (1988) updated.

Years	Countries surveyed	Institutions	Species collected	Location of living collections
1966	Ethiopia	ORSTOM	*C. arabica* L.	Ethiopia Ivory Coast
1960-1974	Madagascar area	MUSEUM IRCC	'*Mascarocoffea*' (50 taxa)	Madagascar
1975	Central Africa	IRCC ORSTOM	'Nana' coffee *C. congensis* FROEHNER *C. liberica* HIERN *C. canephora* PIERRE ex FROEHNER	Central Africa Ivory Coast
1975-1981	Ivory Coast	ORSTOM	*C. canephora* PIERRE ex FROEHNER *C. humilis* A. CHEV. *C. liberica* HIERN *C. stenophylla* G. DON *Psilanthus* spec. HOOK. f.	Ivory Coast
1977	Kenya	IRCC ORSTOM	*C. arabica* L. *C. eugenioides* S. MOORE *C. fadenii* BRIDSON *C. pseudozanguebariae* BRIDSON *C. sessiliflora* BRIDSON	Kenya Ivory Coast
1982	Tanzania	IRCC ORSTOM	*C. mufindiensis* A. CHEV. *C. pseudozanguebariae* BRIDSON *C.* spec. E. *C.* spec. F.	Tanzania Ivory Coast
1983	Cameroon	IBPGR IRCC ORSTOM	*C. brevipes* HIERN *C. canephora* PIERRE ex FROEHNER *C. congensis* FROEHNER *C. liberica* HIERN *C.* spec. nov. *Psilanthus* spec.	Cameroon Ivory Coast
1985	Congo	IBPGR IRCC ORSTOM	*C. brevipes* HIERN *C. canephora* PIERRE ex FROEHNER *C. congensis* FROEHNER *C. liberica* HIERN *C.* spec.	Congo Ivory Coast
1987	Guinea	EEC IRCC ORSTOM	*C. canephora* PIERRE ex FROHNER *C. liberica* HIERN *C. stenophylla* G. DON	Guinea Ivory Coast

3 - The maintenance of coffee reserves for their taxonomic diversity, allows long term conservation of a greater number of *Coffea* species, than that possible in the base collections of the Genetic Resources Units (GRU). If one hectare of forest contains about 30 coffee trees, a total of 300 hectares distributed throughout wet tropical Africa should allow the conservation of 9,000 coffee trees; a number equivalent to our collection today in the Ivory Coast.

3. BIOTECHNOLOGICAL METHODS OF GENETIC CONSERVATION

Germplasm conservation of species, such as the coffee trees, whose seeds and pollen are recalcitrant to dehydration and low temperature is not easy over the long term. In this case, the possibility of storage by *in vitro* methods associated or not with freezing is interesting.

A medium-term storage assay has been carried out by KARTHA et al. (1981), maintaining microplantlets in tubes during 2 years without any subculture. *In vitro* regeneration of plantlets by micropropagation of shoot tips or somatic embryogenesis on leaf explants or calluses has been carried out with cultivated coffees (DUBLIN, 1984; SONDAHL & LOH, 1988).

Cryo-conservation of meristems or zygotic and somatic embryos has already been undertaken for long-term preservation of a few plants. Recently, DESBRUNAIS et al. (1988) obtained the first result of survival and proliferation of *C. arabica* somatic embryos after freezing in liquid nitrogen. This method is now in progress with different coffee species. But, to be feasible, it will be necessary to be sure that *in vitro* propagation methods are adapted to coffee genetic resources and that the following limiting factors are overcome: ability to propagate any genotype, factors limiting growing rate, long-term genetic stability, low-level technology of the developing countries.

D. POTENTIAL VALUE OF COFFEE GENETIC RESOURCES

As a first step, the actual cost of the establishment and maintenance of a wild coffee collection was evaluated. Then the cost was calculated of increasing the value of this plant material for a breeding programme. Such estimates are based on our own research experience in the Ivory Coast but do not take into account the cost of supporting infrastructures. Finally, we will try to measure the impact of the genetic resources on coffee production in comparison with the investment.

1. THE COST OF COLLECTIONS

ORSTOM has carried out explorations in the African forests with French and international support (CEE, IBPGR). Each mission covered only one country and required a large logistic support for the transport of 3 to 5 research teams for two months. The collected plant material was gathered in a single centre for coffee genetic resources in the Ivory Coast. This involved the introduction of several thousand plants and seeds after each mission. It was necessary to transport them rapidly over long distances by aeroplane, to quarantine them in greenhouses, and then to multiply them by grafting and to raise them in a nursery for 1 or 2 years before planting them in the field. The success of this enterprise required technically qualified personnel (BERTHAUD et al., 1984).

The cost price of one exploration and the development of one collecting expedition is in the range of US $ 80,000. During the 8 explorations completed in Africa, 8,000 original genotypes were collected. Each strain in the collection costs about US $ 80.

2. INCREASING THE VALUE OF GENETIC RESOURCES

A wild coffee strain in a collection has only a potential value. Its eventual exploitation in the creation of varieties depends on a long-term evaluation and selection, lasting at least 20 years. For example, the *C. arabica* strain collected in Sudan in the 1940s and called 'Rume Sudan' was used in East Africa in the 1970s-80s to improve the resistance to CBD (Van der VOSSEN, 1985). The Guinean form of *C. canephora* which was found in the 1970s in the Ivory Coast has been used for a short time in a reciprocal recurrent selective scheme which will develop new selected clones at the beginning of the 1990s (BERTHAUD, 1986).

For increasing the value of coffee genetic resources we propose 10 years for studying the diversity of the material, for choosing genomes which have interesting characteristics and for obtaining progenies. Following this would come a period of 10 years for selection and experimentation with new varieties. Practical realization of such research will require at least:

- 15 hectares for evaluation tests and for the study of descendants by two geneticists (cost: US $ 16,700/ha/year).
- 15 hectares of field for selection and varietal trials by two agronomists (cost: US $ 14,400/ha/year).

3. GLOBAL ANALYSIS

On Table 2 are listed the costs for acquiring, maintaining and improving the value of the coffee genetic resources over 20 years, based on the programme carried out by ORSTOM and IRCC in the Ivory Coast.

Table 2. Cost of the coffee genetic resources (US $) (GRU Ivory Coast).

1) Establishment base collection (exploration, introduction) $ 80/genotype	8,000 genotypes	$ 640,000	9%
2) Maintenance in collection $ 10/genotype/year	1 technician 8,000 genotypes 20 years	$ 1,600,000	24%
3) Evaluation (variability, hybridization) $ 16,000/ha/year	2 geneticists 15 ha 10 years	$ 2,400,000	35%
4) Selection (experimental trials) $ 14,400/ha/year	2 agronomists 15 ha 10 years	$ 2,160,000	32%
	Total cost or	US $ 6,800,000 US $ 340,000/year	

This means for the whole programme a total cost of US $ 6,800,000 over 20 years or US $ 340,000/year.

This evaluation over 20 years will allow a better appreciation of the relative size of each operation. The investment to acquire wild coffees and to start a base collection represents less than 10% of the total cost; this percentage will be even lower if collections are obtained by exchange between several regional or continental coffee research centres.

The maintenance of a collection and its conservation over 20 years represents one quarter of the total cost. The possession of such patrimony is not useful unless it is integrated into a programme of varietal improvement, whose cost amounts to 67% of the total.

The whole financial cost of such a programme for exploitation of coffee genetic resources (US $ 340,000/year plus the infrastructure expenses) can be usefully compared to the value of the coffee production. Considering only African countries, the cost of such an improvement programme for coffee trees is about 0.1% (200 tons of coffee/year) of the total production of coffee in Ethiopia (170,000 tons of Arabica) or in the Ivory Coast (250,000 tons of Robusta). The cost would be reduced by half if the coffee genetic resource centre was exploited at a regional level.

4. POTENTIAL INCREASE OF THE VALUE OF GERMPLASM

The potential increase in value of a genetic resource can be based on hypotheses and situations of rather different types. Recall the average level of productivity for one hectare of plantations: it varies from 200 to 500 kg/ha in Africa; from 600 to 800 kg/ha in America, and reaches the exceptionally high level of 1,000 to 1,500 kg/ha in places like Costa Rica. Comparatively, the potential of selected varieties under favourable ecological and agronomic conditions, is in the order of 2 or 3 tons coffee/ha, i.e. 2 to 10 times the present productivity. Evidently, we could attribute a potential value to the new selected varieties linked with the improvement of production obtained in trials at research stations, or the reduction of fungus treatments by using varieties resistant to rust or to the CBD, etc.

But, the increase of productivity depends as much on agronomic practices as on the diffusion of technical and economic progress in agriculture. Coffee production being mostly practiced by small growers in developing countries, it is not realistic to link directly the germplasm value with the productivity of the improved varieties. Its impact is more closely associated with the global annual growth rate of agriculture in these countries. Considering the example of the development of cereal production in Europe since 1945, we obtain a rate of 2 to 3% which means a doubling of production per hectare in 25 to 30 years. Such an hypothesis allows us to determine the average production increase (profit) and to attribute half of it to genetic progress.

Therefore, the increase in value of the germplasm for the coffee producing countries would be in the order of:

- 2,000 tons of arabica coffee/year in Ethiopia;
- 3,000 tons of robusta coffee/year in the Ivory Coast;
- 5,000 tons of arabica coffee/year in East Africa.

Such profit could be considered modest but compared to the cost of collections and selection work, the increase of production due to improved new varieties represents 15 times the cost of the selection programme in Ethiopia and in the Ivory Coast, and 25 times the cost of a regional programme for East or West Africa. The increased value of genetic resources could thus be largely assumed by the major coffee producing countries and by regional programmes.

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