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9

Coffee Germplasm Resources, Genomics, and Breeding

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I. INTRODUCTION
II. GERMPLASM RESOURCES
  A. Wild and Cultivated *Coffea*
  B. Collecting Wild Species
  C. Strategies for Conservation
III. GENOMICS
  A. Developing Linkage Maps
  B. Mapping Genes and QTLs Controlling Biotic Stress and Economic Traits
  C. Construction of Large Insert Genomic Libraries
  D. Coffee Expressed Sequence Tags
IV. BREEDING
  A. Historical and New Trends
  B. Exploiting Genetic Diversity
    1. Disease Resistance
    2. Nematode Resistance
    3. Other Types of Resistance or Tolerance
    4. Low Caffeine Content

Plant Breeding Reviews, Volume 30, Edited by Jules Janick
ISBN 978040171523 © 2008 John Wiley & Sons, Inc.

C. Propagation Systems
1. Seed Propagation
2. Clonal Propagation
3. F_1 Hybrids
D. Future Based on Biotechnology
V. LITERATURE CITED

I. INTRODUCTION

Coffee is the second largest export commodity in the world after petroleum products with an estimated annual retail sales value of US $70 billion in 2003 (Lewin et al. 2004). Over 10 million hectares of coffee were harvested in 2005 (http://faostat.fao.org/) in more than 50 developing countries, and about 125 million people, equivalent to 17 to 20 million families, depend on coffee for their subsistence in Latin America, Africa, and Asia (Osorio 2002; Lewin et al. 2004). Coffee is the most important source of foreign currency for over 80 developing countries (Gole et al. 2002).

The genus *Coffea* (Rubiaceae) comprises about 100 different species (Chevalier 1947; Bridson and Verdcourt 1988; Stoffelen 1998; Anthony and Lashermes 2005; Davis et al. 2006, 2007), and new taxa are still being discovered (Davis and Rakotonasolo 2001; Davis and Mvungi 2004). Only two species are of economic importance: *C. arabica* L., called arabica coffee and endemic to Ethiopia, and *C. canephora* Pierre ex A. Froehner, also known as robusta coffee and endemic to the Congo basin (Wintgens 2004; Illy and Viani 2005). *C. arabica* accounted for approximately 65% of the total coffee production in 2002–2003 (Lewin et al. 2004).

Dozens of *C. arabica* cultivars are grown (e.g., 'Typica', 'Bourbon', 'Catuai', 'Caturra', 'Maragogipe', 'Mundo Novo', 'Pacas'), but their genetic base is small due to a narrow gene pool from which they originated and the fact that *C. arabica* is the only tetraploid species in the genus ($2n = 4x = 44$) and is self-pollinated (Carvalho et al. 1991). In contrast, *C. canephora* is a diploid ($2n = 2x = 22$) and is cross-pollinated. Evidence for a gametophytic system of incompatibility that is controlled by one gene with multiple alleles has been reported by Berthaud (1980). It is estimated that the genetic variation in *C. canephora* is 10 times that in *C. arabica* (Lashermes et al. 2000). Data from Berthou et al. (1980, 1983) and Lashermes et al. (1999) has revealed that *C. arabica* contains two genomes resulting from hybridization between the diploids *C. eugenioides* Moore as maternal donor and *C. canephora* as paternal donor (Anthony and Lashermes 2005). In contrast, Raina et al. (1998) reported *C. congensis* and *C. eugenioides* as the progenitors of *C. arabica*.

Vega et al. (2003) have made a call for the establishment of an international coffee research development program that could coordinate coffee research efforts throughout the world, in an effort to avoid duplication and to better manage scarce research funds. Such an effort would serve to coordinate all coffee research, including pest and disease management, breeding, and molecular biology endeavors. To address the issue of coffee molecular biology, a meeting was held in 2004 at the United States Department of Agriculture (USDA) with scientists from the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD, France), the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE, Costa Rica), and USDA, together with Ted Lingle, Chief Executive Officer of the Specialty Coffee Association of America (currently the Director of the Coffee Quality Institute). The purpose of the meeting was to address the needs within the coffee industry for a better understanding of how molecular biology tools could help the industry.

Subsequently and completely independent of the USDA meeting, a call to establish an International Coffee Genomics Network (ICGN) was made by scientists from over 13 countries at the 2004 Association Scientifique International du Café (ASIC) conference held in Bangalore, India. The ultimate goal of the ICGN would be "to decipher the genetic and molecular bases of important biological traits in coffee tree species that are relevant to the crop." Thus, it is clear that there is an international interest in developing a coordinated effort targeted at coffee molecular biology.

The speed at which DNA is being sequenced is staggering, with one laboratory reporting that the genomes of 15 microorganisms were sequenced in one month (Preuss 2000). So far, over 180 organisms have been sequenced (Genome News Network 2006). The genome for *C. arabica* consists of approximately 1,176 million base pairs (Mbp) compared with approximately 386 Mbp for cacao, 482 Mbp for mango, and 965 Mbp for tomato (Kiehn 1986; Bennett and Leitch 1995). In 2003 the Minister of Agriculture of Brazil announced that Brazilian scientists had decoded the coffee genome (Hay 2004), and a recent paper by Vieira et al. (2006) reports approximately 33,000 genes. Obviously there is a strong interest among the rest of the coffee producers in understanding how the decoding of the coffee genome could help them. What would a coordinated effort on coffee molecular biology provide? This article is aimed at answering this question.

II. GERMPLASM RESOURCES

A. Wild and Cultivated *Coffea*

Natural habitats of wild coffee trees are found in the understory of tropical forests in Africa comprising a wide geographical range from Guinea in West Africa through Central to eastern Africa (Charrier and Berthaud 1985; Davis et al. 2006). Other centers of diversity include Madagascar and the Comoros and Mascarene islands in the Indian Ocean (Davis et al. 2006). Centers of origin and primary diversity of the commercially most important species, *C. arabica*, are located in the highlands of southwestern Ethiopia, the Boma Plateau in southeastern Sudan, and on Mount Marsabit in Kenya (Thomas 1942; Sylvain 1955, 1958; von Strenge 1956; Meyer et al. 1968; Anthony et al. 1987). The tributaries of the Congo River represent a rich center of genetic diversity for *C. canephora* as well as many other diploid coffee species, such as *C. congensis* and *C. liberica* (Dulloo et al. 2001; Davis et al. 2006). Another center of genetic diversity of wild coffee species in East Africa is located in the Eastern Arc Mountain of Tanzania, where Davis and Mvungi (2004) recently described two new endangered species of *Coffea*: *C. bridsoniae* and *C. kihansiensis*. This brings the total number of naturally occurring wild *Coffea* species in Tanzania to 16 (Davis and Mvungi 2004), surpassed only by Madagascar with 59 species (Davis et al. 2006). Chevalier's (1947) infrageneric classification of *Coffea* into four sections (Argocoffea, Eucoffea, Paracoffea, and Mascarocoffea) had many weaknesses and is no longer accepted. The current classification divides the genus in two subgenera: *Coffea* subgen. *Coffea* and *Coffea* subgen. *Baracoffea* (Davis et al. 2006 and references therein).

The narrow gene pool of cultivated *C. arabica* is based mostly on how it disseminated throughout the world. Historical narratives have traced the movement of coffee from southwestern Ethiopia to Yemen; then to India; and from there to Ceylon and Java; and in about 1710 to Amsterdam; from there to Paris; and finally, in about 1718, from Paris a plant was taken to Martinique by the French captain Matthieu de Clieu. It is believed that progeny from this plant, later on referred to as 'Typica', gave origin to plantations throughout South America. Plants were also taken from Yemen to the island of La Réunion in about 1715 and gave rise to a widely used cultivar known as 'Bourbon' (Anthony et al. 2002). Thus, there is a clear genetic bottleneck that gave rise to commercially grown coffee outside its center of origin.

Molecular studies have provided evidence for reduced genetic variability among cultivated *C. arabica* varieties except those in Ethiopia.

Steiger et al. (2002) compared 61 coffee accessions comprising the *C. arabica* cultivars 'Bourbon', 'Catimor', 'Catuai', 'Caturra', 'Typica', and 'Tall Mokka' (previously known as 'Mokka Hybrid') plus *C. canephora* and *C. liberica*. Using a technique known as amplified fragment length polmorphisms (AFLP), which results in specific DNA markers, they found an average genetic similarity of 0.93 (1 being completely identical) among *C. arabica* cultivars based on simple matching coefficient. Their results also indicated that *C. arabica* shares an average similarity of 0.54 with *C. canephora*, in contrast to 0.41 with *C. liberica*, thus providing evidence that *C. arabica* is more closely related to *C. canephora* than to *C. liberica*. The reduced genetic variability in *C. arabica* cultivars was also evident in a study by Moncada and McCouch (2004), where they used a different molecular technique known as simple sequence repeats (SSRs) on 30 *Coffea* accessions from the germplasm collection at the Centro Nacional de Investigaciones de Café (Cenicafé) in Colombia, and in a study by Anthony et al. (2002) using both AFLP and SSRs with 26 *Coffea* accessions. Based on AFLP results, Lashermes et al. (2000) reported that the self-incompatible *C. canephora* is 10 times more polymorphic than *C. arabica*.

In accessions collected from occasional escapes outside cultivated areas in the center of origin and primary diversity of *C. arabica* (i.e., Ethiopia), polymorphism was much higher than in cultivated accessions (Anthony et al. 2002). The use of inter-simple sequence repeat (ISSR) markers revealed high genetic variation between populations from different regions of Ethiopia, while genetic differentiation within the same population or among populations from the same region was much less pronounced (Aga et al. 2005). Greater genetic variation was detected in *C. arabica* germplasm from Africa than in germplasm from America and India using SSRs (G. Graziosi, pers. comm.). These and previous studies raised the awareness that successful *C. arabica* breeding programs depend to a large extent on the availability of genetic diversity from its center of origin. This fact has led to major collecting missions during the past decades (Table 9.1).

B. Collecting Wild Species

For coffee, there have been no alternatives so far to *ex situ* field collections for long-term germplasm conservation. Coffee seeds are recalcitrant, and conventional methods of seed storage cannot extend viability beyond two to three years. Important *C. arabica* field gene banks are located in Africa (Cameroon, Ethiopia, Ivory Coast, Kenya, and Tanzania), Madagascar, India, and the Americas (Brazil, Colombia, and Costa

Table 9.1. Principal collecting missions for *Coffea arabica*.

Year	Country Explored	Organization[z]	Collector	Countries Maintaining Germplasm
1964–65	Ethiopia	FAO	F.G. Meyer, L.C. Monaco, R.L. Narasimhaswamy, L.M. Fernie, and D.J. Greathead	Ethiopia, Costa Rica, India, Peru, Tanzania
1966	Ethiopia	ORSTOM	J.L Guillaumet and F. Hallé	Ethiopia, Cameroon, Ivory Coast, Madagascar
1977	Kenya	ORSTOM, CIRAD	J. Berthaud, J.L. Guillaumet, and M. Lourd	Kenya, Ivory Coast
1989	Yemen	IPGRI, CIRAD	A.B. Eskes	Yemen, Costa Rica, Brazil

[z]FAO = Food and Agriculture Organization of the United Nations
ORSTOM = Office de la Recherche Scientifique et Technique Outre-Mer; renamed Institute de Recherche pour le Développement (IRD) in 1998
CIRAD = Centre de Coopération Internationale en Recherche Agronomique pour le Développement
IPGRI = International Plant Genetic Resources Institute

Source: Adapted from Anthony et al. 1999; Charrier and Berthaud 1985; Eskes, personal communication.

Rica) (Anthony et al. 1999). *C. canephora* is well represented in the field collections of Cameroon, India, Ivory Coast, and Madagascar. The latter also holds a unique collection of approximately 50 species of the section *Mascarocoffea*. Approximately 30 species of diploid African coffee are mainly conserved in Ivory Coast, with, unfortunately, zero accessibility at present due to the ongoing civil war.

Exploration missions for wild *C. arabica* were carried out in its center of origin (Ethiopia and Kenya) and in the secondary center of diversity, Yemen. During 1964–65, a Food and Agriculture Organization of the United Nations (FAO) mission collected coffee germplasm in different locations in Ethiopia (Meyer et al. 1968), followed by an ORSTOM (Office de la Recherche Scientifique et Technique Outre-Mer; renamed Institute de Recherche pour le Développement [IRD] in 1998) mission in 1966 collecting germplasm from 70 different origins (Table 9.1). While some of these materials were obtained from the understory of tropical forests, the majority was collected from cultivated coffee (Anthony et al. 1999). An ORSTOM/CIRAD mission to Kenya collected 80 different materials of *C. arabica* at Mount Marsabit, as well as samples of *C. eugenioides*, *C. zanguebariae* and *C. fadenii* (Table 9.2). Finally, a 1989 International

Table 9.2. Principal collecting missions for diploid coffee species.

Years	Country Explored	Organization[z]	Collector	Coffee Species	Country Maintaining Germplasm
1960–1974	Madagascar, Mauritius, Reunion Island, Comoro Islands	Museum of Paris, CIRAD, ORSTOM	J.F. Leroy, R. Portères, A. Vianney-Liaud, J.L. Guillaumet, and A. Charrier	50 species	Madagascar
1975	Central African Republic	ORSTOM, CIRAD	J.L. Guillaumet and J. Berthaud	*C. congensis*, *C.liberica* var. *dewevrei*, *C. canephora*, *C. liberica*, Caféier de la Nana	Ivory Coast, Central African Republic
1975–1980	Ivory Coast	ORSTOM	J. Berthaud	*C. liberica*, *C. stenophylla*, *C. canephora*, *C. humilis*, *Paracoffea* sp., *Psilanthus* sp.	Ivory Coast
1977	Kenya	ORSTOM, CIRAD	J. Berthaud, J.L. Guillaumet, and M. Lourd	*C. arabica*, *C. eugenioides*, *C. fadenii*, *C. pseudozanguebariae*, *C. fadenii*, *C. sessiliflora*	Kenya, Ivory Coast
1982	Tanzania	ORSTOM, CIRAD	J. Berthaud, F. Anthony, and M. Lourd	*C. costatifructa*, *C. pseudozanguebariae*, *C. mufindiensis*, *C. sessiliflora*, *C.* sp.	Tanzania, Ivory Coast
1983, 1985, 1987	Cameroon	IPGRI (1983), ORSTOM (1985), CIRAD (1987)	F. Anthony, E. Couturon, and C. de Namur	*C. brevipes*, *C. canephora*, *C. congensis*, *C. liberica*, 4 species of *C.* spp., *Psilanthus* spp.	Cameroon, Ivory Coast
1986	Congo	IPGRI, IRD, CIRAD	C. De Namur, E. Couturon, P. Sita, and F. Anthony	*C. canephora*, *C. congensis*, *C. liberica*, 5 species of *Coffea* spp., *Psilanthus* spp.	Congo, Ivory Coast
1987	Guinea	IRD, CIRAD	D. La Pierrès, P. Charmetant, A. Yapo, T. Leroy, E. Couturon, S. Bontems, and H. Tohe	*C. canephora*, *C. humilis*, *C. liberica*, *C. stenophylla*, *Psilanthus* spp.	Guinea, Ivory Coast

[z]CIRAD = Centre de Coopération Internationale en Recherche Agronomique pour le Développement
ORSTOM = Office de la Recherche Scientifique et Technique de Outre-Mer; renamed Institute de Recherche pour le Développement (IRD) in 1998. IPGRI = International Plant Genetic Resources Institute

Source: Adapted from Anthony et al.1999; Charrier and Berthaud 1985; P. Charmetant, personal communication.

Plant Genetic Resources Institute[1] (IPGRI)/CIRAD mission concentrated on Yemen, where samples were collected from coffee plantations from 22 different origins. The mission identified six morphologically different types of coffee plants.

Collecting missions for other *Coffea* species were motivated by the rapid destruction of the tropical forest ecosystems in Africa, Madagascar, and the Comoros and Mascarene islands. These missions (Table 9.3) resulted in the collection of material from 20,000 wild coffee trees representing more than 70 species and in the identification of 300 wild coffee populations (Anthony et al. 1999).

CATIE's International Coffee Germplasm Center at its headquarters in Turrialba, Costa Rica, dates back to the late 1940s and is being maintained on an area of nine hectares under the shade of the leguminous tree species *Erythrina poeppigiana* (Walp.) O. F. Cook (known as poró in Spanish) and, to a lesser extent, *Eucalyptus deglupta* Blume. CATIES's collection is the third largest coffee field gene bank in the world, after those at the Ivory Coast and Cameroon (Monge and Guevara 2000) and comprises to a large extent the entire genetic diversity of *C. arabica*

Table 9.3. Description of genetic material conserved at CATIE's International Coffee Germplasm Center for the Western Hemisphere.

Type of Material	No. Introductions	No. Individuals
1. Wild and Semi-wild Genotypes		
Collected by FAO in Ethiopia	433	1,650
Collected by ORSTOM in Ethiopia	148	420
Collected by IPGRI and CIRAD in Yemen	11	20
Diploid species	288	592
SUBTOTAL 1	880 (44.3%)	2,682
2. Cultivars, Mutants, and Selections		
Cultivars from Ethiopia	191	950
Cultivars of 'Typica' and 'Bourbon'	293	1,810
Introgressed varieties from *C. canephora*	312	1,804
Mutants and other selections	84	650
Unclassified varieties	43	250
SUBTOTAL 2	923 (46.5%)	5,464
3. Hybrids		
Interspecific hybrids	19	90
Intraspecific hybrids	165	820
SUBTOTAL 3	184 (9.3%)	910
TOTAL (1+2+3)	1,987	9,056

(Anthony et al. 1999) with a total of 1987 accessions and over 9,000 coffee trees (Table 9.3). The genetic diversity of *C. canephora* (68 introductions) and *C. liberica* (24 introductions) is represented to a lesser extent.

CATIE's International Coffee Germplasm Center forms part of the registry of base collections, which were established by the International Board for Plant Genetic Resources (IBPGR) in the 1970s. Since May 2004, all major germplasm collections held in trust at CATIE form part of the International Network of Ex-Situ Collections of the Consultative Group on International Agricultural Research (CGIAR) under the auspices of FAO and the International Treaty on Plant Genetic Resources for Food and Agriculture. With the designation of its collections to FAO, CATIE started to use the FAO standard Material Transfer Agreement (MTA) for the exchange of germplasm. Under this MTA, the recipient of the germplasm agrees not to claim ownership over the material, nor to seek intellectual property rights over the same or its genetic parts or components, in the form received. This applies also to related information received. The recipient further agrees to ensure that any subsequent person or institution to which he/she may make samples available is bound by the same provisions. The recipient of germplasm is encouraged to share the benefits accruing from its use, including commercial use, through such mechanisms as exchange of information, access to and transfer of technology, capacity building, and sharing of benefits arising from commercialization.

Within CATIE's International Coffee Germplasm Center, these types of genetic material are conserved (Table 9.3):

1. Wild and semi-domesticated genotypes obtained from the primary and secondary center of coffee origin and diversity, Ethiopia and Yemen, respectively. They comprise a total of 880 accessions or 44.3% of the entire collection and were collected by FAO in 1964 and 1965 (433 accessions) and ORSTOM in 1966 (148 accessions) in Ethiopia. Eleven accessions were obtained from a collecting mission conducted by IPGRI and CIRAD in 1989 in Yemen. In addition, this group of genotypes comprises 288 accessions of various diploid species.
2. The other major part of the collection (923 accessions or 46.5%) is composed of cultivars, mutants, and selections. Some were obtained from Ethiopia, others were derived from the base populations 'Typica' and 'Bourbon'; some represent introgressed lines from *C. canephora* as well as unclassified varieties.

3. Inter- and intraspecific hybrids comprising a total of 184 accessions.
4. Research material (not included in Table 9.3).

C. Strategies for Conservation

The future of coffee improvement in terms of quality, yield, and host resistance depends on the availability and use of the largely untapped genes found in the wild, in farmers' fields and in *ex situ* germplasm collections. The high economic value of coffee genetic resources for breeding purposes was demonstrated by Hein and Gatzweiler (2006). Based on a 30-year discounting period, their cost/benefit calculations resulted in a net present value of genetic resources from Ethiopian highland forests of US $1,458 and $429 million, at discount rates of 5% and 10%, respectively.

Part of the genetic diversity existing in the wild and on farms has been collected in the centers of origin and diversity and is being maintained in *ex situ* gene banks with varying success. Five case studies of coffee gene banks were presented by Dulloo et al. (2001): (1) International Coffee Germplasm Center at CATIE, Costa Rica; (2) Coffee Research Center of Kianjavato and Ilaka Est, Madagascar; (3) coffee gene bank in Jimma, Ethiopia; (4) Coffee Research Foundation, Kenya; and (5) coffee collections at Divo and Mont Tonkoui, Ivory Coast. Among these gene banks, CATIE's International Coffee Germplasm Center is the only one in the public domain due to its designation to the International Network of Ex Situ Collections under the auspices of FAO.

CATIE's International Coffee Germplasm Center is suffering from three main problems: (1) the age of the trees, most of which were introduced before 1970 and are now some 35 years old; (2) the suboptimal climatic conditions at an elevation of 602 meters above sea level combined with excessive precipitation; and (3) the cultivation method, which is suited for cultivated accessions but is lacking adaptation for the wild genotypes, which clearly differ in their needs in terms of shade, pruning, and fertilizer application, among others. Substantial losses of coffee plants have occurred over the years, resulting in the loss of entire accessions. Especially among the wild genotypes, a number of accessions are represented by only one or two individuals and require immediate attention.

Given the public domain status and the high value of CATIE's coffee collection for future breeding programs in Latin America, the renovation and relocation of this international coffee germplasm collection to a new site is of utmost importance. For the rejuvenation, a restructuring of the collection into a base collection, mainly composed of wild and semi-domesticated genotypes from Ethiopia and Yemen, and an active collection, composed of selections, landraces, and varieties, has been proposed. The base and active collections will be established at distinct sites to be able to implement adequate collection management, adapted to the needs of the individuals in each category. Dense and almost permanent shade will be needed for the wild genotypes from Ethiopia, while full sunshine is required for the species from East Africa. The rejuvenation and multiplication of the highly valuable and endangered wild genotypes will be of highest priority and will be carried out first. A rejuvenated and restructured international coffee collection with a functional and easily accessible database of related up-to-date information will serve the entire region as strategic asset for future regional breeding efforts, aiming at the improvement of the competitiveness and sustainability of coffee plantations in Latin America.

Since coffee field collections are very costly to maintain and the conserved material is constantly exposed to biotic and abiotic stress, research staff at CATIE developed a methodology for cryopreservation of coffee seeds in liquid nitrogen (−196°C or −320°F) aiming at long-term storage. In *C. arabica*, seed tolerance to the exposure of liquid nitrogen varies greatly, ranging from zero to 74% seed survival after cryopreservation (Vásquez et al. 2005). This susceptibility of coffee seeds seems to be largely due to damages inflicted on the endosperm, as separate experiments have shown that the viability of cryopreserved embryos always remained high. Careful post-thaw rehydration of seeds over a six-week period using an osmo-conditioning treatment led to a dramatic increase in the viability of frozen seeds. Currently, a core subset of 63 accessions of the coffee germplasm collection at CATIE is being cryopreserved. Further studies are necessary to verify whether plantlets derived from long-term cryopreserved seeds are true-to-type in the field.

Besides these mainly curational issues, it is urgent to further evaluate the large gene pool. While vegetative growth parameters of the coffee plants have been studied in the range of 20 to 50% of all the accessions in the collection, bean size has only been assessed in 16.9%, leaf rust resistance in 27.3%, and nematode resistance in 3.9% of all accessions. Molecular characterization has been carried out in 7.6% of the accessions. Future research efforts will focus on evaluating the coffee germplasm for distinctive quality flavors and sources of host resistance to diseases and pests.

III. GENOMICS

A. Developing Linkage Maps

The development of various types of DNA markers has led to the construction of genetic maps in economically important plant species for crop improvement. Linkage mapping is the first step toward molecular dissection of complex traits such as yield components and quality followed by the cloning of genes or quantitative trait loci (QTLs) controlling these traits, and ultimately, the manipulation of genes to increase productivity and improve quality.

Linkage mapping in coffee requires more effort and is more costly than in annual crops due to the longer generation time, a low polymorphism rate in *C. arabica* with the exception of Ethiopian cultivars and germplasm, and the absence of a large collection of DNA markers and genomic sequences. Genetic maps were first constructed in the diploid *C. canephora*. The first one was developed using a doubled haploid (DH) population derived from female gametes and 147 restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers. This map consisted of 15 linkage groups with a total length of 1402 cM (Paillard et al. 1996). A second *C. canephora* genetic map was constructed using a test cross (TC) population derived from the male gametes of the same clone that was used to generate the DH population. This second map consisted of 11 linkage groups spanning 1041 cM with 160 RFLP, RAPD, AFLP, and microsatellite markers, including more than 40 sequence-tagged site (STS), single-copy RFLP, and microsatellite markers (Lashermes et al. 2001).

These two linkage maps of *C. canephora* revealed a substantial amount of genetic information and provided a set of low-copy DNA markers to be used in coffee genetic research and breeding programs. Segregation distortion has been observed in both the DH and TC populations, but distortion was more severe in the DH population (44% of the markers) than in the TC population (13% of the markers). The most significant finding from comparative analysis of these *C. canephora* maps was that the recombination rates were not distinguishable between the two populations, suggesting similar recombination rates in male and female gametes of *C. canephora*.

Linkage maps were constructed from interspecific crosses that revealed insights on genome structure and the level of heterozygosity among diploid species. A linkage map was constructed from a backcross population between the wild species *C. pseudozanguebariae* (PSE) and a once-cultivated species *C. liberica* var. dewevrei (DEW) as the backcross parent (Ky et al. 2000). This map consists of 167 AFLP and 13 RFLP markers on 14 linkage groups with a total length of 1,144 cM. Segregation distortion was detected on 30% of the markers mapped with 26 loci distorted in favor of PSE and 21 loci in favor of DEW. The entire linkage group (LG) C consisted of distorted markers in favor of PSE while LG H consisted of distorted markers all in favor of DEW. Reduced recombination rate led to the reduction of map size in this interspecific mapping population compared with the linkage map constructed from intraspecific populations of *C. canephora* (Paillard et al. 1996; Lashermes et al. 2001). This reduction of map size is likely due to the lower degree of homozygous regions between these two genomes as observed in potato inter- and intraspecific maps (Bonierbale et al. 1988; Gebhardt et al. 1991). A second interspecific map was constructed from a backcross population derived from *C. heterocalyx* (HET) and *C. canephora* (CAN) with CAN as the recurrent parent (Coulibaly et al. 2003b). This map consists of 190 AFLP and SSR markers on 15 linkage groups. AFLP markers appeared to be randomly distributed on this interspecific map, independent of the AT/GC content of the selective nucleotides. Similar phenomena were observed on a high-density linkage map of the papaya genome except for the male-specific region of the Y chromosome (Ma et al. 2004), but correlations between AT/GC content of the selective nucleotides and the number of AFLP bands have been reported in soybean (Keim et al. 1997), *Alstroemeria* (Han et al. 1999), and Pinyon pine (Travis et al. 1998). Another interesting observation from this second interspecific map was that a higher number of total AFLP bands were amplified from the smaller CAN genome than the larger HET genome. This is likely due to the higher level of genome heterozygosity in the self-incompatible CAN than the self-fertile HET (Coulibaly et al. 2003a).

Linkage mapping of the *C. arabica* genome is hindered by the extremely narrow genetic base and its reproductive nature of self-pollination. A preliminary linkage map was constructed using AFLP markers on a pseudo-F_2 population derived from a cross between the cultivars 'Tall Mokka' and 'Catimor' (Pearl et al. 2004). A total of 456 dominant markers and 8 co-dominant markers were generated from 288 AFLP primer combinations. This map consists of 31 linkage groups with the number of markers ranging from 2 to 21, indicating that this was a partial map as gaps remained on the 22 basic chromosomes this map represented. The total length of the map was 1,802.8 cM with an average distance of 10.2 cM between adjacent markers. This genetic map was used for initial mapping of QTLs controlling source-sink traits in the same population. The dramatic differences in leaf and bean

characteristics and coffee cupping quality between these two parental cultivars make this segregating population an attractive resource for mapping QTLs controlling source-sink traits. A true F_2 population was developed and a new genetic map using the true F_2 population is currently under construction (Nagai and Ming, unpublished data).

B. Mapping Genes and QTLs Controlling Biotic Stress and Economic Traits

Mapping QTLs in coffee is more challenging than in annual diploid crops due to its longer generation time and the difficulty in producing and maintaining large mapping populations with multiple replications in the field. Mapping of major genes controlling disease resistance and self-incompatibility has been successful and has provided linked DNA markers for introgression and marker-assisted selection. Three RAPD markers were tagged to the *T* gene conferring resistance to coffee berry disease caused by *Colletotrichum kahawae* J.M. Waller & Bridge (Agwanda et al. 1997). These three markers were identified by screening 120 RAPD markers with resistant and susceptible cultivars and segregating progenies of the first and second backcrosses between the donor and the recurrent parent. The self-incompatible (*S*) locus in *C. canephora* was mapped on a short linkage group using AFLP markers and backcross populations derived from (*C. heterocalyx* × *C. canephora*) × *C. canephora* (Coulibaly et al. 2002). The *S* locus was 10 cM from a closely linked AFLP marker. Fructification time in coffee is an additive trait, and a major gene, *Ft1*, controlling this trait was located on a linkage group flanked by two tightly linked DNA markers with 1.7 and 3.3 cM, respectively (Akaffou et al. 2003). This gene also showed pleiotropic effect to lower caffeine content and seed weight. A major gene (*Mex-1*) from *C. canephora* conferring resistance to the root-knot nematode, *Meloidogyne exigua*, was identified using a modified bulk segregant analysis (Noir et al. 2003). *Mex-1* was mapped with 14 linked AFLP markers within an 8.2 cM interval. There are nine major dominant resistant genes (S_H–*1* – S_H–*9*) for coffee leaf rust (*Hemileia vastatrix* Berk. & Br.) conferring resistance either individually or in combination. A major leaf rust resistance gene (S_H–*3*) from *C. liberica* was located at the end of a short linkage group co-segregating with an AFLP marker (Prakash et al. 2004). The successful tagging of these major genes with AFLP markers confirmed their pronounced effects on specific traits and generated markers for coffee improvement programs.

Important economic traits, such as yield and cupping quality, are typical quantitative traits affected by accumulated small effects of

multiple genes that require large mapping populations tested at multiple sites and in multiple years. It is technically challenging as well as costly to carry out large-scale experiments fitting these requirements. Nevertheless, attempts have been made to map QTLs in smaller populations that would detect those with relatively large effect. Two QTLs were detected for pollen viability in backcross populations derived from (*C. heterocalyx* × *C. canephora*) × *C. canephora* (Coulibaly et al. 2003b). One QTL contributed to trigonelline accumulation in green coffee beans in reciprocal backcross populations derived from an interspecific cross between *C. pseudozanguebariae* and *C. liberica* var. dewevrei (Ky et al. 2000). As sequencing costs continue to decline and more sequence-specific DNA markers become available and affordable, large-scale QTL mapping experiments will be possible in order to target the complex traits involved in the production of high-quality coffee.

C. Construction of Large Insert Genomic Libraries

Large insert genomic libraries are essential genomic resources for positional cloning, physical mapping, integration of genetic and physical maps, and eventual complete sequencing of the genomes. Two bacterial artificial chromosome (BAC) libraries have been constructed for *C. arabica* and *C. canephora*. The *C. arabica* cultivar 'IAPAR 59' was used for BAC library construction for its resistance to leaf rust and root-knot nematode (Sera 2001). This commercial cultivar, widely cultivated in Latin America, was derived from a spontaneous interspecific hybrid between *C. arabica* and *C. canephora* known as the 'Timor hybrid', and selfed for five generations. The BAC library consists of 88,813 clones with an average insert size of 130 kilobases (kb), representing about eight *C. arabica* genome equivalents (Noir et al. 2004). Fifteen low-copy RFLP probes on 11 chromosomes of the *C. canephora* genome were used to characterize the BAC library. Twelve of the 15 probes hybridized to two loci or two alleles of the same locus from the two subgenomes of the allotetraploid *C. arabica* genome, while the other three probes hybridized to only one allele. This BAC library was obtained from four ligation reactions with different average insert sizes, and 55% of all BAC clones contained insert sizes above 200 kb, which is particularly useful for physical mapping to identify a minimum tiling path for BAC by BAC sequencing of the genome.

The BAC library of *C. canephora* was constructed from genotype 126 and contains 55,296 clones with an average insert size of 135 kb, representing about nine genome equivalents (Leroy et al. 2005). This BAC library was used to study the genomic organization of genes on the

sucrose biosynthesis pathway and to clone one of the two sucrose synthase genes (*CcSUS1*). Additional genes with favorable agronomic and organoleptic characteristics in the genotype 126 can be discovered as coffee genomic research moves forward.

D. Coffee Expressed Sequence Tags

Large-scale genome projects on a variety of plants, animals, and microbes have yielded vast amounts of genomic sequence and expressed sequence tags (ESTs) that have revolutionized genetic and genomic research. In the coffee research community, several EST sequencing projects have been conducted in Brazil, Italy, Colombia, and the United States. The Brazilian EST sequencing project yielded 33,000 unigenes from 214,916 single-pass EST sequences (Vieira et al. 2006). This large collection of ESTs was generated from 37 cDNA libraries of *C. arabica*, *C. canephora*, and *C. racemosa*, and resulted in 130,792, 12,382, and 10,566 clean sequences for each species, respectively. Additional EST resources include 13,175 unigenes from 46,914 ESTs of *C. canephora* from S. D. Tanksley's team at Cornell University in the United States (Lin et al. 2005) and 1,512 unigenes from 2,696 ESTs of *C. arabica* from G. Graziosi's team at the University of Trieste in Italy (www.coffeedna.net).

Detailed comparative genomic analysis revealed a greater degree of similarity on DNA sequences and gene inventory between coffee and tomato than between coffee and *Arabidopsis* genomes (Lin et al. 2005). About 21% of the coffee unigenes had no match in the *Arabidopsis* genome, but 90% of these unmatched unigenes have a match in the Solanaceae unigene collection that represent about 75% of the genes in the Solanaceae genome. Moreover, the DNA sequences of coffee unigenes shared greater similarity with Solanaceae homologs than with *Arabidopsis* homologs. These results are consistent with the close phylogenetic relationship between coffee and tomato that shared a common ancestor 89 million years ago, while coffee and *Arabidopsis* diverged 125 million years ago (Wikström et al. 2001).

IV. BREEDING

A. Historical and New Trends

The history of *C. arabica* introduction into Latin America explains the narrow genetic base of most commercial *C. arabica* cultivars to this

continent. These varieties are characterized by a number of desirable homogenous traits, such as high and stable yield and good quality, but at the same time they also present disadvantages, such as high susceptibility to a wide range of diseases and insect pests and low adaptability to specific agro-ecological conditions. The constant threat caused by the emergence of new strains of existing diseases makes it essential to broaden the genetic base of modern varieties.

For more than a century, coffee has been an export crop of many Latin American countries, accounting for up to one-third of export earnings (Rice and Ward 1996). In the past 20 years, farmers in Latin America were advised to convert to more intensive production systems, based on high-yielding coffee varieties, growing without shade, but requiring high inputs of fertilizers and pesticides. The increase in yield obtained with new varieties, such as 'Caturra' and 'Catuai', was generally accompanied by a slight decrease in coffee quality compared with the traditional varieties 'Typica' and 'Bourbon' (Bertrand et al. 1999). With a clear rise in world coffee production in recent years and concomitant stagnation of consumption, excessively low coffee prices prevailed. These low prices meant that many producers have been barely able to recover their production costs, leading in consequence to a reduction of labor-intensive management practices up to the total abandonment of coffee plantations.

However, the gourmet and specialty coffee sectors have consistently been the fastest-growing international coffee markets and are expected to continue to expand by 5% to 10% per year (Giovannucci 2001). Only a few countries have endeavored to make use of these niche markets, which address consumer preferences for single-origin specialty products, high-grade gourmet blends, flavored coffees, and organically grown coffees. Based on consumer demands, these markets are placing special emphasis on sustainable and environmentally friendly production systems. Organic, shade, and Fair Trade coffees are collectively known as sustainable coffees (Giovannucci 2001); these coffees are rewarded with a substantial premium price for being produced in an environmentally friendly and socially responsible manner. A survey conducted among retailers in the United States, representing the largest specialty coffee market in the world, revealed that purchasing decisions were mainly based on coffee quality rather than price and customer demand. Consistency in supply ranked second in importance (Giovannucci 2001).

Given the high and further increasing labor costs in Latin American countries, such as Costa Rica, a major shift from high-yielding, average-quality coffee varieties to gourmet or specialty coffee varieties, which

are suited for sustainable coffee production systems, is required. To address these market needs, coffee breeding programs have to re-evaluate their priorities, moving away from yield increase as number-one objective toward the selection and creation of varieties with high cup quality and a broader genetic base. The latter is required to enable the integration of resistance genes for the genetic control of prevalent diseases and pests, thus paving the way for more environmentally friendly or organic production systems. According to van der Vossen (2001), coffee breeders are faced with the challenge of making substantial contributions to:

- High bean and cup quality in *C. arabica*, including caffeine-free coffee.
- Lower production costs through easier harvesting (compact growth), higher yields per hectare, and reduced disease and pest control by host resistance.
- Ecologically grown coffee through zero or minimal pesticide use in disease- and pest-resistant varieties.

B. Exploiting Genetic Diversity

The wild and semi-domesticated genotypes collected in Ethiopia and conserved in CATIE's collection present high variability in traits that are of special interest to breeders, such as fertility, bean size, and yield of green coffee (Anthony et al. 1999). Incomplete resistance to coffee leaf rust was also observed in genotypes from Ethiopia (Gil et al. 1990), as well as resistance to *Meloidogyne incognita* (Anzueto et al. 2001). Thus, the wild genotypes and diploid species of CATIE's collection are extremely valuable for the breeding of new varieties: They constitute a reservoir of valuable genes for disease resistance and special flavors and are characterized by a high-combining ability in crosses with traditional commercial varieties. An average heterosis effect of 30% has been reported (Bertrand 2002).

Speciation in the coffee gene pool was not accompanied by the development of strong crossing barriers. This offers good prospects for the introgression of desirable characteristics from wild into cultivated species for the development of new cultivars with host resistance against major diseases and pests or tolerance against drought, low temperatures, or flooding. Markers are already being developed for the identification of genes controlling the resistance to coffee berry disease (Agwanda et al. 1997) and nematodes (Noir et al. 2003).

1. Disease Resistance. As early as 1868, leaf rust disease had devastated coffee plantations in Sri Lanka and led to their replacement with tea (Medina-Filho et al. 1984). The disease reached the northeastern coast of Brazil in 1970 and from there spread rapidly to other South and Central American countries, given the high genetic uniformity of cultivated coffee in the Americas. Leaf rust resistance is frequently observed in *C. canephora* and *C. pseudozanguebariae*, but can also be found in *C. liberica*, *C. eugenioides*, and *C. salvatrix* (Rodrígues Jr. et al. 1975).

As mentioned earlier, nine major dominant resistant genes (S_H–*1* – S_H–*9*) have been identified conferring leaf rust resistance either individually or in combination. The alleles S_H–*1*, S_H–*2*, S_H–*4*, and S_H–*5* have been traced in *C. arabica* germplasm of Ethiopian origin (Medina-Filho et al. 1984), while the S_H–*3* gene seems to have introgressed from *C. liberica* into *C. arabica*, as shown by Prakash et al. (2004) in a F_2 progeny derived from a cross between 'Matari' (*C. arabica*) and the *C. liberica*-introgressed line 'S.288' with a segregation ratio of 3:1, indicating single dominance. Linkage analysis revealed that 23 markers were strongly associated with the S_H–*3* resistance gene and grouped together in a short single linkage group on chromosome fragments introgressed from *C. liberica*. The resistance gene S_H–*6* is linked to the 'Timor Hybrid', the product of a spontaneous natural hybridization between *C. arabica* and *C. canephora*, having the phenotype and genotype from *C. arabica* but showing resistance to all types of leaf rust (Illy and Viani 2005).

Cultivars with horizontal, polygenic coffee leaf rust resistance are progressively replacing traditional cultivars in some countries. For example, cultivars selected from 'Catimor' and 'Sarchimor' populations have resulted in the use of the 'Colombia' cultivar in Colombia, 'IAPAR 59' in Brazil, 'IHCAFE 90' and 'CR 95' in Central America, and 'Cauvery' in India (Illy and Viani 2005). Similarly, 'Icatu', developed in Brazil, originated from an artificial hybrid between *C. arabica* 'Bourbon' and a tetraploid *C. canephora*. This hybrid was backcrossed with the *C. arabica* 'Mundo Novo' and/or 'Catuai amarillo'. 'Icatu' is also considered as a source of resistance for coffee berry disease and several nematodes (Bertrand et al. 1999). 'Ruiru 11' in Kenya and 'Ababuna' in Ethiopia are hybrids carrying resistance genes against coffee berry disease (Illy and Viani 2005; van der Vossen 2001).

Coffee berry disease (CBD) first infected *C. arabica* coffee plantations in Kenya and seems to have originated from *C. eugenioides*, which is indigenous to the mountain forests in western Kenya and Uganda (Illy and Viani 2005). Latin American countries are still free from the disease. The control of CBD by fungicide sprays may account for one-third

of the total production costs, while crop losses due to CBD may still occur at higher elevations during periods of excessive rains (van der Vossen 1987). Thus, only cultivars with durable CBD resistance can provide sustainable cash income to coffee farmers in Africa. Resistance can be found in *C. canephora*, the Kenyan cultivar 'Ruiru 11', 'Rume Sudan', and 'Timor hybrid'. The resistance is controlled by two major genes in 'Rume Sudan' (a dominant *R* and recessive *K* gene) and by one gene (the *T* gene with incomplete dominance) in the 'Timor hybrid' (van der Vossen and Walyaro 1980). Other cultivars, such as 'K7' and 'Blue Mountain', show moderate resistance, which is controlled by the same recessive gene (K gene) of 'Rume Sudan'. Recently, three RAPD markers could be linked to the *T* gene in the 'Timor hybrid', one of the three genes responsible for CBD resistance (Agwanda et al. 1997). Studies on populations of *C. arabica* × *C. canephora* hybrids confirmed the presence of CBD resistance in *C. canephora* coffee (Carvalho et al. 1976). Thus, the resistance gene found in the 'Timor hybrid' might have introgressed from *C. canephora* (van der Vossen and Walyaro 1980).

2. Nematode Resistance. Root-knot nematodes of the genus *Meloidogyne* constitute a major threat in most countries where *C. arabica* is grown. Among the most damaging species is *M. exigua*, which is widespread in Latin America (Campos et al. 1990; Noir et al. 2003). All commercial cultivars grown in Central America ('Typica', 'Bourbon', 'Caturra', 'Catuai', 'Costa Rica 95', and 'IHCAFE90') are susceptible to *M. exigua* (Bertrand et al. 2001). The long-term economic feasibility of nematicide applications is questionable and not a viable option for sustainable and environmentally friendly production systems. The most promising option would be the selection and breeding of resistant genotypes. While *C. arabica* is highly susceptible to root-knot nematodes, resistance has been reported in *C. canephora* accessions (Curi et al. 1970; Bertrand et al. 2001). Studies undertaken by Bertrand et al. (2001) revealed immunity to *M. exigua* in more than 78% of the *C. canephora* plants tested and resistance in 100% of the plants. Similarly, the 'Timor hybrid' was found to be resistant to *M. exigua* (Bertrand et al. 2001). In a multi-institutional effort among IRD, CATIE, and other partners, it was shown that the resistance to *M. exigua* is controlled by a simply inherited major gene (*Mex*-1) with incomplete dominant expression (Noir et al. 2003). This conclusion was based on an extensive segregation analysis carried out in a large F_2 population at CATIE. Inoculation studies conducted in the same institution revealed that nematode multiplication is possible in resistant tissues, but at a very low rate compared with susceptible tissues (Anthony et al. 2005). Fourteen AFLP markers were

found to be associated with the resistance to *M. exigua* (Noir et al. 2003). Resistance to root-knot nematodes can also be introgressed into *C. arabica* from *C. bengalis*,*C. congensis*, *C. dewvrei*, *C. eugenioides*, *C. kapakata*, *C. liberica*, *C. racemosa*, *C. salvatrix*, and *C. stenophylla* (Medina-Filho et al. 1984; Illy and Viani 2005).

For many years, *C. canephora* rootstocks have been successfully employed in *C. arabica* cultivars in Guatemala to control *Pratylenchus* and *Meloidogyne* nematodes (Bertrand et al. 1999). *C. arabica* cultivars grafted on *C. canephora* rootstocks presented four times higher productivity than ungrafted *C. arabica* cultivars (Villain et al. 1999). *C. canephora* rootstocks have been shown to be highly tolerant against *Pratylenchus* sp. (Villain et al. 1996), but seem to be much less efficient against *M. incognita*, which is prevalent in Guatemala (Bertrand et al. 1999).

In recent years, progress has been made in Central America with the selection of specific *C. canephora* rootstocks for *C. arabica* cultivars that are highly resistant against several *Meloidogyne* species (Bertrand et al. 2000). Crosses made between two *C. canephora* accessions of CATIE's collection (T3561 and T3751) resulted in the cultivar 'Nemaya', which is highly resistant against *Meloidogyne* sp. of El Salvador, *M. incognita* of Guatemala, as well as *M. exigua* and *M. arabicida* of Costa Rica (Bertrand et al. 1999). While among unselected *C. canephora* rootstocks only 35% of the plants are resistant against the prevalent nematodes in El Salvador and Guatemala, the 'Nemaya' cultivar raises the level of resistant plants to 80%. Concerning *M. exigua* and *M. arabicida*, 'Nemaya' reaches resistance levels of 90% and 80%, respectively. The 'Nemaya' cultivar is currently being propagated and in high demand in PROMECAFE (Programa Cooperativo para la Protección y Modernización del Cultivo de Café en México, América Central, Panamá y la República Dominicana) member countries. At medium (800–1,000 meters [m]) to high altitudes (>1000 m), the lower temperature range negatively affects *C. canephora*, resulting in yield losses of 10 to 20% in areas with low nematode infestation, and the 'Nemaya' cultivar is therefore not recommended (Bertrand et al. 1999).

Where *Pratylenchus* sp. is not a problem, nematode-resistant 'Catimors' could be used as rootstocks at higher elevation instead, without the negative effects on vigor and yield as observed with 'Nemaya' rootstocks. Selections of the interspecific hybrid 'Arabusta' (developed in the Ivory Coast by crossing a tetraploid *C. canephora* with *C. arabica*) with the aim of improving the quality of *C. canephora* (van der Vossen 2001), could offer another alternative. If 'Arabusta', having one parent from the cultivar 'Nemaya', is crossed with a *M. incognita*– and *M. arabicida*–resistant Ethiopian *C. arabica* tree, the resulting hybrid

should be nematode resistant and could serve at high altitudes as rootstock for commercial *C. arabica* cultivars. Chromosome duplication of the 'Nemaya' parents has already started at CATIE as a basis for crosses with nematode-resistant *C. arabica* genotypes (Bertrand et al. 1999).

3. Other Types of Resistance or Tolerance. Most modern distributed *C. arabica* cultivars, as well as *C. canephora* and *C. congensis*, are susceptible to leaf miner in the genus *Leucoptera* (Medina-Filho et al. 1984). Only the *C. arabica* cultivar 'Mokka' shows limited resistance. Immunity to the leaf miner is found in *C. stenophylla*, while resistance has been reported for *C. racemosa*, *C.eugenioides*, *C. kapakata*, and *C. dewevrei* (Medina-Filho et al. 1984; Illy and Viani 2005). As *C. arabica* cannot be crossed with *C. stenophylla*, *C. canephora* could serve as a bridge to transfer leaf miner immunity from *C. stenophylla* to the *C. arabica* genome.

Global warming has added a new objective to coffee breeding programs: the need to focus on drought tolerance. In Brazil, the largest coffee producer worldwide, drought is considered as a principal environmental stress factor having great impact on coffee production (DaMatta 2004). Drought and high temperature tolerance has been reported for *C. racemosa* (Guerreiro Filho 1992). The species *C. stenophylla* is also well adapted to dry conditions (Illy and Viani 2005). *C. arabica* is characterized by a deeper root system and, therefore, is more drought resistant than *C. canephora* (Illy and Viani 2005). Within *C. arabica*, good candidates for drought tolerance seem to be some East African cultivars like 'SL28' due to a well-developed root system, outstanding plant vigor, and an ability to retain their leaves under water-stress conditions (van der Vossen and Browning 1978). Indian lines of *C. arabica* and crosses of these lines with the modern cultivars 'Mundo Novo' and 'Catuai' are also reported to be drought-tolerant (Medina-Filho et al. 1984). Progenies of *C. arabica* × *C. racemosa* hybrids are highly resistant to drought, highlighting the value of *C. racemosa* germplasm in breeding programs.

Low-temperature tolerance can be found in *C. liberica* (Ahmad and Vishveshwara 1980, cited by Anthony et al. 1999), and flooding adaptation can be found in *C. congensis*. This has been exploited in Madagascar with the 'Congusta' hybrids, resulting from spontaneous hybridization between *C. canephora* and *C. congensis* (Charrier 1972). *C. humilis* is also known to grow well in wet environments (Charrier and Berthaud 1985).

Uniform flowering and fruit ripening can be imparted from *C. racemosa* (Illy and Viani 2005). Under severe drought, this species drops all

its leaves. Regrowth starts with the beginning of the rains, leading to a uniform flowering and berry ripening, which together with compact growth and ease to induce berry abscission, are important selection criteria for mechanical harvesting as practiced in Hawaii.

4. Low Caffeine Content. There are significant differences in the caffeine content among *C. arabica* cultivars. While the leaf caffeine content of *C. laurina* is approximately 23% less than in the commercial cultivar 'Catuai', the difference in the bean caffeine content is approximately 54% less than in 'Catuai' (Illy and Viani 2005). *Coffea salvatrix* also shows low caffeine content (0.71%). Recently, an Ethiopian *C. arabica* cultivar has been reported to have close to zero caffeine content in Brazil (Silvarolla et al. 2004); this plant had been obtained from the CATIE coffee collection, and had been deposited at CATIE after the FAO 1964–1965 coffee mission to Ethiopia. In the study by Silvarolla et al. (2004), three plants out of one accession composed of 12 plants showed an average caffeine content of 0.076 % dry weight compared to 1.2 % dry weight of the widely grown 'Mundo Novo' cultivar. No caffeine synthase activity could be detected in the leaves of mutated plants leading to an accumulation of theobromine, the precursor of caffeine. Genes involved in the caffeine biosynthesis pathway have been identified (Moisyadi et al. 1998; Kato et al. 2000; Ogawa et al. 2001; Uefuji et al. 2003).

Mazzafera and Carvalho (1992) found very low to low seed caffeine content in diploid F_1 hybrids of *C. eugenioides* × *C. salvatrix* and in tetraploid hybrids of *C. arabica* × *C. salvatrix* and *C. arabica* × *C. eugenioides*. The development of low-caffeine or caffeine-free cultivars would be of enormous economic potential. Seven to 10 million bags of green coffee are used annually for the costly process of decaffeination. Water and organic solvents are required to remove caffeine from the coffee beans before they are roasted. Decaffeinated coffee beans are sensitive to molds (Illy and Viani 2005). Uneven bean drying may lead to earthy flavors from microorganism spoilage and to the formation of mycotoxins. Moreover, overdrying renders the beans brittle, leading to high losses during the roasting process.

C. Propagation Systems

1. Seed Propagation. Propagation by seed is common practice in most coffee breeding programs worldwide (van der Vossen 2001). Seed multiplication of *C. arabica* F_1 hybrids requiring hand pollination of previously emasculated and bagged flowers has been successfully applied on a large scale in Kenya for the propagation of the leaf rust– and coffee

berry disease–resistant hybrid 'Ruiru 11'. The production cost of 1 kilogram hybrid seed through emasculation and hand pollination has been estimated at US $55 (Bertrand et al. 1999). Another interesting alternative is the use of male sterility, which has been detected in five coffee trees of CATIE's *C. arabica* germplasm collection. This would significantly lower seed production costs of hybrids. Male sterility is controlled by one recessive gene (Dufour et al. 1997). Introgressive breeding efforts have started to produce pure lines of 'Costa Rica 95' and 'IAPAR 59' based on male sterility (Bertrand et al. 1999). Genetic transformation could be an option to shorten the time required to produce male-sterile female parents for seed production (van der Vossen 2001).

2. Clonal Propagation. A clonal mass propagation system for coffee based on high-frequency somatic embryogenesis and cultivation in liquid medium using temporary immersion has been developed at CATIE (Etienne-Barry et al. 1999; Barry-Etienne et al. 2002a, b). A culture density of 1,600 somatic embryos per 1-L bioreactor and the addition of high levels of sucrose two weeks before direct sowing of the embryos into soil resulted in a 78% plant conversion rate, followed by vigorous plant growth (Etienne-Barry et al. 1999). Barry-Etienne et al. (2002b) reported differences in morphological development from bioreactor-produced embryos that were still evident 40 weeks after planting in the greenhouse. The use of this bioreactor-based clonal mass propagation method allows for large-scale rapid multiplication of high-quality genetic material, as evidenced by the recently completed mass propagation of 100,000 clonal coffee plantlets in CATIE's tissue culture laboratory. Although differences in genotype response were noted (unpublished data), all 19 hybrids of the PROMECAFE-CIRAD-CATIE regional breeding program were successfully multiplied and transferred to the Instituto de Café de Costa Rica (ICAFÉ) for trials on multiple sites. Coffee micropropagation in bioreactors has been recently reviewed by Etienne et al. (2006). Using a different liquid culture propagation method, Ducos et al. (2003) have reported no differences in *C. canephora* plant growth when comparing 5,067 trees derived from liquid culture somatic embryos to 548 trees derived from microcuttings. These were planted in the field in the Philippines and in Thailand.

3. F_1 Hybrids. Hybrid vigor, or heterosis, has been observed in many intra- and interspecific crosses, resulting in increased yield, better yield stability, and improved adaptation of the F_1 hybrids to suboptimal growing conditions (van der Vossen 1987, 2001; Bertrand et al. 1999).

Better performance under suboptimal climate and soil conditions is seen as a major advantage of hybrids over pure lines by Bertrand et al. (1999), confirming earlier reports of Mónaco and Carvalho (1964). The yield superiority over 'Caturra' observed in the 'Catimor' cultivars 'CR 95' and 'IHCAFE 90', resulting from a cross between 'Caturra' (CIFC 19/1) and 'Timor hybrid' (CIFC 832/1), can be explained by a higher number of berries per node in 'CR 95' and increased vigor in 'IHCAFE 90' (Bertrand et al. 1999).

Ameha and Belachew (1985) observed a heterosis effect of 36% to 60% in Ethiopia when indigenous wild genotypes were hybridized. In Costa Rica an average heterosis effect of 30% was observed in 'Sarchimor' and 'Catimor' hybrids when compared with the best parent (Bertrand et al. 1997). However, hybrid vigor often expresses itself also in an increase in total biomass, requiring more space per plant, thus reducing the number of plants per hectare in high-density plantations. Nevertheless, increased plant vigor is a considerable advantage under suboptimal growing conditions. Variations in bean size and cup quality parameters of F_1 hybrids are mainly due to additive genetic effects (van der Vossen 1987). F_1 hybrids tend to produce a higher percentage of defective beans (peaberries and empty berries) than cultivars derived from breeding lines (van der Vossen 1987; Bertrand et al. 1997). Within-family selection of the best plants for clonal multiplication is recommended to minimize this problem.

Intraspecific hybridization was used as strategy to broaden the genetic base of new *C. arabica* cultivars and to accumulate in one genotype resistance genes against various diseases and pests in the context of a regional collaborative breeding program between PROMECAFE, CIRAD, and CATIE, initiated in 1991. This regional breeding program selected 19 F_1 hybrids resulting from crosses made between wild genotypes from CATIE's collection, which originated from Ethiopia, Sudan, Kenya, and Yemen, and commercial varieties ('Caturra', 'Catuai'), as well as 'Catimors' and 'Sarchimors'. After four years of evaluation in Costa Rica, Honduras, El Salvador, and Guatemala, 3 out of the 19 F_1 hybrids are about to be released to farmers for commercial production. Two breeding lines (LI_L13A44 and LI_L12A28) were derived from the same cross T05296 × 'Rume Sudan'; the third breeding line resulted from a cross between 'Caturra' and the Ethiopian genotype T-16725 or ET-41 (FONTAGRO-PROMECAFE 2006). The coffee trees of these three clones are of the dwarf type, but clearly more vigorous than the traditional commercial varieties and show an average yield increase of up to 150%. Bean size of the selected clones is comparable or slightly superior to the traditional varieties, but the percentage of peaberries and empty

grains is higher. At medium to high altitudes, the organoleptic quality of the coffee is equal to the commercial varieties. At lower elevations, there are indications of improved cupping quality of the selected clones, but this needs to be validated in further trials.

D. Future Based on Biotechnology

The progress in coffee genomics has provided a wealth of data and has revealed important characteristics for both *C. arabica* and *C. canephora* genomes. A collection of genomic resources and molecular tools is available to coffee breeders and geneticists to assist and advance their research and development programs. Future advancements and additional EST and genomic sequencing will provide increasingly practical tools for coffee breeders to develop coffee cultivars with improved quality and enhanced disease and pest resistance. Research efforts in these areas will have immediate and lasting impact on coffee genomics programs and in crop improvement:

- Generate low-coverage whole-genome shotgun sequence of the *C. arabica* genome in order to: (1) provide a large collection of sequence-based DNA markers, such as SSRs and SNPs; (2) overcome the low rate of polymorphism among the majority of the *C. arabica* coffee varieties for genetic and QTL mapping and cultivar identification; (3) increase the gene inventory complementing to the EST unigenes; and (4) provide genomic sequences of regulatory elements of expressed genes.
- Identify specific *C. arabica* DNA markers through genome-wide scanning of thousands of markers and apply these markers for cultivar verification using DNA samples extracted from green beans or even roasted beans (Martellossi et al. 2005).
- Construct high-density linkage maps of the *C. arabica* and *C. canephora* genomes using sequence-based anchor markers as frame scaffolds and high-throughput random DNA markers such as AFLPs to fill the gaps.
- Develop the capacity to scan whole coffee genomes for QTLs controlling economic traits through the use of saturated genetic maps and microarrays of unigene collections.
- Integrate coffee genetic and physical maps with most of the abundant EST on the map to generate the ultimate tools for QTL mapping and gene isolation.
- Uncover the principle of genome evolution in allotetraploid coffee and the mechanism causing the DNA content discrepancy between *C. arabica* (1,176 Mbp) and its progenitors *C. canephora* (809 Mbp) and *C. eugenioides* (662 Mbp) (Kiehn 1986; Marie and Brown 1993).

Various interesting questions could be answered with the help of molecular biology tools, including: (1) How does *C. arabica* 'Maragogipe', discovered in the state of Bahia (Brazil), in 1870 (Jones 1956) and believed to be a mutant of *C. arabica* 'Typica', compare to other *C. arabica* varieties? 'Maragogipe' coffee beans are also known as elephant beans due to their enormous size. Could the molecular basis for this attribute be identified?; (2) Do truly "wild" *Coffea* species still occur in Ethiopia (Meyer et al. 1968)? This question might be answered by comparing genetic material from plants found in the forests to that of plants cultivated in nearby areas; (3) How does "wild" *C. canephora* germplasm (e.g., that found at the Kibale National Park in Uganda; Oryem-Origa et al., 2004) differ from cultivated varieties closeby and elsewhere?; and (4) What genes do *C. arabica* share with *C. canephora*?

In terms of coffee germplasm, it is imperative for the coffee industry to realize the vital importance of these collections and to actively search for funding mechanisms aimed at ensuring their long-term survival. A combination of sustained germplasm conservation and genomics research is essential for the future of the coffee industry.

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